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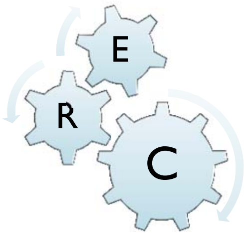
Discipline : Ecologie

Habilitation à Diriger des Recherches



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Comprendre pour Evaluer et Restaurer, Restaurer et Evaluer pour mieux Comprendre

Faculté des sciences de la vie
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TOME 2

Université de Strasbourg

Habilitation à Diriger des Recherches

ERC : Evaluer, Restaurer, Comprendre.
Comprendre pour Evaluer et Restaurer,
Restaurer et Evaluer pour mieux Comprendre

Présentée par :

Isabelle COMBROUX

TOME 2

Ce second volume compile une partie des publications et réalisations listées dans la partie « Curriculum vitae » du tome 1 de mon mémoire d'habilitation à diriger des recherches.

Ces publications reflètent l'évolution et la diversité de mon travail permettront au lecteur d'aller plus loin que les figures présentées dans le tome 1.

Ce volume s'organise en cinq parties :

- **Ecologie des perturbations dans les annexes du Rhône et de l'Ain**
- **Chenalisation de petites rivières de plaine**
- **Restauration du système fluvial Rhénan**
- **Ecologie des rivières et zones aquatiques de la plaine alluviale Ello-Rhénane**.
- **Autres publications**

Certaines publications ne s'insèrent pas directement dans les champs précédemment cités et sont présentées ici

Dessin de couverture: C Heinrich - Terre Sauvage N°251 - juil 2009 – p. 48

☙ Ecologie des perturbations dans les annexes du Rhône et de l'Ain

Combroux I., Bornette, G., Willby, N.J. & Amoros C. (2001). Regenerative strategies of aquatic plants in disturbed habitats: the role of the propagule bank. Archiv für Hydrobiologie,152:215-235.

Combroux I., Bornette, G. & Amoros C. (2002). Plant regenerative strategies after a major disturbance: the case of a riverine wetland restoration. Wetlands, 22:234-246.

Combroux I. & Bornette, G. (2004). Propagule bank and regenerative strategies: a comparison between drought and spate disturbances. JVS,15 : 13-20.

Trémolières M., **Combroux I**., Thiébaut G., Haury J., (2008). Réponse des communautés végétales aux conditions environnementales : perturbations et contraintes. Ingénieries eau Agriculture et Territoire. N° special 63-78.

Arch. Hydrobiol. 152 2 215–235 Stuttgart, September 2001

Regenerative strategies of aquatic plants in disturbed habitats: the role of the propagule bank

I. Combroux[1]*, G. Bornette[1], N. J. Willby[2] and C. Amoros[1]

With 4 figures and 4 tables

Abstract: Recovery of plants from disturbance is frequently associated with the presence of an extensive seed bank. In a cut-off channel of the Rhône River subjected to fluctuating water levels and scouring forces during flash floods, regenerative strategies of aquatic plants were investigated through the study of the sediment propagule bank and its comparison with the established vegetation. Within this channel, the zone disturbed only by scouring floods (zone F) had a small propagule bank (as assessed by greenhouse germination trials), whereas more propagules regrew in zone FE which was subjected to both scouring floods and episodic sediment emersion. There, regrowth was mostly from sexual propagules, with the stonewort *Chara vulgaris* being the dominant component. In zone F, no relationship between propagule bank composition and established vegetation was demonstrated. In zone FE, a strong positive relationship between vegetative propagules (buds + rhizomes + fragments) and the established vegetation occurred. The disturbance history of the site indicated that the flowering and seed production of most species was disrupted by the flood regime, the result being that only one third of the hydrophyte species present in the established vegetation were present in the seed bank. Consequently, in this habitat, resistance type regenerative strategies, involving fragments and rhizomes, were adopted by most species. Resilience regenerative strategies involving seed- or bud-banks, together with resistance strategies involving the development of a terrestrial growth form, were adopted by plants that had to face both floods and sediment emersions. The precise nature of disturbance appears to affect regenerative strategies of aquatic plants and merits greater attention in studies of trait–environment relationship. The diversity of regenerative strategies within and between aquatic plant species is likely to contribute significantly to the underlying stability of vegetation in flood disturbed habitats.

Key words: Recovery of aquatic plants, seed-banks, floods, sediment emersions, Rhône River.

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Introduction

Many studies have recognized that plants evolve sets of life-history traits in response to environmental restraints (Boutin & Keddy 1993, Hills et al. 1994, Shipley et al. 1989). Disturbances are important structuring forces of plant communities (Collins et al. 1995, White & Pickett 1985), and several models predict plant strategies in disturbed habitats (Grime 1979, Kautsky 1988, Noble & Slatyer 1980, Taylor et al. 1990, Weiher et al. 1999). Although these models consider that the ability to recover from disturbances (i.e. resilience) is a key factor for population maintenance in disturbed habitats, studies of regenerative strategies have been poorly developed (Grime & Hillier 1992, Noble & Slatyer 1980, Shipley et al. 1989).

There is a general view that disturbances favour regeneration through sexual propagules (small, light seeds, abundantly produced) (Grime 1979, Noble & Slatyer 1980, MacArthur & Wilson 1967). A slight amendment to this pattern was proposed by Rørslett (1989) for lake macrophytes. He suggested that aquatic ruderals should present high annual seed production, but associated with vegetative reproduction and extensive bud or propagule formation. Kautsky (1988) also pointed out some differences in life strategies between terrestrial and aquatic plants, as for example "the vegetative expansion through clonal growth [that] seems to be the most common regenerative strategy in aquatic phanerogams together with different types of vegetative diaspores, whereas sexual reproduction plays a minor role compared with terrestrial plants". However, in her four strategies model, ruderals were characterized by the absence of vegetative propagules, with perennation occurring through dormant seeds or zygotes and the storage of photosynthetic mineral nutrients confined to seeds or zygotes. Amoros & Bornette (1999) emphasized that vegetative reproduction should be favoured in macrophytes in case of scouring floods because "sexual reproduction remains uncertain, as disturbance events could occur at any time in the year".

Since aquatic plants use both vegetative and sexual propagules (Barrat-Segretain et al. 1998, Kadono 1984, Klein & Carbiener 1989, Spencer & Ksander 1992), they offer an interesting subject for the study of regenerative strategies in disturbed habitats.

Piedmont rivers are characterized by peak floods lasting usually less than 5 days and having a high potential for bed scouring (Müller 1995, Ward et al. 1999). In cut-off channels of such rivers, floods have been demonstrated to act as disturbances, since overflowing water reaches high velocities (>1.5 m/s), breaks and uproots aquatic plants, and scours substratum (Bilby 1977, Müller 1995). These flood disturbances occur stochastically several times during the yearly cycle, and may thus disrupt plants any time in their life-cycle (Bilby 1977, Wiegleb et al. 1989).

The aim of the present study was to assess whether such a disturbance regime favours regenerative strategies involving sexual reproduction and seed banks rather than vegetative reproduction, as is usually expected for ruderal plants. This hypothesis was tested through the comparison of the established vegetation and the sediment propagule bank (both sexual and vegetative propagules, as defined by ABERNETHY & WILLBY 1999) in a flood-disturbed cut-off channel, where strategies to face scouring floods have been established for at least 20 years, and where the patchy vegetation pattern has remained unchanged since 1982 (BORNETTE & AMOROS 1996).

Materials and methods

Study site

The study site was a 2.7 km-long cut-off channel of the Rhône River (45° 48′ N, 5° 10′ E), located 30 km upstream from Lyon (France). When its discharge exceeds 1,000 m^3/s the river overflows into this usually disconnected channel (i.e. nine times a year in average, Table 1 and Fig. 1). The Upper Rhône River is a piedmont river, characterized by peak floods (BRAVARD & GILVEAR 1996, MÜLLER 1995, WARD et al. 1999). The average duration of a flood is four days (Compagnie Nationale du Rhône, unpubl. data, Table 1). The flow velocity can reach 1.6 m/s in the cut-off channel during such events (S. QUIGNARD, unpubl. data).

The downstream part of the cut-off channel (length 520 m, zone F) is never dewatered, whereas the upstream part (length 220 m, zone FE) is episodically submitted to a decrease in the water level that leads to an emersion of the sediment. This sediment emersion occurs when the river discharge falls below 300 m^3/s (Fig. 1). The average duration of such an event is 3.5 days (Compagnie Nationale du Rhône, unpubl. data, Table 1). The duration of this sediment emersion leads to the death of vegetative parts of aquatic plants but is too short to allow the development of a new terrestrial plant community. Scouring floods and/or sediment emersions occur stochastically at any time of the year in this former channel, due to the mixed glacial and rainfall origin of the water of the Rhône and Ain Rivers (Fig. 1). This former channel was thus favourable for testing our hypothesis.

Sampling stations were distributed along the channel, some being only exposed to scouring floods (zone F), and the others exposed to both scouring floods and episodic sediment emersions (zone FE). Physico-chemical characteristics of the two zones do not differ significantly (BORNETTE et al. 1998, ROSTAN et al. 1987).

Propagule bank sampling

Propagules in the bank were quantified by the seedling emergence method (BIGWOOD & INOUYE 1988, GROSS 1990, THOMPSON & GRIME 1979), which is usually considered as the best method to assess the role of the seed bank in a community (HAAG 1983, KAUTSKY 1990). Five stations were sampled in zone F and five in zone FE (Fig. 2) in

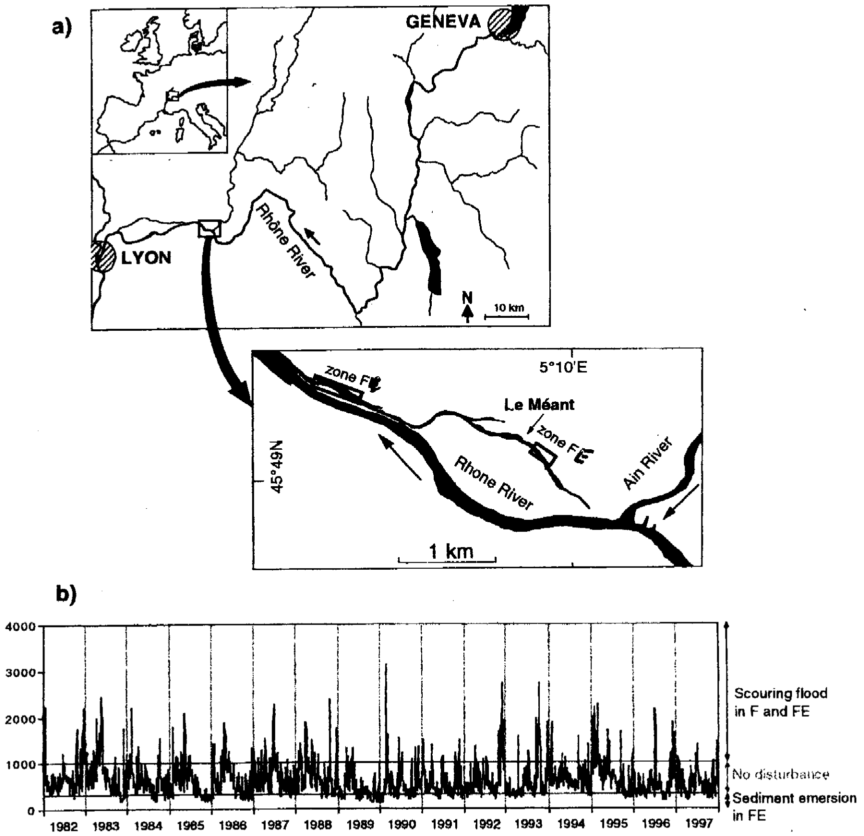

Fig. 1. **(a)** Study site location. **(b)** Daily discharge of the Rhône River from 1982 to 1997. The discharge that cause the overflow of the river in the cut off-channel and the one below which the upstream part of the cut-off channel is dewatered are indicated.

Table 1. Patterns of floods and sediment emersions in the cut-off channel under study (F: flood disturbed zone, FE: zone disturbed by floods and additionally submitted to episodic sediment emersions).

Event	Scouring flood	Sediment emersion
Zone	F, FE	FE
Average length of an event [mean (SD)]	4.2 (4.9) days	3.5 (5.0) days
Average number of events per year [mean (SD)]	9.2 (2.9) events	10.7 (7.1) events
Summed number of days the event occurs on the year-cycle	38.2 (18.4) days	38.1 (33.3) days

mid March 1998, following winter stratification and before spring germination (or sprouting). Because a relatively large number of small, widely-scattered samples provides a better characterization of the seed bank than do a few large samples (ROBERTS 1981, WILSON et al. 1993), in each of the ten sampling stations a set of 15 sediment

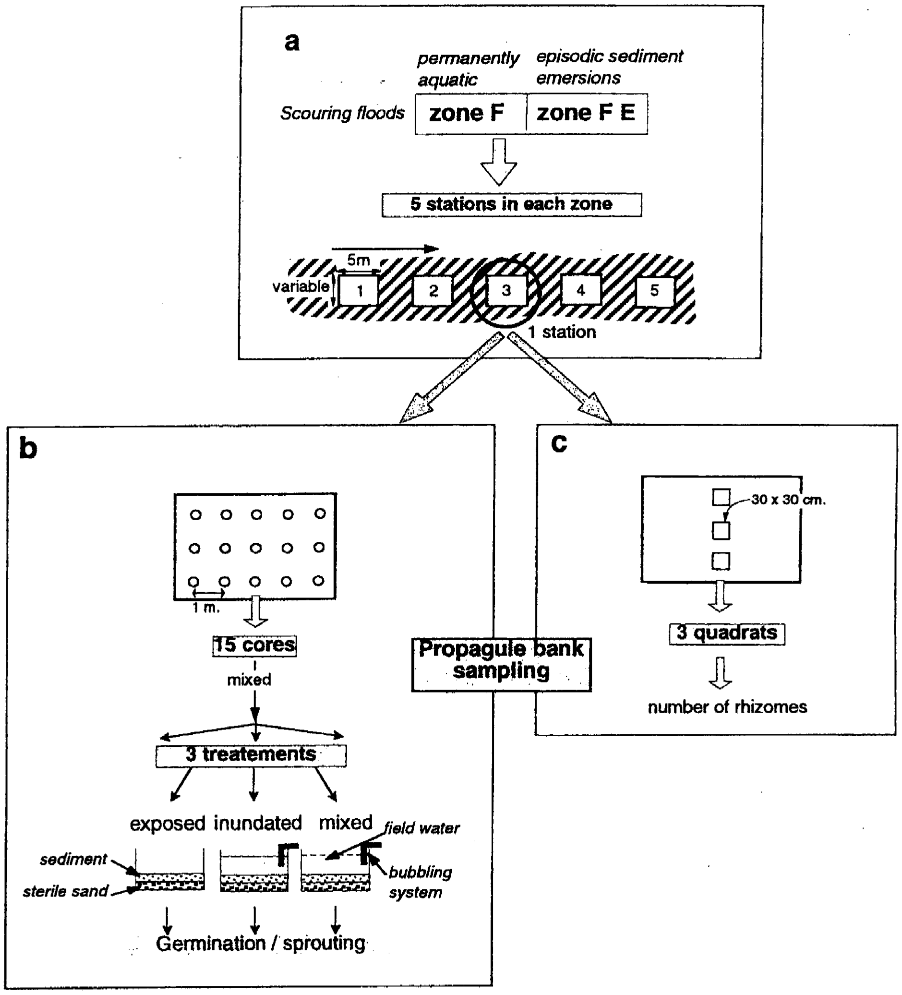

Fig. 2. Sampling design of the propagule bank. (**a**) Five sampling plots were regularly distributed in each zone. (**b**) In each sampling plot 15 sampling cores were collected in March, then mixed and divided into three equal parts cultivated using three different treatments. (**c**) In the same sampling plot, at the beginning of May, all individually rooted perennial plants were identified and their rhizomes were counted in three quadrats. For further details, see text.

cores (5 cm diameter) was collected within a 5 m wide strip crossing the channel (Fig. 2). The sampling of numerous widely-scattered cores allowed us to take into account the patchy sediment heterogeneity in such a disturbed habitat. Indeed, patches were randomly sampled and could thus have been indifferently scoured or silted in their history (MATTHAEI et al. 1999). Only the upper 5 cm of cores were used, because this depth is usually considered as the maximum from which germination will take place and because viable seeds are normally strongly concentrated near the sediment surface (Bonis & Lepart 1994, Roberts 1981). This therefore represents the functional propagule bank from which regeneration may occur in the field and integrates the effects of sediment erosion or aggradation. The 15 cores from each station were thoroughly mixed and divided into three subsamples. Samples were spread in 18×24 cm polystyrene trays (sediment thickness <1.5 cm) (Bonis & Lepart 1994, Leck & Simpson 1987). The rooting layer was first increased by spreading 2 cm of sterile sand (heated to 170 °C for five hours) over the bottom of the trays. Propagules die when heated at a temperature higher than 100 °C for five hours (Muñoz & Fuentes 1989). Lack of germination or sprouting in the reference sand trays demonstrated the efficiency of the sterilisation treatment.

Since macrophyte species require different germination (or sprouting) conditions (Galinato & van der Valk 1986, van der Valk 1981), three treatments were used: (1) inundated: sediment samples were permanently flooded under 4 cm of water collected from the channel, (2) exposed: samples were only moistened, (3) mixed: samples were moistened during one month and then reflooded. Field water was filtered through a 100 µm mesh sieve to prevent any inflow of propagules. Air bubbling maintained high oxygen content (90–100 %) and slow water movements.

Trays were randomly distributed in a conditioned room (natural photoperiod with additional light by Sylvania Daylight Standard 36 W, L/D: 12/12 and air temperature maintained at 10 °C during the night and allowed to rise to 25 °C during the day).

Seedling emergence was monitored for three months (April, May, June), as 1) it was previously demonstrated that macrophytes completely recolonized disturbed gaps within less than three months in this cut-off channel (Barrat-Segretain & Amoros 1996); 2) it has been recognized that "the longer the period of germination, the higher the chance that mosses [and algae] (which impede seedling emergence and survival) cover the tray" (Thompson et al. 1997); 3) several studies have demonstrated that germination is negligible beyond 3 months (Kautsky 1990, Thompson & Grime 1979).

Seedlings were regularly identified and assigned to a source (seeds or oospores, turions or winter buds, rhizomes, unspecialized fragments), and removed from the trays to prevent competition or allelopathy (Gopal & Goel 1993). Seedlings were identified to species level except Poaceae (which represented less than 0.3 % of all seedlings that sprouted from the propagule bank of the two stations). *Veronica* sp. grouped *Veronica beccabunga* and *Veronica anagallis-aquatica* in unknown percentages and *Ranunculus* sp. grouped *R. trichophyllus* and the hybrid *R. trichophyllus* × *circinatus* in unknown percentages.

Seedlings of one Dicotyledone and two Monocotyledones remained unidentified in zone FE (i.e. 0.05 % of seedlings sprouted from the propagule bank in this zone for three months).

The seedling emergence method provides a good idea of the ecologically active component of the seed bank (Abernethy & Willby 1999, Gross 1990) that recolonizes an area from which vegetation has been cleared. However, the core sampling and the emergence method fail to detect large propagules that lie deep in the soil (mainly rhizomes lying below the upper five centimetres). Consequently, rhizome density in the propagule bank was surveyed in mid-spring (May) by counting shoots sprouting from rhizome meristems within three quadrats (30 cm×30 cm) at each station. When a rhizomatous species was detected either by the emergence method or in the quadrat sampling, the highest estimation of rhizome density was used.

Sampling of established vegetation

Vegetation sampling stations, evenly distributed along the zones (seven at F and four at FE) were surveyed during the summer of 1998. Although this survey was only undertaken once, it confirmed the patterns of species relative abundance revealed by repeated sampling during previous years. In each sampling station (2 m-broad strips crossing the channel, i.e. 30 m^2 in FE and 80 to 200 m^2 in F, depending on channel width), aquatic vegetation was surveyed using the BRAUN-BLANQUET (1932) cover/abundance and sociability scales (sociability indicating relative patchiness of distribution of species in the sample plot). The two estimates were then converted into a single value ranging from 1 to 7 for statistical analysis (BORNETTE & AMOROS 1991). The nomenclature followed LAMBINON et al. (1992).

Results

Types and abundance of propagules in the bank

Emergence of propagules was lower in the F than in the FE samples (respectively 4,976 (SE = 2,348) and 104,394 (SE = 63,638) propagules/m^2; Fig. 3 a). This difference remained significant when propagules of *Chara vulgaris* were omitted (respectively 1,654 prop./m^2 (SE = 374) and 5,526 prop./m^2 (SE = 996), Mann Whitney test, $P < 0.01$ in all cases).

The composition of propagules that sprouted was not significantly different in zones F and FE (Fig. 3 b, χ^2, $P < 0.0001$ regardless of the inclusion or not of *C. vulgaris*). Sexual propagules (seeds and oospores) were the most abundant

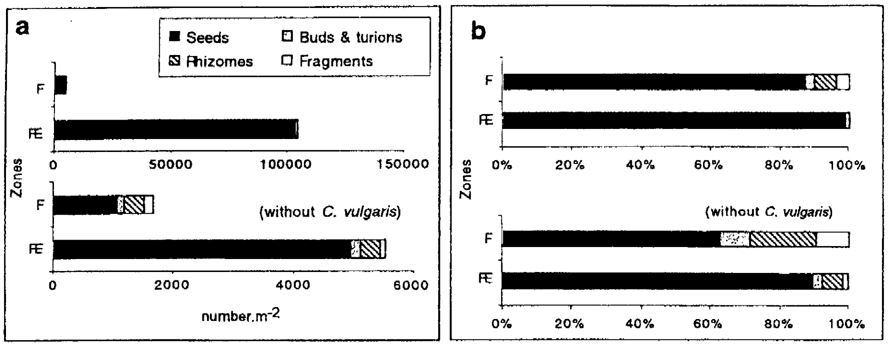

Fig. 3. Abundance and ratio of each propagule type in the two zones. **(a)** Upper part: propagule density. Lower part: propagule density excluding *Chara vulgaris*. **(b)** Upper part: Percentages of propagule types. Lower part: percentages excluding *Chara vulgaris*.

(87 % and 98.5 % of propagules that sprouted in the samples from zone F and zone FE, respectively). Respectively, 17 and 13 species sprouted from propagule bank in zone F and zone FE; of these, nine and ten species occurred as seeds. Vegetative propagules accounted for 13 % of emergence in zone F and 1.5 % in zone FE. Rhizomes were the most abundant vegetative propagules (6.2 % and 0.3 % of total emergence in zones F and FE, respectively), followed by whole individuals and unspecialized fragments (mostly sections of shoot apices) (4 and 1 %), and finally buds (2.9 % and 0.15 %).

Relationship between propagule bank and established vegetation

Despite their abundance in the established vegetation, some species were not recorded in the sediment propagule bank (*Berula erecta, Hottonia palustris* and *Myriophyllum verticillatum* in both zones, *Potamogeton natans* in zone F; see Table 2). Others which occurred only sporadically in the established vegetation were also absent from the bank (*Lemna trisulca, Polygonum hydropiper, Alisma plantago aquatica, Galium palustre* and *Phalaris arundinacea* in zone FE and *Rorippa amphibia* in zone F).

A set of species occurred in both bank and established vegetation in at least one of the two zones: *Elodea nuttallii, Luronium natans* and *Hippuris vulgaris* in zone F; *Myriophyllum spicatum, Ranunculus* sp., *Veronica* sp. and *Juncus articulatus* in the bank of both zones but only in the established vegetation of zone F. *Myosotis scorpioides* and *Nasturtium officinale* appeared in the established vegetation of the two zones but only in the FE bank as seeds.

The Spearman rank correlation test was used to identify the relationships between propagule densities in the bank and the composition of the established vegetation (all combinations of propagule types were taken into account). The test was conducted for hydrophytes only, as they grow in the channel bed and are disturbed by scouring floods whatever their discharge, whereas helophytes, that grow along the channel edge, are only disturbed by the highest floods. Moreover, vegetative parts of hydrophytes are sensitive to sediment emersion, unlike those of helophytes. No relationship between the whole propagule bank and the established vegetation was demonstrated in either zone (Table 3). No significant relationship ($p<0.05$) occurred between any propagule type in the bank and the established vegetation in zone F. Buds and turions were even negatively correlated to the established vegetation ($\rho = -0.41$) at a level of significance of 0.1. Although the whole propagule bank was not correlated to the established vegetation in zone FE, a significant positive relationship occurred between unspecialized fragments and the established vegetation in this zone. All vegetative propagules (buds + rhizomes + fragments) were also correlated with established vegetation.

Table 2. Plant abundance in the established vegetation and in the propagule bank. Average species cover concerned the established vegetation (for the coding of cover see Materials and methods). Abundance of propagules in the bank is expressed as the average number of active propagules per square meter (based on both the rhizome sampling and the treatment that provided optimum germination for each species and propagule type). R = rhizome, F = unspecialized fragment, B = bud or turion and S = seed or spore. Data concerning terrestrial plants and *Poaceae* are not presented.

Taxons	Zone F						Zone FE					
	average species cover	Propagule bank					average species cover	Propagule bank				
		R	F	B	S	Total		R	F	B	S	Total
Aquatic perennials												
Berula erecta	2	0	0	0	0	0	1.3	0	0	0	0	0
Callitriche platycarpa	1.3	0	0	0	528.8	528.8	0.8	0	0	0	2827.1	2827.1
Chara vulgaris	0.4	0	27.1	0	3294.9	3322	2	0	976.3	0	97891.5	98867.8
Eleocharis acicularis	1.9	0	27.1	0	122	149.1	1.8	0	74.6	0	366.1	440.7
Elodea canadensis	0.3	0	27.1	20.3	0	47.5	1.3	0	6.8	0	0	6.8
Elodea nuttallii	1.6	0	13.6	0	0	13.6	0	0	0	0	0	0
Groenlandia densa	0.7	140	0	0	0	140	0.5	291.1	0	0	81.4	372.5
Hippuris vulgaris	1.1	7.4	13.6	0	0	21	0	0	0	0	0	0
Hottonia palustris	0.7	0	0	0	0	0	0.8	0	0	0	0	0
Lemna minor	0	0	20.3	0	0	20.3	1.3	0	0	0	0	0
Lemna trisulca	0	0	0	0	0	0	0.8	0	0	0	0	0
Luronium natans	1.1	0	47.5	0	0	47.5	0	0	0	0	0	0
Myriophyllum spicatum	0.4	1.5	6.8	0	0	8.3	0	22.2	0	0	0	22.2
Myriophyllum verticillatum	0.1	0	0	0	0	0	0.8	0	0	0	0	0
Potamogeton berchtoldii	0.3	0	0	122	81.36	203.4	1	0	0	162.7	20.3	183.1
Potamogeton natans	1	0	0	0	0	0	0	0	0	0	0	0
Ranunculus sp.	1.7	0	0	0	61.02	61	0	0	0	0	305.1	305.1
Sparganium emersum	1.3	162.7	6.8	0	0	169.5	1.3	18.5	0	0	0	18.5
Helophyte annuals												
Polygonum hydropiper	0	0	0	0	0	0	0.3	0	0	0	0	0
Rorippa amphibia	0	0	0	0	20.34	20.3	0	0	0	0	0	0
Helophyte perennials												
Alisma plantago-aquatica	0	0	0	0	0	0	0.3	0	0	0	0	0
Galium palustre	0	0	0	0	0	0	0.5	0	0	0	0	0
Juncus articulatus	0.3	0	0	0	61.02	61	0	0	0	0	61	61
Mentha aquatica	0	0	0	0	20.34	20.3	0.3	0	0	0	0	0
Myosotis scorpioides	1.9	0	0	0	0	0	0.3	0	0	0	40.7	40.7
Nasturtium officinale	0.1	0	0	0	0	0	0.3	0	0	0	122	122
Phalaris arundinacea	0	0	0	0	0	0	1	0	0	0	0	0
Veronica sp.	0.1	0	0	0	81.36	81.4	0	0	0	0	650.9	650.9

Table 3. Spearman rank order correlation coefficients between propagule bank and established vegetation composition. For each propagule type and each zone the correlation coefficient and the level of significance (in brackets) are provided. Significant relationships are in bold figures (n = 17 for F, n = 15 for FE).

	F	FE
seeds	0.16 (0.51)	0.26 (0.34)
buds and turions	−0.41 (0.1)	0.07 (0.80)
rhizomes	0.03 (0.90)	−0.02 (0.94)
unspecialized fragments	−0.06 (0.79)	**0.70 (0.01)**
buds and turions + rhizomes + unspecialized fragments	−0.18 (0.48)	**0.61 (0.03)**
whole propagule bank	0.08 (0.76)	0.33 (0.23)

Species regenerative strategies at the two stations

All hydrophyte species occurring either in the propagule bank or in the established vegetation were potentially perennial (Table 2).

Some species occurred only as sexual propagules in both zones (*Callitriche platycarpa* and *Ranunculus* sp., Fig. 4). Others occurred as both sexual and vegetative propagules (oospores and fragments for *Chara vulgaris,* seeds and fragments for *Eleocharis acicularis,* seeds and rhizomes for *Groenlandia densa,* seeds and turions for *Potamogeton berchtoldii,* Fig. 4). Finally, some species occurred only as vegetative propagules (fragments and buds for *Elodea canadensis,* fragments for *Elodea nuttallii, Lemna minor* and *Luronium natans,* Fig. 4).

Discussion

Seedling emergence under laboratory conditions does not exactly reflect field conditions but is usually considered as the best method to assess the role of seed bank in a community (HAAG 1983, KAUTSKY 1990). During seedling emergence, identified seedlings are regularly removed from the trays thereby avoiding competitive interactions between seedlings and established plants. These conditions are close to the natural situation in frequently disturbed habitats where disturbances create gaps, preventing seedlings from competition. Competitive exclusion should be very low in such habitats because of the high frequency of disturbances (VAN DER MAAREL 1988). Thus, sampled vegetation was presumably that regenerated after scouring floods (BARRAT-SEGRETAIN & AMOROS 1996).

Despite the potential disruption of flowering by the timing of floods, the abundance of sexual propagules in the bank suggests that sexual reproduction is high in this habitat (or that large numbers of seeds are imported by floods). Indeed, the six hydrophyte species that produced seeds or oospores in at least

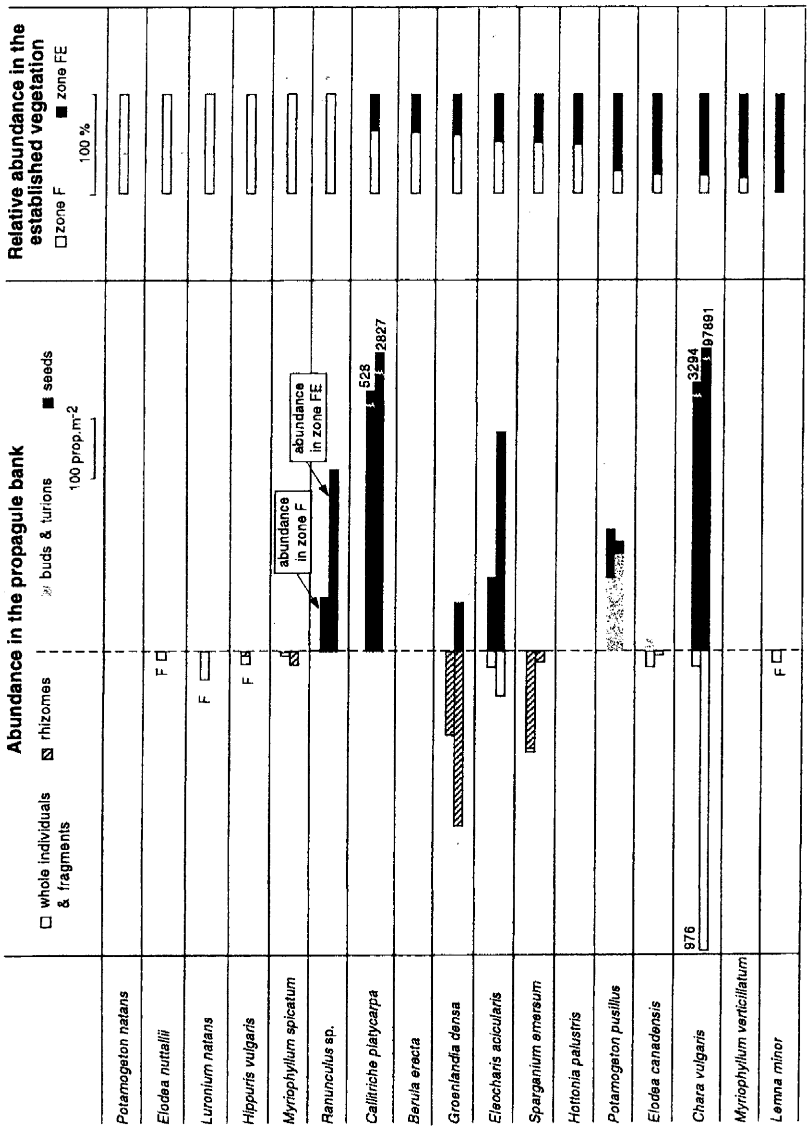

Fig. 4. Type and abundance (number · m^{-2}) of propagules of each species present in the bank (upper bar: zone F, lower bar zone FE, prop · m^{-2}: number of propagules per square meter) and relative abundance of the same species in the established vegetation of the two zones.

one of the two zones (*Callitriche platycarpa, Chara vulgaris, Eleocharis acicularis, Groenlandia densa, Potamogeton berchtoldii,* and *Ranunculus* sp.) are able to reproduce sexually under water (Den Hartog & Segal 1964, Frank & Klotz 1990, Fig. 4) and have a long flowering period (Table 4). As a con-

Table 4. Species flowering periods and potential effect of scouring floods in the cut-off channel under study (Data conserning *Elodea canadensis* are not provided because the reproduction of this species in the area under study is exclusively vegetative).

Species	Period of sexual re-production	References	Average number of scouring floods during the flowering period [mean (SD)]	Percentage of years between 1982 and 1987 with one flood or more during the flowering period (%)	Average frequency of scouring floods per month during the flowering period
Alisma plantago-aquatica	June–Sept.	LAMBINON et al. 1992	2.44 (1.36)	94	0.61
Berula erecta	July–Sept.	GRIME et al. 1990	1.12 (1.09)	75	0.37
Callitriche platycarpa	Apr.–Oct.	LAMBINON et al. 1992	4.5 (2.34)	100	0.64
Chara vulgaris	May–Oct.	CORILLON 1975	3.44 (1.82)	94	0.43
Eleocharis acicularis	June–Oct.	LAMBINON et al. 1992	2.75 (1.57)	94	0.55
Elodea nuttallii	June–Sept.	LAMBINON et al. 1992	2.44 (1.36)	94	0.61
Galuim palustre	June–July	GRIME et al. 1990	1.81 (1.38)	88	0.91
Groenlandia densa	May–Oct.	LAMBINON et al. 1992	3.44 (1.82)	94	0.43
Hippuris vulgaris	June–July	GRIME et al. 1990	1.81 (1.38)	88	0.91
Hottonia palustris	May–June	LAMBINON et al. 1992	2.06 (1.73)	81	1.03
Juncus articulatus	June–Sept.	LAMBINON et al. 1992	2.44 (1.36)	94	0.61
Lemna minor	May–July	GRIME et al. 1990	2.62 (1.82)	94	0.87
Lemna trisulca	June–July	GRIME et al. 1990	1.81 (1.38)	88	0.91
Luronium natans	May–Aug.	LAMBINON et al. 1992	2.69 (1.96)	94	0.67
Mentha aquatica	July–Sept.	GRIME et al. 1990	1.12 (1.09)	75	0.37
Myosotis scorpioides	May–Sept.	GRIME et al. 1990	3.19 (1.76)	94	0.64
Myriophyllum spicatum	June–Aug.	LAMBINON et al. 1992	1.87 (1.54)	88	0.62
Myriophyllum verticillatum	July–Oct.	LAMBINON et al. 1992	1.56 (1.36)	81	0.39
Nasturtium officinale	May–July	GRIME et al. 1990	2.62 (1.82)	94	0.87
Phalaris arundinacea	June–July	GRIME et al. 1990	1.81 (1.38)	88	0.90
Polygonum hydropiper	July–Sept.	GRIME et al. 1990	1.12 (1.09)	75	0.37
Potamogeton berchtoldii	June–Sept.	LAMBINON et al. 1992	2.44 (1.36)	94	0.61
Potamogeton natans	May–Sept.	LAMBINON et al. 1992	3.19 (1.76)	94	0.64
Ranunculus sp.	May–Aug.	LAMBINON et al. 1992	2.69 (1.96)	94	0.67
Rorippa amphibia	June–Aug.	LAMBINON et al. 1992	1.87 (1.54)	88	0.62
Sparganium emersum	June–July	GRIME et al. 1990	1.81 (1.38)	88	0.91
Veronica sp.	May–Aug.	GRIME et al. 1990	2.69 (1.96)	94	0.67

sequence, their sexual reproduction should be less impacted by floods. Other species that occurred as seeds in the bank were marginal helophyte species (e.g. *Veronica* sp. and *Juncus articulatus*) that are less frequently affected by scouring.

The lack of correlation between the propagule bank and the established vegetation in one zone (F) and the few links between some kinds of vegetative propagules and the established vegetation in the other zone (FE) show that (i) the established vegetation is not maintained by sexual propagules alone and that (ii) there is a general lack of dependence on any one particular regenerative strategy. Several regenerative strategies involving both sexual and vegetative propagules are exhibited in both zones.

Propagule bank and established vegetation in the permanently aquatic zone

A few species (e.g. *Callitriche platycarpa*) were abundant both in the established vegetation and as seeds in the propagule bank, suggesting that their populations are maintained by seeds and that the seeds present in the propagule bank are mostly produced in situ (Fig. 4, Table 3). Conversely, many species that occurred in the established vegetation, sometimes abundantly, were not detected either as seeds or as vegetative propagules (e.g. *Berula erecta, Hottonia palustris, Myosotis scorpioides, Myriophyllum verticillatum,* and *Potamogeton natans*), despite their known ability to propagate either by seeds, buds, vegetative fragments (BARRAT-SEGRETAIN 1996, KLEIN & CARBIENER 1989) or rhizomes and turions (e.g. *M. verticillatum* propagules (KLEIN & CARBIENER 1989, WEBER & NOODEN 1976)). *Potamogeton natans* is reported to propagate mainly by rhizomes since regeneration from unspecialized fragments is ineffective in this species (KADONO 1984) and regeneration from turions never contributes much to its maintenance (WIEGLEB & BRUX 1991), while *Myosotis* regenerates extensively by stem and rhizome fragments (GRIME et al. 1990). Patches of these species may have been missed during the rhizome sampling because they were highly localised or still at an early stage of development. *Berula erecta, Hottonia palustris,* and *Myriophyllum verticillatum* propagate by stolons, apical stem fragments or decumbent stems (BROCK et al. 1989, HASLAM 1978, WEBER & NOODEN 1976) but these kinds of structure were not included in our investigation because they are not stored in the sediment and are thus not regarded as part of the propagule bank.

Several species that occurred as vegetative propagules in the bank (*Sparganium emersum, Hippuris vulgaris,* and *Myriophyllum spicatum*) had persistent deeply-anchored rhizomes (6 to 10 cm deep, pers. measurements) allowing them to use the substratum as a refuge (COOK & NICHOLLS 1986). Other species occurred in the bank as dormant apices (e.g. *Potamogeton berchtoldii*).

Dormant apices, especially turions, present a seasonal pattern of production and regrowth that may be vulnerable to the unpredictability of flood disturbances. This might partly explain the low abundance of these species in the established vegetation. It would also explain why stolons or decumbent stems are the main organs of propagation in *M. verticillatum* at this site, whereas this species apparently relies on turions in stable standing water (WEBER & NOODEN 1976).

Finally, unspecialized fragments were frequent across a range of contrasting species, and were produced independently of other kinds of vegetative propagules. Many authors have recognized the importance of vegetative perennation via stem fragments in aquatic plants, even in species such as *Groenlandia densa* (PRESTON 1995) and *Myriophyllum spicatum* (AIKEN et al. 1979) which show very high levels of seed production in stable environments. Four of the species that were rare or absent from the bank are evergreen at our site (*Berula erecta, Elodea nuttallii, Hottonia palustris,* and *Luronium natans*), which allows them to propagate vegetatively throughout the year through fragmentation (BARRAT-SEGRETAIN et al. 1998).

The permanently aquatic zone is thus characterized by (1) species able to reproduce under water, (2) species able to use sediment refugia through deeply anchored rhizomes and (3) evergreen species that regenerate through individuals or fragments produced during the floods. Regenerative strategies involving sexual reproduction and seed banks, as usually predicted for ruderal plants, were only observed for those species that have a high potential reproductive output, a long reproductive period and are able to achieve their whole life cycle (especially flowering and pollination) under water. Regenerative strategies of the other species involved vegetative propagules that could be produced (detached from the mother plant) during disturbances.

Propagule bank and established vegetation in case of episodic sediment emersions

Species in this zone have to cope with two constraints: scouring floods and sediment emersions. This zone is characterized by an abundant seed bank and the presence of numerous helophytes, as usually demonstrated in habitats subject to sediment emersions (SMITH & KADLEC 1983, VAN DER VALK & DAVIS 1978). Some aquatic plants (e.g. *Elodea nuttallii, Hippuris vulgaris,* and *Luronium natans*) did not occur in this zone, whereas several helophytes were restricted to this area (e.g. *Alisma plantago-aquatica* and *Polygonum hydropiper*).

Regeneration from seed bank is frequently linked to a sediment emersion regime (BONIS et al. 1995, BROCK & ROGERS 1998). *Callitriche platycarpa, Chara vulgaris* and *Eleocharis acicularis* were abundant both in the seed bank and the established vegetation and we assume that this reflects the importance of the production of seeds and the regeneration from seeds in maintaining populations of these species within this zone (e.g. Characeae are known to rapidly colonize disturbed water bodies thanks to the longlasting viability of their oospores (WADE 1990)). However, no significant difference concerning the types of propagules occurred between this zone and zone F that was not subject to sediment emersion. Some helophytes were abundant in the propagule bank but remained scarce in the established vegetation (e.g. *Veronica* sp. and *Nasturtium officinale*). These seeds were probably not produced in situ but came with seed rain (from surrounding areas) or drift and then failed to establish in the usually aquatic conditions in this zone (CELLOT et al. 1998).

Some species (e.g. *Elodea canadensis* and *Sparganium emersum*) occurred both in the bank as vegetative propagules and in the established vegetation, suggesting that their propagules tolerate exposure, since sediment emersion can occur at any period of the year in this zone (Fig. 1). *Myriophyllum verticillatum,* that was absent from the bank but abundant in the established vegetation, is likely to present the same pattern (through perennial rhizomes) even though our sampling failed to detect them. Turions of *Potamogeton berchtoldii* were numerous in the bank, suggesting that this species regenerates from winter buds after sediment emersion periods. This result is in accordance with SPENCER & KSANDER (1992), who suggested that subterranean propagules of *Potamogeton* species may allow the plants to survive periods when water is absent from the channel, and that sprouting of such propagules is stimulated by flooding.

Species present in the established vegetation and absent from the bank were helophytes (e.g. *Alisma plantago-aquatica* and *Galium palustre*) or hydrophytes that are able to develop a terrestrial growth form in response to emersion (e.g. *Berula erecta* and *Hottonia palustris*) and thus their maintenance does not depend on their ability to produce propagules.

So the periodically exposed zone is characterized by (1) the same species able to reproduce under water that dominated in the permanently aquatic zone, (2) species with vegetative propagules (mainly rhizomes) tolerant to sediment emersion and (3) traditionally aquatic species able to develop a terrestrial growth form. As already stated for the permanently aquatic zone, regenerative strategies in this zone involved sexual reproduction and seed banks for only a few species, whereas regenerative strategies of most species involved vegetative propagules.

Resistance and resilience of aquatic plants

Scouring floods favour regeneration mainly through rhizomes or fragments. When another constraint, sediment emersion, is added, a shift appears from species occurring in the bank as fragments or rhizomes to species occurring as buds or seeds and species able to develop a terrestrial growth form. Within-species variations between the two zones also occurred as several species did not exhibit the same propagule type in both zones.

Species maintenance through rhizomes, whole individuals or fragments, implies that at least a part of the plant is able to withstand a given constraint (BROCK et al. 1987). Such a maintenance thus imparts a resistance strategy to the species (WEBSTER et al. 1983, WESTMAN 1978). The use of specialized and eventually dormant propagules, such as buds or seeds, can be interpreted as a resilience strategy (WEBSTER et al. 1983, WESTMAN 1978). Aquatic plants able to survive scouring floods possess the ability to resist, either through the use of a refuge, or through regeneration of detached fragments (resistance strategy). Plants that have to face sediment emersions as an additional constraint more frequently use seeds or buds (resilience strategy).

Flood disturbances and sediment emersions are environmental filters that screen species traits (KEDDY 1992). It is usually considered that such filters eliminate species with unsuitable traits and do not alter perennation of species with suitable traits. The present study demonstrates that a sediment emersion regime superimposed on a flood regime (additional filter) reduces the abundance of some species (e.g. *Berula erecta* and *Groenlandia densa*) but also increases the abundance of others (e.g. *Chara vulgaris*).

Unpredictable floods scour substratum and damage plants, thereby impeding both flowering and seed storage. Both zones are submitted to such disturbances several times each year (Table 1). Every aquatic species observed in this study experienced at least one major flood each year during its flowering or fruiting period over the last 16 years (Table 4). Periods between scouring floods were too short (average 35 days) to allow massive seed production (e.g. *Myriophyllum spicatum* needs more than four weeks to achieve seed maturity (HARTLEB et al. 1993)). Drift could be a potential source of seeds in such a disturbed habitat, and according to the high disturbance frequency, one would expect that the seed bank would contain drifting seeds that could have contributed to the recruitment of plants. However, CELLOT et al. (1998) demonstrated that drift inputs in the seed bank (both composition and abundance) were not detectable. They were very low compared to the autochthonous production of propagules. Moreover, many seed-producing species failed to appear as seeds in the bank of the present study, and no hydrophytes present in the seed bank were absent from the established vegetation. Therefore drift does not appear to compensate for any lack of seed production in this site. Frequent scouring in fact favours regeneration through fragments and rhizomes, the latter being the only perennation structure for several species because their potentially deep anchorage allows the plant to escape the scouring of superficial sediment.

The present study has demonstrated that vegetative propagules are an important component of the maintenance of aquatic plants in habitats submitted to unpredictable disturbances, as already noted in several studies of terrestrial plant communities (KOTANEN 1996, MORAVEC 1990), and as suggested for aquatic plant communities (ABERNETHY & WILLBY 1999, RØRSLETT 1989). We also demonstrated that regenerative strategies involving only sexual reproduction do not allow community maintenance in such habitat. The presence of a diversity of regenerative strategies within and between species is likely to be a major factor in buffering communities from disturbance. The present study also implies that the type of disturbance should be considered more systematically as it affects the availability of refuges and regenerative strategies involved in the recolonization processes.

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PLANT REGENERATIVE STRATEGIES AFTER A MAJOR DISTURBANCE: THE CASE OF A RIVERINE WETLAND RESTORATION

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Abstract: In aquatic habitats, both vegetative and sexual reproduction are involved in vegetation maintenance. Restoration projects carried out in such habitats rarely consider the role of both sexual reproduction (seeds) and vegetative (vegetative propagules or clonal fragments) components of propagule banks in vegetation regeneration. Moreover, changes in the seed bank due to the restoration itself have rarely been studied. The aim of this paper is to answer the following questions. Does restoration consisting of sediment removal in a cut-off meander lead to a change in the propagule bank (both sexual and vegetative propagules), and does the vegetation regeneration involve vegetative propagules created by plant breakage during the disturbance? Vegetation was visually surveyed three times during a 15-year period before the restoration and two years afterwards in both the restored zone and an upstream undisturbed zone. The propagule bank was characterized just before and one year after the restoration in the restored zone by sampling of sediment cores and propagule germination in a greenhouse. The impact of restoration on the propagule bank was very pronounced. Sprouting propagules were three times more abundant in post-restoration samples than in pre-restoration samples. The ratio of seeds / vegetative propagules in the bank was lower in the year following restoration. Links between propagule bank and established vegetation were assessed through calculation of correlation and similarity indices. We observed a large difference between established vegetation and the propagule bank before restoration, suggesting that recruitment from the propagule bank was low before restoration. Restoration enhanced the recruitment from rhizomes and vegetative fragments, which were highly correlated with the established vegetation. The pre-eminent role of some vegetative propagules in the recolonization and the increasing abundance of an invasive species are related to the season (June) in which restoration was carried out. Impact of restoration on both the propagule bank composition and environmental conditions (especially those promoting or inhibiting germination or sprouting) thus has to be considered in further vegetation management work.

Key Words: propagule bank, riverine wetland management, seed bank, vegetative propagation, wetland restoration

INTRODUCTION

Regeneration of a plant community after disturbance is often assumed to depend on seeds, especially those coming from seed banks (Noble and Slatyer 1980, Grime 1981, Roberts 1981, Grime and Hillier 1992). However, several authors have demonstrated that seed banks may have only limited involvment in regeneration after a major disturbance (or rare catastrophic event *sensu* Noble and Slatyer 1980) because 1) the soil and the seed bank may be altered and 2) the soil may not contain a persistent seed bank (Belsky 1986, van der Valk and Perderson 1989, Stylinsky and Allen 1999). Community regeneration also involves dispersed seeds (Noble and Slatyer 1980, Bakker and Berendse 1999), dispersed vegetative propagules (van der Valk and Verhoeven 1988, Reed et al. 2000), or even vegetative spread (van der Valk and Verhoeven 1988, Galatowitsch and van der Valk 1996).

The role of seed banks in vegetation regeneration after restoration has been discussed frequently (van der Valk and Verhoeven 1988, van der Valk et al. 1992, Wade 1993, Vivian-Smith and Handel 1996, Wetzel et al. 2001), although very few studies have focused on 1) changes in the seed bank due to the disturbing effect of restoration and 2) the effective role of seed banks in vegetation regeneration after restoration (Davis and van der Valk 1988 in van der Valk et al. 1992). On the one hand, restoration can change environmental conditions (van der Valk and Pederson 1989) or break seed dormancies (Grime 1981) and thus lead to an increase in the active seed bank (seeds lying in the soil and able to germinate as soon as a gap in the vegetation is created, thus increasing, for example, the

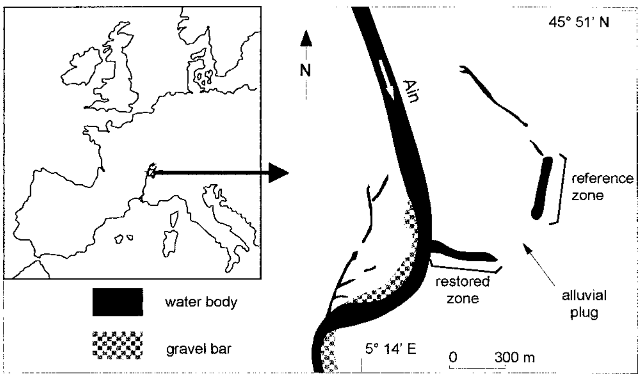

Figure 1. Location of the cut-off meander under study (modified from Piegay et al. 2000).

amount of light or temperature reaching substrate and leading to germination). On the other hand, some restoration work (dredging, tillage, etc.) can alter the superficial soil and thus lead to the loss or burial of at least part of the seed bank.

In aquatic habitats, some species are suspected of being unable to produce seeds or, at least, viable seeds and must therefore propagate mostly by vegetative means (Kadono 1984, Klein and Carbiener 1989, Spencer and Ksander 1992, Barrat-Segretain et al. 1998). Vegetative reproduction could even be favored in cases of disturbance because the timing of such events may be incompatible with the seasonal production or germination of seeds (Raven 1986, Amoros and Bornette 1999, Combroux et al. 2001). Regenerative strategies involved in recolonization after restoration work should, therefore, depend on the restoration work's disturbing action on the whole soil propagule bank (including both sexual and vegetative propagules) and not only on seeds.

The aim of the work described here was to address the following questions. 1) Does restoration lead to a change in the propagule bank, and 2) does the vegetation regeneration involve vegetative propagules created by breakage of plants during restoration? These questions were addressed in a cut-off channel where vegetation dynamics were surveyed five times over a 17-year period through 1) the assessment of changes in the propagule bank and 2) a comparison of the relationship between the propagule bank and the established vegetation both before and after restoration.

MATERIALS AND METHODS

Study Site

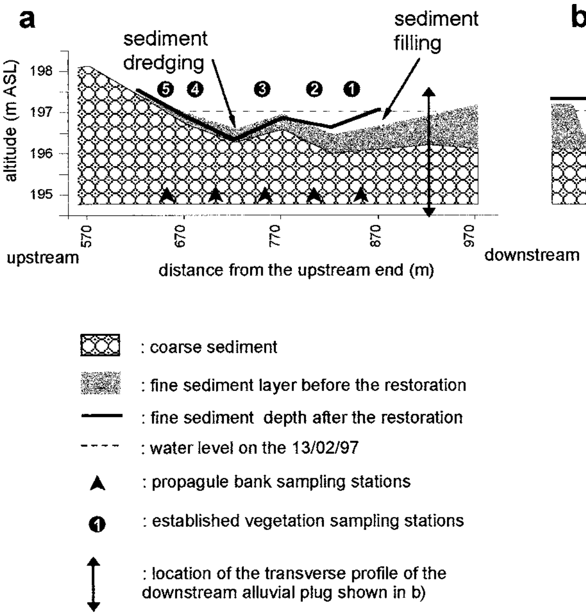

Figure 2. (a) Longitudinal profile of the restored zone. Arrows and numbered discs indicate the location of the propagule-bank stations. (b) Transverse profile of the downstream alluvial plug (where restoration consisted in sediment infilling).

The study was carried out in a cut-off meander of the River Ain (Le Planet, 45° 51′ N, 5° 14′ E) in France. Two sections, separated by an alluvial plug, remained in the former meander bed. The downstream section (250-m long), referred to in the following account as 'restored zone', was connected permanently to the main river through its downstream end. Its sediment was thereby exposed each summer during low river levels. The upstream section (300-m long), referred to in the following account as 'reference zone', was never drained because of its isolation from the main channel (Figure 1). The average water level in the restored zone has decreased dramatically since 1992 probably due to 1) the incision of the river in this area (Bornette et al. 1996), 2) the lateral migration of the main channel (Citterio and Piegay 2000), and 3) the very large withdrawal of ground water for irrigation (Commission Locale de l'Eau, Basse Vallée de l'Ain, unpublished report).

The restoration of a permanent water body in the restored zone was instigated by local managers. The project was also designed to impede the drainage of water during low water level of the river and to decrease river backflows by increasing elevation of the confluence alluvial plug. Indeed, river backflows that entered the channel brought silt and accelerated terrestrialization of the aquatic zone. The restoration work consisted of partial removal of silt deposits that were then used to reinforce the artificial plug at the downstream connection to the river (Figure 2). This work was carried out in late June 1998, since dredgers could only reach the restored zone during periods of very low water levels.

Table 1. Combined scale for the Braun-Blanquet indices of cover-abundance and sociability.

Cover-abundance	Sociability	Combined Index
+	1	1
1	2	2
2	2	2
1	3	3
2	3	3
2	4	3
2	5	4
3	3	5
3	4	5
4	4	5
3	5	6
4	5	6
5	5	7

Established Vegetation

Sampling stations were distributed along the zones according to vegetation variability: seven in the reference zone at 50-m intervals and five in the restored zone (Figure 2). These sampling stations were surveyed during the summer five times over a 17-year period (in 1983, 1992, and 1997 before restoration and in 1998 and 1999 after restoration). In each sampling station (2 m-broad strips crossing the channel, between 50 m and 70 m long, depending on channel width), aquatic vegetation was surveyed by visual observations from a boat. Water depth ranged from a few centimeters to 2 m (greater depth in the upstream section), and water transparency was good enough to see the channel bottom. We used the Braun-Blanquet (1932) cover/abundance and sociability scales (cover: +: isolated individual, 1: vegetation cover (vc) $< 5\%$, 2: $5\% \leq$ vc $< 25\%$, 3: $25\% \leq$ vc $< 50\%$, 4: $50\% \leq$ vc $< 75\%$, 5: $75\% \leq$ vc $<100\%$ and subjective sociability index ranging from 1 to 5 indicating the relative patchiness of the distribution of a species in the sampling station). The two estimates were then converted into a single value for statistical analysis (Table 1, see also Bornette and Amoros 1991, Bornette et al. 1994). Some patches in the established vegetation consisted of *Potamogeton natans* L. and *Potamogeton nodosus* Poiret growing together. These two species were recorded as a single taxon and are referred to as *Potamogeton* in the following account.

Propagule bank

Propagules in the bank were quantified by the seedling emergence method (Thompson and Grime 1979, Bigwood and Inouye 1988, Gross 1990). Although most propagule bank studies in aquatic habitats have focused only on sexually-produced propagules (McKnight 1992, van der Valk et al. 1992, Collins and Wein 1995, Le Page and Keddy 1998), we felt it necessary to quantify both sexual and vegetative propagules, since vegetative propagules such as tubers, winter buds, turions, and rhizomes are recognized to play an important role in the recovery of aquatic vegetation (Kautsky 1990, Lundholm and Simser 1999). Unspecialized fragments (mostly stems with buds and/or roots) were also included as part of the propagule bank, as they can be relatively long-lived (i.e., *Galium palustre* overwinters as small shoots (Grime et al. 1988), and *Myriophyllum spicatum* perennation is possible throughout its root stock (Aiken et al. 1979)).

Five stations (5-m-wide strip crossing the channel, evenly distributed at 50-m intervals) (Figure 2) were sampled in the restored zone March 17, 1998 (just before restoration) and April 5, 1999 (the spring following restoration). Both samplings were conducted after winter stratification and before spring germination (or sprouting). We used a Plexiglas corer with an iron cutter head (Figure 3a). The iron cutter head was very efficient for cutting root stocks and preventing sediment compaction. Samples were immediately stored in plastic bags and brought back to the laboratory. Fifteen sediment cores (5-cm diameter) were collected in each of the five sampling stations. Only the upper 5 cm of the sediment was collected because this depth is usually considered as the maximum from which germination can take place (Roberts 1981, Bonis and Lepart 1994) and could thus be considered as the maximum from which propagules are involved in regeneration. The 15 cores were mixed thoroughly and divided into three sub-samples. Three treatments were carried out: (1) inundation: sediment samples were permanently flooded under 4 cm of water collected from the channel, (2) exposure: samples were maintained at saturation point, and (3) mixture: samples were maintained at saturation point for one month and then re-flooded to a depth of 4 cm (the same as the inundated treatment). Samples were spread in 18 cm x 24 cm x 10 cm polystyrene trays (sediment thickness < 1.5 cm) on top of 2 cm of sterile sand (heated 5 hours to 170 °C). Field water (collected in the study site) was filtered through a 100-μm-mesh sieve to prevent the addition of propagules. Bubbling air (aquarium air pumps and diffusers) maintained a high oxygen content (90–100%) and slow water movement. The trays were distributed in a climate room where the natural photoperiod was supplemented with additional lighting (Sylvania daylight standard 36 W, L/D: 12/12) and the air temperature maintained at 10 °C during the night and allowed to rise to 25 °C during the day.

Seedling emergence was monitored from March to December in the year of sampling. Seedlings were surveyed regularly. Each seedling was identified visually

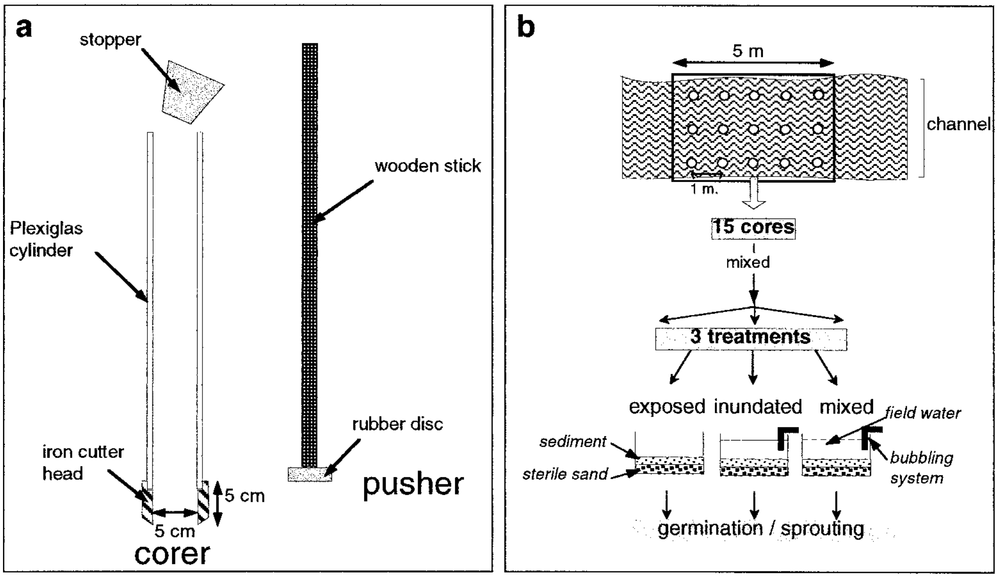

Figure 3. (a) Diagram of the tool used to core aquatic sediment. (b) Propagule bank sampling design: fifteen sediment cores were collected in each of the five sampling stations. The 15 cores were mixed thoroughly and divided into three sub-samples. Three treatments were carried out: (1) inundation: sediment samples were permanently flooded, (2) exposure: samples were maintained at saturation point, (3) mixture: samples were maintained at saturation point for one month and then re-flooded.

and assigned to a source (seeds or oospores, turions or winter buds, bulbs, rhizomes, unspecialized fragments). The identification of the source used either the direct observation of the propagule that generated the seedling (e.g., germinating buds of *Potamogeton berchtoldii* are easily recognized) or the observation of the characters of the young plant (e.g., long hypocotyl intenode and circular cotyledon in seedling issued from a seed of the same species). Identified seedlings were removed from the trays to prevent competition and allelopathy. Seedlings were identified to species level except Poaceae (which represented 4% of all seedlings sprouted from the pre-restoration propagule bank and 3.5% of all seedlings sprouted from the post-restoration propagule bank). *Veronica* spp. grouped *Veronica beccabunga* L. and *Veronica anagallis-aquatica* L. in unknown percentages.

The reference zone was not a potential source of propagules for the restored zone, as the zones are separated by an alluvial plug planted with *Populus* sp. and where *Phragmites* sp. and *Salix* sp. dominate the understory. The propagule bank sampling in this zone was thus not necessary.

Data Analyses

The two matrices containing data of vegetation cover in each zone over the 17-year period were subjected to a special case of partial principal component analyses (ter Braak 1995). Sampling dates were considered as classes, and deviations from the class means were analyzed instead of deviation from the general mean in order to remove the effect of the sampling dates. We used centered PCA to detect differences in species abundance (Vivian-Smith and Handel 1996). This ordination method is called within-class, centered principal component analysis (Dolédec and Chessel 1991). Changes in the spatial structure of the established vegetation over time were analyzed in the restored zone by comparison of established vegetation in five sampling stations. Five data matrices (one per station) were built using the converted value for vegetation cover, and the correlation between every matrix pair was calculated. The ordination analyses were performed using the package ADE version 4 (Chessel and Dolédec 1996). Differences in the propagule abundance measured pre- and post-restoration were tested for each propagule type and each species with a Mann-Whitney test followed by the Dunn-Bonferroni transformation.

Links between propagule banks and established vegetation were analysed using the Spearman's rank correlation tests (vegetation cover *vs.* propagule density) and Sørensen similarity index (Sørensen 1948): $Q = 2c/(a+b)$ where a and b are, respectively, the number of species for the first (propagule bank) and second (mature vegetation) lists and c the number of species present in both lists. Q ranges from 0 to 1.

RESULTS

Dynamics of the Established Vegetation

From 1983 to 1999, the vegetation in the reference zone experienced a reduction in its heterogeneity (Fig-

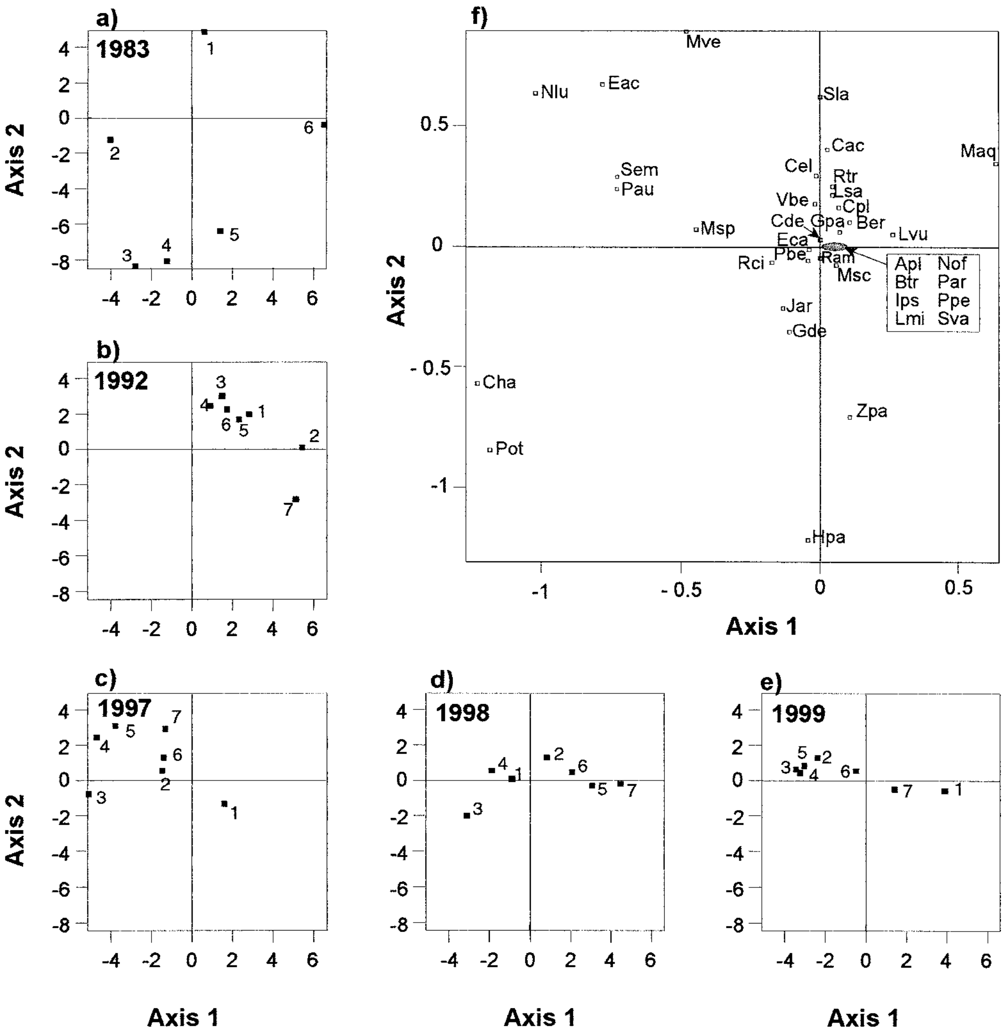

Figure 4. Principal components analysis of the established vegetation data set in the reference zone. Data of the sampling stations concerning all the dates were analyzed together but dispactched per date on several factorial maps (a–e) for increasing readability. (f) ordination diagram of the species in the reference zone (see Table 2 for species codes).

ure 4, Table 2). *Hottonia palustris*, *Potamogeton natans* (together with *P. nodosus*) and *Chara* sp., which were dominant in the vegetation in 1983, disappeared by 1992. From 1992, on the dominant species were *Myriophyllum verticillatum* and *Nuphar lutea*, and included *Eleocharis acicularis* since 1998. These changes explain the convergence of the stations between 1983 and 1999 along the second component of the analysis (Figure 4).

The restored zone was characterized by a decrease in species richness before restoration (24, 30, and 17 species in 1983, 1992, and 1997, respectively). Species with high positive scores on the first component of the analysis (e.g., *Berula erecta*, *Carex elata*, *Rorippa amphibia*, Figure 5), and some species with high positive scores on the second component (*Callitriche platycarpa)* decreased in abundance between 1992 and 1997 (Table 3). Species with high negative scores on the first component of the analysis (*Elodea canadensis*, *Nuphar lutea* and *Sparganium emersum*) increased in abundance during the same period. Number of species increased after restoration (21 and 20 species in 1998 and 1999, respectively). Sampling stations appeared more dispersed along the second component, indicating a higher community heterogeneity after the restoration work. *Berula erecta*, *Elodea nuttallii*, *Nuphar lutea*, and *Sparganium emersum* became the most abundant species after the restoration work.

Spatial Dynamics of the Established Vegetation in the Restored Zone

Vegetation changes over the 17-year period differed among the five sampling stations. Station 1 (where restoration consisted of sediment infilling) appeared poorly correlated with the other stations (where restoration

Table 2. Species codes used in the analyses.

Apl	*Alisma plantago-aquatica* L.	Msc	*Myosotis scorpioides* L.
Ber	*Berula erecta* (Huds.) Coville	Msp	*Myriophyllum spicatum* L.
Btr	*Bidens tripartita* L.	Mve	*Myriophyllum verticillatum* L.
Cpl	*Callitriche platycarpa* Kütz.	Nof	*Nasturtium officinale* R. Brown
Cac	*Carex acutiformis* Ehrh.	Nlu	*Nuphar lutea* (L.) Smith
Cel	*Carex elata* All.	Par	*Phalaris arundinacea* L.
Cde	*Ceratophyllum demersum* L.	Pau	*Phragmites australis* (Cav.) Steud
Cha	*Chara sp.*	Phy	*Polygonum hydropiper* L.
Eac	*Eleocharis acicularis* (L.) Roem. et Scult.	Pbe	*Potamogeton berchtoldii* Fieb.
Eca	*Elodea canadensis* Michaux	Pcr	*Potamogeton crispus* L.
Enu	*Elodea nuttallii* (Planch.) St John	Pot	*Potamogeton* (*natans* + *nodosus*)
Efl	*Equisetum fluviatile* L.	Ppe	*Potamogeton pectinatus* L.
Gpa	*Galium palustre* L.	Rci	*Ranunculus circinatus* Sibth
Gde	*Groenlandia densa* (L.) Fourr.	Rtr	*Ranunculus trychophyllus* Chaix
Hvu	*Hippuris vulgaris* L.	Ram	*Rorippa amphibia* (L.) Besser
Hpa	*Hottonia palustris* L.	Ssa	*Sagittaria sagittifolia* L.
Ips	*Iris pseudacorus* L.	Sva	*Samolus valerendii* L.
Jar	*Juncus articulatus* L.	Sla	*Scirpus lacustris* L.
Lmi	*Lemna minor* L.	Sem	*Sparganium emersum* Rehm.
Ltr	*Lemna trisulca* L.	Tla	*Typha latifolia* L.
Lvu	Lysimachia vulgaris L.	Vbe	*Veronica beccabunga* L.
Lsa	*Lythrum salicaria* L.	Zpa	*Zannichellia palustris* L.
Maq	*Mentha aquatica* L.		

consisted of sediment dredging) (Table 4). Before restoration, *Potamogeton natans*, *Equisetum fluviatile*, *Scirpus lacustris*, and *Typha latifolia* decreased (Table 3, see Table 2 for species codes and authorities), while *Berula erecta* and *Elodea canadensis* increased (Table 3). After restoration, two riparian species, *Mentha aquatica* and *Polygonum hydropiper*, plus a species that tolerates immersion, *Sparganium emersum*, increased in this area.

By contrast, before restoration, stations 2, 3, 4, and 5 (subjected to sediment dredging) were characterized by an increasing abundance of *Elodea canadensis* and *Phalaris arundinacea* (Table 3), together with *Nuphar lutea* in the stations 4 and *Mentha aquatica* in station 2 and *Carex elata* in station 3. After restoration, the changes were due mainly to the increasing abundance of *Elodea nuttallii*. Station 1 (subjected to sediment filling) appeared very different from stations 3, 4, and 5 (Table 4). Station 2, almost as closely correlated to station 1 as it was to stations 3, 4, and 5, was intermediate between these two groups.

Propagule Bank

Propagules in the soil bank were three times more numerous the year after the restoration work (almost 11,000 propagules/m^2) than before (3,350 propagules/m^2, averages densities) (Table 5). All propagules except buds and turions increased in abundance after restoration, but only unspecialized fragments increased significantly (Mann-Whitney test, Table 5). As a consequence, the ratio of sexual versus vegetative propagules was halved after restoration (25.4 before and 12.6 after).

Twenty-five species were found in the propagule bank. Six species showed a significant increase in propagule abundance before Bonferroni adjustment, whereas 1.25 would have been expected by chance (p value of 0.05), leading us to consider that the results are of ecological significance. Twelve species (representing 17 species-type of propagule pairs) did not show any change whatever the propagule type considered (Mann-Whitney test, Table 6). Among these 12 species, seven were characterized by the increase or the appearance of vegetative propagules (*e.g.,* bulbs (*Sagittaria sagittifolia*), fragments (*Berula erecta*, *Rorippa amphibia*), or rhizomes (*Sparganium emersum*)). New types of propagules were observed for two taxa (fragments of Poaceae and seeds of *Potamogeton berchtoldii*).

Five new species (*Berula erecta*, *Bidens tripartita*, *Mentha aquatica*, *Polygonum hydropiper*, and *Polygonum mite*) appeared in the soil propagule bank after the restoration; species content of the propagule bank before and after restoration were thus very close (similarity calculated with the Sørensen index: $Q_{\text{Sørensen}} = 0.81$). The species content changed more in the established vegetation (lower similarity, $Q_{\text{Sørensen}} = 0.74$) than in the propagule bank.

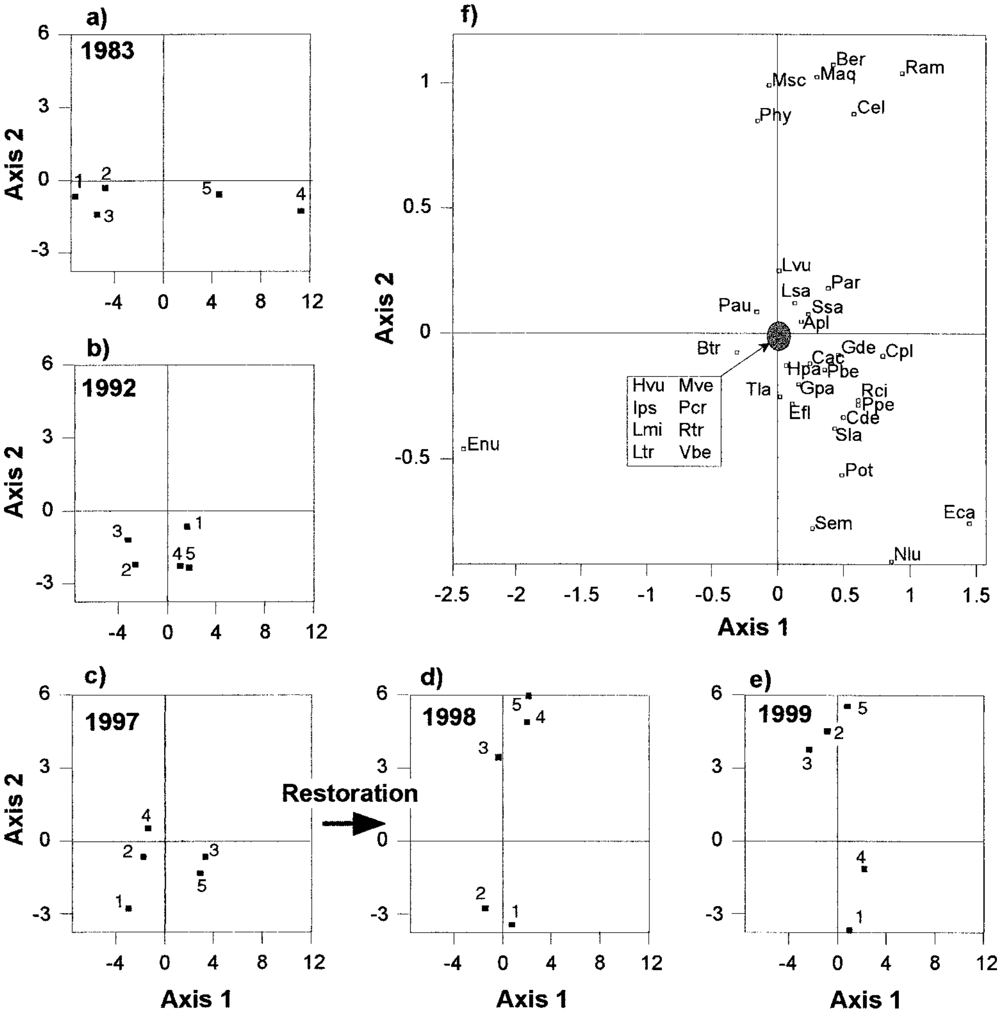

Figure 5. Principal-component analysis of the established vegetation data set in the restored zone. Data of the sampling stations concerning all the dates were analyzed together but dispactched per date on several factorial maps (a–e) for increasing readability. (f) ordination diagram of the species in the restored zone (see Table 2 for species codes).

Links Between the Propagule Bank and the Established Vegetation

Similarity between species content of the propagule bank and the established vegetation was closer after restoration than before ($Q_{Sørensen}$ = 0.60 and 0.41, respectively). No correlation between propagule abundance and vegetation cover occurred before the restoration (Table 7), whereas a positive correlation (even if significance could appear not sufficiently high) was found between rhizomes and fragments in the bank and the vegetation immediately after restoration (ρ = 0.45; p = 0.03) and in the following year (ρ = 0.41; p = 0.05) (Table 8).

DISCUSSION

Vegetation Dynamics

The reference zone was characterized over the study period by the increased abundance of competitive species (*Nuphar lutea*, *Myriophyllum verticillatum*, relatively sensitive to physical disturbance (Amoros et al. 2000)). This led to the dominance of these few species and thus reduced the vegetation heterogeneity (Figure 4). A comparable trend occurred in the restored zone before restoration (decrease in species richness). After the restoration, both competitive species (*Nuphar lutea*, *Sparganium emersum*) and ruderal species tolerant of physical disturbance (*Berula erecta*, *Mentha aquatica*, *Myosotis scorpioides*, *Polygonum hydropiper*, Amoros et al. 2000) occurred in the restored zone, explaining the increase in species richness.

Patterns of vegetation recolonization seemed to depend on the nature of the disturbance (scouring vs. sediment deposition); sediment deposition, which is known to inhibit seed germination of aquatic plants (Dittmar and Neely 1999), disturbed below-ground organs less than did dredging. Consequently, plants were able to regrow from undamaged root systems, and the

Table 3. Changes in species cover of established vegetation in the restored zone from 1983 (83) to 1999 (99). The converted values is provided for the 20 most abundant species (see Table 2 for species codes; the converted value is based on cover/abundance and sociability scales, Bornette and Amoros 1991, Bornette et al. 1994). Data from 1998 and 1999 are post restoration.

Stations	1					2					3					4					5				
Date	83	92	97	98	99	83	92	97	98	99	83	92	97	98	99	83	92	97	98	99	83	92	97	98	99
Ber	—	5	2	3	2	4	3	1	3	3	2	3	5	1	—	6	3	1	3	2	4	3	2	2	2
Cpl	—	2	—	2	—	—	3	—	2	—	—	2	—	1	—	—	6	—	—	1	4	6	—	—	1
Cel	—	3	—	—	—	3	—	—	—	—	—	2	3	—	—	7	5	2	2	3	6	3	2	2	3
Cde	—	—	—	—	—	3	2	—	—	1	3	5	—	1	2	—	1	—	—	—	—	2	—	—	—
Eca	—	2	4	—	—	2	5	7	—	—	—	5	2	—	—	—	3	7	—	—	—	5	4	1	—
Enu	—	—	—	—	—	—	—	—	—	7	—	—	—	6	6	—	—	—	7	—	—	—	—	7	7
Gpa	—	—	1	1	—	—	—	1	1	—	—	2	1	1	—	—	3	—	2	—	—	3	2	2	1
Maq	—	2	1	3	5	—	1	1	—	—	—	—	1	—	—	6	1	—	1	3	—	3	5	1	—
Msc	2	3	1	1	5	2	2	1	—	3	2	1	5	2	2	6	2	—	2	3	2	2	2	2	2
Nlu	6	5	6	5	5	6	5	7	5	7	7	6	7	2	6	—	7	7	1	3	2	6	6	2	5
Par	—	5	—	2	2	—	5	1	1	1	—	2	2	—	—	—	2	2	2	—	—	—	2	1	—
Pau	4	3	6	6	3	4	3	4	6	3	4	3	4	6	3	2	3	3	3	3	7	1	3	1	3
Phy	—	1	—	5	2	—	1	1	2	1	2	2	1	2	1	6	2	—	2	—	2	2	1	3	1
Pot	6	—	—	—	—	6	—	—	—	—	3	3	—	—	—	—	—	—	—	—	—	—	—	—	—
Ppe	3	2	—	—	—	3	5	—	—	—	3	2	—	—	—	—	1	—	—	—	—	—	—	—	—
Rci	2	2	—	—	—	5	2	—	—	—	3	3	—	—	—	—	1	—	—	—	—	—	—	—	—
Ram	—	2	—	—	—	2	3	1	1	1	2	2	5	1	—	6	2	1	—	—	2	3	5	—	—
Sla	6	3	—	—	—	4	2	—	—	—	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sem	3	—	6	3	5	3	5	1	5	2	4	5	—	3	3	—	5	—	3	2	—	5	—	1	1

Table 4. Correlation matrix between the five data tables of established vegetation (one for each station of the restored zone).

Stations	1	2	3	4	5
1	1	0.45	0.22	0.06	−0.02
2	—	1	0.53	0.28	0.37
3	—	—	1	0.26	0.52
4	—	—	—	1	0.47
5	—	—	—	—	1

opportunity for the establishment of new species was rare. Species dominant in station 1 after restoration (where restoration consisted of sediment infilling) were already present, either on the edges of the channel (*Mentha aquatica*, *Polygonum hydropiper*) or in the channel (*Sparganium emersum*). Dredging disturbed the below-ground parts of plants. Consequently, plants were not able to regrow, and new species, such as *Elodea nuttallii*, had the opportunity to establish in gaps. *Elodea nuttallii* became very abundant in this zone after the restoration, despite the fact that it appeared only as small individuals in the bank before the event. The sudden increase of this species could be attributed to (1) the restoration of a permanently aquatic stage and (2) the decreasing competition it had to face (due to decreasing dominance of plants with floating-leaves, mainly *Nuphar lutea*).

Soil Propagule Bank and Regenerative Strategies

Propagule Abundance. The first question addressed by this paper was whether or not restoration work would lead to a change in the active propagule bank (i.e., propagules involved in community regeneration). While few changes in species content were noticed, restoration led to an increase in the propagule abundance. This result was quite unexpected because 1) scouring altered the surface sediment, and 2) other restoration studies where the sediment was not altered (Galatowitsch and van der Valk 1996) reported that seed banks of restored wetlands contained fewer species and fewer seeds than those of natural wetlands. This result could be due to water dispersal of seeds into the site, germination, growth, flowering, and seed set (Baldwin and Derico 1999) or to the breaking of some propagules' dormancies by the disturbance. Indeed, the seedling emergence method only assessed the 'active propagule bank' ('ecologically active component', Gross 1990, Abernethy and Willby 1999). Propagules that needed specific treatment (e.g., scarification) to break their dormancy were not detectable in the samples collected before restoration but were more prone to germinate after the restoration if scouring has served to scarify seeds.

The Role of Vegetative Propagules in Community Regeneration. The second question addressed by this paper was whether regeneration involved vegetative propagules produced by plant breakage during the restoration. The absence of a correlation between the established vegetation and propagule bank before restoration suggests that recruitment from the propagule bank was low. This correlation increased after restoration (due mainly to rhizomes and fragments). New propagules, especially vegetative ones, appeared in the bank after the restoration work. Soon after the disturbance, plants may regrow from vegetative propagules, as indicated by the high correlation between rhizomes and fragments in the bank and the standing vegetation (Table 8). Rhizomes could have survived the disturbance because of deep anchorage. Plant individuals could have been broken into viable fragments that entered the propagule bank, leading to a significant increase in the number of unspecialized fragments that regrew into full individuals.

Most species that dominated after restoration were abundant in the propagule bank as vegetative propagules (*Elodea nuttallii* and *Sparganium emersum*). *Sparganium emersum* was present as rhizomes and fragments. Before restoration, *Elodea nuttallii* occurred only as a few fragments in the propagule bank

Table 5. Propagule abundance in the bank. no/m2 = number of propagules per square meter (Average densities (SE)). U = results of the Mann-Whitney's tests. S/V = ratio of sexual propagules (*e.g.,* seeds) versus vegetative propagules (*e.g.,* buds, turions, bulbs, rhizomes, and unspecialized fragments).

Type of Propagule	Before Restoration (no/m2)	After Restoration (no/m2)	U (p)
Seeds	3,229 (1,606)	10,129 (3,470)	16 (0.14)
Buds, Turions	9 (9)	0	7.5 (0.26)
Bulbs	9 (9)	41 (25)	12.5 (0.46)
Rhizomes	34 (34)	41 (41)	10 (>0.99)
Unspecialized fragments	76 (21)	725 (252)	20 (0.01)
Total	3,356 (1,616)	10,936 (3,491)	16 (0.14)
S/V	25	13	

Table 6. Propagule abundance for each species and propagule type present in the bank (number per m^2). Differences in the propagule abundance measured pre- and post-restoration were tested with a Mann-Whitney test (fifth column). Number of significant values (6) was greater than the number expected by chance (1.25).

Species	Type of Propagules	Before Restoration	After Restoration	U (p)
Alisma plantago-aquatica	seed	8	508	0 ($p \leq 0.05$)
Berula erecta	seed	0	41	NS
Berula erecta	fragment	0	7	NS
Bidens tripartita	seed	0	20	NS
Callitriche platycarpa	seed	136	386	2.0 ($p \leq 0.05$)
Carex elata	seed	8	854	2.5 ($p \leq 0.05$)
Chara sp.	seed	195	3580	2.0 ($p \leq 0.05$)
Elodea nuttallii	fragment	25	386	NS
Poaceae	fragment	0	210	0 ($p \leq 0.01$)
Poaceae	seed	220	651	NS
Mentha aquatica	fragment	0	14	NS
Nuphar lutea	seed	42	20	NS
Polygonum hydropiper	seed	0	1363	0 ($p \leq 0.01$)
Polygonum hydropiper	fragment	0	20	NS
Polygonum mite Schrank	seed	0	20	NS
Potamogeton berchtoldii	bud	8	0	NS
Potamogeton berchtoldii	seed	0	20.34	NS
Rorippa amphibia	seed	153	41	NS
Rorippa amphibia	fragment	42	88	NS
Sagittaria sagittifolia	seed	17	0	NS
Sagittaria sagittifolia	bulb	8	41	NS
Sparganium emersum	rhizome	34	41	NS
Sparganium emersum	fragment	8	0	NS
Urtica dioica L.	seed	8	20	NS
Veronica spp	seed	2441	2603	NS

and was absent from the established vegetation, but many fragments were found in the propagule bank afterwards. This species is known to propagate mainly by vegetative means (Simpson 1990). Restoration created permanently aquatic conditions, allowing *E. nuttallii* fragments to invade the restored area and replace *E. canadensis*. This colonization likely has also involved fragments entering the zone through river backflows (Barrat-Segretain 2001).

The abundance of *Nuphar lutea* increased after the restoration work despite the fact that, as already noted in other studies (Smits et al. 1990, Barrat-Segretain 1996), few seeds were detected in the propagule bank (Table 6). This species is known to propagate inefficiently by seeds but can propagate through rhizomes (Helslop-Harrison 1955, Smits et al. 1990, Barrat-Segretain 1996). All individuals recorded in the first growing season following restoration had floating leaves (pers. obs.). Since individuals issued from seeds do not develop floating leaves during the first growing season (Helslop-Harrison 1955), the recolonizing *N. lutea* population would therefore appear to have sprouted from rhizomes, even though we did not detect any *N. lutea* rhizome in our propagule bank sampling. *Nuphar lutea* rhizomes are deeply anchored (> 5 cm) and have a large diameter (up to 5 cm) (post sampling observations). As we sampled cores of 5 cm in diameter and 5 cm deep, we could hardly sample these rhizomes. We were thus unable to provide a quantitative estimation of the amount of *N. lutea* rhizomes involved in the recolonization processes.

Elodea nuttallii, *Nuphar lutea*, and *Sparganium emersum* are usually considered to be competitive species (Grime et al. 1988, Simpson 1990, Barrat-Segretain 1996), but they appeared to recolonize the restored zone efficiently due to their vegetative propagation and, thus, could also be considered as disturbance-tolerant, even in the case of a rare disturbance event.

Disturbance Timing

The predominance of vegetative reproduction in recolonization was probably influenced by the season in which the restoration work was carried out. Propagule availability depends on the timing of the disturbance (Denslow 1980, Sousa 1984, Kotanen 1996). In June, plants produce many shoots or rhizomes that have a great ability to regrow in case of breakage (Barrat-Segretain et al. 1998, Barrat-Segretain et al. 1999).

The timing could also partly explain the low in-

Table 7. Spearman rank order correlation coefficients between propagule bank before the restoration and established vegetation before and after restoration. For each propagule type, the correlation coefficient and the significance level (in brackets) are provided (none are significant).

Propagule Bank Before Restoration (Sampled in Spring 1998)	Established Vegetation One Year Before Restoration (Sampled in Summer 1997)	Established Vegetation Just After Restoration (Sampled in Summer 1998)
Seeds	−0.20 (0.40)	−0.40 (0.06)
Buds	−0.30 (0.20)	−0.29 (0.17)
Bulbs	−0.04 (0.85)	−0.29 (0.17)
Rhizomes	0.11 (0.64)	0.28 (0.19)
Unspecialized fragments	0.08 (0.74)	0.28 (0.17)
Whole propagule bank	−0.33 (0.17)	−0.23 (0.26)

Table 8. Spearman rank order correlation coefficients between propagule bank after the restoration and established vegetation just after and one year after restoration. For each propagule type, the correlation coefficient and the significance level (in brackets) are provided (*: significant relationships). Correlations between rhizomes + unspecialized fragments and established vegetation were provided, as they were the only significant relationships.

Propagule Bank After Restoration (Sample in Spring 1999)	Standing Vegetation Just After Restoration (Sampled in Summer 1998)	Standing Vegetation One Year After Restoration (Sampled in Summer 1999)
Seeds		
Bulbs	−0.26 (0.22)	−0.30 (0.15)
Rhizomes	−0.29 (0.17)	−0.29 (0.17)
Unspecialized fragments	0.27 (0.19)	0.23 (0.27)
Rhizomes + unspecialized	0.35 (0.09)	0.33 (0.12)
fragments	0.45 (0.03)*	0.41 (0.05)*
Whole propagule bank	−0.14 (0.49)	−0.18 (0.38)

volvement of seeds in the recolonization process, despite their abundance. Seed banks are usually depleted in summer (Thompson and Grime 1979, Roberts 1981, Welling et al. 1988). The seed bank could have been replenished during late summer or early autumn by plants that had recolonized the site just after the restoration, as we sampled the propagule bank almost one year after the restoration (spring1999). Among the species that increased in abundance in the seed bank after restoration (Table 6), all but *Carex elata* were able to flower or bear fruit until September or October (Grime et al. 1988, Lambinon et al. 1992). Propagules that survived the restoration (whatever their type) may have regrown into full individuals and borne fruit during the autumn following restoration. The seed abundance of species that rarely flower after August (*Nuphar lutea*, *Rorippa amphibia*, and *Sagittaria sagittifolia* (Lambinon et al. 1992)) was lower in the bank after restoration than before. *Carex elata* remained on undisturbed banks and could have borne fruit in summer.

Conclusions and Management Implications

This study outlines the important role of vegetative reproduction in recolonization after restoration of an aquatic habitat, especially in the case of sediment dredging carried out during the growing season. The restoration led to an increase in the abundance of an invasive species that is likely to depress native species richness (*Elodea nuttallii)*, which propagated only vegetatively. The propagation of this species was probably enhanced by the timing of the disturbance. In aquatic habitats, recolonization after disturbance often involves vegetative reproduction, and this trend is amplified when the disturbing event occurs during the summer (few seeds immediately able to regrow and many vegetative propagules potentially available). Invasive species with great potential for vegetative propagation have a competitive advantage compared to seedlings (sprouting from seeds), as their vegetative propagules (e.g., fragment) already possess photosynthetic structures. In our study, summer was the only period when dredgers could reach this zone due to ground conditions and flood risk. Knowledge of the propagule bank composition before restoration and the previous pattern of community development did not permit us to predict the recolonization pattern. Consequently, physical restoration may initiate a trajectory of community development not previously seen at that site or in adjacent reference sections. The risks of introducing invasive species as a result of management policies (Smith and Kadlec 1983), restoration work, or disturbance have already been stated, especially in the case of restoration involving imported seed banks (van der Valk et al. 1992, Vivian-Smith and Handel 1996). According to van der Valk and Pederson (1989) ''vegetation management, based on the exploitation of seed banks, will be successful only when (1) the seeds of required or preferred species are present in the seed bank, (2) the seeds of unwanted species are not present or, at least are uncommon, and (3) conditions suitable for the germination of the seeds of preferred species can be established or maintained''. In the case of aquatic habitats, we can add to these statements that vegetation management will be successful only when: (1) the seeds or *vegetative propagules* of required or preferred species are present in the *propagule bank* or *can be created by breakage during the restoration,* (2) the seeds or *vegetative propagules* of unwanted species are not present or are, at least, uncommon *or can be physically excluded,* (3) conditions suitable for seed germination *or the sprouting of vegetative propagules* of preferred species can be established or maintained, and (4) *conditions promoting the germination or sprouting of unwanted species can be avoided.*

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Propagule banks and regenerative strategies of aquatic plants

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Abstract. The role of the propagule bank in aquatic plant maintenance was studied in two riverine wetlands. Four sites were selected, characterized respectively by flooding, drying up, both disturbances operating, and neither operating. Our hypothesis was that recolonization after drying up would mostly involve seeds and buds from the propagule bank, whereas recolonization after floods would mostly involve rhizomes. Dry sites were characterized by a high density of seeds, and a high similarity between seed species and established vegetation. Unspecialized fragments remaining in wet parts of the sediment probably also contribute to species maintenance. Species maintenance in sites subjected to flooding was highly dependent on deeply anchored rhizomes, as indicated by the strong floristic similarity between species that occur in the established vegetation and rhizomes in the bank. Regeneration of the community after scouring floods also involved seeds, some species being able to flower under water. When scouring flooding and drying up were superimposed, regenerative strategies exhibited in the bank did not simply result from the 'addition' of the two disturbance effects. When the disturbances did not occur too closely together in time, species were able to survive either by: (1) producing many propagules under aquatic conditions or (2) coping with the temporal variability by producing several types of propagules.

Keywords: *Chara vulgaris*; Cut-off channel; Disturbance; Drying up; Flooding; Hydrophyte; *Potamogeton pusillus*; Scouring; Seed bank; Wetland.

Nomenclature: Lambinon et al. (1992); Wiegleb & Kaplan (1998) for *Potamogeton* species and Corillon (1975) for *Characeae*.

Introduction

The role of seed banks in vegetation recovery after disturbance has been frequently studied (Thompson 1978; Grelsson & Nilsson 1991; Wisheu & Keddy 1991). Disturbances could have two major effects on propagule banks: (1) the substrate is not altered by the disturbance and recolonization processes could involve the propagule bank and (2) the substrate is altered by the disturbance and recolonization processes rarely involve the propagule bank (Tsuyuzaki 1994; Kotanen 1996).

In wetlands, drying up generally leads to biomass loss in the established vegetation but the substrate is little altered. Indeed, studies have shown that the growth of aquatic vegetation following a dry period generally involves germination from the seed bank (e.g. Brock & Rogers 1998). Seed abundance in the bank decreases with an increased hydroperiod (Poiani & Johnson 1988). These studies mainly involved mudflats, meadows and emergent wetland communities subjected to infrequent (once every several years) and long-lasting (some months to some years) dry periods during which a new plant community may establish itself ('press' disturbance *sensu* Lake 2000). Seed banks of permanently aquatic habitats (true hydrophyte communities) experiencing frequent (i.e. several times a year), unpredictable and very short periods of drying up ('pulse' disturbance *sensu* Lake 2000) have seldom been investigated (Abernethy & Willby 1999). Aquatic vegetation maintenance could, however, involve vegetative structures that can survive dry conditions, e.g. winter buds, turions (that resemble seeds in their ecology and physiology, Abernethy & Willby 1999) or tubers (Spencer & Ksander 1992).

Flooding potentially scours the substrate in riverine wetlands (Bornette et al. 1994; Müller 1995; Ward et al. 1999) and consequently exports or destroys propagules. Frequent scouring favours regeneration through unspecialized fragments and rhizomes.

Combroux et al. (2001) showed that regenerative strategies developed in hydrophyte communities subjected to flooding differed from those developed when subjected to both flooding and drying up.

The aim of the present study is to test hypotheses concerning the effects of these two kinds of disturbance, which occur in riverine wetlands, on regenerative strategies in aquatic species. Involvement of the propagule bank in hydrophyte community regeneration may differ in riverine aquatic habitats subjected to flooding and in habitats subjected to episodic drying up. Lowering of the water level should favour the production of seeds, buds and turions by the aquatic vegetation. These propagules should be more abundant and more firmly linked to the hydrophyte established vegetation than is the case in permanently aquatic habitats. Scouring would be survived by deeply anchored rhizomes. Rhizome species composition should be more correlated with the established vegetation than those found in other sites. Two species, capable of producing various regenerative structures, were studied in detail to determine if different regenerative strategies were favoured by particular disturbances at the species level.

Material and Methods

Study sites

Study sites were located in two riverine wetlands in cut-off channels along the Rhône river. The first, 1.4 km long, was located ca. 90 km upstream of Lyon, France (Fig. 1), the second, 2.7 km long, 30 km upstream of Lyon. When the river discharge falls below 250 $m^3.s^{-1}$ (ca. 12 × per yr) at site 1 and below 300 $m^3.s^{-1}$ (ca. 11× per yr) at site 2, the upstream part of these wetlands is subjected to drying up. The mean duration of such events is 4 d for site 1 and 3.5 d for site 2. This disturbance causes the death of the vegetative parts of aquatic plants but is too short to allow the development of new helophyte or terrestrial plant communities. The Upper Rhône river is a piedmont river, characterized by peak floods occurring stochastically (Müller 1995; Ward et al. 1999). When the river flow exceeds 1000 $m^3.s^{-1}$ (ca. 9 × per yr), the river overflows onto the second wetland. The mean duration of a flood is 4 d (Combroux et al. 2001). Flooding can occur at any time of the year.

Two sites were sampled in each wetland. Downstream, sites which never dry up were referred to as S in wetland 2, subjected to flooding, and O in wetland 1 which is never flooded. Upstream sites subjected to drying up were referred to as SD in wetland 2 and D in wetland 1 (Fig. 1). The main differences between sites (Fig. 1) are due to the differences in scouring regimes. Sites SD and S had a coarser substrate grain size due to the scouring effects. Although both wetlands were isolated in the same period (late 19th century), sites D and O had a thicker layer of fine sediment and more eutrophic species due to the absence of any flood regime (Bornette et al. 1994)

Propagule bank

The propagule bank was quantified by the seedling emergence method (Thompson et al. 1997, see also Boedeltje et al. 2002 for aquatic plants). Sampling was conducted in March 1998 after winter stratification and before spring germination (or sprouting, i.e. a few weeks and four months after the latest drying up in sites D and SD, respectively and two months after the latest flooding in sites S and SD. Sediment cores, 5 cm ∅ (n =15) were collected from each sampling station and five stations were sampled at each site. Sampling stations

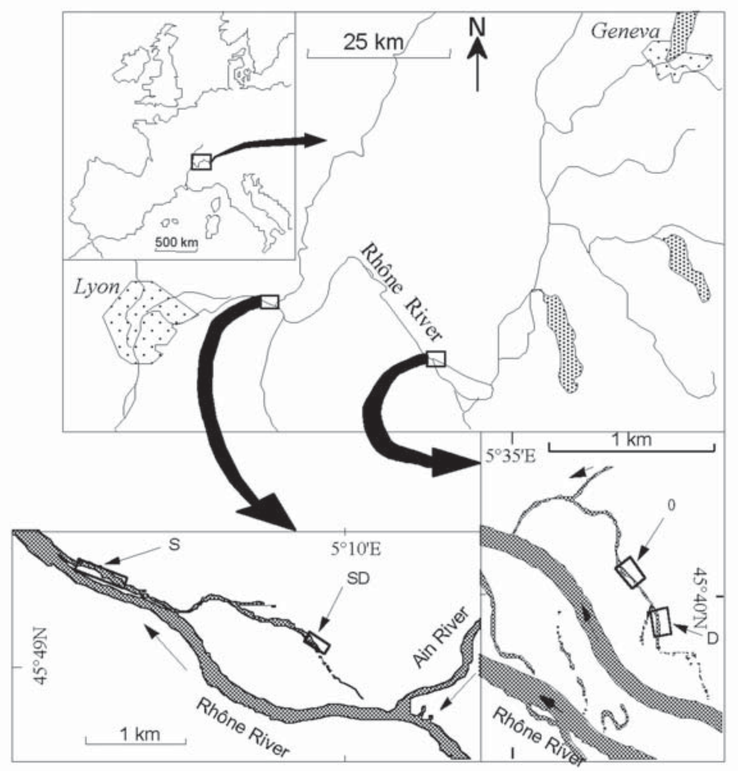

Fig. 1. Location and disturbance regimes in the study sites and site characteristics. S = flooding; D = drying up; SD = S and D combined; O = neither S nor D.

Site	SD	S	D	O
Study site length (m)	215	525	125	140
Mean channel width (m)	15	64	8	9
Mean water depth (m)	0.5	1.3	0.30	0.30
Substrate: gravel (%)	86	72		
Substrate: silt (%)	14	26	100	100
Substrate: sand (%)		2		

The transparency (Secchi depth) is higher than the water depth in every site were evenly distributed along the sites. Only the upper 5 cm of the cores was used because this depth is usually considered as the maximum from which the germination of species involved in short-term vegetation regeneration can take place (Dugdale et al. 2001). The 15 cores were thoroughly mixed and divided into three subsamples which were subjected to three treatments: (1) inundation: sediment permanently flooded under 4 cm of water collected from the wetland, (2) exposure: sediment maintained at saturation point, (3) mixture: sediment maintained at saturation point for one month and then re-flooded under 4 cm of site water. Seedling emergence was monitored in a climate controlled room where the natural photoperiod was supplemented with additional light (L/D: 12/12, at 25/10°C, respectively). Samples were spread on 18 cm × 24 cm × 10 cm polystyrene trays (sediment thickness < 1.5 cm). The rooting layer was first increased by spreading 2 cm of sterile sand (heated for 5 hr at 170 °C) over the bottom of the trays. Site water was filtered through a 100-µm mesh sieve to prevent the addition of propagules. Bubbling air maintained a high oxygen content (90-100%) and slow water movements. Plant emergence was monitored from March to December. Plants were identified and assigned to a source (seeds or oospores, turions or winter buds, bulbs, rhizomes, unspecialized fragments) on a regular basis then removed from the trays to prevent competition and allelopathic effects. Plants were identified to species except for *Poaceae* and some *Characeae* (*Nitella* spp.). *Veronica* spp. included *V. beccabunga* and *V. anagallis-aquatica* and *Ranunculus* spp. included *R. trichophyllus* and *R. trichophyllus* × *circinatus*.

To assign species to a seed bank type (Thompson et al. 1997), germinations were also recorded in the second year after sampling. Species were classified as: (1) transient: species germinating only in the first year (i.e. from March to December 1998) or (2) persistent: species germinating during both years of experimentation (i.e. from March 1998 to December 1999).

The core sampling and emergence method usually fail to detect large propagules deep in the soil (mainly rhizomes lying below the upper 5 cm). Therefore, rhizome abundance in the propagule bank was surveyed in mid-spring (May) by counting shoots sprouting from rhizome meristems within three quadrats (30 cm × 30 cm) at each station. When rhizomes were detected by both the emergence method and quadrat sampling, the highest estimate of rhizome abundance was used.

For each species encountered either in the propagule bank or in the established vegetation, the ability to produce different propagule types (seed, bud or turion, rhizome) was documented (Sculthorpe 1967; Cook 1990; Grime et al. 1990) and compared with propagule types encountered in the field.

Established vegetation

Sampling stations (2 m strips crossing the wetland) were evenly distributed along the sites (7, 4, 6 and 7 stations in sites S, SD, D and O, respectively, the number depending on vegetation heterogeneity) and were surveyed during the summer of 1998 (see Combroux et al. 2001 for sites S and SD). No floods occurred in sites S and SD between the propagule bank and established vegetation samplings. One drying up occurred in site SD a few days after the propagule bank sampling and four dry periods occurred in site D between the propagule bank and established vegetation samplings. At each sampling station, aquatic vegetation was surveyed using Braun-Blanquet cover/abundance scales:

1 = < 5%; 2 = 5-25%; 3 = 25-50%; 4= 50-75%; 5 = 75-100%.

Data analysis

As the species pools were slightly different in the two wetlands, the species were placed into three groups for analysis, according to Raunkiær's life forms (1934): (1) hydrohemicryptophytes (Hemi), (2) hydrogeophytes and hydrotherophytes (Geo/The), (3) helophytes (Helo). To detect differences in propagule abundance and type present in the propagule bank, we used centred Principal Component Analysis (Dolédec & Chessel 1991; ter Braak 1995). Calculations were made with log-transformed abundance data. At the species level, two species were studied: *Potamogeton pusillus* and *Chara vulgaris* that were present at every site. A centred PCA was performed on the *P. pusillus* propagule bank data to determine the effect of the type of disturbance on the abundance of the different types of propagules.

Floristic similarities between established vegetation and propagule bank were calculated with the Sørensen similarity index: $Q = 2c/(a+b)$ where a and b are the number of species in the first (propagule bank) and second (established vegetation) lists, respectively, and c the number of species present in both lists (Pielou 1984). Q ranges from 0 (no species in common) to 1 (every species present in both lists). Similarities between established vegetation cover and the abundance of propagules in the bank were calculated with the Ružička similarity index:

$$RI = 100\frac{\sum \min(x_{i1}, x_{i2})}{\sum \max(x_{i1}, x_{i2})} \qquad (1)$$

where x_{i1} and x_{i2} are the proportions of species i in list 1 (established vegetation) and list 2 (vegetative propagules in the bank), respectively. RI ranges from 0 to 100 (Pielou 1984). The 95% confidence intervals for the means of Q and RI were computed by the bootstrap technique (1000 iterations).

Results

Abundance of propagules and seed persistence in the bank.

Sites that experienced drying up were characterized by higher propagule abundance and higher seed abundance than permanently flooded sites (Fig. 2). Buds were significantly more abundant on site D. The greatest abundance of fragments was found in the propagule bank of site D.

The centred PCA sorted sampling stations according to their propagule bank (propagule types and life forms, Fig. 3). The first component of the analysis (35 % of the inertia) separated the sites subjected to floods (negative scores) from the sites D and O (positive scores). The second component of the analysis (27 % of the inertia) separated the dry sites (positive scores) from sites S and O (negative scores). Hydrogeophyte and hydrotherophyte species did not occur as buds and helophytes did not occur as unspecialized fragments. Consequently, these categories were plotted at the zero point of the factorial map. The analysis separated seeds, buds and fragments (positive scores on axes 1 and 2) from rhizomes (negative scores on axes 1 and 2) of hydrohemicryptophytes. It also separated fragments of hydrogeophytes and hydrotherophytes from helophyte seeds.

Most of the seeds that occurred in the propagule bank were persistent: 97 % (± 1.5), 99.6 % (± 0.2), 99.1 % (± 0.4) and 98.9% (± 0.6) of the seeds that germinated (mean ± s.e.) in sites S, D, SD and O, respectively, originated from species with persistent seed bank. *P. pusillus* had transient seeds, whereas *C. vulgaris* occurred as persistent oospores at every site.

Species distribution in propagule bank and established vegetation

Four hydrophyte species: *Berula erecta*, *Elodea nuttallii*, *Hydrocharis morsus-ranae* and *Lemna trisulca*, that occurred in the established vegetation were absent from the propagule bank (App. 1). Most of the significant differences between the propagule banks were due to species that had greater propagule abundance at the dry sites or were confined to them (buds of *Elodea canadensis*). Seeds of *Callitriche platycarpa,* oogones and fragments of *C. vulgaris* and buds of *P. pusillus* (which was the dominant species in the established vegetation in site D, App. 1) were significantly more abundant in the site D propagule bank. *Groenlandia densa*, which occurred in the propagule bank as seeds and rhizomes, only occurred in the sites subjected to floods, whereas species that occurred in the propagule bank only as buds were absent or poorly abundant in these sites. Some species occurred in the propagule bank as unspecialized fragments (*Lemna minor, Riccia fluitans*. Most of these species only occurred in sites D and O (but also in established vegetation and propagule bank; App. 2). *Ceratophyllum demersum,* that occurred in the propagule bank only as unspecialized fragments and *Elodea canadensis,* that was absent from the propagule bank, were the dominant species in the established vegetation in site O.

Similarities between propagule bank and established vegetation

The similarity between the established vegetation and the propagule bank was significantly higher in the scoured than in the scoured and dry site (Fig. 4). A relatively high similarity was found between established vegetation and seeds, whereas it tended to be lower for buds, rhizomes and fragments. The similarity between vegetation and rhizomes was higher for the scoured than for the other sites.

There was a low similarity between established vegetation cover and the abundance of propagules, regardless of the site. The greatest similarity was observed between bud species composition and established vegetation (17%) at site D.

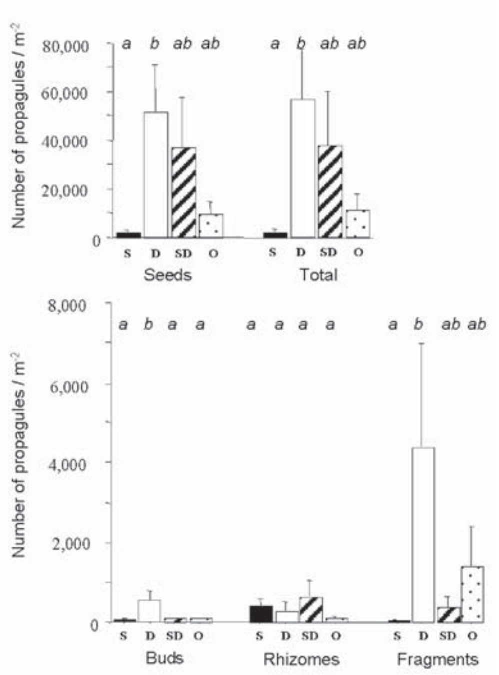

Fig. 2. Propagule abundance (mean no. of propagules per m^2 ± SE; n = 5) at the four study sites. Different letters indicate significant (P < 0.05) differences (Kruskall Wallis test). S = flooding: D = drying up: SD = S and D combined; O = neither S nor D.

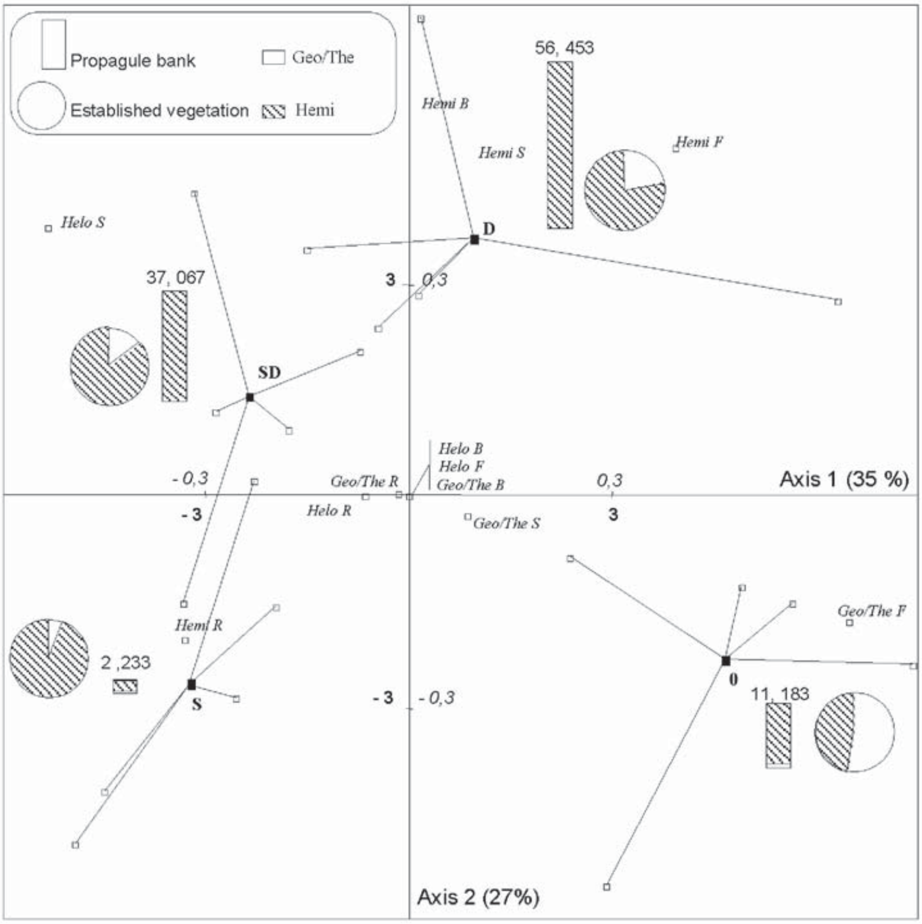

Fig. 3. Dependence of propagule bank content and abundance on disturbance regime. Sampling station biplot based on a group of species-centered PCA of propagule bank data. The inertia of axis 1 (horizontally) and axis 2 (vertically) are 35 % and 27 %, respectively. Hemi = hydrohemicryptophytes; Geo/The = hydrogeophytes and hydrotherophytes; Helo = helophytes; S = seeds; B = buds and turions; R = rhizomes; F = unspecialized fragments (scales in italics). Each sampling station (not labelled) of a site was linked to the centre of inertia of the sites (scales in bold). Close to these centres were plotted bars and discs showing proportion of floating and submerged species in propagule bank and established vegetation, respectively. S = flooding; D = drying up; SD = S and D combined; O = neither S nor D.

Variation of regenerative strategies within-species according to the type of disturbance

P. pusillus can potentially produce seeds, buds and rhizomes. This species had a transient seed bank in every site. The centred PCA performed on the *P. pusillus* propagule bank separated buds (negative scores) from seeds and rhizomes on the first component (64 % of the total inertia, Fig. 5). Sampling stations at site D were all characterized by negative scores on the first component, which correlated with a greater abundance of buds in the bank. S stations were almost all restricted to the positive part of the first component and scattered all along the second component, indicating the dominance of both seeds and rhizomes. Two stations at site 0 were plotted at the zero-point due to the absence of *P. pusillus* propagules in their bank. SD stations were scattered over the whole factorial map; the centre of inertia of site SD was, therefore, plotted near the zero point.

C. vulgaris produces oospores and can also regrow from unspecialized fragments. This species had a persistent bank of oospores at every site. Many unspecialized fragments were found in the bank (App. 2). Oospores were always more abundant than fragments in the bank. Furthermore, the proportion of oospores increased with the intensity of substrate disturbance, as they were 10, 50 and 100 times more abundant than fragments in 0, D and both S and SD sites, respectively.

Discussion

Type of disturbance and regenerative strategies

Regenerative strategies developed by species to survive scouring and drying up mainly concerned the type of propagule involved in regeneration. No differences in seed bank persistence occurred between disturbed and non-disturbed sites, whatever the type of disturbance. Most species had a persistent seed bank at every site.

As hypothesized, seeds were significantly more abundant than vegetative diaspores at sites subjected to drying up (App. 1). The abundance of buds in the banks agreed with the hypothesis (i.e. increasing sites with drying up) only at the unscoured site (site D). Abundance of seeds and buds in the propagule bank suggested that drying up, unlike scouring (Combroux et al. 2001), may not impede flowering and seed set or bud production. Similarities between seeds and established vegetation (either measured with qualitative or quantitative similarity coefficients) were not greater at the D sites than at the others. Recolonization through seeds and buds did not appear to be the only regenerative strategy in habitats subjected to drying up . Unspecialized fragments (mainly from hydrohemicryptophytes) were very abundant in site D. Although able to produce seeds or buds, some species maintained in the established vegetation only through regeneration from unspecialized fragments (e.g. *Ceratophyllum demersum, Utricularia vulgaris*). They may remain in wet parts of the sediment and regrow into full individuals. The ability to regrow from unspecialized fragments has already been noted in aquatic plants (Sculthorpe 1967; Barrat-Segretain et al. 1999) but the effective resistance of such organs to desiccation is poorly understood. Drying up thus favoured regeneration through an abundant bank of seeds or unspecialized fragments. However, availability of these propagules may change during the year. Timing of drying up may affect involvement of propagules in regeneration processes as already noticed in recolonization after a man induced disturbance (Combroux et al. 2002).

Although no flood occurred between propagule bank and established vegetation samplings, established vegetation is close to the recolonizing community since species have been selected by disturbance filters for several decades. Sites subjected to floods were characterized by a high proportion of rhizomes in the propagule bank (Fig. 1, *P. pusillus* Fig. 3) that originate mainly from submerged species (hydrogeophytes and hydrotherophytes, Fig. 2). High floristic similarity between rhizomes and established vegetation at site S suggested that many species present in the established vegetation were also present as rhizomes in the propagule bank. They were supposed to regrow mainly from rhizomes after a flood. Low similarities noticed for the other sites suggested that most species present in the established vegetation did not occur in the bank as rhizomes. Some species do not produce rhizomes (e.g. *Berula erecta, Callitriche platycarpa, Chara vulgaris, Elodea canadensis*) but are present (sometimes abundant) in the established vegetation. Floods can impede sexual reproduction (Combroux et al. 2001) and scour and export seeds and buds stored in the sediment. However, the high floristic similarity between seed composition and established vegetation suggests that some species were able to flower despite scouring conditions. Other propagule species were less correlated with the established vegetation. Some species were totally absent from the propagule bank, despite being abundant in the established vegetation. Among them, *Berula erecta* which can produce seeds, did not occur in the propagule bank at sites SD or S, but this species is evergreen and easily regrows from fragments (Barrat-Segretain et al. 1999). Such species may not be stored in the soil propagule bank but may propagate through fragmentation or from individuals uprooted during floods. Drift and colonization abilities of such propagules have already been noted (Johansson et al. 1996; Cellot et al. 1998; Barrat-Segretain & Bornette 2000).

Chara vulgaris and Potamogeton pusillus

C. vulgaris was the most abundant species as persistent spores at every site, which is consistent with the

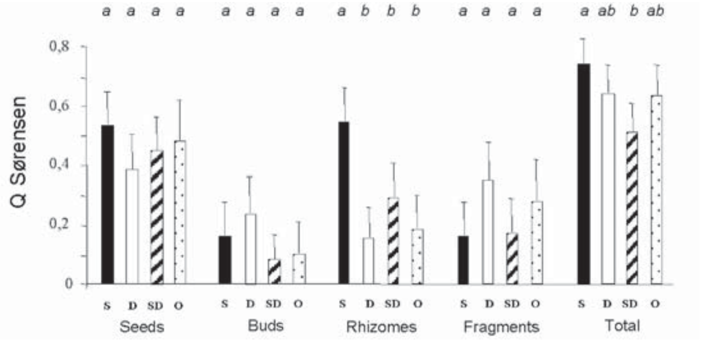

Fig. 4. Floristic similarities (mean Sørensen index ± 95 % confidence intervals calculated by 1000 bootstrap iterations) between species present in the vegetation and in the propagule bank. For each type of propagule, different letters indicate significant ($P < 0.05$) differences. S = flooding; D = drying up; SD = S and D combined; O = neither S nor D.

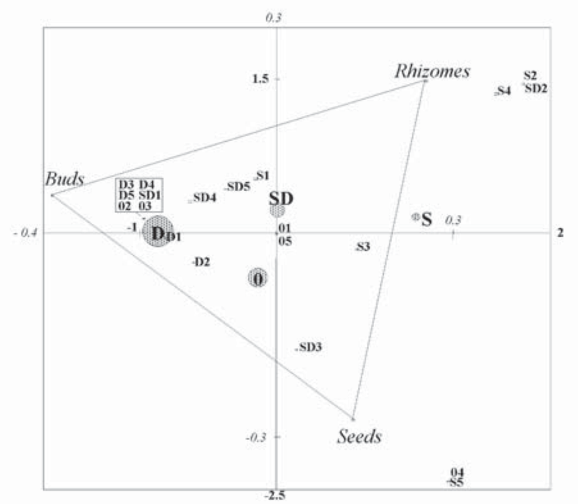

Fig. 5. Dependence of different propagule types of *Potamogeton pusillus* in propagule bank under disturbance. Centered PCA performed on the abundances of the propagules in the propagule banks samples. Inertia of axes 1 = 64%, axis 36%. Scales in italics refer to propagule type; scales in bold refer to sampling station. The centres of inertia of the sites are plotted on the factorial map with circle area proportional to the abundance of *P. pusillus* in the established vegetation. S = flooding, D = drying up, SD = S and D combined; O = neither S nor D.

common characteristics of ruderal species (Grime et al. 1990). The production of oospores can occur over a long period, which could partly explain the high abundance of the oospores in the bank. Although fewer than in the dry site, many *C. vulgaris* oospores were found at the sites subjected to flooding. Seeds and oospores are scoured by the superficial sediment and only a few remain. However, oospores may have persisted in the wetland because they adhered to larger structures (gravel, woody debris, etc.). Reproduction could also be more effective at such sites. Unspecialized fragments may also play a role in recolonization after flood disturbance.

As hypothesized, *P. pusillus* occurred mostly as buds (and seeds) in the dry site and as rhizomes in the sites subjected to flooding. Presence of seeds in the site subjected to floods either suggested that this species managed to set seed within the site or that some seeds produced *ex situ* may have been transported by flood current and deposited (*P. pusillus* seeds may float for more than one day; Praeger 1913).

Combination of two disturbance regimes.

Seed abundance did not differ significantly between the sites subjected to both disturbances (SD) and only drying up (D), but it was higher than in the site subjected to floods (S). In flood meadows of the Upper Rhine floodplain, Hölzel & Otte (2001) found higher seed density associated with frequent flooding. Unlike riverine wetlands, drying up does not represent a disturbance in flood meadows. A slight decrease in the water level of riverine wetlands should be a sufficient trigger to activate hydrophytes seed production. Sediment scouring did not significantly lower the seed stock but the last scouring occurred two months before the propagule bank sampling and probably lowered the seed stock, or the quantity of seeds effectively produced (and exported) at site SD was higher than that produced at site D. Scouring also determined the composition of the propagule bank, as rhizomes were similarly abundant at sites SD and S, and had a high floristic similarity with the established vegetation.

When scouring and drying up are superimposed, regenerative strategies exhibited in the bank do not simply result from the 'addition' of two environmental filters (Keddy 1999). Some types of propagules were filtered out by a single disturbance filter (e.g. seeds in the case of scouring) but they were favoured when this disturbance occurred in combination with another (seeds at site SD). Two types of species were able to cope with the filter resulting from the superimposition of the two disturbances: (1) species such as *C. vulgaris* and *Callitriche platycarpa* that were able to produce large numbers of propagules under aquatic conditions; (2) species such as *P. pusillus* or *Groenlandia densa* that were able to produce several propagule types, increasing their potential to survive these different disturbances as long as they did not occur too closely together in time. *P. pusillus*, for example, may resist scouring through its rhizomes, which regrow into individuals that produce seeds and buds that survive in the sediment during dry periods.

Several findings of this study may have implications in river management and restoration. First, combination of some species traits (e.g. presence of bud and seed bank, ability to regrow from unspecialized fragments) allow hydrophyte communities to persist in wetlands frequently subject to drying up conditions (natural or managed) providing these drying up events are short-time events. This should be taken into account in management policies, for example by choosing a duration of drying up leading (or not) to the death of propagules, or by the collection (or preservation) of plant propagules. Second, the existence of an abundant and persistent seed bank of hydrophyte species in these riverine wetlands indicates the existence of a useful source of propagules for the recolonization of restored wetlands (Hötzel & Otte 2003).

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Réponse des communautés végétales aux conditions environnementales : perturbations ou contraintes

Michèle Trémolières [a], Isabelle Combroux [a], Gabrielle Thiébaut [b] et Jacques Haury [c]

De nombreuses définitions du concept de perturbation ont été publiées. White et Jentsch (2001) distinguent deux types de définition d'une perturbation : une définition relative qui fait référence à une déviation par rapport à la dynamique normale de l'écosystème et une définition absolue basée sur des changements de variables physiques et mesurables (par exemple : biomasse – Grime, 1979 ; disponibilité des ressources – Sousa, 1984). Dans le cas de la définition relative, des événements comme des feux de prairie ou des crues annuelles dans des bras morts ne sont pas considérés comme des perturbations, car ils font partie de la dynamique normale de ces écosystèmes. On parlera alors de contrainte ou stress. Dans le cas de la définition absolue, ces événements sont considérés comme des perturbations car ils entraînent une perte de biomasse, un remaniement des sédiments, etc.

Dans sa définition absolue, la perturbation, en intégrant à la fois la cause et l'effet, est définie comme un événement rare, imprévisible et relativement court (White et Jentsch, 2001), intervenant à diverses échelles de temps et d'espace (Pickett *et al.*, 1989), qui bouleverse la structure de l'écosystème, de la communauté ou de la population (Pickett et White, 1985) et qui modifie qualitativement et quantitativement les propriétés de l'environnement. L'une des conséquences habituelles d'une perturbation est de détruire au moins en partie les individus de l'écosystème qu'elle affecte (défini comme un stress par Grime, 1979 ; Sousa, 1984), en créant ainsi une opportunité pour d'autres individus ou espèces de s'établir (Denslow, 1980). Pour van der Maarel (1993) une perturbation est « *un processus conduisant à une libération de ressources, qui pourront être utilisées soit par les organismes ayant survécu à la perturbation, soit par de nouveaux organismes arrivant dans la communauté* ».

Des pollutions chimiques ponctuelles ou diffuses induisent également des changements souvent rapides de communautés végétales. Par exemple, l'eutrophisation naturelle d'un cours d'eau se traduit par la mise en place d'une zonation des communautés le long du gradient amont aval. Ce gradient naturel est modifié en cas d'apports excessifs de nutriments. Ce changement de conditions se traduit alors par un changement de communauté.

La végétation aquatique, descripteur du fonctionnement des hydrosystèmes : réponse aux perturbations

Dans les hydrosystèmes fluviaux, les crues peuvent constituer des perturbations qui induisent des vitesses d'écoulement suffisantes pour provoquer l'érosion du substrat et l'arrachage des communautés végétales fixées, ou qui favorisent des dépôts de sables ou d'alluvions. Par des modifications géomorphologiques des chenaux, dont la pente, la granulométrie du substrat et le

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degré de connectivité avec le cours principal, les crues engendrent une hétérogénéité spatiale des habitats au sein des zones humides, source de diversification des communautés et de leur dynamique (Klein *et al.*, 1995 ; Bornette *et al.*, 1998a ; Trémolières et Szwab, 2008), dans la mesure où elles modifient les capacités d'érosion par l'eau (Bornette et Amoros, 1996). Toutefois, les échanges avec les eaux souterraines interviennent également dans la définition et la diversité des communautés (Eglin *et al.*, 1997 ; Trémolières et Szwab, 2008), mais aussi dans leur organisation et leur dynamique (Trémolières *et al.*, 1993, 1994a ; Bornette *et al.*, 1998b). L'origine géomorphologique et la position de l'ancien chenal dans la plaine régissent en effet l'action des aquifères (nappe d'accompagnement de la rivière, ou nappe phréatique de versant) sur l'organisation et la dynamique des communautés végétales (Bornette *et al.*, 1998b). Plusieurs travaux ont ainsi pu démontrer qu'il était possible de déterminer, à partir de la caractérisation physico-chimique des eaux dans les zones humides et des phytocénoses induites, les aquifères qui les alimentent, leur pente et leur position altitudinale par rapport au lit de la rivière, mais aussi l'influence des eaux d'infiltration du fleuve (Trémolières *et al.*, 1993 ; 1994a ; Bornette *et al.*, 1996 ; Bornette et Arens, 2002). La chimie des eaux influence en effet fortement la composition floristique et la richesse spécifique des phytocénoses. L'influence fluviale par débordement et reflux peut favoriser la diversité spécifique si elle reste modérée, mais conduit à l'établissement de communautés polluo-tolérantes pauvres en espèces en cas de charge trophique trop élevée. Cette influence dépend donc de la charge trophique des cours d'eau considérés (Trémolières *et al.*, 1993, 1994a ; Bornette *et al.*, 2001). Les apports phréatiques, le plus souvent pauvres en sels nutritifs dans les zones inondables (grâce à l'épuration des eaux de débordement par le système sol-racine, Sanchez-Perez *et al.*, 1993 ; Sanchez-Perez et Trémolières, 1997), sont susceptibles de limiter le processus d'eutrophisation et modifient la composition floristique des phytocénoses (Trémolières *et al.*, 1993 ; Eglin *et al.*, 1997). De plus, l'alimentation des zones humides par les nappes phréatiques provenant d'aquifères de versant peut être suffisante pour arrêter les processus successionnels (Bornette *et al.*, 1994 a-b).

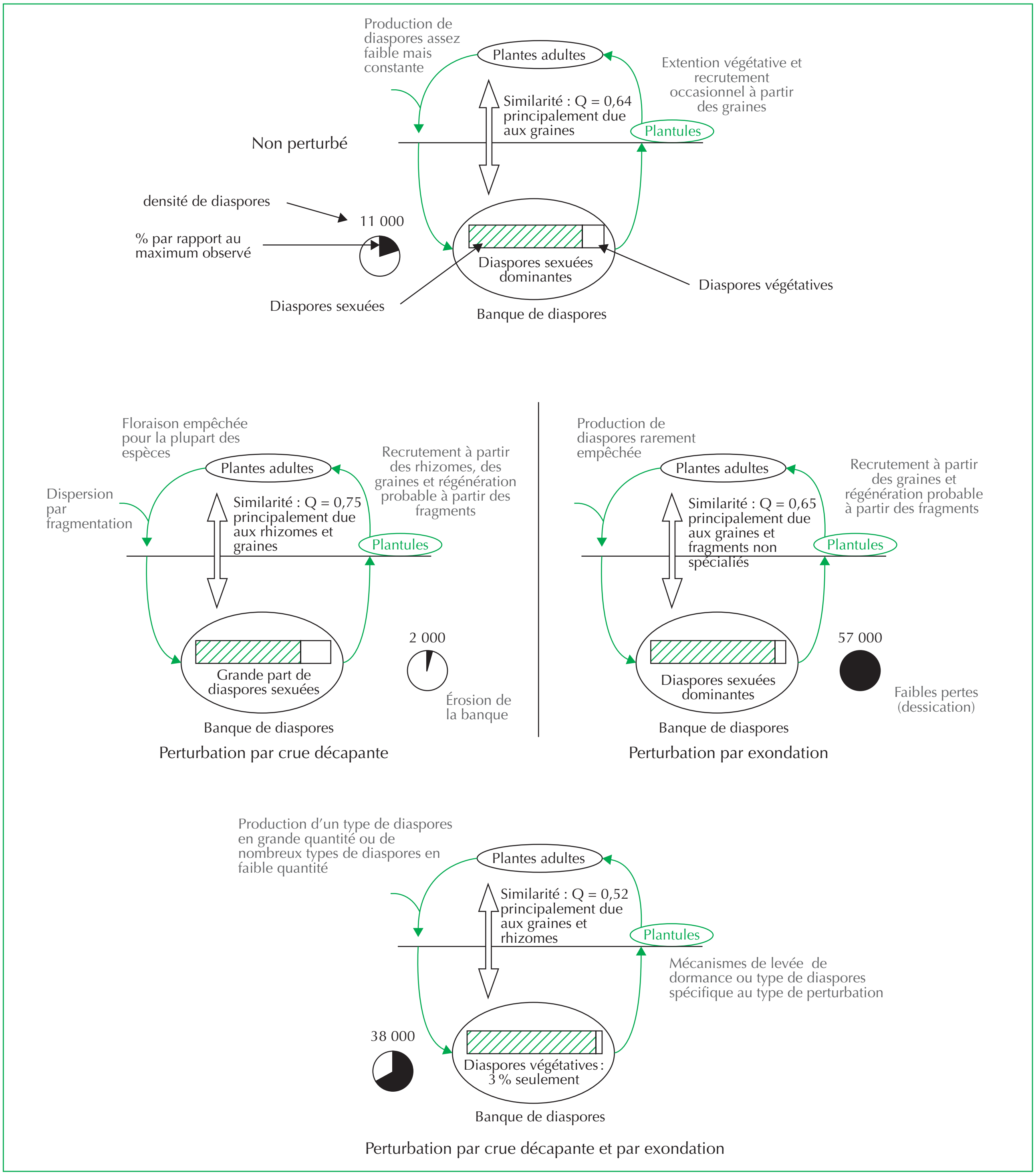
Par ailleurs, dans certaines zones, la fréquence et l'énergie des crues jouent également un rôle dans le ralentissement des processus successionnels, vérifiant ainsi l'hypothèse des perturbations moyennes (Bornette *et al.*, 1994c). Comme le prévoit cette théorie, cette stabilité s'accompagne d'une forte biodiversité au sein des communautés, organisées en mosaïque changeante, remaniée à chaque événement perturbant (Barrat-Segretain et Amoros, 1996 a-b). Il a été montré que les communautés des chenaux du Rhône sont plus diversifiées que celles du Rhin, résultat probablement liée à la dynamique des eaux de crue persistant dans l'hydrosystème rhodanien et arrêtée dans l'hydrosystème rhénan (Trémolières et Szwab, 2008).

Enfin, les effets du surcreusement des cours d'eau sur la connectivité entre les zones humides fluviales ont été abordés au travers d'un modèle de réponse des différents compartiments de la plaine alluviale à l'incision ou à l'exhaussement du cours actif (Bravard *et al.*, 1997), dont plusieurs hypothèses (augmentation du drainage des aquifères de versant par les zones humides, assèchement) ont été validées (Bornette et Heiler, 1994 ; Bornette *et al.*,1996 ; Eglin *et al.*, 1997 ; Trémolières et Szwab, 2008).

Stratégies adaptatives face aux perturbations par des crues

Ces travaux débouchent logiquement sur la nécessité de prendre en compte les traits biologiques des espèces quand on veut établir des prédictions applicables à n'importe quel système fluvial, quel que soit le cortège floristique. Plusieurs études portant sur la régénération des communautés et la maintenance des espèces en milieux perturbés (Bornette *et al.*, 1994d ; Henry *et al.*, 1996) ont montré que l'imprévisibilité forte des perturbations hydrauliques défavorise la reproduction sexuée au profit de la reproduction végétative (fragmentation ou croissance clonale), même en cas de perturbation majeure (Combroux *et al.*, 2001, 2002).

Ainsi, des crues décapantes et/ou des exondations détruisent une partie des communautés végétales aquatiques. La comparaison de la banque de diaspores dans deux écosystèmes, l'un soumis à un régime de crue et l'autre à un régime de crue combiné à un régime d'exondation, a montré des différences significatives dans le contenu de la banque ainsi que dans les relations entre la banque et la végétation recolonisatrice (figure 1). La régénération d'une communauté soumise à



▲ Figure 1 – Effets probables des perturbations par crue décapante et par exondation (Combroux, 2002).
En noir : paramètres mesurés lors de cette étude : banque de diaspores, recouvrement de la végétation établie (toujours supérieure à 100 %) et similarité entre la banque de diaspores et la végétation établie.
En vert : paramètres non mesurés lors de cette étude.
En gris : hypothèses suggérées par les résultats de cette étude.

des crues décapantes semble reposer sur la multiplication végétative (fragments non spécialisés transportés par le courant ou rhizomes profondément ancrés) et, pour un faible nombre d'espèces, sur la reproduction sexuée.

Les communautés soumises à un régime de crue décapante diffèrent principalement des communautés soumises à un régime d'exondation par 1) une floraison impossible pour la majorité des espèces et la formation potentielle de diaspores par fragmentation pendant la perturbation *vs.* une production de diaspores moins affectée par la perturbation ; 2) un décapage d'une grande partie de la banque de diaspores *vs.* une faible conséquence de la perturbation sur la densité de la banque de diaspores ; enfin, 3) bien que non élucidés, les mécanismes de levée de dormance des diaspores dus à l'action de la perturbation sont probablement différents.

Dans le cas d'une communauté soumise à ces deux régimes de perturbations, deux stratégies principales sont sélectionnées :

– le type *Callitriche platycarpa*, rudéral, caractérisé par la production abondante d'un seul type de diaspores quelque soit le régime de contraintes (c'est-à-dire la possibilité de reproduction sous l'eau et en conditions émergées). La grande quantité de diaspores produite permet à certaines diaspores d'« échapper » au décapage de la perturbation par crue (persistance au niveau de refuges). Des mécanismes de levée de dormance permettent certainement une réponse très rapide de ces diaspores après la perturbation et la colonisation des espaces ainsi libérés ;

– le type *Potamogeton pusillus*, caractérisé par la production d'une large gamme de diaspores, chacune pouvant survivre à au moins un type de perturbation. Cette stratégie n'est profitable que si les végétaux peuvent se développer entre chaque perturbation.

De plus les crues participent activement à la dérive de diaspores et leur dispersion vers de nouveaux habitats (Cellot *et al.*, 1998 ; Combroux *et al.*, 2002).

Les stratégies adaptatives des espèces rares inféodées aux milieux perturbés (Greulich, 1998 ; Greulich et Bornette, 1999 ; Greulich *et al.*, 2000 a-b, 2001) ont été également analysées. Ces travaux ont permis de proposer des modèles prédictifs des stratégies adaptatives au sein des communautés végétales en milieu fluvial, qui intègrent les paramètres structurants majeurs des perturbations (nature de la perturbation, intensité, fréquence ; Amoros et Bornette, 1999).

Ces modèles (Amoros et Bornette, 1999 ; Hupp et Bornette, 2003) proposent une gamme de stratégies adaptées à chaque régime de contrainte. Ainsi, lorsque le renouvellement des taches perturbées par les crues diminue en relation avec la diminution de l'intensité des perturbations, la part des processus autogéniques (compétition) dans la structuration et la dynamique des communautés augmente. Dans les situations les plus perturbées, une stratégie opportuniste devrait être favorisée par les espèces végétales. L'hypothèse du modèle est en particulier que les processus d'érosion altèrent les niches de régénération et décapent les banques de diaspores, impliquant dans les situations les plus extrêmes une recolonisation par des sources de diaspores exogènes (recolonisation dépendant de processus de résilience), tandis que des perturbations par dépôts devraient sélectionner des formes de croissance aptes à résister à ces dépôts. Ces prédictions ont été testées et en partie validées sur quarante-sept zones humides du Haut-Rhône, du Doubs et de l'Ain, et dix traits morphologiques et phénologiques documentés sur les trente-quatre espèces constituant le peuplement dominant des espèces végétales aquatiques (travaux de G. Bornette).

Végétation et contraintes physiques : effets des barrages

Les seuils et digues en cours d'eau

Les seuils et digues en petits cours d'eau se traduisent par des modifications des conditions d'écoulement tant à l'amont qu'à l'aval des ouvrages.

Ainsi, sur les rivières armoricaines comme le Scorff, les digues de moulins déterminent des patrons de répartition répétitifs (Haury, 1982, 1995). À l'aval des digues, dans la zone de surverse ou à l'aval des canaux de fuite, les communautés de macrophytes rhéophiles, notamment les bryophytes sont dominantes. À l'inverse, à l'amont des digues, la végétation stagnophile domine, marquée par exemple par des nénuphars. Lorsqu'on examine la répartition du nénuphar jaune sur le cours principal du Scorff, on retrouve l'implantation des moulins : la distribution d'une plante traduit donc les activités humaines.

Mais ces digues jouent aussi un rôle d'écrêtement des crues. Ainsi, sur le Tarn en Lozère, l'implantation d'une base de loisirs, avec création d'une digue, a eu pour conséquence de favoriser l'extension d'une renoncule aquatique (*Ranunculus penicillatus* ssp. *pseudofluitans*), car les sédiments, antérieurement emportés par les crues hivernales, se sont trouvés stabilisés, favorisant le maintien de cette espèce d'une année sur l'autre et donc la progression des herbiers (Codhant *et al.*, 1991).

Barrages hydro-électriques et végétation en cours d'eau de taille moyenne

L'effet des barrages sur les peuplements macrophytiques a été étudié sur trois sites armoricains (Bernez et Haury, 1996 ; Haury, 1996 ; Bernez, 1999 ; Bernez *et al.*, 2002 et 2004 a-b). Il en ressort que les barrages modifient fortement la zonation longitudinale, en fonction des particularités de gestion des ouvrages, mais aussi du contexte géologique et géomorphologique qui prévaut au choix du site d'implantation (Bernez, 1999).

L'effet des éclusées occasionnelles se traduit par des modifications importantes des recouvrements, notamment d'algues filamenteuses, et par des modifications du cycle des espèces situées dans la zone perturbée (par exemple, un faucardage hydraulique des renoncules relançant leur croissance). Sur des populations de potamot pectiné, de myriophylle en épi et de cladophores, l'effet à moyen terme des éclusées du barrage de Rabodanges (Bernez *et al.*, 2007) et d'une augmentation du débit réservé, s'est traduit d'abord par une réduction brutale des recouvrements des deux premiers macrophytes, puis une récupération progressive de leurs recouvrements ; en revanche, les cladophores ont bénéficié de l'augmentation du débit réservé avec des eaux chargées. À l'inverse, si le débit réservé est trop faible, une colonisation par les hélophytes intervient assez rapidement (photo 1).

La rupture de continuité se traduit par une augmentation de l'effet des affluents dans la zone à débit réservé (Haury *et al.*, 1996 ; figure 2). Un suivi de vidange a toutefois permis de noter des colonisations vers l'aval d'espèces initialement uniquement présentes à l'amont du barrage (Bernez, 1999). La cicatrisation après ces opérations semble intervenir assez rapidement si le bouchon vaseux est restreint ou si les boues sont piégées et évacuées, ou bien s'il y a une restauration de cours d'eau.

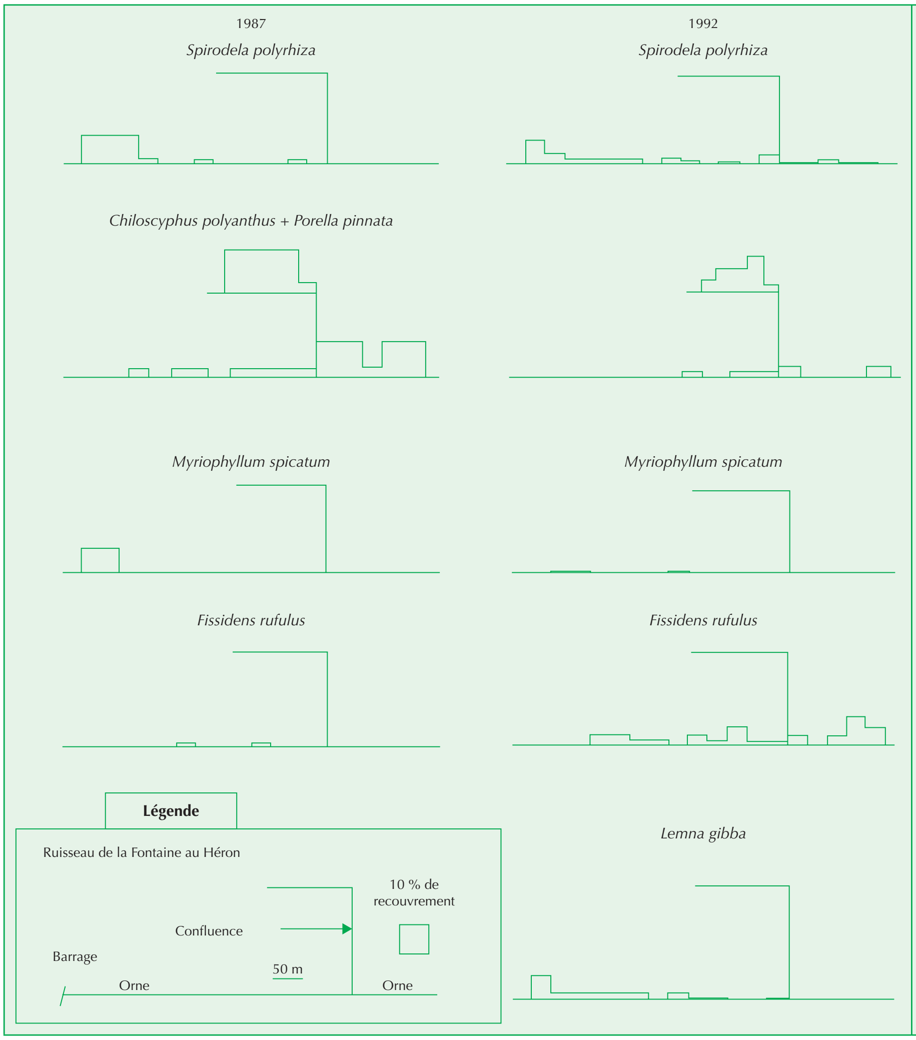
▲ Photo 1 – Effet d'un faible débit à l'aval des barrages dans un lit désormais surdimensionné : envahissement par des hélophytes (oenanthe et faux-roseau) à l'aval du barrage de Rophémel (Côtes-d'Armor) – Photo : Jacques Haury.

Enfin, les barrages étant susceptibles d'être vidangés, ou de subir des marnages importants, différentes communautés de macrophytes des berges peuvent apparaître sur les zones dégagées, en fonction de la banque de graines conservée dans les sédiments (Bernez *et al.*, 1995). On peut ainsi voir apparaître des espèces relativement rares et strictement inféodées à ces zones basses autour des eaux stagnantes, réservoirs ou étangs, comme la limoselle aquatique, le souchet brun, le scirpe ovoïde…

Effets des grands barrages sur les macrophytes

Pour les grands cours d'eau, l'effet de discontinuité due aux grands barrages quant à des différences de colonisation par les macrophytes n'est pas évident (Breugnot *et al.*, 2003, 2004). En effet, la végétation est relativement homogène dans ce type de cours d'eau.

Toutefois, des herbiers de grande taille peuvent se développer localement à la faveur des conditions locales créées par les barrages, en particulier en relation avec la régulation des débits qui réduit les remaniements des sédiments pouvant se produire lors de crues très importantes : un colmatage des sédiments grossiers par des particules fines se produit et facilite l'installation et le maintien de ces macrophytes. C'est le cas de l'herbier d'Argentat, sur la Dordogne, qui a suscité des travaux relativement anciens, en raison des problèmes de gestion induits par le fort développement de *Ranunculus fluitans* (Decamps et Capblancq, 1980). Cet herbier



Commentaire

En 1987, le débit réservé à l'aval du Barrage de Rabodanges sur l'Orne était de 100 l/s. En 1992, il était devenu de 800 l/s.

Sur le ruisseau affluent de la Fontaine aux Hérons, oligo-mésotrophe, la communauté d'hépatiques à feuille associant *Chiloscyphus polyanthus* et *Porella pinnata* colonisait bien le cours principal de l'Orne en 1987.

L'augmentation du débit réservé, correspondant à un flux nutritif plus important, se traduit par une réduction de cette communauté oligo-mésotrophe sur l'aval de la confluence.

À l'inverse, on observe une apparition de *Lemna gibba* et une progression vers l'aval de *Spirodela polyrhiza*, espèces eutrophes à hypertrophes (Haury *et al.*, 1996).

▲ Figure 2 – Différences de distributions spécifiques au « nœud de Rabodanges » (1987-1992).

est d'autant plus développé que les sédiments accumulés à l'aval du barrage ne sont plus du tout évacués par les crues. Il semble, en outre, que le barrage renvoie une eau plus froide qui favorise l'herbier.

Sur le bassin versant du Lot, l'herbier de renoncules d'Entraygues sur Truyère se développe sur la Truyère à l'aval immédiat du barrage EDF[1] de Cambeyrac sur environ six hectares. Depuis les années soixante, il a été considéré comme une nuisance par la commune. Des expérimentations de régulation de cet herbier ont été réalisées en 2002 (Rebillard *et al.*, 2003), l'une consistant en un arrachage des plantes, l'autre en un remaniement des sédiments jusqu'à une profondeur de 50 cm pour déraciner les renoncules et remettre les fractions fines des sédiments en suspension. Un lâcher d'eau nocturne d'une heure à partir du barrage EDF de Cambeyrac a également été réalisé immédiatement après ces travaux pour tenter d'éliminer ces sédiments fins. Les suivis ultérieurs ont montré une recolonisation relativement rapide en trois ans, mais les observations réalisées confirment la relation nette entre la stabilité et le colmatage des sédiments et cette prolifération végétale.

La restauration d'un système de crues dans ces grands cours d'eau aménagés pourrait donc favoriser la réduction de ces développements végétaux.

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[1] Électricité de France.

Il semble donc bien que le « *Serial Discontinuity Concept* » de Ward et Stanford (1985, *in :* Bernez, 1999) se décline de façon particulière dans les grands cours d'eau et pour le compartiment des macrophytes : peu d'effet strict de discontinuité de colonisation, mais des modifications d'écoulement, voire de chimie de l'eau, entraînant des proliférations locales de macrophytes.

Végétation et pollutions chimiques : acidification, trophie

Des activités anthropiques peuvent modifier le milieu, notamment la composition chimique des eaux, et par conséquent la composition naturelle et structurelle des communautés de macrophytes. Ces apports anthropiques concernent des polluants organiques et minéraux que sont :

– les rejets domestiques, fréquents à l'aval des stations d'épuration (Mériaux et Gehu, 1979 ; Thiebaut et Muller, 1998) ;

– les fertilisations agricoles et l'occupation du sol, qui déterminent une eutrophisation préoccupante dans certaines régions, comme en Bretagne (Daniel, 1998 ; Bernez, 1999) ;

– la pisciculture intensive, avec comme conséquences pour le milieu récepteur des rejets d'ammonium et de matières en suspension ainsi que de faibles teneurs en oxygène dissous (Trémolières *et al.*, 1994b ; Daniel et Haury, 1995 ; Thiébaut et Muller, 1998 ; Daniel, 1998 ; Adam, 2000).

La conjonction de la pollution atmosphérique, de la présence de substratum acide et de pratiques sylvicoles inadaptées (enrésinement) peut conduire à une acidification des sols et des cours d'eau, entraînant une modification de la composition floristique et une érosion de la diversité notamment des macro-invertébrés (Thiébaut *et al.*, 1998). La pollution par les xénobiotiques, notamment par les métaux lourds, élimine les végétaux polluo-sensibles et/ou provoque une accumulation des métaux dans les parois cellulaires et les vacuoles, notamment chez les bryophytes et les lichens. Cela permet de les utiliser également à des fins de bio-indication de la contamination métallique (Roeck *et al.*, 1993 ; Chatenet et Botineau, 2001) ou comme bio-accumulateurs (Mouvet et Claveri, 1999). Les herbicides peuvent provoquer des symptômes de phytotoxicité (Giovanni et Haury, 1995).

Les végétaux aquatiques intègrent ces modifications et altérations du milieu. Les communautés végétales répondent aux conditions environnementales naturelles et anthropiques par des changements de composition, de richesse et d'abondance des espèces. De nombreux travaux phytosociologiques mettent en évidence les relations entre la teneur en nutriments et la distribution des phytocénoses dans les eaux courantes (Mériaux et Gehu, 1979 ; Carbiener et Kapp, 1981 ; Carbiener et Ortscheit, 1987 ; Haury, 1985 ; Haury et Muller, 1991 ; Trémolières *et al.*, 1993 ; Haury *et al.*, 1995). Des échelles de bio-indication fondées sur des assemblages de macrophytes et sur la réponse des communautés végétales à l'eutrophisation ont été proposées (Carbiener *et al.*, 1990 ; Eglin et Robach, 1992 ; Robach *et al.*, 1996 ; Muller, 1990 ; Thiébaut et Muller, 1999). Elles représentent une séquence de référence dans des types d'eau définis par leurs caractéristiques lithologiques et chimiques, dans des contextes physiques déterminés.

Des échelles de bio-indication de l'eutrophisation en eau courante

Séquence de communautés dans des eaux faiblement minéralisées

Une échelle de bio-indication de l'eutrophisation basée sur quatre groupements végétaux aquatiques, notés A à D, a été définie dans des ruisseaux du bassin versant de la Haute-Moder dans les Vosges du Nord (tableau 1 ; Thiébaut et Muller, 1995 ; Thiébaut, 1997, Thiébaut et Muller, 1999).

La composition floristique des ruisseaux des Vosges du Nord varie entre l'amont et l'aval. Les têtes de bassin versant de type A et B sont en général peu minéralisées, faiblement acides, oligotrophes à mésotrophes, alors que les stations aval de type C et D sont davantage minéralisées, plus proches de la neutralité et méso-eutrophes à hypertrophes.

Séquence de communautés dans les eaux minéralisées alcalines

Carbiener et Ortscheit (1987), Carbiener *et al.* (1990), Carbiener *et al.* (1995), Robach *et al.* (1996) ont établi, à partir des phytocénoses des rivières phréatiques de la plaine d'Alsace, une séquence de groupements végétaux, déterminés par les phosphates et l'azote ammoniacal, allant de l'oligotrophe A au plus eutrophe F (tableau 2).

▼ Tableau 1 – Échelle de bio-indication du niveau d'eutrophisation des cours d'eau des Vosges du Nord : associations végétales et qualité de l'eau (P-PO$_4^{3-}$, N-NH$_4^+$)[2], d'après Thiébaut et Muller (1999).

				N-NH$_4^+$ (µg/l)		P-PO$_4^{3-}$ (µg/l)	
Espèces caractéristiques ou dominantes	Échelons	Sous association (classification phytosociologique)	Niveaux de trophie	Mean	Std	Mean	Std
Potamogeton polygonifolius	A	*Hyperico-Potamogetonetum polygonifoli*	Oligotrophe	43	13	20	26
P. polygonifolius *Ranunculus peltatus* *Callitriche hamulata* *Callitriche platycarpa*	B	*Callitrichetumhamulatae* sous association à *P. polygonifolius*	Oligo-mésotrophe	49	14	22	11
C. hamulata *E. canadensis* *Oenanthe fluviatilis*, *Myriophyllum alterniflorum* *Potamogeton alpinus*	C	*Callitrichetumhamulatae* typicum	Mésotrophe	86	35	53	19
C. hamulata *C. obtusangula*	D	*Callitrichetum obtusangulae*	Eutrophe	170	109	139	46

▼ Tableau 2 – Échelle de bio-indication du niveau d'eutrophisation des cours d'eau phréatiques de la plaine d'Alsace : associations végétales et qualité de l'eau (P-PO$_4^{3-}$, N-NH$_4^+$), d'après Robach *et al.*, 1996.

				N-NH$_4^+$ (µg/l)		P-PO$_4^{3-}$ (µg/l)	
Espèces caractéristiques ou dominantes	Échelons	Syntaxons (classification phytosociologique)	Niveaux de trophie	Mean	Std	Mean	Std
Potamogeton coloratus	A	*Potamogetonetum colorati*	Oligotrophe strict	14	7,3	7	1,7
Berula erecta	B		Oligo-mésotrophe	22	13,8	13	5,5
Callitriche obtusangula *Berula erecta* *Elodea canadensis*	C	*Callitrichetum obtusangulae*	Mésotrophe	45	27,8	15	6,8
Zannichellia palustris *Groenlandia densa* *Nasturtium officinale*	D	*Zannichellio-Potamogetonetum densae*	Méso-eutrophe	34	31,3	29	23,6
Oenanthe fluviatilis (rare) *Ceratophyllum demersum* *Ranunculus fluitans*	E	*Ranunculetum fluitantis*	Eutrophe	61	40	40	33
Potamogeton. nodosus *P. lucens* *P. pectinatus*	F	*Potamogetonetum pectinati*	Hypertrophe Mésosaprobe	255	107	191	116

[2] Phosphates et azote ammoniacal.

Dans une rivière non perturbée par des apports d’origine anthropique, la séquence normale serait, d’amont à l’aval, A, B, C, (D). Cet agencement longitudinal des associations végétales caractérise la lente et progressive eutrophisation naturelle de la rivière (Carbiener et Kapp, 1981 ; Carbiener *et al.*, 1995). Une eutrophisation plus importante est liée à des rejets dus à des activités humaines. Dans certains cas, les phénomènes d’auto-épuration et d’apport d’eau souterraine dans les ruisseaux peuvent conduire à une amélioration de la qualité de l’eau se traduisant par la séquence inversée « C*B » (Muller, 1990 ; Thiébaut et Muller, 1998) voire « C*B*A » (Trémolières *et al.*, 1994b), avec des processus d’oligotrophisation qui ont par exemple été décrits par Ortscheit *et al.* (1982).

Variation de la séquence d’eutrophisation en fonction du degré de minéralisation des eaux

Une comparaison entre les deux séquences de bio-indication a permis de préciser l’écologie de quelques espèces (Robach *et al.*, 1996). *Callitriche obtusangula, Potamogeton berchtoldii, Oenanthe fluviatilis, Sparganium emersum, Elodea canadensis, E. nuttallii, Potamogeton crispus*, avec le bryophyte *Fontinalis antipyretica*, sont des hydrophytes communs aux eaux calcaires et aux eaux acides. Les deux séquences présentent un gradient trophique différent avec des gammes plus grandes pour les eaux acides. Le type A calcaire correspond à des eaux oligotrophes alors que le groupement végétal A de la séquence acide se développe dans des eaux oligotrophes à mésotrophes. Les espèces inféodées aux eaux mésotrophes à eutrophes des deux séquences (*Elodea canadensis, E. nuttallii, Oenanthe fluviatilis, Potamogeton crispus*...) montrent d’une manière générale une similitude de comportement vis-à-vis de la trophie dans les deux séquences, avec quelques variations d’optimum pour *Oenanthe fluviatilis* et *Callitriche obtusangula*. Le spectre écologique de deux espèces d’élodée est différent dans les deux secteurs étudiés (Thiébaut *et al.*, 1997). Alors que *Elodea nuttallii* pousse dans des eaux plutôt eutrophes en milieu calcaire, elle peut se développer dans des eaux oligotrophes dans les cours d’eau peu minéralisés. Toutefois, son optimum de développement se trouve dans les eaux mésotrophes à eutrophes sur substratum gréseux. Les espèces indicatrices des eaux les plus eutrophisées D et E de la séquence carbonatée (*Zannichellia palustris, Myriophyllum spicatum, Ranunculus fluitans, Ceratophyllum demersum, Potamogeton perfoliatus, P. pectinatus*) manquent totalement dans les échelons correspondants des eaux peu minéralisées.

Échelle de bio-indication de l’acidification

Dès la fin des années quatre-vingt, l’acidification des cours d’eau vosgiens était reliée aux pluies

	Groupe	Aluminium µg/l	Ions calcium mg/l	pH
Dicranella heteromella *Marsupella emarginata* *Jungermannia sphaerocarpa*	I	428-798	1,3-1,8	4,6-5,2
Marsupella emarginata *Jungermannia sphaerocarpa.* *Sphagnum auriculatum*	II	458-881	1,4-2,3	4,9-5,8
Rhynchostegium riparioides *Chyloscyphus polyanthos* *Rhizomnium.punctatum*	III	114-177	2,3-2,5	5,3-5,5
Rhynchostegium riparioides *Dichodontium. pelucidum*	IV	90-165	3,0-3,6	5,8-6,1
Rhynchostegium riparioides *Chyloscyphus polyanthos* *Thamnobryum. alopecurum* *Dichodontium. pelucidum*	V	49-122	4,2-8,5	6,3-6,9

◀ Tableau 3 – Échelle de bio-indication de l’acidification par les bryophytes dans les cours d’eau montagnards : exemple du massif du Donon (d’après Thiébaut *et al.*, 1998).

acides (Massabuau *et al.*, 1987 ; Probst *et al.*, 1990). Dans le nord du massif vosgien, quelques ruisseaux sont acides au niveau de leurs sources (Thiébaut *et al.*, 1995 ; Thiébaut, 1997). L'acidification anthropique des eaux se traduit par la disparition des espèces acido-sensibles telles *Potamogeton polygonifolius* et expliquerait en partie la régression des sites à *Myriophyllum alterniflorum* (Thiébaut, 1997). Des études menées en 1995 sur cinq cents stations du massif du Donon et des Hautes-Vosges montrent que plus de 50 % des ruisseaux présentent un pH inférieur à 5,5 (cours d'eau fortement acidifié). Parmi ceux-ci, 15 % sont caractérisés par un pH inférieur à 4,8 (Guérold *et al.*, 1997). Ces cours d'eau montagnards sont caractérisés par des communautés bryophytiques. Aussi, une échelle de bio-indication par les bryophytes aquatiques du degré d'acidification, à cinq échelons, a pu être mise en évidence dans le massif du Donon (Thiébaut *et al.*, 1998), l'acidification se traduisant par une baisse du pH et une augmentation des protons, du relargage de l'aluminium et une perte en minéraux tels que le calcium et le magnésium.

Conclusion

La chimie de l'eau modifie la composition et l'abondance floristique des communautés. Les altérations physico-chimiques des cours d'eau se traduisent par des modifications de la séquence de végétation observée naturellement dans le gradient amont aval d'un cours d'eau. En revanche, la perturbation, vue comme un événement court et imprévisible, entraîne une destruction souvent totale, parfois partielle de la végétation, et des changements dans les stratégies développées par les espèces telles que leur capacité de régénération qui diffère en fonction des types de perturbation (crues décapantes et/ou exondations, par exemple). Perturbations et contraintes participent à la structure et à la dynamique des communautés.

Dans le cadre des restaurations de bras morts de rivières et de grands fleuves, il devient nécessaire d'analyser la dynamique de recolonisation des communautés, tant en termes de stratégies des espèces (traits biologiques et/ou fonctionnels) qu'en potentialités existantes *via* les banques de graines ou les banques en dérive. ❐

Résumé

Dans les milieux aquatiques, des perturbations peuvent être des crues qui induisent des vitesses d'écoulement suffisantes pour provoquer l'érosion du substrat et l'arrachage des communautés végétales fixées, ou qui favorisent des dépôts d'alluvions. Elles créent ainsi une hétérogénéité des habitats qui se traduit par une grande diversité de communautés végétales dont la composition est liée à la variabilité de l'intensité des échanges hydrologiques, à la qualité et à l'origine des eaux. Les espèces développent des stratégies adaptées à ces fluctuations des niveaux d'eau d'intensité et de fréquence variables.

Dans les cours d'eau non soumis aux crues, les communautés végétales qui s'y développent s'organisent en séquence de végétation selon un gradient naturel du niveau trophique amont-aval. Des altérations physiques (modifications morphologiques du lit, discontinuité créée par les barrages) ou chimiques modifient voire détruisent cette séquence. On montre que les communautés végétales peuvent être des descripteurs du fonctionnement hydrologique du cours d'eau et/ou des bio-indicateurs des altérations physico-chimiques.

Abstract

In aquatic environments, disturbances can be floods which induce the flow velocities sufficient to cause erosion of the substrate and the uprooting of plant communities, or that promote alluvial deposits. Thus they create heterogeneity of habitats which results in a wide variety of plant communities whose composition is related to the variability of the intensity of exchanges hydrological, quality and origin of waters. The species develop strategies adapted to the fluctuating water levels with variable intensity and frequency.

In rivers not subject to flooding, plant communities that are expanding are organized as a sequence of vegetation according to a gradient of natural trophic level upstream downstream. Physical or chemical impairments (morphological changes in the bed, hydroelectric impoundment) change or even destroy this sequence. It shows that plant communities may be descriptors of hydrological functioning of the watercourse and / or biondicators of physical or chemical impairments.

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Chenalisation de petites rivières de plaine

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Does channelization alter spatial and temporal dynamics of macrophyte communities and their physical habitat?

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With 4 figures and 3 tables

Abstract: Channelization is the creation of man-made structures that resect, realign, or enclose aquatic systems in order to prevent flooding or to modify their flows for various land uses. The present study examined the impact of this human pressure on the spatial and temporal dynamics of small streams. The physical and floristic characteristics of seventeen reaches in four French rivers were surveyed six times over the course of two years. Macrophyte communities were divided into three biological groups: vascular plants, macroalgae and bryophytes. We used plant functional traits to understand the effects of channelization on community structure. Our results suggest that channelization affected the spatio-temporal dynamic of physical and floristic composition. Channelized reaches were shallower and narrower than non-channelized reaches, i.e. control reaches. They also exhibited different substrate types and dominant species. Differences were mainly observed at the macrohabitat scale, i.e. the pool/riffle scale, within the selected reaches. Alterations in spatio-temporal dynamics of physical and plant composition could be linked to species biological traits. Vascular plants and macroalgae in channelized reaches used a variety of adaptive strategies (e.g. small *versus* tall size) which allowed them to persist despite environmental differences, whereas plants in control reaches showed a combination of intermediate strategies. Bryophytes were mainly found in control reaches with the exception of *Fontinalis antipyretica*. These findings could serve as guidelines for future channelization projects and for conservation measures to preserve the dynamics of natural streams.

Key words: Biodiversity, functional traits, human impact, heterogeneity, variability.

Introduction

Although in general streams and wetlands provide essential biological and economic services (Brinson et al. 1981, Keddy 2000, Millennium Ecosystem Assessment 2005), they have been seriously threatened by human activities over the last century. Agriculture landscaping and transport planning, for instance, have constrained streams with straight, trapezoidal channel sections which simplify the river bed (Brookes 1988).

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Changes in the physical characteristics of streams world-wide has led to changes in ecosystem functionality and in the composition of biotic communities inhabiting rivers (Boon 1992). Aquatic ecosystems are heterogeneous landscapes with hierarchical levels of habitat 'patchiness' related to spatial or temporal variation in physical or biological factors (Fisher 1994). In streams, even where the physical characteristics lead to a uniform habitat, the distribution of organisms can be distinctly patchy in space and /or time (Neill 1994) due to strong watercourse dynamics and seasonality. Landscape uses, especially stream channelization – i.e. the creation of man-made structures that resect, realign, or enclose the streams in order to prevent them from flooding or to modify their flow for various land uses (e.g. drainage for agriculture, shipping) – usually alter the environmental and biological dynamics of streams. The degree of alteration in river hydrology and plant communities varies across different spatial and temporal scales. One important effect may be the decoupling of interactions among abiotic and biotic factors for ecological systems in general (Brown 2003). Yet, interactions (biotic or not) are important parts of ecosystem functionality and are central to models of community assembly (Drake et al. 1993).

Many taxa are affected by channelization. Benthic macroinvertebrates, for instance, that display a diversity of body forms and habits, are often used as indicators of environmental stressors (Rosenberg & Resh 1993). On the another hand, freshwater macrophytes are well-known for their key role in ecosystem health and the provisioning of services such as water oxygenation, nutrient storage, habitat for other communities, substrate stabilization and increasing structural diversity in watercourses (Haslam 1987, Carbiener et al. 1990, Allan 1995). In lotic environments the distribution of aquatic flora is determined by variations in water quality, dominant substratum, light availability, hydrology (providing that the establishment and growth of plants are also influenced by flow velocity) and depth (Haslam 1978). It has been demonstrated that macrophytes are patchily distributed in relation to spatial heterogeneity (Mouquet & Moore 2002, Stevens & Carson 2002). Several mechanisms related to species operate during the assembly of a biological community, e.g. facilitation, inhibition, tolerance and random colonization (Connell & Slatyer 1977). Anthropogenic impacts may affect these mechanisms and thus, in part, lead to changes in community functionality, e.g. modification of winter persistence (Greulich & Bornette 2003) or age structure of *Luronium natans* (L.) (Szmeja & Bazydlo 2005). To date only a few works have shown an interest in phenology. However, functional groups of species have specific tolerance or vulnerability responses according to different types of disturbances or stressors (Sabbatini & Murphy 1996, Sabbatini et al. 1998). Therefore, it is necessary to explore more precisely the relationships between plant distribution, phenology and spatial and seasonal differences resulting from stream channelization.

The ability of a species to withstand a range of ecological factors usually depends on its life history strategy. A strategy is a combination of biological traits, the key components responsible for spatial and seasonal patterns in species distributions (e.g. the morphological plasticity of *Ranunculus peltatus*, Garbey et al. 2004). Despite their potential role in ecosystem processes (MacGillivray et al. 1995, Grime 1997), these traits have not often been considered in biological conservation studies partly because it is difficult to study them *in situ* (Bornette et al. 1994). Considering that habitat acts as a template (Southwood 1977, 1988), the effects of habitat conditions on the fitness of individual organisms would lead to the selection of traits that are the product of adaptations for survival and reproduction (Townsend & Hildrew 1994).

This study examines the impact of channelization on the spatial and temporal organization and dynamics of physical characteristics and macrophyte community composition (Dobson et al. 1997, Rahel 2000, Brooks et al. 2002). As macrophyte flora is strongly structured by flow velocity, we worked at the macrohabitat scale which corresponds to the pools and riffles found within the banks of the full channel (Muhar 1996). We hypothesized that channelization will attenuate spatial and seasonal variations in physical composition, and thus, lead to different species and /or community phenology (Brown 2003). Our objectives were thus to evaluate the impact of channelization on spatial and temporal dynamics of small streams through the following questions: 1) does channelization attenuate the spatio-temporal dynamics in the stream's physical characteristics or floristic composition at the macrohabitat scale? and if so, 2) could the floristic differences be associated with species biological traits?

Methods

Study site and sampling

This study was carried out in the south of Paris, France and located within the Atlantic biogeographic region where spring (12.2 °C, 50.9 mm) and autumn (13.5 °C, 48.7 mm) are colder

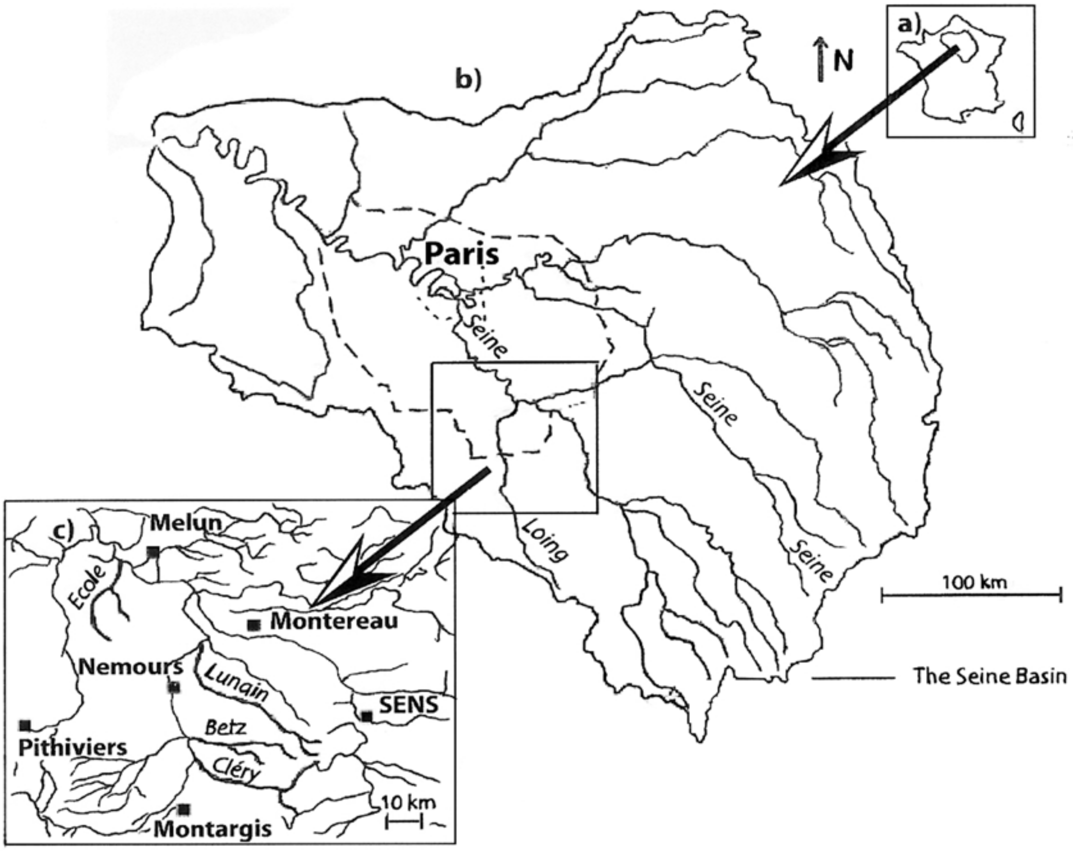

Fig. 1. Location of the four studied streams. **a)** The streams within France; **b**) the Seine and the Loing basins and their networks; **c)** the Ecole, Lunain, Betz and Cléry rivers.

and drier than summer (20.4 °C, 55.8 mm) (Météo-France 2006). Over the last two centuries, landscapes in this region, and specifically running waters, have been severely modified by human activities (Le Marechal & Lesaffre 2000). Streams have been partially channelized by multiple structures designed to regulate water flow, protect human populations and to create water reservoirs for agricultural and domestic usage. We studied four small and calcareous streams found within the Seine and the Loing hydrological basins (78650 and 2300 km^2, respectively, see Fig.1): the Cléry, Betz, Lunain, and Ecole rivers. Each stream was characterized by the same types and intensities of land use (grass-dominated meadows and small forests), similar geomorphologic conditions (chalk geology, low Strahler stream order) and similar chemical characteristics (moderate trophic status and a limited range for: nitrogen total concentration = 4–9 mg/L, phosphate concentration = 0.20–0.50 mg/L, conductivity = 470–550 µS/cm, pH = 7.32–7.84, and temperature = 15.22–18.29 °C) (see also Bornette & Amoros 1991, Robach et al. 1996). The four streams were channelized in the early 1980s via the local addition of longitudinal and transverse channel structures such as dikes, bridges and groynes, as well as by resection (Brookes 1988, Wasson et al. 1995).

We monitored seventeen reaches in all: six control reaches (i.e. unchannelized) and eleven channelized reaches. The reaches were 100 m long and were divided into macrohabitats according to the sequence of pools and riffles *sensu* Frissell et al. (1986). Riffles were shallow areas (10–30 cm depth) with high velocities (40–60 cm s^{-1}). Pools were deep areas (25–80 cm depth) with flow velocities less than 20 cm s^{-1} (Giorgi et al. 2005). Four groups of macrohabitat types were distinguished according to the flow velocity and the presence of channelization: control riffles (CR), control pools (CP), impacted riffles (IR) and impacted pools (IP). We found no significant difference between the size of CR (mean = 56 ± 27.1 m) and IR (mean = 31.91 ± 21.48 m) and between the size of CP (mean = 44 ± 27.1 m) and IP (mean = 68.09 ± 21.48 m). Consequently, our sampling effort was unbiased and the macrohabitat characteristics in control and impacted reaches were comparable (t-test = 2.019, df = 15, $P > 0.05$).

Sampling was undertaken three times per year in 2006 and 2007, i.e. on six different sampling dates. For each sampling date, we insured homogeneous chemical conditions among reaches by collecting 50 ml of water to analyse concentrations of total nitrogen, phosphate, carbonate, magnesium, and pH by means of chemical kits (Reagent case, Visicolor ECO Analysenkoffer, Macherey-Nagel). In addition, we also measured dissolved oxygen concentration, water temperature and conductivity *in situ* using two dataloggers (Dissolved Oxygen Pocket Meter, Oxi315i/SET, WTW; Multi-channel analysers, Consort C533). For each pool and riffle within one reach, physical characteristics such as width, depth, flow velocity, substrate composition and light availability were measured using a graduate rule, decameter and current meter (Table 1). For each sampling date we took one measurement of width, ten random points for

Table 1. Codes for macrohabitat, physical characteristics and floristic species.

MACROHABITATS	CODES	MACROHABITATS	CODES
Control Riffle	CR	*Cinclidotus danubicus* Schiffn. & Baumg.	CIN
Control Pool	CP	*Fissidens crassipes* Wils. ex B., S. & G.	FISc
Impacted Riffle	IR	*Fissidens rufulus* B., S. & G.	FISr
Impacted Pool	IP	*Fontinalis antipyretica* Hedw.	FON
		Lunularia cruciata (L.) Lindb.	LUN
PHYSICAL CHARACTERISTICS		*Pellia endiviifolia* (Dicks) Dumort.	PEL
Depth (cm)	Dep	*Rhynchostegium riparioides* (Hedw.) Card.	RHY
Flow velocity (cm s-1)	FV	*Riccardia chamaedryfolia* (With.) Grolle	RIC
Width (m)	Wid		
Light availability (%) = 100 - Shading (%)	Ligh	**Vascular plants**	
Artificial substratum (%)	As	*Berula erecta* (Huds.) Coville	BER
Cobblestones (%)	Co	*Callitriche obtusangula* Le Gall	CALo
Pebbles and gravel (%)	Pg	*Callitriche platycarpa* Kützing	CALp
Organic detritus (%)	Od	*Callitriche stagnalis* Scop.	CALs
Sand (%)	Sa	*Ceratophyllum demersum* L.	CER
Silt (%)	Sil	*Elodea canadensis* Michx	ELO
Terrestrial sediment (%)	Ts	*Glyceria fluitans* (L.) R. Br.	GLYf
Total cover percentage of vegetation	Ve	*Groenlandia densa* (L.) Fourr.	GRO
		Helosciadium nodiflorum (L.) Lag.	HEL
FLORISTIC SPECIES		*Lemna minor* L.	LEMm
Macroalgae		*Lemna minuscula* L.	LEMi
Batrachospermum sp. Roth	BAT	*Lemna trisulca* L.	LEMt
Cladophora sp. Kützing	CLA	*Mentha aquatica* L.	MEN
Diatoma sp. Bory de St Vincent	DIA	*Myosotis* gr. *Palustris* L.	MYO
Hildenbrandia rivularis (Liebm.) J. Agardh	HIL	*Nasturtium officinale* sl R. Br.	NAS
Melosira sp. C. Agardh	MEL	*Nuphar lutea* (L.) Sibth. & Sm.	NUP
Microspora sp. Thuret	MIC	*Phalaris arundinacea* L.	PHA
Nitella sp.	NIT	*Potamogeton crispus* L.	POTc
Oscillatoria sp. Vaucher	OSC	*Potamogeton obtusifolius* Mert. & Koch	POTo
Phormidium sp. Kützing	PHO	*Potamogeton pectinatus* L.	POTp
Spirogyra sp. Link	SPI	*Potamogeton perfoliatus* L.	POTf
Vaucheria sp. de Candolle	VAU	*Ranunculus fluitans* Lam.	RAN
		Rorippa amphibia (L.) Besser	ROR
Bryophytes		*Sparganium emersum* Rehmann	SPA
Amblystegium fluviatile (Hedw.) B., S. & G.	AMBf	*Veronica anagallis-aquatica* L.	VERa
Amblystegium riparium (Hedw.) B., S. & G.	AMBr	*Veronica beccabunga* L.	VERb
Brachytecium rivulare B., S. & G.	BRA	*Zannichellia palustris* L.	ZAN

depth and five random points for flow velocity for each macrohabitat type in the seventeen reaches. Each reach was thereafter characterized by two physical measurements, the mean value of each physical characteristic along all the pools of the reach and the mean value of each physical characteristic along all the riffles of the reach. During the same sampling dates, we conducted vegetation surveys using a glass-bottom bucket. The total percent cover of vascular plants, bryophytes and macroalgae in each riffle and pool were estimated while walking along the banks or wading in the channel using a decameter. For each species, the plant percent cover per macrohabitat was then standardized by the total riffle or pool size within each reach. Species were identified according to Tutin et al. (1964–1993) for vascular plants, and according to Smith (Smith 1978, 1990) and Coudreuse et al. (2005) for bryophytes. Macroalgae (including *Characea* sp.) were generally identified to the genus level (Bourrelly 1966–70, Corillion 1975) (see Table 1).

Impact of channelization on abiotic and biotic factors at the macrohabitat scale

We previously performed a multivariate analysis of variance (MANOVA) on the chemical characteristics of each reach to verify if any difference existed between control and impacted reaches. Then we used two linear discriminant analyses (LDA) to characterize the four groups of macrohabitat types (CR, CP, IR, IP) based on their physical characteristics and their vegetative composition, using records from the six sampling dates (Legendre & Legendre 1998, Evrendilek & Berberoglu 2008, Robertson & Robertson 2008). In the vegetation analysis, the total percent cover of plants was log-transformed to avoid any excessive influence by dominant species on the results. LDAs involved creating linear combinations of either physical characteristics or floristic cover percentages with standard errors that best discriminate between macrohabitat types (Manel et al. 1999).

Table 2. Mean (m) and standard deviation (sd) of stream physical characteristics and total plant percent cover in the four macrohabitat types across the six study seasons. Spr = Spring, Sum = Summer, Aut = Autumn, 06 = 2006, 07 = 2007. Abbreviations see Table 1.

	Season	Wid		Dep		FV		Ligh		As		Co		Od		Pg		Sa		Sil		Ts		Veg	
		m	**sd**	**m**	**sd**	**m**	**sd**	**m**	**sd**	**m**	**sd**	**m**	**sd**	**m**	**sd**	**m**	**sd**	**m**	**sd**	**m**	**sd**	**m**	**sd**	**m**	**sd**
CR	**Spr06**	8.18	1.59	24.44	5.03	45.08	9.25	21.21	31.94	0.00	0.00	0.25	0.38	0.20	0.34	32.56	28.81	9.12	4.80	13.75	29.50	0.13	0.33	24.74	14.68
	Sum06	8.26	1.67	24.30	8.23	32.13	11.08	20.71	17.04	0.00	0.00	0.25	0.38	0.20	0.34	32.56	28.81	9.12	4.80	13.75	29.50	0.13	0.33	32.79	22.54
	Au06	8.27	1.69	21.38	6.43	39.46	11.08	19.94	23.75	0.00	0.00	0.25	0.38	0.20	0.34	32.56	28.81	9.12	4.80	13.75	29.50	0.13	0.33	25.46	16.23
	Spr07	8.18	1.59	35.21	9.38	40.13	9.13	26.95	27.58	0.00	0.00	0.80	1.02	0.11	0.14	28.98	23.28	22.82	22.05	2.64	6.15	0.16	0.39	30.15	16.48
	Sum07	8.26	1.67	33.19	8.27	37.40	12.06	19.09	22.06	0.00	0.00	2.10	2.78	0.29	0.71	26.01	20.74	22.50	18.75	3.53	8.32	1.07	2.61	27.88	15.84
	Au07	8.27	1.69	33.29	13.09	37.33	11.89	15.90	13.42	0.00	0.00	1.28	2.62	1.55	1.93	29.71	23.24	21.07	22.61	1.37	3.35	0.53	1.31	19.85	13.98
CP	**Spr06**	8.01	2.03	34.52	9.39	24.70	5.10	5.60	9.98	0.00	0.00	0.00	0.00	0.36	0.36	19.22	13.12	9.58	9.60	14.81	18.99	0.03	0.08	9.33	13.76
	Sum06	8.02	2.05	35.11	11.16	19.81	5.80	43.01	87.64	0.00	0.00	0.00	0.00	0.36	0.36	19.22	13.12	9.58	9.60	14.81	18.99	0.03	0.08	18.65	30.42
	Au06	7.97	2.02	32.60	8.02	25.52	5.10	14.87	20.39	0.00	0.00	0.00	0.00	0.36	0.36	19.22	13.12	9.58	9.60	14.81	18.99	0.03	0.08	12.83	21.18
	Spr07	8.01	2.03	43.84	11.63	24.30	4.23	14.96	8.93	0.00	0.00	1.49	3.05	0.86	1.42	21.06	14.52	19.00	18.94	2.09	2.00	0.00	0.00	15.12	15.62
	Sum07	8.02	2.05	41.20	9.31	23.84	5.97	7.90	5.84	0.00	0.00	0.87	0.93	1.14	1.78	21.25	15.98	18.63	18.22	2.61	3.91	0.00	0.00	14.91	14.30
	Au07	7.97	2.02	39.24	7.17	24.76	5.12	12.11	9.59	0.00	0.00	0.88	1.42	1.48	0.57	19.99	19.12	20.65	29.50	1.53	1.43	0.00	0.00	7.82	4.55
IR	**Spr06**	6.91	2.48	26.34	12.76	44.37	18.47	23.48	22.86	0.00	0.00	1.50	1.11	0.47	0.96	25.91	20.19	6.91	11.11	3.38	6.53	0.83	2.50	13.99	17.23
	Sum06	6.91	2.48	17.80	10.95	37.59	9.18	21.36	20.03	0.00	0.00	1.50	1.11	0.47	0.96	25.91	20.19	6.91	11.11	3.38	6.53	0.83	2.50	18.37	23.56
	Au06	6.93	2.44	19.79	8.91	38.27	8.15	17.24	23.06	0.00	0.00	1.50	1.11	0.47	0.96	25.91	20.19	6.91	11.11	3.38	6.53	0.83	2.50	17.61	22.06
	Spr07	6.91	2.48	33.13	15.26	39.15	11.63	14.69	16.76	0.00	0.00	3.23	3.33	1.11	1.33	17.46	15.23	14.28	11.98	1.82	3.94	1.11	3.33	12.62	12.16
	Sum07	6.91	2.48	30.59	13.62	36.53	11.09	20.43	20.53	0.00	0.00	3.01	3.00	0.91	1.69	16.20	13.22	17.60	11.77	1.28	2.68	0.00	0.00	14.56	14.38
	Au07	6.93	2.44	27.67	13.72	40.62	9.42	18.94	20.06	0.00	0.00	1.61	1.57	1.52	1.86	18.63	16.64	15.09	10.67	2.14	4.92	0.00	0.00	8.12	6.42
IP	**Spr06**	7.01	2.38	36.29	8.05	19.66	5.97	24.72	22.59	0.06	0.20	0.56	1.10	2.13	2.76	19.59	21.43	22.40	23.24	23.35	19.68	0.00	0.00	13.58	13.28
	Sum06	7.01	2.38	23.04	12.26	20.00	4.95	23.46	16.79	0.06	0.20	0.56	1.10	2.13	2.76	19.59	21.43	22.40	23.24	23.35	19.68	0.00	0.00	28.87	22.85
	Au06	7.01	2.38	26.99	8.81	18.62	7.47	18.80	13.15	0.06	0.20	0.56	1.10	2.13	2.76	19.59	21.43	22.40	23.24	23.35	19.68	0.00	0.00	15.63	11.85
	Spr07	7.01	2.38	39.23	10.45	20.30	9.50	22.80	12.27	0.00	0.00	3.25	3.45	2.43	2.40	16.87	12.78	37.84	17.22	7.46	14.60	0.24	0.79	16.68	10.59
	Sum07	7.01	2.38	35.75	12.17	20.36	10.43	21.59	12.89	0.07	0.23	0.85	0.86	3.30	2.15	20.84	13.42	36.51	20.61	5.93	14.08	0.60	1.39	22.72	15.59
	Au07	7.01	2.38	37.36	13.15	20.04	8.71	23.21	13.97	0.07	0.23	1.66	2.60	7.35	6.69	16.17	10.89	38.72	21.83	3.91	11.34	0.22	0.49	16.03	9.60

Table 3. Codes for biological species traits. * = Trait specific to bryophytes.

Traits	Attributes	Codes
Growth form	Free-floating, surface	frflsr
	Free-floating, submerged	frflsb
	Anchored, floating leaves	anfle
	Anchored, submerged leaves	ansule
	Anchored, emergent leaves	anemle
	Anchored, heterophylly	anhete
	Encrusting	encru
Vertical shoot architecture	Single apical growth point	siapgr
	Single basal growth point	sibagr
	Multiple apical growth point	muapgr
Leaf type	Tubular	tubula
	Capillary	capill
	Entire	entire
Leaf area	Small (< 1 cm^2)	LA1
	Medium (1–20 cm^2)	LA2
	Large (20–100 cm^2)	LA3
	Extra large (> 100 cm^2)	LA4
Morphology index (score)	(1) 2	MI1
	(2) 3–5	MI2
	(3) 6–7	MI3
	(4) 8–9	MI4
	(5) 10	MI5
Mode of reproduction	Rhizome	rhizom
	Fragmentation	fragmn
	Budding	buddg
	Turions	turion
	Stolons	stolon
	Tubers	tuber
	Seeds	seed
	Propagules*	propagu
Number of reproductive organs year^{-1} individual^{-1}	Low (< 10)	RO1
	Medium (10–100)	RO2
	High (100–1000)	RO3
	Very high (> 1000)	RO4
Perennation	Annual	annual
	Biennal/short lived perennial	shlipe
	Perennial	perenn
Evergreen leaf	/	winter
Amphibious	/	amphib
Gamete vector	Wind	wind
	Water	water
	Air bubble	airbub
	Insect	insect
	Self	self
Body flexibility	Low (< 45°)	BF1
	Intermediate (> 45–300°)	BF2
	High (> 300°)	BF3
Leaf texture	Soft	soft
	Rigid	rigid
	Waxy	waxy
	Non-waxy	non-waxy
Period of production of reproductive organ	Early (March–May)	early
	Mid (June–July)	mid
	Late (August–September)	late
	Very late (post–September)	verlat
Fruit size	< 1 mm	F1
	1–3 mm	F2
	> 3 mm	F3
Rooting at nodes	/	nodal
High below-ground:above-ground biomass	/	root

Temporal analyses

Temporal dynamics were determined using estimates of two values of seasonal variability: one for the physical characteristics and one for plant percent cover. The data used are presented in Table 2. For each group of macrohabitat type we calculated an average distance between the six seasons which corresponds to the average seasonal variability. The Euclidean distance was used for the scaled physical characteristics and the Bray-Curtis distance was used for the vegetation percent cover (Legendre & Legendre 1998, Molnar et al. 2003). These distances were compared among seasons across all macrohabitats by vectorial correlation coefficients (RV coefficients), by *t*-test between controls and impacted pools and between controls and impacted riffles, and finally, within controls and impacted macrohabitats by *t*-test between riffles and pools.

Channelization and species traits at the macrohabitat scale

A double principal coordinate analysis (DPCoA) permitted us to perform an ordination of species percent cover constrained by biological traits in order to understand the role of these traits in channelized reaches. Differences among species with regard to their biological traits helped to identify dissimilarities among communities (Pavoine et al. 2004, Dufour et al. 2006). We selected vascular plant species traits that have been commonly used in the literature and obtained the trait values from published studies (Bornette et al. 1994, Willby et al. 2000), independent of our vegetation surveys. For macroalgae and bryophytes, trait information was obtained from the identification keys of Bourrelly (1966–70) and Coudreuse (2005) and from personal observations. We performed three distinct DPCoA because of the strong differences in the trait composition for the three groups: vascular plants, macroalgae and bryophytes. The seventeen traits used were divided in several attributes and were presented in Table 3. Morphological and reproductive traits were selected based on the assumption that they would be most strongly influenced by physical characteristics and seasonality. The available information was structured using a fuzzy coding technique (Chevenet et al. 1994) with a scale from 0 (no affinity), 1 (moderate affinity) to 2 (high affinity). For each trait, we computed distances among species using Manly's overlap index (Manly 1994) for the twelve multi-level traits and the squared Euclidean metric for the four traits with only one level. The resulting global biological distance among species was obtained by averaging the distances over the percent cover estimates and taking the square root to assure Euclidean properties.

All multivariate analyses and statistical tests were computed using the ade4 package (Chessel et al. 2004) in the R-2.7.0 software (Ihaka & Gentleman 1996, R Development Core Team 2008).

Results

Spatial dynamics

The MANOVA did not reveal any significant differences in chemical characteristics between control and impacted reaches (F = 0.44, df = (7, 43), $P = 0.87$).

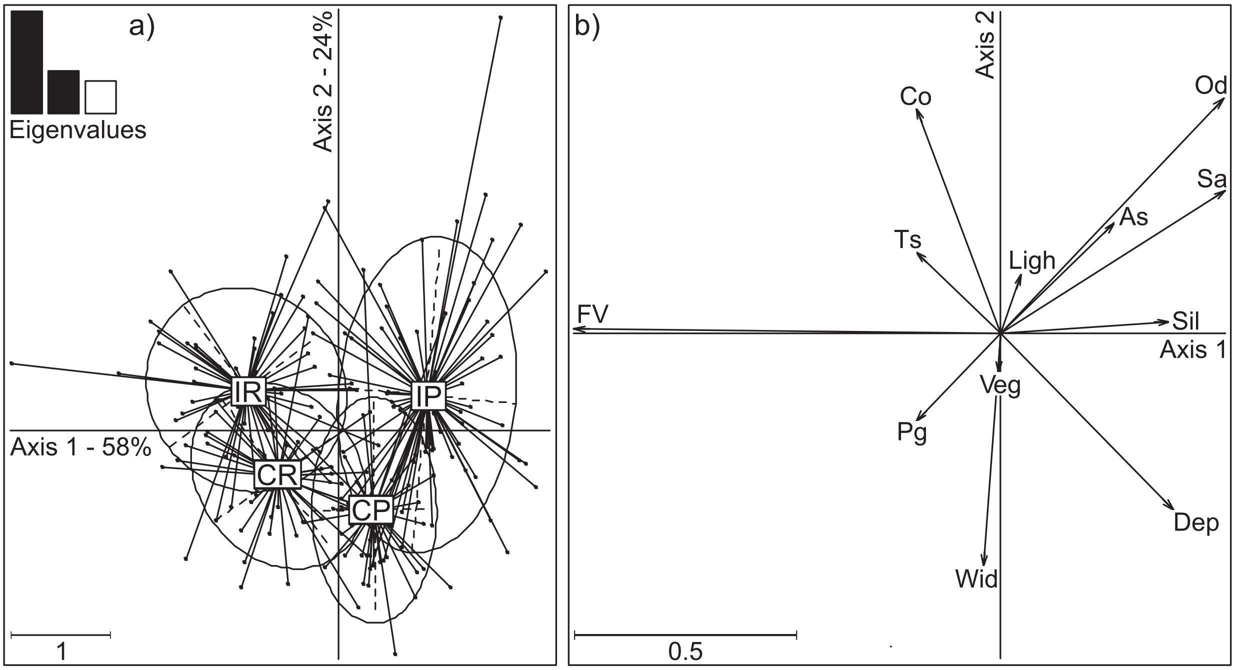

Fig. 2. Linear discriminant analysis of the four macrohabitat types (CR, CP, IR, IP) according to physical characteristics. **a)** Factorial map of the sampling dates grouped by macrohabitat (CR, CP, IR, IP), with the eigenvalue barplot in the upper left-hand corner. Each macrohabitat type is characterized by an average location and an ellipse of point dispersion. The axes of the ellipse are the principal axes of the scatter of points belonging to the focal macrohabitat and their lengths are proportional to the standard deviation of coordinates of the points. **b)** Correlations between the physical characteristics and the first two axes of the linear discriminant analysis. The scale for each panel is given in the left bottom corner. See Table 1 for the codes.

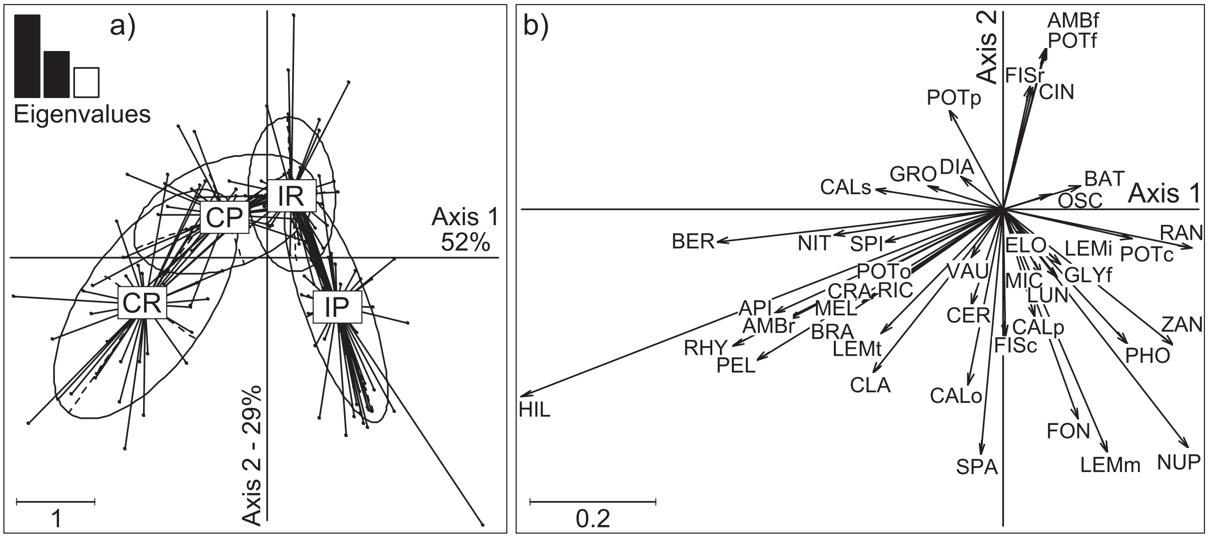

Fig. 3. Linear discriminant analysis of the four macrohabitat types according to floristic cover. **a)** Factorial map of the sampling dates grouped by macrohabitat, with the eigenvalue barplot in the upper left-hand corner. Each macrohabitat type is characterized by an average location and an ellipse of point dispersion as defined in Fig. 2. **b)** Position of species on the first two axes. The scale of each graphic is given in the left bottom corner. See Table 1 for the codes.

However, LDA permitted us to distinguish the macrohabitat types according to physical characteristics (Fig. 2). Unsurprisingly, the LDA first distinguished riffles with high flow velocity from pools with deeper profiles (58 % of the total inertia on the first axis), whereas the second axis (24 %) separated control and impacted macrohabitats. Control reaches were deeper and also larger than impacted reaches irrespective of

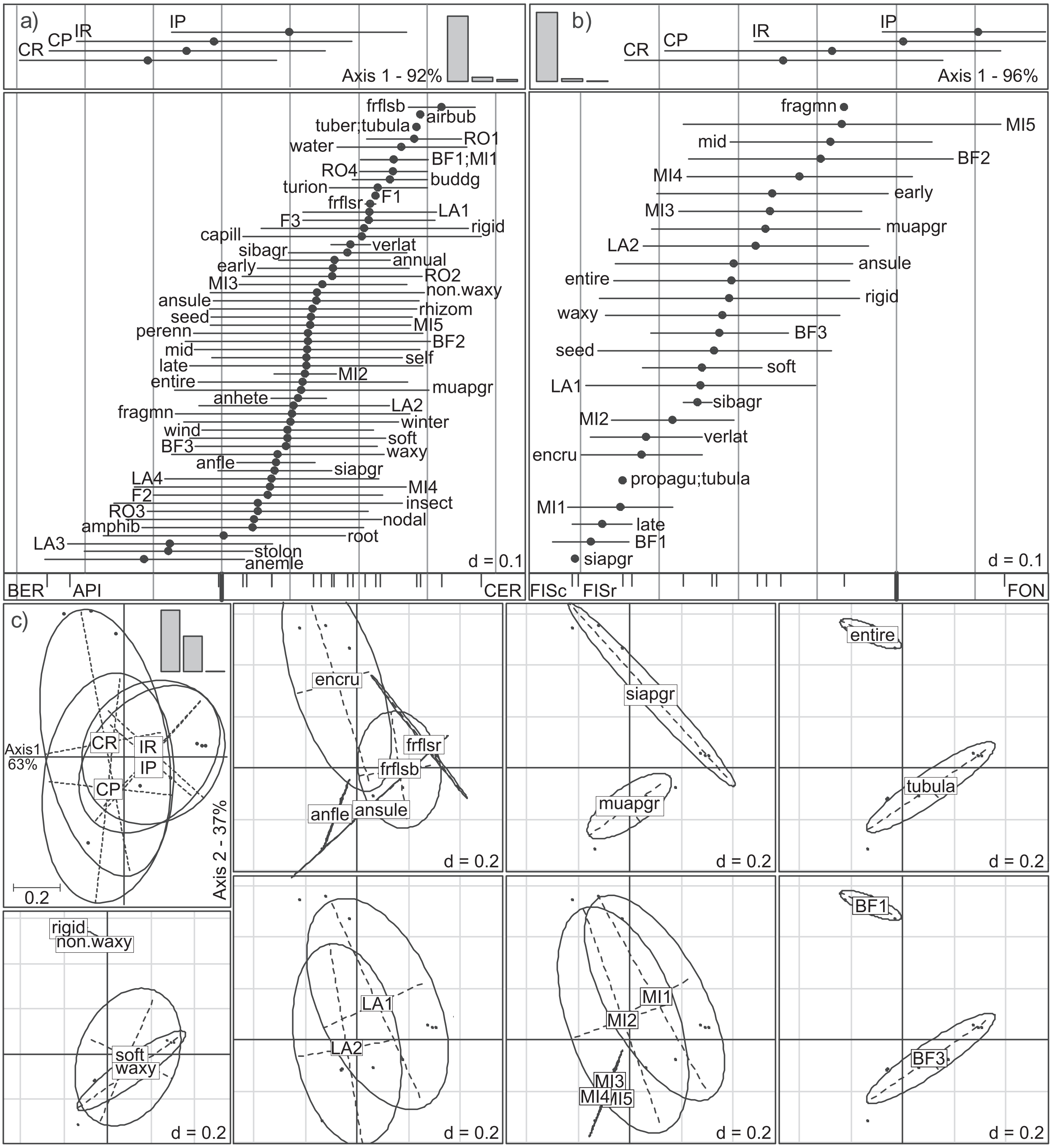

Fig. 4. Representation of the four macrohabitat types according to DPCoA based on species traits for **a)** vascular plants, **b)** bryophytes and **c)** macroalage. A histogram of eigenvalues is showed for each species group. Scales (d value) are indicated by the gap between two adjacent vertical bars for panels a) and b) where a single axis is considered and by the square side on a grid for panel c) where two axes are considered. The mean and standard deviations of species scores, i.e. biological profiles, within each macrohabitat are shown for a) vascular plants and b) bryophytes since only one axis is considered. Each vertical bar in the bottom of panels a) and b) correspond to the position of a species and the longest and darkest vertical bar correspond to the origin (zero) of axis one. The labels of the most extreme positions are given for e.g. *Berula erecta* (BER) or *Fissidens crassipes* (FISc). In panel c), the points in the first figure represent the average position of the macrohabitats by using macroalgal species traits. In the next subpanels of panel c), the points represent species, and the average positions of the levels of the traits are indicated for each trait separately. Ellipses of point dispersion are used as defined in Fig. 2. See Table 1 and Table 3 for the codes.

their pool or riffles status. In terms of the substrate composition, impacted pools were composed mainly of sand, silt and organic detritus, while impacted riffles were composed of cobblestones and control riffles were composed of pebbles and gravels.

Linear discriminant analysis on floristic data (Fig. 3) distinguished on the first axis (52 %) control from impacted macrohabitats and on the second axis (29 %) riffles and pools. Species such as *Berula erecta* (Huds.) Coville, *Hildenbrendia rivularis* (Liebm.) J. Agardh, *Rhynchostegium riparioides* (Hedw.) Card. were correlated with control macrohabitats. Species such as *Fontinalis antypretica* Hedw., *Lemna minor* L., *Nuphar lutea* Sibth. & Sm., *Phormidium* sp. Kützing, *Sparganium emersum* Rehmann, *Ranunculus fluitans* Lam. were found in IP, whereas the IR community was intermediate to CP and IP communities in term of species composition. Furthermore, few dominant species were found in impacted macrohabitats (ten species on the positive part of axis 1 in Fig. 3b), especially in riffles. Most of species were closer to the centre of the figure, meaning that their percent cover was low in impacted macrohabitats.

Temporal dynamics

Seasonal variability among macrohabitats

For stream physical characteristics, vectorial correlation coefficients (RV) among seasons ranged between 0.31 and 0.99 with a mean of 0.67 and a standard deviation of 0.19. For plant percent cover, RV correlations varied between 0.60 and 0.81 with a mean of 0.71 and a standard deviation of 0.06. Consequently, the analysis of the RV coefficients revealed stability in the differences among macrohabitats across seasons for both physical characteristics and floristic percent cover. Differences among reaches were thus mainly due to the degree of impact and the macrohabitat type as shown in Figs 2 and 3.

Seasonal variability within macrohabitats

Despite the low seasonal variability in the patterns of differences among macrohabitats, relatively high seasonal variability in species composition was found within macrohabitats with the distances among seasons varying from 0.31 to 0.73 (Manly's overlap index, bounded between 0 and 1). In terms of the physical characteristics, seasonal variability in control macrohabitats was not significantly different from the variability of impacted macrohabitats. However, within control macrohabitats (*t*-test, $t = 4.75$, $df = 5$, $P < 0.01$), and impacted macrohabitats ($t = 3.93$, $df = 8$, $P < 0.01$), seasonal variability was generally much higher in pools. In terms of the composition of macrophyte species, impacted riffles had significantly higher seasonal variability in total plant cover than controls ($t = 2.527$, $df = 13$, $P < 0.03$). As for physical characteristics, seasonal variability in plant cover within the control macrohabitats was significantly higher in pools ($t = 2.61$, $df = 5$, $P < 0.05$). No difference in this cover was found between the seasonal variability of control and impacted pools, or between riffles and pools within impacted macrohabitats.

Species biological traits

For each vegetative group, we selected biological traits that were variable between CR, CP, IR and IP. The total inertia of DPCoA of vascular plants (Fig. 4a, axis 1, 92 %) and bryophytes (Fig. 4b, axis 1, 96 %) were mainly explained by the first axis whereas the total inertia of DPCoA of macroalgae (Fig. 4c, axis 1, 63 % and axis 2, 37 %) was explained by the first two axes. The first axes of the three analyses separated control from impacted macrohabitats. Vascular plants and macroalgae showed similar trends according to their biological traits in channelized macrohabitats. Species in control macrohabitats were anchored or encrusting to substrate and had an intermediate leaf area between 1 and 100 cm². In contrast, species in impacted macrohabitats developed a floating growth form and small or extra large leaf area which could be explained by slower flow velocities in comparison to the controls. The morphological index of macroalgae (see Table 3), which takes into account the height and lateral expansion of species, ranged between 6 and 10 for controls whereas it was below 5 in channelized reaches. In terms of vegetative multiplication and reproductive traits in control macrohabitats, most vascular plants exhibited stolons, a high annual number of reproductive organs and were perennial species. Channelization was correlated with many attributes of the traits and species were mostly annuals. Bryophytes differed from both vascular plants and macroalgae because they were predominantly found in control reaches except one species (*Fontinalis antipyretica*, corresponding to the point on the positive part of axis 1 in Fig. 4b). Traits related to control reaches were anchored growth forms, small sizes (characteristic of bryophytes) with perennial strategy, water dispersion of gametes and rather late period of reproduction. Whatever the group, species used a lower diversity of trait strategies in controls than in impacted macrohabitats and this trend persisted throughout the seasons.

Discussion

The macrohabitat-level was a useful scale to observe the effects of channelization because riffles and pools exhibited different spatial and temporal trends in both physical characteristics and floristic cover. Our results suggest that spatial effects overwhelmed temporal effects in the differences between control and impacted macrohabitats. The impacts of channelization included a decrease in reach depth and width, as well as changes in substrate composition and shifts in plant species composition. These differences between control and channelized macrohabitats were maintained across seasons as they were relatively stable throughout time. Nevertheless, we noticed a strong seasonal variability within control and channelized macrohabitats, especially in pools. Only the floristic composition in control riffles was stable during the six study seasons. We observed a higher diversity of trait attributes for vascular plants and macroalgae in IR and IP as compared to CR and CP and a contrast in growth form strategies (e.g. floating *versus* anchored species) and time persistence strategies (e.g. annual *versus* perennial species). Bryophytes were mainly found in control reaches, which supports the hypothesis that this species group is strongly affected by channelization.

Channelization and spatio-temporal dynamics

Channelization had a permanent impact on the spatial composition of physical characteristics and plant community. Spatial differences among control and impacted reaches were stable throughout the six field seasons and corresponded to a decrease in depth and width, a shift from pebbles and gravels to sand, silt and artificial material, and many changes in species composition. But channelization did not attenuate the temporal dynamics as we expected. Indeed, the seasonal dynamics of physical characteristics and plant communities were as strong as in control reaches and even stronger for the plant cover in riffles.

A frequent consequence of improving river systems for navigation or human convenience is the narrowing and deepening of channels (Petts & Amoros 1996). In our case, while channelized reaches were narrower than control reaches, they were also shallower. Indeed, the studied streams were small streams that had been channelized to create space for urban construction and to ease the withdrawal of water for irrigation and human consumption leading to low water level. The decrease in width resulting from bank stabilization by longitudinal dikes, i.e. embankment, constrained the channel and lead to a loss of the marginal zone, an area that commonly acts as a buffer against floods. In control reaches, marginal vegetation may direct water flow towards the center of the channel permitting the dissipation of flow energy. Meandering is also responsible for energy dissipation (Petts & Amoros 1996). The loss of depth, marginal zones and natural sinuosity related to channelization could create artificial variability within impacted reaches in riffles as well as in pools. By contrast, the macrohabitat scale seemed to be more informative than the reach scale to explain change in substrate composition, as already demonstrated in a study on macroinvertebrate communities by Beisel et al. (1998). Riffles and pools showed different trends which suggests that the effects of channelization are spatially localized, i.e. the 100 m-long portions studied were not uniformly altered. While pebbles and gravel dominated CP and CR, IP were characterized by fine substrate particles such as sand or silt and organic detritus, and IR by artificial materials probably due to human structures that had been added to the stream bed. Both, sedimentation in pools and rocky substrates in riffles, might disconnect water column from groundwater within the stream bed. It has been demonstrated that a connection with groundwater stabilizes stream temperature and chemistry (Denny 1994). Therefore, a loss of vertical connectivity related to substrate changes could increase the variability in chemical characteristics throughout time. Furthermore, erosion and sedimentation processes related to the fine substrates in IP and the fast flow velocity with hard substrates in IR, explained in part the high dynamic of the impacted reaches. The percent cover of plant species may also contribute to the attenuation of spatial and temporal differences between control and impacted physical characteristics as vegetation tends to retain fine sediments and slow down flow velocity (Sand-Jensen 1998, Schulz & Gücker 2005). Physical differences between control and channelized reaches could be lower during the growth and reproductive seasons of plant species than in the winter when the majority of vegetation has disappeared.

Differences in species composition and biological traits

Changes in stream physical characteristics were also associated with shifts in species composition. Differences were especially important between CR and IP. *Berula erecta*, *Hildenbrendia rivularis*, and *Rhynchostegium riparioides*, for example, were more abundant in CR with fast flow velocity and pebbles whereas

Nuphar lutea, *Lemna minor* or *Fontinalis antypertica* dominated in IP with slow flow velocity and sand. *Ranunculus fluitans* Lam. was surprisingly more developed in IP albeit it is one of the characteristic species of French calcareous streams (Bensettiti et al. 2002). The community of IR consisted of few species with low percent cover and was intermediate between the species composition of CP and IP. The percent cover of species found in IR was highly variable throughout time in contrast to CR suggesting high species turnover and an unstable community composition. IR communities may be similar to CP or IP communities in terms of seasonality. For instance, in summer, after the growth and reproduction period, plant percent cover was greatest along reaches. Riffle and pool vegetation were likely more homogeneous within reaches because frequent dispersal and exchanges between IR and IP led to similarities between their community compositions.

The biological traits of species can be used to explain differences in community composition between control and impacted macrohabitats. The organization of aquatic macrophyte communities depends on the role of the traits exhibited within various types of habitat templates. Indeed, habitats are the templates on which evolution forges characteristic life history strategies (Bornette et al. 1994, Tomanova & Usseglio-Polatera 2007). Plants suffering the effects of channelization do not exhibit the same characteristics as plants growing in the absence of stress. We demonstrated that communities exhibit different trait combinations in control and impacted macrohabitats. The conditions found in control macrohabitats (large and deep stream bed, substrate of pebbles and gravels) are potentially more appropriate for small, slow-growing and weakly competitive plants such as bryophytes, in comparison to tall and fast-growing vascular plants (Sand-Jensen et al. 2008). Rosenzweig (1995) demonstrated that favorable habitats are correlated with specific trait combinations, i.e. specialist species. If we consider specialization as a decrease in the strategy diversity of species, we found that control macrohabitats were colonized by specialist species adopting intermediate strategies. For example, plants in CR and CP exhibited medium leaf area and body flexibility and a relatively high number of reproductive organs. Morphologic adaptations resulting from plasticity or natural selection allows species to maximize their anchorage as well as reduce hydraulic stress on stem due to fast flow velocity. The resultant growth forms improve plant performance in relation to their habitat (Denny 1994). Strong anchorage, reproduction by many stolons and water-based dispersal should permit species to colonize open space and to resist fast flows, especially those found in CR. Perennation was also favoured in these environments which supports the finding of low temporal variability in vegetative cover in CR. Indeed, Greulich & Bornette (2003) have shown that persistence in winter facilitated the development of slow-growing species and poor competitors.

In contrast, in channelized reaches we found that species used several adaptive strategies related to morphology and reproduction to persist despite the spatial and temporal differences in flow as has been shown for *Ranunculus peltatus* in disturbed habitats (Garbey et al. 2004). For instance, a wide diversity of reproductive organs was observed in the species found in channelized macrohabitats as compared with the single reproductive mode found in control macrohabitats, stolon development. It is diversity of reproductive modes that allows for the fast growth and improved competitive abilities necessary for persistence in channelized reaches. Moreover, plants in impacted macrohabitats used contrasting strategies, e.g. little or large leaf area, low or high body flexibility. This diversity of strategies could explain in part the high temporal variation in plant cover observed in IR. Species could invest in a large body size and weak reproductive capacities, or inversely, in a small body size and strong reproductive capacities. Trade-offs between traits should select the most relevant traits to resist against new conditions imposed by channelization. Various trait combinations could lead to a set of dispersal strategies, e.g. plants with long stems that break easily and have weak anchorages, or stress avoidance strategies, e.g. flexible plants with strong anchorages or small plants that remain close to the substrate where drag forces are reduced (Puijalon et al. 2005). Furthermore, species with high functional plasticity and heterophylly such as *N. lutea*, *S. emersum*, *R. fluitans* or *F. antipyretica*, were favored in the impacted macrohabitats to the detriment of specialist species *sensu* Rosenzweig (1995). As a consequence, a diversity of strategies in channelized reaches may increase tolerance to changes in spatial and temporal dynamics (e.g. regenerative strategies, Combroux et al. 2001). Strong competitive capacities are required in the absence of marginal zones and in the presence of hardly colonisable substrate, e.g. artificial material in riffles. Indeed, plant species had a lower available habitat for their establishment. In consequence, communities in channelized reaches corresponded to pioneer and unstable communities with great turnover, whereas communities in control reaches corresponded mostly to stable and well-established communities. An *in situ* experiment by measurements of species traits could be done in control and channelized reaches to confirm these results.

The results of this study are a step towards a general model, based on responses of environmental characteristics and plant species, to predict changes in physical and community composition according to different stresses. Ecological requirements (e.g. depth, flow velocity, substrate) are necessary parameters for this model. We have demonstrated that channelization can lead to totally different spatial organization and seasonality from the one experienced in control reaches. Macrophyte communities exhibited different species composition where species with multiple trait strategies for survival were favored. The observed trends may have implications for restoring the integrity of freshwater streams. Optimizing stream physical structure and species diversity are important strategies to protect the natural spatio-temporal dynamics of stream habitats and should be taken into consideration in future projects involving the management of aquatic systems.

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Relationships between channelization structures, environmental characteristics, and plant communities in four French streams in the Seine–Normandy catchment

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Abstract. We examined the relationships between channelization, environmental characteristics, and plant communities in 4 streams (Betz, Cléry, Lunain, and Ecole Rivers) southeast of Paris, France, with the goal of assessing stream health. Seventeen 100-m-long reaches, each divided into a pool/riffle sequence, were monitored in 2006. Each reach had 0 to 3 channelization structures (i.e., embanking, resectioning, and other man-made structures). Redundancy analysis and mean-comparison tests done at 2 spatial scales (reach scale and pool/riffle sequence scale) indicated that channelization significantly affected flow velocity, depth, substrate type, and number of pools/riffles (4 of 24 variables considered). Physical characteristics of the streams were significantly more affected by channelization than were chemical characteristics. Moreover, different channelization structures affected the streams differently. Resectioning and embanking reduced flow velocity and the number of riffles and increased the number of pools. However, man-made structures either accelerated (downstream) or reduced flow velocity (upstream). Channelization strongly affected floristic richness on the basis of biological type (vascular plants, bryophytes, macroalgae) and ecomorphological type (hydrophytes, helophytes) at the pool/riffle sequence scale. Channelization led to taxonomic shifts and loss of biodiversity. Vascular plant taxa such as *Helosciadium nodiflorum* and *Berula erecta* were replaced by opportunist taxa such as *Potamogeton crispus* in channelized reaches. Combinations of channelization structures affected the stream plant assemblages less than did individual structures, a result that suggested compensatory effects. Our study supports the idea that channelization must be accompanied by measures that preserve the initial physical conditions of streams and the natural plant community composition.

Key words: EU 3260, river, human impact, spatial scale, richness, indicator species.

Stream channelization is widespread and intensive worldwide. 1 Streams have been modified for human convenience, such as for farming, navigation, agriculture, and road building (Maddock 1999). Channelization, or channel modification, is a set of anthropogenic alterations that modify stream hydrology. For instance, addition of man-made structures allows

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resectioning, realignment, or embanking of streams to prevent flooding or to modify channels for various land uses (e.g., drainage for agriculture, shipping). Consequently, channelization affects channel dimension and shape (Brookes 1988) and a stream's physical heterogeneity and connectivity with adjacent lands (Frissell et al. 1986). The physical variation in flow velocity, depth, and substrate that characterizes natural meandering watercourses and provides complex habitat structures also is often altered (Petts 1984, Brookes 1985, 1988). Such changes in the abiotic environment can affect biotic assemblages (Bravard et al. 1997, Thomson et al. 2001), and hydraulic structures can create serious ecological problems (Dynesius and Nilsson 1994, Karr 1994). For example, aquatic biodiversity is still declining because of altered physical conditions and intensive land use despite the improvement in water quality of many European streams during the last 2 decades (Kristensen and Hansen 1994, Haury 1996, Pinder et al. 1997).

Assessment of human influence on the ecological and biological characteristics of the streams is complex because of the large variety of channelization structures. Therefore, several management methods have been developed to assess human impacts, such as channelization of streams (e.g., River Habitat Survey, Raven et al. 1997), and bioassessment techniques have been developed to complement surveys of environmental change (Lenat 1993, Rosenberg and Resh 1993). Bioassessment can involve evaluation of biological communities in terms of taxonomic diversity, number of individuals, biomass, community structure, or a summary index that incorporates >1 metric (Rosenberg and Resh 1993). Benthic macroinvertebrates and fish are the organisms most commonly used to assess the impact of channelization structures on streams because they often integrate effects across large spatial and temporal scales, and therefore are presumed to provide more accurate measures of impact than do environmental characteristics alone (Karr 1987, Gerritsen 1995). However, plant communities also can be used as indicators of stream health. Many plant taxa have distinct preferences for hydrologic variables, such as flow velocity, depth, and substrate size/type (Haslam 1978, Baattrup-Pedersen and Riis 1999, Riis and Biggs 2003, Gurnell et al. 2006, Lacoul and Freedman 2006) and contribute strongly to ecosystem health and services (Gregg and Rose 1985, Sand-Jensen 1998, O'Hare and Murphy 1999, Harrison 2000, Westwood et al. 2006). However, few studies have used aquatic plant communities to assess channelization impacts.

In Europe, concern for stream health and its assessment has been addressed in the European Union's Habitat Directive (92/43 EEC; European Union 1992) and the European Union's Water Framework Directive (00/60 EEC; European Union 2000). Among the European aquatic habitat types, the EU 3260 "Water courses of plain to mountain levels with the *Ranunculion fluitantis* and *Callitricho-Batrachion* vegetation" has been targeted as a priority habitat because of its vulnerability (European Commission 2007). This habitat occurs in chalk streams, which typically are characterized by diverse and abundant plant communities (Butcher 1933, Haslam 1987, Sand-Jensen 1998, Westwood et al. 2006). Assessment of channelization impacts on this habitat is of particular importance and is expected to provide valuable insights for local managers.

We examined possible relationships between the environmental and floristic characteristics of streams to assess the impact of channelization on the health of small French streams dominated by *Ranunculion* and *Callitriche* spp. We studied 3 types of channelization structures: structures for embanking, structures for resectioning, and man-made structures that create obstacles to water flow (bridges, ground sills, groynes) in 4 streams of similar size and geomorphologic characteristics at the reach and the pool/riffle sequence scales. The pool/riffle sequence has been used rarely in impact studies, but in our study it provided information on local-scale processes. Our objectives were to assess the impact of channelization structures on: 1) environmental (physical and chemical) characteristics, 2) floristic richness of different taxonomic groups, and 3) floristic composition of assemblages at the reach and pool/riffle sequence scales. We considered use of several plant taxa as potential indicators of stream health.

Methods

Study sites and sampling

We worked in the Seine–Normandy catchment (100,000 km^2) south of the Ile-de-France region (Fig. 1A–C). This region is flat (mean slope $<1\%$) and consists of plateaus at low elevation (mean = 160 m above sea level) with lowland streams. The geological layer is limestone, and the region lies within a single biogeographical region (Atlantic climate, mean annual temperature = 11°C, mean annual precipitation = 600 mm). Thus, the 4 study streams had comparable geographical and geological conditions.

We considered 3 types of channel structures as defined by Brookes (1988) and Wasson et al. (1995) in

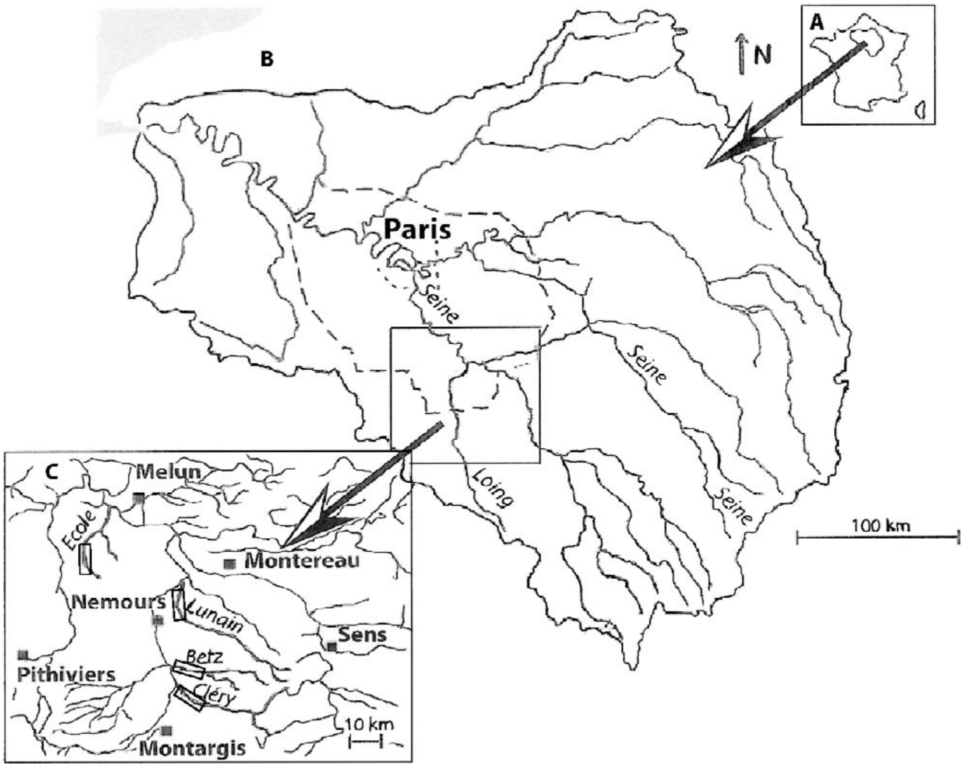

FIG. 1. A.—The study area within France. B.—The Seine–Normandy catchment and its networks. The narrow dashed line corresponds to Paris and the wide dashed line to the Ile-de-France area. C.—The Ecole, Lunain, Betz, and Cléry Rivers and the stream sections studied.

our study: 1) embanking, which increases the elevation of stream banks, 2) resectioning, which changes the channel profile and causes loss of meanders, and 3) addition of rigid man-made structures to the streambed (bridges, ground sills, and groynes). Sills are concrete steps placed in the thalweg or wood logs fixed on the substrate, and groynes are perpendicular wing-dykes. Both structures are used to accelerate water flow.

In 2006, we chose 4 streams (Betz, Cléry, Lunain, and Ecole) where EU 3260 habitat type was well represented. Flow of these streams varied from 0.37 m^3/s (Ecole) to 1.44 m^3/s (Cléry). For 30 y, these streams have been partially channelized by multiple structures to regulate water flow, protect human populations, and create water reservoirs for agriculture and domestic uses. We studied 5- to 6-km sections of each stream (Fig. 1C) that were characterized by chalk geology, low Strahler stream order (1–2), moderate trophic status, and the same type and intensity of land use (grass meadows with small area of forests).

We monitored seventeen 100-m long reaches (3–5 reaches/stream, each 1–2 km apart) along each section (Table 1, Appendix 1; available online from: http://dx.doi.org/10.1899/08-139.1.s1). A succession of pool/riffle sequences sensu Frissell et al. (1986) and Giorgi et al. (2005) was present in each reach. Pools (1–3 per reach) were deep areas (25–80 cm depth) with flow velocities $<$20 cm/s. Riffles (0–4 per reach) were shallow areas (10–30 cm depth) with high velocities (40–60 cm/s). Channelized reaches (n = 11) had 1 to 3 channelization structures (embanking, resectioning, bridge, sill, or groyne) along the 100-m reach studied, whereas control reaches (n = 6) had no channelization structures. No control reaches were present in the Lunain River because of the high degree of human influence along its course.

Physical habitat characterization

We recorded physical habitat characteristics of each reach in spring (May), summer (July), and autumn (September) 2006. We calculated sinuosity (whole reach length/linear distance between the 2 reach extremities) at the reach scale from 1/25,000 Institut Geographique National maps (http://www.ign.fr/). We determined the number and percentage of pools and riffles in each reach in the field. We measured depth, width, flow velocity, and percentage cover of 7

TABLE 1. Codes for streams, reaches, environmental characteristics, channelization structures, and plant taxa. The number associated with each reach code reflects its longitudinal position (1 = upstream to 5 = downstream).

Name	Code
Stream	
Betz	BE
Cléry	CL
Ecole	EC
Lunain	LU
Reach	
Brandard	BE1
Jeandrillonnerie	BE2
Dordives	BE3
Chanteraine	CL1
Boyard	CL2
Gril	CL3
Ferrières D32	CL4
Ferrières Ville	CL5
Oncy	EC1
Milly	EC2
Coudret	EC3
Noues	EC4
Garenne	EC5
Nonville	LU1
Luat	LU2
Villeron	LU3
Gratereau	LU4
Channelization structure	CS
None (control)	N
Embanking	E
Resectioning	R
Man-made structures	S
Environmental characteristics	
Physical	
Sinuosity	Sin
Depth (cm)	Dep
Flow velocity (cm/s)	FV
Width (m)	Wid
Light availability (%)	Ligh
Artificial substrata (%)	As
Cobbles (%)	Co
Pebbles and gravel (%)	Pg
Organic detritus (%)	Od
Sand (%)	Sa
Silt (%)	Sil
Terrestrial sediment (%)	Ts
Number of riffles and pools	RPn
Number of riffles	Rn
Percentage of riffles (%)	Rp
Number of pools	Pn
Chemical	
Conductivity (μS/cm)	Cond
Temperature (°C)	Temp
O_2 (mg/L)	Oxy
Total dissolved inorganic N (mg/L)	Ntot
PO_4 concentration (mg/L)	PO4
$CaCO_3$ concentration (mg/L)	CaCO3
Mg concentration (mg/L)	Mg
Potential H	pH
Taxa	
Hydrophytes	
Macroalgae	
Batrachospermum sp. Roth	BAT
Cladophora sp. Kützing	CLA
Diatoma sp. Bory de St Vincent	DIA
Hildenbrandia rivularis (Liebm.) J.Agardh	HIL
Melosira sp. C. Agardh	MEL
Microspora sp. Thuret	MIC
Nitella sp. C. Agardh	NIT
Oscillatoria sp. Vaucher	OSC
Phormidium sp. Kützing	PHO
Spirogyra sp. Link	SPI
Vaucheria sp. De Candolle	VAU
Bryophytes	
Amblystegium fluviatile (Hedw.) B., S. & G.	AMBf
Amblystegium riparium (Hedw.) B., S. & G.	AMBr
Brachytecium rivulare B., S. & G.	BRA
Cinclidotus danubicus Schiffn. & Baumg.	CIN
Cratoneuron filicinum (Hedw.) Spruce	CRA
Fissidens crassipes Wils. ex B., S. & G.	FISc
Fissidens rufulus B., S. & G.	FISr
Fontinalis antipyretica Hedw.	FON
Lunularia cruciata (L.) Lindb.	LUN
Pellia endiviifolia (Dicks) Dumort.	PEL
Rhynchostegium riparioides (Hedw.) Card.	RHY
Riccardia chamaedryfolia (With.) Grolle	RIC
Vascular plants	
Berula erecta (Huds.) Coville	BER
Callitriche obtusangula Le Gall	CALo
Callitriche platycarpa Kützing	CALp
Callitriche stagnalis Scop.	CALs
Caltha palustris L.	CAH
Ceratophyllum demersum L.	CER
Elodea canadensis Michx	ELO
Glyceria fluitans (L.) R. Br.	GLYf
Groenlandia densa (L.) Fourr.	GRO
Helosciadium nodiflorum (L.) Lag.	HEL
Lemna minor L.	LEMm
Lemna minuscula L.	LEMi
Lemna trisulca L.	LEMt
Mentha aquatica L.	MEN
Myosotis gr. *Palustris* L.	MYO
Nasturtium officinale sl R. Br.	NAS
Nuphar lutea (L.) Sibth. & Sm.	NUP
Phalaris arundinacea L.	PHA
Polygonum amphibium L.	POL
Potamogeton crispus L.	POTc
Potamogeton obtusifolius Mert. & Koch	POTo
Potamogeton pectinatus L.	POTp
Potamogeton perfoliatus L.	POTf
Ranunculus fluitans Lam.	RAN
Reynoutria japonica Houtt.	REY
Sparganium emersum Rehmann	SPA
Zannichellia palustris L.	ZAN

C2

TABLE 1. Continued.

Name	Code
Helophytes	
Vascular plants	
Agrostis stolonifera L. fo. Aq.	AGR
Bidens tripartita L.	BID
Calystegia sepium (L.) R. Br.	CAY
Carex elata All.	CAR
Equisetum arvense L.	EQU
Galium sp. L.	GAL
Glechoma hederacea L.	GLE
Glyceria aquatica (L.) Wahlb.	GLYa
Iris pseudacorus L.	IRI
Lycopus europaeus L.	LYC
Phragmites australis Cav. Trin. Ex Steud	PHR
Poa trivialis L.	POA
Rorippa amphibia (L.) Besser	ROR
Scrophularia auriculata L.	SCR
Solanum dulcamara L.	SOL
Teucrium scordium L.	TEU
Urtica dioica L.	URT
Veronica anagallis-aquatica L.	VERa
Veronica anagalloides Guss	VERl
Veronica beccabunga L.	VERb
Viola sp. L.	VIO

substrate types (artificial [e.g., building material], cobble, pebble and gravel, organic detritus, sand, silt, and terrestrial sediment) in each pool and riffle with a graduated ruler, measuring tape, and electromagnetic current meter for rivers (Mini Air 20, Schiltknecht Messtechnik AG, Gossau, Switzerland). On each sampling date and for each pool and riffle in the 17 reaches, we made 1 measurement of width, established 1 lateral transect and measured depth every 20 cm along the transect and at 10 additional random points, and measured surface flow velocity at 5 random points. We characterized each reach by the mean value of each physical characteristic for all pools in the reach and the mean value of each physical characteristic for all riffles in the reach. We estimated light availability (100 − % canopy cover) by measuring the canopy vertical projection on stream water with a graduated ruler.

Chemical analysis

In each reach, we collected 50 mL of water once each month from May to December 2006. We analyzed water samples <24 h after sample collection with chemical kits for NO_3^-, NO_2^-, NH_4^+, PO_4-P, total hardness, carbonate hardness, and pH (Reagent case, Visocolor ECO Analysenkoffer; Macherey–Nagel, Düren, Germany). We used NO_3^-, NO_2^-, and NH_4^+ concentrations to assess total dissolved inorganic N, and we used total hardness and carbonate hardness to quantify $CaCO_3$ and Mg^{2+} concentrations. Dissolved O_2 concentration, water temperature, and conductivity were measured in situ daily at 1000 h in each reach with 2 data loggers (Dissolved Oxygen Pocket Meter Oxi315i/SET, WTW Wissenschaftlich-Technische Werkstätten, Weilheim, Germany; multichannel analyzers, Consort C533, Consort nv, Turnhout, Belgium) (Appendix 2; available online from: http://dx.doi.org/10.1899/08-139.1.s2).

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Floristic data

We conducted plant surveys in the same reaches and at the same times as the physical surveys (May, July, September 2006). We did not include stream banks, but we did include seasonally dry parts of the streambed. We made observations of submerged plants through a glass-bottom bucket. In pools and riffles, we used a measuring tape to estimate total percentage cover of vascular plant taxa, bryophytes, and macroalgae from the bank and in the channel. Percentage cover provided an estimate of the relative abundance of each taxon in pools and riffles. We identified plants to species with keys in Tutin et al. (1964–1993, vascular plants), Smith (1978, 1990, bryophytes), and Coudreuse et al. (2005, bryophytes). Macroalgae were identified to genus with keys in Bourelly (1966–1970). We calculated floristic richness for the reach and the pool/riffle sequence scales. Richness measures included total taxonomic richness, richness within each biological type (vascular plants, bryophytes, macroalgae), and richness within 2 ecomorphological types (hydrophytes and helophytes; Raunkiaer 1905).

Statistical analyses

We separated environmental characteristics into physical and chemical measures (Table 1). We measured chemical characteristics at the reach scale and analyzed them as means of values from the 8 sampling dates (May–December). We analyzed physical characteristics and percentage cover at both spatial scales as the means of values from May, July, and September for all pools and riffles within a reach. We calculated richness at the reach scale as the total number of taxa recorded in the pools and riffles over the 3 study dates. We calculated 2 values of richness at the pool/riffle sequence scale: the number of taxa found in pools and the number of taxa found in riffles in each reach.

We computed redundancy analyses (RDA) with the ADE4 package (Chessel et al. 2004) in the R-2.7.0 software (Ihaka and Gentleman 1996; R Development Core Team, Vienna, Austria) to explore the relationships among environmental variables and channelization structures at the reach scale and for pools and riffles separately (Ewald 2000, Schröder et al. 2002). Chemical characteristics and sinuosity were the same in both analyses. Principal component analysis and multiple regression models with Akaike Information Criterion (Hastie and Pregibon 1993) indicated that 13 of 37 variables measured in the field were highly correlated. We removed these variables from further analyses. We constrained the ordination of environmental characteristics in RDA in such a way that the resulting ordination vectors were linear combinations of the channelization structures. We centered data on their means and standardized them by column because of nonhomogeneous dimensionality. We used Euclidean distances to relate the data linearly (Legendre and Legendre 1998).

We used *t*-tests comparing floristic richness between unchannelized (control) and channelized reaches at the 2 spatial scales to assess the impact of channelization on floristic richness and to determine which taxonomic groups, biological types, and spatial scales were most affected by channelization. We also tested the relationships between channelization and plant distribution at the 2 spatial scales by RDA (ADE4 package, R-2.7.0 software). We chose RDA instead of canonical correspondence analysis (ter Braak 1986) because of the nature of the data (percentage cover) and the fact that the distribution of percentage cover was skewed (few taxa with high percentage cover and many taxa with very low or 0% cover). We $\log(x + 1)$-transformed and centered the data before running the RDA.

We tested for correlations between the structures of the environmental characteristics and percentage plant cover data sets at the 2 spatial scales by a randomization test (PROTEST) with 999 Monte Carlo permutations (procuste.randtest function in the ADE4 package, R-2.7.0 software; Dray et al. 2002). This analysis allowed us to determine whether differences in environmental characteristics caused by channelization were linked to differences in plant composition.

Results

Environmental characteristics

At the reach scale, environmental characteristics differed among reaches according to their channelization structures (Fig. 2A–C). The sum of the first 2 RDA eigenvalues (37.3% and 31.3%) and the ratio of the variance of predicted values to the variance of observed values (axis 1, $r^2 = 0.76$; axis 2, $r^2 = 0.81$) showed that the differences in environmental characteristics (Fig. 2A) were correlated with differences in channelization structures along these 2 axes. On axis 1, control reaches were separated from reaches with 1 type of channelization structure (Fig. 2B). Control reaches had high percentage riffles, flow velocities, and PO_4^{3-} concentrations, whereas reaches with one type of channelization structure were characterized by high $CaCO_3$ concentrations and fine substrate composed of sand (Fig. 2A, B). On axis 2, control reaches were separated from reaches with only resectioning or embanking (negative end) and from reaches with only man-made structures or combinations of 2 or 3 structures (positive end) (Fig. 2B). Embanked reaches were characterized by a larger number of pools than were other reaches. Reaches with man-made structures and multiple structures were characterized by high temperatures, predominance of coarse substrates such as cobbles, artificial substrates, pebbles, and gravel, and organic detritus (Fig. 2A, B). Thus, embanking and resectioning were associated with morphological changes that affected flow and channel width, whereas man-made structures and their combinations were associated with changes in substrate composition (Fig. 2A, C).

Trends in environmental conditions differed between pools and riffles (Fig. 3A–F). The first 2 axes of the 2 RDAs explained >50% of the total inertia. Thus, we were able to characterize the differences between control and channelized reaches (riffles: axis 1, 44.9%, $r^2 = 0.79$ and axis 2, 23.6%, $r^2 = 0.71$; for pools: axis 1, 32.3%, $r^2 = 0.82$ and axis 2, 26.6%, $r^2 = 0.65$). Axis 3 also had an important eigenvalue (25.4%), but is not shown because it indicated the same trends as axis 2. Pool/riffle sequences were more heterogeneous in control than in channelized reaches, probably because of the larger number of riffles in the control reaches. Control riffles were wider, had finer substrates, and were slightly deeper than channelized riffles (Fig. 3A, B). Riffles with multiple channelization structures were more different from control riffles than were riffles with only 1 channelization structure (Fig. 3B). Control pools and pools with channelization structures were strongly differentiated: control pools were deeper and had greater flow velocities than channelized pools (Fig. 3D, E). Pools in reaches with embanking and resectioning structures were characterized by sand substrate and higher $CaCO_3$ concentrations than were pools with man-made structures and with combined structures (Fig. 3E). Pools with man-made structures and with combined structures had higher light availability, artificial substrata, cobbles, and organic detritus than did pools with embanking and resectioning. These substrates were

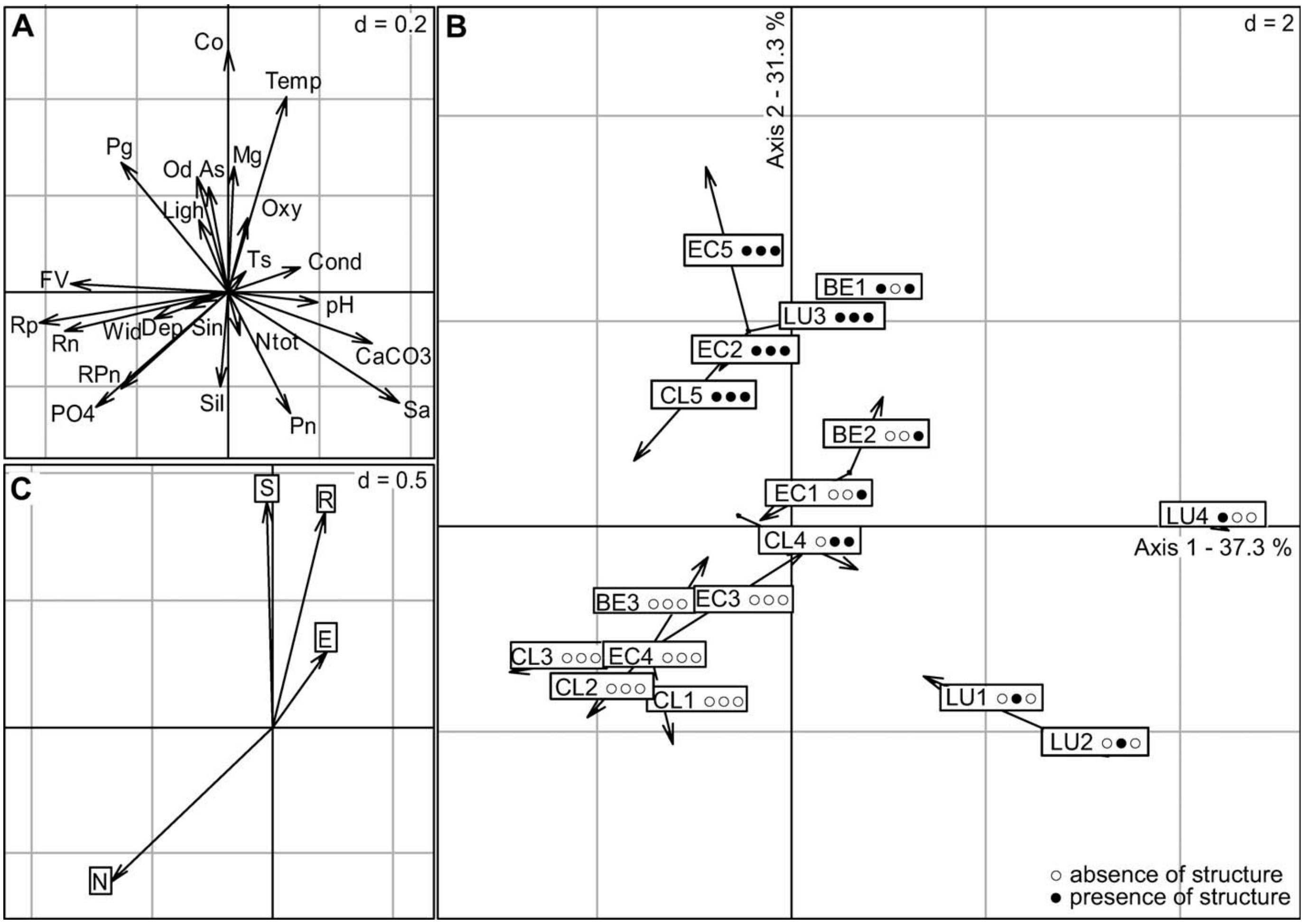

FIG. 2. Redundancy analysis (RDA) of the physical and chemical characteristics of the study reaches showing positions of the environmental characteristics on the 1st (horizontal) and 2nd (vertical) axes of the RDA (A), a representation on the factorial map (axis 1 × axis 2) of the position of each reach (B), and the correlation between the channelization structure (none [N], resectioning [R], embanking [E], and man-made structures [S]) and the 1st and 2nd axes of the RDA (C). The scale of A, B, C is given in the upper right-hand corner (*d* = side length of square). In B, each reach has 2 positions linked by an arrow. The arrow starts from the position predicted by the channelization structures present and points to the observed position. A long arrow indicates a difference between the observed and predicted values of the environmental characteristics in a reach. Each reach code is followed by 3 circles indicating the presence or absence of channelization structures in the order: R, E, and S. Codes for reaches, environmental characteristics, and channelization structures are given in Table 1.

closely related to human impacts, i.e., artificial substrates and cobbles were present as a direct result of streambed modification.

Floristic characteristics

Thirty-three plant families were recorded from the 4 streams. Vascular plant taxa (48 taxa) were much more numerous than bryophytes (12 taxa) and macroalgae (11 taxa). The number of taxa per reach varied between 17 and 35. Hydrophytes were more numerous than helophytes (50 vs 21 taxa, respectively).

The presence of channelization structures was not related to total plant richness at the reach scale (Fig. 4A). However, richness of vascular plant taxa ($t_{94} = -2.05$, $p = 0.04$; Fig. 4B) and helophyte taxa ($t_{92.56} = -4.55$, $p < 0.001$; Fig. 4F) was higher and richness of macroalgae taxa was lower ($t_{94} = 4.52$, $p < 0.001$; Fig. 4D) in channelized than in control reaches. The absence of significant trends in total plant richness at the reach scale was the result of the opposite effects of channelization in riffles and pools. In riffles, total plant richness was significantly lower in channelized than in control reaches ($t_{43} = 2.12$, $p = 0.04$; Fig. 4G), whereas in pools, total plant richness was significantly lower in control than in channelized reaches ($t_{49} = -2.89$, $p = 0.006$; Fig. 4M). In riffles, richness of bryophytes ($t_{28.46} = 3.08$, $p = 0.005$;

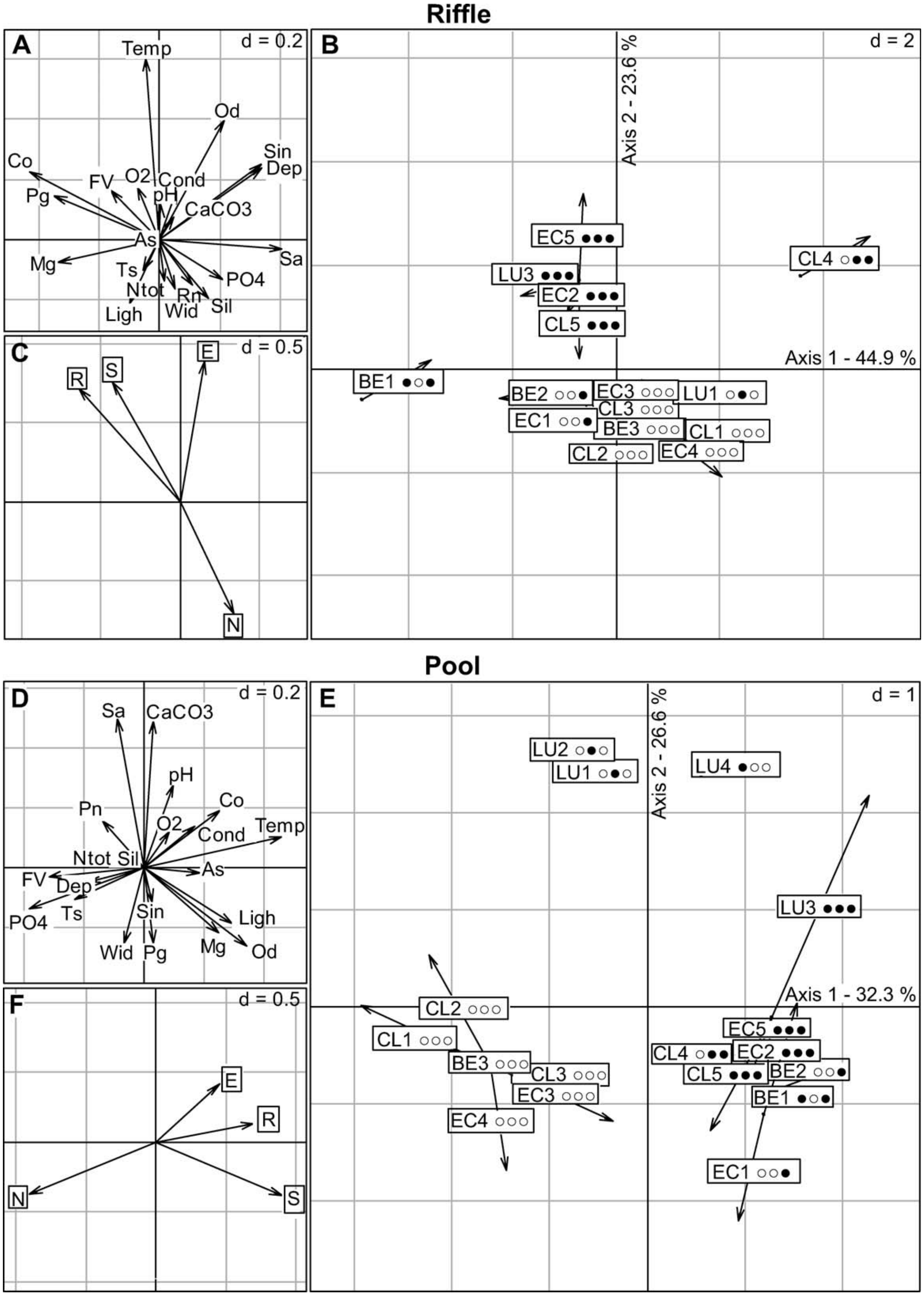

FIG. 3. Redundancy analysis (RDA) of the physical and chemical characteristics in riffles (A–C) and pools (D–F). For each habitat, panel layout, codes, and conventions are identical to those in Fig. 2.

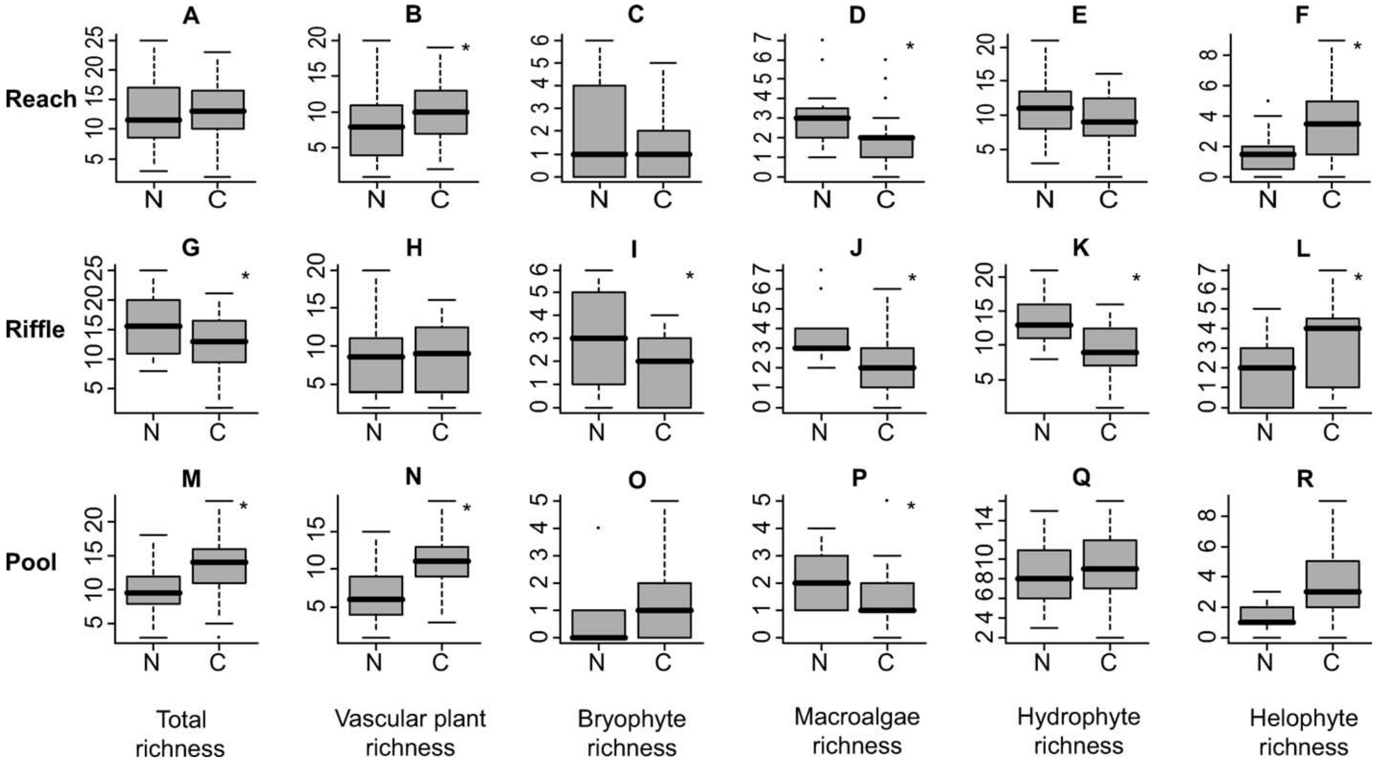

FIG. 4. Box plot of total (A, G, M), vascular plant (B, H, N), bryophyte (C, I, O), macroalgae (D, J, P), hydrophyte (E, K, Q), and helophyte (F, L, R) richness in control (N) and channelized reaches (C) in reaches (A–F), riffles (G–L), and pools (M–R). Lines in boxes are medians, ends of boxes show quartiles, and whiskers show ranges. Asterisks (*) indicate that floristic richness differed significantly (t-test, $p < 0.05$) between N and C reaches. $n = 18$ control reaches, control pools, and control riffles; $n = 33$ channelized reaches and channelized pools; and $n = 27$ channelized riffles (2 reaches had no riffles; Appendix 3; available online from http://dx.doi.org/10.1899/08-139.1.s3).

Fig. 4I), macroalgae ($t_{43} = 4.27$, $p < 0.001$; Fig. 4J), and hydrophytes ($t_{43} = 3.49$, $p = 0.001$; Fig. 4K) was significantly lower in channelized than in control reaches. In contrast, in pools, richness of vascular plants was significantly higher ($t_{49} = -3.33$, $p = 0.002$; Fig. 4N) and macroalgae richness was significantly lower ($t_{49} = 2.18$, $p = 0.003$; Fig. 4P) in channelized than in control reaches.

The RDA for plant taxa at the reach scale underlined the important relationships between channelization and the distribution of plant communities (Fig. 5A–C). The eigenvalue of the axis 1 (56.4%) and the ratio between the variance of predicted values and the variance of observed values (axis 1, $r^2 = 0.84$) showed that the differences in percentage plant cover (Fig. 5A) were correlated with differences in channelization structures along this axis. Control and channelized reaches were separated along axis 1 (Fig. 5B). *Nuphar lutea* (L.) Sibth & Sm, *Ranunculus fluitans* Lam, *Fontinalis antipyretica* Hedw, *Zannichellia palustris* L., *Vaucheria* sp. de Candolle, and *Potamogeton crispus* L. were abundant in channelized reaches (Fig. 5A, B). In contrast, *Helosciadium nodiflorum* (L.) Lag., *Berula erecta* (Huds.) Coville, *Hildenbrandia rivularis* (Liebm.) J.Agardh, *Cladophora* sp. Kützing, and *Nitella* sp. C. Agardh were abundant in control reaches (Fig. 5A, B). Floristic composition in reaches with man-made structures and combined structures was less different from floristic composition in control reaches than from floristic composition in resectioned and embanked reaches (Fig. 5C). As was observed for environmental variables, the effects of channelization structures were compensated by the effects of other channelization structures. On axis 2 (17.9% of the variance, $r^2 = 0.71$), reaches with man-made structures (negative end) were separated from reaches with combined structures (positive end). *Elodea canadensis* Michx, *Lemna minuscula* L., and *Phalaris arundinacea* L. were abundant in reaches with man-made structures, whereas taxa such as *Callitriche obtusangula* Le Gall and *Myosotis* gr. *palustris* L. were most abundant in reaches with combined structures (Fig. 5A, B).

Differences in plant composition also were observed at the scale of riffles and pools (Fig. 6A–F). The first 2 axes of the 2 RDAs explained >50% of the total

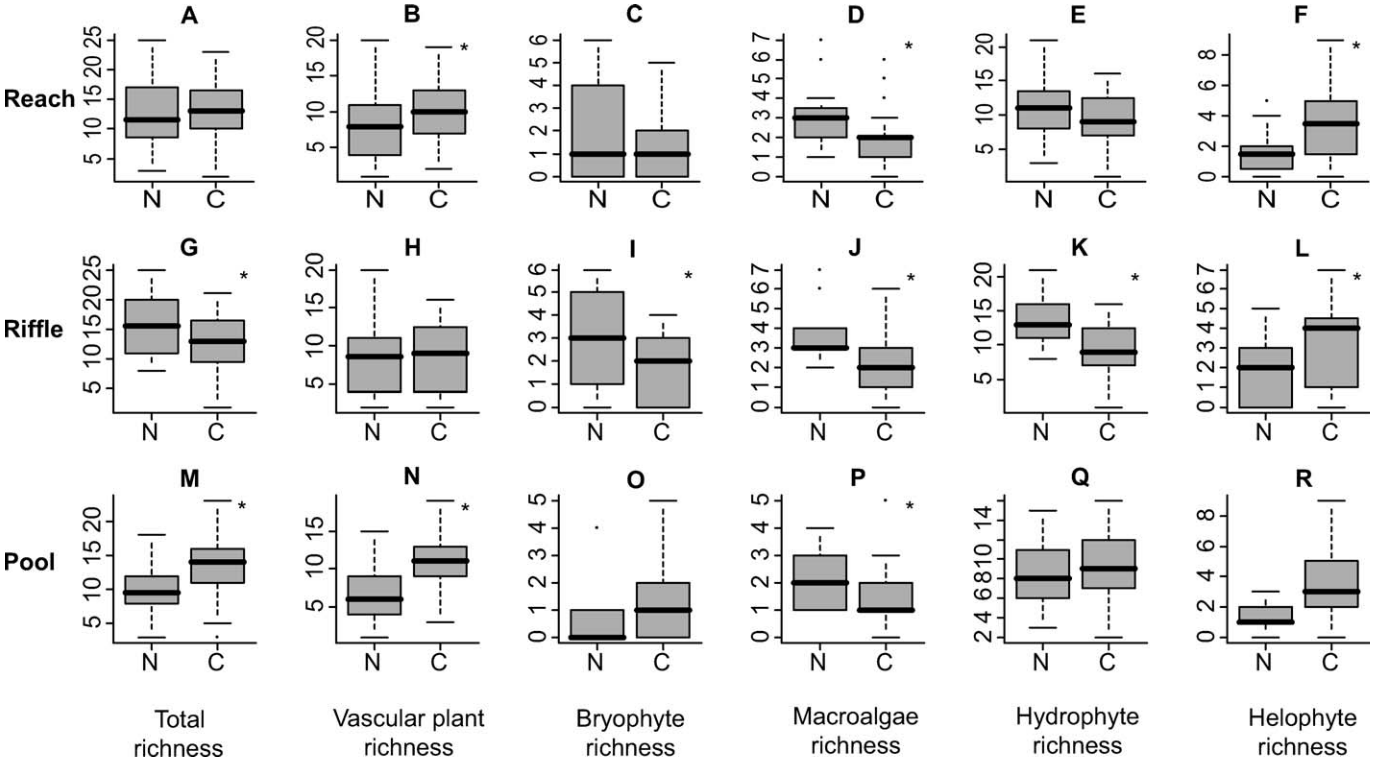

FIG. 5. Redundancy analysis (RDA) of the floristic assemblages in the study reaches (A–C). Panel layout, codes, and conventions are identical to those in Fig. 2. Only the most frequent and abundant taxa are presented in A. The centroid for the remaining taxa is indicated by a triangle (▲). Percentage cover data are presented in Appendix 4 (available online from: http//dx.doi.org/10.1899/08-139.1.s4).

inertia (for riffles: axis 1, 38.4%, $r^2 = 0.66$ and axis 2, 36.6%, $r^2 = 0.83$; for pools: axis 1, 35.5%, $r^2 = 0.86$ and axis 2, 28.3%, $r^2 = 0.71$). Plant communities in riffles in control reaches were dominated by bryophytes (e.g., *Amblystegium riparium* [Hedw.] B., S. & G., *Pellia endiviifolia* [Dicks] Dumort, *Rhynchostegium riparioides* [Hedw.] Card), and macroalgae (e.g., *H. rivularis*, *Nitella* sp.), whereas plant communities in riffles in channelized reaches were dominated by a few abundant helophytes (Fig. 6A, B). Helophytes in riffles with only man-made structures (e.g., *Poa trivialis* L., *Veronica anagallis-aquatica* L., *Solanum dulcamara* L., *Poa arundinacea*) differed from helophytes in the other channelized riffles (e.g., *Veronica anagalloides* Guss, *Teucrium scordium* L., *Phragmites australis* Cav. Trin. Ex Steud). Plant communities in embanked and resectioned pools were composed of abundant taxa such as *Ranunculus fluitans*, *F. antipyretica*, *N. lutea*, *Phormidium* sp. or *Vaucheria* sp., whereas plant communities in pools with several structures were composed of a mixture of hydrophytes (e.g., *C. obtusangula*, *Amblystegium fluviatile* [Hedw.] B., S. & G., *Nitella* sp., *Helosciadium nodiflorum*) and helophytes (e.g., *S. dulcamara* L., *Veronica beccabunga* L.) (Fig. 6D, E).

Physicochemical and floristic data sets were strongly correlated at both spatial scales (PROTEST, $p <$ 0.001). Differences in taxonomic composition among reaches and among riffles and pools were strongly correlated with differences in environmental characteristics. The explanatory (environmental characteristics) and response (floristic) variables showed the same trends with channelization.

Discussion

Channelization and environmental characteristics

Our results indicate 4 key environmental characteristics for assessing the impact of channelization on stream health: the number of pools and riffles in a reach, flow velocity, depth, and substrate type. Thus, the effect of channelization on stream health was strongly linked to physical characteristics, whereas chemical characteristics were poorly associated with channelization. Some chemical characteristics, such as pH or O_2 concentration, fluctuate across time and space or with the degree of human impact. Distinguishing among changes with natural and human-induced causes is often difficult because these

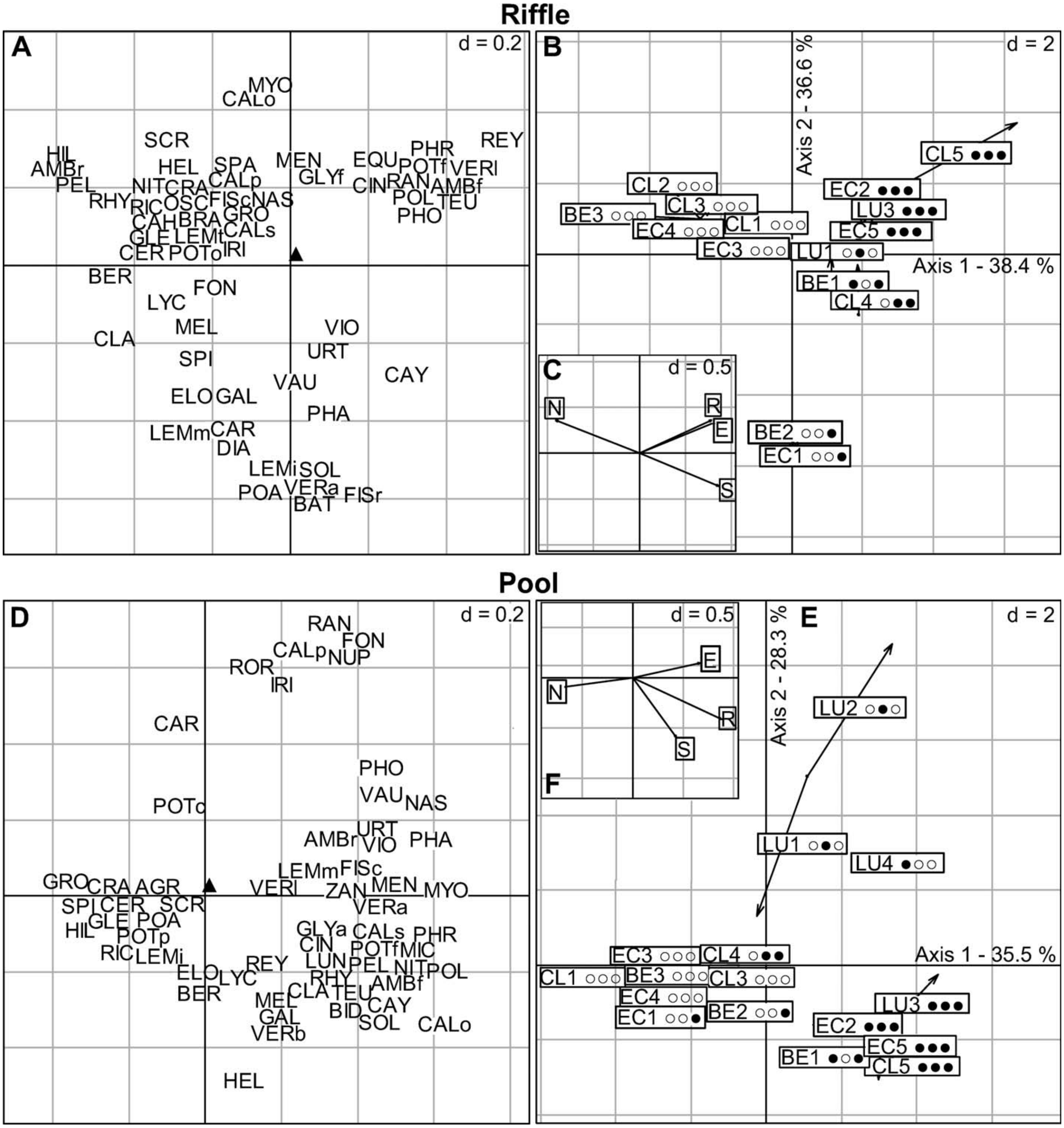

FIG. 6. Redundancy analysis (RDA) of the floristic assemblages in riffles (A–C) and pools (E–G). For each habitat, panel layout, codes, and conventions are identical to those in Fig. 2. Only the most frequent and abundant taxa are presented in A and D. The centroid for the remaining taxa is indicated by a triangle (▲).

variable chemical measures offer little integration over space and time (Norris and Thoms 1999). In contrast, physical characteristics are more stable over time.

Channelization modified environmental conditions in reaches. Physical characteristics related to pool/riffle sequences, such as flow velocity, depth, and substratum type, differed between pools and riffles, but varied with channelization structure. Percentage riffles and flow velocities in pools were lower in channelized than in control reaches, an effect that might lead to a loss of habitat complexity. Canopies were well open in channelized reaches, probably because banks of the reaches near human settlements were likely to have been cleared of riparian vegetation.

The nature and intensity of environmental changes were affected by the type of channelization structure. Resectioning and embanking altered stream morphology at a large spatial scale by changing flow velocity, width, and depth. Pools in reaches modified by these 2 structures had slow flow velocities and fine particle beds across long distances, as previously observed by Raven et al. (2000). The stabilization of stream banks and loss of sinuosity associated with these structures probably were the cause of profile modification. On the other hand, man-made structures, such as bridges, generally had localized effects caused by the addition of artificial materials during their construction. Riffles downstream of man-made structures had fast flow velocities and coarse particle beds, indicating that these structures could accelerate flows and modify fine particles, but upstream waters were stagnant because the structures obstructed flow. Last, reaches with combined structures were less affected by channelization than were reaches with embanking and resectioning alone, a result that indicates that effects of multiple structures might have been compensated at the level of the reach. In channelized reaches, complementary structures appeared to improve local conditions and create habitat diversity when one structure had a strong impact on stream morphology.

Channelization and floristic characteristics

Changes in floristic richness and shifts in plant composition caused by channelization varied according to the channelization structure, scale (reach vs pool/riffle sequence), flow type (pool or riffle), and taxonomic group (vascular plants, bryophytes and macroalgae, or hydrophytes and helophytes). The most significant differences between the control and channelized reaches were found at the pool/riffle sequence scale. Total plant richness was lower in channelized riffles than in control riffles because of a decrease in hydrophyte taxa, especially bryophytes and macroalgae. The observed trends in richness probably reflected the loss of riffles in channelized reaches. In contrast, total plant richness was higher in channelized pools than in control pools because of an increase in vascular plants, especially helophytes.

Channelization affected taxonomic richness of all plant groups, but responses varied with the structure of channelization, taxonomic group, and ecomorphological group. Thus, the taxonomic richness of these groups might be an indicator of the type of stream alteration. Channelization structures, especially embanking and resectioning, prevent flooding by decreasing the frequency and intensity of high flows and stabilize hydrological conditions through time. Moreover, they are associated with tree cutting and bank stabilization, practices that increase light availability. Stable environmental characteristics with low flow velocities and high available light can lead to low community richness and dominance of helophyte taxa. In less stable environmental conditions, high community richness is maintained by plant taxa with diverse colonization (Trémolières 2004) or regeneration (Combroux and Bornette 2004) strategies. Thus, alteration of flow velocities and light in channelized reaches probably affected plant richness by favoring taxa with similar strategies. In presence of man-made structures, flow velocity was locally increased and artificial substrata and cobbles dominated reaches. These changes had a strong negative effect on hydrophyte richness, probably because they restrict propagule flow, establishment of seed banks, or anchorage of plants. Helophyte taxa probably were favored in these reaches because they are more tolerant than strictly aquatic taxa (Dawson et al. 1999, Gantes and Sánchez Caro 2001). Decreased competition from hydrophytes might have facilitated colonization by helophytes, but these effects were evident only at the pool/riffle sequence scale and did not alter the whole reach. Regardless of the channelization structure, macroalgae were the most affected group of plants in both riffles and pools. Addition of physical impediments in the streambed altered the hydrological conditions in ways that might have favored taxa such as vascular plants or other algal taxa that were more competitive than encrusting algae (Sand-Jensen et al. 1987, Sand-Jensen 1998, Gantes and Sánchez Caro 2001).

Most plant taxa were found in all reaches, but their relative abundances differed among reaches and floristic communities differed between channelized and control reaches. Floristic composition in reaches with man-made structures and combined structures was more similar to that of control reaches than was floristic composition in reaches with embankings or resectionings. Taxa in reaches with man-made structures and combined structures tended to be tolerant of a wide range of environmental conditions and to have opportunistic strategies. For example, *E. canadensis* (considered an invasive species; Thiébaut 2007), *C.*

obtusangula, and *M*. gr. *palustris* usually are well widespread because of their efficient dispersal ability and high resistance to disturbance (Grime et al. 1988). Embanking and resectioning strongly affected plant community structure, especially in pools, and seemed to lead to communities with particularly resistant taxa (e.g., *N. lutea*, *P. crispus*, *Z. palustris*). Thus, *N. lutea*, *R. fluitans*, *Z. palustris*, and *P. crispus* might be indicators of channelized habitats, as previously observed for *R. fluitans* (Pedersen et al. 2006) and *Potanogeton crispus* (Baattrup-Pedersen et al. 2003, O'Hare et al. 2006).

Taxa indicative of unimpacted conditions differ strongly among studies, probably because of chemical differences among streams. In the continental region of France that we studied, *H. nodiflorum*, *B. erecta*, and *Hildenbrandia rivularis* appear to be good indicators of unimpacted situations. *Cladophora* sp. also was present in our control reaches, but it is used as an indicator of impacted conditions in water-quality studies. The presence of *Cladophora* sp. in our control reaches might have been related to the higher PO_4^{3-} concentration in control than in channelized reaches. Identification of indicator taxa might be difficult because of variability in patterns of distribution related to regional environmental conditions, the scale used, and the type of channelization. Floristic communities or stenoecic taxa can be more informative than single taxa in comparative stream assessments.

Implications

The aim of our study was to evaluate the impacts of different channelization structures on the environmental characteristics and floristic communities of streams. Our results suggest that physical characteristics, such as composition of riffles and pools, depth, flow velocity, and substratum, could be useful for assessing the physical health or the conservation status of the EU-3260 habitat as indicated under the European Habitat Directive methods (92/43 EEC, European Union 1992). The floristic richness in several taxonomic groups (particularly bryophytes and macroalgae) and morphologic groups (particularly helophytes) highlighted significant differences between control and channelized streams at the pool/riffle sequence scale. The natural sequence of pools and riffles and the natural diversity of environmental and floristic conditions are necessary to maintain physical stream health. Whenever possible, managers should avoid the implementation of channelization because embanking and resectioning could lead to linear watercourses over long stretches. The locations of man-made structures should be strongly regulated to protect stream habitats from irreversible degradation.

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Impact de l'ouverture du milieu et de l'aménagement des berges sur les communautés biologiques des rivières à Renoncules

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INTRODUCTION

Contexte : Améliorer les conditions écologiques pour la Truite Fario (*Salmo trutta fario*) sur une des seules rivières de première catégorie du Loiret.

Rivière d'étude : La Cléry *(Fig. 1)*, un affluent du Loing (Source à St-Loup-d'Ordon (alt. 151 m), Longueur = 43 km, Débit moy. : 1,45 m³/s). Le Paysage environnant est constitué de cultures, pâturages et milieux boisés.

Travaux de restauration sur une station bordant un pâturage : ouverture de la végétation de berges (coupe d'arbres) recul de la clôture du pâturage, construction d'un abreuvoir.

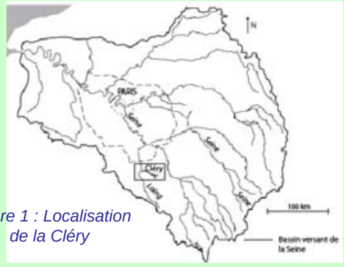


Figure 1 : Localisation de la Cléry

BUT DE L'ÉTUDE

1) **Évaluer l'impact de travaux de restauration sur les communautés biologiques** autres que les communautés piscicoles par la comparaison d'une station de référence et d'une station restaurée proches d'un point de vue géomorphologique et physico-chimique.

macroinvertébrés benthiques

végétaux aquatiques

2) D**iscerner les effets de la restauration et ceux de la saisonnalité**.

MÉTHODOLOGIE

Site d'étude : 2 stations de 100 m distantes d' 1 km : 1 station restaurée (Chanteraine, *Fig.3*), 1 station de référence (Boyard, *Fig.4*) avec des propriétés hydrogéomorphologiques et physico-chimiques comparables.

Protocoles d'échantillonnage : - Transects : vitesse du courant, profondeur, composition en substrats + points contact de végétation

- Liste et pourcentages de recouvrement des taxons végétaux selon le protocole IBMR (Haury *et al.*, 2006)
- Prélèvement et détermination des macroinvertébrés benthiques selon le protocole IBGN (A.F.N.O.R, 1992).

Saisons d'étude : Travaux de restauration : au printemps 2006 (Mai, *Fig. 5*)
Transects et macroinvertébrés : Printemps avant et après restauration, Eté, Automne 2006
Végétation : Printemps, Eté, Automne 2006 et 2007.

Figure 3 : Chanteraine

Figure 4 : Boyard

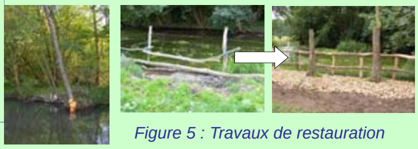


Figure 5 : Travaux de restauration

RÉSULTATS ET DISCUSSION

Figure 6 : Recouvrement total de la végétation sur la station

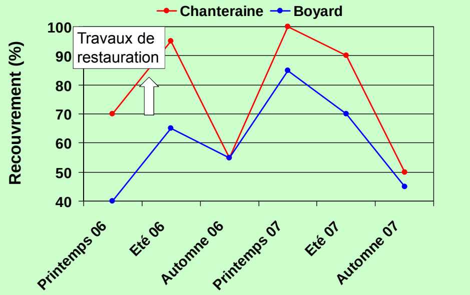


Figure 7 : Nombre total de taxons végétaux sur la station

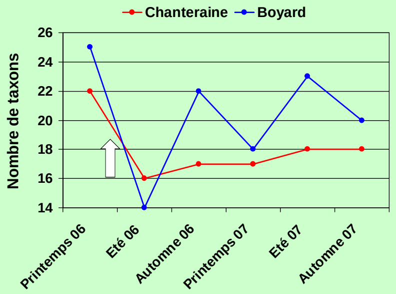


Figure 8 : Nombre de taxons par groupes biologiques sur la station Chanteraine aux différentes saisons de terrain

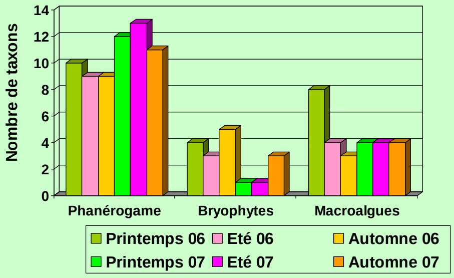


Figure 9 : Nombre total de familles de macroinvertébrés sur la station

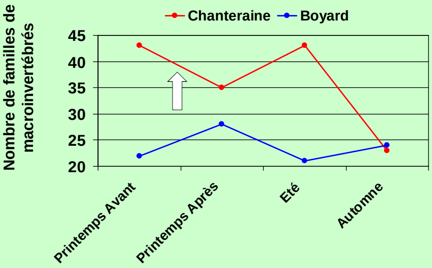


Figure 10 : Diversité en macroinvertébrés sur la station

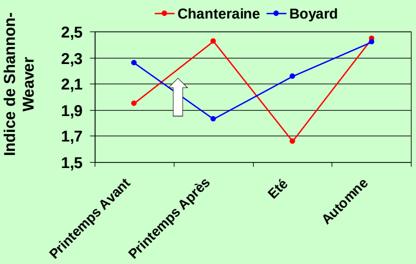
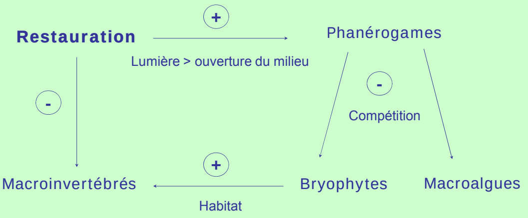


Tableau 1 et Figure 11 : Résumé des résultats et interprétation

	Tendance	Restauration	Saisonnalité
Recouvrement total de la végétation	Très variable		X
Nombre total de taxons	↓	X	
Nombre de Phanérogames	↑	X	
Nombre de Bryophytes	↓	X	
Nombre de Macroalgues	↓	X	
Nombre de macroinvertébrés	↓	X	
Diversité en macroinvertébrés	Très variable		X



Restauration

Phanérogames

Lumière > ouverture du milieu

Compétition

Macroinvertébrés

Bryophytes

Macroalgues

Habitat

Taxons dominants dans la station restaurée :
Helosciadium nodiflorum / Callitriche obstusangula pour la végétation, familles des Chironomidae / Elmidae / Gammaridae pour les macroinvertébrés.

CONCLUSION

Travaux de restauration avec, à court terme un impact négatif se traduisant par une **diminution du nombre de taxons et une simplification des communautés biologiques**.

Forte influence de la saisonnalité → nécessité de réaliser un suivi à plus long terme. Possibilité de récupération pour les communautés biologiques de la station avec le temps.

Plan de gestion à envisager sur l'entretien de la végétation de berges en tenant compte de l'ensemble des communautés biologiques. Coupes d'arbres modérées pour éviter un excès de lumière.

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RESEARCH ARTICLE

Dynamics of Nutrient Contents (Phosphorus, Nitrogen) in Water, Sediment and Plants After Restoration of Connectivity in Side-Channels of the River Rhine

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Abstract

During the last century, canalization of the Rhine river led to disconnection of side-arms, over-sedimentation of these channels, loss of the fluvial dynamics, and aquatic vegetation change or disappearance. Recent restoration projects aim to reconnect disconnected arms to the main channel. The objective of this study was to assess the nutrient dynamics in restored channels during the vegetation colonization process. In spring, summer, and autumn 2009, the phosphorus and nitrogen contents were measured in water, sediment, and plants, sampled in six channels, two reference sites and four restored ones at different dates. Aquatic vegetation was monitored during the same period. Sites were mesotrophic related to the water nutrient concentrations. However, vegetation communities indicated a eutrophic level, as they were dominated by species like *Elodea nuttallii*, *Myriophyllum spicatum*, and *Potamogeton perfoliatus*. Sites were discriminated by P content and mineral nitrogen in the sediment. We showed an effect of species and season on the plant nutrient content, but there was no relationship between plant nutrient content and nutrients in water and sediment. A negative correlation between mean N plant content and the cover of each species was found. Vegetation characteristics (species richness and cover) and bioavailable phosphorus in the sediment were also correlated. In the restored side-arms of the river Rhine, phosphorus-rich sediment seems to be important in the recolonization dynamics, as it was linked to higher species richness, whereas nitrogen played a role in the colonization patterns as a growth limiting factor.

Key words: aquatic, macrophyte, reconnection, river plant, uptake, vegetation.

Introduction

Since the beginning of the last century, large rivers have been intensively channelized worldwide. River beds were strongly modified by human activities such as bed straightening, embankment, and canalization in order to improve river navigability, avoid floods, or produce hydroelectricity (Brookes 1988). These hydraulic works led to the disconnection of side-channels and subsequent floodplain changes, that is, over-sedimentation or terrestrialization of these channels (Bravard et al. 1986; Bornette & Heiler 1994), loss of fluvial dynamics (Hohensinner et al. 2004; Schmitt et al. 2007), and change in trophic status and in macrophyte communities (Trémolières et al. 1993; Bornette & Heiler 1994). Recently, many restoration projects aimed to reflood alluvial zones and reconnect former side-channels to the main channel (program LIFE Nature Rhin Vivant 2002–2006; Schwab et al. 2008). The success of restoration works was usually assessed through the monitoring of ecological changes. Several taxonomic and syntaxonomic groups were monitored to assess ecological changes in restored channels, such as macroinvertebrates (Marchetti et al. 2010), fish (Trexler 1995; Miller et al. 2010), birds (Weller 1995), or plant communities (Small et al. 1996; Combroux et al. 2002).

Plant communities are one of the three favored syntaxonomic groups, when assessing the success of restoration projects (Bernhardt et al. 2007). Aquatic macrophyte communities were shown to be sensitive to many abiotic factors, such as temperature, light availability, flow velocity, substrate types, nutrient availability, and pH, which could be modified by hydraulic works or human activities (Haslam 1978). The importance of these factors depends on the size of the studied area (Lacoul & Freedman 2006). Therefore, within a restored stream, macrophyte communities could be impacted by a subset of these factors. For example, in lowland restored streams in Denmark, Pedersen et al. (2006) showed that bank morphology, bed level, and management intensity exerted a strong influence on macrophyte community diversity and composition. They also showed that macrophyte colonization could

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be controlled by sedimentation. In another context, Henry et al. (1996) showed that dredging sediment in a cut-off channel within the Rhône floodplain increased groundwater supply, which led to a change in the water trophic status, from eutrophic to mesotrophic. Colonization of this channel was then characterized by mesotrophic plant species (Henry et al. 2002). Disconnections usually led to oligotrophication (Bornette & Heiler 1994; Henry et al. 1996) and subsequent reconnection allowed eutrophic water from nearby rivers to feed the restored channels. Nevertheless, links between trophic status within restored streams and macrophyte recolonization processes remain largely unknown. The trophic status of a waterbody is firstly related to phosphorus and in some cases to nitrogen (Clarke & Wharton 2001; Fabris et al. 2009). It strongly influences the composition and distribution of aquatic macrophyte communities (e.g. Robach et al. 1996; Demars & Edwards 2007). Moreover, aquatic macrophytes assimilate more or less nutrients from the sediment and/or from the water column (Chambers et al. 1989; Robach et al. 1995). The source of uptake depends on both species and environmental conditions encountered by those species (Baldy et al. 2007).

In this study, we tested the following hypothesis: nutrient concentrations (phosphorus and nitrogen) in water and/or sediment, and the time elapsed since the restoration works influence the recolonization process. We argued that

1. The expected increase in nutrient concentration in water and/or sediment would lead to the dominance of eutrophic plant species.
2. The nutrient uptake capacity of one species could have an effect on its capacity to colonize newly created sites, to settle and overcome other species.
3. There is a threshold in nutrient concentration in water and/or sediment below which no plant optimal growth can be reached. Phosphorus would be the limiting factor, due to its low bioavailability.
4. Channels reconnected to the same river experience similar temporal changes in vegetation cover and composition.

Methods

Study Sites

The study was conducted in five side-channels in the upper Rhine river floodplain (Fig. 1): Schollengiessen (SCHOL), Schaftheu (SCHA), Eiswasser (EIS), Rossmoerder (ROSS), and Fahrgiessen (FAHR). The Schollengiessen and Schaftheu side-channels are located in seminatural islands created by the Rhine canalization during the seventies. These two channels were considered as reference sites, as they remained connected to the Rhine river after canalization. The other channels, which were disconnected by canalization, were reconnected in the last decade: Eiswasser in 2002, Rossmoerder in 2004, and Fahrgiessen in 2006 (Fig. 1).

Six sites were sampled: two sites on the EIS, one close to the connection waterworks (Eiswasser Upstream; EISUP) and the second ca. 1 km downstream (Eiswasser Downstream; EISDW), and only one site close to the connection on the other channels. The length of the reconnected stretches was

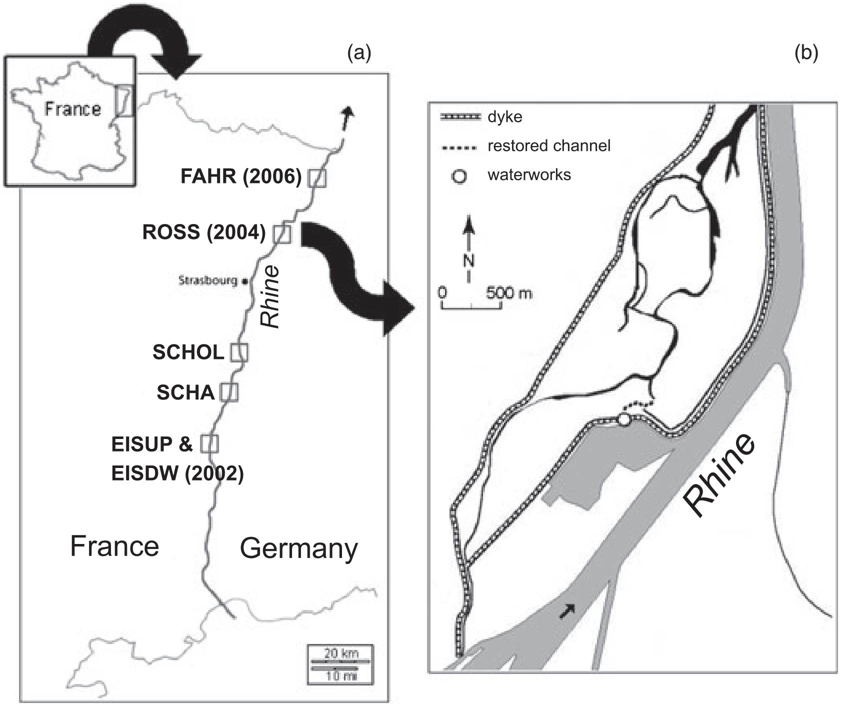

Figure 1. (a) Location of the studied side-channels. The numbers within brackets are the years when the restoration works were carried out. (b) Location of the waterworks and the restored part of the Rossmoerder river.

relatively short, between 300 and 500 m, and their width varied between 5 and 29 m.

In each site, we selected three 5-m wide and evenly distributed cross-sections. The cross sections were considered as one plot. The first cross section was placed near the connection waterworks, except for EISDW.

Sampling Methods

The sites were monitored in 2009 during three campaigns: in spring, summer, and autumn. For each campaign and each site, we estimated the percentage cover of macrophyte species in each cross section. We took two 50 mL water samples and two sediment samples (~120 cm^3; upper layer) per cross section. Three samples (shoots ~100 g fresh weight) of the most common plant species were collected per site close to cross sections.

Chemical Analyses

Water. Water samples were filtered with a 0.45 µm "mesh." Concentrations of N-NH_4^+ (µg/L) and P-PO_4^{3-} (µg/L) were determined by spectrophotometry with a micro flux auto-analyzer. Concentrations of chloride (Cl^-; mg/L), nitrate (N-NO_3^-; mg/L) and sulfate (SO_4^{2-}; mg/L) were determined by ionic chromatography.

Sediment. Exchangeable phosphorus (P_2o_5 Exc-P; g/kg) was measured according to the Joret and Hébert (1955) method. Total phosphorus (TP; g/kg) was analyzed after drying for 24 hours at 70°C and digesting with a mixture of perchloric and nitric acids. TP was determined in the digests as phosphate after formation of a molybdate blue complex (APHA 1985). Total nitrogen (TN; g/kg) was analyzed with the NF ISO 13878 method (1998). Ammonium and nitrate (mineral nitrogen Min-N; mg/kg) were analyzed after extraction with KCl 1 mol/L: ammonium was analyzed by the indophenol blue method and nitrate by ionic chromatography (APHA 1985).

Plant. After drying at laboratory temperature, the samples were ground in a laboratory mill. Then 0.1 g of each sample were digested with 10 mL sulfuric acid and 75 mL ultrapure water at 160°C for 120 minutes, then at 350°C for 200 minutes. Phosphorus (P content; % dried weight [DW]) and nitrogen (N content; % DW) contents were thus measured in the digested solutions as concentrations of P-PO_4^{3-} and N-NH_4^+, using the same procedure as previously described for water and sediment.

Statistical Analyses

All statistical analyses were carried out with the R program (v2.10.0; R Development Core Team 2010). A principal component analysis (PCA) was used to identify the main factors accounting for the differences between the seasons and between the sites. The existence of relationships between all the studied variables was tested by using generalized linear models and correlation analyses. As many data did not follow a Gaussian distribution, the correlations were calculated as Kendall's tau.

Results

Vegetation

Thirty-eight species were identified in all sites. Species richness was lowest at ROSS, whereas FAHR, the most recently restored site, exhibited the highest species richness, with 21 species (Table 1). The four other sites showed an intermediate species richness with ca. 15 species. Six species were found in

Table 1. Vegetation and physico-chemical characteristics of the studied sites.

	Sites	SCHA	SCHOL	EISUP	EISDW	ROSS	FAHR
	Type	Reference	Reference	Restored	Restored	Restored	Restored
	Year of Reconnection	—	—	2002	2002	2004	2006
	Mean Width (m)	22.7	28.2	7.2	11.0	6.3	22.6
	Mean Depth (m)	0.9	2	0.4	0.6	0.8	1.7
Vegetation[a]	S	15 (Au.)	16 (Su.)	14 (Su.)	16 (Su.)	7 (Au.)	21 (Au.)
	Cover (%)	37 (Au.)	30 (Au.)	15 (Au.)	9 (Su.)	1 (Au.)	32 (Au.)
Plants[b]	P content (% DW)	0.34	0.33	0.34	0.44	0.34	0.46
	N content (% DW)	2.58	2.25	2.3	2.44	2.57	2.70
	N	33	36	26	39	15	48
Water[b]	P-PO_4^{3-} (µg/L)	21	21	11	11	23	20
	N-NH_4^+ (µg/L)	29	23	11	13	12	49
	N-NO_3^-(mg/L)	1.39	1.37	1.31	1.33	1.20	1.01
	Chlorides (mg/L)	21.5	20.8	18.5	19.3	23.3	19.7
	Sulfates (mg/L)	33.4	32.6	32.5	33.7	31.0	32.2
Sediment[b]	TP (g/kg)	0.38	0.51	0.66	0.52	0.34	0.72
	Exc-P (g/kg)	0.06	0.08	0.04	0.07	0.04	0.15
	TN (g/kg)	2.64	4.30	5.04	8.58	2.60	9.39
	Min-N (mg/kg)	11.3	26.7	8.7	20.0	13.2	50.9

[a] Maximum data observed in 2009. Seasons of observation are in brackets: autumn (Au.) and summer (Su.); S, species richness.

[b] Average values (for water and sediment: average of every campaign, $N = 18$).

every site (Table 2): the algae, *Cladophora* sp. (Ulvophyceae), the exotic species, *Elodea nuttallii* (western waterweed), two species generally found in eutrophic waters, *Myriophyllum spicatum* (European water milfoil) and *Potamogeton perfoliatus* (redhead grass), the helophyte, *Phalaris arundinacea* (reed canary grass), and the free-floating species, *Lemna minor* (common duckweed). The two species *P. pectinatus* (fennel pondweed) and *Hildenbrandia rivularis* (Hildenbrandiaceae) were found on all the sites except for ROSS (*P. pectinatus*) and FAHR (*H. rivularis*). In the reference site SCHA, the macrophyte community was dominated by three eutrophic species: *P. pectinatus*, *P. perfoliatus*, and *E. nuttallii*, whereas the SCHOL site was dominated by only one species, *E. nuttallii*. Macrophyte communities in ROSS and FAHR were also dominated by *E. nuttallii*, but the macrophyte community in FAHR included six species only found in this site, *P. nodosus* (longleaf pondweed), *P. berchtoldii* (small pondweed), *P. crispus* (curly-leaf pondweed), *P. gramineus* (variable leaf pondweed), *Nuphar lutea* (yellow water-lily) and *E. canadensis* (Canadian waterweed). The macrophyte community in EISUP was dominated by *Fontinalis antipyretica* (willow moss) and *Ranunculus fluitans* (river water crowfoot), whereas the macrophyte community in EISDW was dominated by *Myriophyllum spicatum*.

Water and Sediment Physico-Chemistry

Water N-NO_3^- (~1.3 mg/L), chloride (~20 mg/L), and sulfate (~32 mg/L) were similar in all sites (Table 1). ROSS, FAHR, and the reference sites exhibited similar water P-PO_4^{3-}, close to 22 µg/L, whereas EISUP and EISDW exhibited a lower water P-PO_4^{3-} concentration (11 µg/L). Water N-NH_4^+ highly varied between the sites. The more recently restored channel, FAHR, had a maximum water N-NH_4^+ of ~50 µg/L. The other restored sites (EISUP, EISDW, and ROSS) showed a water N-NH_4^+ of ~12 µg/L, whereas the reference sites exhibited intermediate water N-NH_4^+ (SCHA: 29 µg/L; SCHOL: 23 µg/L).

Sediment TN and Min-N highly varied between the sites. In the sediment of FAHR TP, Exc-P, Min-N, and TN content showed highest values (Table 1), whereas the smallest nutrient contents were measured in ROSS and the reference site SCHA. The two sites EISUP and EISDW showed different nutrient contents in the sediment, despite their similar water chemistry.

A normalized PCA on chemical variables discriminated seasons and sites (Fig. 2). All variables were either significantly linked to the first or the second axis. The first axis accounted for 30.8% of the inertia and was defined by the water chemistry (P-PO_4^{3-}, N-NH_4^+, N-NO_3^-, chloride, and sulfate) and sediment TN, which discriminated the seasons. The second axis accounted for 21.7% of the inertia and was defined by sediment Exc-P, TP and Min-N, and water N-NO_3^-, which discriminated the sites. However, there was no clear distribution according to the date of reconnection. The FAHR site was not included in this analysis, as prior analyses had shown that it differed highly from the other sites by its sediment nutrient content.

Seasonal Variations of Nutrient Concentrations in Water and Sediment

We observed seasonal variations of nutrient concentrations in the water (Fig. 3) and in the sediment (Fig. 4). EISUP, EISDW, and the reference sites SCHA and SCHOL exhibited a stable water N-NO_3^- over the year, whereas there was a minimum in autumn in ROSS and FAHR. Water P-PO_4^{3-} was higher in spring and lower in autumn in all sites except for ROSS. EISUP, EISDW, and the reference site SCHA exhibited similar seasonal variations of water N-NH_4^+, with a maximum observed in summer.

Seasonal changes in sediment Exc-P, TP, TN, and Min-N were generally similar in all sites (Fig. 4). Sediment Exc-P and TP remained stable over the year, whereas TN and Min-N varied over the seasons, with a maximum during spring for TN, and with a minimum during spring and a maximum during autumn for Min-N.

Plant Nutrient Content

Phosphorus content was highest in plants of FAHR and EISDW (~0.45% DW; Table 1), whereas P content was lower in plants of the other sites without differences between sites (~0.34% DW). The plants in FAHR also exhibited the highest N content (2.7% DW), whereas the plants in EISUP and the reference site SCHOL showed the lowest values (~2.3% DW).

Seasonal variations of N and P contents remained low (Fig. 5). The lowest N contents were observed during summer in EISUP, EISDW, and the reference site SCHOL. The lowest P contents were also observed during summer, but in all sites. N and P contents were significantly correlated (Tables 3 & 4). However, N and P contents showed no significant relationship with nitrogen and phosphorus concentrations of water and sediment (Table 3). The generalized linear model showed that plant nutrient contents varied significantly between the species, but not between the sites. N content also varied significantly between the seasons.

Plant species differed in nutrient uptake. *Azolla filiculoïdes* (water fern) and *Nasturtium officinale* (watercress) were the most nitrogen-accumulating species (Table 2), with N contents of around 4% DW. *Veronica anagallis-aquatica* (water speedwell), *E. nuttallii* and *N. officinale* were the most phosphorus-accumulating species, with P contents of 0.55, 0.45, and 0.45% DW, respectively. *Chara vulgaris* (common stonewort) exhibited the lowest phosphorus and nitrogen accumulation, with a P content of 0.16% DW and a N content of 1.18% DW.

Except for *A. filiculoïdes* and *P. gramineus*, all species showed a mean N:P ratio below 10 (Table 2).

Relationships Between Nutrients, Cover, and Richness

Significantly positive correlations were observed in sediment samples of all cross sections between TP and Exc-P, TP and TN, and Exc-P and TN (Table 4). Sediment Exc-P was also positively linked to the vegetation cover and the species richness, whereas water P-PO_4^{3-} was negatively linked to

Table 2. Cover, frequency, and nutrient contents of the plant species.

Species	Frequency	Mean Cover (%)	Cover (%) by site						Nutrient contents (% DW) and N:P ratio			N
			SCHA	SCHOL	EISUP	EISDW	ROSS	FAHR	P Content	N Content	N:P Ratio	
Elodea nuttallii (Planch.) St John	6	6.2	4.8	19.3	≤0.1	0.7	0.7	12.0	0.45 (±0.17)	2.46 (±0.64)	6.1 (±2.5)	38
Potamogeton perfoliatus L.	6	1.6	7.1	2.0	≤0.1	≤0.1	≤0.1	0.2	0.33 (±0.11)	2.59 (±0.82)	8.3 (±3.6)	19
Myriophyllum spicatum L.	6	1.1	0.4	1.7	0.2	3.7	≤0.1	0.7	0.40 (±0.14)	2.40 (±0.60)	6.5 (±2.2)	33
Cladophora sp.	6	0.7	0.5	1.4	0.5	1.3	≤0.1	0.8	0.35 (±0.08)	2.90 (±0.69)	8.8 (±2.8)	7
Phalaris arundinacea L.	6	0.5	0.5	≤0.1	0.4	1.7	≤0.1	≤0.1	0.30 (±0.08)	1.60 (±0.61)	5.4 (±1.8)	4
Lemna minor L.	6	0.2	≤0.1	≤0.1	0.4	0.5	≤0.1	≤0.1	—	—	—	—
Potamogeton pectinatus L.	5	2.1	7.5	0.7	0.7	0.8	—	2.7	0.32 (±0.12)	2.28 (±0.57)	7.8 (±2.9)	23
Hildenbrandia rivularis (Lieb.) J. Agardh	5	0.4	1.0	≤0.1	1.0	≤0.1	≤0.1	—	—	—	—	—
Ceratophyllum demersum L.	4	0.8	—	2.7	—	0.7	0.2	1.0	0.32 (±0.09)	2.31 (±0.77)	7.7 (±2.7)	18
Ranunculus fluitans Lam.	4	0.6	0.2	≤0.1	2.8	—	—	0.7	0.41 (±0.18)	2.32 (±0.62)	6.5 (±2.3)	13
Spirodela polyrhiza (L.) Schleid.	4	≤0.1	≤0.1	0.1	—	≤0.1	—	≤0.1	—	—	—	—
Fontinalis antipyretica Hedw.	3	0.6	—	≤0.1	3.4	—	—	0.2	0.32 (±0.04)	2.10 (±0.38)	6.7 (±1.0)	7
Sparganium emersum Rehm.	3	0.2	≤0.1	—	≤0.1	—	—	1.3	0.36 (±0.03)	2.89 (±1.14)	8.0 (±2.5)	3
Callitriche obtusangula Le Gall	3	0.2	—	≤0.1	0.7	—	—	0.2	0.38 (±0.08)	2.60 (±0.63)	7.2 (±2.7)	4
Hydrodyction reticulatum (L.) Lager.	3	≤0.1	≤0.1	0.3	—	0.3	—	—	—	—	—	—
Vaucheria sp.	3	≤0.1	≤0.1	—	≤0.1	≤0.1	—	—	—	—	—	—
Veronica anagallis-aquatica L.	2	≤0.1	≤0.1	—	—	0.7	—	—	0.55 (±0.19)	2.91 (±0.74)	5.7 (±2.0)	11
Nasturtium officinale R. Brown	2	≤0.1	≤0.1	—	—	0.3	—	—	0.45 (±0.22)	3.99 (±1.27)	9.4 (±1.7)	2
Iris pseudacorus L.	2	≤0.1	≤0.1	—	0.2	—	—	—	—	—	—	—
Berula erecta (Huds.) Coville	2	≤0.1	≤0.1	0.2	—	—	—	—	—	—	—	—
Lemna minuta Humb.. Bonpl. And Kunth	2	≤0.1	—	≤0.1	—	—	—	≤0.1	—	—	—	—
Azolla filiculoïdes Lam.	2	≤0.1	—	≤0.1	—	—	—	≤0.1	0.29	4.09	14.0	1
Myosotis palustris Hill	2	≤0.1	≤0.1	≤0.1	—	—	—	—	—	—	—	—
Rorippa amphibia (L.) Besser	2	≤0.1	≤0.1	—	—	—	—	≤0.1	0.40 (±0.03)	3.09 (±0.56)	7.8 (±1.7)	3
Potamogeton berchtoldii Fieb.	1	0.6	—	—	—	—	—	3.4	0.41 (±0.13)	2.72 (±0.53)	7.0 (±1.6)	6
Chara vulgaris (L.) Wall.	1	0.5	—	3.0	—	—	—	—	0.16 (±0.05)	1.18 (±0.49)	7.5 (±0.9)	2
Potamogeton nodosus Poiret	1	0.5	—	—	—	—	—	3.0	0.42 (±0.15)	3.30 (±1.24)	7.9 (±0.4)	3
Potamogeton gramineus L.	1	≤0.1	—	—	—	—	—	0.3	0.21	3.69	17.8	1
Ranunculus trichophyllus Chaix	1	≤0.1	—	—	—	0.2	—	—	0.28	2.55	9.0	1
Phragmites australis (Cav.) Steud.	1	≤0.1	0.2	—	—	—	—	—	—	—	—	—
Enteromorpha intestinalis (L.) Nees	1	≤0.1	—	≤0.1	—	—	—	—	—	—	—	—
Lemna trisulca L.	1	≤0.1	—	≤0.1	—	—	—	—	—	—	—	—
Potamogeton crispus L.	1	≤0.1	—	—	—	—	—	≤0.1	—	—	—	—
Nuphar lutea (L.) Smith	1	≤0.1	—	—	—	—	—	≤0.1	—	—	—	—
Zannichellia palustris L.	1	≤0.1	—	—	≤0.1	—	—	—	—	—	—	—
Elodea canadensis Michaux	1	≤0.1	—	—	—	—	—	≤0.1	—	—	—	—
Spirogyra sp.	1	≤0.1	—	—	—	≤0.1	—	—	—	—	—	—
Veronica beccabunga L.	1	≤0.1	—	—	—	≤0.1	—	—	—	—	—	—

Cover by site is mean percentage cover calculated for all campaigns (Spring, Summer, and Autumn). Species were classified using their frequency (number of sites where the species occurred), and their mean cover on all sites. The values for the nutrient content and the N:P ratio of each species are means of all samples, ± standard error of the mean (SEM). *N*, number of plant samples.

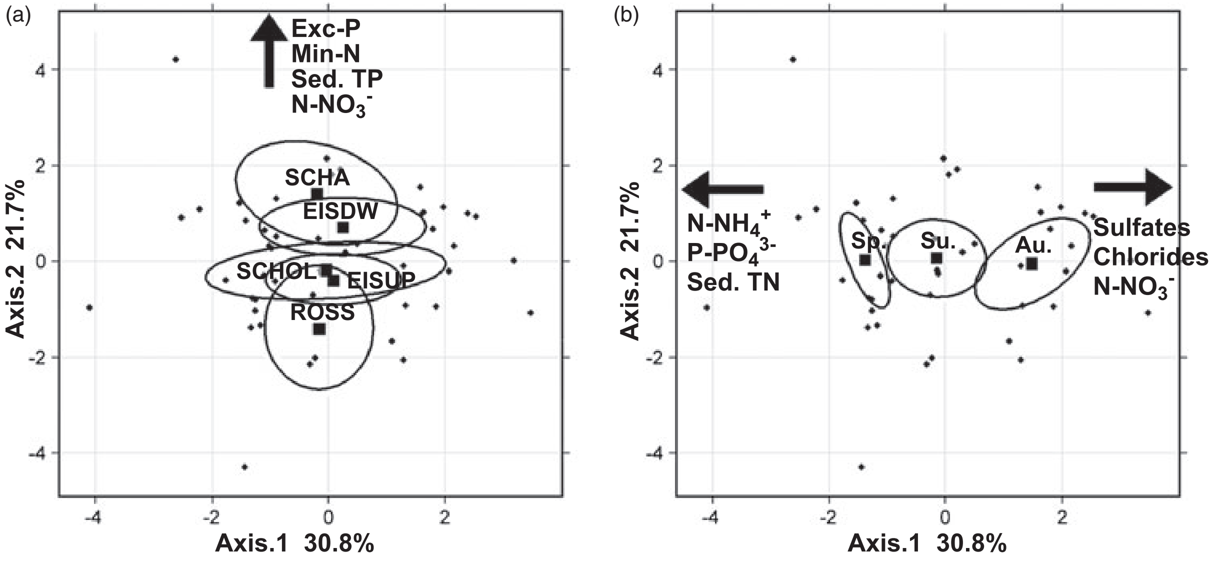

Figure 2. Factorial maps from a normalized principal component analysis (F1 × F2) of the water and sediment chemistry, excluding data from the site FAHR. (a) Factorial map showing confidence ellipses for studied sites. (b) The same factorial map showing confidence ellipses for seasons. Sp., Spring; Su., Summer; Au., Autumn.

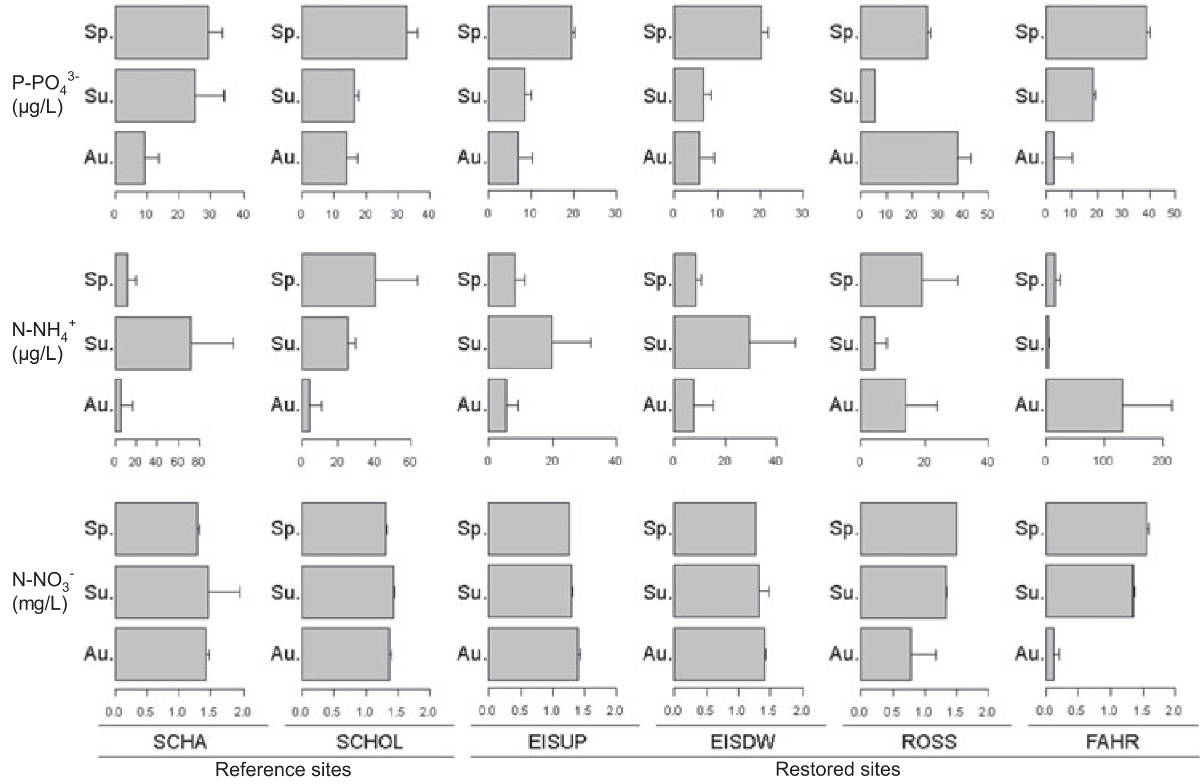

Figure 3. Seasonal variation of the nutrient concentrations in the water in 2009. Mean ± SEM, $n = 6$. Sp., Spring; Su., Summer; Au., Autumn.

Exc-P and the species richness. Sediment TN was positively linked to P and N contents in the plant. Water N-NO_3^- was negatively linked to sediment Min-N.

The mean N content of each species correlated significantly and negatively with their mean cover (Kendall's tau = 0.39, $p < 0.05$). Plant P content was not linked to cover and frequency.

The vegetation cover and the species richness of each section were significantly correlated (Kendall's tau = 0.54, $p < 0.001$).

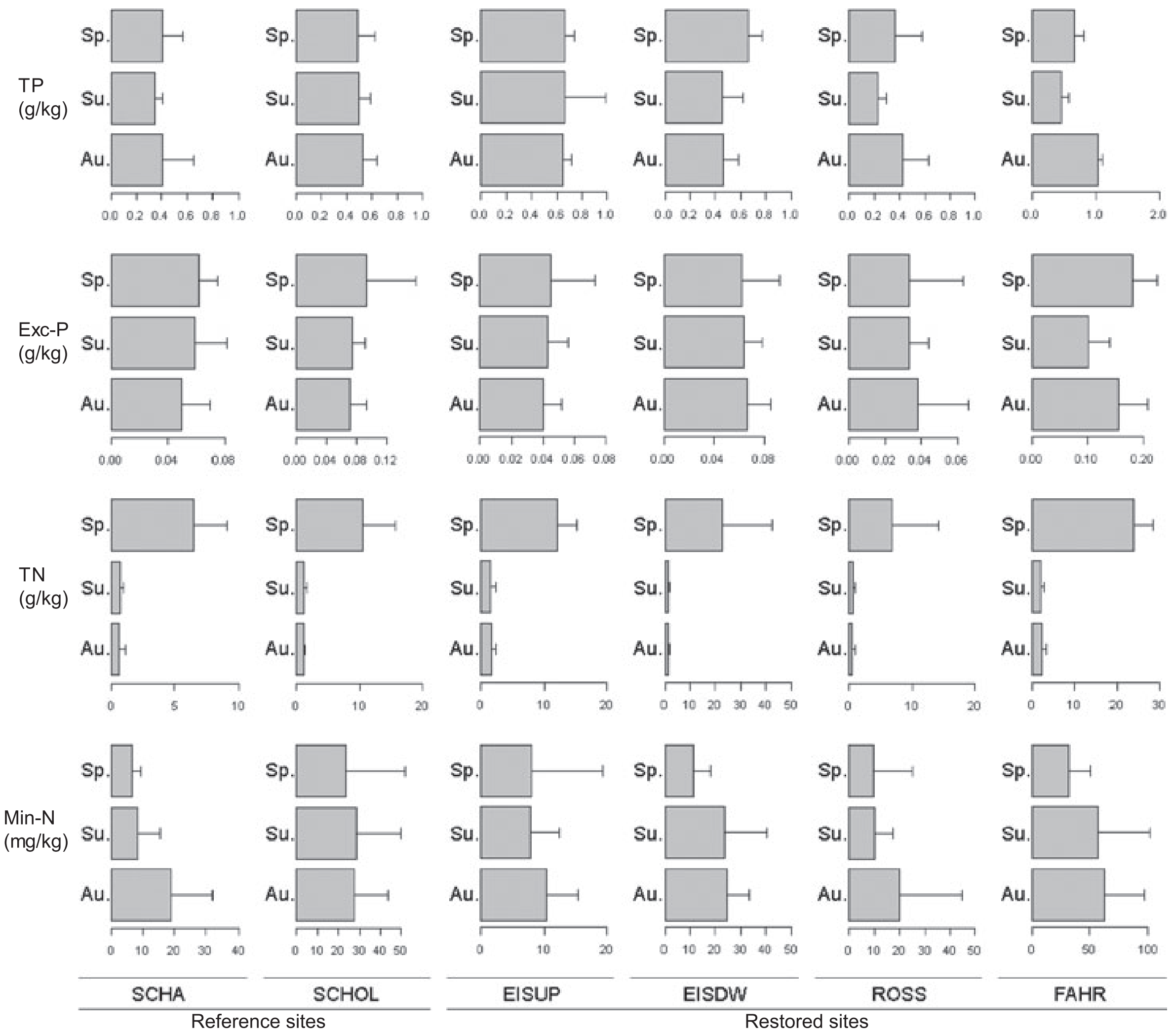

Figure 4. Seasonal variations of the nutrient contents in the sediment over 2009. Mean ± SEM, $n = 6$. Sp., Spring; Su., Summer; Au., Autumn.

Discussion

Although all studied sites were connected to the eutrophic river Rhine (in 2009: ~100 μg/L N-NH_4^+ and 20 μg/L P-PO_4^{3-}; Trémolières et al. 1993; Trémolières & Szwab 2007), they exhibited a relatively low level of nitrogen and phosphorus concentrations in the water, indicating mesotrophic conditions (Robach et al. 1996). However, mainly species typical for eutrophic waters, such as *Elodea nuttallii*, *Myriophyllum spicatum*, and *Potamogeton perfoliatus*, were found in all the sites. Trémolières and Szwab (2007) revealed a discrepancy between the water's trophic level and the macrophyte communities in disconnected side-channels of Rhine floodplain. They concluded that vegetation change may take a long time. In our study, a future vegetation change is unlikely, due to the strong relationship between vegetation structure and sediment richness and to the absence of such vegetation change in the reference sites, despite their mesotrophic waters. Moreover, we observed that only sediment nutrient content discriminated sites, whereas water chemistry did not. These results suggest that the trophic level of the sites is mainly expressed by nutrient stored in sediment and not by water nutrient, and thus it could explain the lack of accordance between the water's trophic status and the vegetation.

At the scale of cross sections, sediment Exc-P was correlated to sediment TP and TN, despite the high seasonal variation of TN. Sediment Exc-P was also positively linked to plant cover and species richness. We thus could conclude that sediments rich in available phosphorus led to an increase in species richness and vegetation cover, despite the low water trophic level. The observed correlation between sediment TN and plant nutrient contents also tended to show that a nutrient-rich sediment supported more species with higher nitrogen content.

The relationship between nutrients in the sediment and vegetation was probably also linked to the substrate texture, and therefore to the morphology of the restored side-channels, which favored the deposition of fine-grained sediment. Other studies (Demars & Harper 2005; Pedersen et al. 2006) pointed

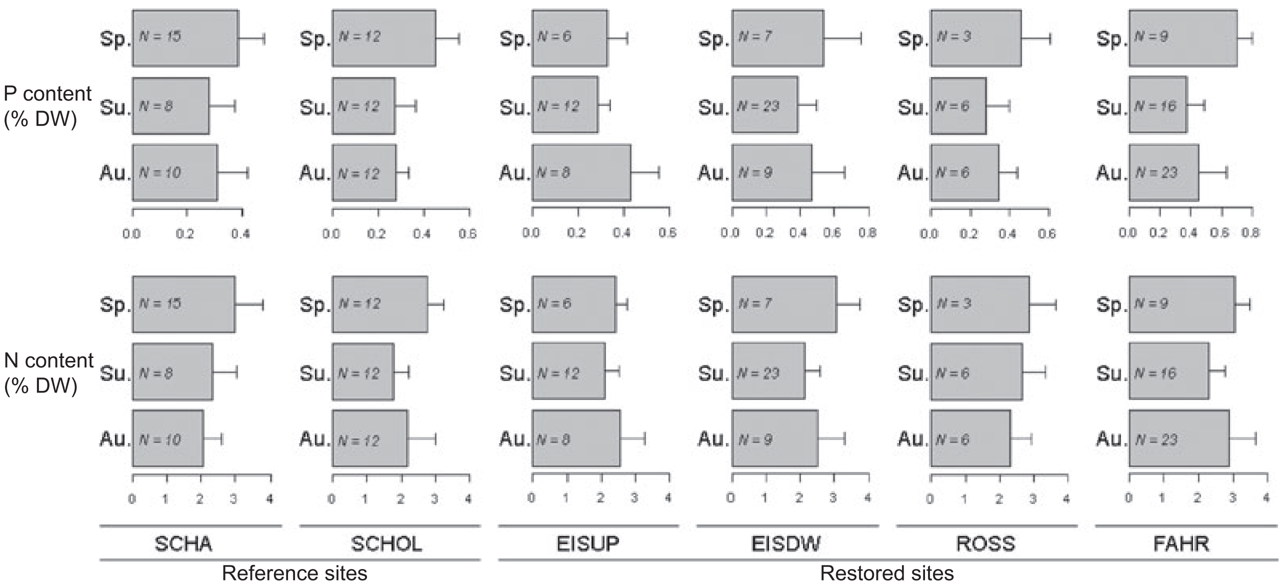

Figure 5. Phosphorus and nitrogen contents of aquatic macrophytes in 2009. Mean ± SEM. Sp., Spring; Su., Summer; Au., Autumn.

Table 3. Effects of nutrient concentrations in plants, water, and sediment, and of sites, seasons and species on the nutrient contents in plants.

		Plants	
	Factor	*P Content*	*N Content*
Plants	P content	—	***
	N content	***	—
Water	$P\text{-}PO_4{}^{3-}$	NS	NS
	$N\text{-}NH_4{}^{+}$	NS	NS
	$N\text{-}NO_3{}^{-}$	NS	NS
Sediment	TP	NS	NS
	Exc-P	NS	NS
	TN	NS	NS
	Min-N	NS	NS
	Sites	NS	NS
	Seasons	NS	**
	Species	**	***

$N = 197$. NS, not significant.
Results are summarized from a Generalized Linear Model analysis.
$^{**}p < 0.01$; $^{***}p < 0.001$.

out the importance of such factors on macrophyte communities. In the Upper Rhine floodplain, Tremp (2007) already showed that physical parameters, such as current, shading, turbidity, and maximum depth, allowed a satisfactory prediction of the distribution patterns of the 25 most frequent hydrophytes. Furthermore, most reconnected sites were largely colonized by *E. nuttallii* (covering ca. one-third of the communities), which could enhance the relationship between sediment nutrient content and vegetation cover. It has been demonstrated that *Elodea* species significantly reduce water velocity within plant patches (Pachuta & Molski 1992), and thus increase the deposition of fine-grained and nutrient-rich sediment (Kleeberg et al. 2010*b*). It has also been shown that the relative growth rate of *E. nuttallii* is positively linked to the nutrient contents in the sediment, whereas species such as *M. spicatum*, which only accounted for 6% of the vegetation cover in this study, did not exhibit such a relationship (Angelstein et al. 2009). With this deposition enhancing effect, *E. nuttallii* favored its own growth and at the same time the nearby colonization by other species.

The species, which colonized the restored sites, showed significantly different nutrient contents. In spite of different environmental conditions, the range of nutrient contents in the plants detected in our study was similar to values found in numerous other studies (Baldy et al. 2007; Demars & Edwards 2007; Badr & Fawzy 2008). *Azolla filiculoïdes* was the species with the highest N content in our study. This is coherent with the fact that *A. filiculoïdes* is known to be an efficient nitrogen-fixer species (Costa et al. 2009). On the other hand, *Chara vulgaris*, a species of low nutritional value (Chambers et al. 1991), was the species that exhibited the lowest nutrient content.

The GLM showed no significant relationship between the nutrient contents of each plant sample and the sediment and water nutrients, while numerous studies suggested a link between the whole plant compartment, water, and sediment compartments (Thiébaut & Muller 2003; Baldy et al. 2007; Badr & Fawzy 2008; Angelstein et al. 2009; Kleeberg et al. 2010*a*). Our finding suggests that such a relationship should not be studied as a relationship between compartments only, as the plant compartment is more complex than expected. Obviously, plant species differ in their relations with water and sediment regarding nutrient accumulation. Aquatic plants may uptake nutrients from either or both water and sediment (Lacoul & Freedman 2006). Some species could indifferently shift from one nutrient source to another, as was demonstrated for *Callitriche obtusangula* and *Berula erecta* (water parsnip) by Baldy et al. (2007). As already shown by Demars and

Table 4. Correlation table (Kendall's tau) between all characteristics measured in the cross sections.

		Water		*Sediment*				*Plants*		*Vegetation*	
		N-NH_4^+	*N-NO_3^-*	*TP*	*Exc-P*	*TN*	*Min-N*	*P Content*	*N Content*	*Cover*	*Species Richness*
Water	P-PO_4^{3-}	−0.18	−0.03	−0.16	**−0.20***	−0.05	0.03	0.04	−0.03	−0.11	**−0.20***
	N-NH_4^+		0.00	0.02	0.13	−0.10	0.04	−0.11	−0.05	0.10	0.14
	N-NO_3^-			−0.17	0.05	−0.02	**−0.20***	−0.07	−0.10	0.15	0.11
Sediment	TP				**0.27****	**0.33*****	0.10	0.00	−0.05	0.07	−0.12
	Exc-P					**0.27****	0.01	−0.09	−0.10	**0.23***	**0.23***
	TN						0.06	**0.20***	**0.23***	0.10	0.03
	Min-N							0.04	0.05	−0.10	−0.05
Plants	P content								**0.66*****	0.09	0.10
	N content									0.16	0.12
Vegetation	Cover										**0.54*****

$N = 54$. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Edwards (2007), N:P ratios of plants were highly correlated and species-dependent in our study and were just weakly influenced by N and P changes in the water and the sediment.

In our study, nearly all species exhibited a N:P ratio inferior to 11, which would indicate nitrogen as a limiting factor (Mainstone & Parr 2002; Güsewell 2004). This result seems to be in contradiction with a common hypothesis that phosphorus could be a limiting factor in freshwater ecosystems due to its low bioavailability (Fardeau & Dorioz 2000; Güsewell 2004). Carr and Chambers (1998), who measured N:P ratios lower than 11, found that macrophyte biomass was primarily affected by sediment phosphorus, but they suggested that if phosphorus was available in sufficient quantities for the growth of aquatic plants, nitrogen may become a limiting factor. Spencer and Ksander (2003) also concluded that sediment nitrogen availability could limit growth and distribution of macrophytes, especially of rooted ones. We observed seasonal variations of sediment TN and plant nitrogen, whereas sediment TP exhibited no variation, which is conform to the nitrogen-limitation hypothesis. Furthermore, the availability of nitrate might be reduced by denitrification which is enhanced by submerged macrophytes and fine-grained deposits (Forshay & Dodson 2011).

All the restored sites have been restored for <10 years. We hypothesized that each restored site should exhibit similar vegetation dynamics. These dynamics should, after an undetermined elapsed time, lead to a species richness and percentage cover similar to those observed in the reference sites. We actually did not observe such similarities. Even after 7 years of reconnection in EISUP and EISDW aquatic vegetation was still not similar to the vegetation of the reference sites, characterized by two dominant species, *P. pectinatus* and *P. perfoliatus*.

The restored and reference sites exhibited similar seasonal nutrient dynamics. The time elapsed since the reconnection seemed to have no effect either on the vegetation colonization rate or on the nutrient dynamics of the restored sites. Our results indicate that a 3-year-old reconnection like in FAHR was sufficient for the site's functioning to reach the targeted nutrient dynamics.

Implications for Practice

- Within large river floodplains, the re-establishment of nutrient dynamics between water, sediment, and plants in reconnected streams is fast and not problematic. Supplementary actions do not seem to be necessary.
- Monitoring macrophyte communities should be carried out over long periods, as a lack of correspondence between the trophic status (water and sediment nutrients) and macrophyte communities may be observed in the first years after a restoration.
- Restoration works which only include reconnection and sediment dredging may lead to the creation of vacant niches for invasive species. Therefore, it could be suitable to introduce target species in reconnected streams and to monitor the effects.

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PRIMARY RESEARCH PAPER

Vegetation dynamics in side-channels reconnected to the Rhine River: what are the main factors controlling communities trajectories after restoration?

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Abstract The hydraulic management of large rivers led to a disconnection of side-channels. Restoration works were to reconnect these side-channels to the main course in order to recover hydrological and ecological functions. The aim of the study was to analyze the vegetation dynamics after restoration and to link it to the change in chemical and hydro-geomorphological characteristics. Changes in species richness, cover and composition of the macrophytes communities were studied in nine side-channels of the Rhine river (France) after reconnection which occurred between 1998 and 2006. Vegetation dynamics was surveyed between 2007 and 2011 and compared to the ones of three target side-channels (never disconnected). Three vegetation communities were identified: one characterized by rheophilic species, a second one by mesotrophic species, and a third one by eutrophic species. Distribution of communities depended mainly on the flow velocity and the sediment texture of the side-channels. The floristic composition of communities evolved rapidly and remained stable a few years after restoration. Changes in species richness and cover remained relatively low in all side-channels over the study period. Time after restoration did not affect the dynamics of colonization. Reconnection allowed the restored side-channels to exhibit vegetation dynamics similar to those of the target side-channels.

Keywords Macrophyte · Hydro-geomorphology · Community · Species richness · Restoration · Trophy level

Introduction

In order to prevent floods, produce hydroelectricity, promote navigation, and/or gain land for farming many large rivers around the world were channelized in the last century. Consequently floodplains and side-channels were disconnected from the main channel, especially in braided and anastomosing river reaches. In some cases such disconnected side-channels were mainly fed by groundwater and were subject to changes in aquatic vegetation, often evolving from eutrophic to oligotrophic communities (Bornette & Amoros, 1991; Trémolières et al., 1993; Bornette et al., 1996, 1998). Moreover, the disappearance of

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flow and fluvial dynamics after a permanent channel disconnection, the decline of aquatic habitats in the floodplain and/or subsequent terrestrialization (Schmitt et al., 2011) usually led to a loss of species and habitat diversity.

For the last 30 years restoration projects have been set up in order to recover the structure and functions of floodplain ecosystems, i.e., diversity of habitats and species, water purification, and flood mitigation. Such projects were conducted, notably, as well as in North America (Bernhardt et al., 2005, 2007), Australia (Brooks & Lake, 2007), or Europe, for the Danube river (e.g., Schiemer et al., 1999), the Rhône river (e.g., Henry et al., 2002), or the Rhine river (e.g., Nienhuis et al., 2002). Monitoring was clearly recommended for every restoration project (Lindenmayer & Likens, 2010). As an example, the benefits of channel reconnections could be assessed through the evaluation of fluvial dynamics recovering, the improvement in fauna and flora dispersal between the floodplain habitats, or the increase in biodiversity (both genetic, species and ecosystems).

Although many side-channel restoration programs have been realized around the world, restoration practitioners highlighted the fact that little or no monitoring usually followed the restoration works (Bernhardt et al., 2007; Brooks & Lake, 2007). It is now obvious that long-term surveys are needed (Stefanik & Mitsch, 2012). Therefore, there is a lack of knowledge about the vegetation change and the factors which induce changes in restored side-channels. The macrophyte compartment is already known to be influenced not only by regional conditions, such as longitudinal connectivity and river basin isolation (Demars & Harper, 2005), but also by local abiotic conditions such as temperature, light availability, flow shear stress or energy, structure of physical habitats, substrate types, nutrient availability, and pH (Lacoul & Freedman, 2006; Hrivnák et al., 2007). According to Pedersen et al. (2006), a higher connectivity enhances the quantity and diversity of propagules supply in restored side-channels. Moreover, with a higher connectivity, a contrasted water supply (groundwater and/or surface water) should favor species diversity and plant cover (Amoros et al., 2005). The increase in habitat diversity usually led to an increase in macrophyte diversity (Pedersen et al., 2007). Some surveys of vegetation after channel reconnections in European rivers showed a rapid colonization, with expanding competitive species, like *Elodea nuttallii*, *Elodea canadensis*, and *Myriophyllum spicatum* (Henry & Amoros, 1996; Biggs et al., 1998; Combroux et al., 2002; Henry et al., 2002; Pedersen et al., 2007). Riis et al. (2004) found that colonization of artificial stream channels (macrophyte growth and arrival of new species) was not affected by flow regime variations, such as high flow events. Near-total colonization (>90% macrophyte cover) of these channels is usually achieved in less than 2 years (between 75 and 98 weeks), whatever the flow regime. On the other hand, Riis & Biggs (2003) showed that macrophyte abundance and diversity were negatively linked to flood disturbance frequency, because of plant uprooting associated with bed sediment erosion. Components of early colonizing communities could thus be explained through the analysis of environmental parameters. However, as few restoration projects are followed by long-term monitoring, very few studies identify the processes that influence post-restoration trajectories of communities.

Restoration projects of the Rhine floodplain consisted of recreating upstream hydrological connections of disconnected side-channels. We hypothesized that the vegetation dynamics within the reconnected side-channels should be characterized by (1) a first stage of rapid vegetation colonization, species richness and cover exhibiting high fluctuations, and an exponential increase in the first vegetation seasons; and (2) a second stage of community stabilization with communities composition and dynamics closely related to the hydro-geomorphological and chemical characteristics of the side-channel. During this second stage, communities composition should be first influenced by the water chemistry as commonly demonstrated (e.g., Carbiener et al., 1990; Haury et al., 2006) and second by hydro-geomorphological characteristics of the restored side-channels.

We thus identify vegetal succession trajectories after restoration and the factors influencing dynamics of colonization. Macrophyte communities dynamics were analyzed in restored side-channels in a comparison with target side-channels. We chose side-channels never disconnected to the main channel as target side-channel sensu Clewell & Aronson (2007), i.e., "actual ecosystems on which restoration planning is based and that can serve as a basis for evaluating the completed restoration project."

Materials and methods

Study sites

Twelve sites in 11 side-channels were monitored. Six were connected to the main channel (Group 1) and six others to a drainage channel flowing along the main channel and mainly fed by groundwater (Group 2; Fig. 1; Table 1).

Group 1 consisted of two target and four restored sites. The Schollengiessen (SCHOL) and the Schaftheu (SCHA) located in artificially fixed (created by the channelization) and regularly flooded islands were considered as target side-channels. Two sites were located on a side-channel restored in 2002; the Eiswasser: one close to the connection to the Rhine river (Eiswasser Upstream; EISUP) and the second one 1-km downstream (Eiswasser Downstream; EISDW). Even though they were situated on a single side-channel, these two sites were geomorphologically different, as the upstream part was narrow and shallow, with a higher current velocity, whereas the downstream part was relatively wider and deeper, with a lower current velocity. The Rossmoerder (ROSS) was reconnected to the Rhine river in 2004, and the Fahrgiessen (FAHR) in 2006.

Group 2 consisted mainly of side-channels connected to the Rhine Drainage Channel (RDC). The

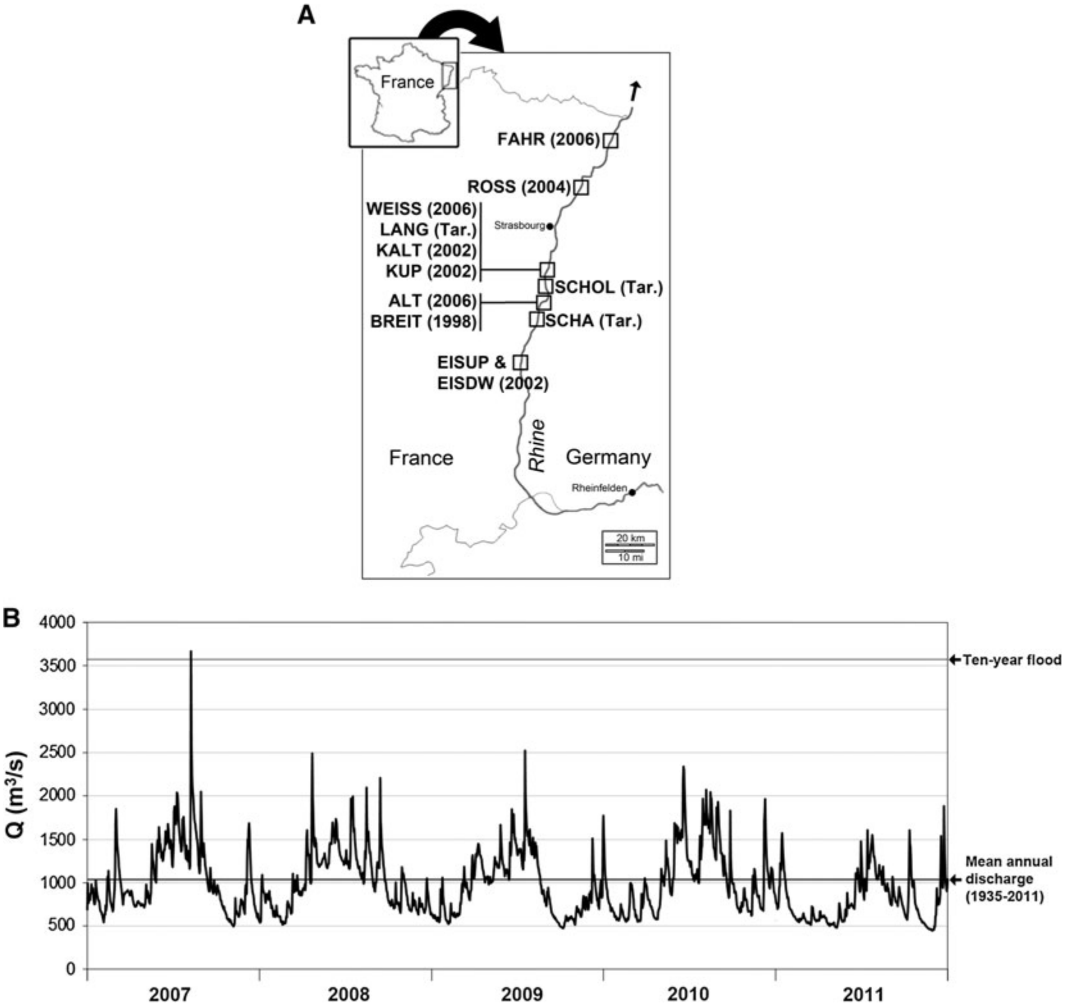

Fig. 1 **A** Location of the studied side-channels. In *brackets* year of restoration. *Tar.* target side-channel. **B** Daily discharge of the Rhine river from 2007 to 2011, measured at Rheinfelden (Switzerland) where the discharge is broadly the same as in the Rhine near the sites due to the absence of large tributaries (OFEV—http://www.hydrodaten.admin.ch/fr/2091.html)

Table 1 Vegetation and physico-chemical characteristics of the side-channels

Sites	River network: Rhine							River network: RDC				
	SCHA	SCHOL	EISUP	EISDW	KALT	ROSS	FAHR	LANG	BREIT	KUP	ALT	WEISS
Type	Target	Target	Restored	Restored	Restored	Restored	Restored	Target	Restored	Restored	Restored	Restored
Year of reconnection	–	–	2002	2002	2002	2004	2006	–	1998	2002	2006	2006
Physical factors[a]												
Width (m)	22.2	22.2	6.5	10.7	12.5	6.5	22.6	7.5	5.3	6.9	6.9	7.2
Depth (m)	0.88	1.99	0.32	0.44	0.59	0.69	1.22	1.11	0.45	0.88	0.48	0.59
Q (m^3/s)	5.73	0.79	0.82	0.91	0.01	0.65	2.07	2.7	0.85	2.37	0.81	0.36
Water velocity (m/s)	0.32	0.02	0.50	0.21	0.00	0.16	0.08	0.36	0.41	0.41	0.31	0.09
Shear stress (N/m^2)	5.61	0.00	2.48	1.95	2.87	2.37	0.00	5.43	2.20	4.32	2.34	2.87
Grain size (mm)	34.80	6.27	8.79	5.76	14.93	14.49	5.13	30.53	28.49	6.37	29.53	1.35
Shade index	1.0	1.0	3.2	2.4	1.8	2.8	1.6	3.2	3.6	2.0	2.6	2.8
Vegetation[b]												
S	17	13	10	16	25	10	23	18	21	21	27	23
H'	2.00	1.48	2.24	1.95	2.10	1.57	2.77	2.30	1.64	2.05	2.17	2.11
Σcover (%)	24.4	39.5	16.8	17.2	39.4	6.9	21.4	22.1	35.0	81.0	35.6	44.7
Water[c]												
P-PO$_4^{3-}$ (μg/l)	24 (±9)	25 (±11)	13 (±5)	15 (±7)	15 (±2)	21 (±11)	19 (±12)	42 (±11)	19 (±1)	11 (±3)	24 (±6)	17 (±2)
N-NH$_4^+$ (μg/l)	19 (±29)	32 (±21)	10 (±11)	9 (±11)	32 (±10)	23 (±24)	33 (±58)	25 (±21)	28 (±8)	30 (±11)	25 (±5)	29 (±25)
N-NO$_3^-$ (mg/l)	1.5 (±0.3)	1.4 (±0.1)	1.3 (±0.1)	1.3 (±0.1)	1.0 (±0.1)	1.3 (±0.3)	1.2 (±0.5)	2.0 (±0.1)	1.2 (±0)	1.4 (±0.1)	1.2 (±0.1)	1.2 (±0.1)
Chloride (mg/l)	21.3 (±6.6)	19.9 (±4.3)	18.2 (±4.8)	19.2 (±4.9)	15.0 (±1.0)	23.5 (±1.6)	20.4 (±1.4)	30 (±1.1)	21.2 (±0.3)	28.3 (±1.2)	20.8 (±0.4)	26 (±2.0)
Sulfate (mg/l)	31.8 (±5.5)	29.7 (±3.8)	31.6 (±2.2)	32.5 (±2.6)	29.2 (±1.0)	30.2 (±1.8)	30.5 (±2.2)	34.7 (±0.9)	30.7 (±0.3)	32.6 (±0.9)	30.5 (±0.3)	30.4 (±0.8)
Sediment[c]												
TP (g/kg)	0.4 (±0.2)	0.5 (±0.1)	0.6 (±0.2)	0.5 (±0.1)	0.5 (±0.1)	0.3 (±0.2)	0.7 (±0.2)	0.6 (±0.1)	0.5 (±0.1)	0.5 (±0.1)	0.6 (±0.2)	0.8 (±0.2)
Exc-P (mg/kg)	56 (±17)	77 (±36)	44 (±17)	65 (±19)	87 (±34)	34 (±21)	145 (±48)	118 (±44)	62 (±30)	52 (±42)	104 (±21)	116 (±57)
TN (g/kg)	2.3 (±3.0)	3.6 (±4.9)	4.2 (±5.0)	7 (±13.4)	2.1 (±0.3)	2.2 (±4.5)	7.9 (±10.0)	4.4 (±1.5)	2.2 (±1.4)	2.7 (±2.6)	4.9 (±1.7)	7 (±2.6)
Min-N (mg/kg)	5.4 (±7.3)	6.4 (±6.1)	6.5 (±5.5)	12.4 (±15.0)	20.7 (±12.3)	2.6 (±5.0)	30.3 (±41.9)	28.5 (±12.1)	14.6 (±10.8)	18.3 (±18.5)	33.3 (±10.8)	51.6 (±36.2)

S species richness, H' = Shannon index, *Σcover* total percentage cover

[a] Mean values calculated over the study period (n = 3)

[b] Values from the 2011 vegetation survey

[c] Mean values ± sd (n = 3)

Langgiessen (LANG), a target site, and the Kuppengiessen (KUP) restored in 2002 were located in the Erstein polder zone, a reflooded sector since 2004. The Breitsandgiessen (BREIT) was reconnected to the RDC in 1998; the Altwasser (ALT) and the Weisswasser (WEISS) were reconnected to the RDC in 2006. The Kaltergiessen (KALT), located in the Erstein polder, is temporarily connected to the Rhine river when the discharge exceeds 1550 m^3/s at the nearest power plant station (Gerstheim hydropower unit).

Methods

On each site, plants were surveyed on a stretch located close to the reconnection. Length of the stretch ranged between 100 and 200 m. Percentage cover was allocated to each taxon, following the Braun-Blanquet phytosociological method (Braun-Blanquet, 1968).

The aquatic vegetation in Group 1 was monitored seasonally (spring, summer, and autumn) between 2007 and 2009, and only in summer in 2010 and 2011. In Group 2, the aquatic vegetation was monitored seasonally in 2010 and 2011. Former surveys, when available (Conservatoire des Sites Alsaciens & Office National des Forêts, 2004), were also used in order to assess mid-term evolution of the vegetation: surveys in 2006 and 2008 in LANG, KALT, and KUP, and one survey in 2005 in BREIT. Surveys raw data are available in the Supplementary Materials.

Geomorphological and chemical parameters were measured in three cross-sections evenly distributed on the surveyed stretch of the side-channel.

Water (50 ml) and sediment (120 cm^3; upper layer) were sampled in the three cross-sections of every side-channel, in 2008 and 2009 for Group 1 and in 2010 for Group 2. Water samples were filtered with a 0.45-µm-mesh filter. Phosphate P-PO_4 (µg/l) and ammonium N-NH_4 (µg/l) were measured by spectrophotometry (protocols follow those described in Meyer et al., 2013). Chloride (Cl, mg/l), nitrate (N-NO_3, mg/l), and sulfate (SO_4, mg/l) were measured by ionic chromatography (Dionex DX120).

In sediment, exchangeable phosphorus (P_2O_5 Exc-P; g/kg) was measured according to the Joret & Hébert method (Joret & Hébert, 1955). Total phosphorus (TP; g/kg) was analyzed after drying for 24 h at 70°C and digesting with a mixture of perchloric and nitric acids. TP was measured as P-PO_4 in the mineralized solution by spectrophotometry. Total nitrogen (TN; g/kg) was analyzed with the NF ISO 13878 method (1998). Ammonium and nitrate (mineral nitrogen Min-N; mg/kg) were analyzed after extraction with KCl (1 mol/l): ammonium was analyzed by spectrophotometry and nitrate by ionic chromatography (APHA, 1985).

Channel width was measured in all cross-sections (Group 1 and 2 side-channels) in 2011. Water depth and substrate type (clay/silt, sand, and pebble/gravel) were determined every 50 cm, along the cross-sections. A mean value of grain size (D_{50}; mm) was calculated for each cross-section by weighting each value by the number of times the substrate type was located along the cross section. Laser granulometry analyses (LS230 COULTER BECKMANN) were conducted in 2010 for clay/silt and sand patches on several samples of Group 1 side-channels and established values were: $D_{50} = 39.36$ µm ($n = 11$) for clay/silt patches and $D_{50} = 210.25$ µm ($n = 12$) for sand patches. Mean grain size of gravel and pebble patches was based on pebble counts (Kondolf, 1997): D_{50} (as mean length of *b* axis) = 46.71 mm ($n = 7$ *samples*, with 100 measured pebbles per sample).

Discharge (Q; m^3/s) was measured with a magnetic field current meter (OTT Sensa Z300) on one cross-section (the most suitable) in each side-channel. Discharge was measured at least twice the same year in each side-channel. Mean water velocity (v; m/s) was calculated as:

$$v = Q/A$$

where Q was the discharge and A was the wetted area on the cross section (m^2).

$$A = wd$$

where w was the width of the water table (m) and d was the mean depth of the water area (m).

Mean boundary shear stress (τ; N/m^2) was calculated as described in Schmitt et al. (2001):

$$\tau = \rho g d S$$

where ρ was the specific weight of water (1,000 kg/m^3), g was the acceleration due to gravity (9.81 m/s^2), d was the mean depth of the water area (m), and S was the slope of the studied side-channel (m/m). Slope was calculated by dividing the slope of the floodplain by the channel sinuosity (Schmitt et al., 2001).

Shade was estimated as the inverse of canopy openness. A shade index was attributed to each cross-section: 1 for 75–100% of canopy openness (co.), 2 for 50–75% co., 3 for 25–50% co., and 4 for 0–25% co. Canopy openness was estimated directly above the whole cross-section.

All the statistical analyses were carried out with the R program (v2.13.0; R Development Core Team, 2011). A principal component analysis (PCA) and a between-class analysis were made on the chemical and hydro-geomorphological parameters of the side-channels in order to find the main discriminating gradients. Vegetation cover were log-transformed ($\log(x + 1)$). A correspondence analysis was used to assess the trajectory (temporal sequences of biotic expression sensu Clewell & Aronson, 2007) of the macrophyte communities. Abbreviations which were used for all species names in all statistical analyses are available in the Supplementary Materials.

Results

Characteristics of side-channels

Water was meso- to eutrophic, with $P\text{-}PO_4$ ranging from 13 to 42 µg/l and $N\text{-}NH_4$ from 9 to 33 µg/l (Table 1). Few differences in the hydrochemical parameters between sites were noticed. Side-channels differed more with regard to physical parameters such as width, mean depth, and grain size. Grain size varied from clay to pebbles. Discharge values were comprised between 0.36 in WEISS and 2.7 m^3/s in LANG (SCHA and KALT excluded). SCHA, a target side-channel, exhibited a higher discharge (max. of 5.7 m^3/s), whereas the mean discharge in KALT was nearly null due to the long periods of disconnection. High differences in aquatic vegetation were observed between sites. Species richness was relatively high in all studied side-channels, ranging between 10 and 27 taxa/site. Alpha biodiversity estimated by Shannon index (H') ranged from 1.5 to 2.8. Total plant cover presented more differences between sites, as it ranged from 7 to 81% in ROSS and KUP, respectively. Restored side-channels globally exhibited higher species richness and Shannon index than target side-channels.

Differences in physical and chemical parameters between the side-channels were analyzed by a PCA (Fig. 2). The first two axes accounted for 47.3% of the total inertia and showed two gradients. A hydro-geomorphological gradient showed a marked opposition between wide and deep side-channels with fine and nutrient-rich sediment and low water velocity (negative scores on axis F1 and positive scores on axis F2) and narrow side-channels with coarse-grained sediment, high energy, and high shade index (positive scores on axis F1 and negative scores on axis F2; Fig. 2A). The sites were mainly distributed along this gradient. There was no clear distinction between the two groups of side-channels (connected to the RDC or the Rhine river). The second gradient was linked especially to water chemical variables. The LANG side-channel characterized by higher nitrate, phosphate and sulfate in the water, together with a high chloride concentration (positive scores on both axes F1 and F2; Fig 2A; Table 1) clearly contrasted with the other sites.

Pairwise correlation (Spearman's rank test; $n = 36$) showed that width was negatively correlated to the shade index ($r = -0.70$; $P < 0.001$). Min-N in sediment was positively correlated to TN ($r = 0.57$; $P < 0.05$), to TP ($r = 0.68$; $P < 0.001$) and to Exc-P ($r = 0.66$; $P < 0.01$). TP was also positively correlated to TN ($r = 0.70$; $P < 0.001$). Nitrate and sulfate were positively linked ($r = 0.65$; $P < 0.01$). Other tested pairs showed no significant correlations.

Post-restoration vegetation dynamics

The ALT and WEISS side-channels were surveyed only in 2010 and 2011 (Fig. 3), which did not allow a correct assessment of the temporal vegetation change. Only FAHR clearly exhibited a rapid increase in plant richness and cover which remained stable 2 years after reconnection. Target side-channels, SCHA, SCHOL and LANG, and some reconnected ones, EISUP, EISDW and ROSS, exhibited a trajectory showing relatively low fluctuations of species richness and cover. The side-channels KALT, BREIT, and KUP exhibited higher richness in the last surveys, in 2011 for BREIT and KUP, and in 2010 and 2011 for KALT, but with high variations of plant cover within the 3 or 4 years surveyed.

The species ordination plan (PCA; Fig. 4) showed that the first axis clearly differentiated mesotrophic communities with *Callitriche obtusangula*, *Berula erecta* and *Sparganium emersum* (negative scores) from eutrophic communities (positive scores) with *E. nuttallii* and species of *Potamogeton* (*P. perfoliatus*,

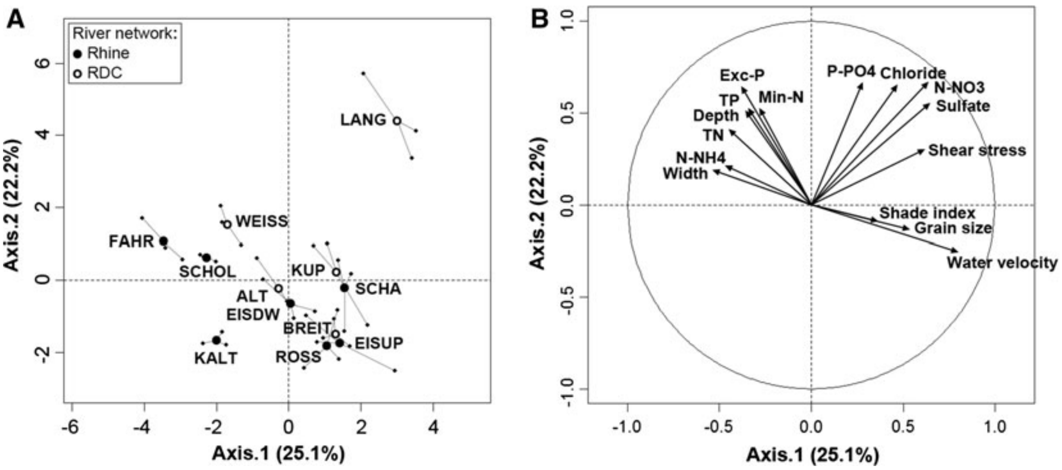

Fig. 2 Factorial maps from a normalized PCA (F1 × F2) of the hydro-geomorphological and chemical parameters. $n = 36$. **A** Projection map of the 36 cross sections and the side-channels. **B** Projection map of the variables

P. pectinatus, *P. lucens*, *P. gramineus*, *P. nodosus*, *P. berchtoldii* and *P. crispus*). The negative scores on the second axis were characterized by rheophilic communities with *Ranunculus fluitans*, *Fontinalis antipyretica* and *Hildenbrandia rivularis*.

The side-channels were roughly divided into three groups. The first group comprised side-channels connected to the RDC and was characterized by mesotrophic macrophyte communities dominated by *C. obtusangula*, *B. erecta* and *S. emersum* (LANG, KUP, and WEISS; Fig. 4A, C). Macrophyte communities in these sites followed a similar temporal trajectory: the dominant species pool shifted from *S. emersum* to *C. obtusangula* and *B. erecta* within few years after restoration. WEISS, for which reconnection was very recent (2006), seemed to support the very beginning of this trajectory, with the cover of *S. emersum* decreasing from 9.5 to 2.0% between 2010 and 2011 and cover of *C. obtusangula* increasing from 20 to around 30% during the same period (cf. Supplementary Materials).

The second group consisted of side-channels connected to the Rhine river or to the RDC with macrophyte communities dominated by rheophilic species (ALT, BREIT, and EISUP; Fig. 4A, C) such as the algae *H. rivularis*, the angiosperm *R. fluitans*, and the bryophyte *F. antipyretica*. These side-channels followed similar temporal trajectories, from a community dominated by species which colonized channels with low energy (*E. canadensis* in ALT, *E. nuttallii* and *C. obtusangula* in BREIT, or algae in EISUP) to rheophilic species such as *R. fluitans* and *H. rivularis*.

The third group comprised side-channels connected to the Rhine river (Fig. 4B, D–F). These side-channels could be further separated by the community observed in 2011. On one hand, ROSS, FAHR, and KALT exhibited vegetation trajectories initiated with different communities but all leading to the establishment of a plant community similar to the one observed in SCHOL, a target side-channel. This one showed a stable state dominated by *P. pectinatus*, *E. nuttallii*, and *C. demersum*, with low fluctuations appearing as a short and circular trajectory on the factorial map. FAHR and KALT exhibited quite similar trajectories. They quickly evolved from a community with *Potamogeton* species to a community dominated by *E. nuttallii*, *C. demersum* and *M. spicatum*. ROSS had an original trajectory from the two species of *Myriophyllum*, *M. verticillatum* (only present in 2007) and *M. spicatum*, to a community characterized by the absence of *Potamogeton* species and a rapid increase in *E. nuttallii* cover.

On the other hand, EISDW exhibited a temporal trajectory toward the community similar to the one observed in SCHA, a target side-channel exhibiting a stable state dominated by *P. perfoliatus* and *P. pectinatus*, with fluctuations appearing as a short trajectory on the factorial map. Actually the trajectory of the restored EISDW was mainly linked to the annual presence (2008 and 2011) or absence (2007, 2009 and 2010) of *M. verticillatum*. The trajectories of EISDW

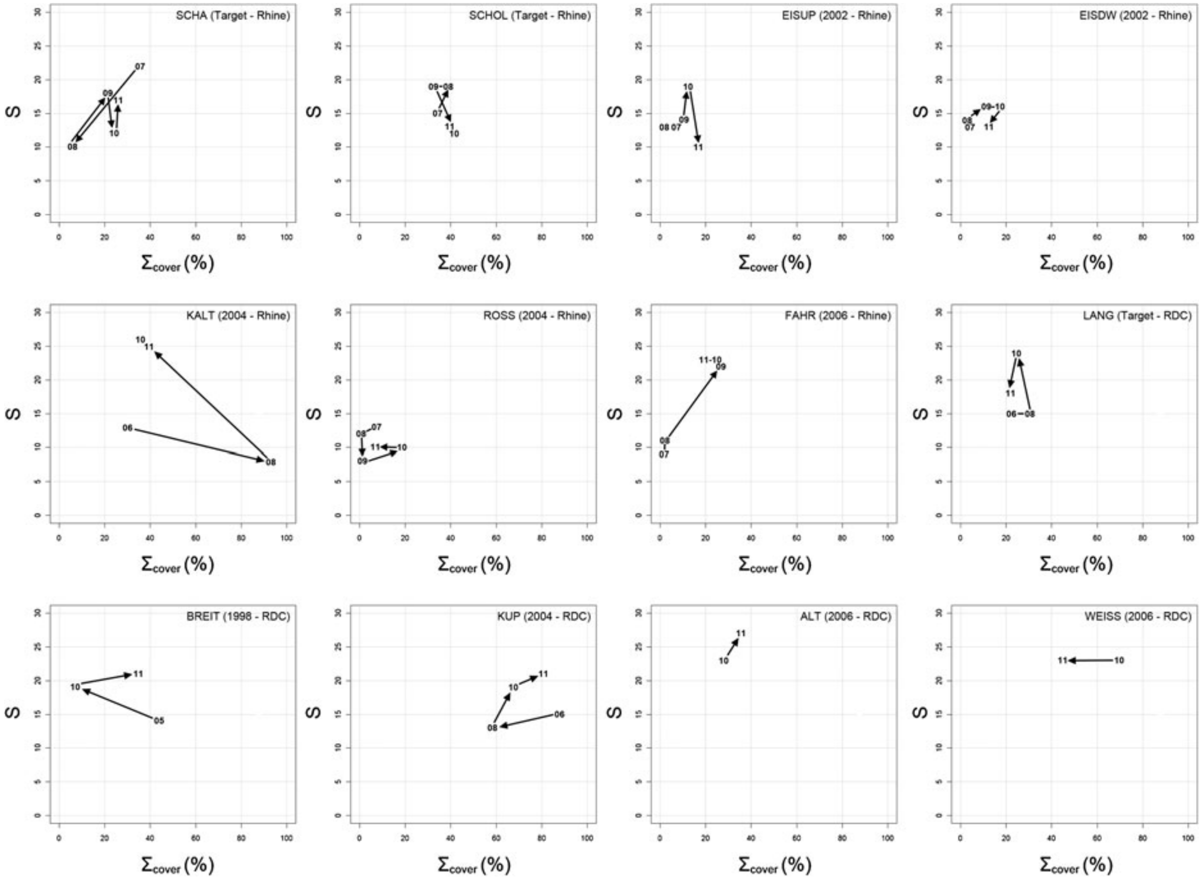

Fig. 3 Temporal variations in the specific richness and total cover in each studied sites. Number corresponds to the year of the survey (i.e., 09 stands for 2009). In *brackets* if restored, year of restoration, and type of hydrological upstream connection. *Σcover* percentage cover of the total vegetation, *S* taxonomic richness

and SCHA were close as these two side-channels were the ones with higher cover of *Phalaris arundinacea*.

Physico-chemical characteristics linked to macrophyte communities

The three communities previously identified were used to a posteriori combine the side-channels:

i. A community dominated by *Potamogeton* sp., *E. nuttallii*, *M. spicatum*, and/or *C. demersum* was found in the side-channels SCHA, SCHOL, EISDW, KALT, ROSS, and FAHR, which is called group RHINE;
ii. A community dominated by *B. erecta*, *C. obtusangula*, and/or *S. emersum* in the side-channels LANG, KUP, and WEISS, which is called group RDC;
iii. A community dominated by *R. fluitans*, *H. rivularis*, and/or *F. antipyretica* in the side-channels ALT, BREIT, and EISUP, which is called group RHEO.

A between-class analysis was used to identify the discriminating physico-chemical characteristics of these groups (Fig. 5). This analysis was carried out on the same dataset used in Fig. 2.

The first axis accounted for 64.6% of the total inertia and differentiated the RHINE group from the RDC one. On this axis the RHINE group was characterized by high width and low shear stress, whereas the RDC group was defined by a little higher nitrogen and phosphate content in the water and the sediment, higher concentrations of chloride and sulfate and high shear stress. The second axis accounted for 35.4% of the total inertia and differentiated the

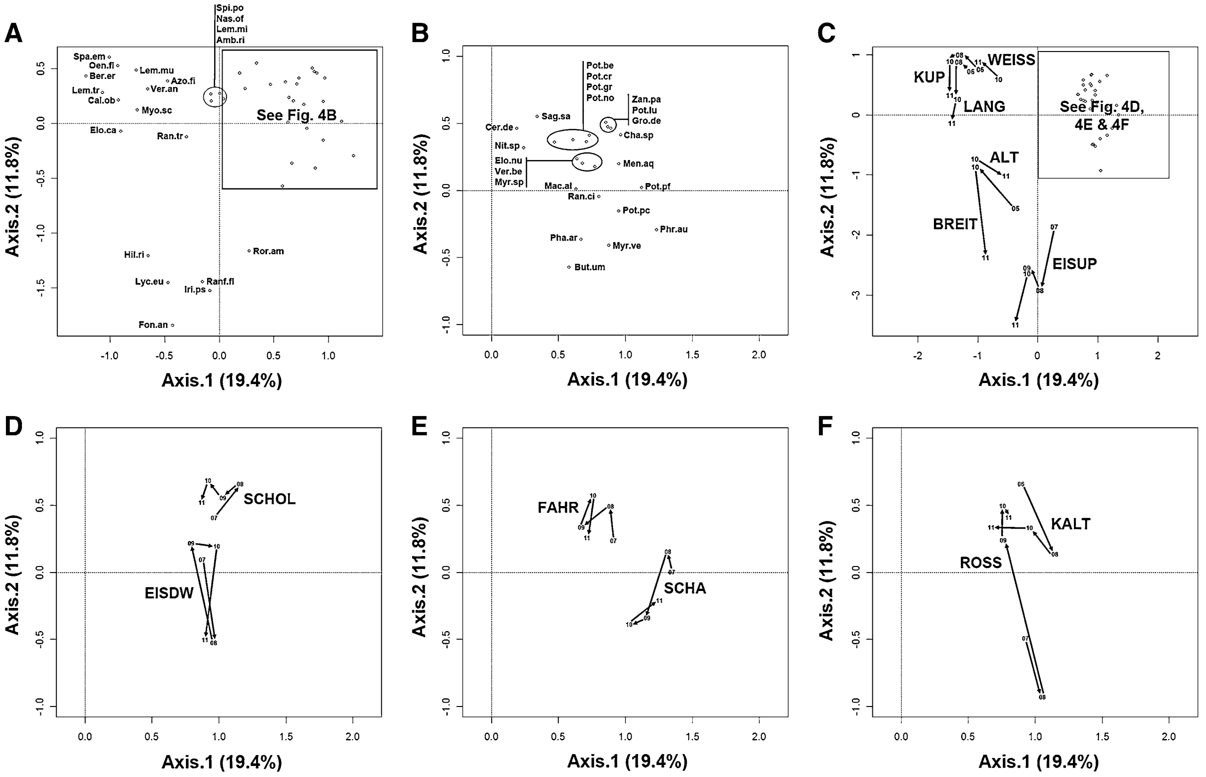

Fig. 4 Temporal trajectories of the macrophyte communities in the side-channels on the F1 × F2 plan of a correspondence analysis. Each point corresponds to a past survey. $n = 49$. For *Abbreviations* see Supplementary Materials. **A**, **B** Species projections. **C**–**F** Trajectories of the studied sites

RHINE and RDC groups to the RHEO one. On this axis, the RHEO group was defined by coarse grain size, high water velocity, and high value of shade index, whereas the RHINE and RDC groups were defined by higher width and depth.

Discussion

Main factors controlling communities in restored side-channels

As the side-channels fed by the Rhine river are supplied by eutrophic waters whereas the side-channels fed by the RDC are supplied by mesotrophic waters, we could have expected some differences in water chemistry (especially N and P) between the two groups of sites. However, phosphorus and nitrogen concentrations in water were similar in these two groups. The main differences between the two groups consisted of the development of different macrophyte communities. The communities of the Rhine were clearly dominated by eutrophic species (Robach et al., 1996), whereas side-channels connected to the RDC exhibited macrophyte communities dominated by mesotrophic species as it could have been expected based on the water supply. But we also identified a third community clearly related to the water velocity. These three communities were already observed by Tremp (2007) in the Upper Rhine floodplain area where they were linked to physical features such as grain size and flow velocity. Baart et al. (2010) also observed that, in rehabilitated wetlands, water velocity and sediment grain size in shallow water area were the best predictors of macrophytes distribution. Our results confirmed that communities composition are first influenced by hydro-geomorphological characteristics rather than water chemistry. We here demonstrated that the vegetation of reconnected streams would be dependent on water chemistry only in reaches with low water velocity and fine sediment grain size. The composition of vegetation in reconnected streams with higher water

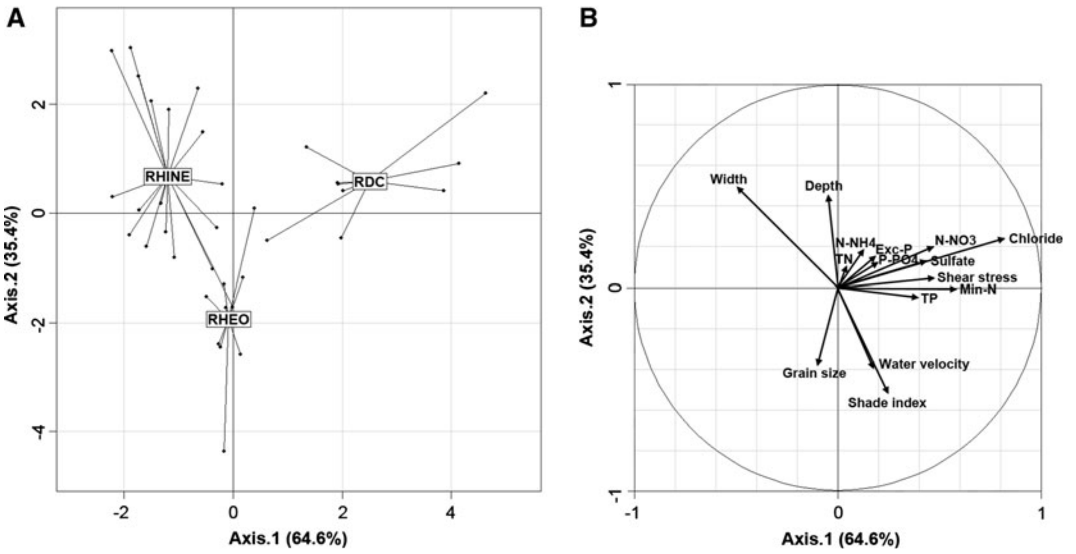

Fig. 5 Between-class analysis of the physico-chemical parameters defining the three identified groups of side-channels. RHINE regroups combined data of SCHA, SCHOL, EISDW, FAHR and KALT, RDC with LANG, KUP and WEISS, RHEO with ALT, BREIT, and EISUP. $n = 36$. **A** Projection map of the groups. **B** Projection map of the variables

velocity was quite similar whatever the water chemistry and the type of supply.

Dynamics of vegetation and link to environmental characteristics

Variations in species richness and total cover remained slight over the 5-year study period in most of the restored and target side-channels. This result suggested that species richness and vegetation cover were already established and stable within the early 5 years after restoration, although equilibrium between species could change (i.e., multiple stable states could exist). According to Biggs et al. (1998), Henry et al. (2002) and Amoros et al. (2005), increase in plant cover happened quickly after restoration works, in less than 4 years. As our observations generally occurred several years after the reconnection, we could not observe whether plant communities exhibited drastic evolutions within a four-year period after the restoration work, except for FAHR and ALT, reconnected in 2006. Actually, the side-channel FAHR exhibited immediate strong colonization by macrophytes. Then the established community remained unchanged over the study period (from 2008 to 2011) which is consistent with results of the previous authors.

The temporal trajectories of the floristic composition, richness, and cover of communities seemed to depend mainly on changes in the hydro-geomorphological characteristics. After restoration works, hydrogeomorphological changes were observed—i.e., fine sedimentation, bedload wave migration, or local bank erosion—but remained weaker than it could occur in some target side-channel (i.e., in SCHA, personal observation).

Vegetation of the eutrophic channels connected to the Rhine river showed a first stage colonized by *Potamogeton* species which evolved rapidly toward two alternative stable states characterized either by the absence of the exotic *E. nuttallii* or by its great abundance. In Danish lowland streams Sand-Jensen et al. (2000) and Riis & Sand-Jensen (2001) showed that *P. crispus*, *P. pectinatus*, and *P. perfoliatus* withstood physical disturbances and remained abundant in these lowland streams, especially in wide ones. Many *Potamogeton* species are also known to prefer slow-flowing side-channels (Janauer et al., 2010). Side-channels with a high presence of *Potamogeton* species in their community—i.e., SCHA, SCHOL, FAHR, and KALT—did exhibit such characteristics, underlying the greater importance of physical conditions than chemical ones in the dynamics of the communities after restoration.

Patches of *P. pectinatus* also occasionally occurred in the RDC, in the transects very close to the connection, but not further in the reconnected side-channel. The RDC is a large channel, ~10 m wide, with only one forested bank and bright lighting. By contrast, side-channels connected to the RDC are narrower (mean width ~7 m) and exhibit a higher shade index as they are situated in forested areas. *P. pectinatus* requires high light availability (Madsen & Adams, 1989) and this preference could explain its absence in the RDC-connected side-channels.

The high variation in species richness and total cover of KALT was unique in this study and could be explained by its temporary connection to the Rhine river depending on a Rhine discharge (upstream connection when the discharge exceeds 1,550 m^3/s), which usually happened 50 days per year (Schmitt et al., 2009). Thus, during the lowwater periods this side-channel was fed by phreatic waters and had a very low discharge and water velocity. In summer 2007, a record flood of the Rhine river, with a return period of 10 years (Fig 1B), may have brought a lot of propagules and/or nutrient-rich sediments in this side-channel. Pedersen et al. (2006) hypothesized that an increase of species richness and total cover within a relatively short period of time is related to a diverse and plentiful propagule supply. Therefore, the variations in flow regime could explain the sudden vegetation cover variations, especially when connections occur during a season when the density of propagules dispersed in the river's water flow is very high. A study on this possible supply is in progress in some of the restored side-channels.

Risk of invasion by exotic species

Colonization by invasive species is a high risk when restoration works create new biotopes opened to every colonizer, as it is the case after reconnection. The two exotic *Elodea* species, *E. canadensis* and *E. nuttallii*, were commonly observed in the restored side-channels. However, *E. canadensis* was only observed in the side-channels connected to the RDC, confirming that this species is now integrated in the local mesotrophic community (Greulich & Trémolières, 2006). Former studies on restored rivers in Europe implied that *E. canadensis* was one of the favored species during recolonization processes (Henry & Amoros, 1996; Biggs et al., 1998). However, *E. nuttallii* introduced in Europe in the middle of twentieth century possessed a higher competitive ability compared to *E. canadensis* (Barrat-Segretain, 2001; Thiébaut, 2005), and replaced *E. canadensis* as the dominant species in side-channels of large rivers (Combroux et al., 2002; Amoros et al., 2005; Greulich & Trémolières, 2006). Based on the characteristics of the side-channels, we hypothesized that variables that could lead to communities dominated by *Potamogeton* or *Elodea* are physical, and that colonization by *Elodea* may be promoted when fluvial dynamics (high flow velocity and shear stress, gravel and pebble mobilization, etc.) disappear. The side-channel SCHA did exhibit hydro-geomorphological dynamics and had a higher cover of *Potamogeton* species and a lower cover of *E. nuttallii*. By contrast, EISDW, submitted to the same Elodea's propagule pressure than SCHA (connected to the same channel) is characterized by a very high cover of *E. nuttallii.* It exhibited sedimentation of fine-grained sediment, which usually favored the colonization by *E. nuttallii* (Demars & Harper, 2005) and we thus observe an increase in *Elodea* cover over the study period. The *E. nuttallii* population was probably at the beginning of its invasive phase in this site, as its cover remained low compared to the available surface of fine-grained sediment.

In the side-channel ROSS, *E. nuttallii* was also the main colonizing species, and its cover increased over the study period. However, in the same time period this side-channel was also colonized by invasive benthic zebra mussels, *Dreissena polymorpha*. In 2011, it covered more than 25% of the side-channel bottom. New questions arose from this situation: will *E. nuttallii* progressively replace *D. polymorpha*, or will its cover progression be impeded by the existing cover of *D. polymorpha*? Competition for benthic encroachment between two invasive species *D. polymorpha* and *E. nuttallii* should be further studied.

Conclusion

This study highlighted that macrophyte recolonization in restored side-channels of large rivers is a process that can occur rapidly. The absence of major changes in the communities during the surveyed years also implied that the community appearing in the early stages after the restoration work may be stable for a relatively long time, including annual fluctuations of dominant species. Restored side-channels globally exhibited plant cover and species richness similar to those observed in the target side-channels. Variations in cover and species richness remained low in most of the side-channels. Only the more recently restored side-channels did exhibit high variations in cover and species richness. The communities which colonized the side-channels depended primarily on the hydrogeomorphological conditions, providing that the trophy level was high enough to be a non-discriminating factor in the side-channels.

Among the exotic species, *E. nuttallii* was essentially observed in side-channels connected to the Rhine river, whereas *E. canadensis* was only present in side-channels connected to the RDC. These observations confirmed that *E. nuttallii* is currently replacing *E. canadensis* as a major colonizing and potentially invasive species. However, the presence of *E. nuttallii* seemed not to impede the presence of *Potamogeton* species, which are declining at least in some part of Europe (Sand-Jensen et al., 2000; Riis & Sand-Jensen, 2001).

In this study most of restored side-channels exhibited similar vegetation dynamics and community to those of the target side-channels. Upstream reconnection can, therefore, be considered as a good way to restore aquatic vegetation communities within the reaches very close to the connection. The hydrogeomorphological factors as the primary factors probably explaining the community composition also highlighted that restoration work should include monitoring of such factors. In order to observe evolution in the community, it is, therefore, necessary to start monitoring immediately after restoration work, as done notably by Henry et al. (2002), and, when possible, long-term monitoring in order to evaluate the stability of communities.

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Les macrophytes sont-ils de bons candidats pour l'évaluation du succès d'une restauration ?
L'exemple des anciens bras du Rhin reconnectés

Lors d'opérations de restauration comme celle menée dans la plaine d'Alsace pour reconnecter d'anciens bras latéraux du Rhin au cours principal, il convient de vérifier comment ces modifications affectent la biodiversité et fonctionnalité des milieux aquatiques. Pour cela, l'étude des macrophytes peut être tout à fait pertinente.

Les nombreux travaux d'aménagement hydraulique menés sur la plupart des grands fleuves européens ont fortement modifié et altéré la structure des habitats alluviaux et le fonctionnement hydro-géomorphologique du fleuve et de sa vallée avec la suppression des champs d'inondation, la déconnexion des bras latéraux et/ou des annexes hydrauliques du fleuve, la modification du pouvoir épurateur des zones alluviales, la diminution de la diversité des habitats et des espèces et par conséquent la perte de services écosystémiques. Or, depuis une trentaine d'années, la prise de conscience de l'ampleur de ces impacts négatifs a conduit les pays riverains des grands fleuves, comme le Rhin, à prendre une série de décisions dans le but de préserver et de restaurer la biodiversité de l'hydro-système fluvial tout en maintenant la possibilité pour les générations futures de bénéficier des apports de la nature et du capital naturel constitué par les milieux fluviaux. Ces décisions ont été concrétisées par la mise en place sur le Rhin de programmes d'action et de recherche, comme le programme d'action Rhin (1987-2000), IRMA (*Interreg Rhine-Meuse Activities*) et le programme Life-Nature « Rhin Vivant » (2002-2006). Ce dernier a permis, outre la définition d'« un référentiel des habitats naturels reconnus d'intérêt communautaire de la bande rhénane » (CSA[1] et ONF[2], 2004), la reconnexion de nombreux bras au cours principal.

Les objectifs des projets de restauration d'écosystèmes dégradés sont de reconstituer la structure et les processus qui leur permettent de retrouver leur fonctionnalité. Sur les grands fleuves, les projets ont souvent pour but de restaurer la connectivité longitudinale et latérale du corridor fluvial. Les suivis de sites restaurés, lorsqu'ils existent, montrent que les restaurations ont pu être des succès mais aussi des échecs. La question posée ici est : comment évaluer le succès d'une opération de restauration ? Existe-t-il des indicateurs plus pertinents que d'autres ?

De nombreux groupes taxonomiques et syntaxonomiques ont été suivis dans le but d'évaluer les changements écologiques survenant après des travaux de restauration d'écosystèmes aquatiques, comme les macro-invertébrés, les poissons, les oiseaux et les communautés végétales. Les communautés végétales font partie des trois groupes syntaxonomiques privilégiés dans l'évaluation du succès de projets de restauration. L'intérêt de suivre les communautés végétales aquatiques réside dans le fait qu'elles participent à certaines fonctions écologiques, comme la rétention des nutriments et de sédiments, qu'elles ne sont pas mobiles, et qu'elles répondent à de nombreux facteurs locaux tels que la nature du substrat, la vitesse du courant, la lumière et la trophie de l'eau. Ces réponses justifient de leur rôle bio-indicateur du niveau trophique et/ou de la pollution organique de l'eau.

Dans la plaine du Rhin en Alsace, d'anciens bras du Rhin ont été reconnectés au cours principal. Les recherches se sont focalisées sur l'étude et l'analyse des processus de recolonisation et de la dynamique de la biodiversité dans ces bras restaurés. Elles ont débuté par une évaluation de l'état écologique avant la restauration de certains de ces bras (Trémolières, 2004). Elles se sont poursuivies par une analyse de l'efficacité et de la réussite des opérations de restauration au travers de l'indicateur « macrophytes aquatiques » (Meyer, 2012), avec l'objectif de montrer non seulement l'état instantané de la colonisation végétale, mais aussi la dynamique d'évolution des communautés de macrophytes.

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[1] CSA : Conservatoire des sites alsaciens.

[2] ONF : Office national des forêts.

Ces travaux sur la restauration des bras morts du Rhin nous ont conduits à poser la question suivante : les macrophytes, déjà utilisés en bio-indication de la qualité de l'eau et du fonctionnement hydraulique, peuvent-ils être des indicateurs du succès de la restauration ? La réponse à cette question s'appuie sur les recherches et observations de terrain menées dans le cadre des travaux cités précédemment.

❶ MÉTHODOLOGIE

Des suivis de la végétation aquatique (identification des espèces et de leur abondance) ont été menés dans douze anciens bras du Rhin dans la plaine d'Alsace : neuf bras reconnectés au fleuve entre 1998 et 2006, et trois bras dits de référence, c'est-à-dire d'anciens bras du Rhin n'ayant jamais été déconnectés du cours principal (photo ❶).

Des relevés phytosociologiques ont été effectués sur cinq transects transversaux situés dans les 200 premiers mètres après la connexion (figure ❶), entre 2007 et 2011, et couplés à des mesures hydro-géomorphologiques (largeur et profondeur du bras, identification et cartographie des substrats, mesures du débit et de la vitesse du courant).

Les relations existant entre les compartiments eau-sédiment-plantes et l'influence de ces relations sur la recolonisation par les macrophytes ont été analysées au travers des mesures des teneurs en nutriments (P phosphore et N azote) dans l'eau, le sédiment et les principales espèces de macrophytes.

Nous avons aussi étudié les flux de diaspores (graines, rhizomes, fragments, bulbes, bourgeons, turions, etc.) dans les bras, flux pouvant ainsi participer à la recolonisation. Les stratégies de recolonisation ont été analysées au travers de treize traits biologiques : quatre traits morphologiques, comme la taille potentielle, la forme de croissance (non ancrée, ancrée avec feuilles flottantes, ancrée avec tissus de soutien…), cinq traits écologiques comme la tolérance aux variations d'humidité (résistance à l'exondation) ou le niveau trophique des espèces, et quatre traits liés à la reproduction et à la dispersion. L'évolution temporelle de ces traits dans les communautés de macrophytes a été suivie en couplant tableau de traits par espèce et composition floristique des communautés.


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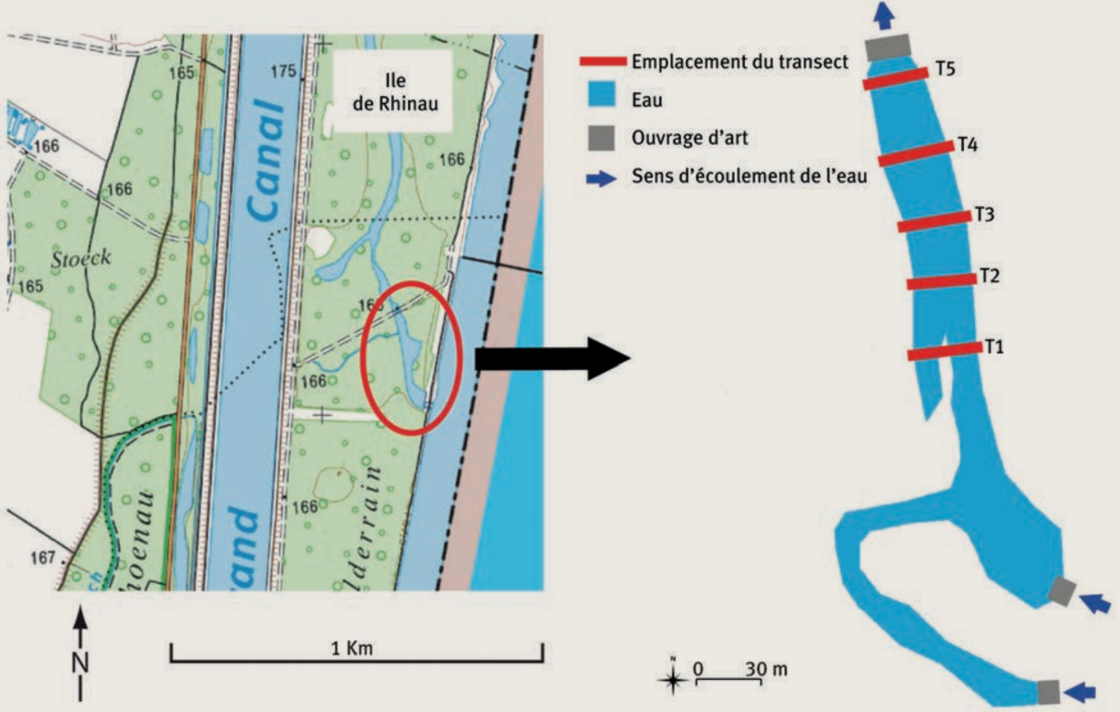
❶ **Le Schaftheu, un des trois bras de référence, situé sur l'Ile de Rhinau.**

❶ **Localisation générale et emplacement des transects sur un des bras de référence, le Schaftheu.**



Communautés végétales et dynamique de recolonisation

En 2011, après quatre années de suivi, trois communautés végétales ont été recensées dans les douze bras étudiés (figure ❷). Deux de ces communautés sont caractérisées, l'une par des espèces eutrophes (*Elodea nuttallii, Ceratophyllum demersum, Myriophyllum spicatum, Potamogeton pectinatus* et *P. perfoliatus*), et l'autre par des espèces mésotrophes (*Berula erecta, Callitriche obtusangula* et *Sparganium emersum*). Elles ont été recensées à la fois dans les bras restaurés et dans les bras de référence. Trois bras se sont différenciés des autres, par l'installation d'une communauté d'espèces préférant des vitesses de courant fortes, indépendamment de leur statut trophique (*Ranunculus fluitans, Fontinalis antipyretica* et *Hildenbrandia rivularis*).

Ainsi un tiers des bras restaurés présente une communauté différente de celles retrouvées dans les bras de référence. La comparaison avec les sites de référence ne permet donc pas de conclure au succès de la restauration sur cette seule base.

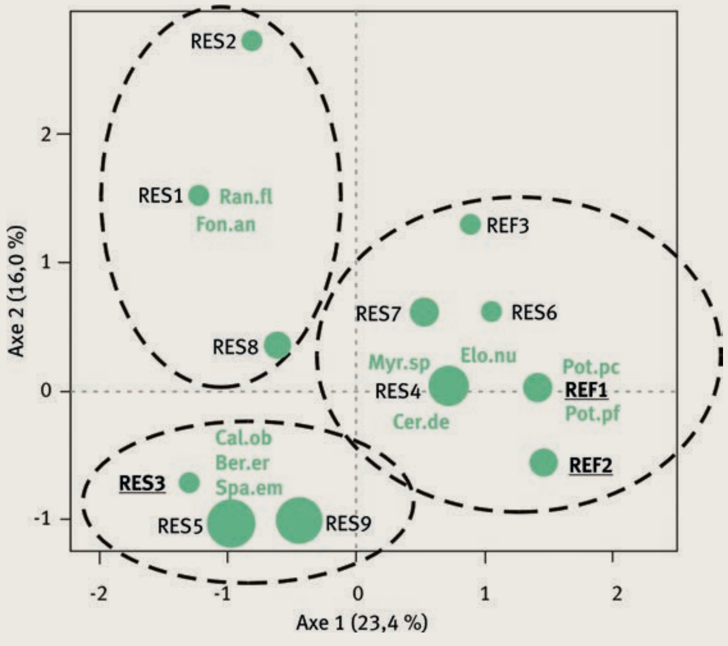
Par ailleurs, le temps écoulé depuis le début de la restauration influence la phase de recolonisation, c'est-à-dire celle durant laquelle la communauté se reconstitue. Après cette phase, la communauté se stabilise et devient indépendante du temps écoulé depuis la restauration. La phase de recolonisation des bras restaurés par la végétation aquatique est un phénomène rapide, en particulier lorsque les conditions sont optimales (présence de substrats adaptés, diaspores abondantes) et dure vraisemblablement moins de trois ans en moyenne. Ainsi Henry *et al.* (2002) ont montré que la reconnexion des bras du Rhône a provoqué une augmentation rapide de la richesse spécifique et du recouvrement végétal des macrophytes, ceci en moins de quatre ans après les travaux de restauration. L'utilisation des macrophytes comme un outil indicateur dans le cadre d'une comparaison avec des communautés de référence nécessite donc *a minima* un délai de trois ans.

Les macrophytes indicateurs de la trophie

La restauration d'une rivière peut provoquer des modifications du niveau trophique. Toutefois, on a vu que les communautés nouvellement installées ne répondaient pas uniquement aux variations du niveau trophique de l'eau. L'évaluation du niveau trophique réel nécessite de prendre en compte les trois compartiments : l'eau qui véhicule des nutriments solubles, le sédiment réservoir de nutriments et la plante bio-accumulatrice qui absorbe à partir de l'eau et/ou du sédiment et relargue ces éléments par décomposition et minéralisation de la matière organique.

La déconnexion des bras latéraux provoque généralement une oligotrophisation de l'eau par des apports d'eau souterraine pauvre en nutriments et la reconnexion assure un retour d'eau eutrophe en provenance du fleuve. La déconnexion a pu alors favoriser le développement des espèces mésotrophes (Henry *et al.*, 2002), alors que

❷ Distribution des espèces (en vert foncé) et des bras (en gris et noir – REF bras de référence, RES bras restauré) dans le plan factoriel F1 x F2 d'une analyse des correspondances réalisée sur les données floristiques. La surface des points indique la richesse spécifique de chaque bras.



Ber.er : Berula erecta ;
Cal.ob : Callitriche obtusangula ;
Cer.de : Ceratophyllum demersum ;
Elo.nu : Elodea nuttallii ;
Fon.an : Fontinalis antipyretica ;
Myr. p : Myriophyllum spicatum ;
Pot.pc : Potamogeton pectinatus ;
Pot.pf : P. perfoliatus ;
Ran.fl : Ranunculus fluitans ;
Spa.em : Sparganium emersum.

la reconnexion devrait provoquer le retour des espèces eutrophes. Dans les bras reconnectés au Rhin, le niveau trophique de l'eau varie faiblement, de mésotrophe à eutrophe selon les bras. En revanche on a observé une différence significative d'accumulation de nutriments dans les sédiments en lien avec la diversité des substrats selon les bras étudiés : un sédiment fin, argilo-limoneux, contient plus de nutriments susceptibles d'être relargués dans l'eau et/ou de matières organiques. Les bras aux sédiments riches en nutriments sont aussi les bras au couvert végétal et/ou à la richesse spécifique les plus élevés. Les espèces les plus abondantes, comme *Elodea nuttallii* et *Myriophyllum spicatum*, privilégient des sédiments fins propices à leur enracinement et riches en nutriments. D'autres macrophytes comme *Callitriche obtusangula* ralentissent la vitesse du courant et accentuent encore les dépôts de sédiments fins. Les interactions sédiments fins/macrophytes expliquent probablement les relations étroites observées entre les teneurs en nutriments dans le sédiment et celles des plantes, alors qu'elles n'existent pas ou peu entre eau et plante. Ainsi les teneurs en phosphore des plantes sont directement liées au phosphore biodisponible du sédiment.

On peut se poser la question du lien entre la capacité d'une espèce à accumuler des nutriments et sa vitesse de recolonisation. Aucune relation significative entre la teneur en nutriments de la plante et son recouvrement n'a pu être observée. Néanmoins, si on étudie le rapport N/P, on constate que les espèces aux rapports N/P les plus faibles sont les espèces les plus abondantes. Un rapport faible, inférieur à 10, indiquerait que l'azote est le facteur limitant de la croissance des macrophytes. Ainsi les espèces qui peuvent croître avec un rapport N/P faible seraient favorisées lorsque l'azote devient limitant.

Les macrophytes indicateurs des caractéristiques hydro-morphologiques

Alors que le niveau trophique des eaux est relativement comparable dans tous les bras, trois communautés différentes ont été observées dont deux se distinguent par l'exigence trophique des espèces. En fait, on a constaté que la communauté « eutrophe » colonise les bras larges et profonds avec une faible vitesse du courant, la communauté « rhéophile » occupe des bras peu profonds et étroits, avec une granulométrie grossière, une vitesse du courant forte et un ombrage important, tandis que la communauté « mésotrophe » colonise les bras présentant globalement des caractéristiques intermédiaires : ce sont des cours d'eau étroits et profonds avec une vitesse du courant moyenne, un ombrage fort, et une granulométrie plutôt grossière. Il apparaît que ce sont prioritairement les caractéristiques physiques des bras qui contrôlent la distribution des communautés.

L'influence des paramètres hydro-morphologiques sur la composition des communautés végétales nécessite de prendre en compte ces paramètres lors des projets de restauration dans les systèmes fluviaux. Quel que soit l'indicateur utilisé, une augmentation de l'hétérogénéité des habitats conduit à l'augmentation du nombre d'habitats, et donc de la richesse spécifique globale du système fluvial. Ainsi des rivières re-méandrées aux Pays-Bas et au Danemark présentent après une dizaine d'années une richesse taxonomique et une abondance accrues des macro-invertébrés et/ou des macrophytes, richesse expliquée par une hétérogénéité (recréée) des substrats plus importante que celle de rivières similaires non restaurées (Lorenz *et al.*, 2009 ; Pedersen *et al.*, 2007).

Dispersion et propagation des diaspores

Outre les facteurs physico-chimiques, les facteurs biologiques tels que les apports de diaspores *via* la reconnexion pourraient faciliter la recolonisation du site nouvellement créé. On a donc vérifié si la composition des communautés pouvait être liée à la composition des flux de diaspores.

Les espèces non ancrées, telles que les lentilles d'eau (des Lemnides) et la fougère *Azolla filiculoides*, sont les espèces ayant la plus forte quantité de diaspores en dérive (84 % de l'ensemble des flux de diaspores). On a ensuite recensé quatre espèces bien représentées sur les sites étudiés, *Ranunculus fluitans, Elodea canadensis, E. nuttallii* et *Callitriche obtusangula*, avec une quantité élevée de diaspores en dérive (jusqu'à 25 000 diaspores/jour/tronçon), alors que cinq autres espèces également fréquentes, *Potamogeton berchtoldii, P. pectinatus, Ceratophyllum demersum, Myriophyllum spicatum, Cladophora* sp., ne présentent qu'une quantité moyenne à faible de diaspores en dérive (moins de 5 000 diaspores/jour/tronçon). Les espèces restantes ont des flux totaux inférieurs à 1 000 diaspores/jour/tronçon. Les bras avec les flux les plus importants sont aussi les bras avec la plus grande richesse spécifique de diaspores. On a noté que les flux amont et aval ont des compositions floristiques plus proches entre eux qu'avec la végétation établie. Par ailleurs, on a constaté que si la composition floristique des flux amont influence peu la composition des communautés, cette dernière influence la composition des flux aval. La végétation établie participe donc à la composition et à l'abondance des diaspores dans le flux aval.

L'étude des flux de diaspores immédiatement après les travaux de reconnexion donne une indication des espèces de macrophytes susceptibles de recoloniser le nouveau milieu. En couplant l'étude des flux avec les caractéristiques hydro-morphologiques du bras restauré, il devient possible de modéliser et donc de prédire la communauté qui recolonisera le bras restauré.

Le rôle des banques de diaspores contenues dans les sédiments apparaît négligeable dans la recolonisation par rapport à celui des flux de diaspores transportées par l'eau. Néanmoins, des diaspores végétatives persistent au niveau du sédiment (rhizomes, bulbes) et participent ensuite à la stabilisation de la communauté « recolonisante ».

Les assemblages de traits biologiques

Les traits biologiques sont un outil de plus en plus privilégié dans l'étude des communautés. Ainsi certains traits biologiques sont maintenant intégrés dans le calcul d'un indice d'évaluation de l'état écologique des cours d'eau fondé sur les macro-invertébrés (nouvel indice I2M2). Les études des traits biologiques des macrophytes sont plus rares, et inexistantes dans les suivis post-restauration. L'analyse des assemblages de traits dans les bras permet de vérifier si la reconnexion peut favoriser certains traits biologiques au cours de la recolonisation végétale des bras restaurés, et de préciser les facteurs en cause.

Les espèces identifiées dans les communautés forment, à partir de leurs traits biologiques, quatre groupes d'espèces qui se différencient essentiellement par leur forme de croissance : hélophytes, hydrophytes strictes, amphiphytes et pleustophytes. Les quatre groupes sont présents dans tous les bras, mais l'abondance de chaque groupe dépend des caractéristiques hydro-morphologiques de ces bras. Ainsi, les bras de référence présentent trois assemblages distincts. Globalement les bras restaurés ont des assemblages de traits similaires à ceux des bras de référence. Cependant des assemblages originaux liés à une surabondance occasionnelle de certaines formes de croissance peuvent être observés. Par exemple, certains bras présentent certaines années une abondance accrue de pleustophytes, signe éventuel de l'absence de dynamique fluviale liée à des variations du débit et du niveau d'eau dans ces bras durant ces années. *A contrario,* la fréquence du trait de résistance aux exondations augmente dans les bras où des fluctuations de débit sont observées.

L'étude des assemblages de traits biologiques au sein des communautés de macrophytes est une approche novatrice qui permet de définir des références, voire des objectifs de restauration, autrement que par la seule définition d'une liste d'espèces-cibles. De plus, les traits biologiques donnent des informations supplémentaires sur le retour des fonctions écologiques. Néanmoins, l'apport des traits biologiques dans des suivis post-restauration et/ou dans des calculs d'indice nécessiterait la mise en place d'une banque de données, à l'instar des banques créées pour les espèces terrestres, banque établissant les liens existants entre traits biologiques et fonctions écologiques.

Les macrophytes : des indicateurs de la restauration de la fonctionnalité écologique

La reconnexion de bras morts au fleuve est une modalité de restauration pour retrouver la continuité écologique au sein de l'hydrosystème fluvial. La fonctionnalité écologique s'évalue, dans ce contexte, par la reprise de la dynamique fluviale, la réinitialisation des transferts avec le cours actif, l'adaptation et/ou la recolonisation des espèces végétales et animales et des communautés proches des communautés-cibles.

Sur une période courte après la restauration, nous avons montré que les bras reconnectés au Rhin sont colonisés par des communautés de macrophytes relativement stables, proches des communautés-cibles définies dans les bras de référence. De plus la richesse spécifique des bras restaurés est comparable, voire dans certains cas, supérieure à celle des bras de référence. Les dynamiques saisonnières des nutriments dans les compartiments eau, sédiment et plantes, ainsi que les flux de diaspores, s'avèrent également similaires entre bras restaurés et bras de référence. Ces résultats révèlent une réinitialisation des transferts depuis le chenal actif. La reprise d'une dynamique fluviale liée à des variations du débit et du niveau d'eau est observée dans certains bras à travers l'évolution des assemblages de traits biologiques mais est peu efficace, les débits d'entrée étant encore trop faibles. Certaines fonctions n'ont pas été encore totalement rétablies, notamment la dynamique hydro-géomorphologique. La reconnexion a ainsi participé au rétablissement de quelques fonctions écologiques d'un système « endommagé ». Elle constitue une première étape dans l'amélioration de l'état écologique des systèmes fluviaux. Les macrophytes, du fait de leur rapidité d'installation, de leurs stratégies adaptatives développées au cours de la recolonisation et de leur capacité intégrative vis-à-vis des facteurs du milieu, notamment la dynamique de l'eau et des nutriments, semblent de bons candidats pour une évaluation du succès de l'opération de reconnexion des bras latéraux dans les hydrosystèmes fluviaux. ■

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RESEARCH ARTICLE

How do instream flow increase and gravel augmentation impact biological communities in large rivers: A case study on the Upper Rhine River

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Abstract

Actions are being developed to address the adverse consequences of engineering works on large European rivers by developing and implementing restoration activities in order to enhance the functionality and biodiversity of fluvial hydrosystems. However, as has frequently been mentioned in the scientific literature, quantitative and qualitative evaluation of the project benefits, if any, and their sustainability are hindered by the difficulty in assessing the responses of aquatic and riparian communities to the methods employed. A case study was conducted on a by-passed section of the Upper Rhine River (France and Germany) to investigate the effects of instream flow increase and gravel augmentation on selected aquatic and riparian communities (macroinvertebrates, macrophytes, and riparian plants). This paper presents the results of a 6-year interdisciplinary, before-after control-impact design monitoring study. The complexity of the study lies in carrying out a separate assessment of the cumulative effects on a site-based, project-specific basis. The results showed that (a) the instream flow increase resulted in greater richness of macrophyte species in the newly created backwaters, (b) the artificial gravel bar favoured the recruitment of pioneer species, including invasive species, although gravel redistribution by floods prevented their development, and (c) gravel augmentation tended to promote the taxonomic richness of macroinvertebrate communities with the appearance of species adapted to the new substrate areas. These findings should help to fill the knowledge gaps in large-scale restoration and contribute key responses to the most frequently arising issues in this area, especially those concerning the efficiency and sustainability of river restoration projects.

KEYWORDS

biodiversity, ecological restoration, invasive species, large rivers, macroinvertebrates, riparian plants

1 | INTRODUCTION

Over the last two centuries, the majority of large European rivers have been significantly affected by engineering works such as channelization, damming, construction of groyne fields, by-passing, and water diversion for the purposes notably of flood protection, navigation, and hydro-electricity production (Petts & Gurnell, 2005; Sparks, 1995). These structural modifications have disturbed the natural hydrological regimes and sediment fluxes (Heiler, Heim, Schiemer, & Bornette, 1995), inducing a loss of spatio-temporal heterogeneity in river habitats (Amoros & Bornette, 2002; Friberg, 2014). Many authors have demonstrated the impact that river engineering has had on ecosystem integrity (Clewell & Aronson, 2010; Malmqvist & Rundle, 2002; Petts, 1984): depletion/loss of alluvial-specific species (pollution-sensitive species, riparian plants), colonization by invasive species, that is, exotic species whose growth rates are not strongly regulated, often resulting in community dominance (David et al., 2017) and thus diminishing the diversity of native plant life forms.

The 1960s and 1970s saw the emergence of the first “corrective” interventions to restore hydrosystem functionalities (Bernhardt et al., 2005; Brookes & Shields, 1996; Poff et al., 1997; Wissmar & Bisson, 2003). Action plans aiming at improving the morphodynamic and ecological processes in large rivers incorporate strategies such as embankment removal, gravel augmentation, instream flow increase, side arm reconnection, or floodplain restoration (Schanze, Olfert, Tourbier, Gersdorf, & Schwager, 2004). In the case of a by-passed river reach, an instream flow increase is needed to ensure the development of riparian plants and macrophytes, which may be influenced by an elevated water table (Naiman, Decamps, & Pollock, 1993). Increasing the instream flow also increases the wetted perimeter (Gurnell, Bertoldi, & Corenblit, 2012), potentially opening up new areas for macrophyte or macroinvertebrate species colonization, thus creating a favourable spawning habitat for fish (Garnier & Barillier, 2015; Gurnell, Van Oosterhout, De Vlieger, & Goodson, 2006).

Another increasingly common practice in river restoration is gravel augmentation, which is the artificial addition of bed material into the channel in order to restore former rates of bedload transport downstream of dams. This type of restoration strategy was first developed in the 1960s in the western USA (Bunte, 2004). In Europe, gravel augmentation projects are more recent, and activities are focused on reducing the downstream propagation and mitigating the adverse ecological impacts of sediment starvation (Klösch, Hornich, Baumann, Puchner, & Habersack, 2011; Schälchli, Breitenstein, & Kirchhofer, 2010; Schanze et al., 2004). Although the intention is generally to increase substrate heterogeneity (Merz, Chan, & Leigh, 2005), gravel augmentation may also improve surface and groundwater water mixing and hence the diversity of benthic and hyporheic zones (Wawrzyniak et al., 2016). However, the results of gravel augmentation have generally been assessed through geomorphological monitoring, which is based, in most cases, on in-channel and bed grain size measurements that aim at tracking changes in channel geometry and grain size distributions (Gaeuman, 2014; Kantoush, Sumi, & Kubota, 2010). Only a few studies have investigated the ecological effects of gravel augmentation (Harvey et al., 2005; Ock, Gaeuman, McSloy, & Kondolf, 2015), and there has been even less feedback from interdisciplinary studies (ecology linked to geomorphology) on large river restorations (Buijse et al., 2002). The complexity of fluvial hydrosystems (notably in terms of temporal and spatial variabilities) and limited interdisciplinary expertise (Darby & Sear, 2008) creates challenges in the assessment of multicompartment, ecological impacts of restorations, including the potential postrestoration colonization of newly created niches by invasive macrophytes or macroinvertebrate species (Combroux, Gudrun, & Amoros, 2002; Paillex, Castella, zu Ermgassen, & Aldridge, 2015; Shea & Chesson, 2002). The majority of large European rivers, such as the Rhine, represent an incredible melting pot of invasive species (Beisel, 2001; Beisel, Peltre, Kaldonski, Hermann, & Muller, 2017; Leuven et al., 2009), which have been introduced from almost all over Europe and around the world.

The purpose of this paper is to investigate the responses of selected aquatic and riparian communities to a restoration programme involving both an instream flow increase and gravel augmentation in a large river. The study was conducted in the channel of the Old Rhine downstream of the Kembs dam (on the French–German border). In a context of a dual-approach and multiscale restoration project, the study objectives were (a) to describe the responses of biological communities to these restoration actions, (b) to measure the permeability of the restored ecosystem to invasive species, and (c) to determine both the individual and cumulative consequences of the restoration actions. The difficulty of evaluating biological responses and consequently of assessing the success or failure of restoration actions is often due to inappropriately short postrestoration follow-ups (Bernhardt et al., 2005; Kondolf et al., 2007). Consequently, a more general aim of the paper is to provide feedback on both the efficiency and the sustainability of the restoration plan based on data collected over a longer (6 years) period, which is crucial for both scientific and operational decision-making purposes.

2 | STUDY AREA

With a length of 1,250 km and a drainage basin of 185,260 km^2, the Rhine is one of the largest European rivers (Figure 1a). The hydrological regime of the Upper Rhine (France and Germany) is nivo-glacial, with high flows occurring in June and July (CHR, 1977). The upstream part of the Upper Rhine River from Kembs (kilometric post (KP) 174), near the Franco-Swiss border, to Breisach (KP 225) is divided by the Kembs diversion dam into two waterways that were subject to regulation works conducted between the mid-19th to the mid-20th centuries (Figure 1b). A maximum flow of 1,400 m^3 s^{-1} is diverted into the "Grand Canal d'Alsace," which encompasses four hydroelectric power plants: Kembs, Ottmarsheim, Fessenheim, and Vogelgrün (Figure 1b). Until December 2010, the flow rate through the by-passed Old Rhine was 20 to 30 m^3 s^{-1}, which is around 1/40 of the mean annual discharge at Basel. Overflows exceeding 1,400 m^3 s^{-1} (the maximum discharge in the Grand Canal d'Alsace) are routed to the Old Rhine. This threshold was exceeded an average of 69 days per year between 1932 and 2011, meaning a minimum flow was maintained in the Old Rhine for 81% of the time. The poor ecological functionality exhibited by the Old Rhine was largely due to hydromorphological alterations, including channel dewatering, bed incision, bed armouring, and static fluvial forms.

The gravel augmentation was carried out within the framework of the European INTERREG programme "Redynamisation of the Old Rhine" (2009–2012). The primary objective was to increase bedform mobility to create hydraulic and habitat diversity, that is, increased channel heterogeneity with new channel bar units, secondary channels, and backwaters (Arnaud et al., 2017). In October 2010, 23,000 m^3 of gravel was supplied to the channel 8 km below the Kembs dam (KP 182.4). This volume corresponds to the mean annual bedload transport capacity estimated on this reach prior to regulation (El kadi Abderrezzak, 2009). Sediments were dumped into the channel in a trapezoidal form: 620 m length, 11 to 15 m width, and 2.0 to 3.5 m height, parallel to, but separated from, the right bank. The 110-m-wide channel is bordered by short groynes and steep, vegetated embankments on the right bank, and by long (100 m), vegetated groyne fields on the left bank. The channel slope is 0.001 m m^{-1}.

Gravel was taken from a flood control area excavated on the nearby floodplain and is shown in Figure 1c. Consequently, the added material was expected to be representative of preriver engineering conditions, with a grain size finer than the present armoured riverbed. Even though the sediments were added without any sorting, it was determined that the in situ material was consistent with the flora and

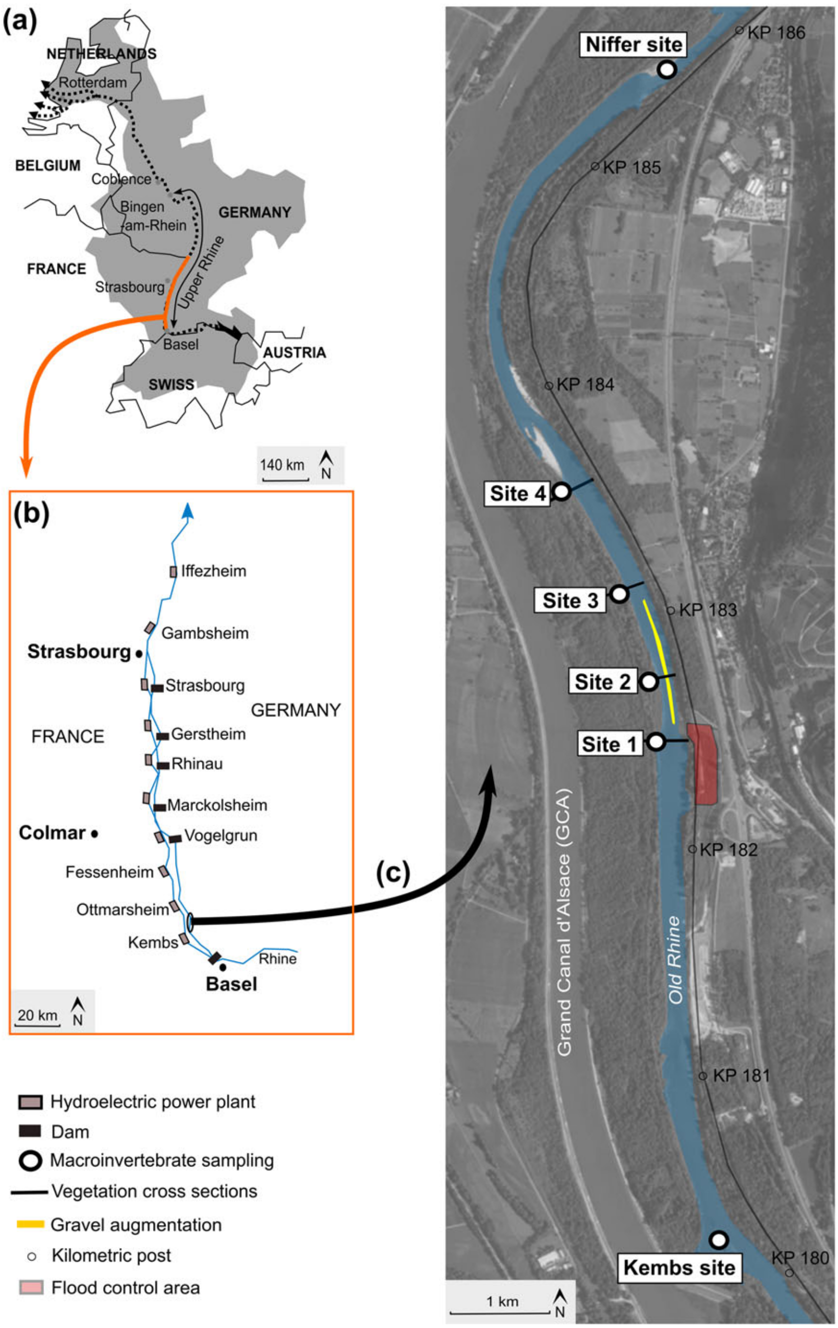


FIGURE 1 (a) Location of the Upper Rhine in the Rhine River Basin. (b) Location of the Old Rhine River, delimited by the Basel and Vogelgrun dams. (c) Location of gravel augmentation (in yellow), with the monitoring sites (Sites 1, 2, 3, and 4) flanking the gravel augmentation and the upstream–downstream sites, respectively, Kembs and Niffer (orthophotography from 2016). [Colour figure can be viewed at wileyonlinelibrary.com]

fauna local species pool, ensuring that there were no unintentional species introductions. Sediment samples taken prior to excavation showed a median particle size (D_{50}) between 12 and 46 mm for the injection material versus 79 mm for the armoured riverbed (Dittrich, Koll, Kunz, & Huppmann, 2010). The fraction of material <2 mm in the sediment samples, and thus presumably in the unsorted supplied sediment, was small (mean value of 15%).

In December 2010, the instream flow was increased from 20–30 to 52–150 $m^3 s^{-1}$ to meet minimum flow requirements for aquatic and riparian communities. A hydraulic model was used to calculate increase values for the entire Old Rhine River. The model results showed that the instream flow needed to vary seasonally with regard to the habitat structure preferenda of the aquatic species (Schneider, Giesecke, Zöllner, & Kerle, 2001). Flow variations start in the spring, the maximum instream flow (150 $m^3 s^{-1}$) is reached early in the summer, and the instream flow decreases in late summer-early autumn.

3 | METHODS

3.1 | Monitoring framework

Arnaud et al. (2017) investigated changes in bed forms and sediment transport over a 5-km-long reach downstream of the gravel augmentation between 2009 and 2014 via topo-bathymetric surveys, radio-frequency particle tracking, and bed grain size measurement. A biological monitoring programme was also implemented along an upstream–downstream gradient between KP 180 and 186. Vegetation cross sections and invertebrate sampling were carried out at the four sites close to the gravel augmentation (Sites 1, 2, 3, and 4; Figure 1c). Additional macroinvertebrate sampling was conducted further upstream, at the Kembs site (near KP 180), and downstream, at the Niffer site, near KP 186 (Figure 1c), in order to complete our analysis. Monitoring data from the upstream and downstream sites was used to discriminate between the impacts of the gravel augmentation (the Kembs site and the Site 1 are upstream and thus not impacted by the gravel augmentation) and those of the instream flow increase that impacted the entire Old Rhine River.

The monitoring programme was based on the before-after control-impact design (Smith, Orvos, & Cairns, 1993), with the spatial arrangement of biological sites defined with respect to the downstream propagation of the added gravel. This enabled biological sites to be classified over time into three categories (Figure 3b): control, not impacted, and impacted. The term “control” is applied to a site that has not been subject to restoration action(s). The term “not impacted” indicates that the sedimentary wave had not yet propagated to the site, whereas the term “impacted” indicates that the sedimentary wave reached the site. For the temporal scale (before-after), three periods were characterized between 2008 and 2014 (see Figure 2): (a) Period “before” (PB): prior to the gravel augmentation (October 2010) and instream flow increase (December 2010), (b) Period 1 (P1): after physical restoration (implementation of both restorative measures) and flood events including an annual flood in December 2010 (instantaneous maximum discharge at Basel, Q_{imax_Basel} = 2,480 $m^3\ s^{-1}$) and 2-year return period floods in June 2012 (Q_{imax_Basel} = 2,740 $m^3\ s^{-1}$) and October 2012 (Q_{imax_Basel} = 2,900 $m^3\ s^{-1}$) but before a 15-year return period flood (Q_{15}) that occurred in June 2013 (Q_{imax_Basel} = 3,880 $m^3\ s^{-1}$), and (c) Period 2 (P2): after the Q_{15} flood.

3.2 | Sampling methods

Sampling campaign periods are shown in Figure 2. Cross sections were used to monitor changes in vegetation over time at Sites 1, 2, 3, and 4, in the main channel, on the river banks and on the artificial gravel bar (black lines on Figure 1c). Vegetation surveys were performed according to the phytosociological method (Braun-Blanquet, 1932) in (a) summer 2010 (PB), (b) summer 2011, and 2012 (P1), and (c) summer 2013 and 2014 (P2) in specific areas: the left bank (LB); Aquatic left side (Aq. LS); on the remains, if any, of the artificial gravel bar (Bar); Aquatic right side (Aq. RS) and on the right bank (RB).

Invertebrate sampling was performed near the left bank (Figure 1 c) at the same four sites, using a 300 × 400 mm high, stainless steel 500 μm Hess Stream Sampler (Hess, 1941) with an attached 1,000 μm dolphin bucket, until the water level became too high, in May 2014 (P2), to wade in the channel. For these sites, an invertebrate sampling is the sum of four samples collected from within the whole range of existing microhabitats, defined as all combinations of substrate types and flow velocity range (Beisel, Usseglio-Polatera, & Moreteau, 2000; Beisel, Usseglio-Polatera, Thomas, & Moreteau, 1998). At the Kembs and Niffer sites, macroinvertebrate sampling was carried out with a Surber sampler (mesh size 500 μm) at three dates that were at least 2 weeks after flood events (May 2008, May 2011, and May 2014) and in accordance with the “Standardized Global Biological Index” (IBGN) protocol (AFNOR, 2004).

3.3 | Data analyses

Structures of the biological communities were evaluated using four diversity indices; specifically, taxonomic richness (S), the Shannon index H′, Simpson index λ, and the Shannon evenness index J'. Taxa considered as pollution-sensitive are denoted EPTC, which signifies *Ephemeroptera*, *Plecoptera*, *Trichoptera*, and *Coleoptera*. Despite the reported use of different methods for invertebrate sampling (Hess and Surber), a comparison assessment of communities can be made with a multivariate approach. Factorial correspondence analysis was used to observe changes in invertebrate communities between all the sites over time and was implemented in the ADE4 library for R software (v3.4.1; R Core

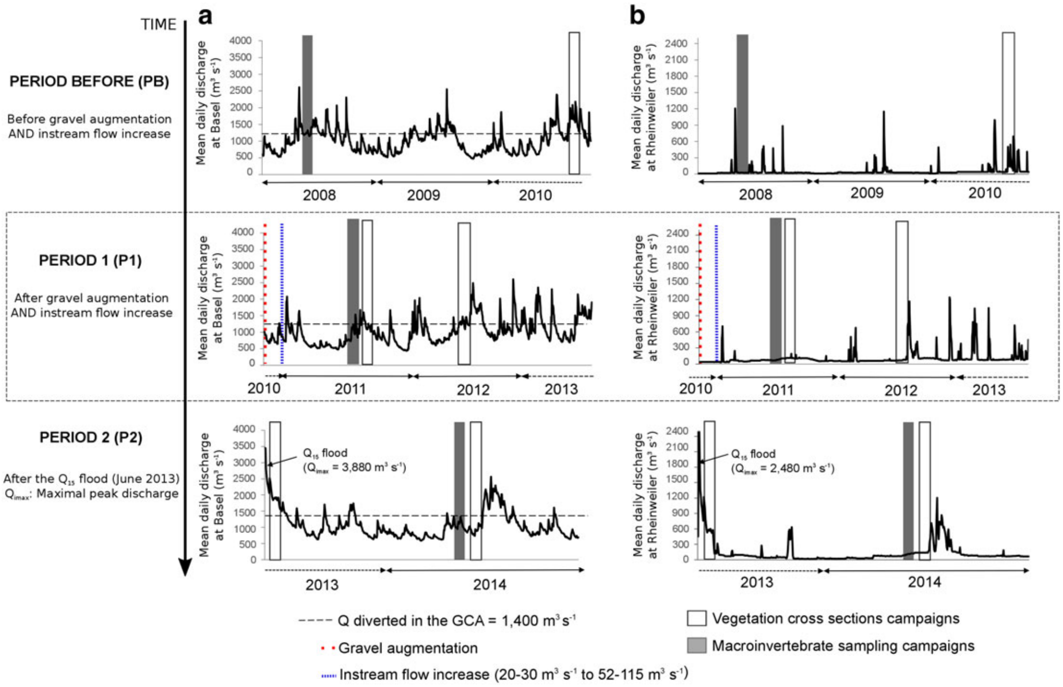
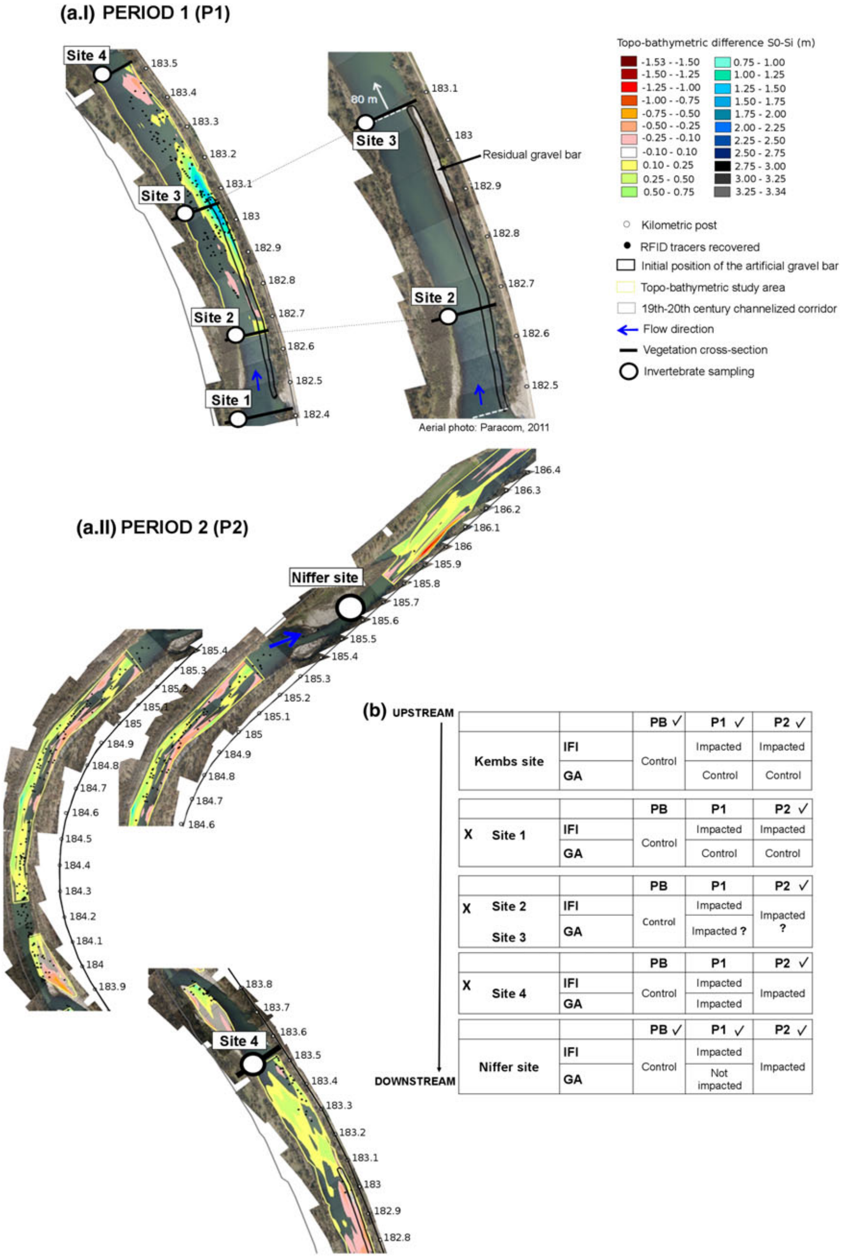


FIGURE 2 Mean daily flow hydrographs from 2008 to 2014 at (a) Basel-Rheinhalle (upstream of the Kembs dam) and at (b) Rheinweiler (15 km downstream of the Kembs dam, in the Old Rhine) illustrated the temporal scale (before-after). [Colour figure can be viewed at wileyonlinelibrary.com]



		PB ✓	P1 ✓	P2 ✓
Kembs site	IFI	Control	Impacted	Impacted
	GA		Control	Control

		PB	P1	P2 ✓
X Site 1	IFI	Control	Impacted	Impacted
	GA		Control	Control

		PB	P1	P2 ✓
X Site 2	IFI	Control	Impacted	Impacted ?
Site 3	GA		Impacted ?	

		PB	P1	P2 ✓
X Site 4	IFI	Control	Impacted	Impacted
	GA		Impacted	

		PB ✓	P1 ✓	P2 ✓
Niffer site	IFI	Control	Impacted	Impacted
	GA		Not impacted	

FIGURE 3 (a) Dispersion pattern of the augmented gravel based on topo-bathymetric surveys (blue/yellow = deposition; red = erosion) and radio-frequency tracking particles over time: (a.I) in 2011–2012 (P1) and (a.II) in 2013 (P2; Arnaud et al., 2017, modified). (b) Classification (control—impacted—not impacted) of the spatially arranged biological sites versus time period and restoration project: The instream flow increase (IFI) and the gravel augmentation (GA). Vegetation surveys were conducted for Sites 1, 2, 3, and 4 (black crosses) in all periods whereas invertebrate sampling was only performed for the time periods ticked off in the column headers. [Colour figure can be viewed at wileyonlinelibrary.com]

Team, 2017). The hierarchical levels of taxa were harmonized for the macroinvertebrate data analyses using faunistic lists of all common.

3.4 | Digitization and modelling

We investigated the effect of the instream flow increase on the water surface area and level as well as on the flow velocity. Using the ArcGIS software, we digitized the aquatic channel boundaries on aerial orthophotos (1 pixel = 20 cm) taken on 1 April 2008 (before instream flow increase) and on 8 April 2011 (after instream flow increase). A 1D hydraulic model (HEC-RAS 5.0.1), based on in situ topographic cross sections and water level surveys, was then used to run simulations for 36.5 $m^3 s^{-1}$ (before instream flow increase) and 73.5 $m^3 s^{-1}$ (after instream flow increase). Seven water level measurements (DGPS Trimble 5800 RTK, XYZ accuracy = 5 cm) spaced every 200 m on the monitoring reach were taken at 40 $m^3 s^{-1}$ for model calibration. A Manning coefficient of 0.5 that corresponds to a gravel-cobble bed river channel (Benson & Dalrymple, 1967) was applied. The maximum difference in elevation observed between in situ measurements and modelling results was ±8 cm and on average ± 5 cm.

4 | RESULTS

4.1 | Hydromorphological changes

Geomorphological monitoring revealed that the trapezoidal deposit was partially dispersed by the first flood event of December 2010. A bar of length 200 m and width 4 to 15 m remained (Figure 3a.I). A sharp deposition front was visible underwater, located 80 m downstream of the end of the initial deposit.

The major part of the residual bar and the sediment wave front disappeared with subsequent floods of June 2012 and October 2012. Tracked particles were found over a distance up to the Site 4 2 years after the gravel augmentation (P1) and downstream of the

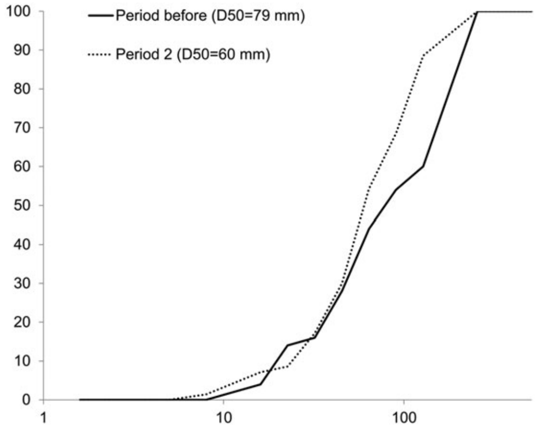


FIGURE 4 Cumulative grain size distributions at Site 4 between PB and P2

Niffer bend (KP 185.9) 4 years after the gravel augmentation (P2; Figure 3a.II). The maximum deposition height measured on the monitoring reach varied from 2.6 (after the first flood) to 1.7 m (P1) and 0.9 m (P2) with the dispersion process over time (Figure 3a.II; see Arnaud et al., 2017 for details on hydromorphological changes). The Site 1 and the Kembs site were not impacted at any time (control sites). The Sites 2 and 3, located close to the left bank, were at the left edge of the gravel dispersion pattern that mainly covered the middle of the channel and the right bank area; the impact of the gravel augmentation on these two sites is therefore questionable. The site most affected by the gravel augmentation was the Site 4, where in-channel grain size measurements on 50 × 200 m in-channel area, revealed a decrease in D_{50} from 79 to 60 mm between PB and P2 (Figure 4).

With regard to the instream flow increase, this corresponded to a measured increase in water surface area of 5,107 m^2 between 1 April 2008 (PB—190.700 m^2–36.5 $m^3 s^{-1}$) and 8 April 2011 (P1—195.807 m^2–73.5 $m^3 s^{-1}$) from KP 182 to KP 184. The 1D hydraulic modelling showed a mean water level increase of 0.41 ± 0.04 m (Figure 5) and a mean flow velocity increase of 0.20 ± 0.06 m s^{-1}. Gentle slopes on the left bank meant the rising water level had a greater impact there than on the right bank, leading to the emergence of new lateral water bodies, such as backwaters (Figure 5d). This rising water level is effectively due to the instream flow increase because the gravel augmentation (trapezoidal deposit) was designed to allow for a 10 cm maximum water level with regard to the functioning of the adjacent flood control area. The artificial deposit was dispersed after the first flood event. As the dispersion process increased through time, we assume that the influence of the gravel injection on the water level decreased and thus was much less than the effect of the instream flow increase.

4.2 | Biological community dynamics

4.2.1 | Plant community

The residual portion of the artificial gravel bar was rapidly colonized by helophytic vegetation, though with a very low coverage. The majority of the vegetation disappeared in P1 following gravel redistribution and all of the vegetation disappeared in P2 (S3, Bar in Table S1). Between P1 and P2 (after the Q_{15} flood), localized deposits (<4 m^2) of fine-sediment (sand and silt) of 20 to 30 cm in thickness were observed at Sites 2 and 3. A vegetated side channel with aquatic macrophytes including a dominant species *Callitriche obtusangula* with associated ones such as *Chara* sp., *Myriophyllum spicatum*, and *Stuckenia pectinata* appeared in P1 on the Site 3 (S3, Aq. LS in Table S1). Similar results were obtained for the Site 2, where a helophyte community took root on this new substrate but with very low cover. The riparian plant phytosociological structure of the Site 4 evolved in 2013 (P2), with a shift in dominance from the *Phalaridetum arundinaceae* community to a *Salicetum-populetum nigrae* association. Most of the riparian and aquatic invasive species, present at low cover in PB and P1, disappeared after the Q_{15} flood (P2). Only *Reynoutria*

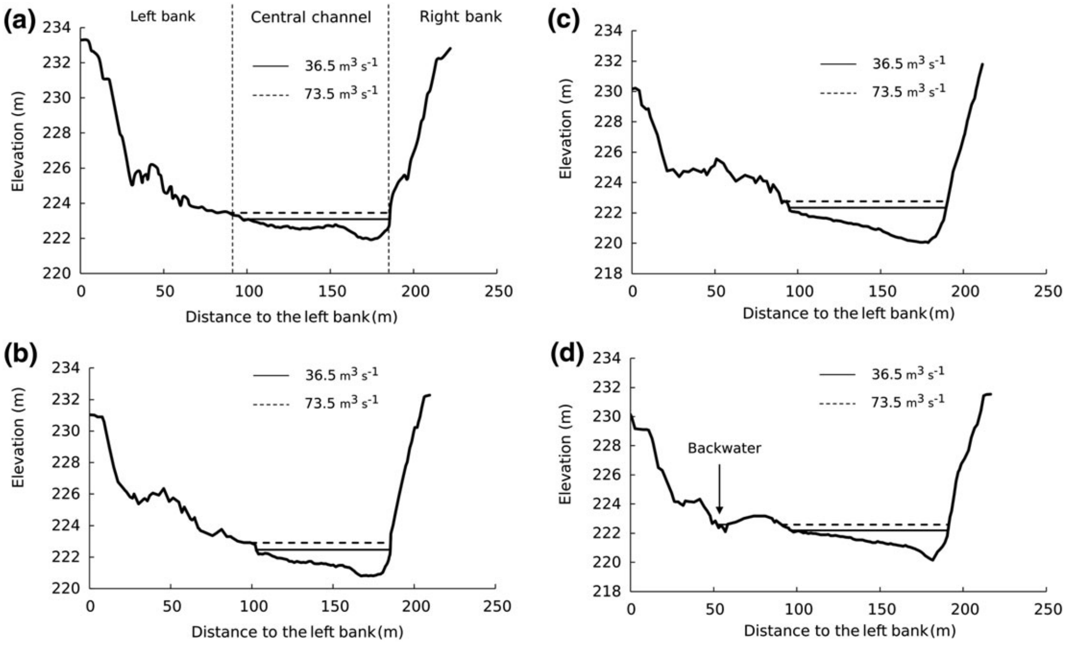


FIGURE 5 Results of water-level modelling based on 2009 cross-sectional data and instream flow increase from 36.5 (PB) to 73.5 $m^3 s^{-1}$ (P1) for Sites (a) 1, (b) 2, (c) 3, and (d) 4

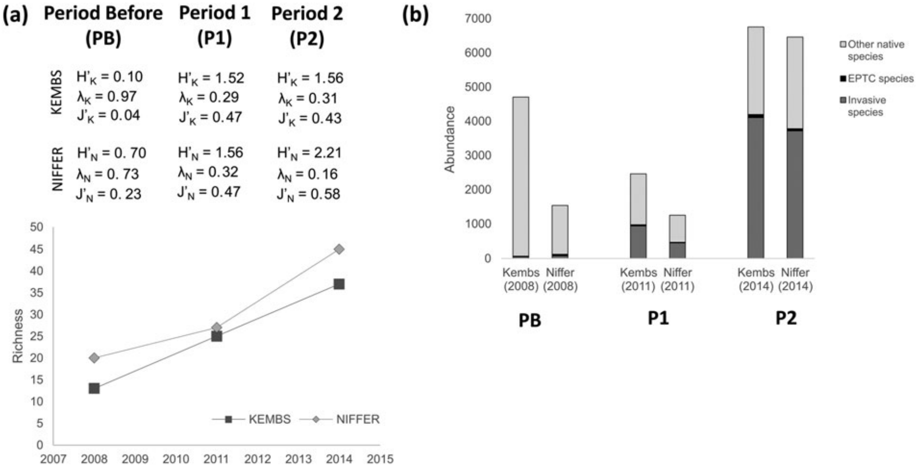


FIGURE 6 Evolution in (a) richness/diversity indices (Shannon index H′, Simpson index λ, and evenness J') and (b) abundance (EPTC, invasive, and native invertebrate species) at the Niffer and Kembs sites

japonica, with a cover of 30%, was still present on the RB at Site 3 in P2 (S3, RB in Table S1).

4.2.2 | Invertebrate communities

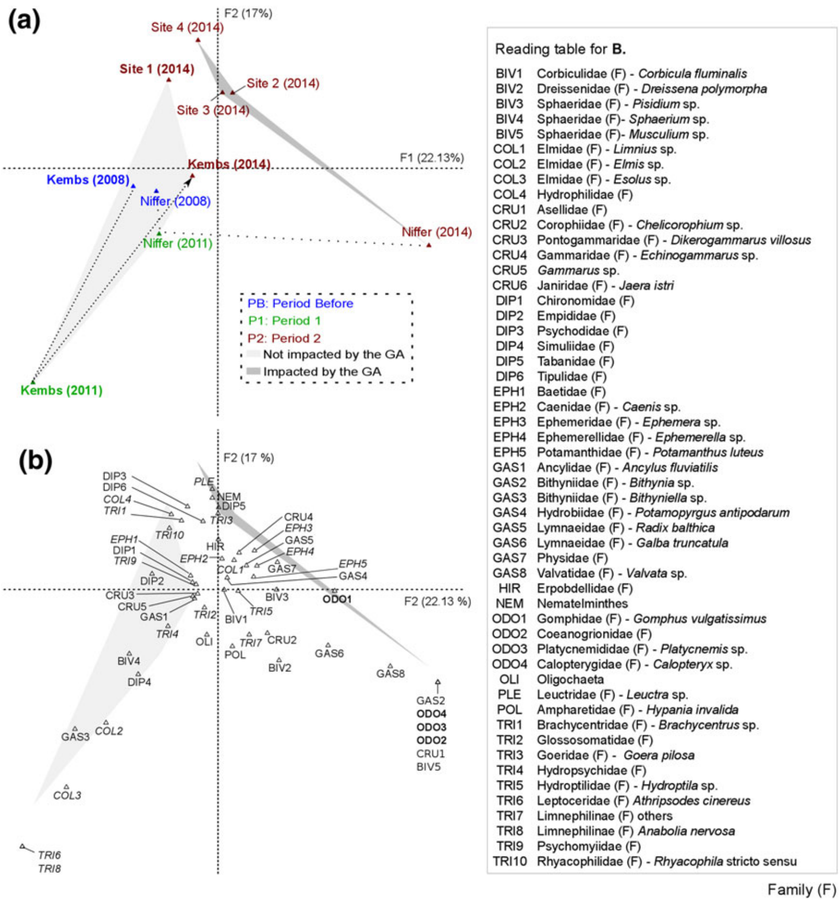
The taxonomic richness (S) of invertebrate groups increased over time at the upstream–downstream sites (Figure 6a). On the Kembs site, there was an increase in the number of species from 13 to 37 (+185%) and on the Niffer site from 20 to 45 (+125%), whereas the total abundance also increased. Both sites showed clearly poorer invertebrate diversity in PB, with existing communities unbalanced by one or two dominant species, in particular Chironomidae (>70%). In P1, communities were already richer in taxa (first observations of Coleoptera) than in PB with a more even distribution between species, albeit a lower total abundance. The level of evenness observed in P2 was in the same range as in P1 despite the increase in abundances of invasive species (Figure 6b). The Shannon index was by far the highest for the Niffer site in P2, with 10 new taxa observed, notably Odonata species including Gomphidae *(Gomphus vulgatissimus)*, Coenagrionidae, Corduliidae *(Cordulia aenea)*, Platycnemididae *(Platycnemis* sp.*)*, and Calopterygidae *(Calopteryx* sp.*)*.

For the Sites 1, 2, 3, and 4, macroinvertebrate samples were only collected during P2. The taxonomic richness was higher at Sites 1 (S = 36) and 4 (S = 30) than at Sites 2 (S = 21) and 3 (S = 25; Table 1). Diversity indices were lowest at Sites 2 and 3, both of which had lower EPTC richness and abundance than elsewhere. However, the *G. vulgatissimus* dragonfly (listed in the IUCN world and European red lists) was only observed at the Site 2. At Site 4, stoneflies (Plecoptera: Leuctridae) were the most abundant (*n* = 46), and caddisfly (*Goera pilosa*, *Brachycentrus* sp., *Rhyacophila* stricto sensu, *Psychomyia pusilla*) densities increased. A marked decrease in invasive species abundance, especially for the amphipod *Dikerogammarus villosus* and *Echinogammarus ischnus* crustacean populations was observed in descending the river: *D. villosus* was 3 times less abundant at Sites 2, 3, and 4 than at the Site 1 (Table 1).

The factorial plane of a correspondence analysis based on invertebrate abundance data is presented in Figure 7. The first and second factorial axes respectively account for 22.13% and 17% of the total inertia of the faunal data matrix. The ordination of the sites along the F1 axis reflects temporal variations (trajectories) and levels (low to high) of habitat heterogeneity. The second (F2) axis position differentiates the sites based on the relative pollution-sensitive taxa composition of their assemblages. Niffer and Kembs are the only sites plotted with trajectories, showing variations over time (Figure 7a and classifications in Figure 3b). Communities at these two sites were broadly similar in PB (Figure 7b); however, a slight variance was observed in P1 with Trichoptera and Coleoptera taxa appearing at the Kembs site but not at the

TABLE 1 Summary of the results of P2 invertebrate sampling at the four sites: Abundances of taxa, density, richness, diversity indices (Shannon index H′; Simpson index λ; and evenness J'), and invasive species and EPTC abundances

	Site 1	Site 2	Site 3	Site 4
Abundance (*N*)	7,766	3,547	4,928	5,643
BIVALVIA	36	88	30	4
COLEOPTERA	13	2	0	10
CRUSTACEA	3,157	760	712	989
DIPTERA	4,260	2,497	3,739	4,180
EPHEMEROPTERA	30	28	40	73
GASTROPODA	143	134	351	229
HETEROPTERA	0	0	0	1
HIRUDINEA	30	6	40	31
NEMATODA	14	9	7	18
ODONATA	0	1	0	0
OLIGOCHAETA	34	0	4	0
PLECOPTERA	4	0	0	46
POLYCHETA	0	0	0	0
TRICHOPTERA	45	22	5	62
Density (m^2)	4,568	2,086	2,899	3,319
Richness (S)	36	21	25	30
Shannon index (H′)	1.35	0.97	0.93	1.16
Simpson index (λ)	0.38	0.54	0.59	0.55
Evenness (J')	0.38	0.32	0.29	0.34
Invasive species abundance (%)	41.5	24.7	15.1	18.1
EPTC species abundance (%)	1.2	1.5	0.9	3.4



Reading table for **B.**

BIV1	Corbiculidae (F) - *Corbicula fluminalis*
BIV2	Dreissenidae (F) - *Dreissena polymorpha*
BIV3	Sphaeridae (F) - *Pisidium* sp.
BIV4	Sphaeridae (F)- *Sphaerium* sp.
BIV5	Sphaeridae (F)- *Musculium* sp.
COL1	Elmidae (F) - *Limnius* sp.
COL2	Elmidae (F) - *Elmis* sp.
COL3	Elmidae (F) - *Esolus* sp.
COL4	Hydrophilidae (F)
CRU1	Asellidae (F)
CRU2	Corophiidae (F) - *Chelicorophium* sp.
CRU3	Pontogammaridae (F) - *Dikerogammarus villosus*
CRU4	Gammaridae (F) - *Echinogammarus* sp.
CRU5	*Gammarus* sp.
CRU6	Janiridae (F) - *Jaera istri*
DIP1	Chironomidae (F)
DIP2	Empididae (F)
DIP3	Psychodidae (F)
DIP4	Simuliidae (F)
DIP5	Tabanidae (F)
DIP6	Tipulidae (F)
EPH1	Baetidae (F)
EPH2	Caenidae (F) - *Caenis* sp.
EPH3	Ephemeridae (F) - *Ephemera* sp.
EPH4	Ephemerellidae (F) - *Ephemerella* sp.
EPH5	Potamanthidae (F) - *Potamanthus luteus*
GAS1	Ancylidae (F) - *Ancylus fluviatilis*
GAS2	Bithyniidae (F) - *Bithynia* sp.
GAS3	Bithyniidae (F) - *Bithyniella* sp.
GAS4	Hydrobiidae (F) - *Potamopyrgus antipodarum*
GAS5	Lymnaeidae (F) - *Radix balthica*
GAS6	Lymnaeidae (F) - *Galba truncatula*
GAS7	Physidae (F)
GAS8	Valvatidae (F) - *Valvata* sp.
HIR	Erpobdellidae (F)
NEM	Nematelminthes
ODO1	Gomphidae (F) - *Gomphus vulgatissimus*
ODO2	Coeanogrionidae (F)
ODO3	Platycnemididae (F) - *Platycnemis* sp.
ODO4	Calopterygidae (F) - *Calopteryx* sp.
OLI	Oligochaeta
PLE	Leuctridae (F) - *Leuctra* sp.
POL	Ampharetidae (F) - *Hypania invalida*
TRI1	Brachycentridae (F) - *Brachycentrus* sp.
TRI2	Glossosomatidae (F)
TRI3	Goeridae (F) - *Goera pilosa*
TRI4	Hydropsychidae (F)
TRI5	Hydroptilidae (F) - *Hydroptila* sp.
TRI6	Leptoceridae (F) *Athripsodes cinereus*
TRI7	Limnephilinae (F) others
TRI8	Limnephilinae (F) *Anabolia nervosa*
TRI9	Psychomyiidae (F)
TRI10	Rhyacophilidae (F) - *Rhyacophila* stricto sensu

Family (F)

FIGURE 7 Factorial plane of a correspondence analysis based on all site data. (a) The three periods are represented by different colours: PB in blue (⮜December 2010), P1 in green (December 2010⮞ i.e., after the gravel augmentation and the instream flow increase) and P2 in red (i.e., after the Q_{15} flood event in June 2013). Time trajectories are given for Niffer and Kembs site scores, whereas Sites 1 (control, in bold), 2, 3, and 4, are each represented by a single point corresponding to the P2 data. (b) Macroinvertebrate species observed in 2008, 2011, and 2014 (abundance data were Log2(x + 1) transformed). EPTC orders are represented in italic and Odonata in bold. This figure is available in colour online at wileyonlinelibrary.com/journal/rra [Colour figure can be viewed at wileyonlinelibrary.com] [Colour figure can be viewed at wileyonlinelibrary.com]

Niffer site (Figure 7b). As neither the site was impacted in P1 by the gravel augmentation, this result could be attributable to the instream flow increase.

The Sites 1, 2, 3, and 4, sampled only during P2, are also represented in the factorial plane. In P2, the gravel redistribution was still impacting the Site 4 and had reached the Niffer site (Figure 3a.II). The invertebrate community observed on the Niffer site appeared more diverse in P2 than in PB and P1, with the notable apparition of Odonata. The Kembs site, by contrast, showed an increase in common species over the same time frame.

In comparison to the Kembs and Niffer sites, which are well separated, the Sites 1, 2, 3, and 4 are clustered closely together on the first factorial axis of the correspondence analysis. The factorial analysis indicates that the gravel augmentation had a minor effect at Sites 2 and 3 despite their left border positions in the first quadrant. The slight difference between the positions of the sites on the factorial map might in fact reflect a greater density of species adapted to sediment conditions (e.g., Odonata) and species that are less sensitive to water quality (e.g., Gastropoda) at Sites 2 and 3 (Figure 7b).

5 | DISCUSSION

5.1 | Biological response indicators

5.1.1 | Riparian plants and macrophytes

The artificial gravel bar was partially dispersed 2 months after the gravel augmentation was completed (December 2010—P1), and it disappeared gradually with subsequent floods (June 2012; October 2012—P1; June 2013—P2). The primary geomorphic objective of increasing bedform mobility was thus achieved. However, with respect to riparian plants and macrophytes, the effects of the gravel augmentation were, as expected, isolated and minor because of the disappearance of pioneer fluvial forms available for vegetation encroachment. The influence of gravel augmentation on vegetation responses was small compared to the instream flow increase. Furthermore, the results showed that the instream flow increase had a stronger impact on the left bank than on the right one (Figure 5). On the left bank, the gentle slopes increased the number of vegetation niches for terrestrial and aquatic organisms (Bornette & Amoros, 1996; Gurnell et al., 2006). Combined with local deposits of fine sediments, this also contributed to successful macrophyte colonization. On the right bank, a high level of remobilization of the artificial gravel bar and steep slopes was clearly responsible for the scarcity of macrophytes observed postrestoration.

The unsorted sediment supplied into the channel contained a small fraction of sand (~15%) that may have been washed out and deposited in low energy areas, for example, at Sites 2 and 3. However, given the location of both sites at the left edge of the gravel dispersion pattern, the observed fine sediment deposits at these two sites may equally well result from sand inputs from upstream. The retention of fine sediments at a smaller spatial scale could also be enhanced by some varieties of riparian plants (*Salix* sp.) or macrophytes (*M. spicatum*) and thus promotes the installation of other riparian plants or macrophytes species (Bornette & Amoros, 1996; Gurnell, 2014).

5.1.2 | Macroinvertebrates

The increase in richness of species adapted to new sediment conditions was observed at Site 2 during P2, such as Gastropoda and the heritage species *G. vulgatissimus*, where local fine-sediment deposits contributed to the creation of a patchy habitat mosaic at a smaller spatial scale. Although specific richness values of fine sediment substrates are low in comparison to coarser substrates, they can host distinctive macroinvertebrate communities (Lancaster & Hildrew, 1993; Yamamuro & Lamberti, 2007).

Species richness increased on the Niffer site, where taxa not previously observed there, such as Odonata, appeared. It is not possible to relate this result directly to the gravel augmentation. Indeed, Arnaud et al. (2017) noted in their analysis that the gravel augmentation effects could not be clearly distinguished from the effects of floods and bed sediment remobilization in the further downstream channel section. However, the impact of the gravel augmentation could be dissociated from the effects of the instream flow increase thanks to the other sites along the before-after control-impact protocol (Figure 3). The instream flow increase resulted in the expansion of ecological niches (which were occupied by other species, especially Coleoptera) whereas the gravel augmentation contributed to increasing particle size diversity notably at Site 4, where a significant decrease in coarse gravel size was measured. The location of this site, that is, in the main channel, at a bar head well exposed to flow, combined with gravel fining and probably improved oxygenation, may have favoured rheophilic taxa (such as Plecoptera and Trichoptera). Merz et al. (2005) reported a similar, positive outcome on macroinvertebrate densities, on seven gravel augmentation sites in the lower Mokelumne River (California) but warned that the site-specific benefits of gravel augmentation are transitory, in keeping with the transient nature of alluvial systems.

5.1.3 | Focus on invasive species establishment

Riparian and aquatic invasive species disappeared from all sites after the Q_{15} flood (P2) except for the Site 3 where *R. japonica* was present on the right bank. The entire restored reach from the Kembs to the Niffer site showed a higher abundance of invasive macroinvertebrate species in comparison to the abundances observed before the restorations. However, the invasive crustacean amphipod *D. villosus* appeared in lower abundance at Sites 2, 3, and 4 than on the Site 1. *D. villosus* is well known for its aggressive and voracious predatory behaviour, which could alter the food web structure and potentially have direct and indirect effects on native populations (Koester, Bayer, & Gergs, 2015). The significant difference in relative abundance between sites could be explained by limited habitat/food resources at Sites 2 to 4 due to the presence of other taxa following the gravel augmentation or by a physiology that is not adapted to high floods. The latter explanation is supported by results obtained on the Rhône River, where populations of dominant predator species declined following an instream flow increase and floods (Paillex, Doledec, Castella, & Merigoux, 2009). The permeability of restored systems to invasive species is widely documented (Friberg, 2014; Leuven et al., 2009), although the ecological constraints of large rivers could prevent or control their settlement.

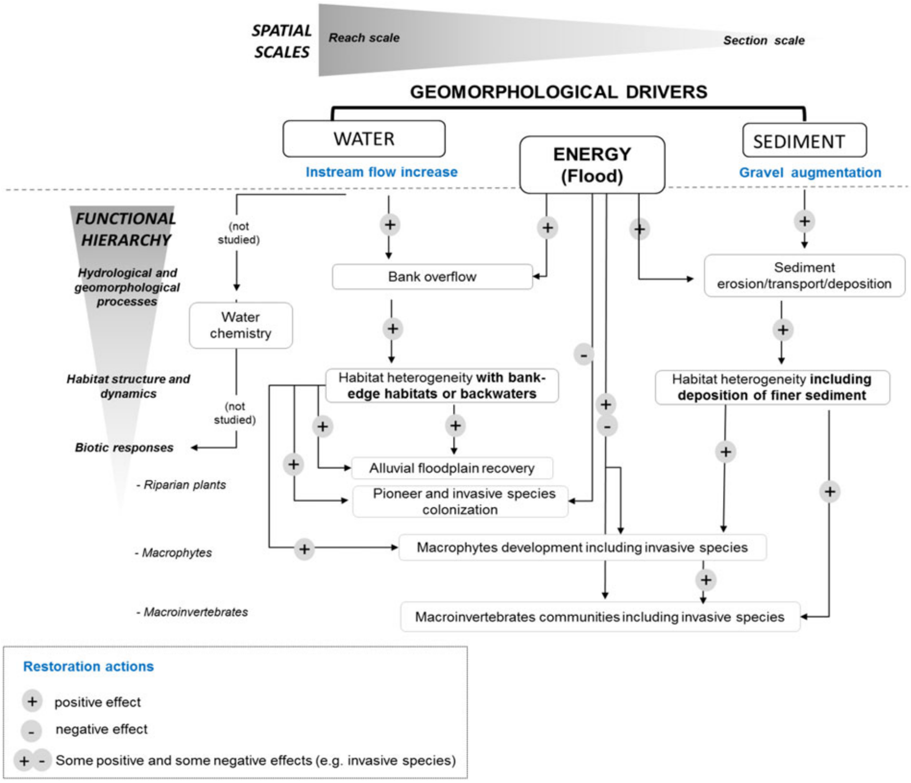


FIGURE 8 Conceptual model illustrating the different relationships/interactions between compartments of the fluvial hydrosystem and the two types of restoration action. Changes in water chemistry were not studied within the scope of this work. Each compartment of the system influences at least one other, resulting in a positive (+), neutral (+/−), or negative effect (−) [Colour figure can be viewed at wileyonlinelibrary.com]

5.2 | Feedback and perspectives

Several studies have highlighted the significance of developing an interdisciplinary understanding of hydromorphological processes and biological communities in river landscapes (Bornette, Amoros, Piégay, Tachet, & Hein, 1998; Elosegi, Diez, & Mutz, 2010; Grabowski & Gurnell, 2016). However, most geomorphological studies investigate the variability of bed mobility at local spatial scales (e.g., riffle-pool-bar sequences) but not at a sufficiently small spatial scale (e.g., local fine-sediment deposition areas) to observe changes perceived at the aquatic organism level. The microhabitats and their spatio-temporal arrangement play a key role in structuring macrophyte communities (Lacoul & Freedman, 2006) and macroinvertebrate assemblages (Beisel et al., 1998, 2000).

Based on the monitoring programme results, a conceptual model of key cause–effect relationships and interactions was developed to describe positive, negative, or mixed effects of both restoration actions (Figure 8). Instream flow increase and gravel augmentation aimed to act on the main geomorphological drivers, that is, water and sediment supply in both integrative and singular pathways. This model stresses the necessity of an interdisciplinary approach to assess ecological restoration efficiency from the microhabitat scale to the entire fluvial hydrosystem, taking into account floods and sediment transport that shape fluvial forms and ultimately biological communities. Some restoration strategies, such as instream flow increases, could lead to the return of an entire ecosystem to its former state through the restoration of geomorphological and biological processes at a global scale (and generally have a good efficiency). Other restoration projects involve singular, targeted actions and must be managed with a long-term restoration strategy that takes into account their generally short-term and transient effects. Regular passive gravel augmentations or a continuous sediment supply from bank erosion are advanced in other studies (Bunte, 2004; Hooke & Mant, 2000). The scientific community of the restoration project discussed in this paper recommended continuing large-scale gravel augmentation and combining it with channel widening (artificially and/or by controlled bank erosion). The latter is expected to promote deposition of a part of the bedload coming from upstream and consequently increase hydraulic and habitat heterogeneity in a more sustainable way than was possible with a single gravel augmentation in a « canal » system (Arnaud et al., 2017). A question remains as to whether this might provide an opening to invasive species introduction or proliferation through the creation of low-energy areas less impacted by floods.

6 | CONCLUSION

Monitoring over several years is necessary, as robust prerestoration and comprehensive postrestoration data are required to establish a baseline and understand the trajectories of change associated with restoration actions (Kail, Brabec, Poppe, & Januschke, 2015; Meyer, Combroux, Schmitt, & Trémolières, 2013). This 6-year study showed that (a) biological communities adapted to new sediment areas and tolerant of low water-quality conditions appeared along the river following the restoration actions, (b) such restored systems were permeable to invasive species; however, the ecological constraints of large rivers (flooding and dominant coarse substrate) could control their establishment, and (c) the sustainability of single gravel augmentation effects remains questionable whereas the instream flow increase has proven to be effective and sustainable. The combination of gravel augmentation with an instream flow increase enhances the habitat complexity and ecological integrity of large rivers, as described in Gurnell et al. (2012) and Harvey et al. (2005). Other process-based river restoration projects are currently ongoing on the Old Rhine River, considering that each restoration action should be selected taking into account external drivers, for example, biological invasions and internal river dynamics, that is, flood occurrence and energy flow.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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Réponses des communautés biologiques à des actions de restauration de grands fleuves (Vieux Rhin, France)

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RÉSUMÉ. – Au cours des deux derniers siècles, l'hydrosystème du Rhin Supérieur a été soumis à des travaux hydrauliques importants. Ces aménagements ont entraîné des pertes de fonctionnalité écologique notamment dans le Vieux Rhin, un tronçon résiduel rectifié de 50 km localisé en aval du barrage de Kembs. Pour pallier ces déficits fonctionnels, plusieurs actions de restauration ont été menées : (i) une augmentation du débit réservé, (ii) des injections sédimentaires, et (iii) une érosion maîtrisée avec implantation d'épis transversaux artificiels. L'érosion maîtrisée a consisté à favoriser l'érosion naturelle de la berge afin d'augmenter le transport solide et diversifier les habitats naturels. L'objectif de l'étude a été de caractériser sur trois ans les réponses de la végétation alluviale et des macro-invertébrés à ce type de restauration. Les résultats obtenus *via* une analyse des zones témoins *versus* restaurées, montrent (i) un gain en hétérogénéité d'habitats naturels, (ii) un retour des milieux pionniers, et (ii) une augmentation de la richesse en espèces floristiques/faunistiques inféodées à des substrats fins. Le rôle majeur des crues, à l'origine du transport solide et de la dynamique des habitats restaurés, a aussi été mis en évidence. Afin d'évaluer au mieux les réponses biologiques, il est important de considérer que le fonctionnement de l'hydrosystème résulte de la combinaison des effets de la restauration avec d'autres déterminants tels que les invasions biologiques. En effet, les nouvelles niches écologiques peuvent favoriser l'installation d'espèces invasives dont l'impact peut être structurant (réseau trophique) sur la biodiversité nouvellement créée.

Mots-clés : biodiversité, érosion maîtrisée de berge, espèces invasives, restauration écologique, Vieux Rhin

Responses of biological communities to restoration actions in large rivers (Old Rhine, France)

ABSTRACT. – Over the last two centuries, the Upper Rhine River was subjected to important hydraulic engineering works which have severely damaged its functioning. This concerned especially the Old Rhine River, a 50-km long by-passed single bed paved and incised channel, located downstream the Kembs dam. Given these functional deficits, restoration actions were initiated *via* (i) instream flow increase, (ii) experimental gravel augmentation and (iii) controlled bank erosion associated to the implementation of artificial transverse groynes. The controlled bank erosion consisted of rip rap protection removing to induce lateral erosion, to feed the main channel in bedload and diversify the fluvial mosaic. The objective of the study was to characterize responses of selected aquatic-riparian compartments (riparian plants, macrophytes and macroinvertebrates) to this type of restoration, thanks to a three years monitoring program. A control-impact analysis of the dynamics of these biological communities allowed to identify both physical and ecological functional changes, and the relationships between them. Results showed (i) an increase in the habitat heterogeneity, (ii) a recovery of riparian biocenosis and (ii) a higher richness of fauna (Odonata) and flora (aquatic plants) species adapted to finer substrate in newly created habitat. Results also stressed the major role of floods which control bedload transport and the habitat dynamics. To better assess how biology responds to restoration actions, it should be considered that the functioning of the fluvial hydrosystem results from the combined effects of restoration actions and other drivers as the arrival of invasive species. Indeed, the new ecological niches created can favor the settlement of invasive species, with a more or less structuring impact (food web changes) on the newly created biodiversity.

Key-words: biodiversity, controlled bank erosion, ecological restoration, invasive species, Old Rhine

I. INTRODUCTION

L'étendue et la gravité des dégradations d'origine anthropique sur les hydrosystèmes fluviaux et leurs biocénoses sont décrites par un grand nombre d'auteurs [Friberg, 2014] : perte d'espèces spécialistes, colonisation par les espèces invasives, perte de l'intégrité de l'écosystème, changement dans la distribution des fréquences d'espèces végétales [Clewell et Aronson, 2013]. Depuis les années 1960-1970, la possibilité de « corriger » ces interventions et, par ce biais, de restaurer ou réhabiliter la fonctionnalité de l'hydrosystème, a suscité un intérêt croissant [Brookes et Shields, 1996 ; Bernhardt *et al.*, 2005]. De nouvelles initiatives ont vu le jour afin de faciliter le retour de processus hydro-morphologiques naturels. Ces programmes intègrent des actions de restauration très variées telles que les recharges sédimentaires, la suppression ou l'arasement de digues, l'implantation de déflecteurs de courant (type épis), le rétablissement de zones inondables ou l'introduction d'espèces végétales [Schanze *et al.*, 2004]. La littérature scientifique offre un nombre important de retours d'expériences quant à ces projets de restauration physique des milieux aquatiques [Schanze *et al.*, 2004], mais peu décrivent l'évolution des biocénoses en réponse à ces actions [Buijse *et al.*, 2002]. La durée souvent trop courte des suivis post-restauration, voire leur absence, mais aussi les difficultés de statuer sur le succès (ou non) d'une restauration, expliquent souvent les incertitudes quant à l'efficacité et la durabilité de tels aménagements [Bernhardt *et al.*, 2005 ; Kondolf *et al.*, 2006]. La complexité de la dynamique des hydrosystèmes fluviaux [Darby et Sear, 2008] ajoute au challenge de répondre à des problématiques récurrentes telles que (i) le manque de connaissances sur les effets biologiques des restaurations, (ii) la sensibilité des systèmes restaurés aux invasions biologiques et (iii) le développement d'indicateurs robustes dans l'évaluation de l'efficacité et de la durabilité des restaurations.

Le Rhin Supérieur a subi d'importants aménagements au cours des deux derniers siècles pour réduire les inondations, favoriser la navigation et l'agriculture, et produire de l'hydroélectricité [Maire, 1997]. Ces aménagements ont entraîné des pertes de fonctionnalité écologique, notamment dans le Vieux Rhin, tronçon résiduel rectifié du fleuve, parallèle au Grand Canal d'Alsace (GCA). Pour pallier ces déficits fonctionnels importants, des actions de restauration par injection sédimentaire et érosion maîtrisée avec implantation d'épis transversaux artificiels ont été menées en aval du barrage de Kembs [Garnier et Barillier, 2015]. Cet article est spécifiquement dévolu aux réponses des compartiments biologiques obtenues au niveau du site d'érosion maîtrisée (site O3), pour lequel le recul temporel est le plus important. Afin d'analyser les effets écologiques de ce type de restauration, la végétation aquatique/rivulaire et les communautés de macro-invertébrés ont été suivies. Cette approche interdisciplinaire et multi-compartiments a pour objectif de répondre aux questionnements suivants : (i) cette restauration permet-elle de rétablir les processus naturels du fleuve ? (ii) quelles sont les réponses des compartiments biologiques ? (iii) quel est le poids relatif de facteurs externes à la restauration tels que les invasions biologiques dans l'évolution des communautés ? Dans cet article, nous privilégions un retour sur l'étude des réponses des compartiments biologiques et ne présentons pas dans le détail les données physiques exploitées par ailleurs [Schmitt *et al.,* 2014 ; Pinte *et al.*, 2015].

II. MÉTHODOLOGIE

II.1. Site d'étude et éléments de contexte

Au siècle dernier, la construction du GCA a court-circuité le Rhin corrigé sur un linéaire de 50 km, créant un tronçon résiduel rectifié, le Vieux Rhin (figure 1A). Ces modifications ont engendré d'importantes altérations hydro-morphologiques impactant la biodiversité et la fonctionnalité de l'ensemble de l'hydrosystème fluvial [Arnaud, 2012]. Au droit du barrage de dérivation de Kembs, la majorité du débit est dirigé vers le GCA, dont la capacité maximale est de 1400 m^3/s. Au-delà, le surplus est déversé dans le Vieux Rhin. En-deçà de ce seuil, le Vieux Rhin était alimenté jusqu'en décembre 2010 par un débit réservé de 20 à 30 m^3/s. Jugé insuffisant pour satisfaire les besoins écologiques du milieu, ce débit a été augmenté à partir de décembre 2010. Le débit a varié au cours de l'étude avec une modulation saisonnière entre 52 m^3/s et 115 m^3/s (tableau 1).

Le projet d'érosion maîtrisée et d'implantation d'épis transversaux artificiels, mis en œuvre en avril-mai 2013 entre les PK 191.3 et 191.6, a consisté à favoriser l'érosion naturelle de la berge gauche (française) pour permettre un apport sédimentaire au chenal principal afin de diversifier les habitats naturels [Garnier et Barillier, 2015]. Des modélisations physiques et numériques des itinéraires techniques possibles ont été menées au Laboratoire National

Tableau I : Descriptif des travaux de restauration menés depuis 2010 sur le site O3 (Vieux Rhin). La localisation du site est renseignée sur la figure 1A.

Secteur concerné	***Type de restauration***	***Objectif***	***Date de mise en œuvre***	***Aspects techniques (pK : point kilométrique)***	***Attendus écologiques* [Garnier et Barillier, 2015]**
Secteur du Vieux Rhin	Augmentation du débit réservé	Compenser partiellement la dérivation d'une partie du débit dans le GCA	Fin décembre 2010	Passage de 20-30 m^3/s à 52-115 m^3/s Modulation saisonnière de mai à septembre	1. Accroître les milieux de frayères pour les poissons. 2. Maintenir la dynamique de paysages alluviaux typiques (saulaies) 3. Etendre des annexes hydrauliques pour la végétation aquatique et des insectes.
Site O3	Travaux d'érosion maîtrisée	Provoquer une érosion maîtrisée de la berge gauche pendant les crues et diversifier la mosaïque alluviale et réactiver des processus morphodynamiques	Avril-Mai 2013	Démantèlement local de digue submersible de Tulla [Pinte *et al.,* 2015] et implantation de deux épis transversaux artificiels - pK 191,2 à 191,6 [Die Moran, 2012]	1. Favoriser la croissance et la reproduction des poissons 2. Permettre l'installation d'une végétation pionnière 3. Gagner en hétérogénéité d'habitats pour favoriser les autres organismes aquatiques 4. Rétablir la fonctionnalité écologique initiale

d'Hydraulique et d'Environnement – LNHE – EDF [Die Moran, 2012 ; Clutier *et al.*, 2012]. Sur la base de ces modèles, l'ensemble de la berge gauche du site O3 a été dérocté et défriché, à l'exception de quelques arbres. Trois épis datant des aménagements du début du XX$^{\text{ème}}$ siècle ont été remaniés : les deux plus éloignés ont été remodelés et celui en position intermédiaire a été arasé. L'objectif de la présente étude est de montrer si les attendus écologiques de la restauration ont été atteints (tableau 1) *via* un suivi post-restauration de la végétation aquatique/rivulaire et des communautés de macro-invertébrés. Les résultats d'un suivi piscicole mené par ailleurs [Ecotec, 2016], ont été pris en compte dans la discussion.

II.2. Suivi écologique

La végétation aquatique et rivulaire a été suivie en réalisant une cartographie des habitats du site O3 au 1/1000$^{\text{ème}}$ de 2013 à 2016. Cette cartographie a été complétée par 7 transects transversaux (relevés de végétation - coefficients de Braun-Blanquet pour la végétation rivulaire et pourcentages de recouvrement pour la végétation aquatique). Onze stations d'étude des macro-invertébrés ont été définies (20 mètres de linéaire de berge sur 30 à 50 mètres vers le chenal central) pour leur représentativité en termes de combinaisons HVS (hauteur d'eau, vitesse et substrat). Les macro-invertébrés ont été échantillonnés au printemps et en automne de 2014 à 2016, à l'aide d'un cylindre de Hess (filet avec un vide de maille et un embout de précision de 500 µm) [Hess, 1941]. Chaque point d'échantillonnage des macro-invertébrés a été caractérisé : géolocalisation, mesures physiques (hauteur d'eau, vitesse, substrat) et physico-chimiques (température, oxygène, conductivité ; sonde multi-paramètres WTW). Les réponses des compartiments biologiques ont été étudiées selon un plan d'échantillonnage comparant stations témoins *versus* stations restaurées [Smith *et al.*, 1993]. Trois grandes zones d'étude ont été définies : (i) zone 'Amont' : stations/transects situés à l'amont de la zone d'érosion maitrisée – linéaire témoin non restauré à mosaïque hétérogène et à faciès lotique dominant, (ii) zone des 'Epis' : stations/transects au niveau de la zone d'érosion maitrisée – linéaire restauré - et (iii) zone 'Aval' : stations/transects à l'aval de la zone d'érosion maitrisée – linéaire à mosaïque monotone, témoin du linéaire pavé et incisé du Vieux Rhin. La figure 1B présente les stations d'étude des macro-invertébrés et les profils transversaux de végétation associés à chaque zone.

Un suivi géomorphologique a permis de caractériser l'évolution morphologique de la zone des 'Epis' et de définir les gains physiques potentiels de la restauration *via* (i) le traçage sédimentaire des matériaux érodés de la berge et charriés par le fleuve, (ii) un suivi granulométrique fin par prélèvement manuel et par photographie du fond du chenal (profils tous les 10m), (iii) un suivi topographique de la berge par LiDAR terrestre et du chenal par bathymétrie mono-faisceau et par LiDAR topo-bathymétrique [Schmitt *et al.*, 2014].

III. RÉSULTATS

III.1. Évolution du milieu physique et définition des effets

Le lit du Vieux Rhin pré-restauration (avant 2010) présentait des écoulements très homogènes et une faible diversité des unités morphodynamiques. La comparaison des orthophotographies pré- (avril 2011) et post-érosion maîtrisée (mars 2016 ; 3 ans après la restauration) met en évidence une modification de la mosaïque fluviale (figure 2A). Le

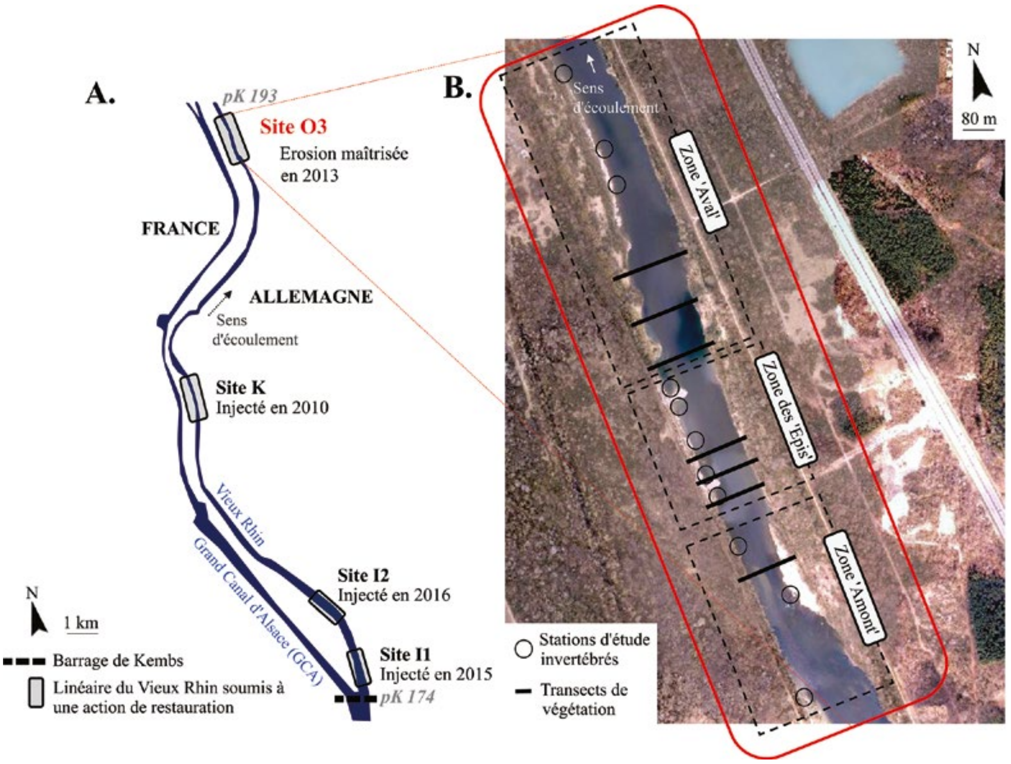


Figure 1 : *A. Localisation des sites de recharges sédimentaires (site K, octobre 2010 ; site I1, Février -Mars 2015 ; site I2, Janvier 2016) et d'érosion maîtrisée avec implantation d'épis transversaux artificiels (site O3), B. Focus sur le site O3 avec la localisation des stations d'étude des macro-invertébrés et les profils transversaux de végétation associés à chaque zone.*

suivi topographique et bathymétrique au profil transversal situé en aval de l'épi amont (PT10), confirme et complète les observations faites à partir des orthophotographies (figure 2B).

Après la restauration par érosion maîtrisée (avril-mai 2013) et une crue Q_{15} de période de retour de 15 ans (juin 2013 – $Q_{max\_Bâle}$ = 3456 m³/s), d'importantes évolutions morphologiques ont été observées, notamment (i) une érosion de berge, majoritairement au droit de l'épi amont sous la forme d'une encoche d'érosion (volume total érodé de la berge = 1500 m³ / encoche d'érosion = 1070 m³) et (ii) la formation de deux langues sédimentaires à l'aval des épis transversaux (dépôts compris entre 0,1 m et 3 m d'épaisseur) avec un affinement granulométrique estimé de 30 mm [Schmitt *et al.*, 2014]. Les évolutions morphologiques ont été moins contrastées entre 2014 et 2016, avec de légères remobilisations partielles des langues sédimentaires qui se sont globalement érodées en aval immédiat des épis (de quelques dizaines de cm et jusqu'à 1m localement), et au contraire de faibles dépôts dans les deux mouilles principales (quelques dizaines de cm dans les deux cas). La construction des épis transversaux a ainsi entraîné la création de nouvelles formes fluviales, pour lesquels une dynamique subsiste même après la crue importante de 2013.

III.2. Réponses des compartiments biologiques

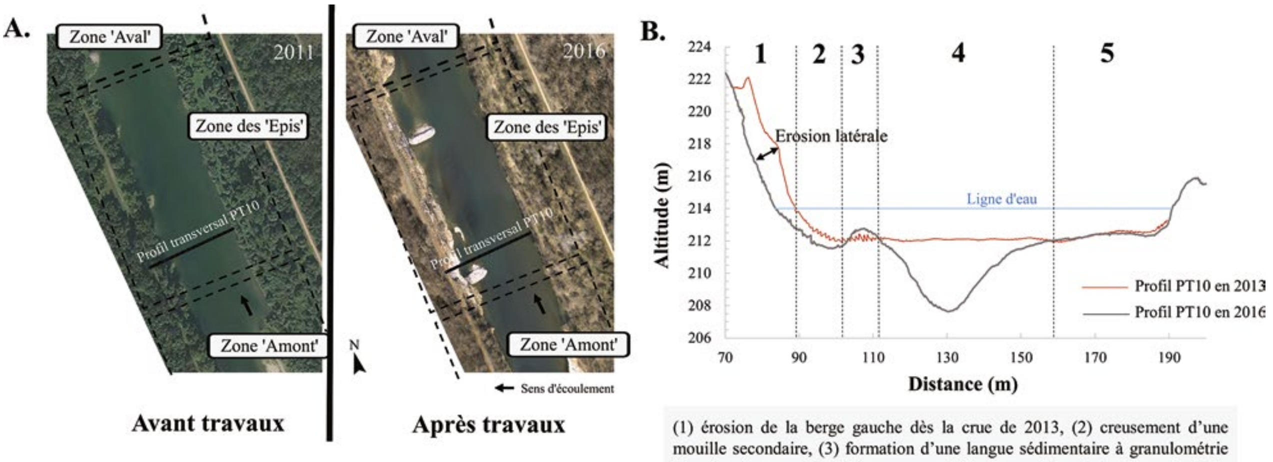
Les peuplements de macro-invertébrés étaient dominés en abondances par des crustacés et/ou des diptères tout au long de l'étude. L'analyse factorielle du tableau faunistique montre une composition des communautés sensiblement différente selon les grandes zones d'étude : 'Amont', 'Epis' et 'Aval' (figure 3A). Cette analyse révèle que les compositions automnales en macro-invertébrés des trois zones ont évolué selon une même trajectoire au cours du temps, mais avec une légère différenciation. La zone 'Amont', témoin à mosaïque hétérogène et faciès lotique dominant, était ainsi dominée par des taxons rhéophiles (surtout des Trichoptères) typiques des milieux lotiques. La zone des 'Epis' était caractérisée par des communautés de macro-invertébrés retrouvées préférentiellement au sein de substrats de sédimentation (figure 3B), ce qui a entraîné une hausse de l'abondance (i) de taxons peu polluo-sensibles et fouisseurs et (ii) de taxons remarquables (Odonates). Ces résultats sont en cohérence avec les changements du milieu physique et l'apparition de nouveaux habitats à substrat fin (*e.g.* graviers, sables) au sein de la zone d'érosion maîtrisée.

Le tableau II montre que la zone des 'Epis' a contribué à une augmentation de la richesse spécifique en végétation aquatique. Un gain de 6 espèces végétales a été constaté entre 2014 et 2016 (tableau 2). Les communautés végétales aquatiques ont évolué d'un groupement à algues filamenteuses (2013-2014) vers des tapis localisés à fort recouvrement de *Myriophyllum spicatum* (à partir de 2015). De nouveaux taxons tels que *Nitella sp.* et *Stuckenia pectinata,* ont été nouvellement recensés en zone 'Aval' à partir de 2016 (tableau 2).

La restauration par érosion maîtrisée a également favorisé la colonisation des berges par des communautés herbacées pionnières, dominées par *Phalaris arundinacea*. Les langues sédimentaires en aval des épis transversaux artificiels, bancs nus à leur formation, se sont végétalisées au cours du temps. Les germinations de saules (*Salix sp.*) et de peupliers (*Populus sp.*) n'ont pas supporté les contraintes hydrauliques du Rhin, cédant ainsi l'espace nu à une communauté herbacée identique à celle de la berge érodée ainsi qu'à quelques espèces invasives (*e.g. Reynoutria japonica, Coniza canadensis*). La dynamique végétale a été semblable au niveau des épis transversaux artificiels. Les zones 'Amont' et 'Aval' n'ont pas montré de changements notables au sein des communautés végétales. Les anciens épis de régularisation du lit corrigé y sont caractérisés par des saulaies (*Salix sp.*) créant des milieux quasi-monospécifiques.

III.3. Invasions biologiques

Le fonctionnement de l'hydrosystème fluvial résulte des effets combinés des actions de restauration avec d'autres déterminants tels que l'arrivée ou la variation de densités des



(1) érosion de la berge gauche dès la crue de 2013, (2) creusement d'une mouille secondaire, (3) formation d'une langue sédimentaire à granulométrie fine (gravier meuble), (4) creusement d'une mouille principale au droit de l'épi amont (une mouille similaire s'est formée au droit de l'épi aval), (5) partie orientale du chenal et rive droite : aucun changement

Figure 2 : *A. Orthophotographies 2011 et 2016 de la zone des 'Epis' et localisation du profil transversal 10 (PT10, situé à faible distance en aval de l'épi amont) avant et après les travaux d'érosion maîtrisée. B. Evolution topographique/bathymétrique entre 2013 (avant érosion maîtrisée) et 2016 (après érosion maîtrisée) au droit du PT10. La ligne d'eau a été modélisée pour un débit de 52 m³/s.*

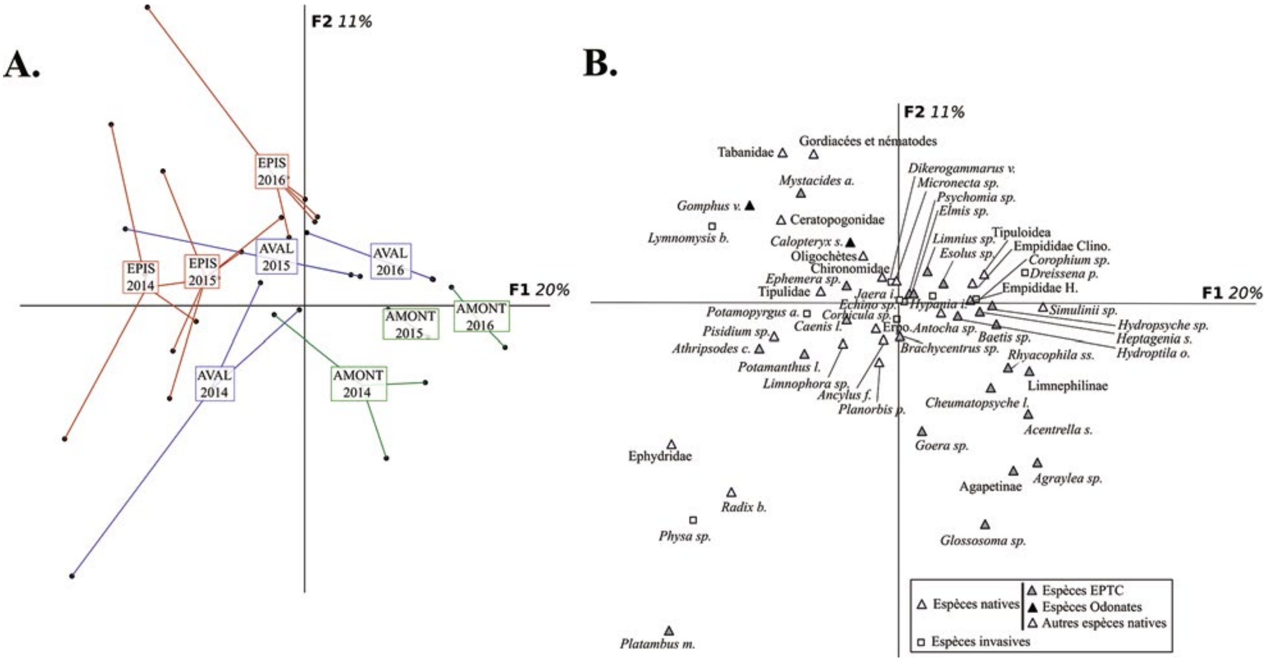


Figure 3 : *Résultats de l'analyse factorielle des correspondances (plan F1 x F2) basée sur des données d'abondances d'automne 2014, 2015 et 2016 par station (11 stations) - transformées en Log2(x+1) et après élimination des espèces rares (i.e. dont l'abondance est égale à 1). Plans factoriels (A) des stations/dates (trajectoires), (B) des taxons de macro-invertébrés.*

espèces invasives. Au cours du suivi écologique, les assemblages faunistiques de macro-invertébrés étaient composés d'une part importante d'espèces invasives (figure 4) dont des bivalves (*Corbicula fluminea, Dreissena polymorpha*), des crustacés (*Chelicorophium curvispinum, C. robustum, Dikerogammarus villosus, Echinogammarus ischnus, E. trichiatus, Gammarus roeselii, Jaera istri, Limnomysis benedeni*) et des gastéropodes (*Potamopyrgus antipodarum, Physa acuta*). Le crustacé amphipode *Dikerogammarus villosus* a dominé en partie les peuplements et sa présence dans pratiquement tous les échantillons a démontré son caractère ubiquiste (figure 4). La zone 'Aval' était dominée par des espèces invasives, contrairement à la zone 'Amont', plus favorable à l'installation d'espèces natives et plutôt polluo-sensibles. La proportion d'espèces invasives était très légèrement plus faible dans la zone des 'Epis' que dans la zone 'Aval'. *In fine*, il est à noter qu'en 2016, la fréquence relative des espèces invasives était bien inférieure aux fréquences observées en 2014 et 2015.

Six espèces végétales invasives dont cinq terrestres (la renouée du Japon – *Reynoutria japonica*, le robinier faux-acacia – *Robinia pseudoacacia*, le solidage géant – *Solidago gigantea*, l'érable negundo – *Acer negundo,* l'érigéron du Canada – *Conyza canadensis)* et une aquatique (l'élodée de Nuttall – *Elodea nuttallii*) ont été relevées sur le site O3 de 2013 à 2016. L'espèce végétale invasive *R. japonica* a été relevée en faible recouvrement sur les épis transversaux artificiels, mais celui-ci n'a pas évolué au cours du temps. Les relevés de végétation aquatique, sur cette même période, ont montré un fort recouvrement d'*E. nuttallii* (> 80 %) dans une petite mare latérale localisée en zone 'Aval'. Cette espèce a été quasi-absente du chenal principal jusqu'en 2015 (< 0.01 %). Le pourcentage de recouvrement a légèrement augmenté (environ 1 %) en 2016.

Tableau II : Composition spécifique des communautés macrophytiques au niveau des trois zones d'étude 'Amont', 'Epis' et 'Aval' en 2014 et 2016.

	AMONT 2014	**AMONT 2016**	**EPI 2014**	**EPI 2016**	**AVAL 2014**	**AVAL 2016**
Algues filamenteuses	X	X	X	X	X	X
Ceratophyllum demersum				X		
Elodea nuttallii				X	X	X
Fontinalis antipyrectica	X	X		X	X	X
Lemna minor						X
Myriophyllum spicatum			X	X	X	X
Nitella sp.						X
Potamogeton crispus				X		
Stuckenia pectinata				X		X
Potamogeton perfoliatus				X		
Ranunculus circinatus				X		
Ranunculus fluitans				X		
Nombre de taxons présents	2	2	2	10	4	7

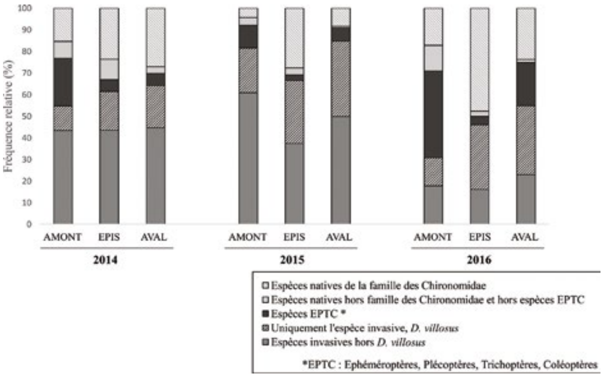


Figure 4 : *Fréquences relatives en automne 2014, 2015 et 2016 des espèces de macro-invertébrés invasives, EPTC et natives (hors EPTC) selon le regroupement spatial en zones 'Amont', 'Epis' et 'Aval'. La part de l'espèce dominante invasive* Dikerogammarus villosus *a été ajoutée ainsi que celle de la famille Chironomidae (taxon natif de Diptères).*

IV. DISCUSSION

Le suivi des milieux après l'initiation d'un processus d'érosion maîtrisée avec implantation d'épis transversaux artificiels montre une augmentation de l'hétérogénéité et de la dynamique des unités hydro-morphologiques. Le panel des niches écologiques disponibles s'y est globalement élargi du fait des apports sédimentaires par érosion latérale et de la présence des épis, ces derniers ayant induit le dépôt des deux langues sédimentaires constituées de sédiments relativement fins. Le suivi des macro-invertébrés a été réalisé avec une méthode d'échantillonnage originale, aucune norme française respectant la Directive Cadre sur l'Eau et utilisant les macro-invertébrés n'étant adaptée aux grands cours d'eau. L'utilisation du cylindre de Hess s'est avérée pertinente au sein du lit dynamique du Vieux Rhin, présentant l'avantage de pouvoir échantillonner des substrats grossiers y compris dans des zones de fortes vitesses de courant. La méthode utilisée a permis d'évaluer peu de temps après la restauration, des densités plus fortes d'espèces faunistiques et floristiques inféodées spécifiquement à des milieux de sédimentation. L'implantation des épis transversaux artificiels semble avoir favorisé les populations de certaines espèces, notamment des odonates comme cela a déjà été observé en République Tchèque [Buczyński *et al.,* 2017]. De nouvelles espèces de macrophytes sont aussi apparues, alors que le secteur étudié près de Bâle a été évalué en 2013 par la Commission Internationale pour la Protection du Rhin (CIPR) comme présentant des déficits macrophytiques marqués [CIPR, 2015]. Cette conclusion est issue d'une évaluation récente basée sur le jugement d'experts à partir de données collectées sur des sites de prélèvement situés sur le cours du Vieux Rhin. Quatre critères ont été utilisés pour définir un indicateur global : (1) le nombre de formes de croissance, (2) le nombre d'espèces de macrophytes, (3) le nombre d'espèces indicatrices de qualité et (4) le pourcentage de surface occupée par les macrophytes [CIPR, 2015]. L'application de cette méthode sur les données de 2016 oriente ce même secteur vers un statut plus favorable, *i.e.* présentant de légers déficits. L'apparition de milieux nouveaux contribue à maintenir la dynamique des paysages alluviaux et favorise l'installation dans le lit mineur et sur les berges d'espèces végétales pionnières (*Phalaris arundinacea*). Les langues sédimentaires, situées à l'aval direct des épis, favorisent la germination de *Salix sp.*, dont la croissance est cependant entravée par les crues [Karrenberg *et al.,* 2002]. Le suivi piscicole a permis de mettre en évidence les effets positifs des épis transversaux artificiels : une augmentation du nombre de frayères et de juvéniles. Or, il est aussi à noter la prolifération rapide de poissons invasifs d'origine ponto-caspienne sur le site d'étude : le gobie à tache noire (GTN) et le gobie de Kessler (GKS). Les effectifs des gobies capturés sur le site O3 ont été multipliés par 9,3, passant de 3,6 % à 33,4 % de l'effectif total des poissons capturés entre 2014 et 2016 [Ecotec, 2016]. Sa prédation importante sur les macro-invertébrés natifs [Lederer *et al.,* 2008] et son comportement agressif [Kornis *et al.,* 2012] peuvent influencer le devenir des communautés en place. Des études complémentaires sur le réseau trophique sont actuellement en cours afin d'évaluer l'impact potentiel de ces espèces invasives sur l'interprétation des effets de la restauration [Staentzel *et al.*, en prep.].

Les secteurs avals des grands fleuves sont fortement impactés par des espèces invasives désormais intégrées dans le pool régional d'espèces [Beisel, 2001 ; Beisel *et al.*, 2017]. La présence ou l'introduction d'espèces invasives dans un milieu restauré représente un déterminant important dans l'évolution de la composition de la communauté locale et interfère inévitablement sur l'interprétation des effets de la restauration (figure 5). Le maintien des populations natives peut être menacé [Leuven *et al.*, 2009]. Les nouveaux habitats issus de l'action de restauration sont situés en bordure de chenal et peuvent faciliter l'installation d'espèces végétales invasives [Combroux *et al.*, 2002]. Plusieurs espèces végétales à caractère invasif ont effectivement été observées mais avec de faibles surfaces de recouvrement. L'élodée de Nuttall, seule espèce végétale strictement aquatique qui ait été observée, n'a pas montré de caractère proliférant à ce stade. La colonisation du linéaire du Vieux Rhin lui a été difficile, sans doute du fait d'une granulométrie grossière et de la stabilité latérale du lit. Les nouveaux habitats à granulométrie fine pourraient être des niches potentielles pour l'expansion de l'Elodée de Nuttall. Le crustacé amphipode *Dikerogammarus villosus*, connu pour présenter un comportement agressif et vorace [Van Riel *et al.*, 2006 ; Koester *et al.*, 2016], pourrait contribuer aux changements de communautés sur le site O3 malgré un effet de prédation évalué comme faible sur des sites envahis [Koester *et al.*, 2016]. La proportion en espèces invasives de macro-invertébrés a globalement diminué entre 2015 et 2016. Un réarrangement des communautés en place a pu survenir suite à des évènements pouvant être liés à des facteurs environnementaux comme des évènements hydrologiques (crue moyennement intense et particulièrement longue en 2016 : $Q_{max\_Bâle}$ = 3021 m^3/s, déversement dans le Vieux Rhin durant trois mois).

Dans un contexte de restauration écologique, de nombreux auteurs décrivent la nécessité de coupler l'analyse de l'évolution physique à celle de la réponse biologique [Elosegi *et al.*, 2010 ; Grabowski et Gurnell, 2016], mais aussi d'intégrer des concepts d'écologie théorique [Lake *et al.*, 2007] et fonctionnelle [Mouillot *et al.*, 2013]. Les règles d'assemblages des communautés locales, décrites dans les années 1980-1990 [Tonn, 1990 ; Keddy *et al.*, 1992], et unifiées pour l'ensemble des communautés biologiques [Lortie *et al.*, 2004 ; Friberg *et al.*, 2016], intègrent plusieurs types de filtres, notamment le filtre environnemental (figure 5). Les conditions de passage de ce filtre par une espèce dépendent

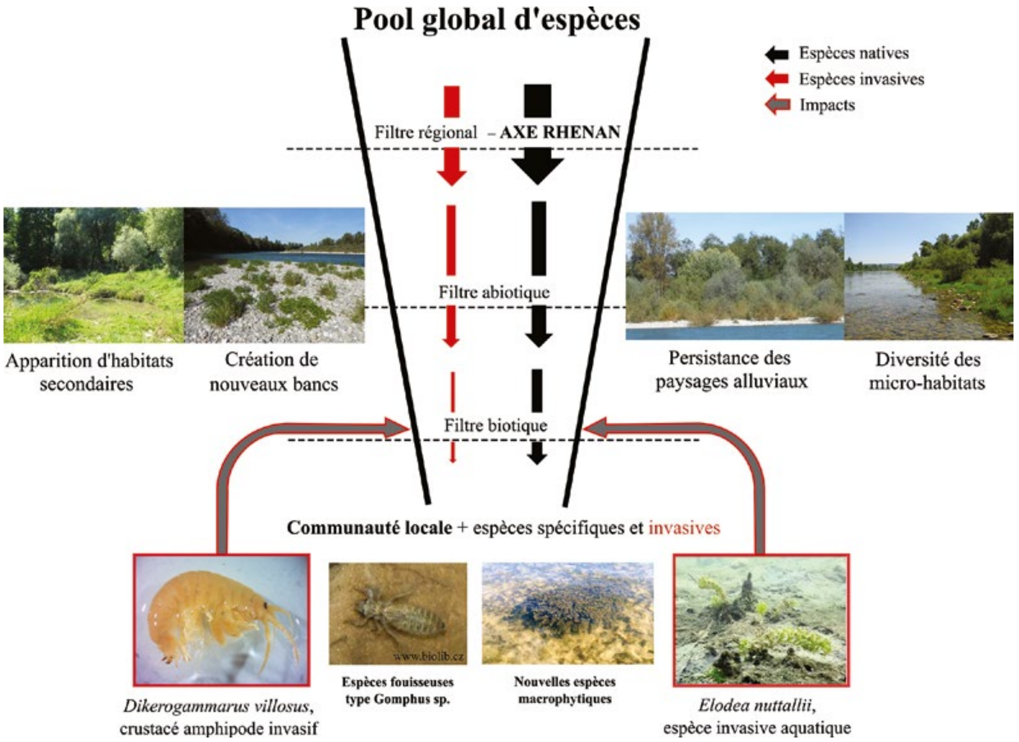


Figure 5 : *Schéma résumant la contribution écologique des actions de restauration à la constitution d'une communauté locale nouvelle.*

des variables hydrauliques et granulométriques des habitats. Cette étude a mis en évidence la pertinence de l'échelle la plus fine de mesure de ces variables, qui doit correspondre au paysage de l'espèce étudiée afin d'évaluer au mieux les réponses biologiques [Beisel *et al.*, 2000]. *In fine*, la restauration écologique par érosion maîtrisée et implantation d'épis transversaux artificiels contribue à rendre ce filtre environnemental plus perméable au pool global d'espèces natives et invasives (figure 5). La durabilité de la diversification des habitats et des effets écologiques associés est clairement dépendante du maintien des épis transversaux artificiels.

V. CONCLUSIONS

L'action de restauration décrite dans cet article, une érosion maitrisée de berge avec implantation d'épis transversaux artificiels sur un grand cours d'eau, est inédite. Il s'agit par conséquent d'une approche expérimentale pour laquelle le retour d'expérience est important. L'analyse des résultats issus de ces suivis est à la fois exploratoire tout en s'inscrivant en partie dans une démarche hypothético-déductive, dans la mesure où un certain nombre de prédictions des changements de biodiversité ont pu être éprouvées. L'étude interdisciplinaire de ces restaurations est primordiale pour mettre en évidence les trajectoires hydro-morphologiques post-restauration du milieu [Arnaud, 2014] et comprendre la réponse des compartiments biologiques associées [Staentzel *et al.,* soumis]. Le suivi écologique est prévu jusqu'en 2017. Les perspectives de cette étude sont (i) d'affiner les résultats en se focalisant sur le gain en diversité fonctionnelle, (ii) de développer des outils/indicateurs d'évaluation d'une restauration écologique et (iii) de proposer une étude comparative avec les réponses biologiques observées suite à des recharges sédimentaires.

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Original Articles

A multiscale assessment protocol to quantify effects of restoration works on alluvial vegetation communities

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ABSTRACT

Vegetation mapping is a legal obligation in environmental monitoring prompting the need for easy-to-read methods of quantifying changes in vegetation dynamics. Transition matrix modelling provides an alternative approach to qualitative assessment promoting quantification for revealing current complex changes effects on the trajectory of ecosystems. Transition matrix models (TMMs) and two newly developed metrics, the pixel change (PCI) and zonal change (ZCI) indices, were combined into a methodological scheme that provides a multiscale assessment protocol. This protocol was applied during a field study along the Old Rhine River in order to assess complex shifts in alluvial vegetation communities in relation to restoration works and natural processes. The restoration works aim to restore lateral mobility through instream flow increase and controlled bank erosion with artificial groynes implementation. Characterisation of spatial and temporal pathways was carried out using a 'before-after control-impact' (BACI) design, and a fuzzy coding approach has shed further light on shifts in aquatic vegetation functional traits. The multiscale assessment protocol highlighted (i) an increase in alluvial habitat types, including habitats of European concern (Natura 2000), and (ii) low time-scale aquatic vegetation recovery. Both ZCI and PCI recorded high values along the restored section with controlled bank erosion, indicating strong ecosystem change. Use of functional traits detected three requirements for the successful establishment of aquatic vegetation in the restored river section, i.e. high degree of flexibility, flow variation tolerance, and fine-sediment adaptability. Subject to the reliability and availability of vegetation mapping, the method opens the possibility of an efficient tool for precisely monitoring alluvial vegetation communities and identifying pathways. It also discriminates event effects, e.g. natural process effects vs. human-induced effects. At full potential, such a protocol may reveal community responses to disturbance during conservation, restoration and management decision-making projects.

1. Introduction

Current trends in nature environmental public policies are usually based on surveys using habitat type classifications, e.g. the European Nature Information System (Louvel et al., 2013), the Corine Biotope classification (Bissardon et al., 1997) and the phytosociological approach (Bardat et al., 2004; Bensettiti et al., 2000; Braun-Blanquet, 1932). Vegetation characteristics and environmental conditions have long been recognised as convenient and reliable habitat type descriptors. Vegetation surveys, habitat classifications and multivariate statistical analysis allow the description of vegetation characteristics and identification of possible ecological succession pathways (Khan et al., 2016; Meyer et al., 2013; Řehounková and Prach, 2008; Van Geest et al., 2005).

The concept of trajectory is connected to that of ecological successions, based on the idea that an ecosystem can travel along different pathways (Hobbs and Norton, 1996). The main idea of the restoration ecology is to take into account such pathways, and spatial and temporal dimensions (Clewell and Aronson, 2013). During their summary of how restoration success has been evaluated in restoration projects, Ruiz-Jaen and Aide (2005a) found that vegetation characteristics were one of the main attributes for evaluation success (Ruiz-Jaén and Aide,

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2005b). Although qualitative description of vegetation characteristics is useful for illustrating restoration trajectories, it rarely allows quantification and prediction of restoration success (Anand and Desrochers, 2004). Despite this, managers frequently request quantification of potential biological community dynamics at newly restored sites or ongoing restoration projects, especially along large rivers (Jungwirth et al., 2002; Palmer et al., 2005; Pander and Geist, 2013; Woolsey et al., 2007).

Gallet and Sawtschuk (2014) recently described transition matrix modelling as a new approach for highlighting restoration effects on habitat types using vegetation maps. Such maps are currently produced for most managed and restored sites due to a legal obligation to map sites during ecological monitoring (Hearn et al., 2011). If such maps are produced repeatedly over time, and have good reliability, they can be used for transition matrix modelling to analyse trajectories (Godron and Lepart, 1973; Sawtschuk and Bioret, 2012; Turner, 1990). Transition matrix models (TMMs) are based on calculating the probability that a piece of land will change from one state to another (Usher, 1992). This approach has mostly been used on (i) well-understood ecosystems in terms of plant ecology and environmental factors, and (ii) sites that have a rate of vegetational change high enough to be observed over time (Balzter, 2000; Hobbs and Legg, 1984; Lippe et al., 1985). Large rivers and their associated alluvial landscapes satisfy most of these criteria. Few studies have yet used the method on fluvial hydrosystems, partly due to their biocomplexity (Amoros and Bornette, 2002). More than many other ecosystems (White and Pickett, 1985), fluvial hydrosystems are usually subjected to internal patch dynamics that create an on-going turnover of different states that together define the stable state (Beisner et al., 2003; Hughes et al., 2005). Fluvial hydrosystem complexity is also a result of the high degree of natural processes, i.e. internal river dynamics operating at different spatial and temporal scales (Amoros and Bornette, 2002), that are frequently combined with human impacts, e.g. restoration actions or disturbance. Viewing fluvial hydrosystems as a mosaic of patches and investigating mechanisms of the spatial and temporal dynamic at different scales could prove a useful approach for examining interrelationships (Pringle et al., 1988). Many patch-to-patch variations defined over small areas could potentially have important ramifications at the landscape scale (Baker, 1989).

We thus developed a multiscale assessment protocol for evaluating whether changes in vegetation dynamics defined over small areas (patch or pixel) necessarily lead to changes at the landscape scale, thereby acting as a multi-scale indicator of the ongoing stable state. The methodological scheme of this protocol combined TMMs used by Gallet and Sawtschuk (2014) with two newly proposed metrics for this study, the pixel change (PCI) and zonal change (ZCI) indices. This protocol was applied during a field study monitoring an experimental restoration programme of controlled bank erosion with artificial groynes implementation, aimed at restoring lateral mobility along the Old Rhine River (Pinte et al., 2015). The field study was incorporated into a monitoring framework based on the "before-after control-impact" (BACI) protocol (Smith et al., 1993). The field study had two main aims, (i) to undertake a dry run of the multiscale assessment protocol along a fluvial hydrosystem, and (ii) to assess the effect of controlled bank erosion on riparian and aquatic vegetation dynamics, also using an additional approach on shifts in aquatic vegetation functional traits as recommended by Cadotte et al. (2011).

2. Material and methods

2.1. Methodological scheme of the multiscale assessment protocol

The methodological scheme (Fig. 1) was based on two vegetation maps taken at date 1 (d1) and date 2 (d2), displaying three main habitat types (A, B and C). Habitat types were defined as phytosociological syntaxa (e.g. *Phalaridetum arundinaceae* Libbert 1931, *Potamion pectinati* Carstensen 1955) or other natural biotopes (e.g. unvegetated river gravel banks). Phytosociological syntaxa were identified by determining vegetation relevés. Vegetation mapping was undertaken using commonly available GIS softwares (ArcGis 10.3, ESRI, Redlands, US; QGIS 2.14 Development Team).

Mapping software transformation tools were used to convert the maps to raster format, as explained by Gallet and Sawtschuk (2014) and to convert raster images into ASCII files. Before running (McGarigal et al., 2012), prerequisites such as pixel size were made consistent with field survey precision. Raster transformation was used to develop indices of change in vegetation dynamics at two different scales: (i) at large-scale, the zonal change index (ZCI) and (ii) at the local scale, the pixel change index (PCI).

The ZCI was based on the PCh_i, percentage of change in total area filled by each habitat type i within the landscape between d1 and d2. ASCII files promoted the development of the ZCI with Fragstats software (McGarigal et al., 2012). The ZCI was calculated between two dates-states, where N is the number of habitat types, and P_i is the percentage of total area filled by habitat type i on each date (d1 and d2). The 'Class' scale PLAND metric in Fragstats software (McGarigal et al., 2012) was useful to obtain the $P_{i.}$ The ZCI provides the sum of PCh_i within the landscape between d1 and d2 (loss or gain). This sum was divided by two in order to account for loss-gain in each habitat (Eq. (1)).

$$Zonal\ Change\ Index,\ ZCI\ (\%) = \frac{\sum_{i=1}^{N} PCh_i}{2} = \frac{\sum_{i=1}^{N} |P_{i(d1)} - P_{i(d2)}|}{2} \quad (1)$$

We defined the pixel change index (PCI) as the relative frequency of pixel number (PN) that changed from one habitat type to another between d1 and d2 (Eq. (2)). The latter was obtained thanks to the combination of the two raster images. This generated a transition matrix (d1 × d2) informing on both stable and dynamic transitions from one habitat type to another (or the same) between two dates (d1 and d2), with the surface area concerned for each type of transition.

$$Pixel\ Change\ Index,\ PCI\ (\%) = \frac{\sum_{i=1}^{N} PN\,(changed)}{\sum_{i=1}^{N} Total\ PN} \quad (2)$$

The PCI decreases if the system gains in stability between two dates. This provides an add-on index to the potential stability index described in Gallet and Sawtschuk (2014). Output results from the TMMs and PCI permit to identify the role of spatial heterogeneity and temporal variability, by focusing on changes from one pixel to another.

Reading both PCI and ZCI together is of fundamental importance, allowing definition of both local- and large-scale potential effects (Fig. 1). A high ZCI alongside a high PCI (with approximately equal values) reflects an ecosystem trajectory change towards a novel stable state. A PCI value higher than the ZCI value indicates the current stable state, with low zonal variation and natural dynamics maintained, while a low ZCI and a low PCI (with approximately equal values) reflect a lack of internal patch dynamics.

2.2. Application in the field

2.2.1. Study site and description

Since the mid-19th century, the upper Rhine River has been strongly modified by engineering works (Uehlinger et al., 2009). The original 3 km wide braided and anastomosed channel has been transformed into a 200 m wide stable channel, inducing bed degradation and sediment coarsening (Dittrich et al., 2010). These improvements resulted in the purging of part of its coarse load (Maire, 1997), leading to a loss of ecological functionality, especially along the Old Rhine River, a 50 km by-passed single-bed paved channel located at the border between France and Germany. The upstream part of the Rhine from "Village-Neuf" (Kilometric point – KP 174), near the border between

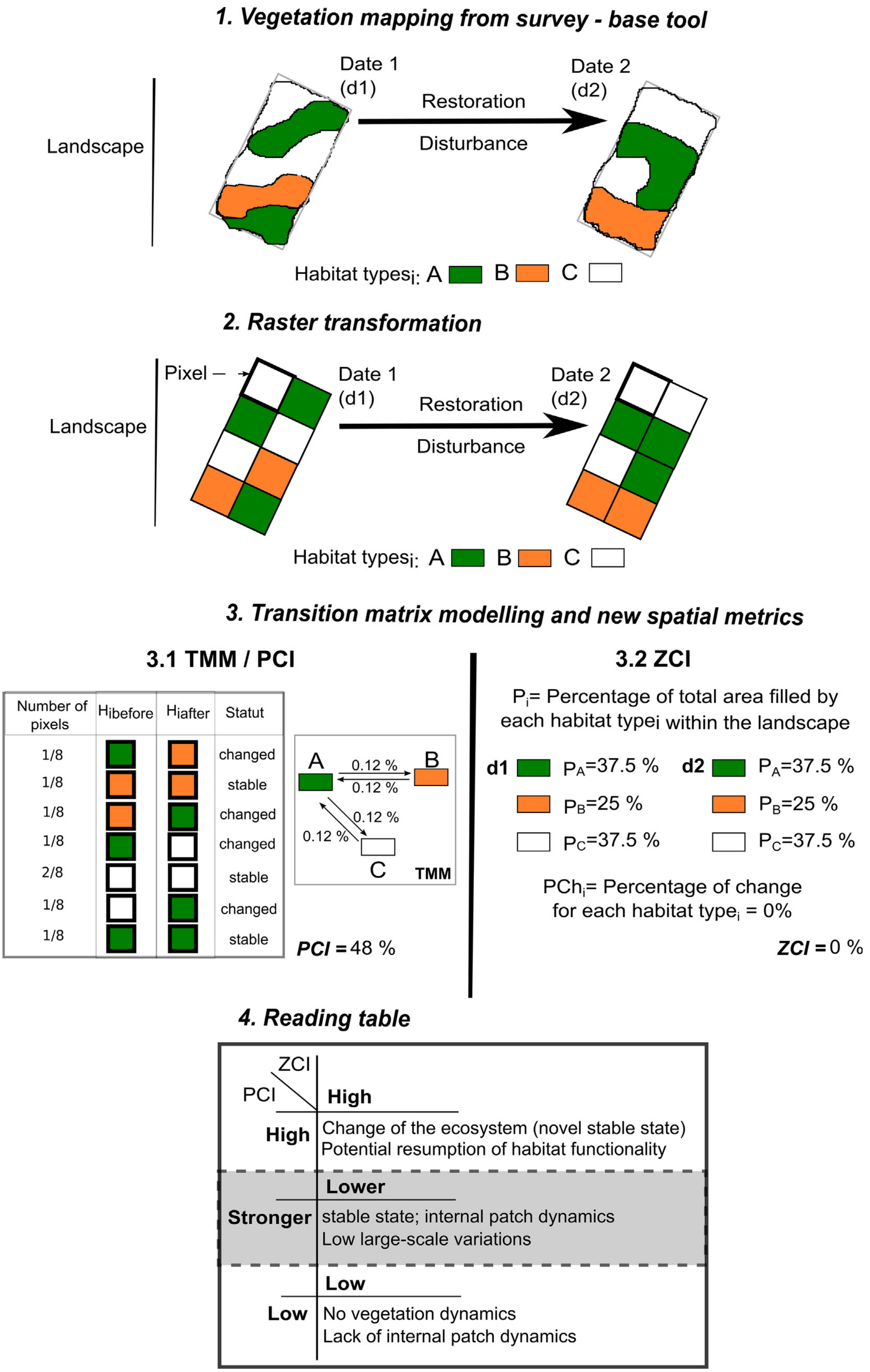

Fig. 1. Methodological scheme of a multiscale assessment protocol for revealing the complex nature of ecosystem change.

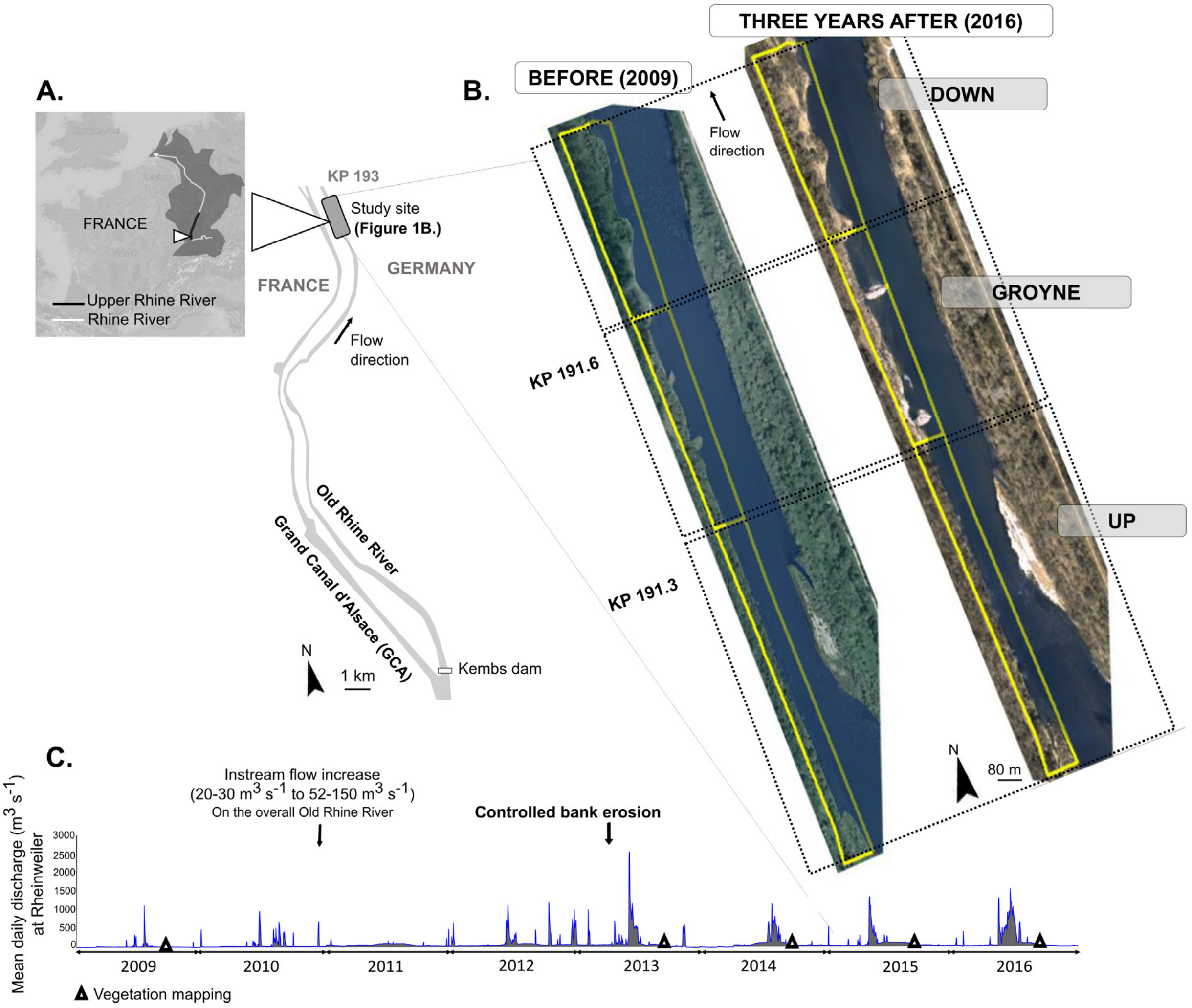

Fig. 2. A. Rhine catchment and study site locations within the Kembs diversion reach. B. Before-after orthophotos of the study site in 2009 and 2016, respectively. The 2016 orthophotograph shows each section (UP, GROYNE and DOWN) delimited by a yellow line, used for mapping standardisation. C. Mean daily flow hydrograph from 2009 to 2016 at Rheinweiler (15 km downstream the Kembs Dam, on the Old Rhine).

Switzerland and France, to "Vogelgrün" (KP 225), is divided by the Kembs diversion dam into two waterways, regulated in the mid-20th century (Fig. 2A). A flow of 1400 $m^3.s^{-1}$ is permanently diverted into the "Grand Canal d'Alsace", overflows exceeding 1400 $m^3.s^{-1}$ being routed to the Old Rhine.

The study site is located on the Old Rhine in southern Alsace (47°45′03·36″N, 7°32′27·41″E), about 2 km upstream of the Ottmarsheim Power Plant (power plant located on the Grand Canal of Alsace). In December 2010, the instream flow was increased within the Old Rhine from 20–30 $m^3.s^{-1}$ to 52–115 $m^3.s^{-1}$ (increase in May, 115 $m^3.s^{-1}$ reached by June–August, decreasing in September) with the aim of improving lateral mobility, and thus aquatic and riparian biodiversity (Garnier and Barillier, 2015). Over April–May 2013, a section of the study site located between KP 191.3 and KP 191.6 (Fig. 2B) was subjected to experimental restoration through controlled bank erosion with artificial groynes. In our study, the term "restoration works" for the GROYNE section includes the combined effects of instream flow increase and controlled bank erosion with artificial groynes. For the UP and DOWN sections, the same term represents the effects of instream flow increase only.

Controlled bank erosion provided lateral mobility to the Old Rhine by favouring the natural erosion of the left bank (French bank), which feeds the main sediment channel, by flooding (Garnier and Barillier, 2015). The restoration action was carried out in April 2013, after modelling and tests conducted on a reduced model at the EDF's National Laboratory for Hydraulics and Environment (Clutier et al., 2012). At the end of these simulations in March 2013, the entire left bank of the study site (with the exception of a few trees) was cleared prior to the removal of bank protection by mechanical destabilisation of the Tulla groynes (Pinte et al., 2015). Three groynes dating from the early 20th century already present at the study site; one of which was removed and the other two mechanically remodeled with bank doffing materials to form two artificial transverse groynes (Fig. 2B). During the study (1st June 2013), a Q_{15} flood (i.e. return period of 15 years) occurred. The intensity and duration of floods was lower in 2014 than 2015 and 2016 (Fig. 2C).

Vegetation mapping took place on the left bank only, and covered an area of 6.14 ha, with 1.7 km framed (see yellow line, Fig. 2B). Three homogeneous sections were delimitated for spatial scale analysis: (i) the UP section, located along the upstream stretch, restored by instream flow increase only (38% of the study site - 2.36 ha); (ii) the GROYNE section, located along the middle stretch, restored by both instream flow increase and controlled bank erosion with artificial groynes (29% of the study site – 1.73 ha); and (iii) the DOWN section, located along the downstream stretch and restored by the instream flow increase only. From April–May 2013, the latter section may also have been subject to slight effects of controlled bank erosion from the GROYNE section (33% of the study site – 2.05 ha).

2.2.2. Implementation of the multiscale assessment protocol

Vegetation mapping was undertaken during autumn in 2009, and from 2013 to 2016, when flow discharge was 52–60 $m^3.s^{-1}$. Mapping was performed at a scale of 1: 500 using phytosociological relevés (Braun-Blanquet, 1932) for riparian plants and a percentage cover estimate for aquatic vegetation. The same habitat typology was used for all maps, which were then integrated into ArcGis software v.10.3 (ESRI, Redlands, US). The aquatic channel was only mapped where expected restoration effects would be greatest, i.e. near the bank. Habitat types were defined according to standardised references such as the European Nature Information System (Louvel et al., 2013) or the Corine Biotope classification (Bissardon et al., 1997). For most habitat types, the more precise level of phytosociological classification was retained using local synsystems (Bardat et al., 2004; Ferrez et al., 2009; Hoff, 1975). Habitats of European concern were also identified using the French Habitats Classification (Directive 92/43/EEC, 1992). Habitat typology is synthesised in Appendix A. In order to construct the TMMs, fifteen maps were converted from vector data to raster format, with each pixel equal to 2 × 2 m. Maximum area option was used to consider the dominant vegetation type in a heterogeneous pixel. Twenty-one TMMs were built to provide before-after restoration works models (2009/2013, 2009/2014, 2009/2015, 2009/2016). The role of internal river dynamics was highlighted by TMMs 2013/2014, 2014/2015 and 2015/2016. Only percentages of surface area that have changed equal to or higher than 0.1% were considered. The TTMs allowed calculation of the PCI, following which the ZCI was calculated using Fragstats software v.3 (McGarigal et al., 2012).

2.2.3. Functional diversity endorsement

Thirteen semi-quantitative and qualitative traits were selected for the study i.e. size, growing form, flexibility (resistance to stem torsion), phenology, flowering, vegetative reproduction, reproduction period, dispersion, drying tolerance, fine-sediment affinity, eutrophication tolerance, organic matter and flow variation. Macrophyte trait preferences were defined according to Bornette et al. (1994), Henry et al. (1996), Meyer (2012) and Willby et al. (2000), with each taxon assigned a score describing its affinity to each modality of the trait: no affinity (0), low affinity (1) and high affinity (2). The affinity scores were then transformed into a relative use frequency distribution by dividing the scores for trait modalities by their sum (Appendix B). The trait tables were analysed by fuzzy correspondence analysis (Chevenet et al., 1994), while functional diversity indices (Petchey and Gaston, 2002) were calculated with the R-package *cati* (Taudiere and Violle, 2016) using the '*SumBL*' function with UPGMA classification in R software v.3.3 (R Core Team, 2017).

3. Results

3.1. Qualitative description of vegetation dynamics

The main changes were observed in the GROYNE section (Fig. 3). Restoration works encouraged the establishment of pioneer and dynamic assemblages at the expense of poplar and false acacia plantations. The *Phalaridetum arundinaceae* Libbert 1931 association evolved from 0.02 ha in 2009 to 0.60 ha in 2013, and remained stable over 2014. The drier *Rubo caesii – Populion nigrae* Passarge 1985 alliance occupied the top part of the eroded bank from 2015 to 2016. Aquatic vegetation cover was very sparse at the beginning of the colonisation process (3% of water surface area in 2013) but reached 15% of water surface area in vegetated zones by 2016.

The DOWN section showed a stable percentage of occupation (Fr) over time in each habitat type (Fig. 4). Similar observations were made for the UP section; however, we noticed a few changes after the instream flow increase, including a gain in pioneer habitats in the upstream part of this section (Fr_{2009} = 17.9%; Fr_{2016} = 19.1%).

The GROYNE section included a high area percentage of river gravel banks and pioneer habitats ($Fr_{2009\_\text{GRAVEL BANK + PIONEER HABITATS}}$ = 1.4%; $Fr_{2016\_\text{GRAVEL BANK + PIONEER HABITATS}}$ = 22.8%; Fig. 4). Although the water part decreased (Fr_{2009_WATER} = 65.8%; Fr_{2016_WATER} = 53.5%), aquatic and semi-aquatic vegetation appeared and increased strongly in 2016 (Fr_{2009} = 0%; Fr_{2016} = 9.3%). Restoration works favoured pioneer and willow shrub habitat types at the expense of mature post-alluvial forest and flood sward ($Fr_{2009\_\text{FORESTS + FLOOD SWARD}}$ = 26.8 %; $Fr_{2016\_\text{FORESTS + FLOOD SWARD}}$ = 3.2%).

Invasive species such as *Reynoutria japonica, Robinia pseudoacacia, Buddleja davidii, Solidago gigantea, Coniza canadensis* and *Stenactis annua* were more often observed on river gravel banks than on the artificial groynes. *R. japonica* cover was mainly fragmented over time, with many small plant shoots occurring along the riverbank associated with *S. gigantea, C. canadensis* and *S. annua*. Other invasive species were mostly located on the top of the bank. The invasive aquatic species *Elodea nuttallii* was first observed in a disconnected waterhole that connects with the main channel during high floods, in 2009. This species became established at the GROYNE section over 2015 to 2016, though with a low cover value (< 1%).

3.2. Quantitative description of vegetation dynamics

The 2009/2013, 2009/2014, 2009/2015 and 2009/2016 before-after restoration works TMMs only included instream flow increase effects for the UP and DOWN sections. In contrast with the UP and DOWN sections, the GROYNE section showed high ZCI cumulating with high PCI (Fig. 5). At this section, the highest values of both metrics were obtained in 2016 ($PCI_{2009/2016}$ = 43.7%; $ZCI_{2009/2016}$ = 36.2%). A stronger PCI than ZCI value was observed on all sections (Fig. 5). ZCI and PCI values were low and basically equivalent at the DOWN section. Both metrics were slightly higher at the UP section and increased over time, while those at the DOWN section remained low and stable.

Changes induced by internal river dynamics were quantified by TMMs 2013/2014, 2014/2015 and 2015/2016. PCI and ZCI values were lower at the UP and DOWN sections than at the GROYNE section, while both metrics increased at the GROYNE section for TMMs 2014/2015 and 2015/2016 ($PCI_{2013/2014}$ = 9.5 %; $ZCI_{2013/2014}$ = 6.8%; $PCI_{2015/2016}$ = 15.5%; $ZCI_{2015/2016}$ = 13.7%; Fig. 5). The main trajectories of the GROYNE section were shown by TMM 2009/2013, 2013/2014, 2014/2015 and 2015/2016 (Fig. 6). The TMM 2009/2013 had a low stability index value (63%), caused by a high percentage of *Carici albae – Tilietum cordatae* (Issler 1924–1925) Muller & Gors, 1958, EU 9170, later changing to *P. arundinacea* (Koch 1926) Libbert 1931 association (9.6%).

Aquatic vegetation first appeared in 2013 (Fig. 6), with the *Charetum vulgaris* Krause 1969, EU 3140 association (0.8%) and the *Potamion pectinati* (Koch) Libbert 1931, EU 3260 alliance (0.4%).

Fewer transitions from one habitat type to another were observed in TMM 2013/2014, with a stability index of 95% (Fig. 6). The main change in 2015 (Fig. 6) concerned expansion of the *R. caesii – P. nigrae* Passarge 1985 alliance (11.4%). Despite the high stability of TMMs 2014/2015 and 2015/2016 (stability index = 84%), structural and compositional habitat type changes were observed (Fig. 6), e.g. emergence of the *Batrachion fluitantis* Neuhausl 1959 EU 3140 alliance (9.3%) and a natural shift of sparsely vegetated gravel banks towards pioneer habitats (4.1%).

3.3. Aquatic vegetation and functional diversity

Functional diversity index values were high in 2016 (FD_{2016} = 3.50) in contrast to the preset value (FD_{2013} = 1.93).

Richness and cover of aquatic vegetation species displaying high flexibility and drought tolerance increased in 2016 (Fig. 7). In 2013, only two modalities of the drying tolerance trait (no and low tolerance) were represented within the aquatic vegetation community, whereas all four modalities were represented in 2016, increasing functional

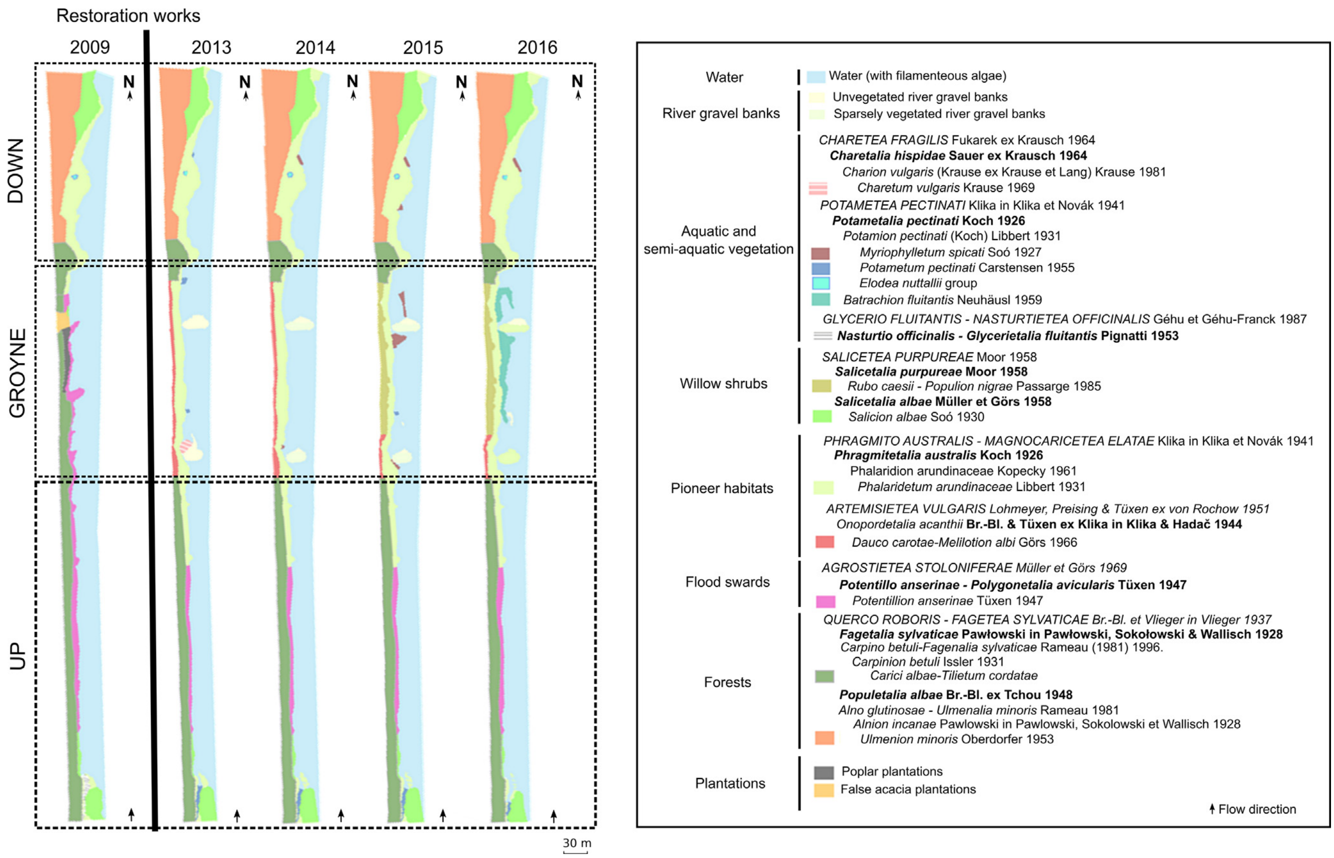

Fig. 3. Habitat type mapping for 2009 and from 2013 to 2016 at UP, GROYNE and DOWN sections.

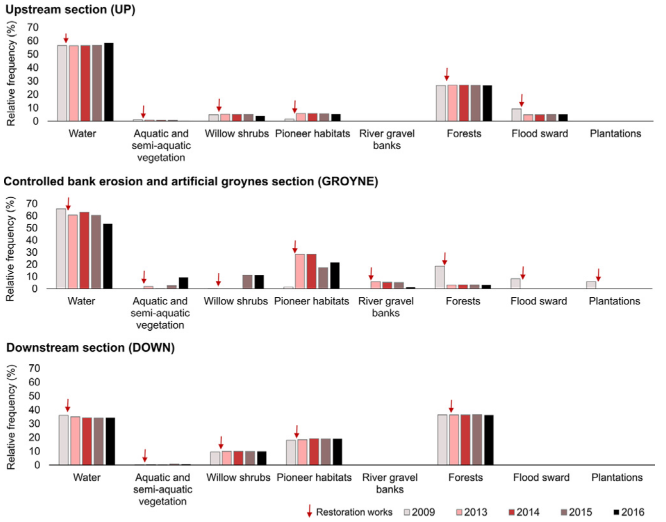

Fig. 4. Terrestrial and aquatic habitat occupation for 2009 and from 2013 to 2016 at each section.

A. Upstream area (UP)

	2013	2014	2015	2016
2009 PCI	8.1	8.4	8.2	9.4
ZCI	5.7	5.4	5.3	6.2
2013 PCI		0.6	×	×
ZCI		0.3		
2014 PCI			0.4	×
ZCI			0.3	
2015 PCI				2.6
ZCI				1.9

C. Downstream area (DOWN)

	2013	2014	2015	2016
2009 PCI	2.6	3.5	3.3	2.8
ZCI	1.1	1.9	2.0	1.9
2013 PCI		0.97	×	×
ZCI		0.98		
2014 PCI			0.7	×
ZCI			0.29	
2015 PCI				0.9
ZCI				0.37

B. Artificial groynes area (GROYNE)

	2013	2014	2015	2016
2009 PCI	37.3	35.3	37.6	43.7
ZCI	30.9	32.4	34.6	36.2
2013 PCI		9.5	×	×
ZCI		6.8		
2014 PCI			16.7	×
ZCI			13.7	
2015 PCI				15.5
ZCI				13.7

Fig. 5. Pixel change index (PCI) and Zonal Change Index (ZCI) values (%) for A) UP, B) GROYNE and C) DOWN sections, obtained by analysis of TMM 2009/2013, 2009/2014, 2009/2015, 2009/2016 and Fragstats outputs, and by analysis of TMM 2013/2014, 2014/2015, 2015/2016 and Fragstats outputs (black crosses: values not determined). Instream flow was increased in December 2010 along all sections. Controlled bank erosion with artificial groynes was undertaken at the GROYNE section in April–May 2013. Intensity and duration of flooding increased from 2014 to 2016.

richness. Running-water species, i.e. *Fontinalis antipyrectica*, *Ranunculus circinatus* and *Ranunculus fluitans*, emerged in localised areas in 2016. Over time, aquatic species showed variation in fine-sediment affinity and flow variation tolerance (Fig. 7).

4. Discussion

4.1. Effects of restoration works on vegetation dynamics

Before restoration works, the study site had a characteristic channelised river bank pattern with species common for altered riparian systems (Nilsson and Berggren, 2000). It was surrounded by an alluvial forest heading towards mature post-alluvial forest. Just a few of the pre-restoration habitat types were identified as habitats of European concern (EU 9170, EU 91E0); however, the number of habitats (Natura 2000) was increased with the emergence of three alliances, *R. caesii – P. nigrae* (EU 91E0), *P. pectinati* and *B. fluitantis* (both EU 3260), after

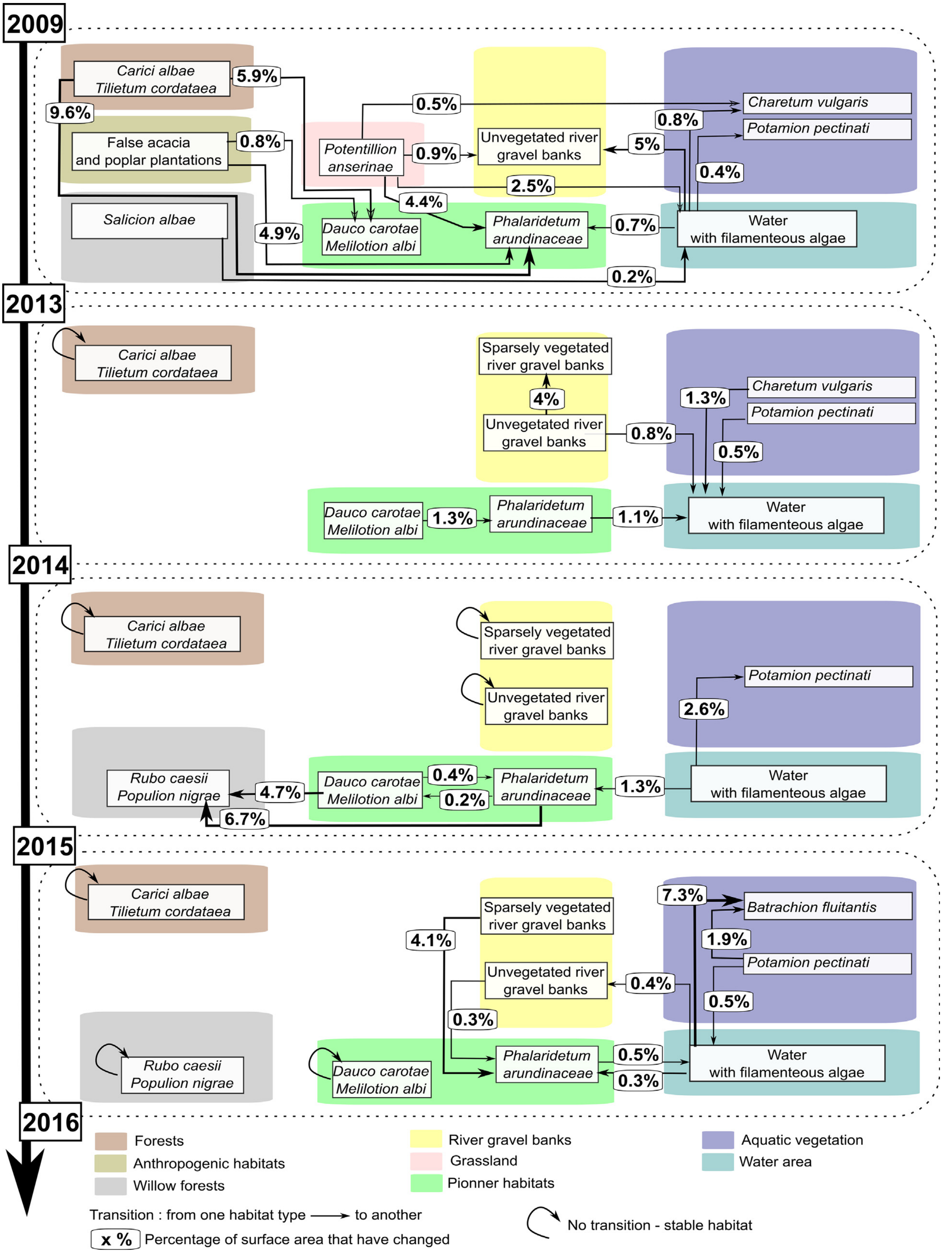

Fig. 6. Schematic box-and-arrow diagrams of transition matrix models for vegetation dynamics along the GROYNE section from TMMs 2009/2013, 2013/2014, 2014/2015 and 2015/2016.

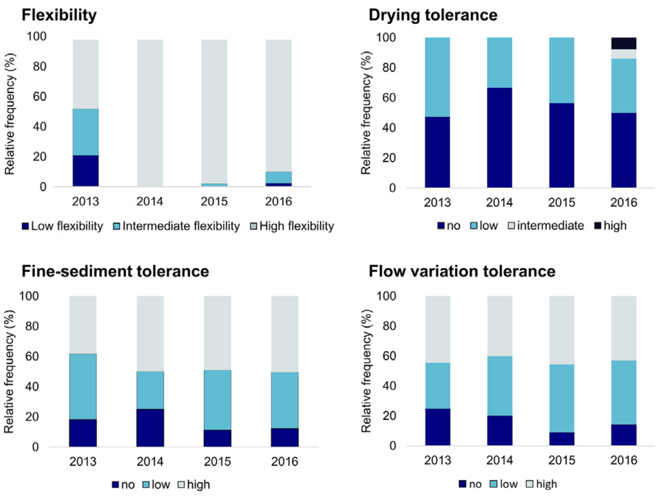

Fig. 7. Relative frequency of trait modalities at the GROYNE section from 2013 to 2016. Affinity scores were weighted by the mean percentage cover of aquatic species.

restoration. The most interesting outcome of the restoration was the increase in aquatic vegetation (EU 3260, EU 3140) and a progressive return to a high diversity plant assemblage, commonly reduced by channelisation (Rambaud et al., 2009).

Göthe et al. (2016) reported method of restoration as having an overriding role on plant community responses, with strongest effects attributed to stream channel widening, which reproduces a mosaic of habitats typical of natural floodplains (González et al., 2017). The effect of channel widening was reproduced through an instream flow increase combined with controlled bank erosion with artificial groynes. Both resulted in riparian system recovery and wider availability of aquatic vegetation niches, resulting in terrestrial and in-channel patchy habitat mosaic. Such a result would favour other biological communities, such as fish or macroinvertebrate diversity, with the emergence of species such as dragonfly larvae (Buczyński et al., 2017). The range of flow velocity values (predominantly low) and grain-size refinement produced along the GROYNE section resulted in an atypical environment for the active channelised Rhine floodplain. The cumulative effects of controlled bank erosion with artificial groynes and instream flow increase led to a global transformation at the landscape scale, as indicated by high PCI and ZCI values. Both metrics also highlighted the transient aspect of newly created fluvial forms, with high ZCI and PCI for TMMs 2014/2015 and 2015/2016 along the GROYNE section. This showed that the restoration works have multiplied the effects of internal river dynamics, i.e. the restored fluvial forms were more easily shaped than in channelised river patterns (UP and DOWN sections). The interdependence between physical processes, fluvial landforms and vegetation dynamics has been emphasised in a number of studies (Corenblit et al., 2007; Elosegi et al., 2010; Gurnell et al., 2012, Staentzel et al., 2018).

Three years after restoration works, the emergence of lentic habitats (low energy areas) contributed to the coexistence of contrasting alliances, e.g. *P. pectinati* and *B. fluitantis*, within the in-channel patchy habitat mosaic. Transient riffles in the inner groynes area facilitated settlement of running-water species such as *F. antipyrectica* and *R. fluitans*. Other new species with a lentic profile appeared, though they performed the same function as those present since 2013 (redundant species). A relatively long period was needed in order to see an increase in functional richness along the GROYNE section, as also noted in other studies (e.g. Meyer et al. 2013). Functional diversity index values showed that functional diversity increased strongly in 2016. Fine-sediment affinity and flow variation tolerance emerged as requirements for settling in the GROYNE section. The functional approach suggests increased spreading of the invasive aquatic species *E. nuttallii* in a few years, having the previous required aptitudes. Controlled bank erosion also encouraged the development of terrestrial invasive species although cover remained low and stable over time. A decrease in flood occurrence and energy flow, however, could lead to an increase in both terrestrial (e.g. *S. gigantea, C. canadensis, R. japonica*) and aquatic (*E. nuttallii*) invasive species. One major role of internal river dynamics could be the regulation of invasive taxa in such ecosystems (Greet et al., 2015).

PCI values were slightly higher than those for ZCI at the UP and DOWN sections, but both were lower than at the GROYNE section. Steep slopes and a channelised river pattern at both sections limited vegetation dynamics and the positive effects of instream flow increase (Gilvear, 1999). Following the instream flow increase, a single side-channel appeared at the upstream part of the UP section. Local fine substrate areas were observed near the left bank at the DOWN section, which encouraged *Myriophyllum spicatum to* settle. These effects observed are unlikely to be due to controlled bank erosion with artificial groynes; hence those effects were concentrated at the GROYNE section.

4.2. Relevance of the multiscale assessment protocol

Models for assessing landscape change have a limited ability to capture multiscale processes (Baker, 1989). Despite the multi-faceted context of fluvial hydrosystems (Amoros and Bornette, 2002; Camporeale et al., 2013; Gurnell et al., 2016), our protocol was able to quantify multiscale changes in vegetation dynamics. Considering the UP section in our field study, changes at a small scale not necessarily led to changes at the landscape scale. In comparison, we observed ecosystem changes in the GROYNE section and no internal patch dynamics in the DOWN section. Further vegetation mapping between 2009 and 2012 could have provided more PCI and ZCI values to evaluate ongoing river dynamics, including the effects of instream flow increase on the GROYNE section (2009/2011; 2009/2012; 2010/2011). Nevertheless, the BACI study design (Smith et al., 1993) allowed us to integrate spatial and temporal variations, and to discriminate the main event effects, i.e. restoration works or internal river dynamics, on alluvial vegetation communities.

The highly dynamic nature of the landscape was slightly underestimated by the TMMs due to the dominant percentage of water area in the mapping frame (Fig. 3). Percentage of change from one habitat type to another were lower than those in some previous studies focusing on more terrestrial ecosystems (Gallet and Sawtschuk, 2014). This could be explained by the structure of the fluvial hydrosystem, the dominant central channel remaining stable throughout the analysis. The TMMs allowed us to identify the main trajectories along the GROYNE section, the results indicating that most of the changes in vegetation dynamics were due to the combined effects of restoration works and internal river dynamics. Bare restored terrestrial soils represent pioneer habitats that change following flooding degree, with (i) high flooding resulting in habitat type homogenisation with persistence of pioneer habitat, (ii) low flooding leading to establishment of willow shrub, and (iii) no flooding favouring brushwood progression and colonisation by invasive species. Bare restored in-channel soils were first colonised by lentic communities. Later, shaping of fluvial forms by flooding resulted in changes to the taxonomic and functional richness of aquatic vegetation, favouring species with a lotic profile at the smaller scale. If such results can be generalised, the clear understanding of restoration-dependent habitat trajectories provided would prove a useful tool for managers, allowing the prediction of trajectories in multi-faceted habitats mixing human disturbance and natural processes.

4.3. Conclusions and prospects

In recent years, significant efforts have been dedicated to quantifying the efficiency and the sustainability of restoration actions (Friberg, 2014; Jaunatre et al., 2013; Wortley et al., 2013). This paper, promoting the use of easy-to-obtain data i.e. vegetation mapping, as a base tool for the multiscale assessment protocol, represents part of those efforts. This protocol provides a multiscale quantitative inference, which enriches classical approaches that usually provide large-scale descriptive evaluations as remote sensing methods (Rogan et al., 2002; Tong et al., 2017). As both ZCI and PCI can be aligned with the emergent property consideration (Loreau et al., 2003), they could be easily correlated with other multi-scale quantitative indicators as the Shannon index or the functional diversity (Beisel et al., 2003; Mason et al., 2005; Villéger et al., 2008). It could thus reinforce the understanding of interrelationships between vegetation dynamics and other biological communities, especially as regards shifts related to biological invasions or climate change. The method also represents a powerful tool for decision making and modelling of restoration actions, not just for post-restoration ecosystem changes monitoring, but also for pre-restoration. A further step would be to go beyond the evaluation of the restoration success and assume the multiple predictable trajectories (Choi, 2004) thanks to scenarii analyses e.g. using past learnings to simulate vegetation dynamics acknowledging the ever-changing environments.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind.2018.03.050.

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Effects of a river restoration project along the Old Rhine River (France-Germany): Response of macroinvertebrate communities

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ABSTRACT

The rise of restoration projects on large rivers is a response to the increasing human-induced pressures on these ecosystems. Despite this, there is a relative lack of data documenting restoration success using macroinvertebrate communities in such environments, with those existing frequently producing contrasting results. Here, we examined post-restoration responses of macroinvertebrates following a unique experimental restoration approach based on controlled bank erosion and artificial groyne implementation, initiated in 2013 on the Old Rhine River (France-Germany). We investigated how macroinvertebrate communities have responded to restoration-induced variations in three main abiotic parameters, i.e. water depth, flow velocity and substrate type, by comparing the restored section with unrestored ones. The Eco-hydro-morphological index (EHMID), a modified version of a hydro-morphological diversity index, showed a gain in mesohabitat heterogeneity along the whole site. Newly created mesohabitats with low flow velocity and finer substrate were dissimilar to those along the rest of the Old Rhine channel, favouring burrowing taxa such as Odonata. The presence of such insect larvae was related to the post-restoration emergence of typical alluvial terrestrial-aquatic border connectivity, and the rise in macrophytes over time. On the whole site, changes in composition or in functional profile diversity were highly related to the high degree of mesohabitat heterogeneity from the restored section, which would persist as long as groynes remain. The main inter-annual effect concerned the decrease in invasive taxa abundance that also varied according to any changes in fluvial forms. Our findings confirmed that macroinvertebrate responses are highly influenced by hydrological events and are dependent on the study-scale monitoring, clearly putting forward fine-scale hydromorphological gradients. Biological results from this restoration project should approach those obtained in smaller rivers restored using deflectors, suggesting a potential application of the hydraulic law of similarities. However, the accuracy of biological prediction using said application is limited by the distance from source populations, biological invasions and internal river dynamics.

1. Introduction

The growing deterioration of natural habitats has received special attention as human-induced pressures and impacts have increased, e.g. through resource overexploitation, pollution, biological invasions, fragmentation, hydro-morphological alteration, changes in water flow regime and climate change (Friberg, 2014; Grizzetti et al., 2017). Freshwater ecosystems are among the most affected (Vörösmarty et al., 2010; Smith and Chadwick, 2017) and are experiencing unprecedented declines in biodiversity (Dudgeon et al., 2006; Decamps, 2011). Large rivers are influenced by the upstream drainage network, the surrounding land, the riparian zone and the legacy of two centuries of engineering works. Since the 19th century, engineering works in large rivers, such as channelisation, rectification and damming, have caused a loss of lateral mobility and failures in active sediment transport (Wohl, 2012; Kondolf et al., 2014). This loss of functionality reduces fluvial hydrosystem complexity and modifies the river's geomorphological functioning, resulting in modifications to the three primary attributes of biocenoses, i.e. composition, structure and function (Franklin et al., 1981; Noss, 1990; Duan et al., 2011).

In an effort to reverse such impacts, ambitious hydro-morphological restoration actions have been initiated in many rivers throughout the

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world (Kondolf et al., 2014, Wohl et al., 2015). The primary objective of such restoration actions is generally focused on generating higher mesohabitat heterogeneity (Friberg et al., 2017). Macroinvertebrates are reliable indicators of temporal and spatial changes in aquatic habitats, widely used as bio-indicators of stream condition and water quality (Resh and Rosenberg, 1993; Resh, 2008; Kenney et al., 2009; Carter et al., 2017). For this reason, they are commonly used to monitor and assess river restoration efficiency (Peterson, 2015; Rubin et al., 2017). However, among the rise in restoration projects aiming to increase mesohabitat heterogeneity (Morandi et al., 2017), a strong positive response by macroinvertebrate assemblages has not been systematically observed (Jähnig and Lorenz, 2008; Louhi et al., 2011; Lepori et al., 2005; Jähnig et al., 2010; Palmer et al., 2010; Haase et al., 2013; Friberg, 2014). Verdonschot et al. (2016) and Pilotto et al. (2018) assumed that other external drivers or stressors, such as a depleted regional species pool, degraded water quality, insufficient hydro-morphological change or poor riparian zone habitat quality, could explain the lack of aquatic biota response to physical restoration. It has also been demonstrated that macroinvertebrates respond strongly to fine-scale hydro-morphological gradients, i.e. at the macroinvertebrate organism perception scale, inducing scale-dependent biological responses (Beisel et al., 2000; Lepori et al., 2005). Therefore, the lack of results from some studies regarding the link between macroinvertebrate responses and mesohabitat heterogeneity may also be due to the inappropriate scale of the study design, which prevents assessment of the effects of hydro-morphological river restoration (Jähnig et al., 2010).

In this study, we investigated macroinvertebrate changes following a unique experimental restoration programme along a 50-km stretch of by-passed single-bed paved channel on the Old Rhine River (France-Germany), located downstream of the Kembs dam. The project, which took place in April 2013, made use of controlling bank erosion in order to provide increased lateral mobility to the river by favouring natural erosion by floods. This process aims to feed the main channel with sediment from the eroded bank and potentially diversify the natural habitats (Garnier and Barillier, 2015). Our objective was to identify relationships between macroinvertebrate responses and hydro-morphological changes within the framework of solid transport and erosion processes. We specifically developed a mesohabitat heterogeneity index adapted to the study of macroinvertebrates by modifying the hydro-morphological index of diversity (HMID) originally proposed by Gostner et al. (2013) through the inclusion of a substrate-related element. The case study was included in a control-impact protocol (Smith et al., 1993) using spatial comparisons between the restored section and its upstream and downstream sections. Potential improvements resulting from the restored section were measured as a contribution to the whole site (restored + upstream + downstream sites). This contribution might lead to positive effects e.g. through higher mesohabitat heterogeneity, increased diversity indices, changes in community structure, i.e. variation in the relative abundance of pollution-sensitive taxa, new taxa and functional profile emergence, or neutral ones e.g. through redundancy with existing unrestored sections. Two main hypotheses were thus investigated: (i) restoration by controlled bank erosion enriches the channel with new geomorphic units that lead to a wider range of mesohabitats, i.e. combinations of substrate type, flow velocity and water depth; and (ii) any gain in mesohabitat heterogeneity will induce a positive response in the three primary attributes of biocenoses i.e. composition, structure and function. There is a clear current need for the assessment of post-restoration effects in biological structure and function for a better understanding of restoration consequences. In this study, a functional approach supplements the classical use of diversity indices in the assessment of restoration actions (Paillex et al., 2009; Friberg, 2014; Schmera et al., 2017; Frainer et al., 2018). Rather than simply recording a species loss or decrease in abundance, the use of a taxa trait approach can indicate potential causal mechanisms (Dolédec et al., 2006; REFORM D4.3, 2014; Friberg, 2014; White et al., 2017; Pilotto et al., 2018). Research on these issues provides an opportunity to identify reliable and sensitive indicators for improving the assessment of restoration on macroinvertebrate assemblages.

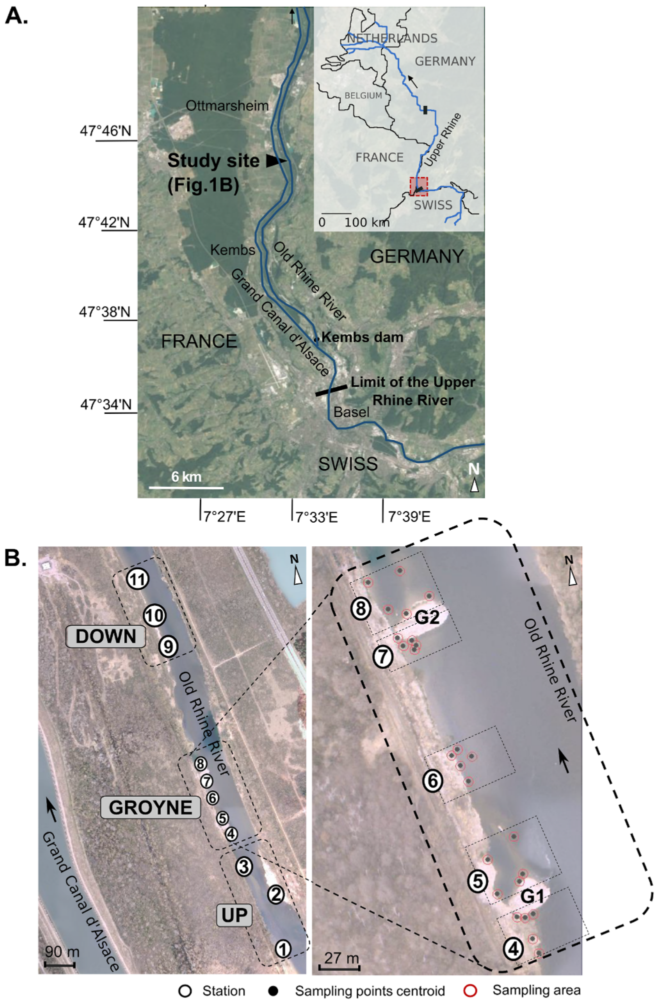

Fig. 1. A. Location of the study site (black arrow) on the Upper Rhine River (Google Earth, 2016). The grey box indicates the Rhine River watercourse, the two thick black lines framing the Upper Rhine River. B. Location of stations S1 to S11 along the three study sections: (i) the upstream section (UP), (ii) the restored section, including groyne implementation (GROYNE) and (iii) the downstream section (DOWN). At each station, five sampling points were selected that best represented the available mesohabitats (black dots on the orthophotography, 2015). The red circles correspond to sampling effort, i.e. five Hess cylinders by sampling point. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Material and methods

2.1. Study site

The study site was located on the upstream stretch of the Old Rhine River in southern Alsace (France) (Fig. 1A). Since the mid-19th century, the Upper Rhine has been strongly modified by engineering works (Uehlinger et al., 2009). The Kembs diversion dam divides the main course into two waterways from Kembs to Vogelgrun (near Basel): the Grand Canal d'Alsace (GCA) and the Old Rhine River, a 50 km by-passed single-bed paved channel. These works have undercut active sediment transport (Dittrich et al., 2010), especially in the Old Rhine River. The poor ecological functionality exhibited by the Old Rhine was largely due to hydro-morphological alterations, including bed incision, bed armouring with homogeneous coarse substrate, and static fluvial

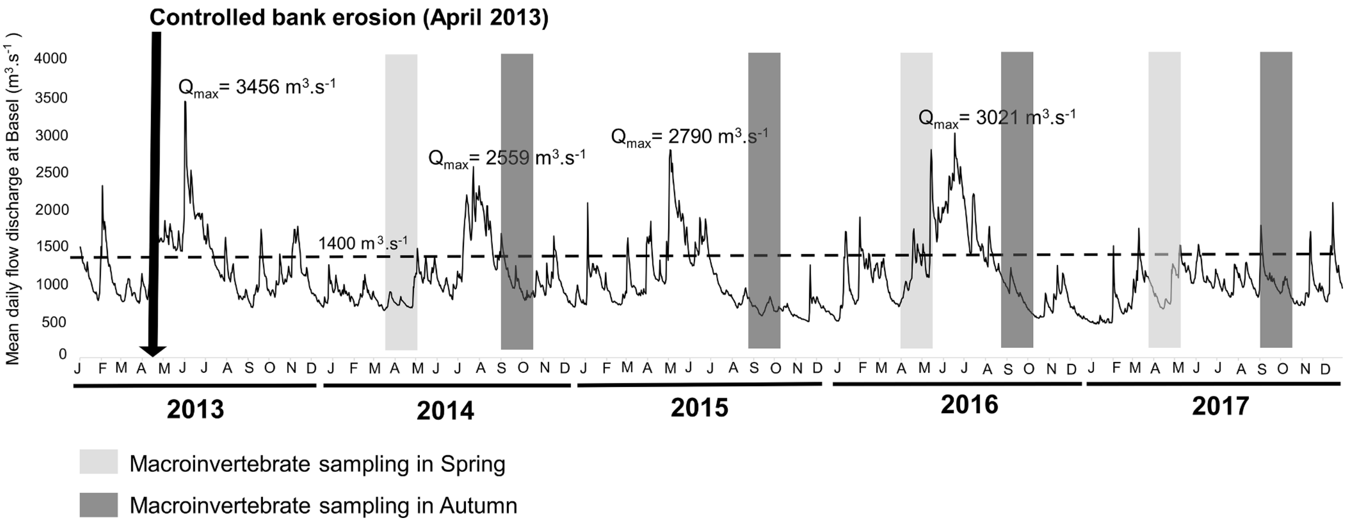

Fig. 2. Mean daily flow discharge at Basel ($m^3.s^{-1}$), hydrological constraints (i.e. flooding history) and periods of macroinvertebrate sampling (autumn/spring) at one year (2014), two years (2015), three years (2016) and four years (2017) after the restoration action (black arrow).

forms where macrophytes were scarce (CIPR, 2015; Staentzel et al., 2018a,b). Experimental restoration through controlled bank erosion was carried out in April 2013 after modelling and tests conducted on a 1/40th scale model (Clutier et al., 2012, Die Moran, 2012). The project aimed to provide increased lateral mobility to the Old Rhine by favouring natural erosion of the left (French) bank by flooding. Aside from a few trees, the entire left bank of the target site was cleared of vegetation in March 2013, prior to the removal of bank protections. A groyne constructed in the early 20th century was removed, and two others remodeled (Pinte et al., 2015; see G1 and G2 in Fig. 1B).

About two months after the restoration action (June 2013), a Q_{15}-flood (i.e. flow with a return-period of 15 years) occurred with a Q_{max} at Basel equal to 3456 $m^3.s^{-1}$ (Fig. 2).

A flow up to 1400 $m^3.s^{-1}$ is permanently diverted into the GCA, with any overflow exceeding 1400 $m^3.s^{-1}$ rerouted to the Old Rhine (Fig. 2). As the instream flow in the Old Rhine is less than 1400 $m^3.s^{-1}$, the flow is maintained at 52–60 $m^3.s^{-1}$ in order to meet minimum flow requirements for aquatic and riparian communities. The instream flow is subjected to modulation in its flow; increasing step by step in the spring to reach 115–150 $m^3.s^{-1}$, and decreasing as well in late summer-early autumn towards 52–60 $m^3.s^{-1}$. The duration and intensity of maximum energy flow was lower in 2014 and 2015 than in 2013. 2016 was characterised by a long period of flooding from March to July (Q_{max} at Basel = 3021 $m^3.s^{-1}$) and 2017 by a very low water level without main flood (Fig. 2).

2.2. Study design and sampling methodology

Macroinvertebrate sampling was performed during both spring and autumn in 2014, 2015, 2016 and 2017 (Fig. 2). The study site was divided into three sections (Fig. 1B); the upstream (UP) and downstream (DOWN) sections framing the restored section (GROYNE) and serving as spatial comparisons for evaluating changes related to the restoration action along a control-impact protocol (Smith et al., 1993). Three stations were located along the UP section (S1, S2 and S3), five along the GROYNE section (S4–S8) and three in the DOWN section (S9, S10 and S11). A thin gravel bar bordered the left bank of the DOWN section and the right bank of the UP section, while the waterbed consisted of a single-bed paved channel with reduced interstitial zones for macroinvertebrates.

Severe flooding halted the total spring campaign of 2015 and the spring campaign for the DOWN section in 2016. Hence, aside from the identification of spatial and temporal sources of variability, major analyses have undertaken on the autumn campaign datasets only. Each autumn faunistic dataset provided biological responses at one year (autumn 2014), two years (autumn 2015), three years (autumn 2016) and four years (autumn 2017) after restoration works (Fig. 2).

Macroinvertebrate sampling was performed using a Hess stream sampler (330 mm Ø × 400 mm high, 500 µm mesh; Hess, 1941) with an attached 500 µm dolphin bucket. For each of the eleven stations, five sampling points were collected within the range of mesohabitats observed, defined as a combination of flow velocity, water depth and substrate type (Beisel et al., 1998; Beisel et al., 2000). Each sampling point represented the sum of five Hess cylinder samples. The sampling area for each station, therefore, was equal to 2.1 m^2 (five sampling points = 25 Hess cylinders per station). Taxa were usually identified to genus taxonomic level or even species one, except for Oligochaeta and Nematoda, which were recorded as such.

In order to appreciate potential gains resulting from the restoration action, we examined abiotic and biotic metric differences considering (i) each individual section (UP, GROYNE and DOWN), and (ii) the pooled UP and DOWN sections, representing unrestored sections *versus* the pooled UP, GROYNE and DOWN sections, representing the whole site.

2.3. Abiotic measures and quantification of heterogeneity

River bottoms represent a mosaic of mesohabitats consisting of combinations of flow velocity (v), water depth (d) and substrate type (D_{50}). Substrate type was described through a visual estimation of the percentage covered by particle size classes based on a modified Wentworth grade scale (Malavoi and Souchon, 2002), i.e. silt [< 0.0625 mm], sand [0.0625–2 mm], gravel [2–16 mm], small pebbles [16–32 mm], coarse pebbles [32–64 mm], cobbles [64–256 mm] and boulders [> 256 mm]. The particle size distribution was defined using the visual estimation of the percentage covered by each particle size classes. Cumulative percentage distribution of the total number of all particle size classes permitted to obtain the D50, i.e. the particle size value or diameter at 50% in the cumulative distribution. For flow velocity and water depth, three repeated measurements were completed at each sampling point location (n = 165 by autumn campaign). Section-to-section differences in abiotic measurements were assessed using Kruskal-Wallis tests, with post-hoc multiple comparisons performed using the ‘pgirmess’ package (Giraudoux, 2012).

Gostner et al. (2013) developed the hydro-morphological index of diversity (HMID) as a straightforward tool for use in river engineering projects to assess quantitative mesohabitat heterogeneity. The HMID at a site is calculated as the product of partial diversities resulting from two hydraulic variables (Eq. (1)), flow velocity (v) and water depth (d).

$$HMID\ (\text{Gostner et al. , 2013}) = V(v)^2.\ V(d)^2 \tag{1}$$

$$HMID\ (\text{Gostner et al. , 2013}) = (1 + (\sigma_v/\mu_v))^2.\ (1 + (\sigma_d/\mu_d))^2$$

Each partial diversity (V) integrates into its formulation the coefficient of variation where σ is the variance and μ is the mean. While it has been demonstrated that the HMID approach sufficiently represents the hydro-morphological heterogeneity of stream reaches (Gostner et al., 2013), its application has to be integrated with evaluations of long-term streambed evolution at the catchment scale and is strongly related to the sediment regime of the stream under study (Gostner et al., 2013). We thus modified the HMID of Gostner et al. (2013) by adding a third abiotic parameter, i.e. the D_{50} of substrate particle size, in order to create the EHMID, the Eco-hydro-morphological index of diversity (Eq. (2)).

$$EHMID = V(v)^2.\ V(d)^2.\ V(D50)^2 \tag{2}$$

$$EHMID = (1 + (\sigma_v/\mu_v))^2.\ (1 + (\sigma_d/\mu_d))^2.\ (1 + (\sigma_{D50}/\mu_{D50}))^2$$

A total of eight abiotic indices were analysed for each station: HMID, EHMID, each partial diversity index $V(v)^2$, $V(d)^2$ and $V(D_{50})^2$ and the mean values of flow velocity (μ(v)), water depth (μ(d)) and median particle size ($\mu(D_{50})$).

2.4. *Spatiotemporal sources of variability on macroinvertebrate composition*

The relative importance of spatial (stations: from S1 to S11; sections: UP, GROYNE and DOWN) and temporal (years: from 2014 to 2017; seasons: spring and autumn; campaigns: autumn 2014 – AUT14, autumn 2015 – AUT15, autumn 2016 – AUT16, autumn 2017 – AUT17, spring 2014 – SPR14, spring 2016 – SPR16 and spring 2017 – SPR17) sources of variability in macroinvertebrate composition were assessed by comparing percentages of inertia explained by between-class correspondence analyses (CA), resulting from (i) a global factorial correspondence analysis on both seasons datasets [73 taxa × 370 sampling points] and (ii) a global factorial correspondence analysis on autumn datasets [59 taxa × 220 sampling points]. The inertia partitioning method is described in Dolédec and Chessel (1989).

2.5. *Diversity, composition and functional analyses*

Macroinvertebrate community structure was examined using densities, taxonomic richness (S) as well as classic diversity indices such as the Shannon diversity index (H′; Shannon and Weaver, 1963), the Simpson diversity index (D; Simpson, 1949) and Hurlbert's evenness ($E_{Hurlbert}$; Hurlbert, 1971). Looking at both indices of diversity was interesting in that the Shannon diversity index is more sensitive to rare taxa while Simpson diversity index is more sensitive to dominant taxa (Magurran, 2013). Reading both indices helped to clearly assess the gain or loss in taxa diversity between sections and years. The Hurlbert's evenness is a better evenness index than the usual Pielou index as it always ranges from 0 to 1, which simplifies the interpretation of ecological data (Beisel and Moreteau, 1997; Beisel et al., 2003). Five composition metrics were also defined: (i) the richness of EPTC (*Ephemeroptera*, *Plecoptera*, *Trichoptera and Coleoptera*), corresponding to pollution-sensitive taxa, (ii) the relative abundance of invasive taxa, corresponding to all invasive taxa present at the study site, (iii) the richness in Odonata taxa, (iv) the relative abundance of Chironomidae, and (v) the GOLD index, corresponding to the relative abundance of Gasteropoda, Oligochaeta, and Diptera (Pinto et al., 2004).

We performed nonmetric multidimensional scaling ordination (NMDS) of stations (Clarke, 1993) with the 'vegan' and 'MASS' packages to examine taxa composition pattern based on a Bray-Curtis similarity index. Here, each station × campaign event was treated as a basic unit of faunal analysis giving a [59 taxa × (44 stations × years)] where only macroinvertebrate abundance datasets from the autumn campaigns were used. Macroinvertebrate abundance data were log-transformed log2(x + 1) to downweight high abundances, while rare taxa (single individuals) were removed. Nematoda were removed from analyses because of their potential parasitic life cycle.

The five composition metrics were regressed on scores of the NMDS axes as well as 87 modalities from 15 bio/ecological traits describing biological, physiological and ecological preferenda (Appendix B) using the 'vectorfit' function to calculate the strength of association and determine the significance of R^2 (permutation test with 999 simulations; $p < 0.001$). The functional trait table was analysed using a biological traits analysis (Bremner et al., 2006). Each trait modality was weighted by relative taxa abundances at each sampling points over the years, providing a value of the trait modality representation at each sampling point (n = 5 by stations). At the end, the mean of these values was used to create a [(stations × years) × traits] table. Data on traits were derived from available biological information on taxa (including expert knowledge, particularly as regards invasive taxa; Usseglio-Polatera et al. (2000); Tachet et al., 2010; Beisel, pers. com.). Each abiotic parameter and index of spatial heterogeneity (see Section 2.3) were also regressed on scores of the NMDS axes. The 'ade4' and 'vegan' packages from R Studio software (1.0.143) were used to perform multivariate analyses.

Graphics and indices developed by Mouillot et al. (2013) for assessing functional changes after disturbance were applied in this study focusing on functional divergence (FDiv). For a first step, we realized a fuzzy PCA (FPCA) from the trait table of [87 modalities × 59 taxa]. The coupling of this output and the log-abundance taxa database permitted to run the function 'FD change' (Taudiere, 2015) based on the function 'FSECchange' from Mouillot et al. (2013). In order to maximize differences, the 'before' statement corresponded to the UP section and the 'after' statement corresponded to the GROYNE section. The functional divergence between statements reflected the shift or the overlapping (circles) that occurred in the functional space.

The functional eco-typology from Usseglio-Polatera et al. (2000) provides uniform functional groups of taxa that should allow a more effective use of macroinvertebrate biological and ecological traits. They used multivariate analyses to examine separately the relationships among 11 ecological traits of 472 benthic macroinvertebrate taxa (mainly genera). The Shannon index of diversity calculated on the seven defined ecological groups (Usseglio-Polatera et al., 2001) was used to explore the contribution of the restored section to the functional diversity at the whole site.

3. Results

3.1. *Mesohabitat heterogeneity*

On average, autumn flow velocity (v) was significantly lower in the GROYNE section than in the UP and DOWN sections (Table 1). While a similar trend was observed with the D_{50}, significant differences were only recorded for the GROYNE and UP sections in 2016. However, a high partial diversity in D_{50} was recorded (Table 1).

The HMID and EHMID showed a high sensitivity to intermittent extreme differences in variables. In our study, the GROYNE section displayed a higher EHMID than the two other sections, resulting in a patchy-mesohabitat mosaic (Fig. 3). The HMID, based on flow velocity and water depth variations, showed no such differences between sections (Table 1). The contribution of the GROYNE section to the whole site was assessed with differences in EHMID between the pooled UP, GROYNE and DOWN sections i.e. the whole site, and the pooled UP and DOWN sections i.e. unrestored sections (Fig. 3). On the whole site, the higher EHMID informed that the newly created mesohabitat pattern

Table 1
Mean values ± standard deviations of water depth, flow velocity ($n_{UP} = 45$; $n_{GROYNE} = 75$; $n_{DOWN} = 45$) and of the median particle size ($n_{UP} = 15$; $n_{GROYNE} = 25$; $n_{DOWN} = 15$) in each section. Means with different letter (a, b) were significantly different in section pairwise comparisons (post-hoc Kruskal-Wallis tests; $p < 0.05$).

	UP (n = 3 stations)	GROYNE (n = 5 stations)	DOWN (n = 3 stations)
2014			
Flow velocity (m/s)	0.29 ± 0.29^{a}	0.09 ± 0.12^{b}	0.22 ± 0.14^{a}
Water depth (m)	0.23 ± 0.10^{a}	0.35 ± 0.14^{b}	0.39 ± 0.16^{b}
Median particle size (D_{50})	42.96 ± 27.11^{a}	22.34 ± 16.77^{a}	31.17 ± 12.66^{a}
HMID/EHMID	8.40/22.34	12.69/38.92	5.32/10.51
Partial diversity $V(v)^2$	3.91	6.42	2.70
Partial diversity $V(d)^2$	2.15	1.97	1.96
Partial diversity $V(D50)^2$	2.66	3.06	1.97
2015			
Flow velocity (m/s)	0.31 ± 0.34^{a}	0.08 ± 0.10^{b}	0.20 ± 0.18^{b}
Water depth (m)	0.28 ± 0.11^{a}	0.42 ± 0.15^{b}	0.44 ± 0.21^{b}
Median particle size (D_{50})	45.78 ± 30.52^{a}	25.96 ± 22.86^{a}	34.90 ± 13.58^{a}
HMID/EHMID	9.63/26.75	10.25/36.24	7.90/15.25
Partial diversity $V(v)^2$	4.64	5.41	3.64
Partial diversity $V(d)^2$	2.07	1.89	2.16
Partial diversity $V(D50)^2$	2.78	3.53	1.93
2016			
Flow velocity (m/s)	0.48 ± 0.58^{a}	0.07 ± 0.10^{b}	0.36 ± 0.17^{a}
Water depth (m)	0.27 ± 0.11^{a}	0.39 ± 0.16^{b}	$0.24 \pm 0.19^{a,b}$
Median particle size (D_{50})	38.55 ± 11.90^{a}	19.85 ± 18.17^{b}	$31.55 \pm 13.24^{a,b}$
HMID/EHMID	14.29/24.49	12.23/44.92	7.29/14.69
Partial diversity $V(v)^2$	7.33	6.00	3.27
Partial diversity $V(d)^2$	1.94	2.03	2.23
Partial diversity $V(D50)^2$	1.71	3.67	2.01
2017			
Flow velocity (m/s)	0.31 ± 0.36^{a}	0.07 ± 0.11^{b}	0.24 ± 0.18^{a}
Water depth (m)	0.22 ± 0.09^{a}	0.30 ± 0.14^{b}	0.38 ± 0.10^{b}
Median particle size (D_{50})	47.10 ± 30.57^{a}	21.9 ± 26.59^{a}	29.97 ± 15.83^{a}
HMID/EHMID	9.76/26.54	13.90/67.96	5.44/12.71
Partial diversity $V(v)^2$	4.64	6.19	3.31
Partial diversity $V(d)^2$	2.10	2.24	1.64
Partial diversity $V(D50)^2$	2.72	4.88	2.33

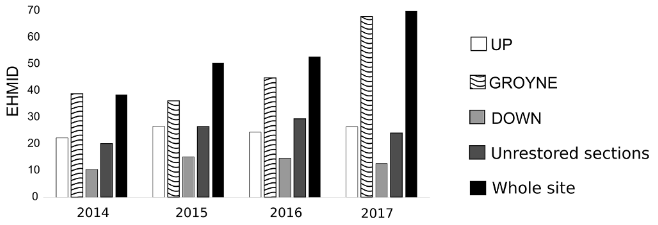

Fig. 3. Inter-annual Eco-hydro-morphological index (EHMID) for each section (UP, GROYNE and DOWN) and pooled sections i.e. unrestored sections as the pooled UP and DOWN sections, and the whole site as the pooled UP, GROYNE and DOWN sections.

with lentic geomorphological units and finer substrate areas were added to the existing mesohabitats (Fig. 3).

3.2. Relative importance of spatiotemporal sources of variability on macroinvertebrate composition

The between-class inertia distribution produced by the global CA [73 taxa × 370 samples] (Fig. 4) showed that the effect of seasonal factor (season: 2.39%) was lower than that of spatial factors (section: 3.92%; station: 9.19%), though the percentage of inertia explained by field campaigns (CAMP as [year × season]) was equal to 11.15%.

The highest percentage of inertia explained was by stations × campaigns (39.38%). The low effect of seasonal sources of variability confirms the potential use of autumn datasets only for the follow-up analysis. The restricted global CA on autumn datasets reflects the need for a spatial approach based on stations, using a control-impact protocol (Smith et al., 1993) on the basis of an inter-annual post-restoration approach (years: 6.32%, station × years: 39.38%).

3.3. Restoration-induced effects on biological structure, composition and function

Overall richness for the whole site was higher in 2014 (+4), 2015 (+7), 2016 (+4) and 2017 (+3). Owing to the variation in taxa richness, the Shannon diversity index and the Hurlbert's evenness varied only slightly between the unrestored sections and the whole site. The lowest value of the Shannon diversity index was recorded in the GROYNE section in 2016, though this was not a consequence of invasive taxa, which play an important role elsewhere in the lack of evenness within macroinvertebrate communities.

Data for macroinvertebrate composition indicated a higher richness on the whole site than in unrestored sections, indicating that new taxa were observed in the restored section (Table 2). In contrast to EPTC, whose relative abundance (RA) increased strongly over time in the unrestored sections, the abundance of Odonata remained low. The opposite pattern was observed at the GROYNE section, which had low EPTC ($RA_{\_2014} = 5.81\%$; $RA_{\_2015} = 2.65\%$; $RA_{\_2016} = 4.05\%$, $RA_{\_2017} = 1.49\%$) values and the highest richness in Odonata taxa. In 2017, the GROYNE section showed a relative abundance of 2.32% for Odonata taxa compared to 2014 (0.06%), increasing the whole site relative abundance in Odonata taxa to 0.51% (2014: 0.02%). While invasive taxa accounted for up to 60% of macroinvertebrate composition at the whole site in 2014 and 2015, values were lower in 2016 and 2017 ($RA_{\_2016} = 42.43\%$; $RA_{\_2017} = 41.82\%$).

The NMDS ordination showed that the best representation of observed distances between stations was achieved at two dimensions with a final stress value of 0.19 (Fig. 5). This value ensured a good representation at reduced dimensions (stress value > 0.3 indicate weak solutions). The DOWN section was located at an intermediate position on the NMDS ordination while the GROYNE section was located at the

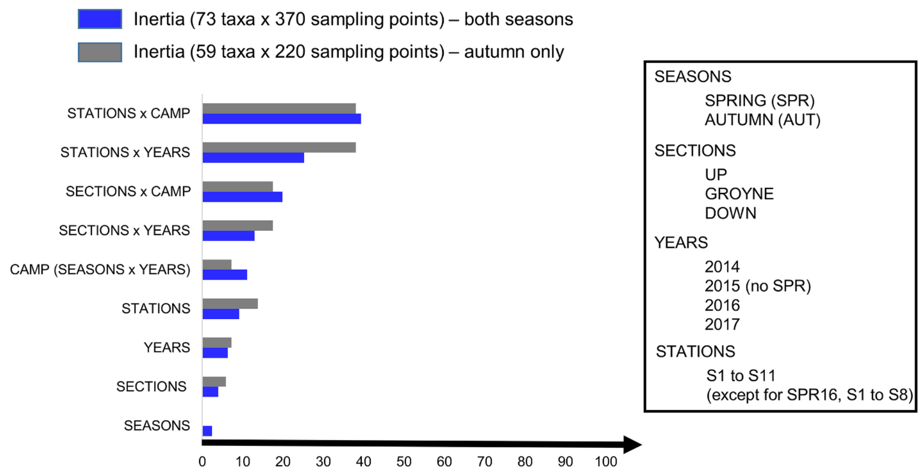

Fig. 4. Percentage of inertia for each between-class analysis from (i) the global CA with both seasons datasets [73 taxa × 370 sampling points] and (ii) the CA for autumn datasets only [59 taxa × 220 sampling points].

Table 2

Table of structural indices at each year (2014, 2015, 2016 and 2017) for individual sections and pooled ones (NR: unrestored sections, WS: whole site): total taxonomic richness (S), density (ind.m^{-2}) details on invertebrate composition using pollution-sensitive (EPTC), invasive (INV) and Odonata (ODO) relative abundance, and diversity indices (Shannon diversity index H′; Simpson index D and Hurlbert evenness $E_{Hurlbert}$).

	Year	S	Density (ind.m^{-2})	EPTC–INV–ODO (%)	H′–D–$E_{Hurlbert}$
UP	2014	36	1483.65	22.15 – 54.74 – 0.00	1.94 – 0.22 – 0.53
GROYNE	2014	28	642.66	5.81 – 64.42 – 0.06	1.86 – 0.20 – 0.50
DOWN	2014	32	1091.42	5.61 – 64.24 – 0.00	1.82 – 0.23 – 0.49
NR	2014	40	1287.54	15.14 – 58.77 – 0.00	1.98 – 0.20 – 0.54
WS	2014	44	994.41	12.39 – 60.43 – 0.02	1.99 – 0.20 – 0.55
UP	2015	30	2821.10	10.62 – 81.57 – 0.00	1.53 – 0.33 – 0.42
GROYNE	2015	35	1009.70	2.65 – 66.60 – 0.03	1.66 – 0.24 – 0.45
DOWN	2015	32	3859.20	6.22 – 84.79 – 0.01	1.51 – 0.28 – 0.41
NR	2015	37	3340.20	8.07 – 83.43 – 0.007	1.55 – 0.29 – 0.42
WS	2015	44	2280.90	6.98 – 80.04 – 0.01	1.64 – 0.27 – 0.45
UP	2016	33	1770.63	40.21 – 30.79 – 0.008	1.99 – 0.18 – 0.54
GROYNE	2016	28	484.00	4.05 – 46.24 – 0.09	1.37 – 0.34 – 0.37
DOWN	2016	31	1405.87	20.04 – 54.89 – 0.02	1.82 – 0.20 – 0.49
NR	2016	40	1588.25	31.28 – 41.46 – 0.01	2.03 – 0.16 – 0.55
WS	2016	44	1086.32	25.77 – 42.43 – 0.03	1.97 – 0.17 – 0.54
UP	2017	26	1334.60	32.92 – 35.92 – 0.35	2.16 – 0.14 – 0.59
GROYNE	2017	29	300	1.49 – 39.43 – 2.32	1.49 – 0.35 – 0.40
DOWN	2017	35	2363.30	24.61 – 45.65 – 0.21	1.96 – 0.17 – 0.53
NR	2017	39	1849.00	27.61 – 42.14 – 0.26	2.08 – 0.15 – 0.57
WS	2017	42	1144.90	24.50 – 41.82 – 0.51	2.06 – 0.16 – 0.56

opposite right-bottom position from the UP section, mainly characterised by a large representation of Trichoptera: *Hydropsyche* sp. (TRICHO6: *H. exocellata*, *H. incognita/pellucida*, *H. angustipennis*, *H. siltalai*), *Rhyacophila sensu stricto* (TRICHO13), *Hydroptila occulta* (TRICHO7), Limnephilidae (TRICHO11), *Agraylea* sp. (TRICHO8) and Glossosomatidae/Agapetinae (TRICHO2/3) (Fig. 5A).

A significant negative correlation was observed between richness in EPTC and the first NMDS axis ($R^2 = 0.60$, $p < 0.001$; Table 3). The high $V(D_{50})^2$ ($R^2 = 0.55$, $p < 0.001$, Table 3) and EHMID ($R^2 = 0.33$, $p < 0.001$, Table 3) recorded in the restored section did not result in colonisation by pollution-sensitive taxa, possibly due to the low flow velocity values ($R^2 = 0.42$, $p < 0.001$, Table 3). Gains in mesohabitat heterogeneity and changes in abiotic parameters favoured a greater density of taxa adapted to low flow velocity and, to a lesser extent, finer substrates ($\mu(D_{50})$; $R^2 = 0.35$, $p = 0.002$; Table 3). The restored section hosted a number of common taxa (GOLD_RA, $R^2 = 0.40$, $p < 0.001$), such as Dipteran Chironomidae (Chiro_RA, $R^2 = 0.43$, $p < 0.001$). Among new taxa observed in the restored section, we observed *Calopteryx splendens*, *G. vulgatissimus*, *Onychogomphus* sp., *Platycnemis* sp., and *Pyrrhosoma nymphula*. However, only a weak correlation was observed between richness in Odonata ($R^2 = 0.22$, $p = 0.005$) and the first NMDS axis, though just a few individuals (n < 10) were recorded during campains apart from 2017 where 73 individuals were found in the restored section compared to UP (n = 30) and DOWN (n = 31) sections. No significant correlation was found for invasive relative abundance ($R^2 = 0.19$, $p = 0.018$), which were high in all macroinvertebrate communities (> 30%). Here, the HMID was not correlated to taxa composition across stations ($R^2 = 0.02$, $p = 0.66$, Table 3). Overall, the direction of vectors showed a shift along the first NMDS axis from rheophilic taxa to limnophilic taxa from the UP section to the GROYNE section (Fig. 5B). Vector length is proportional to the correlation between the variable and the ordination axis (Table 3). The highlighted inter-annual effect was that the three sections presented similar compositions in 2014. Over the years, a real dissociation took place. While the evolutionary trajectories remained parallel, taxa composition from the restored section appeared further and further away from unrestored sections (Fig. 5).

Modalities of functional traits were also regressed on the NMDS ordination in order to shed further light on the functional contribution of the restored section at the whole site (Fig. 5C). This concerned taxa having an affinity to fine-sediment as mud (SUBS9, $R^2 = 0.54$, $p < 0.001$; Table 3) or silt (SUBS4, $R^2 = 0.53$, $p < 0.001$; Table 3). Communities at GROYNE section were characterized by burrowing taxa (LOCOM5, $R^2 = 0.42$, $p < 0.001$), taxa living in macrophytes (FOOD5, $R^2 = 0.34$, $p < 0.001$), taxa with clutches free or in vegetation (REPRO5, REPRO6, $R^2 = 0.44/0.37$, $p < 0.001$) and parasite (FEE8, $R^2 = 0.45$, $p < 0.001$) (Table 3). Their dispersion is mainly aerial passive (DISP3, $R^2 = 0.39$, $p < 0.001$; Table 3). At the opposite right-bottom position, the UP section was mainly characterised by taxa having a high affinity for cobbles/boulders (SUBS1, $R^2 = 0.37$, $p < 0.001$; Appendix C) and twigs/roots (SUBS7, $R^2 = 0.50$, $p < 0.001$; Appendix C).

The displacement of circles in Fig. 6A indicates a slight shift in the functional space between the before (UP) and after (GROYNE) statements, resulting in an overlapping of the functional divergence (Fig. 6A). The diversity in eco-profiles was higher in the unrestored sections than at the whole site (Fig. 6B). However, the disparity in eco-profiles diversity between mesohabitats increased over time at the whole site (Fig. 6B). The latter result was thus similar to the EHMID change over time (Fig. 3).

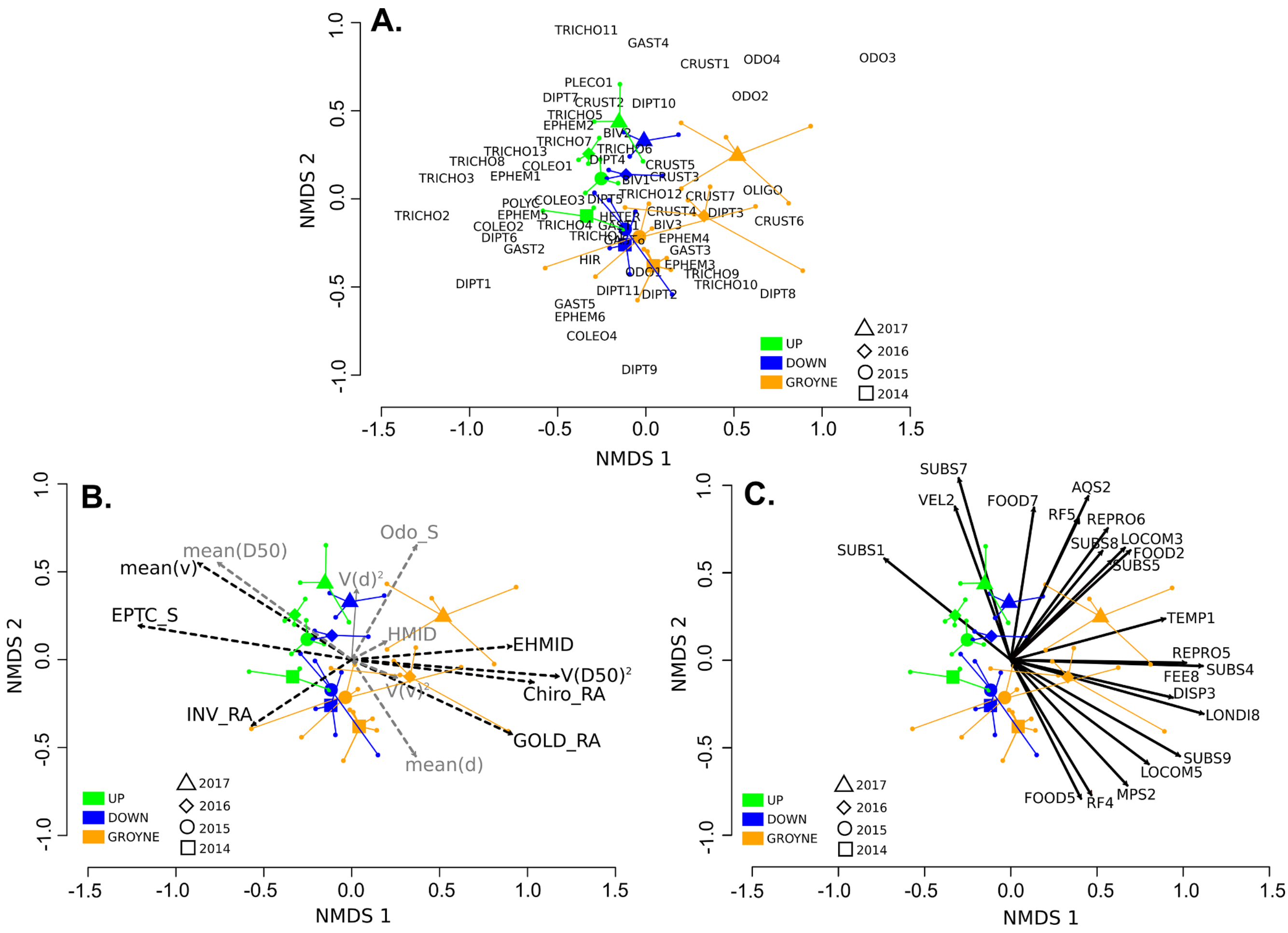

Fig. 5. A. Community composition pattern (the coding system of taxa is available in Appendix A) of stations colored by their section affiliation (UP, DOWN and GROYNE) resulting from non-metric multidimensional scaling ordination (NMDS) based on autumn datasets only, B. Setting of thirteen environmental and structural metrics where metrics in black showed the highest effect on community composition across stations ($p < 0.001$). C. Community composition pattern of stations colored by their section affiliation (UP, DOWN and GROYNE) resulting from non-metric multidimensional scaling ordination (NMDS) based on autumn datasets only. Only modalities showing the highest effect ($p < 0.001$) on community composition across stations were represented (see Appendices A and B with respectively coding for taxa and modalities, and the Appendix C for R^2 and p-value).

4. Discussion

4.1. Goal attainment regarding mesohabitat heterogeneity

Important morphological evolutions were highlighted after the extreme hydrological episode from the 1st June 2013, inducing new morphological patterns of natural habitats (Garnier and Barillier, 2015; Chardon and Schmitt, 2018) as emerged sedimentary benches, emergent deposits, and the appearance of a notch of erosion at the right of the upstream groyne (Fig. 1B). While morphological evolution was consistent with initial modelling studies, high lateral mobility and an increased sediment erosion budget were not achieved (Die Moran, 2012; Chardon et al., 2016; Chardon and Schmitt, 2018) as flooding uncovered historical embankments that strongly limited lateral erosion, and thus sediment feeding for self-restoration. The volumes eroded laterally at the bank are mainly attributable to the erosion notch at right upstream (Chardon and Schmitt, 2018). On the whole site, Chardon and Schmitt (2018) showed a clear substrate refinement with a significant diversification of the substrate after restoration works. This prompted a rise in macrophytes including those of an aquatic invasive plant species, *Elodea nuttallii*, three years after restoration works within the inner-groynes section (Staentzel et al., 2018b).

For enhancing biotic communities, restoration projects should aim to increase (and monitor) habitat diversity at a range of spatial scales that are ecologically relevant for the target organism groups, especially for those that have contrasting responses (Beisel et al., 1998; Muhar et al., 2016). The spatiotemporal sources of variability analysed in this study highlight the need to describe physical changes at a fine-scale in order to perceive changes in the macroinvertebrate community. At the station-scale used in this study, near-bed hydraulic and physical variables of control (i.e. flow velocity and water depth) were just one of the major determinants in influencing post-restoration mesohabitat distribution and heterogeneity. The patchy-substrate mosaic appeared to have greater relevance for the landscape perception of macroinvertebrates (Beisel et al., 1998) and, therefore, macroinvertebrate response may be limited by the type and dynamics of substrate favoured by the restoration project (Mueller et al., 2014; Verdonschot et al., 2016). The EHMID was higher in the GROYNE section than in the UP or DOWN sections due to higher partial diversity in flow velocity V $(v)^2$ and $V(D_{50})^2$ than elsewhere. Following restoration, small areas of fine-substrate were quickly added to the bottom mosaic of the Old Rhine, though these did not replace all of the mesohabitats previously in place. Our first hypothesis was thus appropriate, confirming that the addition of fine-substrate areas contributed to a gain (i.e. positive contribution of the GROYNE section) in mesohabitat heterogeneity over the whole site. Some localised fine-sediment deposition was observed locally in the DOWN section near the left bank, though this was not necessarily attributable to the restoration action.

Table 3
Correlation coefficients of the thirteen environmental and structural metrics (code and details) with p-values ($^{***} < 0.001$, $^{**} < 0.01$, $^{*} < 0.05$).

Code	Details	R^2 (*p-value*)
Structural metrics		
Mean (v)	Mean of flow velocity (v)	0.42^{***}
Mean (d)	Mean of water depth (d)	0.17^{*}
Mean (D50)	Mean of median particle size (D50)	0.35^{**}
V(v)^2	Partial diversity of flow velocity (v)	0.03
V(d)^2	Partial diversity of water depth (d)	0.06
V(D50)^2	Partial diversity of median particle size (D50)	0.55^{***}
HMID	Hydro-morphological index of diversity (Gostner et al., 2013)	0.02
EHMID	Eco-hydro-morphological index of diversity	0.33^{***}
EPTC_S	Richness in Ephemeroptera, Plecoptera, Trichoptera and Coleoptera taxa	0.60^{***}
Odo_S	Richness in Odonata taxa	0.22^{**}
GOLD_RA	Index GOLD: relative abundance of Gasteropoda, Oligochaeta and Diptera taxa	0.40^{***}
Chiro_RA	Relative abundance of Chironomidae taxa	0.43^{***}
INV_RA	Relative abundance of invasive taxa	0.19^{*}
Function approach with the most significant trait modalities		
MPS2	Maximum potential size type 2: $> 0.25–0.5$ cm	0.41^{***}
AQS2	Aquatic stage type 2: larvae	0.46^{***}
REPRO5/REPRO6	Reproduction type 5: clutches, free	0.44^{***}
	Reproduction type 6: clutches, in vegetation	0.37^{***}
DISP3	Dispersal type 3: aerial passive	0.39^{***}
RF4/RF5	Resistance form type 4: diapause or dormancy	0.35^{***}
	Resistance form type 5: none	0.34^{***}
LOCOM3/LOCOM5	Locomotion type 3: full-water swimmer	0.36^{***}
	Locomotion type 5: burrower	0.42^{***}
FOOD2/FOOD5/FOOD7	Food type 2: detritus < 1 mm	0.37^{***}
	Food type 5: living macrophytes	0.34^{***}
	Food type 7: living microinvertebrates	0.33^{***}
FEE8	Feed type 8: parasite	0.45^{***}
LONDI8	Longitudinal distribution type 8: outside river system	0.57^{***}
SUBS1/SUBS4/SUBS5/	Substrate type 1: flags/boulders/cobbles;	0.37^{***}
	Substrate type 4: silt; Substrate type 5; macrophytes	$0.53/0.28^{***}$
SUBS7/SUBS8/SUBS9	Substrate type 7: twigs/roots;	0.50^{***}
	Substrate type 8: organic detritus/litter; Substrate type 9; mud	$0.29/0.54^{***}$
VEL2	Velocity type 2: slow	0.37^{***}
TEMP1	Temperature type 1: psychrophilic	0.37^{***}

Fine sediment patches represent very selective natural mesohabitats, with low stability and poor biogenic potentiality (Beisel et al., 1998). Previous studies have suggested that finer substrates contribute to a reduction in the density of macroinvertebrates, especially those sensitive to pollution (e.g. Buendia et al., 2013), while others argue that fine-sediment favours specialist species such as burrowers (e.g. Lancaster and Hildrew, 1993; Yamamuro and Lamberti, 2007). In order for restoration projects to be successful, it is important that they not only promote a wider range of mesohabitat types (e.g. mesohabitat diversity) but also restore specific mesohabitats of special importance (REFORM D4.3, 2014) as each has its own features and the sum of these features maximises biodiversity.

Staentzel et al. (2018b) applied transition matrix modeling and spatial metrics to highlight changes in vegetation dynamics and an increase in aquatic vegetation richness along the same study stretch. Bank erosion permitted the establishment of riparian plants (pioneer species) that probably provided decreased distances within the patchy-mesohabitat mosaic favouring better connections that allow successful life-cycle completion (Beisel et al., 2000). Castella (1987) showed that Odonata larval populations can be used as indicators of local hydrosystem features characterising flow influence, e.g. groundwater resurgence. Presence of aquatic vegetation in the GROYNE section confirmed river bottom diversification in the main channel increasing mesohabitat heterogeneity, potential relationships at the water-riverside interface and, thus macroinvertebrate biodiversity. Previous restoration projects promoting groyne implementation have also noted an increased population of Odonata (Buczyński et al., 2017) and Trichoptera (Buczyńska et al., 2018) taxa. Modiba et al. (2017), however, highlighted the importance of plant composition in riparian systems, with invasive plant species potentially disrupting Odonata life cycle completion.

4.2. Community structure and function in restored rivers by controlled bank erosion and groynes implementation

Macroinvertebrate responses were studied by [stations × years] along a control-impact protocol (Smith et al., 1993), superimposing community structure, environmental parameters and trait modalities. The richness showed differences between the unrestored sections and the whole site, highlighting the gain in new taxa when pooling unrestored section with the restored one. It further highlighted that restoration favoured taxa not seen elsewhere or nor observed in large abundances i.e. Odonata.

In restoration case studies, the functional approach is prescribed using measurements of functional groups, desired community structure, and careful consideration of community-level attributes, not focusing on single species or clusters of 'desirable' species only (Palmer et al., 1997; Maddock, 1999; Merz et al., 2005). To date, relatively few studies have considered the response of river ecosystem function and functional metrics to restoration (e.g. Lepori et al., 2005; Friberg et al., 2014; Kupilas et al., 2017). One such study, REFORM D4.3 (2014), showed that the effect of restoration on community structure, traits and functional indicators was more pronounced than the effects on basic richness and diversity. This was reflected by the NMDS analysis that was more informative than diversity indices. Composition metrics and trait modalities helped to characterize the nature of the shift in the whole community structure across stations over the years. The taxa trait approach through changes in taxa abundance and in the relative frequency of trait modalities developed by Mouillot et al. (2013) indicated that this was a slight shift in community functional attributes. It is likely that the restored section shared a part of similar pattern of traits with the UP section, resulting in a certain level of functional redundancy. However, some modalities of traits were promoted in the restored section, favouring the settlement of burrowers living in macrophytes or in fine-sediment substrate. Indeed, the eco-typology of Usseglio-Polatera et al. (2000) and its application showed a high variation in the diversity of functional eco-profiles in the GROYNE section. Such results informed that the restored section sheltered a wide range of mixed mesohabitats, some of which showed low and others high functional diversity of eco-profiles (Fig. 6B). Overall, the evolution in variation of eco-profile diversities exhibited the same pattern than the EHMID.

The prevalence of invasive taxa in macroinvertebrate communities has been also shown in our study, especially crustaceans with the amphipods *D. villosus*, *E. ischnuus*, or the isopod *J. istri.* These invasive taxa were less favoured in the GROYNE section compared to the unrestored sections, mainly located on the transitory submerged sedimentary benches within the inner-groyne area. Such transitory and poorly represented mesohabitats were composed of a high moving layer of gravels without fine sediment (sand, silt). Moreover, the inter-annual analysis showed that their relative abundance decreased in 2016 and 2017 in favour of more common taxa, in conjunction with the rise in macrophytes (Staentzel et al., 2018a). Both campaigns were also performed after the annual flood what may lead to the instability of invasive species populations. The 4-year monitoring was here sufficient to assess restoration-induced effects on mesohabitat heterogeneity, and taxa composition spatial and temporal changes. However, a longer monitoring would bring more information e.g. on the potential continuous decrease in invasive species.

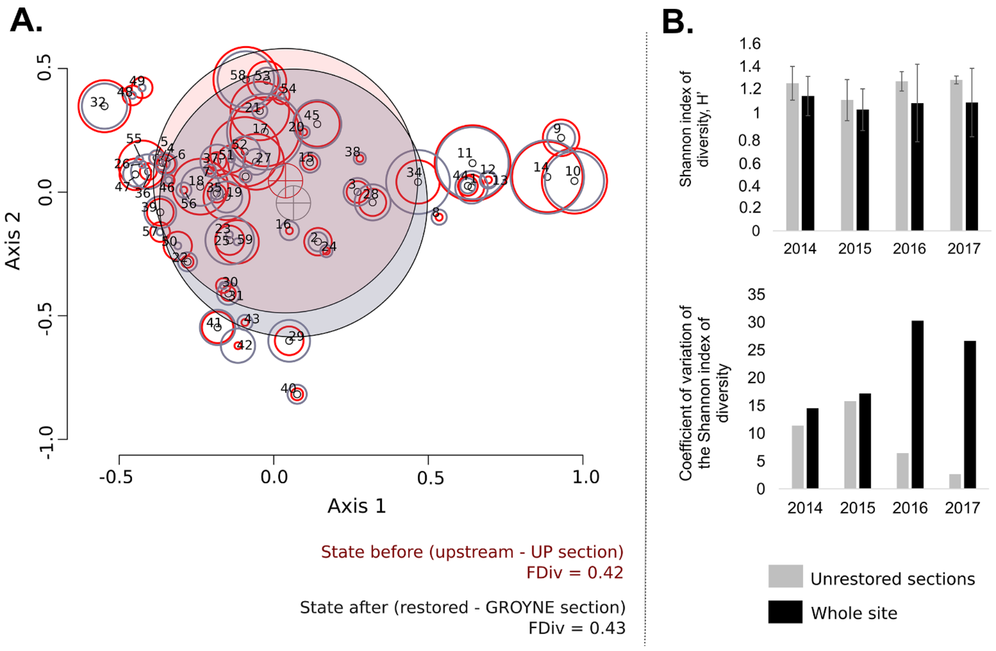

Fig. 6. A. Shift in the functional structure of macroinvertebrate communities between the UP section (state "before") and the restored one (state "after"), i.e. functional divergence (circles). Taxa (dots) are plotted in a two-dimensional functional space according to their respective trait values, while the axes are extracted from an FPCA analysis. Circle sizes are proportional to taxa relative abundance at the state "before" (UP) and the state "after" (GROYNE), and are coloured red and grey, respectively. Coding for taxa is described in Appendix A. B. Shannon index of diversity and its coefficient of variation from functional ecoprofiles issued from Usseglio-Polatera et al. (2000). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Hydro-morphological conditions undergo constant changes as rivers adjust to changing environmental conditions in the catchment and valley floor (Dufour and Piégay, 2009). Ongoing river dynamics influence aquatic biodiversity *via* several interrelated mechanisms that operate over different spatial and temporal scales. These include habitat complexity, life history patterns, connectivity and biological invasions (Bunn and Arthington, 2002). Restoration on the Old Rhine affected these interrelated mechanisms by emphasising habitat complexity, influencing life history patterns, improving lateral connectivity and favouring invasions. Although the restoration action increased subsidies that often drive macroinvertebrate density, extreme hydrologic events limited the impact of potential structuring species, including invasive species (Bunn and Arthington, 2002; Moore, 2006). The important weight of 'years' in spatial and temporal sources of variability may be explained by internal river dynamics that induce a re-setting of biological communities, i.e. each year is defined by a host of specific factors.

5. Conclusions and prospects

Controlled bank erosion had positive ecological effects on both in-channel (this study) and riparian compartments (Staentzel et al., 2018c). Although morphological changes were not as important as expected (low eroded volume, Chardon et al., 2016; Chardon and Schmitt, 2018), the EHMID reflected a gain in mesohabitat heterogeneity along the restored section at the landscape perception of macroinvertebrates. This concurs with Beisel et al. (1998), who stressed that the degree of macroinvertebrate community response is scale-study dependent. The artificial groynes favoured deposition of fine-deposits downstream during floods, slowing flow velocity and enhancing bank erosion when not joined to the bank. Such newly created mesohabitats were scarce on the Old Rhine River before restoration and their rise increased macroinvertebrate biodiversity on the whole studied site. This result could be considered as an unusual outcome for large river restoration projects, mainly focusing on lotic geomorphological units as on the Rhône River, France (Lamouroux et al., 2015).

The ecological conclusions from our study are in accordance with those of the EU-funded research project 'REFORM' (www.reformrivers.eu), where channel widening was carried out in the majority of twenty hydro-morphological river restoration projects. Such passive restoration projects result in more sustainable ecological and morphological effects than other one-off far-reaching hydro-morphological restoration projects based on gravel augmentation, with the proviso that groynes stay in place. In-channel structure management provide similar physical results in smaller rivers using deflectors or large woody debris, reducing current flow velocity and diversifying mesohabitats (Tockner et al., 2003; Biron et al., 2004). The hydraulic law of similarities assumes that either coarse-grain or fine-grain, two objects could have the same dynamic feature if they have the same length-scale ratio (geometric), time-scale ratio (kinematic) and force-scale ratio (dynamic) (Binder, 1973; Chanson, 2009; Kline, 2012; Heller, 2011). This opens up a broad range of possibilities for transposition and prediction of effects regarding changes in physical features and biological responses. The greatest difficulty that lies in potential biological prediction is the embedding of the river bottom, the internal river dynamics or climate change, and biological phenomena such as biological invasions or the distance of available habitats from source populations (Friberg, 2014; Hulme, 2017; Nilsson et al., 2017).

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Appendices A, B and C. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoleng.2018.10.024.

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Conservation status assessment of aquatic habitats within the Rhine floodplain using an index based on macrophytes

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The programme LIFE Nature "Conservation and restoration of habitats of the Rhine fringe" (2002-2005) focussed on the assessment of the conservation status of terrestrial and aquatic habitats. Along the Rhine fringe, the degree of connection between waterbodies and the river main channel determined the water flow (surface or phreatic waters, flow velocity) and induced flood disturbances (hydromorphological quality, scouring effect). A new typology based on metrics characterizing the conservation status, was proposed for the upper Rhine floodplain. Seven physical and biological metrics were selected: specific richness, presence or absence of rare, polluto-tolerant and exotic species, species abundance, degree of connection, and trophy level defined by the macrophyte community. Aquatic macrophytes surveys were conducted at 315 sites along the Rhine French fringe. Multiple correspondence analysis and discriminant analysis were used to define metrics scores in a preliminary network of 129 macrophytes surveys stands. Metrics were combined to give a measure of the conservation status of the 315 sites. Five classes of the conservation status have been defined from degraded to excellent status. The mapping of the conservation status was realized to answer the question addressed by the program "Life Nature". We discuss on the choice of the metrics and their justification in the definition of the conservation status. The method was tested in the fluvial hydrosystem of Ill river, the main tributary of the Rhine river in the Alsace floodplain, and compared to the French trophic index based on macrophytes, IBMR.

Keywords: biological index, conservation status, macrophytes, running waters, Rhine floodplain

Introduction

In aquatic systems, both running and standing waters, high physical (current velocity, substrate, light…) and chemical (organic matter and nutrient content) fluctuations were commonly observed (Dodkins et al. 2005). Although they show high ecological plasticity, species and plant communities cope with these fluctuations (Barrett et al. 1993, Garbey et al. 2003). Thus their use as bioindicators can be discussed due to their high adaptability in changing environment. Bioindication is based on the tolerance of certain species to high levels of mineral nutrients or organic pollution and conversely to a high sensitivity to pollution or adaptation to low level of nutrient of others. Affected waters will tend to support an increased abundance of more nutrient-tolerant species associated with an overall loss of species diversity.

Nowadays, macrophytes are commonly used as indicators of the nutrient status and their use lead to the proposal of numerous European typologies (Grasmück 1995, Robach et al. 1996) or indices (MTR Mean Trophic Rank of Holmes 1995, RPM Relative Pflanzen Menge of Kohler & Janauer 1995, TIM Trophic Index of Macrophytes of Schneider & Melzer 2003, IBMR Indice Biologique Macrophytes en Rivière in France 2003, Haury et al. 2006).

Aquatic systems are more and more considerably altered by organic pollution and then become eutrophic, and sometimes hypertrophic depending on the phosphorus content (SRP) and nitrogen, as a consequence of degradation of organic matter. A 'damage rate' based on macrophytes community relative to an undisturbed reference was proposed by Haslam (1982). Recently, the Water Framework Directive (WFD 2000, transposed in France 2004) required the good ecological status of surface waters to be reached, and the reduction in the release of dangerous and toxic substances. Application of WFD needs (1) to find indicators to estimate ecological quality (*e.g.* structure and function of habitats) based on physical, chemical and biological quality of water bodies and (2) to establish a reference system. In another hand, the Habitats (Natura 2000) Directive focussed on the definition of conservation status of habitats and species. In the Alsace

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Rhine floodplain (Eastern France), the program LIFE Nature "conservation and restoration of habitats" (2003-2005), aimed (1) the definition of a reference for a good conservation status of habitats (terrestrial and aquatic habitats according to the Natura 2000 definition) and (2) mapping of this status in order to identify sites or stretches to preserve, restore or rehabilitate.

A bioindication scale of eutrophication based on macrophyte communities was established in the phreatic streams of the Alsace floodplain, a scale which covered the whole gradient of trophy level, from oligotrophic to eutrophic (Carbiener et al. 1990, Robach et al. 1996) as that formerly proposed by Kohler (1975). At each trophy level, corresponded a plant community (defined by the phytosociological method according to Braun-Blanquet 1964). However, this method was not validated neither for standing waters (lakes, ponds) nor for marshes habitats; it defined more precisely a trophic level than a conservation status.

To assess the conservation status of water bodies of the Rhine fringe (as defined in the programme LIFE), both running (streams, connected lateral arms) as standing waters (cut-off channels, ponds, marshes), we proposed a new tool based on macrophytes. This tool was set-up in the former lateral arms of the Rhine, in order to identify sectors exhibiting good and bad conservation status and specify the ones that could be restored.

Methodology

Three steps were used 1) vegetation sampling and identification of the plant community, 2) definition of the conservation status through a notation based on 7 metrics, and 3) mapping of the conservation status of aquatic habitats of the Rhine fringe.

Study sites

The upper Rhine floodplain was drastically managed until the 19th century, by straightening and canalisation. As a consequence, many water bodies were cut-off from the river Rhine. Both hydrological connection and exchange between river and water bodies and exchange with the groundwater were highly modified by the hydraulic management of the river. However, some indirect hydrological relationships, *i.e.* seepage from the river to the groundwater were preserved and thus influenced the water quality of disconnected former lateral arms which were still supplied by groundwater. The water nutrient content of water bodies along the river fringe, depends on the type and degree of connection with the river main channel (Trémolières et al.1993, Eglin et al.1997, Bornette et al.1998). The water bodies which are supplied by surface water (Rhine waters) are eutrophic, whereas the disconnected ones are most of the time mesotrophic. Where water is supplied by a mixture of ground- and surface-water, waterbodies can be mesotrophic to eutrophic, depending on the surface water quality.

In the Alsace floodplain (Eastern France), along the Rhine fringe, we selected aquatic sites or stretches belonging to the five aquatic habitat types according to Natura 2000 classification (Table 1, Bensettiti et al. 2002). Study sites were composed of water bodies, 50 to 100 m length in running waters and 10 m^2 minimal surface in standing waters.

Table 1. Definition of the five aquatic natural habitat types whose conservation requires designation of Natura 2000 sites present in the Rhine fringe

Natura 2000 Codes	Characteristics, phytosociological name
3130	Oligotrophic to mesotrophic standing waters with vegetation of the *Littorelletea uniflorae* and/or of the *Isoëto-Nanojuncetea*
3140	Hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp.
3150	Natural eutrophic lakes with *Magnopotamion* or *Hydrocharition* – type vegetation
3260	Water courses of plain to montane levels with the *Ranunculion fluitantis* and*Callitricho-Batrachion* vegetation
3270	Rivers with muddy banks with *Chenopodion rubri* p.p. and *Bidention* p.p. vegetation

Vegetation sampling

Macrophyte surveys (N = 315) were carried out between mid-June and mid-September, according to the phytosociological method of Braun-Blanquet (1964). Vascular plants and bryophytes were identified to the species level, whereas algae were identified to the genus level. According to this macrophyte survey, we designated a community according to Oberdorfer (1992) and the trophy scale proposed for the Rhine floodplain by Robach et al. (1996). This latest scale identified six communities named A to F, corresponding to a gradient of trophy, from oligotrophic to eutrophic level.

Each survey was characterized by a phytosociological community and a ‘trophic’ community coded in the Table 2.

Table 2. Correspondence between phytosociological community, trophic community and Natura 2000 habitat code, and number of surveys per community used in the statistical analyses with the proposed conservation status. N= total number of surveys; *according to Oberdorfer (1992), **according to Robach et al. (1996)

N	Conservation status	Code	Phytosociological community or alliance*	Community in the trophic scale**	Habitat code (Natura 2000)
56		calo	*Callitrichetum obtusangulae*	C	3260, 3150
26		cerd	*Ceratophylletum demersi*	E	3260, 3150
13	good	char	*Charion*	C	3140
59		elod	*Elodeetum*	E	3260, 3150
15	moderate	lemt	*Lemnetum trisulcae*	C	3260, 3150
20		lmsp	*Lemno minoris- Spirodeletum polyrrhizae*	E	3150
1	high	myri	*Myriophyllo-Nupharetum*	C	3150
25		potl	*Potamogetonetum lucentis*	D	3150
63	poor	potp	*Potamogetonetum pectinati*	E	3260, 3150
37	bad	ranf	*Ranunculetum fluitantis*	F	3260

Definition of the metrics

Seven metrics were defined for the 315 surveys. Five simple ecological metrics were proposed to assess the conservation status:

(1) specific richness with four modalities : 1= 0- 4, 2=5 - 7, 3=7 - 10, 4=11 – 23.

(2) presence of rare species. They were the species of the regional red list such as *Sparganium minimum, Potamogeton trichoïdes, P. Friesii,* and two less frequent species *Nymphea alba* and *Najas marina*.

(3) presence of polluto-tolerant species. Species considered as polluto-tolerant which grow in the Alsace floodplain were *Potamogeton pectinatus, Ranunculus fluitans, Potamogeton nodosus,* and *Lemna gibba*.

(4) presence of exotic species (*Elodea* sp.).

(5) vegetation cover percentage with 4 modalities : 1=0 - 30%, 2=30 - 60%, 3=60 - 80%, 4=80 - 100%.

The classes of specific richness and cover were defined as medians and quartiles of these variables.

Two additional physical and chemical metrics were used:

(1) connection, that involves fluxes of material (nutrient and sediments) and living organisms, occurs both between cut-off channels and main channel, and between channels and groundwater. Three modalities were considered for the degree of connection: 1 = very high (permanent connections), 2 = intermediate (depending on frequency and magnitude of floods) and 3 = very low (infrequent connections).

(2) trophy level with 3 modalities: mesotrophic, eutrophic, hypertrophic. This metric was obtained from the plant community survey according to the bioindication scale (Robach et al. 1996), independently from the degree of connection.

We selected 129 surveys, representing the 5 phytosociological communities which status was known according to experts opinion (ranging from good to bad) in order to calibrate the measure of conservation status (Table 2). Then, the index of the conservation status was calculated for the remaining 186 surveys representing five other phytosociological communities. Conservation status of these sites were not defined *a priori*. The index of conservation defined for a set of 619 surveys (including the 315 surveys) located along the Rhine fringe was mapped with a GIS (Geographical Information System- geoconcept software).

Statistical analyses

The data used in this analysis was obtained from surveys of 315 aquatic sites, along the upper Rhine left bank. This set was analysed by using the SAS software (Version 9.1, 2002-2004, SAS Institute Inc., Cary, NC, USA).

Physical and biological data were coded into classes. We obtained a numerical representation of each site by projection on the first two principal axes through a multiple correspondence analysis (MCA) used in case of qualitative data (Greenacre 1984). This step allows us to transform mixed data (qualitative and quantitative) in normalized numerical data. The first two principal axes explain 30.47% of inertia which can be estimated to be sufficient to classify sites into classes of conservation status.

A canonical discriminant analysis (CDA) was then applied on transformed data (Hand 1981). By this way a linear function of the two principal components was built in order to classify the 129 calibration surveys into communities of known conservation status. A single linear function is sufficient in the case of two components. This linear function gives a score for each modality of coded data. So we are able to classify a site by using the coding of the first step. This procedure was used to classify the 186 supplementary test surveys and to validate the first classification.

Results

Floristic composition of habitats

At first we observe an unequal distribution of Natura 2000 habitats in the Rhine fringe: habitats 3130 and 3270 are present in less than 1% of waterbodies, habitat 3140 in less than 7% and habitats 3150 and 3260 are the most common habitats with approximatively 45% of the whole studied stations. Habitats 3130 and 3270 are colonised by helophytic vegetation with preference for the bank and the zone of tidal range. Habitat 3140 with Characean occurs in recently opened habitats with clear waters.

Ten phytosociological communities of hydrophytes (named according to Oberdorfer 1992) are found in the three aquatic habitats of running waters (3260), standing waters (3150) and Characean habitat (3140). Fourteen phytosociological communities are found in total in the Rhine fringe whether we include marsh habitats (3170 and 3230). All these communities correspond to the mesotrophic and eutrophic levels: C, D and E in the scale of bioindication (Robach et al. 1996) (table 2). In the connected sectors, four phytosociological communities were found: *Ranunculetum fluitantis* and *Potamogetonetum pectinati* in running waters, and *Ceratophylletum demersi, Potamogetonetum lucentis* and again *Potamogetonetum pectinati* in standing waters. These four communities are the richest with up to around 30 species and correspond to eutrophic D and E communities in the bioindication scale. In the disconnected sectors, the growing vegetation is dominated by *Callitriche obtusangula, Berula erecta* and *Lemna trisulca*. These species defined two communities: the *Callitrichetum obtusangulae* and the *Lemnetum trisulcae*. These two communities are attributed to the mesotrophic C community in the bioindication scale (Robach et al. 1996). In oligotrophic conditions (rarely found in the Rhine fringe), the *Myriophyllo-Nupharetum* with *Myriophyllum verticillatum* and *Nymphea alba*, as characteristic species, is growing in standing waters. The new area opened by the reconnection to the river main channel are often colonised in mesotrophic conditions by pioneer *Characean* or by exotic species such as *Elodea nuttallii* in more eutrophic conditions.

Distribution of communities according to their conservation status

The distribution of macrophyte communities within the Alsace floodplain exhibits a characteristic pattern as shown by the multivariate analysis in Fig. 1: the first axis of the factorial plan (MCA) is strongly associated with the trophic gradient: hypertrophic level and the presence of polluto-tolerant species are associated with negative values, whereas the presence of rare species is associated with positive values on axis 1. Figure 2 showed the distribution of communities, whose conservation status is already known, in the factorial plan F1xF2 of the discriminant analysis. The first axis, representing trophic gradient, can be considered as an ecological gradient, high values of conservation status representing a good status. All the sites with the communities of good conservation status are located towards the positive values of F1 axis and the communities of a less good quality presented negative values. The *Myriophyllo-Nupharetum* (Myri) community, exhibiting the highest value on the first axis, was characterized by the presence of rare species such as *Nymphea alba*, and the absence of polluto-tolerant species in an oligotrophic water. The *Lemnetum trisulcae* (Lemn) characterized a “moderate” conservation status with mesotrophic water, medium specific richness, around 7 species and the absence of polluto-tolerant and exotic species. On the opposite way, the *Ranunculetum fluitantis* (Ranf) and the *Potamogetonetum pectinati* (Popt) communities present both polluto-tolerant and exotic species. So, they are in a poor to bad conservation status with a high trophic level. Figure 3 showed a factorial plan F1xF2 with the 186 supplementary individual surveys. The mesotrophic community *Callitrichetum* (Calo) had positive values on the axis 1 and the eutrophic community *Potamogetonetum lucentis* (Potl) negative values, confirming the first axis as a trophic gradient.

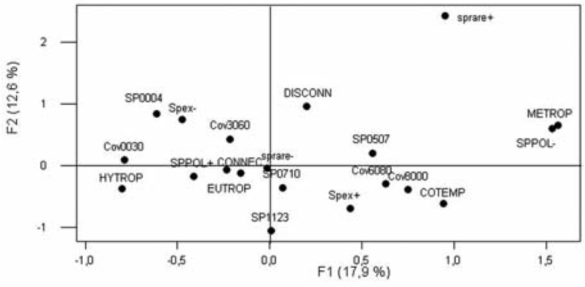

Fig. 1. Distribution of modalities of metrics in the Factorial plan F1xF2 (MCA Multiple Correspondence Analysis). Specific richness : SP0004: 0 – 4 species, SP0507: 5 – 7 species, SP0710: 7 – 10 species, SP1123: 11 - 23 species; rare species : presence sprare+, absent :sprare-; polluto-tolerant species : presence, SPPOL+, absent , SPPOL- ; exotic species : present, Spex+, absent, Spex- ; cover : 0 - 30%, Cov0030, 30 - 60%, Cov3060, 60 - 80%, Cov6080, 80 - 100%, Cov8000 ; Connection : connected, CONNEC, temporarily connected, COTEMP, disconnected, DISCONN ; Trophic level : mesotrophic, METROP, eutrophic, EUTROP, hypertrophic, HYTROP.

Table 3. Scores of modalities of the metrics for the definition of the conservation status

Metrics	Modalities	Value	*Myriophyllo-Nupharetum*
Specific richness	0 - 4	-0.13	
	5 - 7	0.72	
	7 - 10	-0.14	
	11 - 23	-0.65	-0.65
Rare species	Presence	2.52	2.52
	Absence	-0.04	
Polluto-tolerant species	Presence	-0.55	
	Absence	2.08	2.08
Exotic species	Presence	0.04	
	Absence	-0.04	-0.04
% of cover	0 - 30%	-0.78	
	30 - 60%	0.04	
	60 - 80%	0.49	
	80 - 100%	0.56	0.56
connection	connected	-0.24	
	Temporarily connected	0.62	
	disconnected	0.81	0.81
Trophy	mesotrophic	2.01	2.01
	eutrophic	-0.29	
	hypertrophic	-1.08	
Index of conservation status			7.29

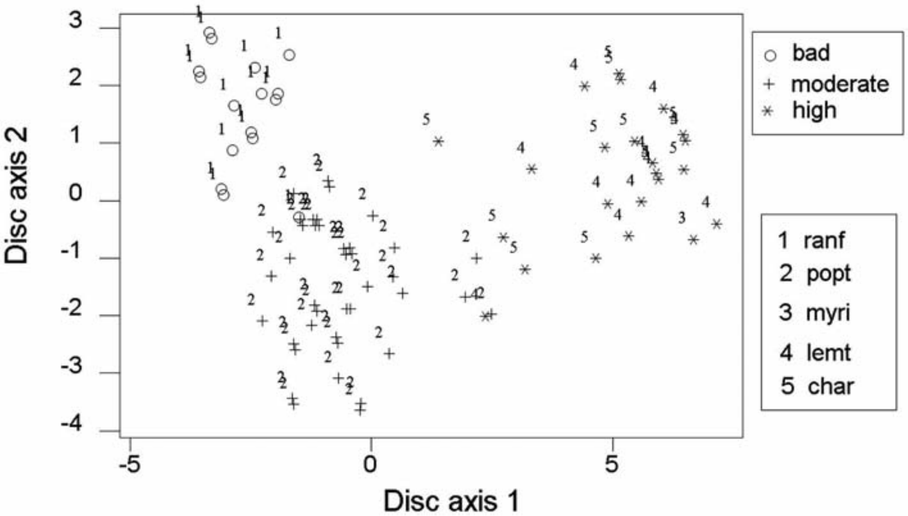

Fig. 2. Distribution of the 5 plant communities classified according to their conservation status on the factorial plan F1xF2 (CDA Canonical Discriminant Analysis). The code of community is given in the table 2.

The coordinate on the first axis in the discriminant analysis (main discriminatory axis) of each modality of the metrics was used as a score (Table 3). All the scores corresponding to the modality of the survey under study were added to calculate an index for the survey (see the example of the community *Myriophyllo-Nupharetum*, Table 3). Classes were determined according to the classification of the individuals of the 5 known communities : > 6 very good conservation state (high), 3-6 good, 1-3 moderate, 1 to -2 poor, < -2 bad. The extreme values corresponded respectively to the communities *Potamogetonetum pectinati* (Potp) in a poor status and *Charetum* (Char) in a good status (Fig. 3). *Callitrichetum* and *Lemnetum trisulcae* were in moderate status.

Application to the fluvial system of the Ill river

The classification of conservation status proposed in this study was tested in a system close to the Rhine fringe, the ILL river which is the main tributary of the Rhine in the Alsace floodplain. The system studied

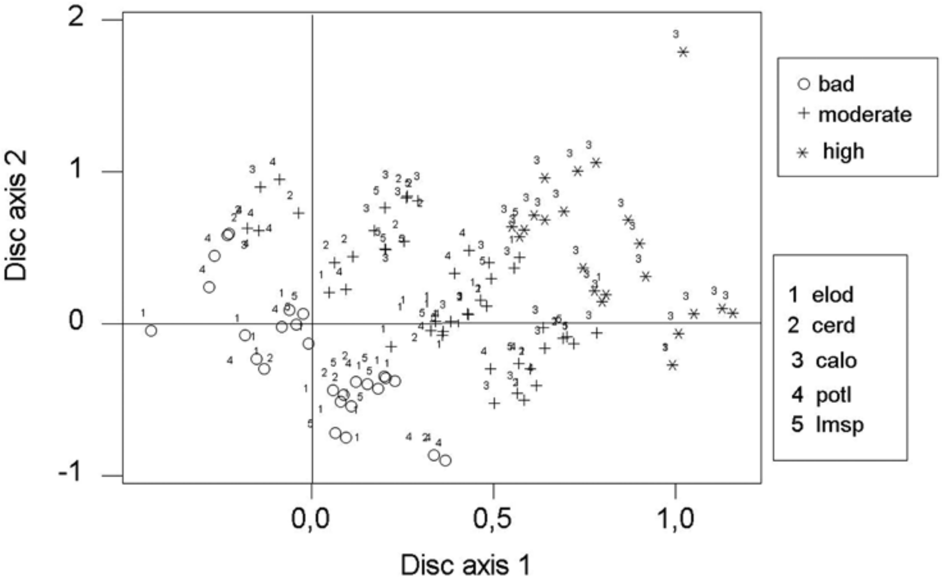

Fig. 3. Distribution of 5 supplementary plant communities (not classified) in the factorial plan F1xF2 (CDA Canonical Discriminant Analysis). The code of community is given in the table 2.

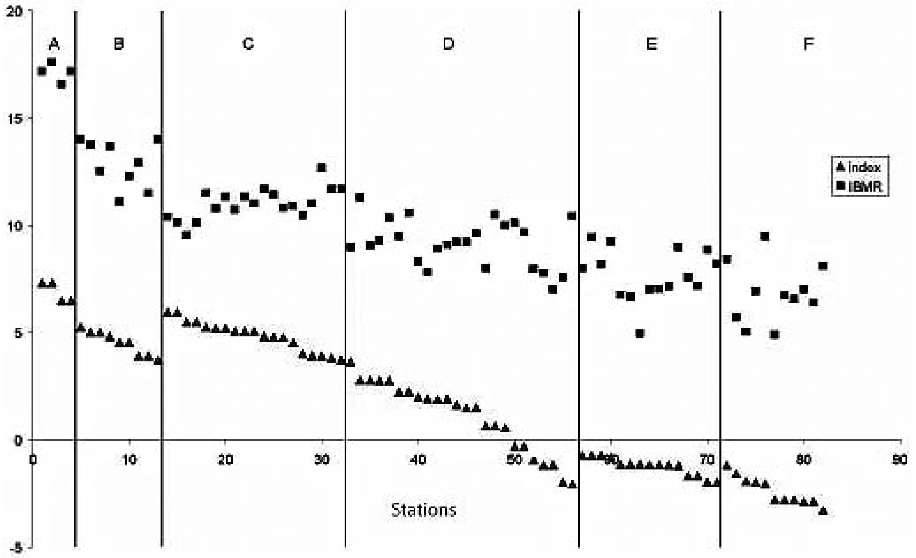

Fig. 4. Correspondence between communities of the trophic scale (named A,B,C,D,E,F from oligotrophic to eutrophic level), IBMR (Biological Macrophyte Index in River, AFNOR 2003) and index of conservation status in the hydrological network of the ILL floodplain (Eastern France).

concerns a flooded forest of the ILL, through which flows a large hydrological network (52 km in a forested surface of 1500 ha). This network encompasses connected, disconnected streams still fed by groundwater, and streams temporarily connected by floods. In this sector, there is no seepage of ILL waters into the groundwater (Trémolières et al. 1994). So the disconnected streams fed by groundwater are either oligotrophic, characterized by a plant community with *Potamogeton coloratus*, a species characteristic of a very low trophy level, or oligo mesotrophic, with a single species *Berula erecta*.

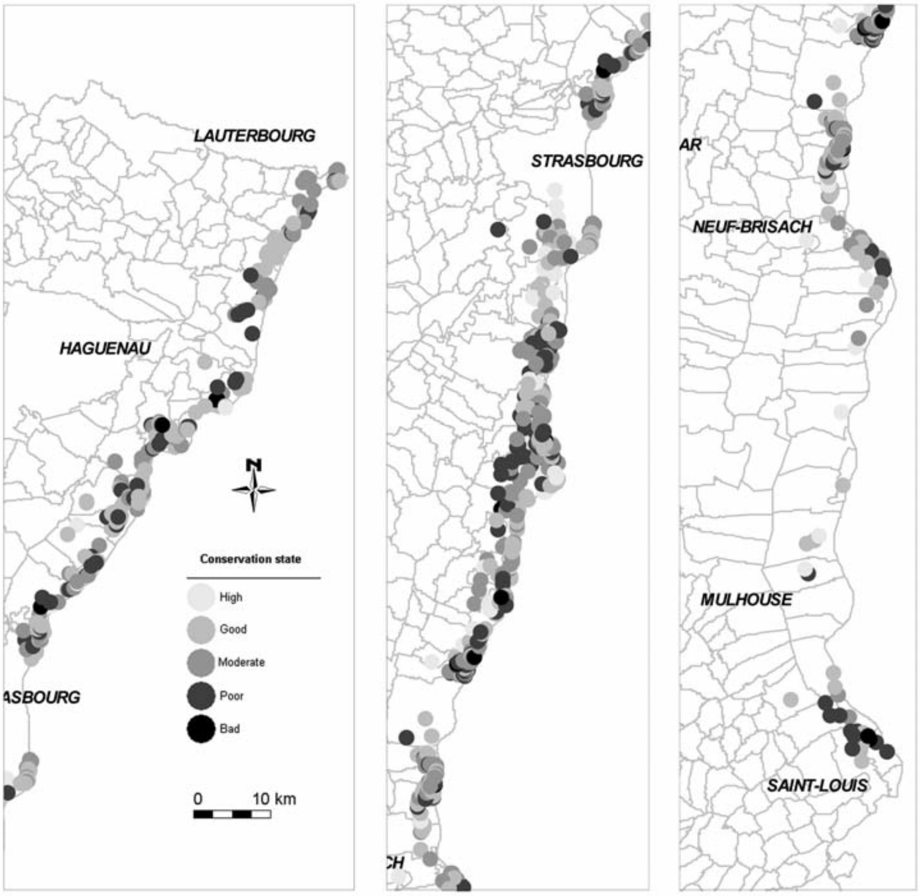

Fig. 5. Mapping of the conservation status of aquatic habitats in the Rhine fringe based on the conservation index.

Table 4. Correspondence between IBMR (Biological Macrophyte Index in River, AFNOR 2003) and conservation status index in the streams of Ill floodplain. In brackets: number of surveys.

Community	A(4)	B(9)	C(19)	D(24)	E(15)	F(11)
IBMR	17.1	12.8	11	9,2	7.7	6.8
std	0.42	1.08	0.7	1.1	1.2	1.7
Index of conservation status	6.9	4.5	4.8	1.05	-1.24	-2.38
Std (index)	0.47	0.55	0.69	1.10	0.43	3.03

The trophic level of 86 stations, are defined according to the bioindication scale and to the IBMR index, the normalized French macrophytes based index (AFNOR 2003, Haury et al. 2006) ; the corresponding conservation status is then calculated at all the stations. The average index per 'trophic' community is given in Table 4. There is a relatively good adequacy between the trophic level founded on the plant communities and the IBMR. The high correlation between IBMR and conservation index confirms the trophic level as a key factor of the conservation status (Fig. 4). However communities B and C have similar indices, IBMR and conservation status (Table 4). The species composition of these two communities is also very close (Carbiener et al. 1990). Groups D and E have also similar IBMR, but contrasted index of conservation status. In the group D, the conservation status varies strongly from good to poor (+3 to –2, Fig. 4), whereas group E and F present lower variations respectively from -0.7 to -2 and -1.6 to -3.3. The group D has low or high specific richness (3-10 species), but with a high cover. The lowest values of conservation status of this group are mostly related to the presence of polluto-tolerant species, such as *P. pectinatus* or *Ranunculus fluitans*. In the groups E and F, the variations of index seem to be related to the specific richness and cover percentage, all the other metrics being similar.

Discussion

Distribution of communities with regard of environmental factors

Up to nine communities of hydrophytes were found in the Rhine fringe. This relative high number may be due to the high diversity of habitats created by the dynamics of the river (as shown by Bornette et al. 2001) in spite of hydraulic management (straightening and canalisation). The aquatic habitats of the Rhine fringe are characterized by a broad range of vegetation communities: from mesotrophic to eutrophic vegetation (Trémolières et al. 1993, Eglin et al. 1997). In the Rhine cut-off channels conditions, nutrient level was highly related to the connectivity because of the specific hydrological functioning of large rivers as shown in Rhône and Danube (Bornette et al. 1998, Bornette et al. 2001, Janauer & Kum 1996). In river floodplain, the river main channel provides eutrophic waters to the lateral arms during connections (floods) and induces scouring effect due to flow velocity. Such condition may induce highest species richness in waterbodies (Bornette et al. 1998). In the Rhine waterbodies, the nutrient level is actually very low due to an improvement of water quality of the Rhine river (IKSR Rheinatlas 2001). However the growing vegetation remains eutrophic, with species such as *Potamogeton pectinatus, P. nodosus, Ranunculus fluitans*. The nutrient level of water bodies has to be related to the whole aquatic system (water-plant-sediment) (Carignan & Kalff 1980, Moore et al. 1994). Many authors consider that the nutrient level is not a discriminant parameter in the macrophytes distribution because they consider only the water nutrient content, *i.e.* SRP soluble phosphorus (Demars & Harper 2005). In fact macrophytes growth can depend on the bioavailabilty of both phosphorus as SRP and interstitial P (Carpenter & Adams 1977, Carignan & Kalff 1980). The change in macrophytes colonisation remains lower than change in nutrient content (hysteresis effect) (Kohler et al.1989, Debold 1997, Trémolières & Szwab in press).

The disconnected sectors exhibited meso-oligotrophic waters and the growing vegetation is dominated by mesotrophic species such as *Callitriche obtusangula, Berula erecta* and *Lemna trisulca*. However, because of seepage from the Rhine river to the groundwater, the former lateral arms close to the Rhine river present meso-eutrophic waters, characterized by eutrophic species such as *Ceratophyllum demersum, Potamogeton pectinatus* or *P. lucens* and related phytosociological communities. In standing waters the *Myriophyllo-Nupharetum*, a species-poor community, is growing in oligotrophic conditions, because of an exclusive groundwater supply of good quality: groundwater quality has been improved by feeding with waters which were purified during the transfer through the soil-root system of the alluvial forest (Sanchez et al. 1991, Sanchez & Trémolières 2003).

The new areas opened by a recent reconnection (2002-2004) with the river are colonised by the exotic species *Elodea nuttallii* in eutrophic waters (Weber 2005, Bornet 2005). In fluvial hydrosystems, the connectivity and the related trophy factor appear therefore as discriminant factors in the distribution of communities and species (Bornette et al. 1998, 2001, Demars & Harper 2005). These last authors attribute the major role of connectivity to the exchange and input of propagules into a network of connected rivers rather than to an increase of nutrient level. This contradiction could be attributed to a scale change of the analysis (at river level or channel level).

Metrics analyses

Positive values of the proposed index represent a good quality, negative values characterized a degraded state (Table 3). Two important metrics are the presence of rare species, indicating good status, and the presence of polluto-tolerant species, indicating a degraded status with high values of correlation with discriminant axis.

The specific richness and the cover percentage (or abundance of species) are inversely related, and together provided an idea of the opening of the habitat and the risk of colonisation by exotic species. High specific richness fails to be an indicator for a good conservation status, the best status being given by a medium richness (5-7 species). The conservation status is good where high cover corresponds to a large specific richness, whereas the same cover with a low richness corresponds to a poor to bad status. Moreover a high nutrient content in water ensures a high development of biomass linked to a low or a high specific richness. Amoros & Bornette (2002), Amoros (in press) and Bornette et al.(1998) observed a higher diversity for an intermediate degree of connection, which corresponds to an intermediate level of trophy in the case of established vegetation.

Connection and trophy level (nutrient P and N content), are narrowly linked to the functioning of the Rhine floodplain (Trémolières et al. 1993, Eglin et al. 1997). Connected stretches are eutrophic to hypertrophic depending on the eutrophication of the river. Disconnected stretches fed by groundwater are mesotrophic, due to the seepage of the river waters through the banks or bed into the groundwater. High trophic level or strong connection to a high trophic level waterbody can relegate a site to a bad status. In our study, a high specific richness as a result of an increase of trophy level leads to a poor status. In fact species which grew in very nutrient -rich waters, are polluto-tolerant *P. pectinatus* and *Ranunculus fluitans* species (Haslam 1978, Janauer & Dokulil 2006). They then become dominant and exclusive, and lead to a degraded status.

However, surperficial connection between a water body and river main channel cannot be reduced to the phenomenon of eutrophication. Such contributes to inputs of sediments and propagules (Combroux et al. 2001), which can enhance the specific richness or decrease it by scouring effect of overflow or input of turbid nutrient-rich waters (see also Bornette et al. 1998). By this way, it appears necessary to associate the assessment of conservation status with both connectivity and biological metrics (such as polluto-tolerant species) as a response to complex effects of connectivity.

Conservation status of Rhine habitats

The phytosociological surveys allowed us to establish a classification of the macrophytes based conservation status of watercourses along the Rhine fringe. On this basis we can propose stretches or water courses to preserve, restore or abandone.

The five aquatic natural habitat types whose conservation requires designation of Natura 2000 sites as regards to the Habitat Directive (92/43/EEC) are present in the Rhine fringe. However, the distribution of sites characterized by a majority of UE 3260 (running waters) and UE 3150 habitats (standing waters) is probably linked to the degradation and artificialisation of aquatic zones by hydraulics works of the river Rhine. These works removed the floods and isolated all the lateral arms. The scarcity of marsh habitats UE 3130 and UE 3270 is relative to the removal of floods and as a consequence, lowering of water fluctuations. They are exclusively located in the areas where floods still occur. These habitats persist in the Northern Alsace plain and on the artificial islands of the Rhine which are still flooded, and where the variations of water level can occur.

The aquatic habitats of the Rhine fringe are in a good to poor conservation status (Fig. 5). More than 32% of the habitats exhibit a good status, and 34% a poor status. Habitats in a high to a good conservation status (respectively 2.1% and 21.5% of 619 stations) are in opposition to habitats in a bad conservation status (around 7%). Along the Rhine French fringe, three sectors were distinguished from the south to the north. These sectors correspond to geomorphic zonation and degradation of many aquatic habitats (Fig. 5).

The Southern sector ranges from Neuf-Brisach to St Louis in the south of Alsace. It presents a lower density of water courses than the northern and central sectors that can be explained by the low groundwater level (around 7 m) and the consequence of the canalisation of the Rhine. The groundwater sources are rare and water courses are often contaminated by village effluents, and thus become largely eutrophic. This sector is considered to be in poor to bad conservation status. One exception is the natural reserve of the "petite Camargue alsacienne", located in the more Southern area which still presents some habitats of “moderate” conservation status.

The central part of the plain (from Strasbourg to Neuf-Brisach) corresponds to the braided and anastomosed sector of the Rhine. It is characterized by a large network of phreatic streams, due to the proximity of the groundwater from the soil surface. This part included disconnected, temporarily connected by floods and connected sites. The trophic level which is linked to the degree of connection in our study case was reflected by the type of vegetation. Hydrological functioning is characterized by a specific distribution of communities. The disconnected mesotrophic sites which are colonized by the community of *Myriophyllo Nupharetum* with the relatively rare species *Nymphea alba*, are in a moderate to high conservation status. Temporarily connected sectors are dominated by the mesotrophic *Callitrichetum obtusangulae* community. The sites connected to eutrophic waters are characterized by two communities: an eutrophic community, *Potamogetonetum pectinati* which is in a poor conservation status and the hyper-eutrophic *Ranunculetum fluitantis* community which is in a bad conservation status. This latest community is observed in some disconnected sectors such as the drainage canal flowing along the Rhine river and more and less contaminated by the seepage of Rhine waters (Trémolières et al. 1993). This sector is characterized by a high diversity of habitats related to a diversity of modalities of connection and thus of different conservation status.

The Northern sector from Strasbourg to Lauterbourg, is characterized by waters flowing from the Northern Vosges (Moder, Sauer, Lauter) into the Rhine. It corresponds to a sector of anastomoses and the beginning of a meandering river. These waters are neutral, lowly mineralized and often highly eutrophicated, they are mixed with phreatic waters in the plain. The Rhine habitats of this sector are in good to poor status. The poor status dominates, due to a high level of trophy and a decrease of flow, compared with the previous sector.

Some waterbodies become shallow with muddy substrate. Under these conditions, water temperature which is not buffered by groundwater inputs, varies with the air temperature and is highly increased during summer. High temperature has a degrading effect on aquatic habitats (Welsh et al. 1998).

Comparison with other biological methods

The comparison between two indices in the Ill system shows that the index IBMR related to the trophy level is also linked to the conservation status. However the within-community and between-community variations of IBMR are relatively regular whereas we observe a gap between communities of the conservation index along the trophic gradient. The meso-eutrophic group D has low or high specific richness (3-10 species), but a high cover, which can explain higher variations of the conservation index. The lowest values of conservation status of this group are mostly related to the presence of polluto-tolerant species, such as *P pectinatus* or *Ranunculus fluitans*. In the more eutrophic groups E and F, the variations of index seem to be related to the specific richness and cover percentage, all the other metrics being similar.

The presence of rare species, such as *Potamogeton coloratus* due to the scarcity of the oligotrophic habitats, is also a determinant metric although they are species from species-poor habitats (Carbiener et al. 1990). In another way, the presence of polluto-tolerant species deteriorates the conservation status. Commonly a high specific richness was recognized as an indicator of good ecosystem functioning. In our study case it seems not to be a good indicator of conservation status as well in the Rhine sector as the ILL system. We show that at highest trophic level, there are high variations of specific diversity often related to a high level of disturbance (high connectivity, see Bornette et al. 1998, Bornette et al. 2001). Amoros et al. (2000) showed that these two phenomenons, nutrient tolerance and disturbance, can interfere in some cases. An example is the oligotrophic species *P. coloratus* which is tolerant to physical disturbances, that is why this species could survive in connected sectors but under nutrient - low conditions.

Concluding remarks

The proposed index of the conservation status of aquatic habitats was founded on both physical and biological metrics. The metrics proposed have to be simple for users. Due to the context of numerous cut-off channels after the canalisation of the river Rhine and the demand of restoration by reconnection to the main course, the connectivity was integrated as a metric of change in hydrological functioning of the fluvial hydrosystems, and indicator of reversibility. The connection contributes to inputs of nutrient-rich waters, fine sediments, and propagules. The trophic level a metric mostly related to connection in this study case, highly influences the conservation status. The biological response to different degrees of connectivity consists in a change in specific richness and occurrence of exotic and rare species. Superficial connectivity modifies specific richness, by changing propagules pool and favouring the propagation of exotic species. High connection if it is related to a high trophic level combined with absence of rare species and presence of exotic species leads to a poor or bad conservation status. In cases where high connection was linked to low nutrient input with oligotrophic rare species, the conservation status is good to high.

The upper Rhine floodplain seems to be a particular case of good adequacy between hydrological functioning (connectivity), trophic level and conservation status. This relationship between connection and trophy explains the relative good correspondence between the trophic index IBMR, and the index of conservation status in the case of the ILL fluvial system. The objective of determination of conservation status concerns potential for restoration, and consequently reversibility depending on succession stage of terrestrialization of aquatic systems. According to this study, the question is whether preservation of hydrological dynamics (by connection to the river) which favour disturbances, but also often inputs of nutrient-rich waters could be a guarantee of maintenance of communities of interest and of their diversity.

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BOTANY LETTERS
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Performance, genetic and ecological insights for the conservation of the endangered large pink, *Dianthus superbus* L. (Caryophyllaceae) in semi-natural grassland

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ABSTRACT

The traditional management of hay meadows has allowed them to maintain a notable part of biodiversity currently threatened by recent changes in agricultural uses. In the North Eastern region of France, the large pink, *Dianthus superbus* L. (Caryophyllaceae), mainly grows in hay meadows, and its protected status implies a conservation strategy merging information about its demography, its genetic diversity and its ecology. This study has revealed that the population size of *D. superbus* is correlated with the trophic status of meadows, with a decrease from mesotrophic to eutrophic meadows. No relation has been established between population size and habitat size, spatial isolation or habitat connectivity. Moreover, AFLPs genetic fingerprints indicated equivalent genetic diversities among meadows, without impact of population size or habitat characteristics. The absence of genetic structure and the lack of population differentiation suggest a large genetic admixture at the regional scale. The most crucial issue for the conservation of *D. superbus* in the North Eastern region of France seems to be the modification of its habitat due to the eutrophication of hay meadows.

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Arable species; hay meadows; mowing; semi-natural grasslands; conservation genetics; mesotrophic species

Introduction

Modern changes in agricultural practices constitute an important pressure for biodiversity conservation (Tilman et al. 2002; Henle et al. 2008). Natural taxa co-evolving with agriculture for millennia are now threatened by intensive farming and herbicides' abuse (Wesche et al. 2012). In addition, farmland abandonment for decades has led to habitat modifications with natural closing by woody vegetation, or even habitat destruction due to urbanization. As a consequence, many arable weed species, i.e. species linked to agricultural practices in their life cycle, have dramatically declined during the last decades (Rühl et al. 2015). These alarming observations concern segetal species, i.e. annual species taking advantage of crop harvest for their seed dispersion (e.g. Le Corre et al. 2014), but also many other perennial herbaceous taxa from rural opened habitats like grass stripes, pastures and hay meadows.

Hay meadows, i.e. grasslands which are annually mown for hay, represent a key habitat for biodiversity conservation in European agricultural landscapes. These semi-natural ecosystems combine agricultural, patrimonial and natural values, promoting their conservation for both their taxonomic diversity and ecosystem services (De Groot et al. 2010). However, the over-fertilization of these agrosystems tends to favor competitive species (mainly for light; Hautier, Niklaus, and Hector 2009), resulting in a change in community composition and a global loss of biodiversity (Wesche et al. 2012). In addition, these meadows are currently threatened by landscape fragmentation reducing their ecological connectivity (Krauss et al. 2010). At the community level, habitat fragmentation limits species dispersal and recolonization compensating local extinctions (Krause et al. 2015). At the infraspecific level, this progressive isolation could induce a lack of gene flow among populations, and coupled with the reduction of habitat size, an increase of genetic drift over time (Hooftman et al. 2004). The resulting inbreeding and low-genetic diversity can progressively bring a weakened fitness, a decrease of population sizes and a lack of genetic adaptability leading to local extinction of populations (Leimu et al. 2006; Brütting et al. 2012). To prevent this waterfall effect, several studies based on both demographic and genetic data have advised to increase the habitat size and connectivity (Aavik et al. 2013; Lamy et al. 2013). However, these theoretical guidelines for habitat and genetic restoration are challenged by several studies failing to link spatial isolation to population size or

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Supplementary data for this article can be accessed here.

genetic diversity (Broeck et al. 2015; Putz, Schmid, and Reisch 2015).

The fringed pink or large pink, *Dianthus superbus* L. (Caryophyllaceae), is a perennial herb with a -scattered Euro-Siberian distribution. This species was initially described from low-altitude grasslands, with an unresolved infraspecific taxonomy adding an alpine, a forest and an East-Asian subspecies. *Dianthus superbus* is emblematic of mesotrophic European grasslands, specifically of hay meadows. This species is protected in several countries, as in France where it is considered vulnerable to regionally extinct in several regions. Its lifecycle is well-adapted to mowing perturbations which stimulate its regrowth twice (hay cutting in June and September), preventing light competition with tall grasses and multiplying its seed production (Bernard 1970, 1971). This perennial hemicryptophyte can persist several winters, but it also produces up to 30 capsules per individual, each one including a hundred seeds. This species has coevolved with nocturnal pollinators such as hawkmoths (Sphingidae), as suggested by its long calyx tubes and its large amounts of emitted scent (Jürgens, Witt, and Gottsberger 2003). Consequently, its ratio of sexual reproduction and vegetative growth remains a challenging estimation. Nevertheless, it is possible that mowing may advantages vegetative regrowth against seedling establishment.

This study was initiated with the setting up of a regional plan for the conservation of *D. superbus* in the North Eastern region of France. The remaining populations of this area have been increasingly impacted by changes in land use. The main aim of this study was to investigate how spatial and ecological parameters defining its habitat could influence its biological parameters such as population size, genetic diversity and genetic differentiation. More specifically, we asked the following questions: (i) Are species occurrence and abundance higher in mesotrophic meadows? (ii) Is genetic diversity higher in large population and/or in suitable environmental conditions? (iii) Is there genetic structuring and isolation among a contrasted ecological landscape?

Material & methods

Study area and species prospection

This study focused on the Zorn floodplain (Alsace, NE France), an area of 18,000 ha, 30 km long and 10 km wide. This area is naturally delimited by the Forest of Haguenau on the North (48°49′02”N, 7°47′19”E), by the metropolitan area of Strasbourg on the South (48°34′24”N, 7°45′08”E), by the first hills of the Vosges Mountains on the East, and by the Rhine River on the West. The Zorn River flows into the former alluvial plain of the Rhine River, with a remaining proximity of its groundwater table. These natural ecosystems have been deeply impacted by agricultural practices, currently dominated by croplands isolating smaller hay meadows. These opened environments are interspersed with forests patches, but they are also highly fragmented by artificialized lands (Figure 1).

During field-work, 784 hay meadows were prospected during the summers 2013 and 2014 covering 1,290 ha in the Zorn floodplain. As many herbaceous perennial species, the spatial delimitation of individual genets was sometime debatable. Despite its vegetative shoots, the laxly caespitose habit of *D. superbus* enabled an efficient, cost- and time-effective counting of populations. In a few doubtful cases, a basal distance of 30 cm was used as an arbitrary threshold to delimit two putative genets. Overall, these parameters tended to underestimate population size, in particular for large populations. In addition, seedlings were also deliberately underestimated due to their small size and their morphological similarity with the sympatric *D. carthusianorum*. The individuals of *D. superbus* from 45 meadow plots were counted in 2013 and 2014 to estimate a rate of inter-annual demographic variation, computed as a ratio between the inter-annual variation (the difference of individuals between 2013 and 2014) and the initial population size (i.e. the number of individuals in 2013). Meadows were localized using GIS layers from the regional database GéoGrandEst (http://www.cigalsace.org/). In each meadow, every individual was geolocated using a GPS device. GIS data and spatial analyses were handled in QGIS v.2.4 (QGIS Development Team 2017). The *a priori* delineation of population units for plant sampling is generally a first bias input in the representation of species dynamics. In this study, a meadow plot was arbitrary considered as a suitable surrogate of population unit for *D. superbus*, based on the hypothesis that individuals growing on a same meadow share the same dynamics under a same chain of homogeneous treatments (e.g. nutrient and water inputs, abiotic stresses, perturbations). Consequently, population statistics were estimated using effective meadows delimitation.

Ecological data and analyses

To understand which environmental parameters affect *D. superbus* in the Zorn floodplain, four explanatory variables were studied, i.e. the habitat size (meadow area), the number of mesotrophic species (explained below), the spatial isolation between meadows (as the mean distance of a meadow centroid from the others) and the habitat connectivity between meadows (explained below). We also tested the effect of these four explanatory variables on genetic diversity and divergence, in addition to the population size and the spatial isolation between individuals (as the mean distances between all individuals averaged for each meadow; an analogue of the spatial isolation between meadows weighted by population size). Spatial and demographic data were compiled using Quantum GIS 2.4 (QGIS Development Team 2017). The significant contribution of each explanatory variables was tested with linear and logit generalized linear models using *stats* R-package in R environment (RCore Team, 2017).

The trophic status of grassland, i.e. their richness in nutrients, has a deep impact on the composition and dynamics of plant communities. However, the soil analysis of hundreds of meadow plots was out of the scope of this study. In order to manage a trophic index during meadow prospection, we chose to use the species richness in mesotrophic plant species as a suitable estimation of trophic status. Thus, about 80 taxa were selected on the basis of their affiliation to six phytosociological alliances characterizing mesotrophic plant communities: *Mesobromion erecti* (Braun-Blanq. & Moor 1938) Oberd. 1957 nom. cons. propos.; *Arrhenatherion elatioris* W.Koch 1926; *Cynosurion cristati* Tüxen 1947; *Bromion racemosi* Tüxen in Tüxen & Preising 1951 nom. nud.; *Molinion caeruleae* W.Koch 1926; *Juncion acutiflori* Braun-Blanq. in Braun-Blanq. & Tüxen 1952. *Flora Gallica* (Tison and de Foucault 2014) was used as a reference flora for taxonomy and nomenclature.

Habitat connectivity between meadows was quantified using a graph-based landscape approach using Graphab 1.1 software (Foltête, Clauzel, and Vuidel 2012; Clauzel et al. 2016), modelling the spatial relationships among patches of habitats. The contribution of each meadow in their habitat connectivity was assessed as parts of the variation in connectivity probability (dPC), by adding three components (dPCintra, dPCflux and dPCconnetor), as developed in Saura and Rubio (2010). We developed a set of nodes using all meadows under the Zorn floodplain (Figure 1). Links between meadows were generated using least-cost path geometry. Resistance values were arbitrarily attributed to each landscape element (see Zeller, McGarigal, and Whiteley 2012) in order to represent the different dispersal capacities of *Dianthus superbus*: a resistance value of 1 (weaker resistance) was attributed to meadows, 10 for agricultural surfaces, 50 for forests, woods and other semi-natural elements of the landscape, and 100 for artificialized zones and hydrosystems (higher resistance). The probability of connectivity index associated to each meadow was then used in comparative analyses.

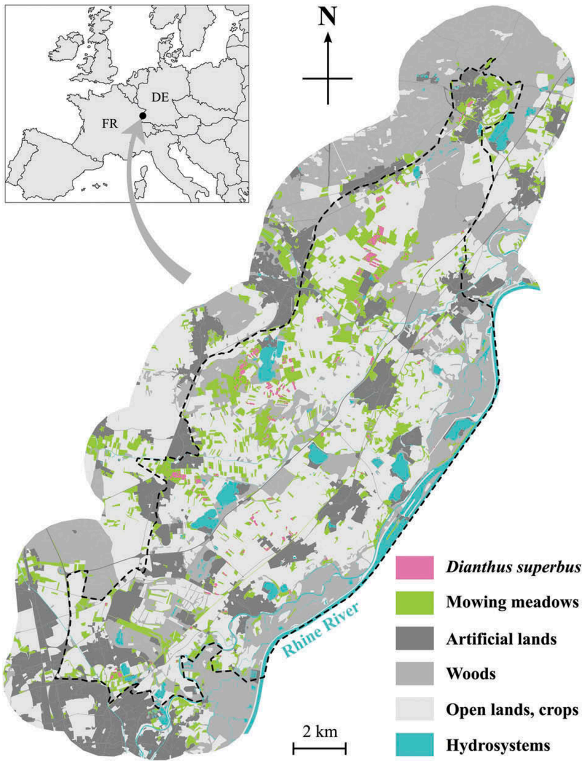

Figure 1. Distribution of the 29 825 individuals of *Dianthus superbus* in 194 of the 784 prospected hay meadows in the Zorn floodplain, and land use. Dashed line distinguishes the study zone from the buffer zone (2 km wide). FR, France; DE, Germany.

DNA extraction, AFLP protocol and genetic analyses

A total of 22 populations were sampled for genetic analyses, by covering the whole species distribution in the Zorn floodplain. In addition, eight NE French populations out from the Zorn floodplain (seven from Alsace and one from Franche-Comté) were sampled to add a broader scale of genetic diversity. For each sample, material was deposed in the Herbarium of the University of Strasbourg (STR). DNA extraction was performed following the procedure of Doyle and Doyle (1987), with the following modifications: *c.* 50 mg of grinded dried leaf material were suspended in 0.7 mL of cetyl-trimethyl-ammonium bromide (CTAB) at 50°C and incubated 1h at 65°C. An equal volume of biophenol: chloroform: isoamylic alcohol (25:24:1) was then added. After 30s of vortex mixing, samples were centrifuged 10 min at 13 000 rpm at room temperature. The aqueous phase was transferred into new tubes with an equivalent volume of 0.6 M NaAc: 95% ethanol at −20°C, and gently mixed for DNA precipitation at −20°C overnight. The DNA pellets were centrifuged 15 min at 13 000 rpm at 5°C, then washed in 70% ethanol, centrifuged again 5 min, dried and suspended in TE-buffer with RNase. The DNA concentrations were measured using a NanoDrop ND-1000 spectrophotometer (Labtech, Uckfield, UK), and diluted to 50 ng/µL in 1X TE-buffer.

The amplified fragment length polymorphism (AFLP) is a molecular method of multilocus fingerprints able to generate hundreds of highly polymorphic markers with an optimized cost-benefit balance. Contrary to other genotyping methods such as microsatellites, AFLPs do not need prior investigation for primers design, but they do not reveal locus heterozygosis. The AFLP was carried out following Vos et al. (1995) with the following modifications: 500 ng of DNA was digested with 10 units of *Eco*RI and 4 units of *Tru*9I in a final volume of 25 µL, incubated during 3 h at 37°C and 3 h at 65°C. Ligation was performed at room temperature for 8 h, by adding 0.5 units of T4 DNA ligase, 12.5 mM of ATP, 3.125 units of Eco adaptors and 31.25 units of Mse adaptors to the digested DNA. Preamplification was performed with 0.2 ng ligation product, 1X of GoTaq Flexi Buffer, 0.25 µM of Eco+ A and Mse+ C primers, 1.0 mM of $MgCl_2$, 0.2 mM of dNTP and 0.5 units of GoTaq G2 Flexi DNA polymerase (Promega, Fitchburg, Wisconsin, US) in a final volume of 20 µL. Preamplification cycles were 94°C for 2 min, followed by 20 cycles at 94°C for 45 s, 56°C for 45 s, 72°C for 1 min and 72°C for 10 min. Selective amplification was performed using Eco+ AAC primer dyed with 6-FAM fluorescent at 5′ end, and Mse+ CAA. Selective amplification was performed in 20 µL volumes with 5 µL of 10× diluted preamplification, 1X of GoTaq Flexi Buffer, 0.25 µM of each primer, 1.0 mM of $MgCl_2$, 0.2 mM of dNTPs, 0.5 unit of GoTaq polymerase. The amplification thermocycle profile was 94°C for 2 min, 10 cycles of 94°C for 30 s, 65°C for 30 s (−0.7°C touchdown per cycle), 72°C for 1 min, followed by 20 cycles at 94°C for 30 s, 56°C for 30 s, 72°C for 1 min and 72°C for 5 min. Lengths of PCR products were obtained on an ABI Genetic Analyzer 3130 (ThermoFischer Scientific, Waltham, MA, USA) at the IBMP genomic platform (CNRS, Unistra). PCR product sizes were calibrated using the size standard SM594, fluorochromed with 5′ATTO 633 (Mauger, Couceiro, and Valero 2012).

Electrophoretograms were semi-automatically transformed in presence-absence dataset using GeneMapper v.4.1 (ABI), and non-repeatable markers were discarded using 10 replicate samples. AFLP analysis was made by defining putative loci as any fragment with minimal amplitude of 200 relative fluorescent units (RFU) from 50 to 500 bp, and automated scoring was manually checked to correct any misinterpreted RFU signal and to discard any overlapping or ambiguous markers. The following genetic analyses were led on both types of fingerprinting. For each population, descriptive statistics such as percentage of polymorphic fragments (%P), Nei's gene diversity (*D*) and frequency downweighted marker value (*DW*, using here as an estimation of genetic divergence; Schönswetter and Tribsch 2005) were estimated using the AFLPdat R-scripts (Ehrich 2006). The *DW* index is estimated as the number of occurrences of each marker in each population divided by the number of occurrences of this marker in the whole sampling. This index should be higher in long-term isolated populations where rare markers should accumulate due to mutations. The differentiation between populations was tested with an analysis of molecular variance (AMOVA) generated in GenAlEx v.6.5 (Peakall and Smouse 2012), in order to show the parts of genetic diversity distributed between populations, and between individuals within populations. In order to test specifically the impact of geographical isolation on genetic differentiation, Mantel correlogram compared the genetic and geographical distances between every pair of individuals, using the R-function mantel.correlog() from the R-package *vegan* (Dixon 2003). A principal coordinates analysis was generated with the R-package *ade4* (Dray and Dufour 2007).

Results

Inventory and distribution

Field prospection in the 784 meadows identified 29,825 individuals of *D. superbus* in 174 meadows representing 410 ha (that is 22.2% in number and 31.8% in surface of prospected meadows). Among all the 784 prospected meadows (with or without *D. superbus*), 133 meadows didn't hold any mesotrophic species, 367 meadows held two or less mesotrophic species, and only 155 meadows held more than five mesotrophic species, with a maximum of 16 mesotrophic species for one meadow. The number of mesotrophic species significantly contributed to explain the occurrence (p-value < 0.001; logistic regression in Table 1) and the population size (p-value < 0.001; linear model in Table 1) of *D. superbus* in the hay meadows of the Zorn floodplain (Table 1), with a mean richness of 6.61 ± 2.82 mesotrophic species in meadows with *D. superbus vs.* 2.39 ± 2.13 mesotrophic species in meadows without *D. superbus*. The habitat size also significantly contributed to explain the variability of population size (p-value < 0.01; linear model in Table 1). However, no significant contribution was found for spatial isolation indices and habitat connectivity (p-value > 0.05; logistic regression and linear model in Table 1).

Dianthus superbus was mainly found on loam-clayey black soils including a high amount of organic matter in the upper horizons, and with a strong hydromorphic character due to the closeness of water table. The floristic relevés collected on the meadows with *D. superbus* mainly described mesotrophic meso-hygrophilous communities from the *Arrhenaterion elatioris* and oligotrophic hygrophilous

Table 1. Contributions of explanatory variables in the variability of species occurrence (logistic regression), population size (log value) and genetic parameters (linear models).

	Occurrence			Population size [a]			Genetic diversity			Genetic divergence		
	Coef.	z-value	*p*-value	Coef.	t-value	*p*-value	Coef.	t-value	*p*-value	Coef.	t-value	*p*-value
Habitat size [a]	0.02	0.30	0.767	0.14	2.76	< 0.01 **	0.03	1.39	0.185	9.39	0.85	0.410
Isolation bet- ween meadows	0.45	0.09	0.930	7.35	1.31	0.191	0.12	2.45	0.027	1 070	0.45	0.662
Mesotrophic taxa	0.62	12.7	< 0.001 ***	0.22	5.12	< 0.001 ***	−0.01	−0.54	0.600	−1.07	−0.10	0.920
Habitat conectivity	17.6	1.80	0.072	16.2	1.70	0.091	−5.44	−1.19	0.254	463	0.20	0.844
Isolation bet- ween meadows	-	-	-	-	-	-	−0.14	−0.07	0.948	1 200	1.10	0.288
Population size [a]	-	-	-	-	-	-	$7.18e^{-5}$	0.62	0.545	$-9.81e^{3}$	−0.17	0.869

[a], log value

meadows from the *Molinion caeruleae* alliances, with some transgressions toward meso-xerophilous grasslands (*Festuco valesiacae – Brometea erecti*), woodland edges (*Trifolio medi – Geranietea sanguinei*) and thickets (*Crataego monogynae – Prunetea spinosae*). Among the 80 species surveyed, the five most common species were *Sanguisorba officinalis* L. (Rosaceae), *Galium verum* L. (Rubiaceae), *Pimpinella saxifraga* L. (Apiaceae), *Silaum silaus* (L.) Schinz & Thell. (Apiaceae), *Achillea ptarmica* L. (Asteraceae).

The mean population size estimated on the 174 meadows with D. superbus reaches 154 ± 869 individuals, with a median value of 27 individuals. The discard of extreme values (one plot with 11,160 individuals, and nine between 1,700 and 500 individuals) decreased the mean value to 80 ± 45 individuals. A mean of 78 individuals per hectare was estimated, decreasing to 48 after discarding the 11 extreme values previously cited. However, this mean abundance must be considered with the relatively strong aggregative distribution of individuals within meadows. Across the Zorn floodplain, two close centers of distribution gathered most of the individuals, located in the west-central part of the study area (Figure 1). The mean inter-annual rate of demographic variation was estimated to 1.7 ± 3.2, ranging from 0.15 to 15.41 (considering only absolute values).

Table 2. Genetic diversities and divergences among different populations from the Zorn floodplain, the south Alsace, and from one distant population from a neighboring region (Bannans, Franche-Comté).

Locality	Lat	Long	n	% PF	*D* ± SD	SF	DW
Zorn floodplain (North Alsace)							
Reichstett	48.637	7.757	5	33.2	0.221 ± 0.019	2	416.1
La Wantzenau	48.663	7.812	5	15.3	0.102 ± 0.015	0	601.2
La Wantzenau	48.669	7.820	5	28.0	0.187 ± 0.018	0	197.1
Kilstett	48.678	7.843	5	33.2	0.221 ± 0.019	0	298.4
Kilstett	48.686	7.837	5	30.2	0.201 ± 0.019	4	393.2
Hoerdt	48.705	7.795	5	20.9	0.209 ± 0.025	2	384.1
Gries	48.745	7.842	5	26.9	0.179 ± 0.018	0	269.2
Schirrhein	48.796	7.911	5	31.0	0.206 ± 0.019	0	313.1
Reichstett	48.639	7.760	5	26.9	0.193 ± 0.019	0	311.3
Reichstett	48.640	7.778	5	28.7	0.192 ± 0.018	3	342.3
La Wantzenau	48.673	7.872	5	28.0	0.187 ± 0.018	0	227.8
Gambsheim	48.702	7.893	5	21.6	0.216 ± 0.025	0	294.3
Offendorf	48.714	7.898	5	23.1	0.231 ± 0.026	0	316.9
Offendorf	48.718	7.929	5	28.4	0.189 ± 0.018	0	231.2
Herrlisheim	48.746	7.929	5	30.6	0.204 ± 0.019	0	281.9
Drusenheim	48.760	7.915	5	28.0	0.187 ± 0.018	0	283.6
Soufflenheim	48.782	7.954	5	22.4	0.224 ± 0.026	0	346.4
Soufflenheim	48.826	7.963	5	24.3	0.162 ± 0.017	0	185.4
Oberhoffen/ moder	48.775	7.898	5	32.1	0.214 ± 0.019	1	312.2
Herrlisheim	48.740	7.903	5	29.9	0.199 ± 0.019	1	261.2
Bischwiller	48.734	7.861	5	30.6	0.204 ± 0.019	0	221.7
Gries	48.733	7.849	5	25.7	0.172 ± 0.018	0	203.0
South Alsace							
Petit Ballon	48.006	7.149	5	23.1	0.154 ± 0.017	0	237.5
Sundhouse	48.270	7.633	5	32.8	0.219 ± 0.019	1	413.6
Innenheim	48.489	7.587	5	32.1	0.214 ± 0.019	3	408.8
Ottrott	48.468	7.403	5	16.4	0.164 ± 0.023	1	296.5
Huttenheim	48.344	7.595	5	17.5	0.175 ± 0.023	0	296.8
Ohnenheim	48.204	7.486	5	31.0	0.206 ± 0.019	1	345.0
Petite Camargue	47.630	7.540	5	20.5	0.137 ± 0.016	1	226.7
Franche-Comté							
Bannans	46.900	6.250	5	35.1	0.234 ± 0.019	2	423.5

Lat, latitude; Long, longitude; %PF, percentage of polymorphic markers; *D*, Gene diversity; SF, population specific markers; *DW*, frequency down-weighted marker value (index of genetic divergence).

Genetic diversity and divergence

The 83 AFLP fingerprints generated 272 analysable markers of which 264 (97%) were polymorphic. At the population scale (Table 2), this variability decreased from 35.1 to 15.3%, and most of the 30 populations did not show private markers (n = 18) or just one (n = 6). In each population, the genetic diversity is weak to medium, with Nei gene diversity (*D*) ranging from 0.102 to 0.234 (Figure 2). The most differentiated population (indicated with the *DW* index) is not the outgroup population selected in a neighboring region (Bannans, Franche-Comté), but one population located in the Zorn floodplain which also presents the lowest values for genetic diversity (Table 2).

The analysis of molecular variance found the most important part of genetic variability within populations, with only 10.3% and 11.9% at the scales of the Zorn floodplain and the NE France, respectively (Table 3). This lack of genetic structuring between populations was also found in the principal

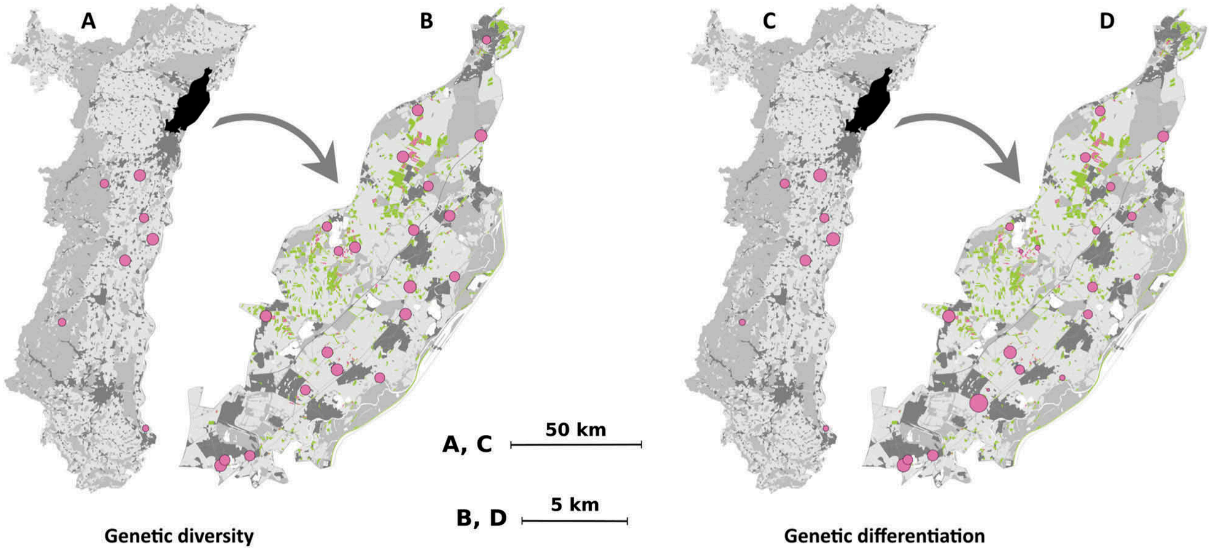

Figure 2. Geographical distribution of population genetic diversity (Nei Gene Diversity; A, B) and divergence (frequency down-weighted marker value; C, D) in the Alsace Region (regional scale; A, C) and Zorn floodplain (more local scale; B, D). Green area indicates species suitable area, i.e. mowing meadows.

Table 3. Analysis of molecular variance (AMOVA) within the Zorn floodplain (and within the Alsace region).

	df	SS	MS	Var	%Var
Among populations	21 (29)	687.7 (864.2)	31.3 (32.0)	2.7 (3.2)	10.3% (11.9%)
Within population	88 (115)	991.7 (1162.0)	23.6 (23.2)	23.6 (23.2)	90.7% (88.1%)
Total	109 (144)	1679.4 (2026.2)	54.9 (55.3)	26.3 (26.4)	100% (100%)

df, degree of freedom; SS, sum of square; MS, mean square; Var., estimated variance

coordinates analysis, with the two first components explaining only 3.41 and 3.3% of the total inertia (Supplemental data). At the population level, no significant contribution of explanatory variables was found to explain genetic diversity and divergence (Linear models in Table 1), except a slight effect of isolation between meadows on genetic diversity (p-value = 0.027). Between individuals, the Mantel correlogram detected a significant positive correlation between genetic and geographical distances only concerning individuals separated from less than 1.17 km (p-value < 0.001). Beyond this range of geographical distances, there was no correlation between genetic and geographical distances.

Discussion

Meadow management drives spatial distribution and abundance of D. superbus

This study has underlined that the main factor limiting the occurrence and abundance of *D. superbus* is the trophic status of meadows, indirectly considered here through the richness in mesotrophic species. Indeed, this number is higher in meadows with *D. superbus* than in meadows without *D. superbus*. In hay meadows, this trophic status is under the major influence of agricultural practices. Other threatened species (red lists or protected taxa) as *Veronica longifolia* L. (Plantaginaceae), *Peucedanum officinale* L. (Apiaceae) and *Allium angulosum* L. (Amaryllidaceae) were observed during field prospection. They are suffering from the same habitat degradation. Among current land use techniques, meadow fertilization has largely been recognized as a threat for mesotrophic species, leading to soil eutrophication, habitat degradation and local extinction of patrimonial species (Hautier, Niklaus, and Hector 2009; Ceulemans et al. 2014). In addition, hay meadows are becoming more and more isolated in an agricultural landscape dominated by monospecific cultures. This habitat fragmentation limits dispersal in such meta-population systems (Krause et al. 2015). In this way, land use practices and habitat fragmentation have been designated as the main environmental factors influencing the performance and the genetic diversity of *Dianthus seguieri* Vill. (Caryophyllaceae), another pink from European semi-natural meadows (S Germany; Busch and Reisch 2016). However, our results failed to find a correlation between the occurrence-abundance of *D. superbus* and the spatial distribution or the habitat connectivity of meadows. Nevertheless, all these results have to be put in perspective of the strong inter-annual variation of population size, underlining the short-term answer of *D. superbus* distribution to environmental changes.

In addition, several environmental parameters were not estimated in this study despite their potential impact on species growth and persistence, such as soil texture and hygromorphy, or the historical differences in land use for each meadow.

Genetic diversity without spatial structuring

In the Zorn floodplain, the populations of *D. superbus* showed a homogeneous amount of genetic diversity. The mean Nei gene diversity of *D. superbus* from NE France (0.192 ± 0.029) was equivalent to other comparable pink species: slightly weaker than *Dianthus gratianopolitanus* Vill. (Caryophyllaceae) from southern Germany (0.217) and from Switzerland (0.206; Putz, Schmid, and Reisch 2015), and slightly stronger than *D. seguieri* from southern Germany (0.170; Busch and Reisch 2016). All sampled populations of *D. superbus* were composed of clearly distinct genotypes, without clonal similarities. This result shows that the two mowing perturbations per year do not limit sexual reproduction and genetic admixture. However, no correlation was shown between genetic diversity and population size. Such a correlation was found for other *Dianthus* taxa, such as *Dianthus callizonus* Schott & Kotschy (Caryophyllaceae) in Carpathian alpine meadows (Gabel, Sattler, and Reisch 2017), and for *D. seguieri* from German grasslands (Busch and Reisch 2016), but not for German populations of *Dianthus carthusianorum* L. (Caryophyllaceae) (Reisch et al. 2017).

The lack of genetic structuring for *D. superbus* over a regional scale was supported by the absence of significant correlation between genetic and geographical distances and by the low amount of genetic variation located at the inter-population scale in the AMOVA. Such a lack of spatial genetic structure was already found for other *Dianthus* species living in agropastoral ecosystems, such as *D. carthusianorum* in German grazed meadows (Rico and Wagner 2016), but not for a *Dianthus* taxon living in more natural ecosystems such as *D. callizonus* from Carpathian alpine meadows (Gabel, Sattler, and Reisch 2017). This lack of genetic structuring was also found for arable species such as *Cyanus segetum* Hill. (Asteraceae) (Le Corre et al. 2014; Petit, Arnal, and Darmency 2015), *Papaver rhoeas* L. (Papaveraceae) (Kati et al. 2013) and *Alopecurus myosuroides* Huds. (Poaceae) (Menchari, Délye, and Le Corre 2007). For these species, agricultural practices were often mentioned as a recurrent vector of dispersal limiting genetic structuring (e.g. unintentional dispersal on agricultural machinery, hay transfer and seeding). For *D. superbus*, this human-mediated dispersal for centuries may have generated an artificialized metapopulation. Such a human-mediated dispersal was already shown on *D. carthusianorum* in southern Germany, where the rotational shepherding significantly increased dispersal and gene flow of grazed populations compared to ungrazed populations (Rico, Boehmer, and Wagner 2014; Rico et al. 2014; Rico and Wagner 2016).

Regarding the highest values of genetic differentiation (DW), another interesting result was the highest value of a small population (n = 22) located in the Zorn floodplain (La Wantzenau). This outlier did not seem to be due to a methodological bias since it was supported by every sample of this population. In addition, this population is not notably isolated from the other populations. Their genotypes were relatively similar but quite differentiated from the remaining sampling. One hypothesis to explain this outlier value could be an ancient event of hybridization with another *Dianthus* species, followed by recurrent introgression with *D. superbus* parents (Andersson-Kotto and Gairdner 1931). Indeed, interspecific hybridizations are well-known in the genus *Dianthus* and the resulting hybrids could be fertile and crossable with parental species, promoting morphological convergence of hybrids toward one parent species in two generations (Williams 1893). The hybrid with the other *Dianthus* species native from French hay meadows, *D. carthusianorum*, was not mentioned in floras. However, it was formerly generated *in vitro* as a sterile hybrid (Föcke 1881; Carolin 1957). The second most differentiated population was the outgroup population collected outside the NE France region (Bannans, Franche-Comté Region). This result was expected since this population was the more geographically distant from the remaining sampling. This inter-regional differentiation may suggest a genetic structuring at a broader geographical scale.

Conclusions

This study underlined the negative impact of meadow eutrophication on the persistence and fitness of *D. superbus* in NE France. Because this habitat modification could be partly explained by human-induced fertilization, conservation policies focusing on *D. superbus* have to promote habitat suitability in terms of management and nutrient amounts. The homogeneous amounts of genetic diversity without structuring tone down the consideration of genetic parameters for the conservation of *D. superbus* in NE France. However, this study may underline the putative role of agricultural practices as a major dispersal vector between hay meadows, promoting genetic mixing and species recolonization.

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Laboratoire GESTE
Gestion Territoriale de l'Eau et de l'Environnement

APPORTS À L'ÉVALUATION D'UNE OPÉRATION DE RESTAURATION : APPROCHE MULTICRITÈRES (ÉCOLOGIQUES ET ÉCONOMIQUES)

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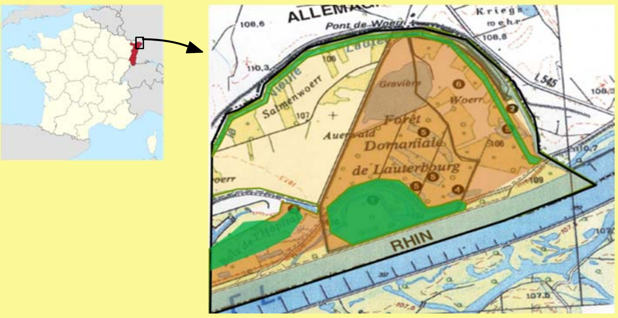
Contexte : Restauration du Woerr

Le Woerr se situe sur la commune de Lauterbourg (Bas-Rhin), en plaine alluviale du Rhin et présente une mosaïque de milieux caractéristiques de la plaine alluviale anthropisée : forêt alluviale, ancienne gravière, pelouses, roselières et depuis quelques années petits plans d'eau.
Le Site du Woerr est un espace naturel sensible du Conseil Général du Bas – Rhin (CG67) ; une partie du site est également en réserve de l'ONF. Enfin, certaines parcelles appartiennent au Conservatoire des sites Alsaciens (CSA).

A partir de 2000, l'ONF a commencé des travaux **d'adoucissement des berges de la gravière** sur sa rive ouest. A partir de 2011, le CG67 a continué ces travaux sur les rives Est (80 % des berges de la gravière ont été réhabilitées) et a créé plusieurs **mares** en périphérie de la berge nord–est. Dans la continuité de ces restaurations, deux « **étangs expérimentaux** » ont été créés en 2012 en bordure de la gravière afin de créer des zones de faible profondeur. Ces zones devraient permettre l'établissement de communautés végétales émergentes. Elles devraient également permettent de tester l'efficacité de méthodes de restauration écologique : **transfert de banque et de transplantation d'espèces et lutte contre l'invasive *Elodea nuttallii***.



RHEP : Méthode d'évaluation du gain environnemental sur un site restauré :

- Méthode HEP + notion de **l'évaluation de l'état de conservation** (EC).
- Indicateurs de l'EC des habitats d'intérêts communautaires (modifiés si besoin) calculé pour chaque polygone : **structure et la fonctionnalité** de l'habitat + **altérations** de cet habitat.
- Ajout d'indicateurs en fonction des objectifs de la restauration du site : exple, un critère « **espèce rare** » ajouté pour quantifier l'apparition ou la disparition d'espèces rares par polygone d'habitat.

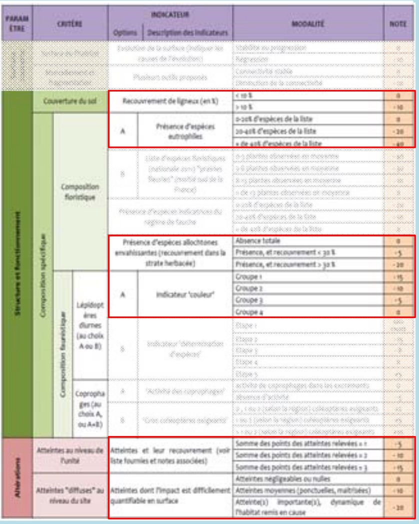
Habitat 1			polygones			
			Surfaces (ha)			
indicateur	**paramètres**	**critères**	**modalité**	**scores**	score intermédiaire	note finale
Etat de conservation	structure et fonctionnement	critère 1	modalité 1	score 1		
			modalité 2	score 2		
		critère 2	modalité 1	score 1		
			modalité 2	score 2		
	altérations	critère	modalité 1	score 1		
			modalité 2	score 2		
	biodiversité	présence d'espèces rares	modalité 1	score 1		
			modalité 2	score 2		
			modalité 3	score 3		

Ajout dans RHEP

Habitat H	**H 1**	**H 2**	**H 3**	**Surface totale**	**HSI**	**UH**
Surface S	**S1**	**S2**	**S3**			
Espèce a	X	X		S1 + S2	HSI a	UH a
Espèce b		X		S2	HSI b	UH b
Espèce c	X		X	S1 + S3	HSI c	UH c
Score EC 1	X			S1	HSI ec1	UH 1
Score EC 2		X		S2	HSI ec2	UH 2
Score EC 3			X	S3	HSI ec3	UH 3

HEP adaptée

Exemple de calcul de l'indicateur EC pour les habitats prairie dans la méthode RHEP.
Le tableau présente les paramètres utilisés par la méhtode Maciejewski *et al.*, 2013.
Seuls les indicateurs entourés en rouge ont été utilisés ici. La note théorique maximale obtenue avec ces indicateurs serait de 120, le seuil pour HSI = 1 est donc de 85 (optimal pour 70 % de la note maximal dans la méthode)



PARAMÈTRE	CRITÈRE	INDICATEUR Options	INDICATEUR Description des indicateurs	MODALITÉ	NOTE
Structure et fonctionnement	Couverture du sol		Recouvrement de ligneux (en %)	< 10 %	0
				> 10 %	-10
	Composition spécifique – Composition floristique	A	Présence d'espèces eutrophiles	0-20% d'espèces de la liste	0
				20-40% d'espèces de la liste	-20
				> de 40% d'espèces de la liste	-40
			Présence d'espèces allochtones envahissantes (recouvrement dans la strate herbacée)	Absence totale	0
				Présence, et recouvrement < 30 %	-5
				Présence, et recouvrement > 30 %	-20
	Composition faunistique – Lépidoptères diurnes (au choix A ou B)	A	Indicateur 'couleur'	Groupe 1	-15
				Groupe 2	-10
				Groupe 3	-5
				Groupe 4	0
Altérations	Atteintes au niveau de l'unité		Atteintes et leur recouvrement (voir liste fournies et notes associées)	Somme des points des atteintes relevées = 1	-5
				Somme des points des atteintes relevées = 2	-10
				Somme des points des atteintes relevées = 3	-15
	Atteintes "diffuses" au niveau du site		Atteintes dont l'impact est difficilement quantifiable en surface	Atteintes négligeables ou nulles	0
				Atteintes moyennes (ponctuelles, maîtrisées)	-10
				Atteinte(s) importante(s), dynamique de l'habitat remis en cause	-20

Méthode HEP adaptée (Dumax & Rozan, 2011) :

Méthode adaptée de la procédure d'évaluation des habitats (HEP : habitat evaluation procedure, USFWS 1980) pour quantifier **l'impact d'un projet d'aménagement** sur des habitats naturels en utilisant des **indicateurs de la qualité du milieux.**

- Cartographie SIG des **habitats** du site => **surfaces** (indice quantitatif).
- Identification des **services écosystémiques** (SE) et **espèces** présentes sur le site = proxy habitat – SE.
- Calcul **HSI** (Habitat Suitability Index : indice qualitatif) = Rapport entre conditions observées et conditions optimales fixées pour un habitat.

Habitat H	**H 1**	**H 2**	**H 3**	**S disponible par espèce**
Surface S	**S1**	**S2**	**S3**	
Espèce a	X	X		**Sa**
Espèce b		X		**Sb**
Espèce c	X		X	**Sc**

Habitat H	**H 1**	**H 2**	**H 3**	**HSI**
Espèce a	X	X		**HSI a**
Espèce b		X		**HSI b**
Espèce c	X		X	**HSI c**

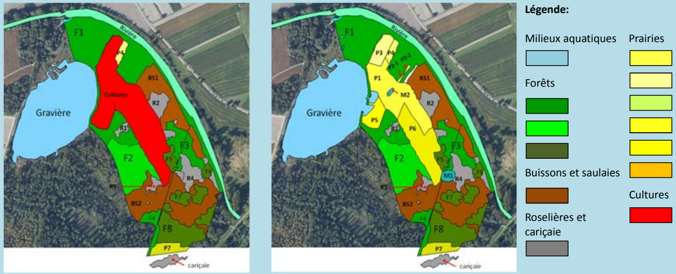
Calcul d'**Unitées d'habitat** (UH) pour chaque habitat.
UHi = Si * HSI i

Habitat H **Surface S**	**S disponible par espèce**	**HSI**	**UH**
Espèce a	**Sa**	**HSI a**	**UH a**
Espèce b	**Sb**	**HSI b**	**UH b**
Espèce c	**Sc**	**HSI c**	**UH c**

Impact de l'aménagement sur le milieu :
Somme UHavant <-> Somme UHaprès.

Identification des habitats avant et après restauration (et gestion) :

État initial pré-restauration (1994 – 2003)   État final post-restauration (2014)



Estimation du gain environnemental sur le Woerr

1996

Restauration
+
Gestion ENS

2014

Uh total = 16,70 UH

Uh total = 37,74 UH

Gain d' UH observé :
37,74 – 16,70 = 21.04

- Augmentation de plus du double d'UH
- Gain environnemental important lié au techniques de restauration et à la gestion du site

Conclusions - Perspectives

- **Méthode HEP adaptable à la restauration : RHEP**

- **Evaluation du gain environnemental sur l'ensemble du site du à des travaux de restaurations ponctuels**

- **Utilisation d'indices choisis en fonction :**
  - du site
  - du niveau de connaissance et de la capacité de collecte de données,
  - des données disponibles,
  - des objectifs de gestion et de restauration

- **Méthode actuellement en cours d'amélioration** (méthodes d'évaluation de l'EC pour des habitats hors Directive européenne, données manquantes, etc.)

- **Utilisation possible dans des objectifs de compensation**

Références : **Dumax N., Rozan A., (2011) :** Using an adapted HEP to assess environmental cost, *Ecological Economics, 72, 53-59*
Maciejewski L., Seytre L., Van Es J., Dupont P., Ben-Mimoun K. (2013) : État de conservation des habitats agropastoraux d'intérêt com munautaire, méthode d'évaluation à l'échelle du site Natura 2000. *Rapport SNP, mai 2013. 184p.*
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Colloque Rever 6 :
REVER et Dynamiser

Restauration fonctionnelle et durable
6èmes journées atelier

3 – 4 Mars 2015
Strasbourg

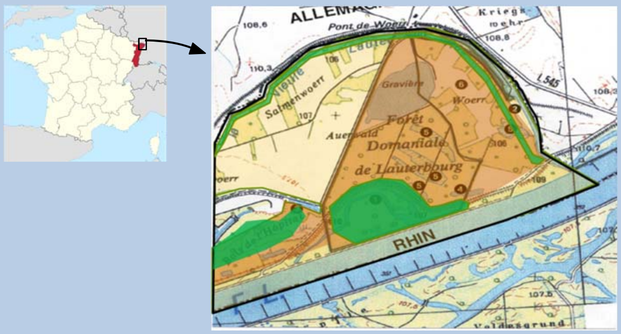
TRANSFERT DE BANQUE, TRANSPLANTATION, DÉCONNEXION TEMPORAIRE : MESURES EFFICACES EN RESTAURATION DE MILIEUX AQUATIQUES ?

Isabelle COMBROUX[1], Cybill STAENTZEL[1], Antoine PERRIER[1], Sébastien KERN[2]
1. UMR CNRS 7362 LIVE, Strasbourg ; 2. Conseil Général 67, Strasbourg

Contexte : Restauration du Woerr

Le Woerr se situe sur la commune de Lauterbourg (Bas-Rhin), en plaine alluviale du Rhin et présente une mosaïque de milieux caractéristiques de la plaine alluviale anthropisée : forêt alluviale, ancienne gravière, pelouses, roselières et depuis quelques années petits plans d'eau.
Le Site du Woerr est un espace naturel sensible du Conseil Général du Bas – Rhin (CG67) ; une partie du site est également en réserve de l'ONF. Enfin, certaines parcelles appartiennent au Conservatoire des sites Alsaciens (CSA).

A partir de 2000, l'ONF a commencé des travaux **d'adoucissement des berges de la gravière** sur sa rive ouest. A partir de 2011, le CG67 a continué ces travaux sur les rives Est (80 % des berges de la gravière ont été réhabilitées) et a créé plusieurs **mares** en périphérie de la berge nord–est. Dans la continuité de ces restaurations, deux « **étangs expérimentaux** » ont été créés en 2012 en bordure de la gravière afin de créer des zones de faible profondeur. Ces zones devraient permettre l'établissement de communautés végétales émergentes. Elles devraient également permettre de tester l'efficacité de méthodes de restauration écologique : **transfert de banque et de transplantation d'espèces et lutte contre l'invasive *Elodea nuttallii***.



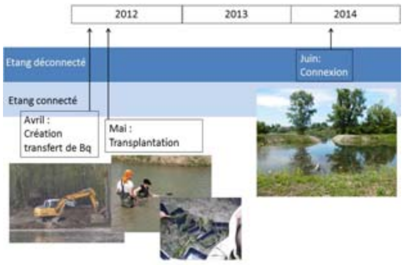
Objectifs de la restauration :

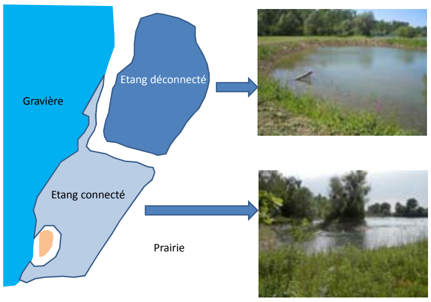
\# Retour à des écosystèmes alluviaux fonctionnels :
- Création de mares, roselières…
- Création de berges en pente douce
- Limitation des invasives

\# Restauration d'habitats pour les amphibiens
\# Restauration d'habitats pour la cistude

Dispositif expérimental : tests de méthodes :

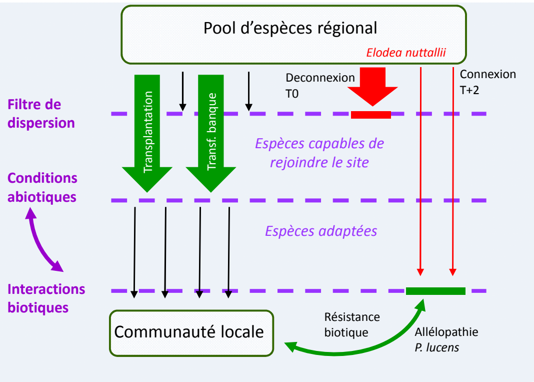
Création de zones expérimentales en berge Est de la gravière :
- Déconnexion / reconnexion
- Transplantation
- Transfert de banque





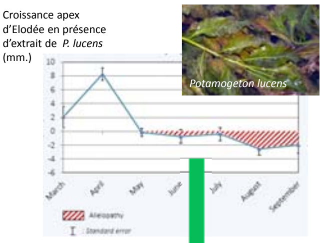
Cadre théorique :

Filtres hiérarchiques (Lortie 2004)



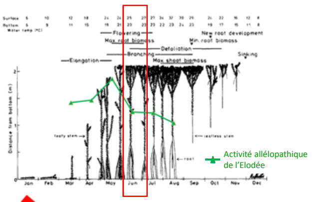
Limitation de l'Elodée

Allélopathie chez *Potamogeton lucens*



Elodée sensible à *P. lucens* à partir de **Juin**

Allélopathie chez *E. nuttallii*



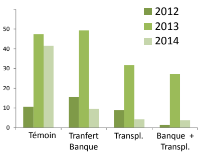
Juin : Risque minimal % Elodée (chute allélopathie, avant dispersion)

Etang déconnecté

Connexion en **Juin** 2014
Efficacité : aucune Elodée installée à l'automne 2014… à suivre…

Etang connecté

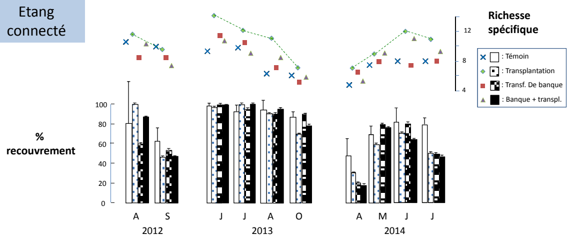
***Elodea nuttallii*
% recouvrement moyen (étang connecté)**



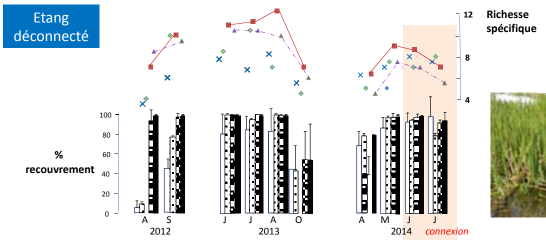
Transfert de communauté :
- Sédiment riche et fin : favorable à *Elodea*.
- Transplantation :+)

Colonisation végétale

Etang connecté



Etang déconnecté



Richesse et % recouvrement max à partir de Mai-Juin

- Transplantation : Efficacité immédiate en Etang connecté, faible en étang déconnecté
- Transfert de sédiment : rapide homogénéisation en étang connecté, efficace en étang déconnecté (communauté + sédiment)
- Esp. Rare *Hippuris vulgaris* sur l'étang déconnecté

Conclusions - Perspectives

Méthodes de transferts de communauté efficaces suivant les situations (↗ % recouvrement et résistance biotique)
Déconnexion temporaire efficace
Possibilité d'utilisation des interactions biotiques négative (phytotoxicité) et de leur phénologie.

? : Efficacité à long terme ? … à suivre…

Colloque Rever 6 :
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Wet meadows restoration within an industrial wasteland conversion: what are the potential sources of seeds ?

Guillaume JACEK & Isabelle COMBROUX
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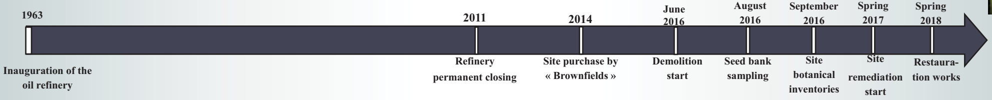
UMR CNRS LIVE 7362, University of Strasbourg, Strasbourg, France

Oil refinery site before rehabilitations work

Context: The study area, a 450 ha highly pertubated wasteland of an old oil raffinery site, is situated 5km north from Strabourg, France. This industrial wasteland is abandonned since 2011, and will be transformed in a 85 ha « ecological » business park with restorated wetland meadows areas (10.3 ha) as compensatory measures. The site present lot of artificialised polluted areas (roads, pipes, tanks) and is dominated by ruderal vegetation (cf: vegetation map). Invasive species, like *Solidago gigantea, Senecio inaequidens* and *Erigeron annuus* cover 75% of the total area. Due to high site pollution and high invasive vegetation cover, a special interest has to be set up to evaluate the potential sources of seeds to restore these wetlands. The aim of this study, was to evaluate the site restoration potentiality by studying different source of seeds from site seed bank to dispersion from local and surrounding vegetation, and how they could colonized the different created areas.

Oil refinery site after rehabilitations work (model)



1963	2011	2014	June 2016	August 2016	September 2016	Spring 2017	Spring 2018
Inauguration of the oil refinery	Refinery permanent closing	Site purchase by « Brownfields »	Demolition start	Seed bank sampling	Site botanical inventories	Site remediation start	Restauration works

The seed bank study

Due to site demolition agenda, sampling had to be done in August 2016 instead of end of winter. This led to some bias compared with classic seed bank studies:

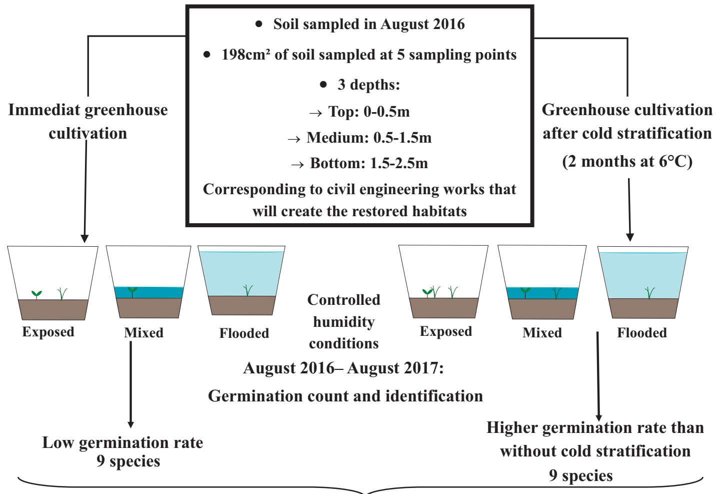
➡ 1) Underestimation of species that need a cold period to be activated before germination under seedling emergence method

➡ 2) Underestimation of the top soil seed bank particularly for species that fructificate after August

1) Soil seed bank estimation

Seedling emergence method

(adapted from Combroux et al. 2001)



- Soil sampled in August 2016
- 198cm² of soil sampled at 5 sampling points
- 3 depths:
  - → Top: 0-0.5m
  - → Medium: 0.5-1.5m
  - → Bottom: 1.5-2.5m

Corresponding to civil engineering works that will create the restored habitats

Immediat greenhouse cultivation

Greenhouse cultivation after cold stratification (2 months at 6°C)

Controlled humidity conditions

August 2016– August 2017:
Germination count and identification

Low germination rate
9 species

Higher germination rate than without cold stratification
9 species

Layer depth	Seed density (seeds/m²)
Top : 0-50 cm (active seed bank)	4242
Medium : 0.5 -1.5m	2121
Bottom : 1.5 - 2.5 m	1515

SEED BANK ESTIMATION

Total of 13 species identified

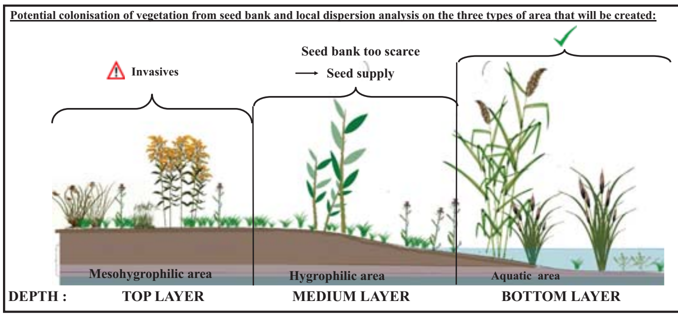
➡ Poor and really disturbed seed bank

Weak seed sources for restoration

3) Analysis and perspectives

Analysis of species biological traits to evaluate their potential establishment in the different created area and soil humidity level.

Potential colonisation of vegetation from seed bank and local dispersion analysis on the three types of area that will be created:



⚠ Most of the species underestimated by seedling emergence method are invasive species
eg. : *Solidago gigantea*

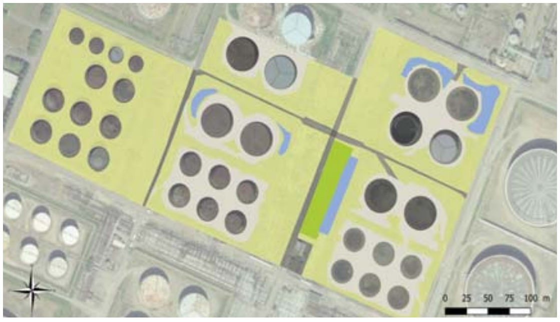
Recommendations :

- **Mesohygrophilic area:**
- Top layer (50 cm) removal ➡ invasive species seed bank (mostly transient) removed
- **Hygrophilic area:**
- Local hay transfert to bring or direct seed supply with selected and local wet meadows species, to accelerate target communities establishment and limit invasives species re-establishment
- **Aquatic area:** No action needed

2) How to overcome bias due to a summer sampling of the seed bank?

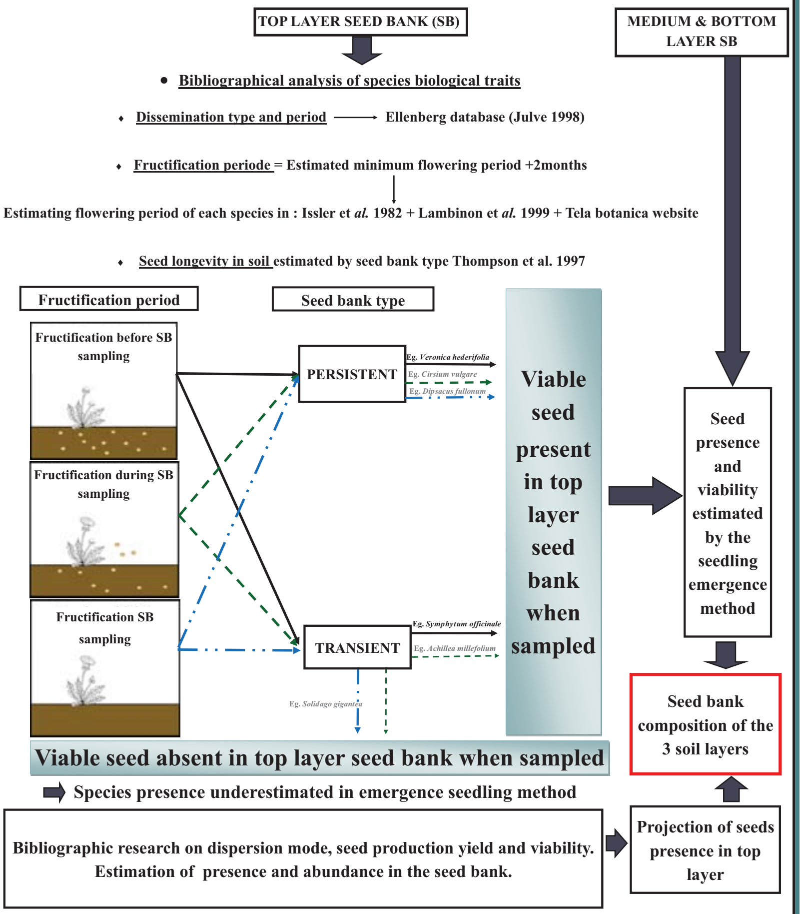
- Estimation of species not detected in the top layer seed bank due to sampling in August 2016

Vegetation map before restoration works (based on the site botanical inventories).



Vegetation type
🟩 Wood composed of young willows and poplars
🟦 Hydrophilic vegetation
🟨 Ruderal vegetation less than 75% vegetation cover
⬜ Ruderal vegetation less than 25% vegetation cover
⬛ Artificialised area

➡ List of the total site species pool



TOP LAYER SEED BANK (SB)

MEDIUM & BOTTOM LAYER SB

- Bibliographical analysis of species biological traits
  - Dissemination type and period ⟶ Ellenberg database (Julve 1998)
  - Fructification periode = Estimated minimum flowering period +2months

Estimating flowering period of each species in : Issler et *al.* 1982 + Lambinon et *al.* 1999 + Tela botanica website

  - Seed longevity in soil estimated by seed bank type Thompson et al. 1997

Fructification period

Seed bank type

Fructification before SB sampling

Fructification during SB sampling

Fructification SB sampling

PERSISTENT

TRANSIENT

Viable seed present in top layer seed bank when sampled

Seed presence and viability estimated by the seedling emergence method

Seed bank composition of the 3 soil layers

Viable seed absent in top layer seed bank when sampled

➡ Species presence underestimated in emergence seedling method

Bibliographic research on dispersion mode, seed production yield and viability. Estimation of presence and abundance in the seed bank.

Projection of seeds presence in top layer

References :

Thompson K., Bakker J., Bekker R.,(1997) The soil seed banks of North West Europe: methodology, density and longevity. Cambridge University Press.

Lambinon J., De Langhe J.E., Delvosalle L., Duvigneaud J. (1999) Nouvelle Flore de Belgique, du Grand-Duché de Luxembourg, du Nord de la France et des Régions voisines. Edition du Jardin botanique national de Belgique

Issler E., Loyson E., Waler E. (1982) Flore d'Alsace. Société d'étude de la flore d'alsace.

Tela botanica access : http://www.tela-botanica.org/site:accueil

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Julve, Ph., 1998 ff. Baseflor. Index botanique, écologique et chorologique de la Flore de France. Version [2016]. http://perso.wanadoo.fr/philippe.julve/catminat.htm

Nasstec international conference 25-29 september 2017
Jodrell laboratory lecture theatre, Royal botanic Gardens Kew
Richmond, Surrey, UK

Calico crayfish (*Faxonius immunis*) a new invasive species in France: From biological traits to preventive measures

FRANCOIS Marie[1], GRAC Corinne[2] & COMBROUX Isabelle*[3]

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Calico crayfish is coming…

Non-indigenous crayfish species are now more numerous than indigenous ones in Europe. Escaped from aquaria in southern Germany in the late 1990's, Calico Crayfish (*Faxonius immunis* ex - *Orconectes immunis*) (Fig. 1) is actually spreading in the North Eastern part of France through the Upper Rhine Valley. Once in a waterbody, it can quickly develop a massive population as it can mature within its first summer. The ecosystem, then rapidly turn into a "milky coffee" water system, leading to the vegetation disappearance. As *F. immunis* as an omnivorous regime (Fig. 2), its invasion lead to strong reduction of amphibian and macroinvertebrate communities, as its. Calico crayfish also digs deep burrows, which allow it to occupy shallow and temporary water bodies and colonize a wide range of alluvial ecosystems.

Figure 1 *: Faxonius immunis ex - Orconectes immunis*

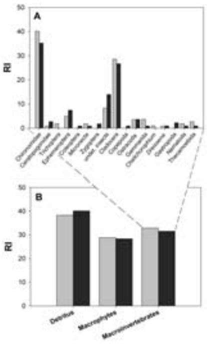


Figure 2 : *Relative importance (RI) of prey items (A; top) and food categories (B; bottom) in the diet of O. immunis. Crayfish shorter than 27 mm CL (juveniles) are depicted in light grey, crayfish longer than 27 mm CL (adults) are shown in dark grey.*
From C. Chucholl et al. KMAE 2012

… in a protected area…

In the Upper Rhine Valley, F. Immunis entered the Woerr protected area (Fig. 3) in the early 2010's …

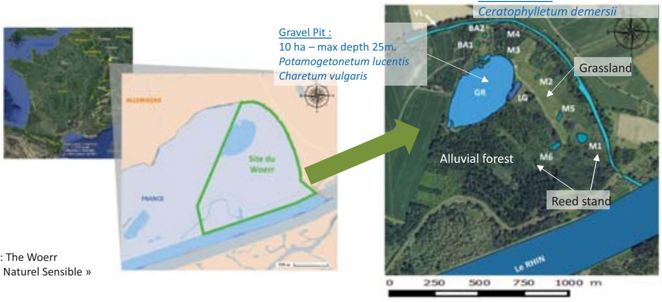


Figure 3 : The Woerr
« Espace Naturel Sensible »

⇒ « milky coffee » water in the restored ponds
↘ macroinvertebrates and macrophytes

… What can we do ?

In summer 2019, we studied some biological traits of this *F. immunis* population to propose some preventive / curative measures

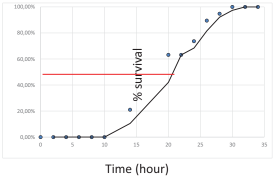
Biological traits

Dispersal

Walking speed measurements

Faster on bare ground

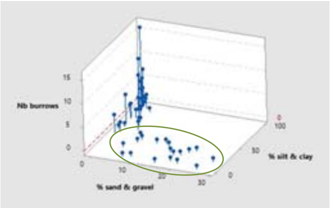
Desiccation survival estimation



50 % population can survive outside water more than 20 h. at ca. 26°C (= more than 1 km. Walking)

Burrows and sediment types

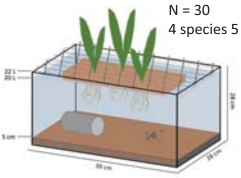
Sediment sampling & Number of burrows /m2 estimated on shorelines



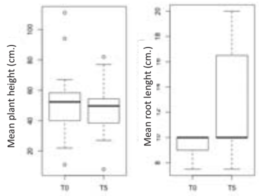
Floating vegetation consumption

Does crayfish eat plants (or roots) growing on floating beds ?

Mesocosm experiment in greenhouse



N = 30
4 species 5 weeks



Proposed measures

Limit dispersion ?

Every ponds and waterbodys are potentially connected

⇒ Dispersal limited by tall grasses and forbs

⇒ Practice late and high mowing, where in accordance by management plan

Limit burrows digging?

Favor gravel and sandy shoreline

=> Civil engineering works where possible

introduce floating beds ?

No impact on vegetation growing on floating beds

=> re-introduce vegetation in ponds through floating beds installation

… More ?

Université de Strasbourg
Faculté des sciences de la vie Université de Strasbourg
CONSEIL DÉPARTEMENTAL BAS-RHIN
Image Ville Environnement
CNRS
ENGEES

☙ Autres publications

Combroux I., Vandijk, P. & Rozan A. (2013). Evaluation du risque invasif lié à l'utilisation de bandes végétales comme technique d'interception des coulées boueuses. 'Des ingénieries par et pour le vivant, écologiques et agro-écologiques'. Séminaire d'animation scientifique du 19 décembre 2013- INRA, CIRAD, CNRS, et IRSTEA.

Guittony-Philippe, A, Masotti, V., **Combroux, I.** Malleret, L., Boudenne J.-L., Petit, M.-E. Monnier, Y., Coulomb, B.., Viglione, J. & Laffont-Schwob, I. (2015). Proposal of a new method to assess industrial effluent ecotoxicity reduction by wastewater treatment systems: the Helophyte Development Index. Ecological Eng 77 : 180-188.

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Perrier A., Hardion L., Rozan, A., Staentzel, C. & **Combroux I**. (in press - 2019). *Miscanthus x giganteus* crop fields hide a genotype of the invasive *M. sacchariflorus*; Weed Research.

Laboratoire GESTE
Gestion Territoriale de l'Eau et de l'Environnement

EVALUATION DU RISQUE INVASIF LIÉ À L'UTILISATION DE BANDES VÉGÉTALES COMME TECHNIQUE D'INTERCEPTION DES COULÉES BOUEUSES

PROJET GERIHCO 3 – 2014 - 2017

Isabelle Combroux[1], Paul Vandijk[2], Anne Rozan[3]

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2 : ARAA - Association pour la Relance Agronomique en Alsace. 3 : GESTE UMR IRSTEA-ENGEES - Gestion Territoriale de l'Eau et de l'Environnement

Le Contexte

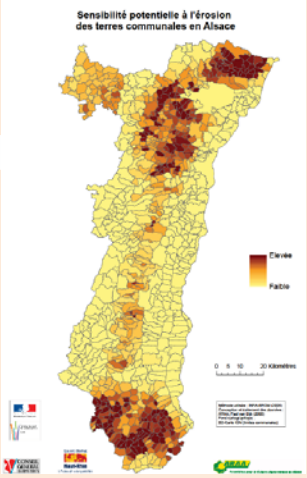

Figure 1 : Sensibilité à l'érosion des terres agricoles en Alsace

- Orages violents
- Sols (limoneux, faible stabilité)
- Cultures peu couvrantes (maïs)

Coulées d'eaux boueuses


DNA / Strasbourg et sa région / Temps forts

Eckwersheim / Après le violent orage de la nuit de jeudi à vendredi

La boue des champs dans les rues

… et dans les villages entourés de terres arables sur collines (notamment les collines limoneuses)

… une solution envisagée - des bandes végétales :

Le Projet GERIHCO 3

Pérennisation des comportements préventifs, étude globale du génie végétal et analyse paysagère

Axe 1 : Agir en amont : changer les comportements et les pérenniser

Sociologie
Economie
Droit

Axe 2 Analyse multicritère des dispositifs de génie végétal pour l'interception des sédiments

Site expérimental Schwindratsheim → Structures existantes

Efficacité
Biodiversité
Risque invasif

Axe 3 – Analyse paysagère des systèmes de haies

Un dispositif expérimental :

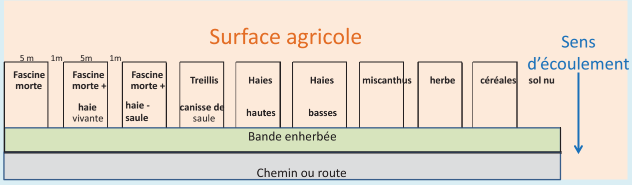

Figure 3 : Dispositif (provisoire) de la vitrine des fascines et des haies (Chambre d'agriculture Alsace).

Figure 4 : Pièges à ailettes. Dispersion anémochore

Evaluation du risque invasif

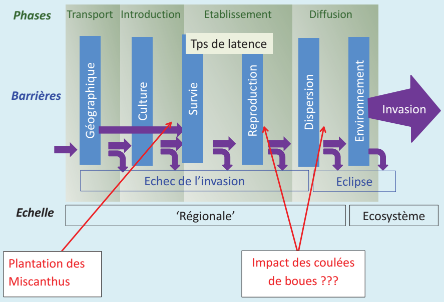

Figure 2: Adaptation du modèle unifié des processus d'invasions biologiques dans le cas du *Miscanthus* et des coulées de boues (* : Blackburn *et al.* 2011 – *TREE* 26(7) 333-339)

Différentes méthodes :

Protocoles	Résultats attendus
Placettes de suivi sur la zone expérimentales	- Surveillance : *Miscanthus* ou d'autres espèces invasives en « aval » de chacun des dispositifs testés. - Résistance biotique des communautés face à une invasion biologique.
Graines	- Validation de la stérilité des souches de Miscanthus utilisées
Piégeage de graines à dispersion anémochore sur la zone expérimentale	- Impact des dispositifs (dont les bandes de Miscanthus) sur la dispersion de graines anémochores et notamment sur la dispersion d'autres espèces invasives.
Fragments de rhizomes de Miscanthus produits sur la zone expérimentale	- Estimation du potentiel de fragmentation et de dispersion des rhizomes de Miscanthus par des coulées de boue. - Estimation de la viabilité de ces fragments de rhizomes. - - Estimation de la taille minimale d'un fragment de rhizome viable
Inventaire des Miscanthus proches de plantations existantes	- Estimation du risque invasif. - Identifications de paramètres favorisant le risque invasif.
Test des effets allélopathiques de Miscanthus	- Mise en évidence de phénomènes d'allélopathie chez Miscanthus

Des ingénieries par et pour le vivant, écologiques et agro-écologiques

Séminaire d'animation scientifique du 19 décembre 2013 organisé conjointement par l'INRA, le CIRAD, le CNRS, et IRSTEA

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Proposal of a new ecotoxicity evaluation tool based on morphological responses of five helophytes to mixtures of pollutants: The Helophyte Development Index

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ABSTRACT

Industrial effluents discharged into the environment may have ecotoxic effects even if they come up to regulatory standards. Chemical evaluation of treatment performance by end-of-pipe treatment systems is thus not sufficient, especially when mixtures of metallic and organic contaminants are concerned. Given that contamination may alter biological characteristics of the environment, biomonitoring studies may provide information on integrated ecotoxical effects. However, there is a need for bioassays purpose-designed for direct use at industrial sites. Many biomonitoring tools already exist and have been proved to be efficient for evaluating the ecotoxicity of contaminated waters, but most of them require laboratory equipment. In this study, an experiment in microcosms under controlled conditions of pollution was carried out to assess the morphological responses of five helophytes exposed to mixtures of organic and/or metallic pollutants. The criteria of plant growth and development, i.e. aerial elongation and leaf senescence, that were the most relevant for reflecting the ecotoxicity of contaminant mixtures and that could be monitored on-site with a user-friendly method, were then selected. Focusing on these selected criteria, a new bioindicator tool, named the Helophyte Development Index (HDI), was created. Our results suggest that the HDI is a promising tool to use on-site for assessing the ecological state of waters released in aquatic environment by industrial factories, following the recommendations of the European Water Agency.

1. Introduction

Member states of the European Union have to achieve good chemical and ecological status of water bodies by 2015 (European Council, 2000). To this purpose, chemical regulation levels are defined for industrial wastewaters released in the environment (European Union, 1976). Nevertheless, industrial wastewaters are characterized as complex mixtures with varying concentrations of pollutants (Soupilas et al., 2008) and given that interactions between contaminants frequently occur (Chen et al., 2004; Millward et al., 2004), the ecotoxicity of purified industrial wastewaters may not always be equal to the sum of the ecotoxicity of each contaminant. Effluents discharged into the environment may thus have ecotoxical effects even if each chemical is present at a level below regulatory standards (Charles et al., 2011). This stresses the importance of complementing the chemical approach with the ecotoxical one to better assess the quality of industrial wastewaters (Hoshina and Marin-Morales, 2009; Mendonça et al., 2009; Zhou et al., 2008) before their release in aquatic bodies. While effective off-site tools do exist to assess the chronic and acute toxicity of wastewaters (e.g. whole effluent toxicity test

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methods, US EPA, 2000), user-friendly bioassays that could be used directly on-site are still needed (Guittonny-Philippe et al., 2014; Jones et al., 2010; Libralato et al., 2010) for a more widespread use.

To this end, several organisms could be used (Sims et al., 2013), e.g. macroinvertebrates (Mondy et al., 2012), bryophytes (Bleuel et al., 2005), mussels (Monirith et al., 2003), daphnia (Martins et al., 2007), lichens (Monnet et al., 2005), microalgae (Araújo and Souza-Santos, 2013), fishes (Yeom et al., 2007), bacteria (Soupilas et al., 2008) or aquatic plants (Bonanno, 2012; Lewis, 1995; Haury et al., 2006; Trémolières et al., 2007). Among these organisms, macrophytes integrate temporal, spatial, chemical, physical, and biological qualities of their ecosystem (Lacoul and Freedman, 2006; Rambaud et al., 2009) and simple measurements based on morphological observations may indicate harmful effects of exposure to contaminants (Zhou et al., 2008). Aquatic plant species exhibit multifaceted responses to industrial pollutant mixtures, that are dependent on the species exposed (Deng et al., 2006; Kearney and Zhu, 2012; Zhang et al., 2010) and on the characteristics of pollutants, including their concentrations, their chemical types and the potential interaction between them (Babu et al., 2001; Lin et al., 2008; Zhang et al., 2011). For these reasons, macrophytes could be appropriate bioindicators in industrial context, for an *in situ* use.

The aim of our study was to assess five helophytes' morphological responses towards mixtures of pollutants mimetic of industrial effluents (in a full factorial design) and to create a user-friendly index based on these responses. In this article, we report a new methodology – named the Helophyte Development Index (HDI) – which could have potential further applications for ecotoxicity assessment in European industrialised catchments.

2. Material and methods

2.1. Planted microcosm set up

Plantlets from five helophyte species commonly found in European water bodies and exhibiting different biological traits, i.e. *Alisma lanceolatum* With. (Alismataceae), *Carex cuprina* (Sandor ex Heuff.) Nendtv. ex A. Kern. (Cyperaceae), *Epilobium hirsutum* L. (Onagraceae), *Iris pseudacorus* L. (Iridaceae) and *Juncus inflexus* L. (Juncaceae), were collected from a polluted wetland (South of the Berre lagoon, South-East France; WGS 84 GPS coordinates: longitude: E 6,426519; latitude: N 43,359009, Guittonny-Philippe et al., 2015a) and maintained in the greenhouse for 4 months of vegetative reproduction before experiment. The experiment has been designed in order to distinguish metallic and organic pollutant effects on plants and the possible interactions between both types of contaminants in a full factorial design, as recommended by Lewis et al., 1999. Twenty microcosms consisting of rectangular plastic tanks (413 × 345 × 294 mm, length × width × depth) filled up with pozzolan were implemented, as previously described (Guittonny-Philippe et al., 2015a). The microcosms were planted with six plant individuals per species X condition. For each species, one microcosm was kept without contamination and served as control and three other microcosms were independently exposed to three different pollutant mixtures.

2.2. Chemicals and exposure phases

Three types of pollutant mixtures mimetic to industrial effluents were added in the microcosms, as previously described (Guittonny-Philippe et al., 2015a):

- A metallic pollutant mixture (MPM) consisting of an aqueous mixture of eleven metallic salts, i.e. $AlCl_3{\cdot}2H_2O$; AsO_3; $CdSO_4{\cdot}8H_2O$; $K_2Cr_2O_7$; $CuSO_4$; $Fe_2O_{12}S_3$; $MnSO_4{\cdot}4H_2O$; $NiSO_4{\cdot}7H_2O$; $Pb(NO_3)_2$; $SnCl_2$; $ZnCl_2$.
- An organic pollutant mixture (OPM) composed of total hydrocarbons (THC) (i.e. Blend Arabian Light petroleum topped at 250 °C (BAL 250)), as well as phenanthrene (PHE) and pyrene (PYR) obtained in reagent quality from Merck (Germany) and an anionic detergent linear alkylbenzene sulfonate (LAS) named CARPHEM®.
- An organic and metallic pollutant mixture (OMPM) containing both types of contaminants at concentration levels identical to the ones used for the MPM and the OPM.

Two main criteria were considered for the choice of contaminants: ubiquity of selected chemicals in industrial context (Haritash and Kaushik, 2009; Megharaj et al., 2011; Wasi et al., 2013) and their potential ecotoxicity in mixtures (Banat et al., 1974; Hernández-Soriano et al., 2011; Thavamani et al., 2012; Radić et al., 2010; Zhang et al., 2011).

After 40 days of plant acclimatisation in microcosms (Guittonny-Philippe et al., 2015a), exposure to the artificial effluents was conducted in the microcosms for 113 days in three successive pollution events. In the first phase, pollutant concentration levels in the artificial effluents were set at the European environmental quality standards (Table 1, European Union, 1976). In the second and third phases (that began on days 35 and 70, respectively), pollutant concentrations in the artificial effluents were ten times higher than the European environmental quality standards, except for the anionic detergent LAS whose concentration was equal for the three pollution phases. In all the microcosms, 25 mL of an organo-mineral fertilizer (NutriActiv®, NF U 42-001 produced by FLORENDI JARDIN SAS) containing 3% of total nitrogen, 3% of total P_2O_5 and 3% of water-soluble K_2O was also added at the beginning of the first test-phase.

2.3. Physico-chemical and plant parameter monitoring

In each microcosm, pH was monitored with a portable pH meter (Hanna Instruments®). Electrical conductivity (EC, µS/cm), dissolved oxygen level (DO, percentage of saturation % 0_2/L), and temperature (*T*, °C) were monitored with a WTW® device. These measurements were repeated at least once per month. Aerial height (height of the longest leaf or shoot) and number of leaves (green, senescent or dead) of each plant individual were monitored at least every two weeks during the experiment. A leaf was considered senescent when at least one third of its surface was yellow or brown (Holopainen et al., 2010). Heights were measured

Table 1
Metal and organic pollutant concentrations that industrials are authorised to release in aquatic bodies (European Union, 1976).

Chemicals	European environmental quality standard (authorized concentrations in released effluents) (mg/L)
Al	2.5
As	0.05
Cd	0.2
Cr	0.5
Cu	0.5
Fe	2.5
Mn	1
Ni	0.5
Pb	0.5
Sn	2
Zn	2
PHE	0.05
PYR	0.05
THC	10
Anionic detergent LAS	10

from the level of the pozzolan surface. The biomonitoring data were recorded in 13 times of measurements along the experiment.

2.4. Chemical analysis

Before enriching microcosms with the artificial effluents, three water aliquots were taken in the pollutant mixtures in order to determine the real value of initial concentrations of pollutants added in the microcosms (Guittonny-Philippe et al., 2015a). At the end of each test-phase, water samples were collected in the contaminated microcosms to determine the residual concentrations of pollutants. Rhizospheric pozzolan (pozzolan in contact with plant roots) samples were taken at the end of the third test-phase in each microcosm in order to analyse metals (in all microcosms) and organic pollutants (Guittonny-Philippe et al., 2015a). At this time, five plant individuals per microcosm were harvested for metal analysis in plant biomass (Guittonny-Philippe et al., 2015b).

Samples were analysed for Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn content by inductively coupled plasma-atomic emission spectrometry (ICP-AES; Sn was not quantified because of analytical constraints linked to the presence of spectroscopic interferences with other elements and to the low sensitivity obtained with the available apparatus), as previously described (Guittonny-Philippe et al., 2015a).

The concentration of anionic detergent LAS was monitored by MBAS analysis according to EPA 425.1 method (Clesceri et al., 1998).

Briefly, for the analysis of phenanthrene (PHE), pyrene (PYR) and total hydrocarbons (THC), water samples were extracted by liquid–liquid extraction with *n*-hexane. Sample extracts were reduced to 1 mL and analysed using a gas chromatograph (7890A GC System, Agilent Technologies, USA) coupled to a 7000 Triple Quad mass spectrometer, equipped with an HP-5MS silica fused capillary column (30 m × 0.25 mm inner diameter × 0.25 μm film thickness). The quantification of PHE and PYR was performed by using chrysene-D12 as surrogate and phenanthrene-D10 as internal standard. The amount of THC was determined as the sum of resolved and unresolved components eluted from the GC capillary column between the retention times of *n*-decane and *n*-tetracontane. The specific conditions used for extractions together with the chromatographic and mass spectrometry parameters have been previously detailed (Guittonny-Philippe et al., 2015a).

2.5. Statistical analysis

For each species in control and OPM, MPM or OMPM conditions, growth traits (aerial height and proportion of green leaves) were analysed using an univariate analysis of repeated measures (rmANOVAs) since models were set up of independent orthogonal components (Von Ende, 2001). Two-tailed Student's *t*-tests (for comparison of two means with equal variances assessed by F-test) were performed to test significant differences between control and MPM, OPM or OMPM plants in growth traits, at each date of plant measurement. In case of unequal variances, two-tailed *t*-test with Welch's correction was carried out.

Table 2
Contaminant concentrations in water of control microcosms at the end of the experiment (mean of microcosms, $n = 5$). n.m.: not measured.

Chemicals	Concentrations in control microcosms (mg/L)
Al	0.24 ± 0.20
As	<0.03
Cd	<0.01
Cr	0.025 ± 0.001
Cu	0.02 ± 0.01
Fe	0.19 ± 0.29
Mn	0.15 ± 0.29
Ni	0.03 ± 0.03
Pb	<0.04
Sn	n.m.
Zn	0.04 ± 0.03
PHE	$0.27 \times 10^{-3} \pm 0.03 \times 10^{-3}$
PYR	$0.10 \times 10^{-3} \pm 0.003 \times 10^{-3}$
THC	0.23 ± 0.03
Anionic detergent LAS	n.m.

Data were analysed statistically using GraphPad Prism version 6.00 for Windows, GraphPad Software.

3. Results and discussion

3.1. Fate of pollutants in microcosms during the test-phases

In every microcosm and for all the contaminants, mean aqueous concentrations in water at the end of each test-phase (Tables 2–5) were below the water regulatory limits that industrial factories are authorized to release in natural environments (Table 1, European Union, 1976), except for Mn in OMPM microcosms that was slightly over 1 mg/L at the end of phases 2 and 3 (Table 3). In MPM microcosms, mean removals of metals varied from 52% for Mn to 98% for Cu in the first test-phase, while mean removals of metals were all over 88% in the second and third test-phases. In OPM microcosms, mean removals of organic pollutants were comprised between 64% for THC to 99% for PHE in the first test-phase, and were all over 87% in the second and third test-phases. In OMPM

Table 3
Contaminant concentrations in water of OMPM microcosms at the end of the test-phases (mean of microcosms, $n = 5$). n.m.: not measured.

Chemicals	Organic pollutant and metal concentrations (mg/L) in water of OMPM microcosms		
	Phase 1	Phase 2	Phase 3
Al	0.33 ± 0.34	0.34 ± 0.21	0.25 ± 0.27
As	<0.03	<0.03	<0.03
Cd	<0.01	0.017 ± 0.017	0.005 ± 0.02
Cr	0.013 ± 0.03	0.021 ± 0.007	0.018 ± 0.004
Cu	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
Fe	0.28 ± 0.22	0.54 ± 0.32	0.17 ± 0.07
Mn	0.59 ± 0.23	1.70 ± 0.38	1.21 ± 1.11
Ni	0.02 ± 0.01	0.08 ± 0.05	0.28 ± 0.20
Pb	<0.04	<0.04	<0.04
Sn	n.m.	n.m.	n.m.
Zn	0.06 ± 0.04	0.23 ± 0.08	0.17 ± 0.11
PHE	$0.28 \times 10^{-3} \pm 0.06 \times 10^{-3}$	$3.53 \times 10^{-3} \pm 1.61 \times 10^{-3}$	$0.85 \times 10^{-3} \pm 0.24 \times 10^{-3}$
PYR	$1.66 \times 10^{-3} \pm 0.15 \times 10^{-3}$	$2.55 \times 10^{-3} \pm 0.45 \times 10^{-3}$	$0.76 \times 10^{-3} \pm 0.11 \times 10^{-3}$
THC	3.42 ± 2.76	0.28 ± 0.07	0.79 ± 0.30
Anionic detergent LAS	0.44 ± 0.14	0.61 ± 0.14	0.22 ± 0.06

Table 4
Organic pollutant concentrations in water of OPM microcosms at the end of the test-phases (mean of microcosms, $n = 5$).

Chemicals	Organic pollutant concentrations (mg/L) in water of OPM microcosms		
	Phase 1	Phase 2	Phase 3
PHE	$0.29 \times 10^{-3} \pm 0.03 \times 10^{-3}$	$3.53 \times 10^{-3} \pm 2.12 \times 10^{-3}$	$0.68 \times 10^{-3} \pm 0.23 \times 10^{-3}$
PYR	$1.64 \times 10^{-3} \pm 0.09 \times 10^{-3}$	$3.62 \times 10^{-3} \pm 1.05 \times 10^{-3}$	$1.18 \times 10^{-3} \pm 0.58 \times 10^{-3}$
THC	2.53 ± 1.83	0.73 ± 0.45	0.55 ± 0.29
Anionic detergent LAS	0.44 ± 0.11	0.59 ± 0.19	0.24 ± 0.05

microcosms, mean removals of metals were comprised between 53% for Mn to 98% for Cu, and mean removals of organic pollutants were between 52% for THC and 96% for PYR in the first test-phase. In the second and third test-phases, mean removals of metals were all over 85%, and mean removals of organic pollutants were all over 87%.

The strong decrease of aqueous metal concentrations is attributable to a set of geochemical reactions (e.g. metal precipitation as oxides, sulfides or carbonates, co-precipitation, complexation) depending on physico-chemical conditions, even if for some metals like Al, Fe or Mn, sorption in plants also significantly contributed in metal removal (Guittonny-Philippe et al., 2015b). The strong decrease of aqueous organic pollutant concentrations may have been caused by adsorption in plants (Simonich and Hites, 1995), biodegradation by plants and/or rhizospheric microorganisms (Atlas, 1981; Cerniglia, 1993; Gramss et al., 1999; Imfeld et al., 2009; Thoumelin, 1995), or adsorption in plastic tank sides or pozzolan (Dordio and Carvalho, 2013; Temmink and Klapwijk, 2004).

3.2. Morphological responses of helophytes exposed to the pollutant mixtures during the test-phases

The OMPM and the OPM limited the aerial elongation of two out of the five species, i.e. *I. pseudacorus*, *J. inflexus* (Figs. 1A and 2A) from the second test-phase. The OMPM and the MPM provoked an acceleration of the leaf senescence in three out of the five helophytes, i.e. *A. lanceolatum*, *E. hirsutum*, *J. inflexus* (Figs. 1B and 2B), from the second test-phase. These results are consistent with previous studies concerning impacts of organic pollutants (Adieze et al., 2012; Alkio et al., 2005; Chaîneau et al., 1997; Liu et al., 2004; Ma et al., 2010; Yu et al., 2006) and metals (Briat and Lebrun, 1999; Kabata-Pendias, 2011; Rascio and Navarri-Izzo, 2011) on plant growth and development. Consequently, for revealing the global health status of plants exposed simultaneously to metals and organic pollutants, we created the species development values "sdv" calculated on the basis of two morphological criteria: green leaves' proportion and relative size of plants.

Table 5
Metal concentrations in water of MPM microcosms at the end of the test-phases (mean of microcosms, $n = 5$). n.m.: not measured.

Chemicals	Metal concentrations (mg/L) in water of MPM microcosms		
	Phase 1	Phase 2	Phase 3
Al	0.55 ± 0.49	1.71 ± 2.66	2 ± 0.71
As	<0.03	<0.03	<0.03
Cd	<0.01	0.018 ± 0.02	0.10 ± 0.03
Cr	<0.02	0.024 ± 0.018	0.07 ± 0.05
Cu	0.01 ± 0.01	0.04 ± 0.04	0.11 ± 0.03
Fe	0.46 ± 0.26	0.66 ± 0.36	0.57 ± 0.12
Mn	0.61 ± 0.04	0.39 ± 0.29	0.09 ± 0.07
Ni	0.03 ± 0.00	0.27 ± 0.16	0.43 ± 0.16
Pb	<0.04	0.05 ± 0.02	0.05 ± 0.01
Sn	n.m.	n.m.	n.m.
Zn	0.09 ± 0.03	0.43 ± 0.35	0.93 ± 0.25

3.3. Development of the ecotoxical index on the basis of plant morphological responses

3.3.1. Calculation of the species development values "sdv"

Green leaves proportion of the plant individuals (Glp_i) is calculated as the number of green leaves of the plant individual (gl_i) divided by the total number of leaves (including senescing and dead leaves still attached to the stem) of the same plant individual (tl_i): $\mathrm{Glp}_i = \mathrm{gl}_i/\mathrm{tl}_i$. On the basis of the results previously described, the Glp_i should traduce the alteration of plant development linked with the ecotoxicity of metals.

Relative size of the plant individuals (Rs_i) is calculated as the height of the plant individual (h_i; corresponding to the height of the longest shoot for *E. hirsutum* and of the longest leaf for the four other species) divided by the height of the plant individual of reference at the corresponding time of measurement (hr_i; corresponding to the height of the highest individual among control individuals at the time of measurement): $\mathrm{Rs}_i = h_i/\mathrm{hr}_i$. On the basis of the results previously described, the Rs_i should traduce the alteration of plant growth linked with the ecotoxicity of organic pollutants.

The species development values of each individual (sdv_i) are then calculated at each time of measurement, as follow: $\mathrm{sdv}_i = (\mathrm{Glp}_i + \mathrm{Rs}_i) \times 10$.

The multiplication by a factor 10 enables obtaining scores of sdv_i on a scale of 20.

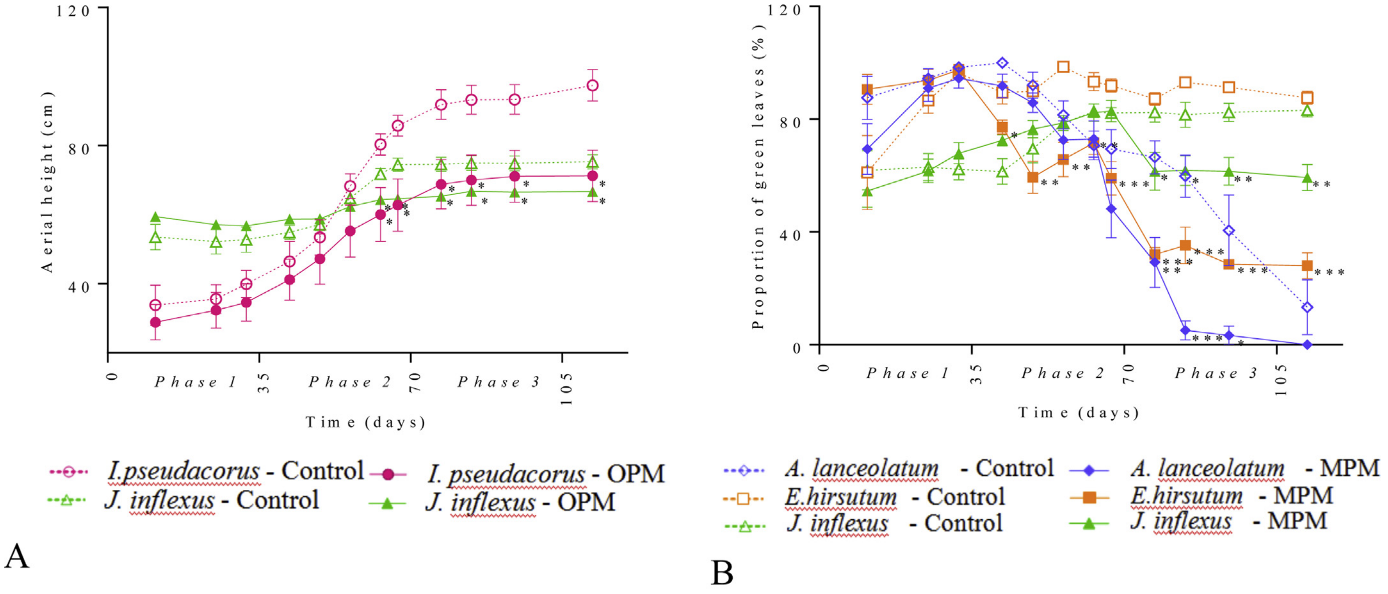
Then, the mean of sdv_i for each "species × condition" is calculated, to obtain the species development values (sdv) at each time of measurement which is expected to reflect the ecotoxicity of both metals and organic pollutants on the considered species.

3.3.2. Calculation of the HDI

In order to reveal ecotoxicity of pollutant mixtures, we created an index based on global responsive aerial traits and called it "Helophyte Development Index (HDI)". The HDI is calculated at each time of biometric measurement, by summing, for the n species considered ($n = 5$ in this study), the differences of sdv between control and contaminated conditions, when significant (non-parametric Mann–Whitney U-tests; P-value < 0.05):

$$\mathrm{HDI} = \sum\nolimits_{n} \quad {}_{\text{species}} \left[\mathrm{sdv(control)} - \mathrm{sdv(contaminated)}\right]^{*}.$$

The theoretical maximum ecotoxical value of the HDI calculated with five species is +100 and is reached when all the plants in contaminated environment are dead while all the plants in control environment both reached the maximum height and do not have any dry or senescent leaves. In order to reveal the ecotoxicity of the OMPM, we calculated the HDI on the basis of the five species used in this study (Table 6).



	Proportion of green leaves						Aerial height			
	E. hirsutum		***A. lanceolatum***		***J. inflexus* (control vs MPM)**		***I. pseudacorus***		***J. inflexus* (control vs OPM)**	
Source of Variation	**F**	**P value**	**F**	**P value**	**F**	**P value**	**F**	**P value**	**F**	**P value**
Time X Treatment	20,57	< 0,0001	4,908	< 0,0001	9,185	< 0,0001	6,095	< 0,0001	4,48	< 0,0001
Time	15,2	< 0,0001	62,1	< 0,0001	13,51	< 0,0001	123,9	< 0,0001	21,47	< 0,0001
Treatment	224	< 0,0001	11,98	0,0072	1,76	0,2142	4,022	0,0727	0,7071	0,4201
Subjects (matching)	0,9445	0,4959	5,105	< 0,0001	13,87	< 0,0001	47,25	< 0,0001	14,74	< 0,0001

C

Fig. 1. (A) Impact of the OPM on aerial elongation of *I. pseudacorus* and *J. inflexus* (means ± SEM); (B) impact of the MPM on leaf senescence of *A. lanceolatum*, *C. cuprina*, and *E. hirsutum* (means ± SEM); and (C) results of the repeated measures ANOVA performed on the proportion of green leaves of *E. hirsutum*, *A. lanceolatum* and *J. inflexus* and on the plant aerial height of *I. pseudacorus* and *J. inflexus*. Asterisks associated with values at a given time indicate a significant difference (***$p \leq 0.001$; **$p \leq 0.01$; *$p \leq 0.05$) between the control and the contaminated plants (two-tailed Student's *t*-tests).

3.4. Use of the sdv and HDI for revealing the ecotoxicity of the OMPM

3.4.1. sdv of the five helophytes in control and OMPM microcosms

The sdv of *A. lanceolatum* in control and OMPM conditions became significantly different (Mann–Whitney *U*-tests, *P*-value ≤ 0.05) immediately after the second addition of the pollutant mixture (Fig. 3A). For this species, significant differences of sdv were observed on 6 out of 9 measurement times performed during the two last test-phases (Table 4).

No significant difference of sdv could be observed between control and OMPM conditions for *C. cuprina* on the 13 measurement times performed throughout the experiment, and sdv stayed quite constant, at a level over 15/20 (Fig. 3B).

The sdv of *E. hirsutum* in OMPM condition became significantly lower compared to the sdv in control condition, from the middle of the second test-phase, until the end of the experiment (Fig. 3C).

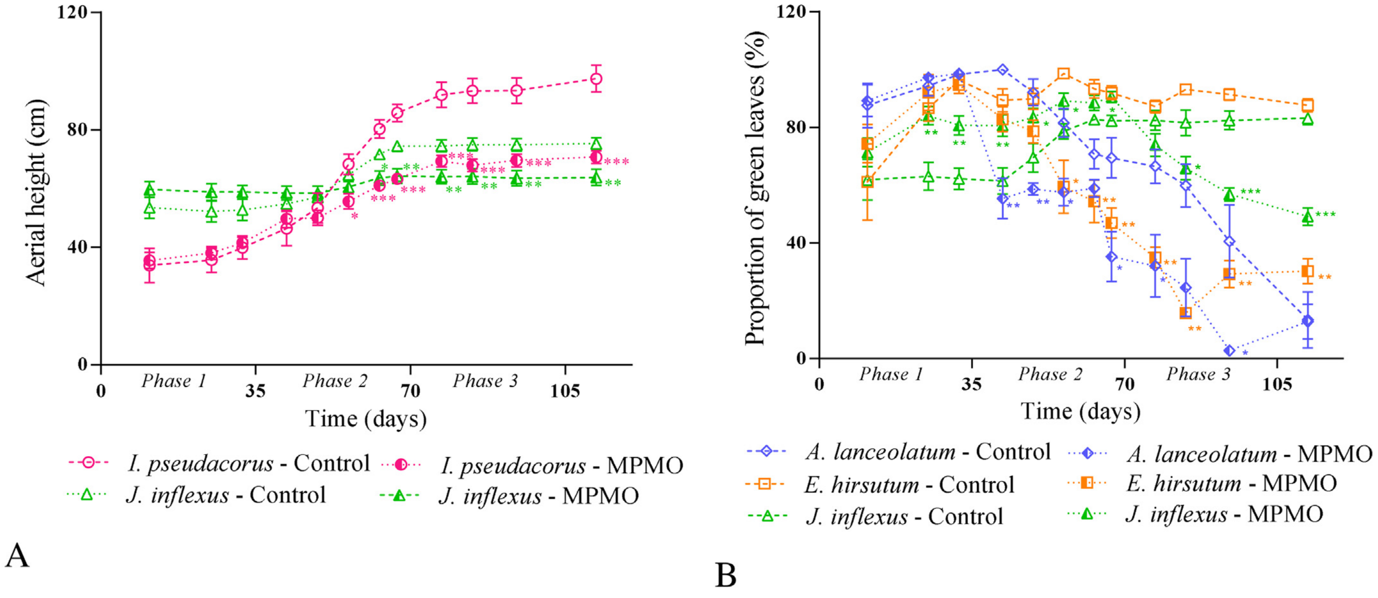
In control and OMPM conditions, *I. pseudacorus* had similar sdv from the beginning of the first test phase until the end of the second test-phase (Fig. 3D). Then sdv in control condition became significantly higher than that in OMPM condition (Table 4).

For *J. inflexus*, during the end of the first test-phase and the beginning of the second, sdv in the OMPM condition was significantly higher than that in the control condition (Fig. 3E). After the third addition of artificial industrial effluent, *J. inflexus* in the OMPM condition had a significantly lower sdv than in the control condition, until the end of the experiment.

Significant sdv differences between the control and the OMPM conditions were always positive for *E. hirsutum*, *A. lanceolatum*, *I. pseudacorus* and *J. inflexus* after the 30th April, during the second and third test-phases. This means that the OMPM altered the development of these helophytes compared to tap water.

3.4.2. Use of HDI as ecotoxical index of OMPM water

The HDI, calculated on the basis of the significant sdv differences of the five plant species used in this study, reached a positive value from the moment pollutant concentrations in the OMPM went over regulation levels (after the beginning of the second test-phase, day 35), and it rose remarkably following a linear trend between the second phase and the beginning of the third phase (Fig. 4). Consequently, the HDI variation range revealed the harmful effects on the helophytes exposed to the OMPM, with pollution over the regulation levels. At the end of experiment, the HDI reached a plateau *ca.* +20 points. On one hand, the plateau may be due to the fact that we did not renew the feeding of the



	Proportion of green leaves						Aerial height			
	E. hirsutum		*A. lanceolatum*		*J. inflexus*		*I. pseudacorus*		*J. inflexus*	
Source of Variation	**F**	**P value**	**F**	**P value**	**F**	**P value**	**F**	**P value**	**F**	**P value**
Time X Treatment	17.11	< 0.0001	4.967	< 0.0001	23.49	< 0.0001	12.14	< 0.0001	13.23	< 0.0001
Time	14.08	< 0.0001	46.14	< 0.0001	13.60	< 0.0001	112.2	< 0.0001	35.46	< 0.0001
Treatment	311.6	< 0.0001	20.33	0.0015	0.2574	0.6229	11.85	0.0063	1.612	0.2329
Subjects (matching)	0.7220	0.7022	3.950	0.0003	11.44	< 0.0001	11.96	< 0.0001	21.25	< 0.0001

C

Fig. 2. (A) Impact of the MPMO on aerial elongation of *I. pseudacorus* and *J. inflexus* (means ± SEM); (B) impact of the MPMO on leaf senescence of *A. lanceolatum*, *C. cuprina*, and *E. hirsutum* (means ± SEM); and (C) results of the repeated measures ANOVA performed on the proportion of green leaves of *E. hirsutum*, *A. lanceolatum* and *J. inflexus* and on the plant aerial height of *I. pseudacorus* and *J. inflexus*. Asterisks associated with values at a given time indicate a significant difference (***$p \leq 0.001$; **$p \leq 0.01$; *$p \leq 0.05$) between the control and the contaminated plants (two-tailed Student's *t*-tests or Mann–Whitney *U*-test).

Table 6
Differences of sdv between control and OMPM conditions for the 5 helophyte species and HDI. Values in bold correspond to significant difference between the sdv ($p \leq 0.05$). n.r.: not relevant.

Date of measurement	Phase	Differences of sdv (sdv control – sdv OMPM)					HDI
		A. lanceolatum	*C. cuprina*	*E. hirsutum*	*I. pseudacorus*	*J. inflexus*	5 Species
19/03/2012	1	−5.2	0	−0.1	−0.6	−0.3	n.r.
30/03/2012		−4.0	0.3	1.4	−0.9	−1.9	n.r.
13/04/2012		−1.7	0.3	2.3	0.3	**−3.2**	−3.2
20/04/2012		−0.3	0.9	2.4	−0.3	**−2.8**	−2.8
30/04/2012	2	**4.5**	1.1	0.9	−0.6	**−2.5**	2
07/05/2012		**3.2**	1.0	1.7	0.8	−1.6	3.2
14/05/2012		**2.2**	1.2	**4.7**	0.5	−0.4	6.9
21/05/2012		1.1	1.1	**5.0**	**2.8**	0.5	7.8
25/05/2012		**3.5**	1.0	**5.5**	**3.0**	0.4	12
04/06/2012	3	**5.1**	1.1	**6.7**	**3.0**	**2.2**	17
11/06/2012		4.8	1.2	**9.4**	**2.8**	**2.9**	15.1
21/06/2012		**3.5**	0.9	**7.4**	**5.6**	**3.9**	20.4
09/07/2012		1.5	0.7	**5.8**	**8.4**	**4.8**	19

microcosms with artificial effluent. On the other hand, summer high temperatures may have induced the senescence of control plants, thereby, hiding the differences between control and OMPM conditions. All these results demonstrated that the HDI, as described here, provided a means of revealing the ecotoxicity of the artificial industrial effluent containing both organic and metallic contaminants. For a more polluted effluent, we could expect a supplementary increase of the HDI, given that some of the

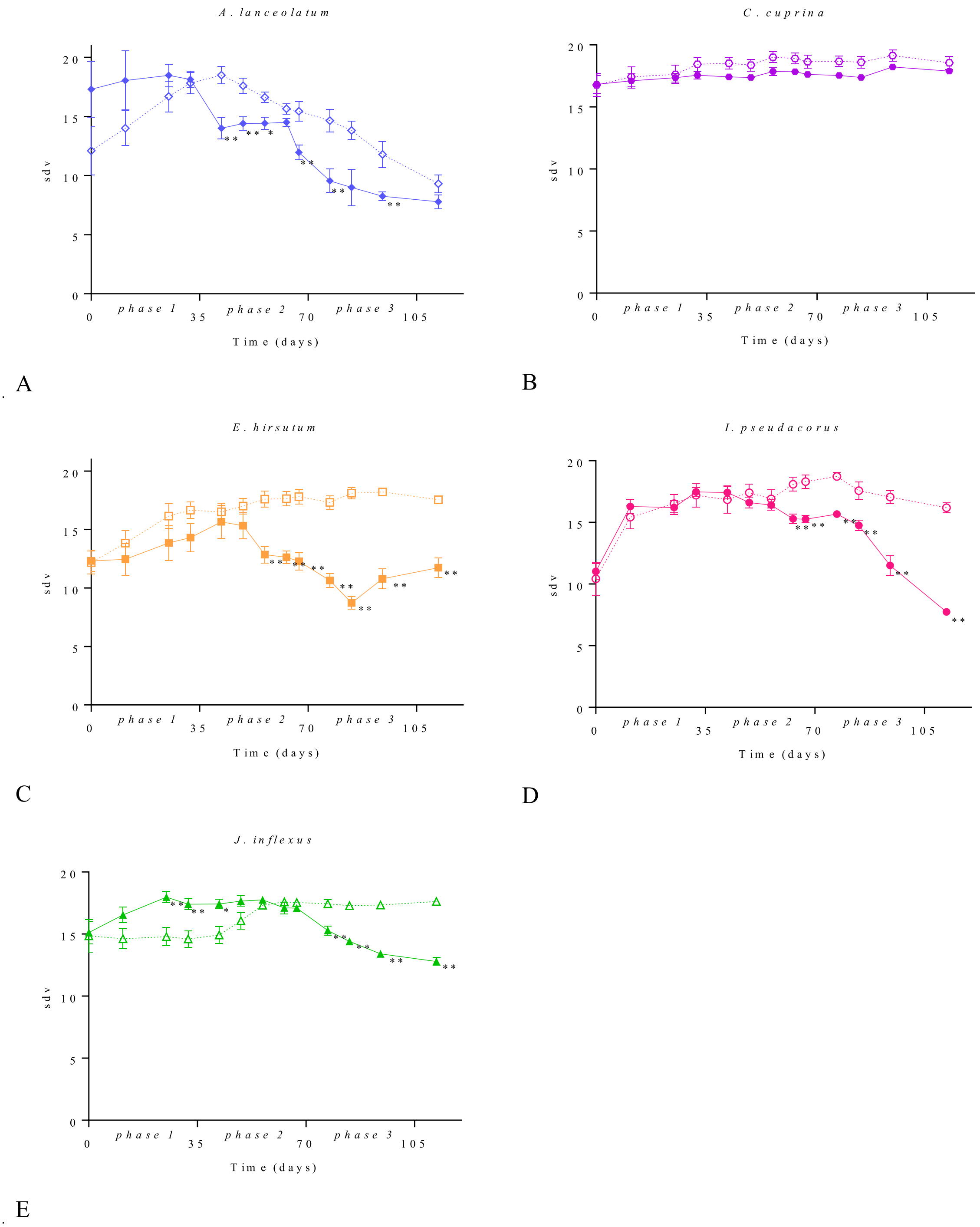


Fig. 3. sdv of control and OMPM conditions for (A) *A. lanceolatum*, (B) *C. cuprina*,(C) *E. hirsutum*, (D) *I. pseudacorus* and (E) *J. inflexus*. Control sdv are represented with empty symbols and dotted lines, OMPM sdv are represented with full symbols and continuous lines. Asterisks associated with values at a given time indicate a significant difference (**$p \leq 0.01$; *$p \leq 0.05$) between the sdv of control and OMPM conditions (Mann–Whitney *U*-tests).

used helophytes did not exhibit any change in their health status (no sdv differences between OMPM and control conditions) and none of the plants died in OMPM condition during the experiment.

3.4.3. Perspectives for using the HDI

Each species exhibits its own responses to a given contaminant, with various levels of sensitivity and reaction time. This highlights the benefit of using multiple species for assessing the ecological state of water (Bae and Park, 2014). The present study shows that the use of *A. lanceolatum*, *I. pseudacorus* and *E. hirsutum* species seems to be well suited for providing information on the ecotoxicity of industrial pollutant mixtures in ranges of concentrations up to 10 times higher than those authorised for industrial releases in aquatic environment. *J. inflexus* and *C. cuprina* species, that appeared to be more tolerant to pollutant mixtures, can also be used in the case of conditions of higher contamination.

Given that ecotoxicity of an industrial effluent may vary in time depending on the entrant effluent, performance of the treatment station or rainfall, it is appropriate to have at least five species in the HDI calculation to cover the various ranges of ecotoxicity. This

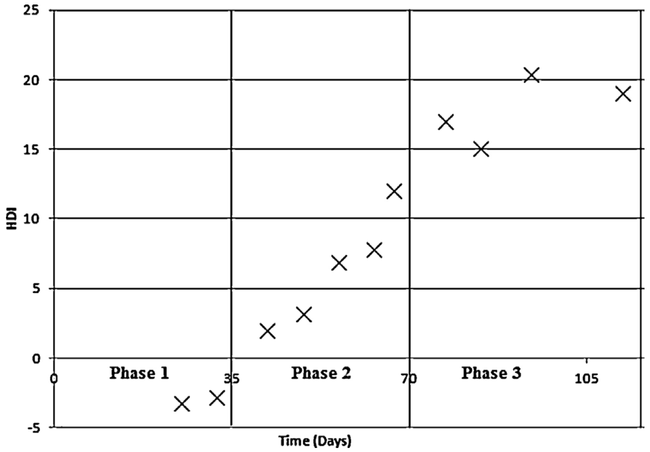


Fig. 4. Evolution of the HDI calculated on the basis of the 5 helophytes, during the three test-phases.

is one other benefit provided by this multi-specific bioindication tool in comparison with the majority of existing ecotoxical tests that are based on a single species (De Laender et al., 2009).

The HDI has been tested in microcosms with artificial effluents containing metals and organic pollutants and should now be tested on-site to estimate the ecotoxicity of several types of effluents (e.g. leachates from ultimate waste storage plants or effluents from recycling plants of used cars) in view of confirming its relevance and reliability.

4. Conclusion

We assessed the morphological responses of five helophytes of different biological types in order to create a new bioindicator tool, the Helophyte Development Index (HDI), which may provide relevant information on the ecotoxical potential of industrial wastewaters, following the recommendations of the European Water Agency. The HDI tool has the potential to be routinely used to check the ecotoxicity of industrial discharges, but it is first necessary to accumulate *in situ* data in order to validate this method under various real environmental and contamination conditions.

For the purposes of Water Framework Directive implementation, direct ecotoxicity assessment of wastewater treatment plant discharges is a way of attaining or maintaining ecological quality objectives in water masses. Calculation of the HDI is a promising tool to be used on-site for assessing the ecological state of waters released in aquatic environment by industrial factories and this new tool addresses strong expectations of engineering consulting firms for their environmental diagnoses. The HDI could be also well-suited to assess the ecotoxicity of other types of waters (e.g. containing biotoxins, pesticides). We advocate testing the HDI suitability in other contexts, taking care to adapt the choice and the number of species to the objectives.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecoleng.2015.01.022.

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Restoration Ecology
THE JOURNAL OF THE SOCIETY FOR ECOLOGICAL RESTORATION

OPINION ARTICLE

Promoting ecological restoration in France: issues and solutions

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Ecological restoration has developed greatly over recent decades. Promoting harmonious relationships between scientists and practitioners, between restoration ecology and ecological restoration, is essential to improving restoration projects. These relationships are difficult to achieve at a global scale, although international action remains essential. Therefore, regional and national networks are attempting to take up the challenge. With several European countries planning to create their own network in the coming years, insights from current practice are helpful. Here, we (1) describe the context in which ecological restoration is developing in France and (2) present the French restoration network, Réseau d'Echanges et de Valorisation en Ecologie de la Restauration (REVER). Most public policies related to restoration in France are derived from European Union (EU) directives, such as those on water, ecological networks, biodiversity, and protected species and natural habitat. Restoration can also be undertaken through Environmental Impact Assessment (EIA) or subsequent to damage. Following the model of the International Society for Ecological Restoration, the French network for ecological restoration (REVER) aims at accompanying and promoting restoration by facilitating relationships between the various stakeholders: practitioners, scientists, site managers, etc. To encourage exchange of knowledge and experience, REVER manages a website, organizes workshops, and provides links with SER-Europe and Society for Ecological Restoration International (SERI). This article provides information that will be of interest to other countries trying to meet the Aichi targets of the convention on biological diversity: the restoration of 15% of degraded ecosystems by 2020.

Key words: **applied science, compensate or offset impacts on biodiversity, horizontal communication, knowledge sharing, mitigation hierarchy**

Implications for Practice

- Implementing European Union (EU) directives linked with the environment has helped promote ecological restoration in France and should do so in other EU countries.
- National policies on environmental impact studies and environmental liability further promote ecological restoration sensu lato before and after damage.
- Agro-environmental schemes and local initiatives carried out by public institutions or site managers significantly contribute to the implementation of restoration.
- Annual workshops have been REVER's most valuable tool to improve communication between restoration stakeholders and to initiate collaboration and exchange.

Introduction

It has taken only a few decades for ecological restoration to become an essential part of the response to various environmental issues, such as habitat and biodiversity conservation, ecosystem service rehabilitation, or sustainable development of human societies (Roberts et al. 2009; Aronson & Alexander 2013). Improvements to restoration quality and technical feasibility are still needed, however, and substantial efforts will

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have to be made over the coming years to fund and implement large-scale ecological restoration (Aronson & Alexander 2013; Cortina-Segarra et al. 2016). Better communication between the various restoration stakeholders, and particularly between scientists and practitioners, should help. Cabin et al. (2010) reported that only 26% of stakeholders surveyed at the 2009 SERI (Society for Ecological Restoration International) conference considered their scientist–practitioner relationships "generally mutually beneficial and supportive of each other." One of the issues most commonly cited for the improvement of restoration science and practice was the science–practice gap.

One of the main objectives of SERI, created in 1989, is to promote harmonious relationships between scientists and practitioners and between restoration ecology (i.e. the scientific process of developing theory to guide restoration) and ecological restoration (i.e. the practice of restoring degraded ecological systems) (Clewell 1993). Because it is particularly difficult to reach this objective at a global scale, regional chapters and national networks are developing to take up the challenge. Three networks were recently created, the Brazilian Network for Ecological Restoration (REBRE) in 2010 (Isernhagen et al. 2017), Sociedad Ibero-Americana y del Caribe para la Restauración Ecológica (SIACRE) in Latin America in 2013 (Echeverría et al. 2015; Zuleta et al. 2015), and Réseau d'Echanges et de Valorisation en Ecologie de la Restauration (REVER) in France in 2008.

This article (1) describes the context in which restoration is developing in France; (2) presents the French restoration network REVER; and (3) concludes on how national networks are helping to improve restoration.

Background to Ecological Restoration in France

The first documented "restoration" in France dates back to the 1860s, when the Department of Mountain Land Restoration—*RTM: Restauration des Terrains de Montagne*—carried out large-scale tree planting to combat heavy soil erosion. However, these actions do not meet the current definition of restoration (Society for Ecological Restoration International Science & Policy Working Group 2004), as some exotic species were used, such as Austrian black pine (*Pinus nigra* ssp. *nigra*). Follow-ups 120 years later, however, showed that the pine could serve as a nurse species enabling native species to establish if appropriate silvicultural practices, such as thinning, were used (Vallauri et al. 2002). With related objectives, between the 1940 and 1980, soil defense and restoration—*DRS: Défense et Restauration des Sols*—were developed by foresters around the Mediterranean Basin to face up to droughts, reservoir silting, soil erosion, and degradation (Roose 2004). Much later (1970), the restoration of open ecosystems, such as grasslands, wetlands, and marshes, started mainly by reintroducing extensive grazing with rustic breeds and continued on in the 1990s with seeding or other techniques aimed at reducing agricultural intensification (fertilization, early cutting, etc.) (Muller et al. 1998). Starting in the mid-1980s, coastal environments have also been the focus of many restoration projects. Between 1984 and 2007, 35 projects were carried out to restore Atlantic coast cliffs following years of excessive visitation (Bioret & Gallet 2015).

A century after the first mountain land restoration, the French law on Nature Conservation (1976) provided for Environmental Impact Assessment (EIA) prior to land use planning actions potentially affecting the quality of the human environment (Table 1). The law provided for a three-step mitigation hierarchy procedure: avoid, reduce, and offset (i.e. ecological measures implemented outside the impacted site to compensate for residual losses). However, it did not meet expectations on ecological restoration, partly because the mitigation procedure was not enforced before 2012 (Lucas 2009).

Other incentives also contributed to the development of restoration in France, particularly the promotion of research supported by the French Department of the Environment, the CNRS & Irstea research centers, etc. (Fig. 1; Appendix S1, Table S1; Gallet et al. 2017). In 2008, a research program funded the creation of the French-language restoration network REVER (Appendix S1). REVER became a French non-profit organization in 2011, its objectives inspired by SERI. Its main aim is to organize and promote relationships between land managers, practitioners, students, and researchers working in ecological restoration and/or restoration ecology. As recently recommended by Meli et al. (2017), REVER is based on non-hierarchical knowledge spreading. The year 2008 also saw the first attempt to create mitigation banking out of a restoration project in France: the restoration of a Mediterranean dry grassland, La Crau area, in southeastern France (Dutoit et al. 2015). Inspired by the U.S. wetland mitigation bank, it aimed at anticipating restoration by creating compensatory mitigation credits ahead and independently of land use planning actions. Mitigation banking opened new perspectives for restoration in France, as did the release in 2007 of the order related to the environmental code protected species section. This updated the EIA three-step mitigation hierarchy procedure, which until then had scarcely been implemented. On 1 January 2017, a new public institution, *Agence Française pour la Biodiversité* (French Agency for Biodiversity), was created, principally to contribute to the protection, management, and restoration of biodiversity in terrestrial, aquatic, and marine environments.

The Driving Forces Behind the Development of Restoration in France

In France, the increase in the number of protected areas over the second half of the twentieth century, and the creation of protected species lists in 1979, reduced pressures on certain species and ecosystems. Yet, it is now clear that in France and in Europe, this must be accompanied by measures to increase habitat areas, to restore ecological functions or to recreate ecological networks (Mose 2007). Restoration, an essential partner to conservation, has grown substantially since the turn of the twenty-first century due to the evolution of conservation practices, input from research, social demand, and the evolution of the European Union (EU) and French regulatory framework (Fig. 1; Appendices S1 and S2; Gallet et al. 2017).

Table 1. Summary of legal tools promoting ecological restoration in France. Many texts in the table have been amended since publication. EIA is a decision-making tool that describes the environmental effects of the land use planning actions. The hierarchical mitigation procedure is a three-step procedure (avoid, reduce, and offset) which when it is impossible to avoid environmental impacts, permits to reduce, restore, and offset the residual impacts through compensatory measures.

		Regulation Text (year)				
	Regulation Fields	*European or international*	*French law*	*Competent Authority*	*Links With Restoration*	*Details*
Restoration implemented when a project is expected to have environmental impacts	Environmental Impact Assessment (EIA)	Directive 1985/337	1976, 2016	Administrative authorities	EIA decrees the hierarchical mitigation procedure	
	Facilities classified for environmental protection (EIA)		1976	Administrative authorities		
	Aquatic impacts (EIA)		1992	Administrative authorities		
	Protected habitat (EIA)	Directive 1992/43	2001, 2007	Administrative authorities	EIA exception for protected habitats: the offset of residual impacts is one of the three conditions to obtain derogation from the prohibition on destruction of protected species habitats (other conditions: absence of alternative solutions, imperative reasons of overriding public interest)	French ministerial decree of 19 February 2007
Public policy on restoration	Protected species and natural habitats	Directive 1992/43	2001	Management committees of Natura 2000 sites	Natura 2000 sites must be maintained or restored to favorable conservation status (management plans)	
	Water	Directive 2000/60	2006	Water agencies, national authority	Good water quality cannot be achieved without restoring watersheds and wetlands	
	Ecological network	Paneuropean ecological network, 2010	2009	Local authority	The 2009 Grenelle 1 law, article 23 sets out the following government goals, to halt the loss of wild and domestic biodiversity and to restore and maintain their evolutionary capacities: (1) setting up, by 2012, a green and blue infrastructure network (action plan for ecological continuities—*plan d'actions pour la restauration de la continuité écologique*) funded by water agencies; (2) implementing measures for protection, for natural habitat and species restoration and for offsets due to environmental damage	

Table 1. Continued

		Regulation Text (year)				
	Regulation Fields	European or international	French law	Competent Authority	Links With Restoration	Details
	Biodiversity	Aichi objectives 2010	2016	National authority		Restoring at least 15% of degraded ecosystems by 2020 (thereby contributing to climate change mitigation and adaptation and to combating desertification, as well as including green infrastructures in land use planning)
Restoration following environmental damage	Facilities classified for environmental protection (damage expected at the end of the exploitation)	Directive 96/61	1976	Administrative authorities		Increased restoration opportunities, especially for quarries and mines Where polluted soils are being rehabilitated, rehabilitation measures are designed to meet quality requirements needed for the future use of the site; most of the time a very low level of environmental quality is set (public health or security).
	Biodiversity, water, soil (accident, severe damage)	Directive 2004/35	2008	Administrative authorities		Polluted water or biodiversity degradation: restoration in kind as a priority (no application in France since 2008 because no case of severe damage, but application methodology already set in the law)
	Environmental liability (ecological damage, accident)		2016	Judge		Compensate ecological damage with restoration (instead of financial compensation)

At the European scale, several EU directives encourage restoration activities (EU directives set out results that all EU Member States must achieve, with national authorities then choosing the forms and methods of intervention). Directives are then translated into national laws that should reflect common policy (Table 1). Some prescribe restoration objectives, some aim at anticipating future impacts and the need for restoration and offsets, whereas others provide for restoration following structural or accidental damage due to human activities.

European and National Policies Anticipating Impacts

The first policy connected with restoration is the EIA (Table 1). EIA requires entities implementing land use planning actions subject to administrative permits to avoid environmental impacts, and, when impossible, to reduce, restore, and offset the residual impacts through compensatory measures; these three steps (avoid, reduce, and offset) are called the hierarchical mitigation procedure. As mentioned previously, this procedure was included in the French 1976 Nature Conservation law, and later within European directive 85/337 in 1985. Despite the fact that the "no net loss" notion was introduced in U.S. laws in 1987 and applied in other European countries in the 1990s (Rundcrantz & Skärbäck 2003), guidelines, as well as the implementing decree, were released in France only in 2012 (MEDDE 2012). EIA also concerns projects which are likely to have a significant impact on any kind of habitats within a Natura 2000 site or on a protected species or habitat inside or outside a Natura 2000 site (Table 1). The 1992 Habitats directive also provides a means of derogating from the prohibition on destruction of protected species habitats, through effective compensatory measures (Table 1; Regnery et al. 2013). The July 2016 law on Biodiversity, Nature and Landscape Recovery (*loi pour la reconquête de la biodiversité, de la nature et des paysages*) could also encourage ecological restoration, notably by setting performance obligations and by creating the French Agency for Biodiversity.

European and National Policies With Restoration Objectives

In addition to EIA policies, many legislative texts set ecological goals (Table 1), a key example being the above-mentioned Habitats directive. This European directive ensures the conservation of a wide range of rare, threatened or endemic animal and plant species, inciting EU Member States to "maintain or restore, at favorable conservation status, natural habitats and species of wild fauna and flora of Community interest" (article 2). This means resorting to ecological restoration when the

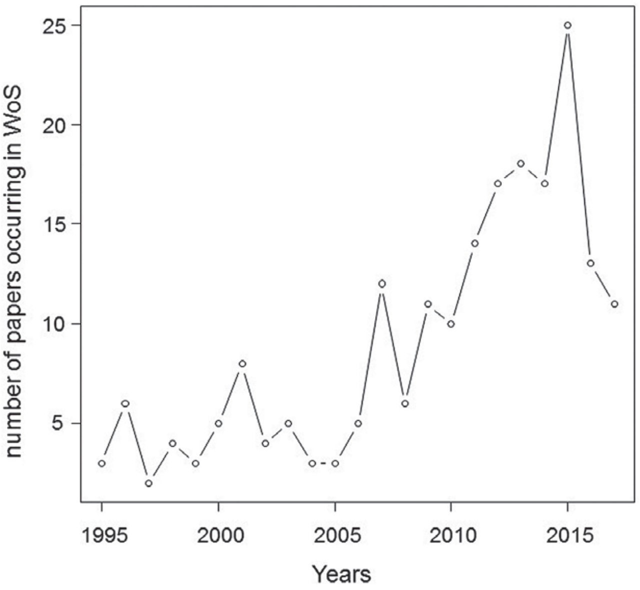

Figure 1. The increase in restoration ecology research in France is shown by the increasing number of papers published between 1995 and 2016 and indexed by the Web of Science (thus excluding most papers written in French). Source: Web of Science, searching for the keywords "restoration" and "ecology" and "France" ($n = 194$).

conservation status of a species or a habitat is not favorable, particularly within Natura 2000 sites (Table 1).

Adopted in 2000, the Water Framework directive 2000/60/EC also underpins European regulations favoring restoration. This directive does not directly concern natural habitats, but sets mandatory targets in terms of water quality that cannot be achieved without restoring watersheds and wetlands and their associated ecosystem services. French legislation adopts the principles of this directive in the 2006 law on Water and Aquatic Ecosystems, which stipulates that ecological restoration is to be used, funded by the six water agencies located throughout France, to meet EU targets (Table 1; Appendix S2, Tables S2, S3). Recently, the French environmental summit (*Grenelle de l'environnement*) incited the development of ecological networks in land use planning (Table 1). Thus, whether by establishing networks or by recreating damaged corridors, ecological restoration is used to increase or improve ecological continuities.

More recently, in May 2011, the EU adopted the 2020 Biodiversity Strategy which aims to halt the loss of biodiversity and improve the state of Europe's species, habitats, ecosystems, and the services they provide, by 2020. Among other goals, it implements the (1) 1992 Habitats directive 92/43/EEC and (2) October 2010 Aichi Biodiversity Targets, whereby Europe committed to restoring at least 15% of degraded ecosystems by 2020. All Member States were to deliver a sound national restoration prioritization framework by the end of 2014, which none did (Cortina-Segarra et al. 2016) contrary to other countries of the world, such as Colombia, Ecuador, and Guatemala (Meli et al. 2017).

European and National Policies Following Damage

Finally, some policies seek to restore degraded ecosystems following legal activities or accidental damage (Table 1). For example, the 1977 Act on Facilities Classified for Environmental Protection was designed to ensure public safety and aesthetically improve and stabilize the terrain following mining. This increased restoration opportunities, especially for quarries and mines. The 2004 European directive on Environmental Liability (transposed into French law in 2008) introduced the notion of reparation for environmental damage (i.e. damage to protected species and natural habitats, damage to water and damage to soil). It is the first attempt in France to repair accidental ecological damage and favors concrete restoration measures, under the auspices of the public authorities. Although the scope of application appears limited and no restoration has yet taken place in this context, the French Department of the Environment is now working on a national method of biophysical assessment of less severe damage.

Although not exhaustive, the above list of legislative texts highlights key EU and French environmental laws promoting the use of restoration.

Other Incentives and Policies

In addition to legally enforceable regulations, other measures also support the development of restoration. One example is the "no net loss of biodiversity" objective included in the July 2016 law on Biodiversity, Nature and Landscape Recovery & the Environmental Code, which incites public and private stakeholders to implement restoration actions. The French National Strategy for Biodiversity 2011–2020 also prioritizes the restoration of natural habitats and of ecological continuities (Appendix S1; French Department of Environment (FDE) 2017).

Various incentives led by the French Department of the Environment, such as *Opération Grands Sites*, target restoration on heritage sites or natural sites of major importance, within a concerted framework (Pára 2013). Agro-environmental schemes, implemented within the framework of EU Common Agricultural Policy, also favor ecological restoration of natural habitats (European Commission (EC) 2013). Both the Coastal Conservancy (which protects coastal areas through land acquisition and adequate site management) and the departmental council (under their sensitive natural areas policy) can also initiate restoration actions on their sites (Conservatoire du littoral (CL) 2015; Départements de France (DdF) 2015).

Restoration can thus be required by law or promoted by incentives. Moreover, local voluntary actions implemented at more or less large scale (notably in nature reserves) by local or regional authorities or non-governmental organizations (NGOs) should not be underestimated.

Barriers to Ecological Restoration

Although the French and European contexts appear to favor ecological restoration, various barriers need to be recognized. The implementation of restoration may encounter barriers linked to local factors, such as (1) laws and administrative procedure or (2) the socioeconomic context, particularly local practices and local stakeholders' perception of the site's state of conservation.

Legal and Administrative Barriers

Environmental laws can paradoxically complicate or even prevent the implementation of ecological restoration. Some restoration projects may even be considered as potentially damaging to the environment and to protected (although degraded) habitats and areas. In such cases, complex administrative procedures have to be initiated before any action can be taken. Wetland restoration is a particular problem, as any intervention on river beds, modification of hydrological regimes, or creation of ponds requires administrative authorization under the Water and Aquatic Ecosystems law. Similarly, any action planned on heritage sites (*sites classés, sites inscrits au titre de la loi de 1930 sur les paysages*) has to be declared by the project planner and is subject to ministerial authorization.

Moreover, sites considered for restoration may contain populations of protected animal or plant species that could be impacted by restoration, thus requiring EIA or special procedures for the manipulation, transfer or destruction of protected species (Table 1). It has to be proved that despite a potential temporary negative impact, the final state will be more favorable to the species involved. Similarly, before a protected species can be reintroduced, the French Nature Conservation Council needs to be consulted and a derogation obtained for its manipulation and transportation. These procedures are cumulative, and although a favorable outcome is often reached, the process can be discouraging.

Stronger legal barriers can durably impede restoration actions. For example, various ecosystem types, such as sand dunes and heathlands, which underwent tree planting in the 1960s–1970s can be considered degraded and in need of restoration. Legally, however, they are considered as forested and thus subject to forest governance. Because restoration of open ecosystems and shrub lands is regarded as deforestation, another permit is required. This also illustrates how the current designation of land devoted to tree planting as compensatory afforestation (Forest Code article L341-6) has to be considered very carefully. Similar cases are reported in other European countries; for example, Bottin et al. (2005) showed that in Belgium, restoration of calcareous grasslands were pine trees were planted is in conflict (1) with restoration to beech woodlands as natural beech regeneration can be observed in the pine understory, and Natural Beech Forests are an EU protected habitat (Natura 2000 code: 9150), and (2) with a bird species of EU interest, the Black Woodpecker (*Dryocopus martius*) which forages in these pine woods. Manning et al. (2006) also show that human memory fades in time and provide a "shifting baseline" for restoration.

Soils also present complications. French and European regulations do not consider soils as an ecosystem component, which reduces the scope for restoration (Bispo et al. 2016; Desrousseaux et al. 2016). Where polluted soils are being rehabilitated, it is usually only to ensure public health or security (see Table 1 for details). Although the French law is in accordance with the European legislation, and most Member States set equivalent obligations, new approaches should be developed to better take into account soils, an important element of biodiversity (Desrousseaux et al. 2016; Heckenroth et al. 2016). Germany and Belgium do benefit from a more detailed and "soil-based" legal framework (Desrousseaux et al. 2016).

Social Barriers

The success of ecological restoration also depends on social acceptance of the project locally (Meli et al. 2017). There can be local opposition to projects that do not take account of local practices, where ecological gains are not perceived by local stakeholders or where the project is perceived as too radical or difficult to visualize (Manning et al. 2006; Menozzi & Pellegrini 2012). Take the above case of afforested grasslands, of which the artificiality is generally not recognized and which are considered part of the natural landscape. Restoration through deforestation can therefore provoke strong local opposition. Strong opposition generally appears where ecological restoration implies restriction (e.g. foot traffic control) or prohibition of access or a ban on certain local practices, unless dialogue is previously established with stakeholders to reconcile restoration objectives with local practices. Destruction of dams and weirs that entail major landscape changes and destroy connected fishing ponds is another example of a restoration project requiring extensive local dialogue well ahead of realization (Germaine & Lespez 2014).

Finally, economic considerations, like budget cuts, are sometimes insurmountable barriers for restoration projects, often requiring adaptations or reductions in scope (Manning et al. 2006). Public acceptance is contingent on recognizing differences between stakeholders, in language and in restoration goals. Both local dialogue during restoration planning and solid technical and scientific arguments appear essential to project success. Two of the aims of the REVER network are to (1) promote nonhierarchical knowledge sharing and (2) make allowance for the specific expectations and needs of all stakeholders.

REVER: The French Restoration Network

REVER was created in 2008, after several years of brainstorming among French-speaking researchers and practitioners at SER-Europe conferences highlighted a need to strengthen relationships. As a rising and evolving discipline, restoration requires efficient channels of communication between stakeholders, to share fundamental knowledge, experience, and concrete field issues. However, most French practitioners, land managers, and policy makers do not have access to scientific literature, do not participate in SER conferences, or do not speak English, all of which limits their access to information (Amano et al. 2016).

REVER's main aim is to organize and promote relationships between the various French-speaking restoration stakeholders. Meli et al. (2017) highlight that to face up to the stakes of

Table 2. Membership of the French restoration network REVER. Created in 2008, it only became a French non-profit organization (*association loi 1901*) in 2011.

Year	*2011*	*2012*	*2013*	*2014*	*2015*	*2016*	*2017*	*Average*
Number of members	62	27	69	69	55	53	84	60
% Researchers	38.7	25.9	23.2	23.2	25.5	26.4	26.2	25
% Practitioners	43.5	59.3	27.5	27.5	21.8	15.1	20.2	29
% Students	17.7	14.8	49.3	49.3	52.7	58.5	53.6	46

ecological restoration in a context of global change, there is a need to improve communication between stakeholders who should be gathered in a community. REVER mainly achieves this aim by organizing annual workshops. Using shared definitions, stakeholders can discuss the evolution of the field and conduct joint actions (Prola et al. 2015). Researchers have somewhere to test ecological theories against on-the-ground reality, to find sources of information and new field work opportunities, to develop new investigations addressing land managers' and practitioners' issues, and to present the results of their research. Land managers and practitioners can exploit their empirical knowledge, talk about and compare restoration methods, promote best practices, and meet researchers interested in further exploring aspects of their methods (Prola et al. 2015). Communications within the REVER network are intended to be horizontal (as opposed to *top/down* or *bottom/up*) (Meli et al. 2017), to avoid exclusively promoting certain types of knowledge. This is partly guaranteed by the board's composition and is reflected in both membership and participation in REVER workshops (Appendix S3, Tables S4, S5 and Figs. S1 to S6). The average number of members is 60 members with 25% researchers, 29% practitioners, and 46% students (Table 2), which is quite different from the average number and proportions of participants at REVER workshops (144 participants per year with 25% researchers, 47% practitioners, and 28% students; Appendix S3).

REVER uses several tools to provide access to basic information at any time and to increase the visibility of the network and of restoration: a website (Réseau d'Echanges et de Valorisation en Ecologie de la Restauration (REVER) 2011), social media accounts, a biannual newsletter, partnerships, an emailing list (408 subscribers), and annual workshops (Appendix S3). Although all these tools are complementary, annual workshops have been REVER's most valuable tool to improve communication, and to initiate collaboration and exchange (voluntary entries in the database are rare; Appendix S2). While maintaining all these activities in the future, REVER will also develop closer links with SER-Europe and its affiliated structure and subchapters.

Conclusion

Recently, different regional networks linked to ecological restoration were created. Each of them was designed to meet its local context and is the outcome of different construction processes. For example, in Latin America and the Caribbean (LAC), national networks were created first. Thus, when SIACRE was created in 2013 (Echeverría et al. 2015; Zuleta et al. 2015), one of its aims was to increase capacity building, education, and outreach that will strengthen pre-existing networks. Because many LAC countries share a common language (Spanish), SIACRE allows easy exchange of experiences from countries with various restoration policies. In Europe, the situation is quite different. Indeed, SER-Europe was created before the national networks (Table 3). Exchanging experiences at the European level completely makes sense as European countries share a common legal framework. However, it is clear that national networks are also needed notably because of the language barrier, especially in the world of practitioners (EU has 24 official languages). Five already created national networks have signed a memorandum of understanding with SER-Europe: France's REVER in 2014, and since then Italy, Finland, Spain, and the Netherlands (Table 3). In the coming years, several European countries (Germany, Hungary, the Czech Republic, and Portugal) plan to create their own network (or SER-Europe subchapter) in order to communicate better at national level, in their own language, and to network on national advances with SER-Europe and other European countries.

Table 3. Creation date of SER-European chapter and European national restoration networks. When two dates are written, the first date is the date of informal creation and the second is that of official registration as an NGO or association.

Name of Organization	*Country*	*Year of Creation*
SER-Europe	NA	1998/2012
Asociación Española de Ecología Terrestre	Spain	2004
Ennallistamisen ja Luonnonhoidon Ohjausryhmä	Finland	2007
Ontwikkeling + beheer natuurkwaliteit	Netherlands	2006
Società Italiana di Restauro Forestale	Italy	2012
Réseau d'échanges et de valorisation en écologie de la restauration	France	2009/2011

France and REVER are just one example of how restoration can be developed and such networks implemented. More restoration networks should communicate on how they were set up and operate, providing useful input for other countries. Although each country has its own restoration history, EU directives mean that all European countries are likely to see their restoration practices converge. Sharing approaches will help everyone meet common targets, countries to better monitor their restoration efforts and the EU to assess progress.

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Supporting Information

The following information may be found in the online version of this article:

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Miscanthus x *giganteus* crop fields hide a genotype of the invasive *M. sacchariflorus*

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Summary

Despite their putative invasive potential, *Miscanthus* taxa have been increasingly viewed as promising crops for bioenergy production. *Miscanthus* x *giganteus* is cultivated worldwide due to its high productivity, and its non-spreading growth limits its risk of invasion. However, the genetic uniformity of its unique cultivated clone has recently been questioned. We used morphometry, nuclear microsatellites, AFLP fingerprints and nrDNA ITS barcoding to assess *Miscanthus* genotypes cultivated in eastern France. As expected, the globally cultivated clone was the main morphotype and genotype of our samplings; however, we also identified a second genotype, assigned to *M. sacchariflorus*, in the sampled fields. Morphological differentiation partially overlaps genotypic assignation, probably due to disturbance at the crop edges. We also show that *Miscanthus* taxa are misidentified in commercial nurseries. Our study confirms previous reports of the occurrence of other accessions in fields of *M.* x *giganteus*. These taxonomic confusions limit the certainty that a single clone of *M.* x *giganteus* is cultivated. In this context, *M.* x *giganteus* crop fields may actually promote the dispersal of invasive *Miscanthus* taxa.

Keywords: bioenergy crops, invasive weeds, AFLP, microsatellites, ITS barcoding, *Miscanthus*.

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Introduction

The depletion of known sources of fossil energy resources and the increased consumption and demand for energy have fuelled interest in renewable energy and, in particular, towards plant crops as bioenergy sources (Hujerová *et al.*, 2017). Some of the most promising candidates for biofuel production are members of the genus *Miscanthus* ANDERSSON (Poaceae: Panicoideae) that originate mostly from Southeast Asia. Due to their C4 metabolism, *Miscanthus* species have high carbon sequestration rates, photosynthesis activity and water use efficiency (Lewandowski *et al.*, 2000). The most studied taxa of this genus are *Miscanthus sinensis* ANDERSSON (*Msi*), *Miscanthus sacchariflorus* BENTH. (*Msa*) and their sterile hybrid *Miscanthus* x *giganteus* GREEF & DEUTER ex HODK. & RENVOIZE (*Mxg*). In addition to their wide ecological tolerance and high biomass production, *Msi* and *Msa* produce large amounts of seed; this ensures a low investment cost for planting (Quinn *et al.*, 2010). However, these characteristics also favour the risk of field escapes through anemochory or rapid rhizome growth. Therefore, the culture of these two

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exotic taxa as bioenergy crops is limited in Europe and North America where they are deemed as invasive species (Jørgensen, 2011).

Across their native range, (China, Korea and Japan) *Msi* and *Msa* have naturally formed several hybrids with different ploidy levels (Clark *et al.*, 2018). The worldwide cultivated *Mxg* is a naturally occurring triploid hybrid ($2n = 3x = 57$) between *Msi* ($2n = 2x = 38$) and *Msa* ($2n = 4x = 76$). It was introduced from Japan to Denmark in 1935 and is commonly referred to as *M.* x *giganteus* 'Illinois' or '1993–1780' – in reference to the first institution to study it and its type specimen at the Kew Royal Botanic Gardens Herbarium respectively. Over the last years, it has gradually replaced its parents as a key alternative bioenergy feedstock. This hybrid is of particular interest due to its high productivity rate and its low invasive risk, given that it is restricted to a single sterile clone (Linde-Laursen, 1993) with limited clonal expansion (*c.* 10 cm per year; Jørgensen, 2011). Consequently, experimental, cultivated and sold individuals of *Mxg* have, until recently, shown a low genetic diversity (Greef *et al.*, 1997; De Cesare, 2012; Oladeinde, 2012; Hodkinson *et al.*, 2013; Cichorz *et al.*, 2014).

Nevertheless, agronomists have recently developed polyploid and GMO variants of *Mxg* to restore the fertility and increase the cost efficiency of *Mxg* crops (Yu *et al.*, 2009; Głowacka *et al.*, 2015). If these new fertile genotypes escape towards natural areas, they could severely damage local biodiversity (Baute *et al.*, 2016; Miriti *et al.*, 2017). In addition, current field research involving new hybrids also called *Mxg* could increase confusion regarding the genetic identity of cultivated accessions (Clark *et al.*, 2018). Recent molecular studies have revealed misidentified sold samples of *Mxg*, *Msi*, *Msa* and even *Miscanthus floridulus* (LABILL.) WARB. ex K. SCHUM. & LAUTERB. (*Mflo*) as mislabelled *Msi* accessions that correspond to *Msa*, or mislabelled *Mflo* that are closely related to the *Mxg* 'Illiniois' genotype (Oladeinde, 2012; Cichorz *et al.*, 2014). A pair of studies also found distinct genotypes in horticultural and experimental collections of *Mxg* (Chouvarine *et al.*, 2012; Tang *et al.*, 2015). This confusion in the taxonomic assignation of commercially sold *Miscanthus* accessions adds to the risk of introducing new and potentially invasive genotypes within European and North American crop fields. Despite these risks, there is little knowledge of how to prevent and manage this invasion threat. Agronomic research involving *Miscanthus* has exploded since 2008, with 1952 studies associating '*Miscanthus*' and 'biomass' in the title, keywords or abstract from 1950 to 2018, representing 60.7% of all *Miscanthus* studies (www.webofknowledge.com, 15 September 2018). However, only a few have focused on the invasion potential of these taxa, with 70 studies associating '*Miscanthus*' and 'invasive', representing 2.2% of all *Miscanthus* studies.

We have recently observed in *Mxg* crop fields in France some *Miscanthus* individuals morphologically different than the nominate plant, growing at – and even spreading out of their field margins. This study aims to test the genetic uniformity of *Mxg* crop fields in eastern France; that is, how many different genotypes are cultivated in *Mxg* crop fields? We used three genetic approaches to identify potential different genotypes: nuclear microsatellites and AFLP fingerprints – also applied to a reference *Miscanthus* sample – and nrDNA ITS barcoding used against a reference sequence database generated from sequences deposited in GenBank. In addition, we performed a morphometric characterisation of the same samples to characterise the different morphotypes and to test their congruence with the potentially different genotypes identified in the field.

Materials and methods

Sample sites for this study include eight crop fields of *Mxg* located in the Bas-Rhin Department (Alsace, Grand Est, France). Although crop fields of *Mxg* remain rare in France (*c.* dozens of km^2 in 2018) and located mainly in western France, the Alsace region encompasses by itself *c.* 1 km^2 of *Mxg* surfaces (in 2014) across 59 cultivations and 40 towns (French Ministry of Agriculture). We duplicated the sampling effort in the five crop fields presenting patches of individuals bearing a different morphology, with three of them presenting individuals spreading outside of the field margins. In addition, we added seven accessions of *Mxg* as reference material from botanical gardens and plant nurseries as well as from laboratories that have previously described *Mxg* based on molecular markers (Table 1). Some closely related species – *Msi*, *Msa* and *Mflo* – were also analysed to demonstrate a phylogenetic framework.

DNA sampling and extraction

We studied eight *Mxg* crop fields using molecular biology techniques; sampling included at least two samples per patch for a total of 29 field samples. In addition, we also analysed four accessions identified previously as *Mxg* genotypes (De Cesare, 2012; Teagasc Crops Research Oak Park; generously provided by S. Barth & M. Klaas), along with other accessions and taxa from the Botanical Garden of Strasbourg and botanical stores; thus, we had 16 reference samples in total. We assessed

Table 1 Sampling details, taxonomic, morphotype and genotype assignations, GenBank accessions for nrDNA ITS sequences and herbarium vouchers deposited in the Herbarium of Strasbourg University (STR). Taxon *a priori* corresponds to the name provided by the supplier. Taxon *a posteriori* corresponds to the taxon assignation after genotyping

	Taxon *a priori*	Taxon *a posteriori*	Code	Locality	Morpho types	Geno types	ITS*
Crop fields	*Mxg*	–	S1a	Boersh, France (Boe)	M1	G1	(1)
	Mxg	–	S1c	Boersh, France (Boe)	M1	G1	–
	Mxg	–	S2a	Wittersheim, France (Wit)	M1	G1	–
	Mxg	–	S2c	Wittersheim, France (Wit)	M1	G1	–
	Mxg	–	S3a	Rhinau, France (Rhi)	M1	G1	–
	Mxg	–	S3c	Rhinau, France (Rhi)	M1	G1	–
	Mxg	–	S3bisa	Rhinau, France (Rhi)	M2	G1	–
	Mxg	–	S3bisc	Rhinau, France (Rhi)	M2	G1	–
	Mxg	–	S4a	Oberschaefolsheim, France (Obe)	M1	G1	–
	Mxg	–	S4c	Oberschaefolsheim, France (Obe)	M1	G1	–
	Mxg	*Msa*	S4bisa	Oberschaefolsheim, France (Obe)	M2	G2	(2)
	Mxg	*Msa*	S4bisb	Oberschaefolsheim, France (Obe)	M2	G2	–
	Mxg	*Msa*	S4bisc	Oberschaefolsheim, France (Obe)	M2	G2	–
	Mxg	–	S4bisd	Oberschaefolsheim, France (Obe)	M2	G1	–
	Mxg	–	S4bise	Oberschaefolsheim, France (Obe)	M2	G1	–
	Mxg	*Msa*	S4bisf	Oberschaefolsheim, France (Obe)	M2	G2	–
	Mxg	*Msa*	S4bisg	Oberschaefolsheim, France (Obe)	M2	G2	–
	Mxg	–	S5a	Landersheim, France (Lan)	M1	G1	–
	Mxg	–	S5c	Landersheim, France (Lan)	M1	G1	–
	Mxg	–	S5bisa	Landersheim, France (Lan)	M2	G1	–
	Mxg	–	S5bisc	Landersheim, France (Lan)	M2	G1	–
	Mxg	–	S6a	Brumath, France (Bru)	–	G1	–
	Mxg	–	S6c	Brumath, France (Bru)	–	G1	–
	Mxg	–	S7a	Haguenau, France (Hag)	–	G1	–
	Mxg	–	S7c	Haguenau, France (Hag)	–	G1	–
	Mxg	–	S8a	Duntzenheim, France (Dun)	–	G1	–
	Mxg	–	S8b	Duntzenheim, France (Dun)	–	G1	–
	Mxg	*Msa*	S8bisa	Duntzenheim, France (Dun)	–	G2	–
	Mxg	–	S8bisb	Duntzenheim, France (Dun)	–	G1	–
References	*Mxg*	–	MxgSTR	BG of Strasbourg University, France	M1	G1	–
	Mxg	–	Tea-74	TCROP, Carlow, Ireland	–	G1	–
	Mxg	–	Tea-81	TCROP, Carlow, Ireland	–	G1	–
	Mxg	–	Tea-82	TCROP, Carlow, Ireland	–	G1	–
	Mxg	–	Tea-83	TCROP, Carlow, Ireland	–	G1	–
	Mxg	–	SCO1	Dehner GmbH, Saarbrücken, Germany	–	G1	–
	Mxg	–	NEU	BG of Neuchâtel, Switzerland	–	G1	–
	Mflo	*Mxg*	MfloSCO2	Dehner GmbH, Saarbrücken, Germany	–	G1	–
	Mflo	*Mxg*	MfloTHO1	Toom GmbH, Kehl, Germany	–	G1	–
	Mflo	–	MfloSTR	BG of Strasbourg University, France	–	MfloSTR	(3)
	Msi	–	MsiSTR	BG of Strasbourg University, France	–	MsiSTR	(4)

Table 1 (Continued)

Taxon *a priori*	Taxon *a posteriori*	Code	Locality	Morpho types	Geno types	ITS*
Msi 'Zebrinus'		Msi Zeb	BG of Strasbourg University, France	–	Msi Zeb	(5)
Msi f. *gracillimus*		Msi gra	BG of Strasbourg University, France	–	Msi gra	(6)
Msi 'Strictus'		Msi Stri	Dehner GmbH, Saarbrücken, Germany	–	Msi Stri	(7)
Msa	*Msi*	MsaPAV	Avettes nursery, La Genête, France	–	'Msa'PAV	(8)
Msa ssp. *lutarioriparius*		Msa lut	BG of Strasbourg University, France	–	Msa lut	(9)

Mxg, *Miscanthus* x *giganteus*; *Msi*, *M. sinensis*; *Msa*, *M. sacchariflorus*; *Mflo*, *M. floridulus*; BG, Botanical Garden; TCROP, Teagasc Crops Research Oak Park.

*ITS GenBank accessions and STR herbarium vouchers: (1) KU297965, STR75132; (2) KU297966, STR75133; (3) KU297958, STR75134; (4) KU297962, STR75135; (5) KU297961, STR75136; (6) KU297963, STR75137; (7) KU297964, STR75138; (8) KU297959, STR75139; (9) KU297960, STR75140.

45 *Miscanthus* accessions via molecular analyses: 36 *Mxg*, 4 *Msi*, 2 *Msa* and 3 *Mflo* (Table 1). DNA extraction followed the procedure of Doyle and Doyle (1987) with the following modifications: *c.* 50 mg of ground dried leaf material was suspended in 0.7 mL of cetyl-trimethyl-ammonium bromide (CTAB) at 50°C, incubated 1 h at 65°C and then mixed with 0.7 mL of biophenol:chloroform:isoamylic alcohol (25:24:1). After centrifuging for 10 min at 18 000 ***g***, the aqueous phases were mixed with 0.5 mL of 0.6 M NaAc: 95% ethanol to precipitate DNA and left at −20°C overnight. DNA was centrifuged for 15 min at 18 000 × ***g*** at 5°C then washed in 70% ethanol, recentrifuged 5 min, dried and suspended in TE buffer with RNase. DNA concentrations were estimated using a NanoDrop ND-1000 spectrophotometer (Labtech, Uckfield, UK) and diluted to 50 ng μL^{-1} in 1× TE-buffer.

AFLP fingerprints

AFLP followed Vos *et al.* (1995) with the following modifications: 500 ng of DNA was digested with 10 units of *Eco*RI and 4 units of *Tru*9I (an isoschizomer of *Mse*I typically used in AFLP) in a final volume of 25 µL incubated 3 h at 37°C and 3 h at 65°C. Ligation was performed at room temperature for 8 h by adding 0.5 units of T4 DNA ligase, 12.5 mM of ATP, 3.125 units of Eco adaptors and 31.25 units of Mse adaptors to the digested DNA. Preamplification was performed with 0.2 ng of ligation product, 1× of GoTaq Flexi Buffer, 0.25 µM each of Eco+A and Mse+C primers (Eurofins Scientific, Luxembourg), 1.0 mM of $MgCl_2$, 0.2 mM of dNTP and 0.5 units of GoTaq G2 Flexi DNA polymerase (Promega, Fitchburg, WI, USA) for a final volume of 20 µL. Preamplification cycles were 94°C for 2 min, followed by 20 cycles at 94°C for 45 s, 56°C for 45 s, 72°C for 1 min and 72°C for 10 min. Selective amplification was performed using an Eco+AAC primer dyed with a 6-FAM fluorescent at the 5′ end and a Mse+CAA primer in 20 µL volumes having 5 µL of 10× diluted preamplification, 1× of GoTaq Flexi Buffer, 0.25 µM of each primer, 1.0 mM of $MgCl_2$, 0.2 mM of dNTPs and 0.5 unit of GoTaq polymerase. The thermocycle profile of selective amplification was 94°C for 2 min, 10 cycles of 94°C for 30 s, 65°C for 30 s (−0.7°C touchdown per cycle), 72°C for 1 min, followed by 20 cycles at 94°C for 30 s, 56°C for 30 s, 72°C for 1 min and 72°C for 5 min.

SSR fingerprints

SSR analysis was carried out using 16 primer pairs selected from Głowacka *et al.* (2015), Hodkinson *et al.*

(2013) and Oladeinde (2012) based on the primer pairs' positive amplification and polymorphism for *Mxg*. PCR amplifications were performed by multiplexing these primers four by four, using the 'three primers' PCR method proposed by Schuelke (2000) that follows the protocol described by Culley *et al.* (2013). The four tail primers M13(-21), M13modA, M13modB and T7term were used, labelled on their 5′ ends with 6-FAM, YakimaYellow, ATTO550 and ATTO565 dyes respectively (Eurofins Scientific). The forward primer of each primer pair was then modified by adding as a tail one of the four universal sequences to the 5′ end. Overlaps in fragment sizes were limited by carefully choosing the tail labelling and multiplex grouping (Table S1). Primers were then mixed together in four 100× primer mixes with 0.5 μm of each four forward tailed primer, 2 μm of each corresponding reverse primer and 2 μm of each corresponding tail primer in a final volume of 50 μL. PCRs were performed in 10 μL volumes with 5 μL of the QIAGEN Mastermix (QIAGEN, Venlo, the Netherlands), 1 μL of 10× primer mix (10× diluted as in the original protocol) and 100 ng of DNA. Thermocycle profiles followed indications from Culley *et al.* (2013): 94°C for 15 min, 45 cycles of 94°C for 30 s, 57°C for 90 s, 72°C for 1 min followed by a final step at 60°C for 30 min.

Fingerprint analyses

For both fingerprinting techniques, lengths of PCR products were obtained on an ABI Genetic Analyser 3130 (ThermoFischer Scientific, Waltham, MA, USA) at the IBMP genomic platform (CNRS, Unistra). PCR product sizes were calibrated using the size standard SM594, 5′-dyed with ATTO633 (Mauger *et al.*, 2012). Electrophoretograms were then transformed in a presence–absence data set using GeneMapper v.4.1 (ABI), manually for SSRs and semi-automatically for AFLPs. Non-repeatable markers were discarded using 10 replicate samples. AFLP analysis was performed by defining putative loci as any fragment having a minimum amplitude of 200 relative fluorescent units (RFU) from 50 to 500 bp, and automated scoring was checked manually to correct any misinterpreted RFU signal and to discard any overlapping or ambiguous markers. Numbers of genotypes were identified using the Clones () R-function from the AFLPdat R-scripts (Ehrich, 2006), as binary profiles that differed in the percentage of polymorphic fragments (%P) and Nei gene diversity (*D*), both calculated in the R programming environment v.3.2 (R Core Team, 2019). Neighbour-joining tree and bootstrap values were obtained using the *ape* package in R (Paradis *et al.*, 2004) and the neighbour-net network using SplitsTree4 (Huson & Bryant, 2006).

ITS sequencing and barcoding

Following Hodkinson *et al.* (2002), the nuclear ribosomal internal transcribed spacer (nrDNA ITS) is a suitable and cost-effective marker to verify the hybrid identity of *Mxg*, based on the manual interpretation of the chromatogram. This distinction would not be possible on a plastid sequence as *Mxg* is highly similar to its maternal parent *Msa*. The ITS of each genotype detected by molecular fingerprinting was amplified in PCR using the primers ITS5 (5′-GGAAGGAGAAGTCGTAACAAGG; Sang *et al.*, 1995) and ITS4 (5′-TCCTCCGCTTATTGATATGC, White *et al.*, 1990), following the standard protocol suggested by Promega (Madison, USA) for the GoTaq G2 Flexi DNA Polymerase. The protocol for one sample has, in a final volume of 25 μL: 1× of GoTaq Flexi Buffer, 1 mm of $MgCl_2$, 0.2 mm of dNTPs, 0.1 mm of each primer, 0.625 units of polymerase and 2.5 μL of template DNA at 50 ng μL^{-1}. Amplicons were then purified and sequenced by Eurofins Genomics (Luxembourg). The nrDNA ITS is the most commonly used nuclear sequence for plant DNA barcoding (CBOL Plant Working Group, 2009), and we specifically chose this region because it is the most documented sequence in GenBank database for *Miscanthus* with 436 nucleotide accessions for 26 *Miscanthus* taxa. In addition to our ITS sequences, the alignment of this GenBank data allowed us to conserve 429 well-aligned sequences. This constitutes a custom database for megablast queries (http://blast.ncbi.nlm.nih.gov/Blast.cgi). MEGA6 (Tamura *et al.*, 2013) was used for verifying the chromatogram, alignment and access to the NCBI web interface. For each ITS genotype and new GenBank accession, a herbarium specimen was deposited at the Herbarium of the University of Strasbourg (STR).

Morphometric analysis

Five crop fields were selected for the morphometric analysis of individual plants (Table 1). In three fields, the occurrence of patches that had escaped from the initial field boundary led us to replicate our measurements. Thus, we had a morphological characterisation of two different patches within the same field. Seven morphometric variables measured previously (De Cesare, 2012) were estimated from ten 30 × 30 cm plots in each patch. The variables were the number of culms, the number of inflorescences, maximum height, maximum diameter, height and diameter at shoulder height of a randomly selected culm, and width of a leaf at shoulder height. Principal component analysis (PCA) was used to visualise the morphological differences between the different patches of the *Mxg* fields. PCA was run using the dudi.pca() function in R in the *ade4* package (Dray & Dufour, 2007). Hierarchical clustering with Ward's criterion based on the PCA results was also used to identify morphotypes. Clustering was run using the HCPC() function of the package *FactoMineR* run using R (Lê *et al.*, 2008).

Results

AFLP & SSR fingerprinting

The AFLP selective amplification of the 45 samples generated 104 analysable fragments with lengths of 67–392 bp and having 89.4% polymorphic fragments (Table S2). The error rate for the genotyping was 0.025 based on 10 replicates. We used this rate as a threshold to convert the binary-converted chromatograms to genotypes, using the Clones() R-function. Nine different genotypes were identified within all analysed samples (Table 1). Two different genotypes were identified from the crop field samples (Fig. 1). Genotype G1, representing the majority of field samples (23/29), was found in all sampled fields. Genotype G2 was found in only two of the three fields that had escaping individuals (6/29 field samples). The two genotypes had marked differences, sharing 51.9% of the polymorphic fragments and having a mean Nei genetic distance of 0.123 (Table S2). *Mxg* accessions from reference collections (Botanical Garden of Strasbourg University, Teagasc Crops Research Oak Park; De Cesare, 2012) were assigned to the G1 together with the *Mflo* '*Giganteus*' accessions from plant nurseries (*Mflo*SCO2 and *Mflo*THO1; Table 1). The other nine reference accessions were grouped into seven genotypes: two for *Msa*, four for *Msi* and one for *Mflo*.

Among the 16 tested SSRs, 13 primer pairs showed reliable amplification; three pairs (ESSR_008, GSSR_035 and GSSR_054) were removed from the

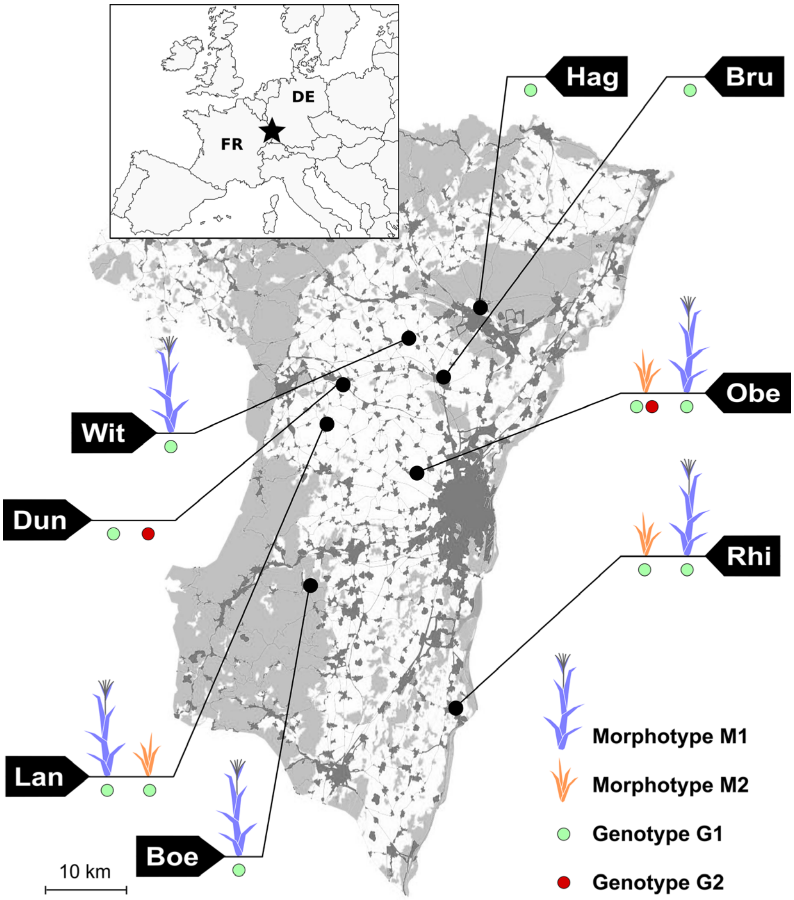

Fig. 1 Map of studied crop fields of *Miscanthus* x *giganteus* with distribution of morphotypes and genotypes (consensus of AFLP and SSR markers showing similar results).

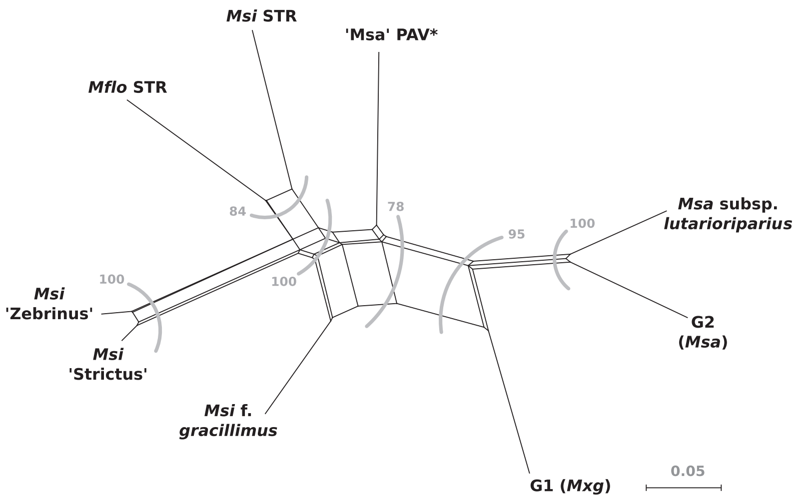

Fig. 2 Neighbour-net network based on AFLP and SSR markers of the nine identified genotypes. Grey values indicate bootstrap percentages that support nodes from the neighbour-joining tree (not shown) as estimated by 1000 replications. *'Msa' PAV is a misidentified sample from a commercial plant nursery corresponding in reality to an *Msi* individual, as determined by ITS barcoding (KU297959).

analysis due to poor reliability. The SSR amplifications of the 45 *Miscanthus* samples generated 111 analysable fragments that had lengths of 108–293 bp and 93.7% polymorphic fragments. By setting an error rate of 0.00, estimated from 10 replicates, the Clones() R-function identified nine genotypes from all the analysed samples (Table 1). Again, we identified two different genotypes from the crop field samples, with identical patterns as the AFLP outcomes (Fig. 1). These genotypes were highly different, having 42.3% of polymorphic fragments between them and a mean Nei genetic distance of 0.066 (Table S2). Finally, *Mflo* '*Giganteus*' was again grouped with the genotype G1.

Because the AFLP and SSR datasets generated similar phylogenetic patterns, we merged the two datasets to produce the neighbour-net network (Fig. 2). The network produces a bipolar shape between the two groups. On the left side of the figure, one group includes the four *Msi* genotypes and the genotype of *Mflo* and well as a sample from a commercial plant nursery, the plant named erroneously as '*M. sacchariflorus*'. To the right side of the figure lies the genotype of *Msa* subsp. *lutarioriparius* and the genotype G2. The genotype G1 has a middle position between the *Msi* and *Msa* genotypic clusters. These groups are also well defined in the neighbour-joining phenogram (tree not shown) with nodes supported by high bootstrap values (78–100%; Fig. 2).

ITS barcoding

Eight of the nine genotypes characterised by AFLP and SSR were successfully sequenced for the nrDNA ITS spacers. However, the chromatogram corresponding to genotype G1 showed variable levels of signal reliability for nucleotide distinction, with poor values in the second part of the chromatogram (orange shading; Fig. 3). We can discern two overlapping sequences with a shift of 2 bp in their alignment (Fig. 3). This shift is due to a 2-bp insertion–deletion (indel) that distinguishes the *Msa* and *Msi* ITS sequences. As the hybrid genotype G1 possesses the two parental copies, this shift leads to an overlap of the two sequences during the sequencing process, causing the loss of nucleotide signal reliability. In addition, on the other few sites distinguishing *Msa* from *Msi* before the indel, the ITS of genotype G1 shows overlapping nucleotide signals for each allele (e.g. 'A' and 'G' for nucleotide site 165; Fig. 3). The G1 sequence was not used in the following megablast queries as the low quality of the chromatogram prevents the interest of such a barcoding approach. Megablast queries against the custom ITS database of 438 sequences allowed us to identify the ITS of genotype G2 as being identical to an ITS sequence of *Msa* from GenBank (HQ822018; Table 2). The other closest sequences were a cloned ITS copy of *M.* x *purpurascens*, a diploid hybrid between *Msa* and *Msi* (Jiang *et al.*, 2013), and another sequence of *Msa*. Megablast queries of the other genotypes confirmed their taxonomical identification, except for the '*Msa*' sample from a commercial plant nursery ('*Msa*' PAV, KU297959) that was more closely related to the ITS sequence of *Msi* (data not shown).

Morphometric analysis

The seven morphometric variables clearly distinguished two morphotypes, evidenced by the first axis of the PCA (52.4%; Fig. 4A). This axis was correlated mainly with the height variables and the number of inflorescences. Hierarchical clustering also divided the samples into the two morphotypes (Fig. 4B). Morphotype M1 found in every field that we studied was larger in form than morphotype M2 found in three fields. Morphotype M1 had a maximum height of 3.2 m (range 1.8–4.5 m), whereas the maximum height of morphotype M2 was 1.1 m (0.7–1.6 m). The random height of M1 was 2.6 m (1.4–4.2 m), whereas it was 0.9 m (0.4–1.4 m) for M2. The culms of M1 were also slightly thicker than those of M2, having a random diameter at shoulder height of 8.5 mm (6.0–10.5 mm) for M1 in comparison with 6.1 mm (4.0–8.2 mm) for M2. The morphotype M2 has never been observed with inflorescences, contrary to the M1 morphotype. The second axis of the PCA (15.2% of the total variation) correlates with random diameter variables and leaf width and highlights differences between some extreme samples. Morphotype M1 was strictly associated with the genotype G1, whereas morphotype M2 was associated with genotypes G1 and G2.

Discussion

The hybrid *Mxg* is well established as a biofuel crop in Europe and North America. Its sterility, low dispersal abilities and clonal reproduction ensure a lower invasive risk into other crop fields compared to its parents *Msi* and *Msa*. In this study, we questioned the existence of only one single clone in *Mxg* crop fields from eastern France. Based on three kinds of genetic markers, including two highly resolutive multilocus fingerprint methods, we identified two distinct genotypes of *Miscanthus* and found both within several *Mxg* crop fields. Our results contradict the assumption that all crop fields consist solely of the *M.* x *giganteus* (*Mxg*) ‘Illinois’ clone that is cultivated worldwide. Compared to reference samples also used in a genetic characterisation study of *Miscanthus* (De Cesare, 2012), the genotype G1 corresponds fully to the legacy cultivar of *Mxg* ‘Illinois’. Based on the large prevalence of the morphotype M1 in fields and its strict assignation to the genotype G1, we assume that *Mxg* ‘Illinois’ is largely predominant, even in fields marked by the co-occurrence of both genotypes. The occasional occurrence of the genotype G2 within *Mxg* ‘Illinois’ fields is worrisome due to its genetic assignation to the invasive *Msa*. We remain cautious regarding its taxonomic identity due to our limited number of outgroup taxa and the great genetic diversity within *Msa* (Clark *et al.*, 2018). However, our analysis of the nrDNA ITS sequences also supports the existence of two distinct genotypes. This identification was possible despite the heterogeneous nucleotide signals on the chromatogram of the G1 ITS. This heterogeneity revealed the hybrid origin of *Mxg* and the co-occurrence of two distinct copies of ITS derived from *Msa* and *Msi*, marked by several dinucleotide sites, as initially demonstrated by Hodkinson *et al.* (2002). Also, the ITS sequence of *Mxg* produces a stronger nucleotide signal for the ITS

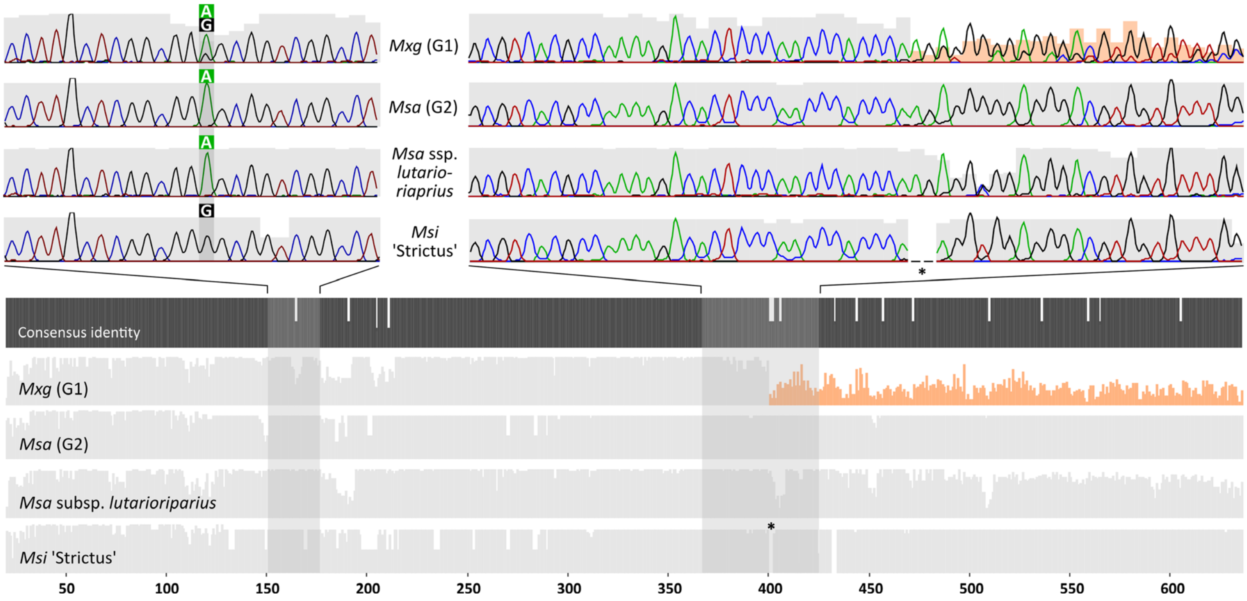

Fig. 3 Alignment of nrDNA ITS sequences illustrating the diagnostic parameters for *M.* x *giganteus* (*Mxg*). Grey bars indicate the reliability of the nucleotide signal. Lower values of consensus identity (black bars) indicate polymorphic sites between sequences. Curves represent nucleotide signals from sequence chromatograms (Sanger sequencing). The poor signal reliability of the second part of the in *Mxg* chromatogram is due to the occurrence of two ITS sequences inherited from *Msi* and *Msa* and misaligned due to an indel (*).

Table 2 Megablast query for the nrDNA ITS sequence of the genotype G2 (642 bp) against an ITS database of 438 sequences from GenBank

% Identity	Taxon	Site mismatches	GenBank accession	Length (bp)	Query cover (%)
100	*M. sacchariflorus* (*Msa*)	0	HQ822018	683	100
99.8	*M. sacchariflorus* (*Msa*)	1	KF163650	634	94.2
99.5	*M.* x *purpurascens**	3	JN544339	661	98.8
99.5	*M.* x *purpurascens**	3	JN544336	661	98.8
99.5	*M. sacchariflorus* (*Msa*)	3	JN544311	661	98.8

*ITS sequences of *M*. x *purpurascens*, a diploid hybrid between *Msa* and *Msi* (Jiang *et al*., 2013). Sequence isolated using bacterial cloning methods.

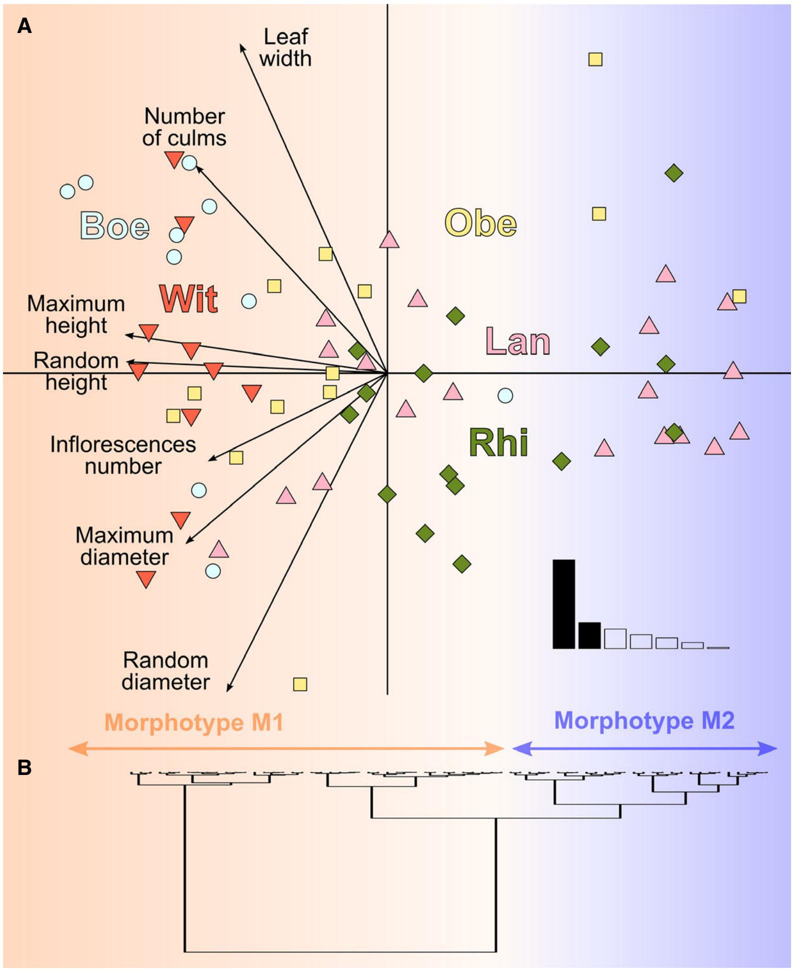

Fig. 4 (A) The two first axes of the principal component analysis (PCA) based on seven morphometric variables. Axes 1 and 2 represent 52.4% and 15.2% of total variability respectively. Each point corresponds to a plot, and symbols represent the various sampled fields. (B) Hierarchical clustering with Ward's criterion based on principal components from the PCA.

allele of *Msa* due to the occurrence of two genome copies of *Msa* and only a single *Msi* in the triploid *Mxg* ($2n = 3x$; Linde-Laursen, 1993; Rayburn *et al*., 2009). The occurrence of genotypic diversity among horticultural samples of *Mxg* has been observed previously in Poland via a microarray-based genotyping method (Tang *et al*., 2015). The authors also underlined that one of these two *Mxg* clusters was more related to some *Msa* samples than to the other *Mxg* cluster. However, they did not investigate the taxonomic identity of this second cluster and treated the cluster as a sublineage of *Mxg*. Tang *et al*. (2015) did not use ITS sequencing to compare their samples to known accessions; however, we suspect that our two genotypes are the same pair of genotypes as found by Tang *et al.* (2015). Consequently, we heartily recommend the cost-effective use of ITS barcoding in further molecular investigations of *Miscanthus* clones to allow comparative approaches with the literature, even in addition to innovative genotyping methods.

The most likely reason for this repeated occurrence of a *Msa* genotype in the *Mxg* crop fields could be the genetic contamination of the commercialised bundles of rhizomes of *Mxg* 'Illinois' sold in Europe. To resolve this problem, it is essential to retrace the commercial pathway to the contamination source. Such a process is difficult due to the lack of legal control in regard to the genotypic identity of crop species, even when they pose an invasion risk. Another possibility for explaining the occurrence of this *Msa* genotype could be its *in-situ* formation from monoploid gametes of *Mxg*. However, even if *Mxg* is comprised of two genomes of *Msa* and one genome of *Msi*, the meiotic mixing of such chromosomes limits the possibility of this scenario. Nevertheless, Clark *et al.* (2018) showed recently that naturally formed hybrids between *Msi* and Msa are often fertile. The sterility of *Mxg* 'Illinois' is therefore an exception, and this sterility is even questioned (Linde-Laursen, 1993). In addition, Clark *et al.* (2018) also demonstrated several introgression events between wild *Mxg* and *Msa* in Japan and putative gene flow between *Msi* and *Msa* by a rare haploid gamete produced by *Mxg*.

While genotype G2 is only associated with the morphotype M2, the later – characterised by shorter culm heights and a lack of inflorescence production – is also found in several patches associated with genotype G1. This mismatch may be due to disturbance along the margins of *Mxg* fields, especially during the harvesting of adjacent corn fields; the cut of individuals at the edge of the *Mxg* field could lead to a reduced regrowth and delayed flowering of these individuals. The occurrence of small individuals of *Mxg* at crop field edges could also be explained by the rhizomatous growth and underground colonisation that leads to the emergence of younger hence shorter forefront individuals compared to the taller culms from older rhizomatous parts. Consequently, if morphometric parameters and specifically culms heights can offer insight into the taxonomic diversity within *Mxg* crop fields, these parameters alone cannot be used as diagnostic criteria in the field. Although not systematically observed in our study, sheath pubescence appears to be a putative better character to distinguish *Mxg* (densely pubescent sheaths) from *Msa* (sparsely pubescent or glabrous sheaths; Sun *et al.*, 2010). However, before applying this character difference to field surveys, it must be evaluated across a larger taxonomic sampling and tested under laboratory conditions to assess how environmental conditions affect pubescence.

The hidden occurrence of *Msa* in the crop fields of *Mxg* in France and potentially Europe (Tang *et al.*, 2015) raises serious questions regarding its invasiveness (Schnitzler & Essl, 2015). As described previously, the abundant and aggressively spreading rhizomes of *Msa* were distinguishable from *Mxg* in fields. Associated with its putative seed dispersal, this would increase the risk of field escape and invasion into other crops (Bonin *et al.*, 2014). The appearance of *Msa* within various *Mxg* crop fields may indicate an initial contamination of commercial bundles of *Mxg* rhizomes prior to cultivation. This study also revealed the taxonomic misidentification of three of five *Miscanthus* accessions from commercial plant nurseries. Both molecular fingerprinting methods reassigned *M. floridulus 'Giganteus'* (*Mflo*SCO2 and *Mflo*THO1) and *M.* x *giganteus* (*floridulus*) (SCO1) to the *Mxg* legacy cultivar, a nomenclatural and taxonomical confusion that had been suggested previously (Chouvarine *et al.*, 2012; Cichorz *et al.*, 2014). Additionally, a commercial individual (*Msa*PAV), initially identified as *Msa*, was reassigned to *Msi* based on molecular fingerprints and ITS barcoding. These nomenclatural uncertainties between taxa and cultivars represent another potential source of the accidental introduction of *Msa* and *Msi* into the crop fields of *Mxg*. Finally, the recent commercialisation of the cultivar *Mxg* 'PowerCane' must be monitored carefully, as its fertility represents a potential future invasive risk (Bonin *et al.*, 2017; Miriti *et al.*, 2017).

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 List of microsatellite primers, expected size of amplicons and multiplex combinations.

Table S2 Genetic diversity within species and genotypes.