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THÈSE

présentée par :

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Discipline/ Spécialité : Géochimie de l'Environnement

Transport and Degradation of Pesticides in Wetland Systems : A Downscaling Approach

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“Look deep into nature, and then you will understand everything better.”

Albert Einstein

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Curriculum Vitae

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Educational Background

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Research Experience

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Ph.D. thesis: "**Transport and degradation of pesticides in wetland systems: A downscaling approach**" (Supervisor: Dr. Gwenaël Imfeld)

- Experimental design – Field and lab samplings
- Hydrochemical analyses
- Pesticide extraction from soil, plants, water and air samples **(SPE)**
- Pesticide quantification **(GC-MS-MS)** and chiral analyses **(chiral GC-MS)**
- Compound-specific isotope analyses **(GC-C-IRMS)** in collaboration with the Helmhotz Centre for Environmental Research – UFZ – Leipzig, Germany (Department ISOBIO)
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Scientific collaborator (Oct. – Nov. 2009)

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Master internship (Apr. – Sep. 2009)

Master Thesis: **"Hydrochemical variability in an artificial wetland treating non-point source pesticide pollution."** (supervisor: Dr. Gwenaël Imfeld)

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Bachelor's degree internship (Mar. – Jun. 2008)

Bachelor's degree thesis in **Hydrogeology**: "Mineralization of water retained by the hydroelectric dam of Laparan (Ariège, France)" (Supervisor: Pr. Jacques Mudry)
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List of publications

• **Maillard E.**, Imfeld G., Pesticide mass budget in a stormwater wetland. Environmental Science and Technology, *submitted.*

• **Maillard E.**, Elsayed O.F., Millet M., Imfeld G., Transport and biodegradation of three chloroacetanilide herbicides in lab-scale wetlands: A comparative study. Environmental Pollution, *submitted.*

• Elsayed O.F., **Maillard E.**, Vuilleumier S., Nijenhuis I., Richnow H.H., Imfeld G., 2013, Using compound-specific isotope analysis to assess the degradation of chloroacetanilide herbicides in lab-scale wetlands. Chemosphere, *in press.*

• Lefrancq M., Payraudeau S., García Verdú A. J., **Maillard E.**, Millet M., Imfeld G., 2013. Fungicides transport in runoff from vineyard plot and catchment: contribution of non-target areas. Environmental Science and Pollution Research, *in press*.

• Imfeld G., Lefrancq M., **Maillard E.**, Payraudeau S., 2012. Transport and attenuation of dissolved glyphosate and AMPA in a stormwater wetland. Chemosphere, 90, 1333-1339.

• **Maillard E.**, Payraudeau S., Imfeld G., 2011. Removal of dissolved pesticide mixtures by a stormwater wetland receiving runoff from a vineyard catchment: an inter-annual comparison. International Journal of Environmental Analytical Chemistry, 92, 979-994.

• Martin S., Bertaux A., Le Ber F., **Maillard E.**, Imfeld G., 2011. Seasonal changes of macroinvertebrate communities in a stormwater wetland collecting pesticide runoff from a vineyard catchment (Alsace, France). Archives of Environmental Contamination and Toxicology, 62, 29-41.

• **Maillard E.**, Payraudeau S., Gregoire C., Imfeld G., 2011. Removal of pesticide mixtures in a stormwater wetland collecting runoff from a vineyard catchment. Science of the Total Environment, 409, 2317-2324.

Conferences and awards

• **Maillard E**., Imfeld G., Pesticide transport, partitioning and distribution in a stormwater wetland collecting runoff from a vineyard catchment. Wetpol, 13-17 October 2013, Nantes, France / Oral presentation.

• **Maillard E.**, Elsayed O., Millet M., Vuilleumier S., Imfeld G., Transfer and biodegradation of chloroacetamide herbicides in lab-scale wetlands. 9th INTECOL international Wetlands Conference, 3-8 June 2012, Orlando, Florida, United States / Poster.

• **Maillard E.**, Babcsanyi I., Payraudeau S., Imfeld G., Transfer of pesticides and copper in a stormwater wetland receiving contaminated runoff from a vineyard catchment. European Geoscience Union General Assembly, 22-27 April 2012, Vienna, Austria / Oral presentation.

• **Maillard E.**, Elsayed O., Millet M., Vuilleumier S., Imfeld G., Transport and attenuation of chloroacetanilide herbicides in lab-scale wetlands. Eurosoil, 1-8 July 2012, Bari, Italy / Oral presentation.

• **Maillard E.**, Elsayed O., Vuilleumier S., Imfeld G., Transfer and biodegradation of chloroacetanilide herbicides in lab-scale wetlands treating agricultural runoff water. Joint meeting of society of wetland scientists, Wetpol and biogeochemistry symposium. 3-8 July 2011, Prague, Czech Republic / Poster. (Best student poster presentation award).

• **Maillard E.**, Payraudeau S., Imfeld G., Removal of pesticide mixtures in a stormwater wetland collecting runoff from a vineyard catchment. XIV symposium in pesticide chemistry, 30 August – 1 September 2011, Piacenza, Italy / Poster. (“ Paolo Cabras” best poster award).

Abstract

A fundamental understanding of transport and transformation of modern agricultural pesticides in biogeochemically dynamic environments is critical for predicting their fate, especially that of chiral molecules. 30% of currently used pesticides are chiral molecules consisting of a mixture of two (or more) enantiomers, which can undergo enantioselective biological processes. Wetlands are dynamic ecosystems harboring a wide spectrum of biogeochemical conditions that promote the co-existence of different pesticide transformation pathways governing their capacity to improve water quality and related ecosystem services. However, quantitative knowledge on pesticide partitioning, distribution and degradation in the various environmental compartments in relation to the biogeochemical and hydrological conditions is currently very limited. In this work, wetland systems were used as 'natural laboratories' to investigate at different spatial scales the transport and the fate of widely-used pesticides in terrestrial/aquatic interface ecosystems.

In a downscaling approach, three different wetland systems receiving agricultural runoff were investigated: i) a stormwater wetland (320 m^2) receiving agricultural runoff to assess the partitioning of a pesticide mixture and its distribution among the different compartments using a mass balance approach, ii) planted beds (7 m^2) to investigate the influence of hydraulic and redox patterns on the removal of the herbicide *S*-metolachlor, and iii) lab-scale wetland columns (0.02 m^2) to compare the transport and removal of three herbicides of the chloroacetanilide family, i.e. acetochlor, alachlor and metolachlor. Enantiomeric and compound-specific isotope analyses (CSIA) were combined for assessing pesticide biotransformation. The results showed that i) pesticides partitioning in runoff varies according to the properties of the molecules and pesticides mainly accumulated in sediments and plants in spring and late summer, with a larger pesticide mass attenuation in summer (weekly removal ranged from negative to 99.9% in spring and from 71.9 to 100% in summer), ii) batch flow conditions were more efficient in removing *S*-metolachlor (mass removal rate: 93 ± 3.4%) than flooded conditions (mass removal rate: 27 ± 3.5%), iii) the three chloroacetanilide herbicides were preferentially biodegraded under anoxic conditions even though metolachlor was more persistent possibly due to a greater steric hindrance during microbial degradation. Change in carbon isotope composition between the inlet and outlet of the wetland columns ($\Delta\delta^{13}C$) were 2.6, 2.5 and 0.7‰ for acetochlor, alachlor and metolachlor, respectively. The mean enantiomeric excess (EE) for metolachlor was -3.8% in the anoxic zone compared to -0.6% at the wetland inlet, indicating a preferential biodegradation of the *S*-enantiomer compared to the *R*-enantiomer.

Overall, our results provide quantitative knowledge on the transport and the attenuation of a wide range of pesticide molecules in different biogeochemical conditions, with further implications for the ecotoxicological risks associated with pesticide runoff and transport in wetlands. While large-scale studies provide integrative information on pesticide transport and distribution patterns with respect to wetland functioning, small-scale investigations are necessary to characterize underlying molecular processes governing pesticide transport and transformation. Dynamics of redox conditions characterizing wetlands are closely linked to hydrological conditions. The interplay of hydro-biogeochemical conditions eventually influence pesticide adsorption/desorption mechanisms and thus their distribution patterns over time within wetlands compartments, thereby controlling microbial processes involved in pesticide degradation. We anticipate our results to be a starting point for better understanding the transport and transformation of pesticides and pharmaceuticals in biogeochemically active environments by the combination of novel methods such as CSIA methods and enantiomer analyses.

Résumé étendu en français

Les pesticides, encore appelés produits phytosanitaires sont des contaminants actuels majeurs, fréquemment détectés dans tous les compartiments de l'environnement, à savoir les sols, l'atmosphère, les organismes vivants, les eaux de surface et les eaux souterraines. Cette contamination massive par les pesticides menace grandement l'état de santé des écosystèmes, ainsi que la qualité de la ressource en eau et la santé humaine et requiert de ce fait une attention toute particulière. Le développement de l'industrie agrochimique conduit à l'arrivée sur le marché de molécules à la structure de plus en plus complexe, dont le devenir environnemental et la toxicité sont encore méconnus. Parmi ces molécules émergentes, les pesticides dit chiraux représentent environ 30% des molécules actuellement commercialisées et consistent en une mixture de deux (ou plus) énantiomères (Figure 1).

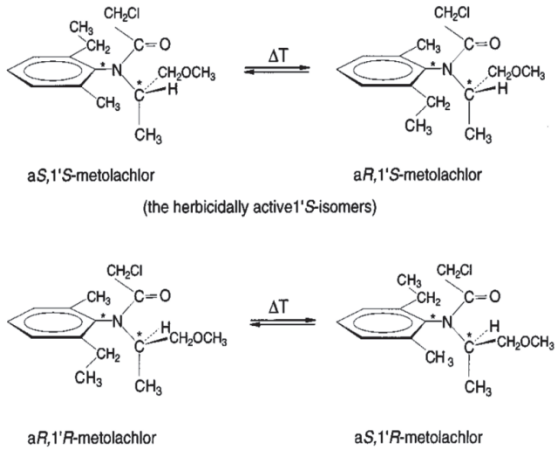


Figure 1 : Structure des 4 stéréoisomères du métolachlore (en haut : énantiomères S et en bas : énantiomères R.

Les énantiomères sont des molécules identiques, étant l'image l'une de l'autre dans un miroir. Les énantiomères d'un même pesticide possèdent des propriétés physico-chirniques identiques et ont de ce fait un comportement similaire dans l'environnement au regard des processus abiotiques (solubilité, sorption, photodégradation). En revanche, les énantiomères peuvent subir des processus biologiques dits énantioséléctifs, conduisant à la biodégradation préférentielle d'un des énantiomères lors de réactions enzymatiques. En d'autres termes, l'énantiomère présentant la configuration spatiale la plus favorable aux réactions enzymatiques est généralement préférentiellement absorbé ou dégradé par les plantes ou les microorganismes tandis que l'énantiomère présentant un encombrement stérique plus important tend à

s'accumuler dans l'environnement. Cependant, le devenir des pesticides émergents, incluant les pesticides chiraux, dans l'environnement demeure peu étudié. La compréhension des mécanismes régissant le transport et la dégradation des pesticides agricoles émergents est donc un enjeu actuel majeur pour pouvoir prédire leur devenir et leur impact environnemental.

Dans les zones agricoles, les zones humides peuvent collecter des eaux de ruissellement contaminées par les pesticides ou être connectées à un aquifère et ainsi intercepter des eaux souterraines contaminées durant les périodes de crue. Les zones humides sont des zones d'interface entre un écosystème terrestre et un écosystème aquatique ayant la particularité d'être des milieux biogéochimiquement actifs dans lesquelles coexistent des conditions hydrologiques, physico-chimiques et biologiques très variées. Cette particularité confère aux zones humides une fonction de bioréacteur qui permet le traitement des contaminants, dont les pesticides par le biais d'un large panel de voies de dégradations (Figure 2).

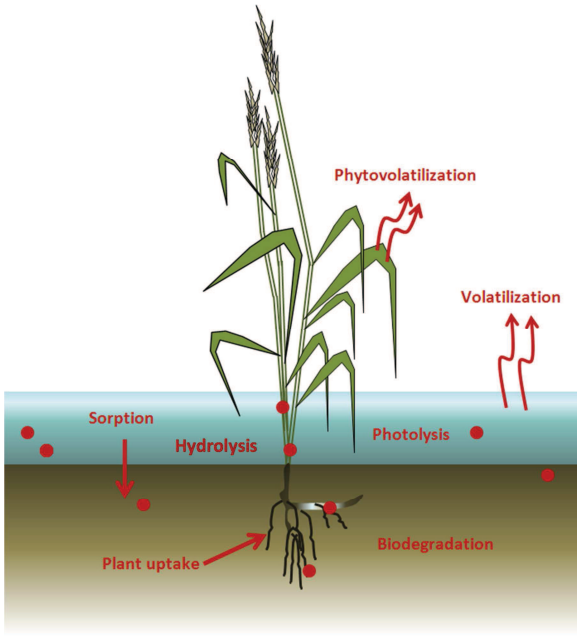


Figure 2 : Processus de dissipation des pesticides dans les zones humides

Les zones humides peuvent donc être utilisées comme des laboratoires naturels, pouvant servir à l'étude des processus de transport et de dégradation des pesticides dans l'environnement. Les processus de transport et de dégradation des pesticides dans les zones humides sont extrêmement complexes et sont régis par un très grand nombre de facteurs, comme la fréquence et l'intensité des pluies, la végétation, les propriétés physico-chimiques des molécules de pesticides ou encore les conditions biogéochimiques. L'influence de ces différents facteurs sur

les mécanismes de transport et de dégradation des pesticides demeure encore peu connue et rarement quantifiée. Durant les 20 dernières années, de nombreuses études ont démontré que les zones humides étaient des systèmes très efficaces pour traiter les pesticides. Cependant, les mécanismes régissant les processus intrinsèques de dissipation des pesticides dans les zones humides sont rarement investigués, considérant ces systèmes comme des boites noires. Le manque de connaissances qui en résulte réside en particulier dans le fait qu'il est très difficile de lier les données de dégradation obtenues en laboratoire, avec les observations de terrain. De ce fait, la compréhension du devenir des pesticides dans les zones humides requiert la combinaison de différentes approches et l'intégration de plusieurs échelles.

Tandis que les études à large échelle (études de terrain) permettent de comprendre les dynamiques de stockage et de dégradation des pesticides en lien avec le fonctionnement global des zones humides, les études à petite échelle permettent d'appréhender les processus moléculaires régissant les réponses observées à plus grande échelle. Le recours à une approche multi-échelles présente donc l'avantage d'obtenir des informations mécanistiques sur le transport et la dégradation des pesticides dans les zones humides et de les transposer à plus large échelle afin de comprendre le devenir des pesticides dans l'environnement (Figure 3).

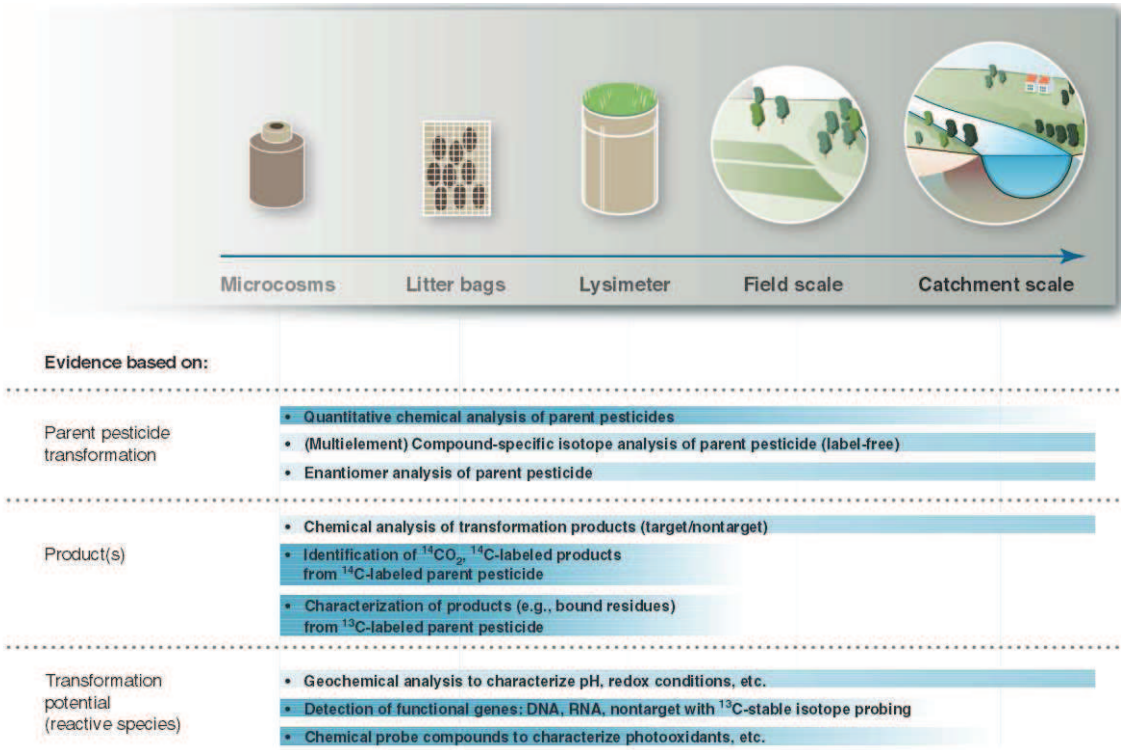


Figure 3 : Méthodes analytiques disponibles pour l'étude de la transformation des pesticides.

De plus, la combinaison de différentes techniques analytiques incluant les analyses des

composés parents, des produits de dégradations ainsi que des analyses isotopiques ou énantiomériques permet de distinguer les différents processus et voies de dégradation impliqués dans la dissipation des pesticides dans les zones humides.

Le but principal de cette thèse est de comprendre le transport et la dégradation des pesticides dans les zones humides en lien avec le fonctionnement du système (hydrologie, développement biogéochimique). Dans une approche multi-échelles, trois zones humides différentes recevant des eaux contaminées par les pesticides ont été étudiées afin de répondre aux objectifs suivants:

• Un bassin d'orage (320 m^2) a été étudié (Chapitre III) afin de:

i) Evaluer la capacité du bassin d'orage à dissiper un cocktail de pesticides provenant d'un bassin versant viticole durant plusieurs saisons agriculturales successives;

ii) Evaluer l'influence de la répartition des pesticides entre la phase aqueuse et les particules de sol sur leur dissipation par la zone humide;

iii) Distinguer les processus de dissipation et quantifier la rétention et la dégradation en effectuant des bilans de masse, et lier cette dissipation au fonctionnement de la zone humide;

iv) Evaluer la distribution des pesticides parmi les différents compartiments de la zone humide et leurs propriétés de puits/source durant une saison agricole;

• Des lits plantés (7 m'') ont été étudiés (Chapitre IV) afin de:

i) Comparer le transport et la degradation d'un herbicide chiral, le S-métolachlore (produit commercial Mercantor Gold®) à ceux de traceurs fluorescents photosensibles et hydrophobes et déterminer si ces traceurs peuvent être utilisés comme des analogues du S-métolachlore et plus généralement du comportement des pesticides;

ii) Comprendre l'influence des changements hydrologiques et biogéochimiques sur la dissipation du S-métolachlore;

iii) Evaluer la biodégradation *in-situ* du S-métolachlore en utilisant des techniques innovantes (analyses énantiomériques et isotopiques);

• Des colonnes de laboratoire (0.02 m^2) ont été étudiées (Chapitre V) afin de:

i) Comparer le transport et la dissipation de 3 herbicides de la famille des chloroacétanilides, à savoir l'acétochlore, l'alachlore et le métolachlore;

ii) Evaluer la biodégradation *in-situ* des chloroacétanilides par la combinaison d'analyses isotopiques et énantiomériques.

Dans le chapitre III, un bassin d'orage a été étudié afin d'évaluer le potentiel de cette zone humide recevant de l'eau de ruissellement contaminée par les pesticides à traiter ces polluants pendant plusieurs saisons agriculturales successives (sections 1, 2 et 3) (Figure 4).

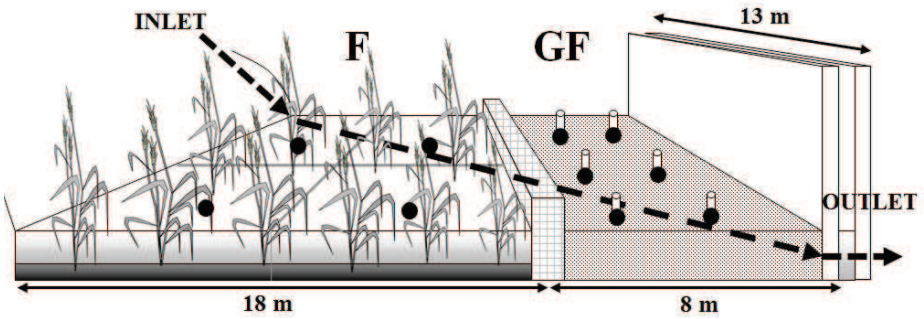


Figure 4 : Schéma du bassin d'orage d'étude.

Les résultats ont montré que le système dissipe efficacement les pesticides dissous et particulaires (efficacité totale: 73%) et que la dissipation varie au cours du temps, avec une atténuation globale plus importante lorsque les conditions sont oxiques au printemps, comparé à l'été (section 1). Les fluctuations du régime hydrique entrainant des changements drastiques des conditions redox et le développement de la végétation impactent grandement la dissipation des pesticides dans le bassin d'orage et en particulier leur potentiel de biodégradation. De plus, des taux de dissipation variables ont été observés en fonction des caractéristiques physico-chimiques des molécules de pesticides, allant de 39% pour la simazine à 100% pour le cymoxanil, gluphosinate, kresoxim methyl et terbutylazine (section 1).

Dans une seconde étude (section 2), le partitionnement, le transport, le stockage et la dégradation d'un cocktail de 12 pesticides utilisés en viticulture ont été systématiquement quantifiés dans la phase aqueuse, les matières en suspension, les sédiments, la végétation et les invertébrés du bassin d'orage durant une saison agriculturale. Nos résultats ont montré i) que les pesticides dissous représentaient plus de 95% de la charge totale entrant dans le bassin, ii) que le partitionnement était très différent selon les molécules et iii) que plus de 90% de l'AMPA et des dithiocarbamates étaient stockés dans les sédiments fins ‹ 250 um) au début du printemps et à la fin de l'été. L'atténuation des pesticides n'était pas significativement influencée par le régime hydrologique et la charge entrante, mais variaient en fonction des molécules et des conditions biogéochimiques prévalentes dans le système. La végétation contribue à la dégradation des pesticides à la période de développement de la végétation, en permettant en particulier la dégradation microbienne au niveau de la rhizosphere mais contribue également à un relargage de pesticides durant la dégradation des végétaux. Les dithiocarbamates sont

préférentiellement degradés en conditions oxiques au printemps, alors que la dégradation du glyphosate et de l'AMPA ont lieu principalement en conditions anoxiques en été. Cette seconde étude a permis de déterminer les propriétés puits et source du bassin d'orage concernant le transport des pesticides au cours d'un cycle végétatif des macrophytes. Elle a également permis d'identifier les compartiments de la zone humide jouant un rôle prépondérant dans le stockage des pesticides (sédiments et racines des macrophytes) et l'évolution du stockage au cours du temps. La dégradation, quantifiée d'après le bilan de masse, est le processus dominant de dissipation dans cette étude, en particulier durant la période d'application des pesticides (section 2) (Figure 5).

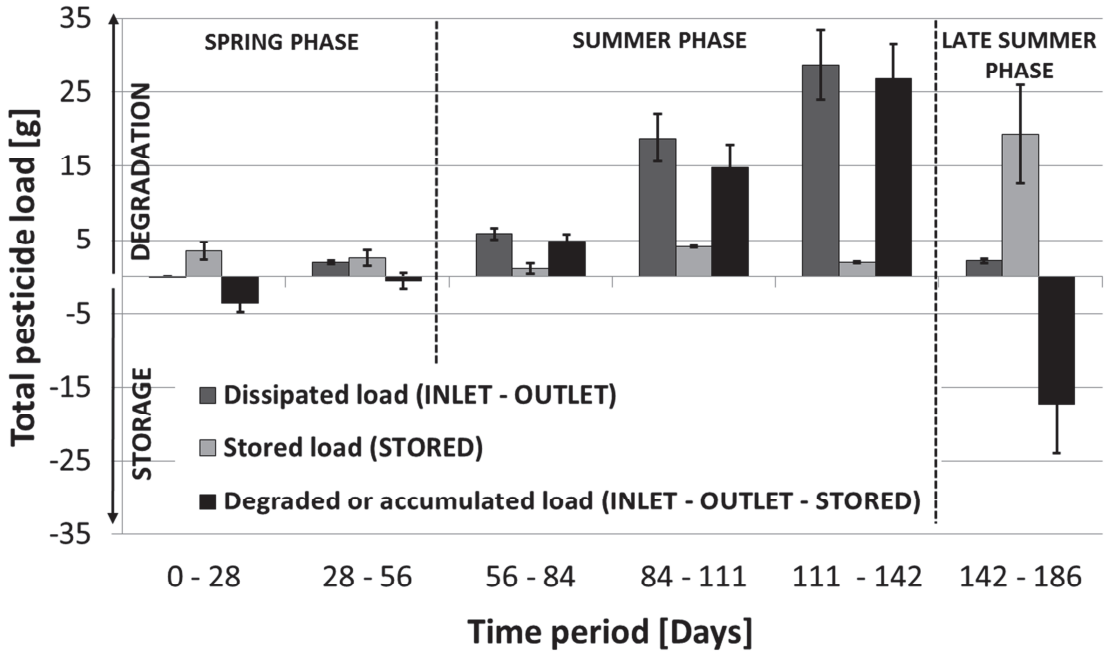


Figure 5 : Dynamiques de degradation et de stockage des pesticides totaux dans le basin d'orage au cours d'une saison agriculturale.

Afin de comprendre plus spécifiquement les processus de dégradation des pesticides ayant lieu dans la zone humide, une troisième étude a visé à caractériser le transport et la dégradation d'un composé modèle, le glyphosate et de son principal produit de dégradation, l'acide aminométhylphosphonique (AMPA) durant 3 saisons agriculturales consécutives (section 3). L'abattement massique total du glyphosate et de l'AMPA variait annuellement de 75% à 99%. Le glyphosate et rAMPA ont été très peu détectés dans les sédiments du bassin d'orage indiquant que la sorption n'était pas le processus de dissipation dominant dans cette étude. La dissipation de ces 2 substances est majoritairement attribuée à la biodégradation et à la sorption sur les macrophytes qui augmente au cours du temps. La biodégradation du glyphosate en AMPA était principalement liée au développement de la végétation, la végétation étant un support important

de l'activité microbienne au niveau de la rhizosphere (section 3). Le pourcentage d'AMPA par rapport au glyphosate très élevé à la sortie comparé à l'entrée traduit la dégradation du glyphosate dans le bassin d'orage par la voie de l'AMPA (Figure 6).

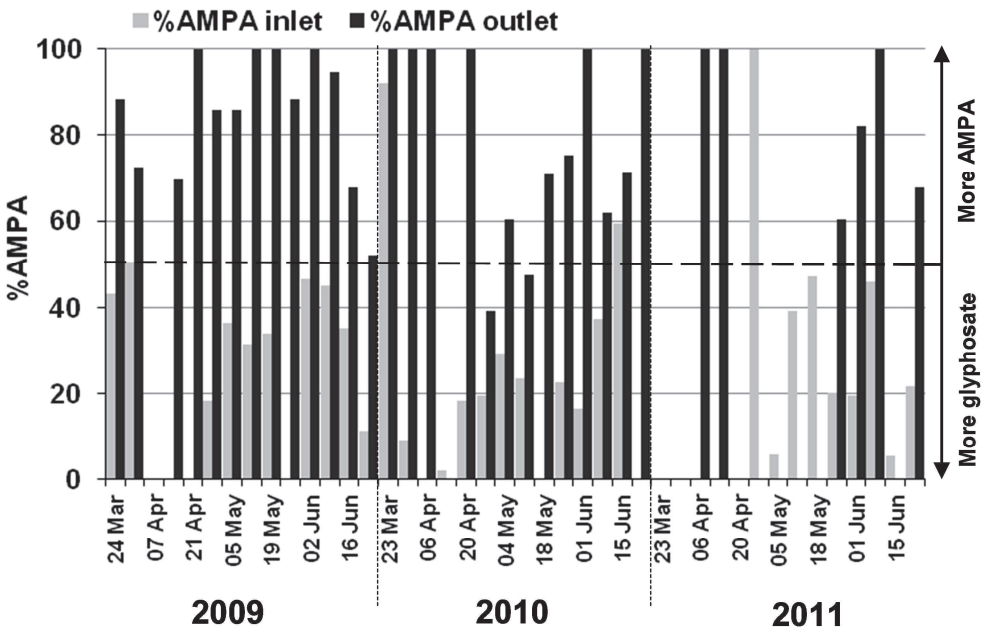


Figure 6 : Evolution temporelle de la proportion relative d'AMPA comparé à la masse totale de glyphosate et d'AMPA à l'entrée et à la sortie du bassin d'orage.

Pour résumer, les études du chapitre III ont permis d'obtenir des informations sur la dissipation des pesticides dans une étude de terrain à grande échelle. II a été montré que la dégradation est un processus de dissipation important en été, pendant la période d'application des pesticides, tandis que le stockage a majoritairement lieu au printemps et en fin d'été, lorsque la végétation est peu développée ou commence à décliner. Cependant, les processus de dissipation des pesticides sont difficilement distinguables et quantifiables dans les conditions de terrain dû à la complexité des facteurs environnementaux influençant ces processus. Afin de comprendre l'influence des conditions environnementales sur la dissipation des pesticides, le transport et la dégradation d'un composé modèle, le S-métolachlore ont été étudiés à l'échelle de mésocosmes, dans des lits plantés (chapitre IV), et dans des zones humides de laboratoire (chapitre V). Le S-métolachlore est l'un des herbicides les plus utilisés au monde et a la particularité d'être chiral, pouvant potentiellement être dégradé de façon énantioséléctive. Dans les études suivantes, des analyses des produits de dégradation couplées à des techniques émergentes d'analyses isotopiques et énantiomériques ont été utilisées pour caractériser les processus de biodégradation.

Dans le chapitre IV, l'influence de différents régimes hydrauliques sur la dissipation du S-métolachlore a été étudiée. L'étude a porté sur le transport et la dissipation du S-métolachlore dans des lits plantés, alimentés respectivement à l'aide d'un flux continu et de « batchs » (Figure 7).

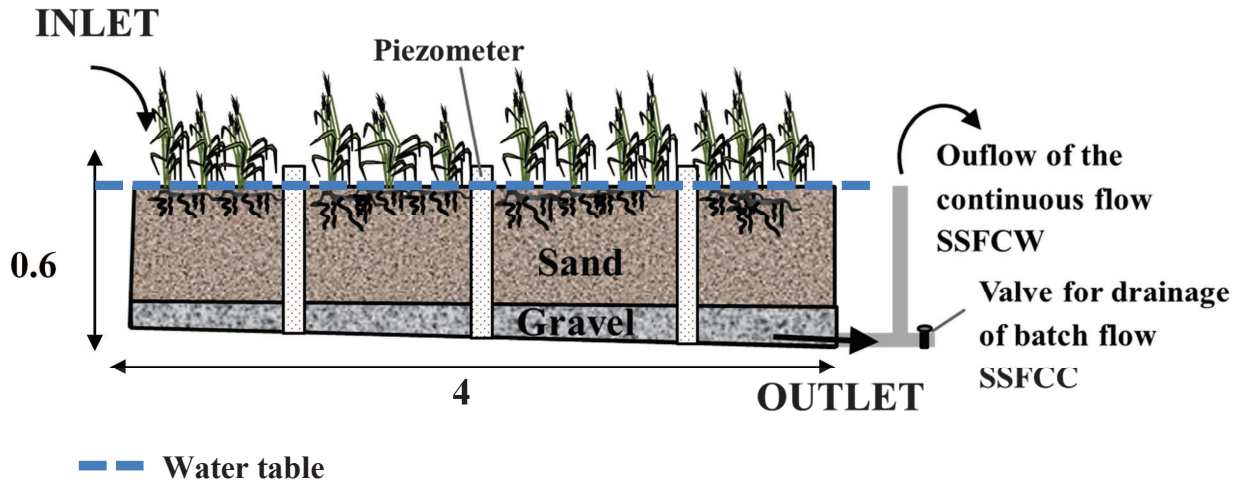


Figure 7 : Schéma du design expérimental des lits plantés étudiés.

Deux traceurs fluorescents, l'uranine qui est photosensible (UR) et la sulphorhodamine B (SRB) qui est hydrophobe, ont servi de traceurs de référence pour comprendre le comportement du S-métolachlore dans les deux systèmes. La biodégradation a été évaluée en combinant des analyses i) des composés parents et des produits de dégradation, ii) des énantiomères iii) des isotopes stables du carbone. Les conditions d'oxydoréduction étaient plus élevés dans le système en batch (concentration en oxygène: 3,1 ± 1,8 mg L^{-1}; Eh: de -440 à 320 mV) par rapport au système alimenté par un flux continu (concentration en oxygène: 1,0 ± 1,3 mg L^{-1}; Eh: de -560 à -190 mV). Les résultats ont montré que les conditions de batch étaient plus efficaces pour dissiper le S-métolachlore (taux de dissipation du S-métolachlore : 93 ± 3,4 %) ainsi que les traceurs UR et SRB, comparé au flux continu (taux de dissipation du S-métolachlore : 27 ± 3,5 %), soulignant la dégradation préférentielle de ces substances en conditions plus oxiques (Figure 8).

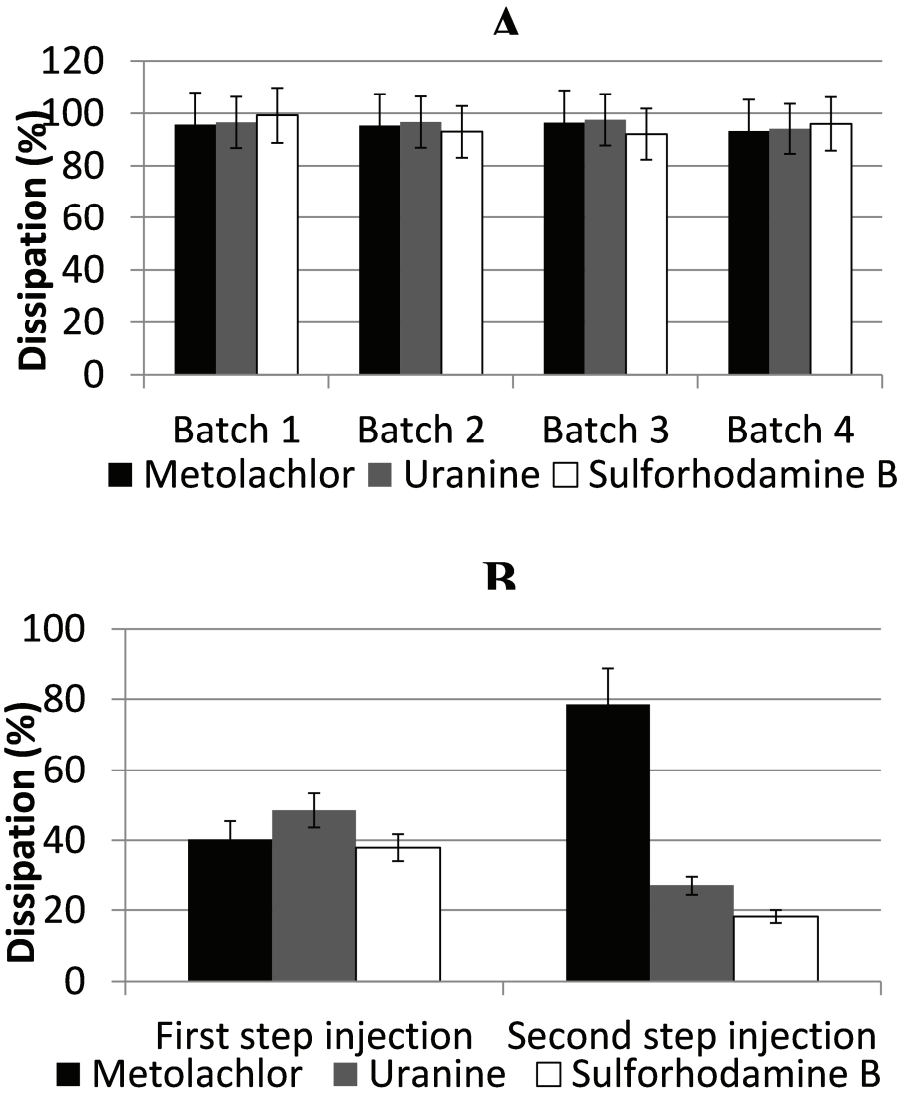


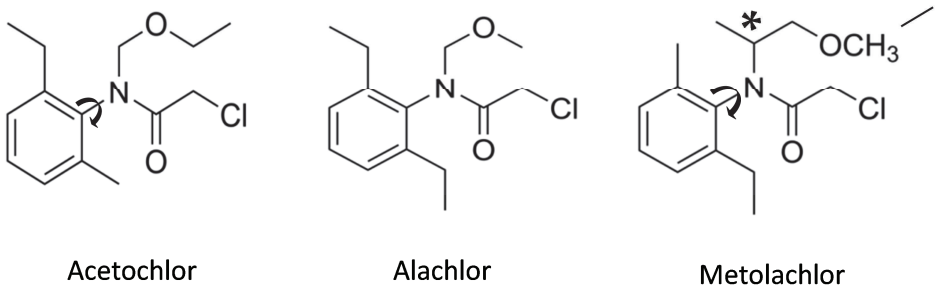
Figure 8 : Dissipation du métolachlore, de l'uranine et de la sulphorhodamine B dans les 2 lits plantés.

Les analyses isotopiques ont confirmé la présence de biodégradation *in-situ* du S-métolachlore dans le système fonctionnant en batch en conditions moins réductrices ($\Delta\delta^{13}C_{inlet\text{-}outlet}$ = 1,2‰) alors qu'aucun shift isotopique n'a été observé dans le système alimenté par un flux continu. Le fractionnement énantiomérique (EF, représente l'excès de l'énantiomère S par rapport au R) varie au cours du temps, de 0,71 à 0,81 et de 0,72 à 0,85 dans le flux continu et le système en batch, ce qui indique une alternance de dégradation des énantiomères R et S en fonction des conditions biogéochimiques mais pas de dégradation préférentielle de l'un des énantiomères. Ce résultat traduit la potentielle co-existence d'enzymes dégradant préférentiellement l'un et l'autre des énantiomères, conduisant à la non-détection d'une dégradation prédominante. Dans le lit en batch, les taux de recouvrement similaires entre les traceurs et le pesticide ont montré que UR et SRB pouvaient servir de proxy pour le transport du métolachlore dans les zones

humides. Cette étude souligne l'importance et la nécessité de combiner des outils innovants afin de mieux comprendre les processus de biodégradation des pesticides dans les zones humides.

Cette deuxième étude a montré que la biodégradation du produit commercial de S-métolachlore a principalement lieu dans le lit en batch, comparé au système en flux continu, plus anoxique. Pour diminuer le niveau de complexité de l'étude, une dernière expérience a été réalisée dans des zones humides de laboratoire dans des conditions contrôlées, avec des substances actives pures d'herbicides. Le transport et la dégradation du métolachlore racémique (contenant 50% d'énantiomère S et 50% d'énantiomère R) ont été comparés à deux autres herbicides de la famille des chloroacétanilides, l'alachlore et l'acétochlore dont les structures chimiques sont très proches de celle du métolachlore. Les chloroacétanilides sont largement utilisés aux États-Unis et en Europe sur une variété de cultures, comme le maïs, la betterave à sucre et le tournesol et sont des contaminants environnementaux majeurs. L'objectif du chapitre V est de comparer le transport et la biodégradation de chloroacétanilides présentant des similitudes structurelles mais possédant des groupements fonctionnels différents, dans des zones humides de laboratoire afin d'évaluer l'influence de la structure chimique des pesticides sur les processus de biodégradation.

Dans le chapitre V, le transport et la dégradation de trois herbicides de la famille des chloroacétanilides, le métolachlore, l'alachlore et l'acétochlore (Figure 9) ont été étudiés dans des colonnes de laboratoire plantées (Figure 10), en combinant des analyses hydrochimiques et biomoléculaires classiques à l'analyse isotopique et énantiomérique.



Acetochlor Alachlor Metolachlor

Figure 9 : Structure des 3 herbicides chloroacétanilides étudiés.

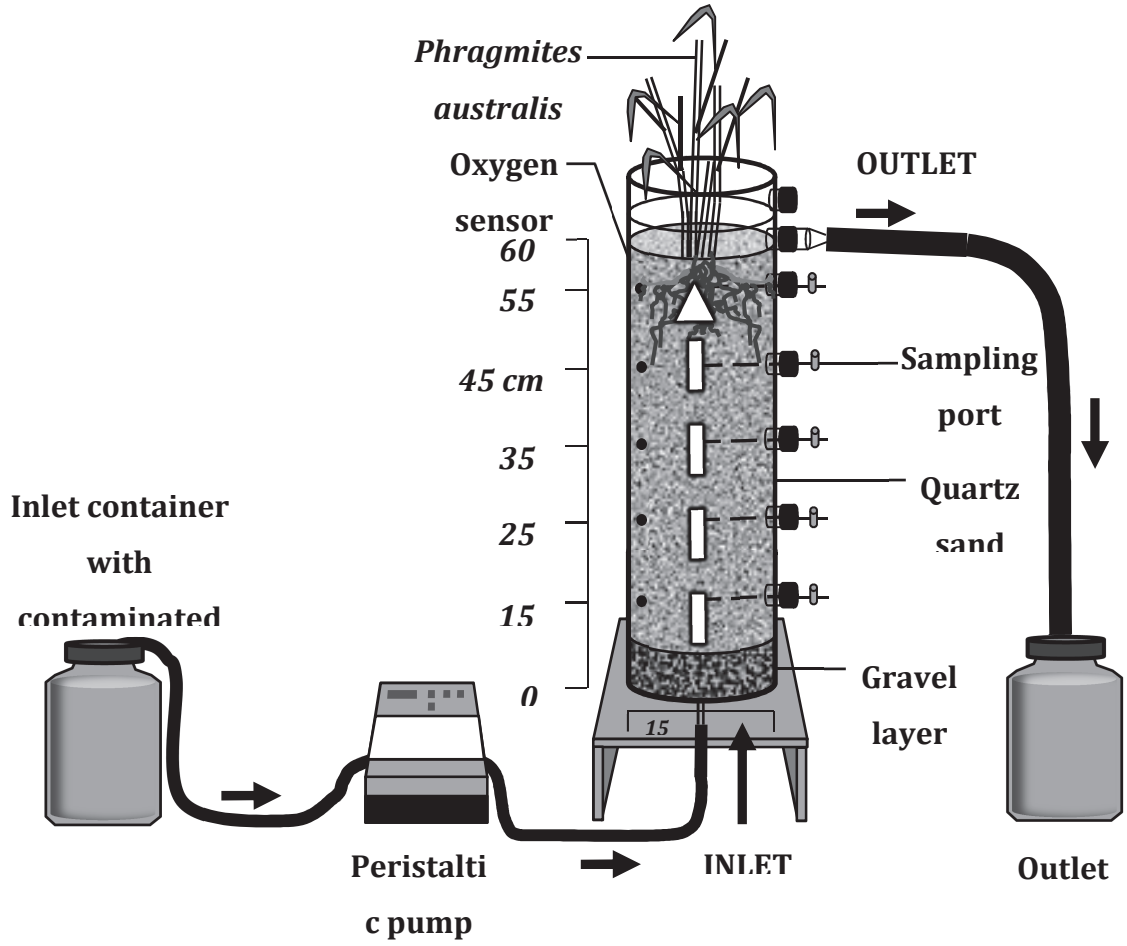


Figure 10 : Schéma du dispositif expérimental.

Les changements de conditions hydrochimiques ont été évalués en utilisant des espèces redox-sensibles. Les concentrations en oxygène variaient de 6,8 ± 0,8 mg L^{-1} à 0,7 ± 1,3 mg L^{-1}, respectivement à l'entrée et à la sortie de chaque zone humide indiquant un gradient d'oxydo-réduction le long du flux, allant de conditions oxiques à la base des colonnes à des conditions anoxiques au niveau de la rhizosphere. Les trois herbicides sont préférentiellement biodégradés dans la zone racinaire, où une activité microbienne accrue est attendue due à la présence d'exsudats racinaires, sources de carbone nécessaires à la croissance bactérienne. Une réduction des nitrates a été observée dans la rhizosphere de toutes les colonnes. Une dissipation importante a été observée pour l'alachlore et l'acétochlore (52 ± 12% et 61 ± 14% respectivement) alors que le métolachlore était plus persistant (29 ± 19%). La grande persistance du métolachlore peut être expliquée par la substitution d'une chaîne latérale alkoxyméthyle, présente dans les molécules d'alachlore et d'acétochlore par une chaine alkoxyéthyle plus volumineuse dans le métolachlore, ce qui a pu conduire à un plus grand encombrement stérique autour de la liaison chlorure-carbone et donc à une diminution de la biodégradation microbienne. De plus, il a été démontré dans des études précédentes que le

métolachlore était potentiellement toxique pour les bactéries, ce qui expliquerait également son faible taux de biodégradation (section 1). La modification de la composition isotopique du carbone entre l'entrée et la sortie des colonnes de zones humides *($\Delta\delta^{13}C$)* étaient de 2,6, 2,5 et 0,7‰ (erreur standard: 0.5‰), pour l'acétochlore, l'alachlore et le métolachlore respectivement indiquant une biodégradation similaire de l'acétochlore et de l'alachlore comparé au métolachlore, plus persistant (section 2). La dégradation des chloroacétanilides est supposée être principalement co-métabolique, par la voie de l'enzyme Gluthation-S- Transférase (GST), d'origine végétale ou microbienne. La voie de la GST conduit à la formation de 2 métabolites ioniques principaux, l'acide éthane sulfonique (ESA) et l'acide oxanilique (OXA) pour chacun des trois chloroacétanilides. Les produits de dégradation ESA et OXA ont principalement été détectés à partir du jour 70, en particulier pour l'alachlore et l'acétochlore, confirmant une nouvelle fois la persistance du métolachlore. L'ESA a été le produit de dégradation le plus fréquemment détecté au cours de l'étude. La prédominance de l'ESA comparé à l'OXA peut être due à la présence de sulfites dans les colonnes qui a pu conduire à la dégradation abiotique des chloroacétanilides par substitution de l'atome de chlore par le sulfite, comme démontré dans des études précédentes. Un fractionnement énantiomérique (EF) significatif du métolachlore a été observé au niveau de la rhizosphère (EF = 0.480 ± 0.005), indiquant une biodégradation préférentielle de l'énantiomère S par rapport à l'énantiomère R, alors qu'aucun fractionnement n'a été observé dans la zone oxique (EF = 0.494 ± 0.006) (Figure 11).

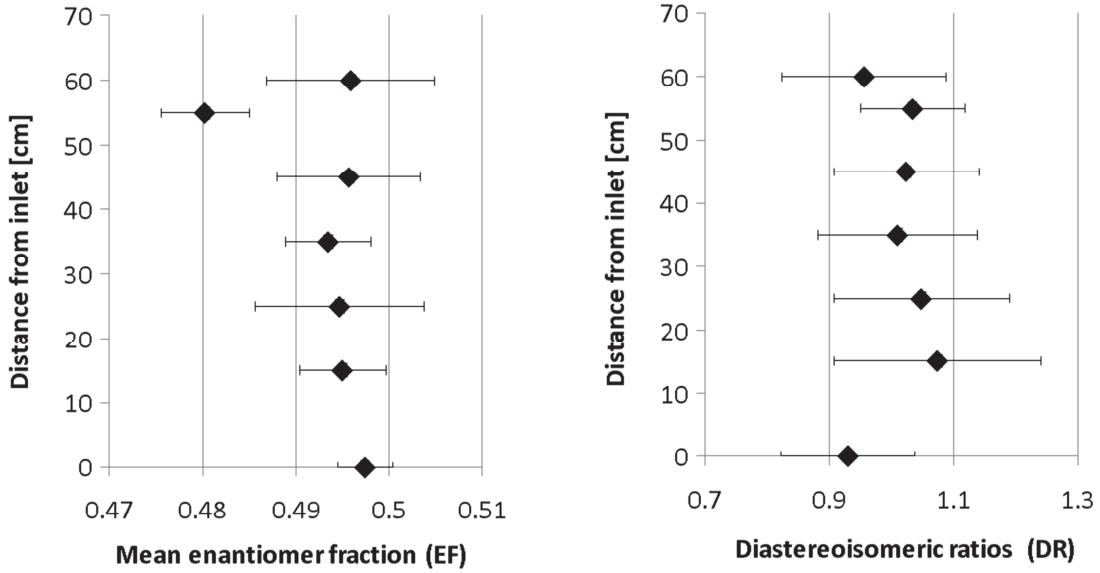


Figure 11 : Evolution spatiale de la fraction énantiomérique et du ratio diastéréoisomérique du métolachlore.

Ce résultat démontre que l'énantiomère R peut persister et s'accumuler au fil du temps dans les zones humides, entraînant des risques écotoxicologiques potentiels sur les organismes non-cibles. Des études précédentes ont en effet montré une plus grande toxicité de l'énantiomère R pour de nombreux organismes, incluant les vers de terre ou les invertébrés aquatiques. Cette étude a montré l'utilité de combiner les analyses énantiomérique et isotopique pour évaluer le transport et la biotransformation *in-situ* des chloroacétanilides dans l'environnement.

Pour conclure, les études de terrain ont montré que le partitionnement influençait la dissipation des pesticides dans les zones humides avec un possible relargage de pesticides dissous durant les périodes où le bassin d'orage est moins efficace, au printemps et surtout en automne, lorsque la végétation commence à dépérir. La dégradation et le stockage des pesticides sont étroitement liés au développement biogéochimique de la zone humide. En particulier, la végétation joue un rôle important dans la dégradation des pesticides en été en augmentant la dégradation microbienne dans la zone racinaire, mais peut également agir comme une source de pesticides pendant la période de sénescence des plantes. L'interaction des conditions hydro-biogéochimiques peuvent influencer la distribution des pesticides au fil du temps dans les différents compartiments, contrôlant ainsi la dégradation des pesticides et leur rétention dynamique. Cette étude est la première à évaluer le fonctionnement puits-source d'une zone humide vis-à-vis de la contamination par les pesticides.

Bien que les études à grande échelle fournissent des informations intégratives sur le transport des pesticides et leur distribution dans les zones humides, des études à petite échelle sont nécessaires pour caractériser les processus moléculaires sous-jacents qui régissent le transport des pesticides et leur transformation. Cette thèse met en évidence la nécessité de diminuer la complexité des systèmes d'étude en contrôlant des paramètres essentiels tels que le flux de contamination et la charge hydraulique, la température ou l'éclairage afin d'avoir un meilleur aperçu des processus qui régissent le transport et la dissipation des pesticides.

L'étude en mésocosmes (lits plantés) a montré que les conditions hydrologiques ont grandement influencé la dégradation de l'herbicide modèle chiral S-métolachlore. La dynamique des conditions redox qui caractérisent les zones humides est étroitement liée aux conditions hydrologiques et joue un rôle essentiel dans la dégradation des pesticides dans les zones humides. La combinaison des analyses énantiomériques et isotopiques est apparue comme une approche pertinente pour mieux comprendre les processus de biodégradation des chloroacétanilides dans les zones humides, et pourrait être mise en œuvre dans d'autres systèmes hydrologiques.

L'étude à l'échelle du laboratoire a fourni des informations sur l'influence de la structure chimique des pesticides sur leur biodégradation. Les variations structurelles entre les composés de la même famille chimique a conduit à d'importantes variations en termes de biodégradation. Le plus grand encombrement stérique de la molécule de métolachlore par rapport à l'alachlore et à l'acétochlore peut avoir induit des vitesses de dégradation plus faibles.

Les résultats obtenus au cours de cette thèse sont un point de départ pour mieux comprendre le transport et la transformation de polluants émergents organiques dans des environnements biogéochimiquement actifs par la combinaison de nouvelles méthodes telles que les méthodes isotopiques et énantiomériques. Cette étude montre également que les zones humides sont des laboratoires pertinents pour l'étude des processus de transport et de dégradation fondamentaux de nouveaux contaminants organiques dans l'environnement. Bien que les études à grande échelle permettent de déchiffrer les processus de dégradation et de rétention des pesticides, les études à fine échelle sont nécessaires pour comprendre les processus intrinsèques de dégradation et de transport des pesticides.

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Chapter I. General Introduction

1. Pesticides in the environment: the current challenges

1.1. Definition and usage of pesticides

Pesticides, also called phytosanitary products or more recently plant protection products, are defined according to the European legislation (regulations (EC) No 283/2013 and 284/2013) as "*active substances and preparations containing one or more active substances intended to i) protect plants or plant products against all harmful organisms or prevent the action of such organisms, ii) influence the life processes of plants, other than as a nutrient (e.g. growth regulators), iii) preserve plant products, in so far as such substances or products are not subject to special Council of commission provisions on preservatives, iv) destroy undesired plants, or v) destroy parts of plants, check or prevent undesired growth of plants*" (European commission, 2013).

About 2.4 million tons of pesticides are yearly applied worldwide, with herbicides accounting for the largest portion of the total use (39%), followed by other pesticides that include nematicides and fumigants (33%), insecticides (18%) and fungicides (10%) (US EPA, 2011). In 2003, the total amount of pesticides used in Europe was 219,729 tons, including 107,561 tons of fungicides (49%), 83,934 tons of herbicides (38%), 7,809 tons of insecticides (4%) and 20,425 tons of other pesticides (9%) (Eurostat, 2007). It is estimated that about 90% of the total pesticides are used in agriculture worldwide, whereas 10% are used in urban areas or for non-agricultural purposes (e.g. railway or cimentery weed control).

France is the fourth world's largest consumer and the first European consumer of pesticides with 62.700 tons traded in 2011, including 48.800 tons of organic pesticides and 13.900 tons of copper sulfate (used as a fungicide, mainly in organic farming) (Chauvel, 2012; UIPP, 2013). French field crops represent 45.7% of the total cultivated area and use 67.4% of commercialized pesticides, being the most important pesticide-consuming cultures. Vineyard is the second type of agriculture that consumes pesticides, representing 14.4% of the total pesticide use whereas it only concerns 3.3% of the total French cultivated area (Butault et al,

2011). Since they are massively applied worldwide, pesticides are considered as one of the main factors involved in environmental contamination of today's world (Mostafalou and Abdollahi, 2013).

1.2. Pesticide transport in the environment

During and after application on target areas (field crops), pesticides become dispersed in the environment through several transport processes and can thus contaminate non-target compartments, i.e. soils, air, surface and groundwater (Figure I-1). During application, it is assumed that the main portion of the applied active substance is lost in the atmosphere because of spray drift (Carlsen et al., 2006; Doruchowski et al., 2013; Hilz and Vermeer, 2013). After application, pesticides losses typically range between 50 and 60% due to volatilization and can sometimes reach up to 90%, depending on the molecules (Grégoire et al., 2009). Once on the soil, several processes of pesticides transport are likely to occur (Tang et al., 2012). Groundwater contamination is mainly due to pesticide leaching through infiltration whereas surface water contamination comes from surface runoff or tile drainage water (Grégoire et al., 2009). Leaching is the process whereby dissolved pesticides are carried downward through the soil profile to groundwater, mainly by rainwater. Runoff refers to water flowing over the ground surface that does not infiltrate the soil. Leaching and surface runoff are the most important transport processes contributing to the contamination of non-target water bodies. In 2011 in France, pesticides were found in 93% and 63% of the surface and ground-water sampling points, in particular where agriculture predominates (French Ministry of Environment and Sustainable development, 2013). Pesticide widespread contamination raises critical issues regarding ecosystems sustainability, drinking water quality and human health (Vorosmarty et al., 2010).

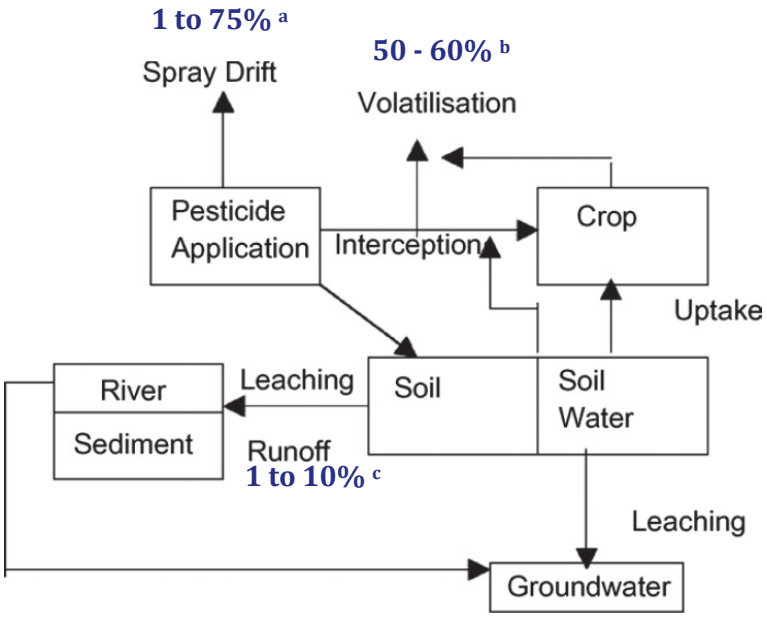

Figure I-1. Pesticides transport pathways after application (adapted from Arias-Estévez et al, 2008) ([a]Barbash, 2007; [b]Grégoire et al., 2009; [c]Schulz, 2004)

The ecotoxicological effects of pesticides and their degradation products on various organisms, such as earthworms (Spurgeon et al., 2011), soil or sediment bacteria (Widenfalk et al., 2008) or aquatic invertebrates (Beketov et al., 2013), have been demonstrated recently. Ecotoxicological effects can be determined directly in the field or more frequently in laboratory tests, at the individual, population, community or ecosystem level. Ecotoxicological tests have demonstrated the acute and/or chronic toxicity of a wide range of currently used pesticides. For example, the herbicide *S*-metolachlor showed a LC_{50} (Lethal Concentration = concentration at which half of the population died after an exposure of 14 days) to *Daphnia magna* of 51.2 mg L^{-1} (Liu et al., 2006). The herbicides acetochlor and alachlor that have an EC_{50} (Effect Concentration = concentration causing a response in 50% of the tested parameter) for green algae of 2.5 and 6.7 µg L^{-1} (Souissi et al., 2013), whereas the insecticide carbofuran presents a lower LC_{50} of 0.49 mg kg^{-1} for earthworms (Saxena et al., 2014). In a recent study, Beketov et al. (2013) showed a significant ecotoxicological effect of pesticides on the richness of stream invertebrates in France and Germany, at concentrations that current legislation considers environmentally-protective. The bioavailability of pesticides plays a key role in toxicity. In ecotoxicological studies, the bioavailability is defined as the extent to which a xenobiotic is available to living organisms. Bioavailability is closely linked to sorption/desorption processes of pesticides on soil mineral or

organic particles. Sorption is known to decrease contaminant bioavailability and thus their toxicity (Yu et al., 2006).

Concerning the relationship between pesticides and human health, there are today growing evidences that human long-term exposure to pesticides via dietary (drinking water and food) and atmospheric exposure (dust, breathing air) is associated with an elevated rate of chronic diseases, including different cancers and neurodegenerative disorders such as Alzheimer and Parkinson (Parrón et al., 2013). Organophosphates, organochlorines, carbamates, pyrethroids, which are the major groups of pesticides, have been reported to be carcinogenic in various models and studies (George and Shukla, 2011). Most of them, and in particular old organochlorine pesticides such as DDT or chlordane, which are still present in the environment, have been recognized as endocrine disruptors (Mostafalou et al., 2013).

Since the 1960s, pesticide use has been regularly called into question for environmental, toxicological and agronomical reasons (Chauvel et al., 2012). Pesticides and their degradation products are today among the most relevant environmental micro-contaminants and the control of their release in the environment has become of utmost importance.

1.3. Modern pesticide molecules: an increasing complexity

About 1000 different active substances were available on the market worldwide before 1993. The majority (67%) was removed from the market after their evaluation by the European commission, with only 250 passing the harmonized EU safety assessment. Due to their high persistence in the environment, a large range of banned pesticides are still detected in all the environmental compartments, at concentrations that can be comparable to those observed for currently used molecules. For example, even 40 years after its ban, residues of one of the most used insecticide worldwide, DDT are still present in soils of agricultural regions (Huang and Wang, 2013). In addition, along with the ban of a large range of active substances, new substances continuously appear on the market. Currently, there are more than 1,055 active substances registered as pesticides that are found in 16,000 pesticide commercial products (Sierka, 2013). More emerging compounds with more complex structures are commercialized today along with the development of agrochemical industries.

Among them, chiral pesticide molecules account for 30% of currently used modern pesticides and consist of a mixture of two (or more) enantiomers, which are non-superimposable mirror images of each other (Ye et al., 2010). Among chiral pesticides, 34% are insecticides, 27% are herbicides and 18% are fungicides (Ulrich et al., 2012). Newly developed chiral compounds are supposed to be more "environmentally-friendly" molecules because their formulation is enriched in the active enantiomer(s) and minimize the presence of the inactive enantiomer(s) that can, in some cases, be even more toxic. Enantiomers of chiral pesticides have identical physico-chemical properties and are expected to undergo similar abiotic transport and transformation processes in the environment. However, it is well known these increasingly commercialized molecules can undergo enantioselective biological processes (Celis et al., 2013). As this large panel of formerly and currently used pesticides is found in all the environmental compartments, there is a need to understand their transport and transformation to better predict their fate and anticipate associated adverse effects in the environment.

There are a number of challenges regarding pesticides in the environment that remain unsolved. First, despite the important amount of data provided by pesticide research, it remains difficult to predict the extent and pathways of pesticide transport and degradation under specific field conditions (Fenner et al., 2013). Besides, difficulties are mounting along with the large spectrum of available molecules and their increasing complexity. Finally, degradation of commercialized molecules (source substances) results in the formation of degradation products that are for the most part unknown. The identification of degradation products and of the main degradation pathways that cause their formation is thus an important challenge to understand pesticide fate from source (fields) to sink (non-target receptor) areas.

Non-target ecosystems receiving pesticides fluxes, such as ground- or surface water bodies, may act as pesticide sinks, intercepting contaminated runoff or leaching. Among them, wetlands are potential sink ecosystems that received growing attention since the last decades. Wetlands are being prominent systems for the dissipation of various inorganic and organic pollutants, including pesticides, although ongoing processes affecting pesticide transport remain poorly known.

2. Wetland systems diversity and ecological functioning

2.1. Wetlands definition, types and classification

Wetlands are transitional interface systems, wich represent typical 'ecotones' in ecology, between terrestrial (dry lands) and aquatic environments (deeper aquatic ecosystems like rivers or lakes). Wetland ecosystems are shallow water habitats, or saturated areas characterized by wet soils at or near land surface but not necessarily covered by water, with reduced conditions and an accumulation of organic matter (Mitsch and Gosselink, 2007; Brinson et al, 2002; Kadlec and Wallace, 2009). The RAMSAR convention, which is an intergovernmental treaty (1971, Iran) providing the framework for national action and international cooperation for the conservation and wise use of wetlands, defines these ecosystems as "*areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six metres*" (Ramsar, 2009). Wetlands currently occupy a total surface area of >207 millon hectares, corresponding to 6% of the world's land surface (RAMSAR, 2009; Junk et al., 2013). They represent one of the most biologically active and productive ecosystems, comparable to tropical evergreen forests, with an annual net primary productivity of about 2,000 g C m^{-2} yr^{-1} (US EPA, 2011).

Along with the large range of climate, hydrological, and biogeochemical conditions existing on earth, there is a large diversity of wetland systems that differ according to their morphology, fauna, vegetation, water chemistry or hydrological functioning. Consequently, various wetlands classifications are co-existing. Wetlands can be split into 2 major groups: the natural wetlands and the constructed or artificial wetlands. Although the formation of natural wetlands results from natural processes, constructed wetlands are anthropogenic ecosystems, which are designed to mimic conditions and/or processes occurring in natural wetlands (Vymazal and Kropfelova, 2008). One simplified classification for natural and constructed wetlands is based on wetlands hydrology, as this parameter is considered as the primary driving force of wetlands functioning (Dahl et al., 2007).

Concerning natural wetlands, 3 main groups can be defined according to their water supply, i.e. mainly precipitation-dependents (bogs), groundwater-dependents (fens), and surface

water-dependent wetlands (swamps, marshes and shallow open water) (Dahl et al, 2007) (Figure I-2).

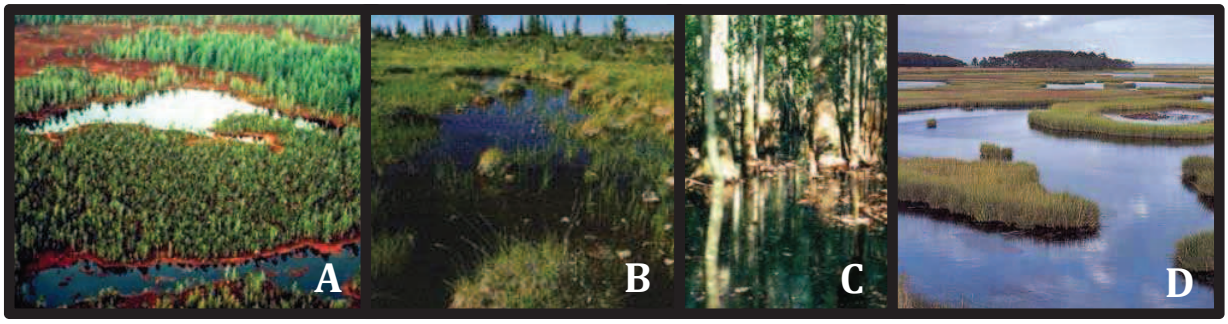

Figure I-2. Main natural wetland types. A. Bog, B. Fen, C. Swamp, D. Marsh. Source: US EPA.

Briefly, bogs are ombrotrophic i.e. solely precipitation-dependent ecosystems that produce peat, characterized by an accumulation of organic matter over long periods (Vitt, 2006; Poulíčková et al, 2013). Bogs are thus extremely acid and oligotrophic wetlands where vegetation is often dominated by *Sphagnum sp.*

The second group of natural wetlands is represented by fens. Fens are also peat producing wetlands but which are mainly groundwater-dependent habitats (Dahl et al., 2007; Johansen et al., 2011). Fens are less acidic and often alkaline due to the supply of nutrient-poor but calcareous-rich groundwater, which lead to an important biodiversity dominated by orchids, sedges or mosses.

Swamps are forested wetlands that are often located around large rivers or freshwater lakes. They are ecosystems characterized by low peat accumulation, except peat swamp forests where the waterlogged nature of soils prevent from complete decomposition of plant material, which cause the formation of a peat layer (Aselmann and Crutzen, 1989; Mao et al., 2013).

Marshes are wetlands ecosystems often found at the edges of lakes or streams that are frequently or permanently inundated. They are dominated by herbaceous plant species such as grasses, sedges, rushes or reeds. They are mostly supplied with surface water, but can also be fed by groundwater (Sun et al., 2013).

Shallow open water bodies are designated as open water bodies of standing or slowly flowing water that are transitional stages between lakes and marshes. Shallow open water wetlands vary greatly and are represented by ponds, pools, oxbows or channels. Their depth is usually less than 2 meters. They are often vegetation-free, except for aquatic macrophytes (Aselmann and Crutzen, 1989; Rooney and Bayley, 2012).

Concerning constructed wetlands, two groups can typically be distinguished:

- the *artificial wetlands* constructed for other purposes than water quality improvement (for a recreation or aesthetic purpose for example);
- the *treatment wetlands* that are designed to mimic physical, chemical and biological processes that occur in natural wetlands to remove contaminants from polluted water (Fonder and Headley, 2013).

Treatment wetlands are typically classified into 3 major types, which are the free water surface wetlands (FWSWs), the subsurface flow constructed wetlands (SSFCWs) and the vertical flow wetlands (VFWs) (Mander et al., 2014, Kadlec, 2009; Stottmeister et al., 2003) (Figure I-3). Surface flow wetlands have generally low water depths (about 0.3 m) and are densely vegetated with macrophytes. Subsurface flow constructed wetlands use saturated beds of gravel or soil as a substrate for vegetation growth (0.6 m) (García et al., 2010) that is typically composed of common reed (*Phragmites australis* (Cav.)), cattail (*Typha* spp.), and bulrush (*Schoenoplectus* spp.).

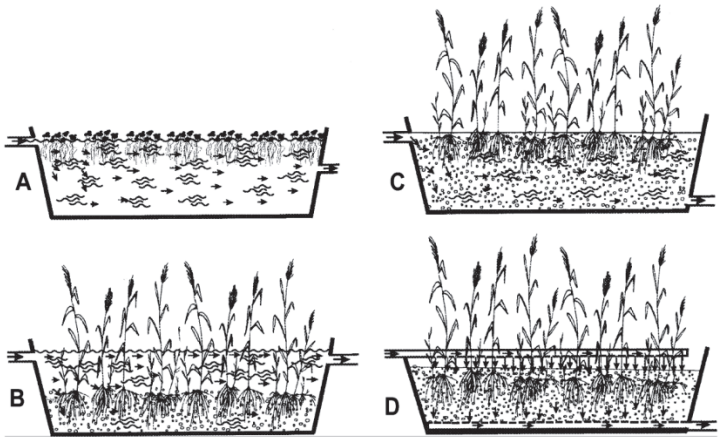

Figure I-3. Constructed wetland systems for wastewater treatment (A. Pond with free-floating plants; B. Horizontal surface flow wetland or pond with emergent water plants; C. Horizontal subsurface flow wetland; D. Vertical flow wetland) (Stottmeister et al., 2003).

2.2. Wetlands ecosystem services and biogeochemical functioning

Wetlands are valuable ecosystems that are essential part of the human civilization being of critical social, economic and environmental importance (Junk et al, 2013). They provide a wide range of essential ecosystem services to humankind, including flood control, groundwater replenishment, recreation, food and water quality control, and harbor a large part of the earth's

biodiversity (Junk et al, 2013). Despite their numerous benefits, natural wetlands are seriously threatened because of extensive resource exploitation, drainage, invasive species, diffuse pollution and global climate change. 30 to 90% of the world's wetlands have already been destroyed or dramatically modified in the world, depending on the regions (Junk et al., 2013).

Wetlands, and especially constructed wetlands, are used since decades to improve water quality (Kadlec, 2009). Many studies have shown that wetlands can efficiently dissipate or trap a wide range of contaminants, including total suspended solids (TSS) (Dunne et al., 2012), nutrients (Vymazal, 2005), heavy metals (Sheoran and Sheoran, 2006) or organic contaminants (Matamoros et al., 2007). In addition to the use of wetlands to treat water, recent studies aim to investigate the capacity of wetlands to act as carbon sinks and long-term store carbon dioxide from the atmosphere in the context of global climate change (Mitsch et al., 2013).

Ecosystem services provided by wetlands result from the interplay of the wetlands components, i.e. water, sediment, plants and microorganisms that regulate their biogeochemistry. Wetlands biogeochemical functioning is mainly driven by the hydrological component and in particular by fluctuations of the water table that controls the establishment of a redox gradient in wetland bed sediments (Alewell et al., 2008). Anoxic conditions are prevailing in wetland sediment, due to water saturation. However, diurnal oxygen release from plant roots creates the co-occurrence of oxic and anoxic conditions in the rhizosphere (Nikolausz et al., 2008). Redox gradients ranging from oxic to anoxic conditions regulate microbial communities that in turn regulate oxido-reduction reactions. Microbially-mediated reactions control the distribution of redox determining species, gradients and interfaces in wetlands (Groffman and Crossey, 1999). In the absence of O_2, microorganisms preferentially reduce a variety of alternative Terminal electron acceptors (TEAs) for respiration. Depending on redox potential, pH, and availability of electron acceptors, the following microbial reduction processes can occur after O_2 depletion: NO_3^- (denitrification), Fe(III) (iron reduction), Mn(III, IV) (manganese reduction), SO_4^{2-} (sulfate reduction) and finally CO_2 reduction (Burgin et al., 2011). Reduction of these TEAs is coupled to oxidation of organic matter to CO_2. This consecutive sequence of terminal electron-accepting processes (TEAPs) is defined as the sequential reduction chain (Figure I-4). However, due to heterogeneous redox conditions occurring at microscale and at larger scale, these TEAPs can occur simultaneously in wetlands (Faulwetter et al., 2009; Groffman and Crossey, 1999; Keller et al., 2009).

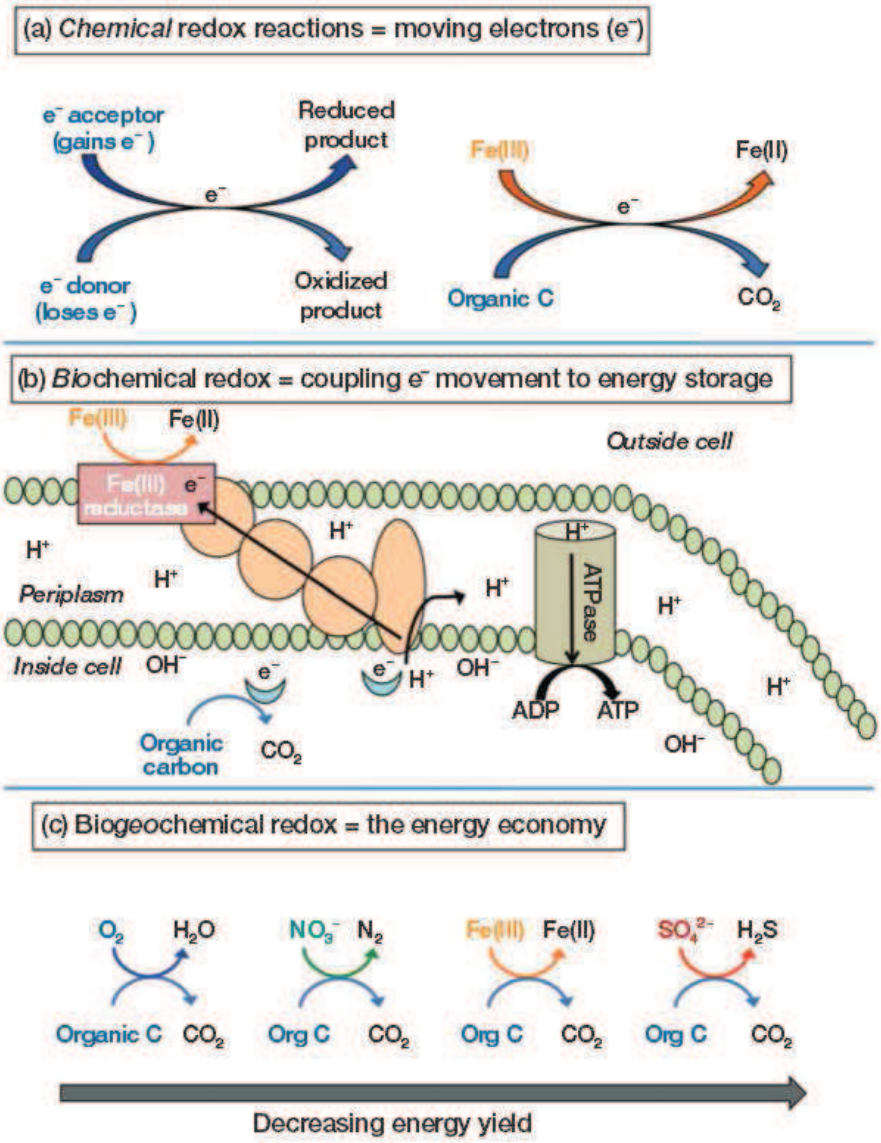

Figure I-4. Redox reactions. (a) Coupled redox half-reactions and anaerobic respiration coupled to iron reduction. (b) Biochemical coupling of proton and electron transport from organic carbon across the bacteria cell membrane to yield reduced iron. (c) The sequential decreasing energy yield of potential terminal-electron-accepting processes. (from Burgin et al., 2004).

Microbially-mediated reactions involved in element cycles can be implied in contaminant degradation processes. TEAs significantly impact organic contaminants, including pesticides, via both oxidative and reductive transformation processes in wetlands (Borch et al., 2009).

3. Wetlands as 'natural laboratories' to investigate pesticides

In agricultural landscapes, wetlands can receive pesticide-contaminated runoff or can be hydrologically connected to an aquifer and intercept pesticide-contaminated discharge during flood periods. Wetlands are dynamic ecosystems harboring a wide spectrum of biogeochemical conditions that promote the co-existence of different pesticide transformation pathways governing their capacity to improve water quality and related ecosystem services. Therefore, wetlands can be used as 'natural laboratories' to investigate the transport and the fate of pesticides at biogeochemically active interface ecosystems. Pesticides transport and degradation in wetland systems are governed by various factors including the intrinsic physico-chemical properties of pesticide compounds, soils and vegetation characteristics, rainfall patterns and biogeochemical conditions.

3.1. Pesticide intrinsic physico-chemical properties

Intrinsic properties of pesticides molecules control their transport, and their partitioning among the different environmental compartments and their degradation in wetlands (Grégoire et al., 2009). Among these physico-chemical properties, the Henry constant (Pa m^3 mol^{-1}, 25°C) stands for the ability of a molecule to volatilize into the atmosphere and hence to be transferred from the aqueous compartment to the atmosphere (Imfeld et al., 2009). This partitioning coefficient is one of the main partitioning processes that follow pesticide use on crops (Grégoire et al., 2009).

The solubility of pesticides (mg L^{-1} in water, generally at 20°C) is also very important as it determines its behavior towards the aqueous phase (Stottmeister et al, 2003). Thus, pesticides which present a high solubility constant have an important affinity for the aqueous compartment. Hence, such molecules are generally easily leached out from the field during rainfall/runoff events, being potentially rapidly transported to non-target aquatic ecosystems.

The soil-water partition coefficient (K_d, L kg^{-1}, 20°C) results from properties previously quoted. K_d is calculated as the ratio of the concentration of the pesticide in soil and its concentration in water and represents a measure of sorption of a pesticide to soil. In a larger extent, K_d is used to determine pesticide mobility in the soil (Barbash, 2007). The more K_d is high

and the more the affinity of the molecule for soil is high (Gevao et al., 2000; Grégoire et al., 2009). K_d is expected to largely vary among the different soils in relation with their organic carbon content (f_{oc}). Thus another index was used to take into account this variability, the organic carbon – water partition coefficient (K_{oc}). K_{oc} is calculated as the ration of K_d and f_{oc}. K_{oc} presents a good estimation of the mobility of the pesticide when it is adsorbed on the organic matter. Thus, the more a pollutant has a high affinity for the organic matter, the more it will be adsorbed on this phase (Dabrowski and Balderacchi, 2013). The water-octanol partition coefficient (log K_{ow}) is also used to describe the hydrophobicity of pesticide molecules (Rose et al., 2008). K_d, K_{oc} and K_{ow} are extensively used indices for the characterization of pesticide sorption and mobility in the environment.

Another important property to consider concerning pesticide molecules is their half-life time (DT_{50}, days). It corresponds to the time which is necessary to the degradation of the half quantity of molecules in water or soil. This property highlights the persistence of the pollutant into the environment. A molecule is considered as persistent if its half-life time exceeds 100 days (Poissant et al., 2008).

Finally, as previously mentioned, pesticides are also characterized by their ecotoxicological properties, such as their lethal dose (DL_{50}) or their no-effect concentrations (NOEC) for specific organisms (e.g. aquatic invertebrates, fishes, mammals). The more the DL_{50} and the NOEC are low and the more the substance is toxic. All these physico-chemical properties are essential to take into account to understand the behavior and the fate of pesticide molecules in wetland systems.

3.2. Processes affecting pesticide transport and degradation in wetlands

Several studies have demonstrated that wetland systems can efficiently dissipate runoff-related pesticides (Blankenberg et al., 2006; Lizotte et al., 2009; Moore et al., 2002; Schulz et al., 2003). The interplay between water, sediment, vegetation and microorganisms in wetlands govern a succession of processes involved in contaminants dissipation (Stottmeister et al., 2003). Pesticide dissipation involves destructive processes i.e. degradation (photolysis, hydrolysis, biodegradation) and non-destructive processes i.e. volatilization, phytovolatilization, plant uptake and sorption (Imfeld et al., 2009) (Figure I-4). While destructive processes lead to

the breakdown of the molecule into degradation products, non-destructive processes are partitioning processes that will determine the distribution of pesticides among the different environmental compartments.

Among non-destructive processes, plant uptake was found to be closely linked to the octanol–water partition coefficient (K_{ow}) of the pesticide molecule and the nature of the vegetation. Globally, compounds with log K_{ow} < 1 hardly penetrate the lipidic root epidermis because of their hydrophilic property. Compounds having log K_{ow} > 3 are not expected to be uptaken by plants because they are increasingly retained by the root epidermis and the surrounding organic matter of the soil. Thus, pesticide compounds with log K_{ow} between 1 and 3 can be translocated into plants and can then be metabolized or just stored in roots, leafs or stem tissues (Schröder and Collins, 2002; Verkleij et al., 2009). Pesticide sorption is mainly governed by physico-chemical characteristics of pesticides and the nature of runoff-related particles, including their size, shape, polarity or charge distribution (Spark and Swift, 2002).

In wetland systems, sorption/desorption processes control pesticide retention and degradation (Reid et al., 2000). Sorption is a kinetic-driven process that varies from complete reversibility to total irreversibility depending on the nature of the bond and the contact time of pesticide-particle interactions, namely the ageing (Gevao et al., 2000). A recent constructed wetland owns a high sorption rate because the adsorption capacity of the soil is maximum. The sorption capacity gradually decreases over time until the sorption/desorption equilibrium is achieved. Particle trapping in wetlands can also lead to an improvement of water quality by reducing the solid load and by immobilizing nutrients or pollutants associated to the suspended matter (Haarstad and Braskerud, 2005). Sorption of hydrophobic pesticides enhances their retention in wetlands through TSS settling (Budd et al., 2009; Moore et al., 2009) while lowering their bioavailability and biodegradation (Rodriguez-Liebana et al., 2011).

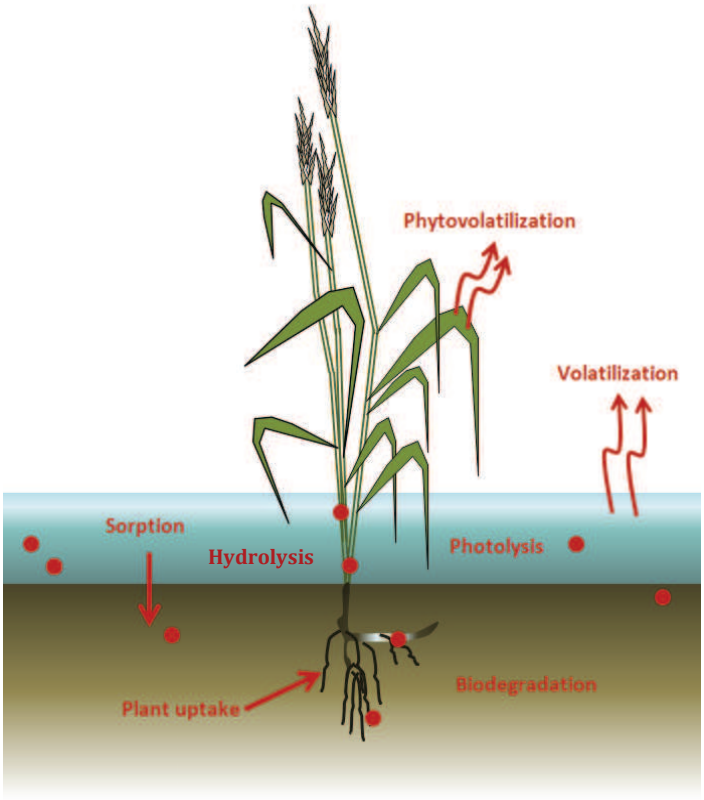

Figure I-5. Pesticide dissipation processes occurring in wetland systems

Pesticide degradation processes can be biotic or abiotic. Abiotic transformation brings into play physico-chemical or photochemical processes (Syversen and Bechmann, 2004), such as hydrolysis or photolysis. Pesticides can undergo direct photolysis in water but are more likely to be degraded through indirect photolysis, as the result of the reaction with another species which play the role of oxidant such as fulvic and humic acids (Barbash, 2007; Fenner et al., 2013). For instance, Mathew & Kahn (1996) showed that metolachlor photodegradation was enhanced in presence of montmorillonite or fulvic acids. Although abiotic hydrolysis can occur in aqueous solutions, this process is often mediated by microorganisms, bringing into play the intervention of enzymes (Fenner et al, 2013). Biologically-driven hydrolysis is more expected to occur in water. Thus, biotic processes play an important role in pesticide degradation, including that of chiral pesticides.

Chiral pesticides can undergo enantioselective biological processes (Celis et al., 2013). In other words, enantiomers of a chiral compound can behave differently during biological processes, such as plant uptake or microbial degradation. Enantioselective degradation can greatly differ, with respect to the pH, redox conditions or type of matrices that are considered (Gámiz et al., 2013). Enantiomers of chiral pesticides have identical physico-chemical properties and are expected to undergo similar abiotic processes such as sorption or volatilization. Differences in enantiomers behavior in the environment induce also differences in term of toxicity. For example, Xu et al. (2010) showed that *rac*-metolachlor was more toxic than *S*-metolachlor for earthworms. Therefore, there is a need to understand biological mechanisms underlying the transport and transformation of modern chiral compounds to predict their fate in the environment. Biological processes include non-destructive processes, such as plant uptake and destructive processes such as biodegradation.

Biodegradation is known to be the most important degradation pathway for pesticides and can involve plants, animals, fungi and bacteria. Phytodegradation consists in the metabolic degradation by enzymes of plants. Pesticide metabolization by plants is often fortuitous, being catabolized by large-spectrum enzymes for a detoxification purpose. Among degradation processes, microbial degradation, which includes degradation by bacteria or fungi, is the major degradation route of organic contaminants in constructed wetlands (Fenner et al., 2013; Hijosa-Valsero et al., 2010). Contrary to other organisms, bacteria commonly metabolize pesticides for assimilation and energy. However, pesticide degradation can also be attributed to cometabolism, where the degradation occurs fortuitously via non-specific enzymes and where pesticide molecules are not used by the bacteria as a source of energy of nutrient (Nzila, 2013). Co-metabolism is suspected to be a major biodegradation pathway for alachlor for example (Słaba et al., 2013). Except in case of complete mineralization, the degradation of pesticides lead to the release in the environment of degradation products that can be more harmful and/or persistent than the parent compounds, as illustrated by the degradation of chloroacetanilide herbicides (Figure I- 6).

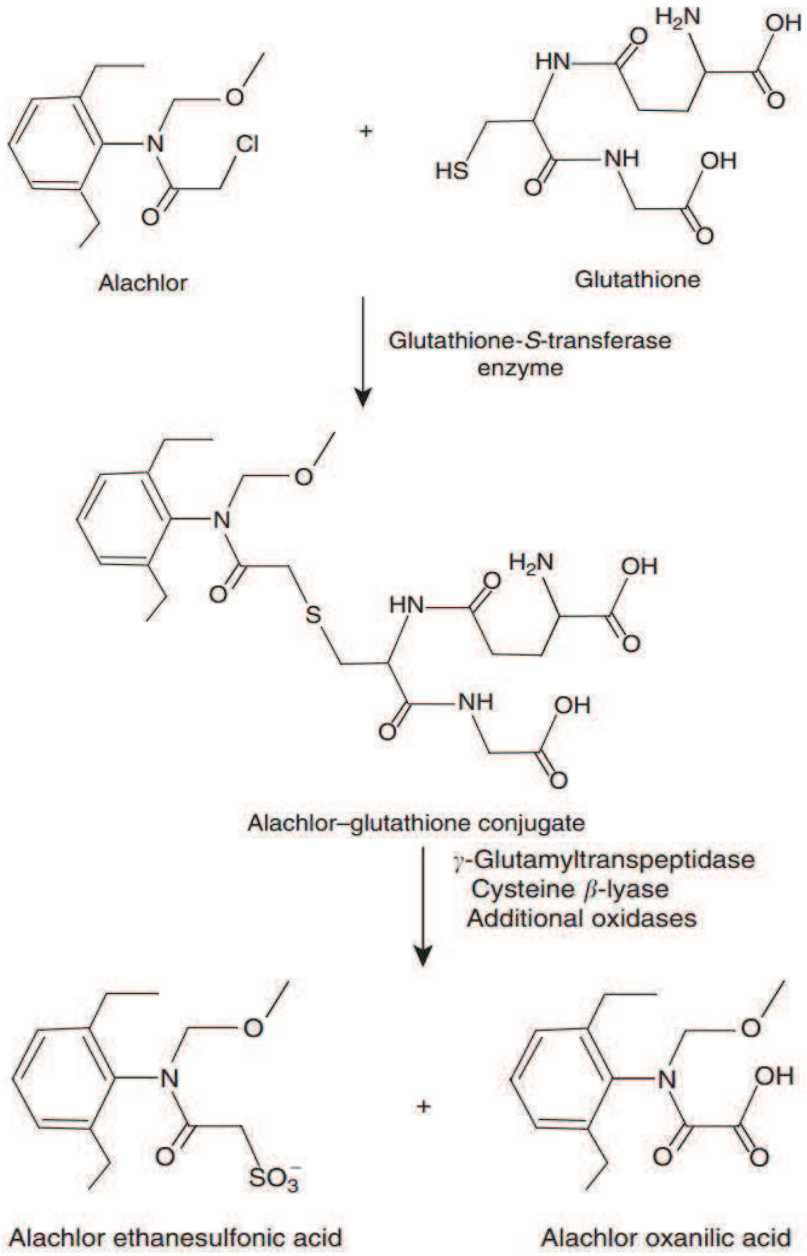

Figure I-6. Formation of ethanesulfonic acid (ESA) and oxanilic acid (OXA) metabolites from the reaction of the chloroacetanilide herbicide alachlor with glutathione and other enzymes (from Field & Thurman, 1996 in Barbash, 2007). Degradation products of chloroacetanilide herbicides (especially ESA) have been shown to be persistent in groundwater.

3.3. Biogeochemical indicators of prevailing degradation processes

Pesticide degradation processes are closely linked to the redox potential that govern biogeochemical cycles in wetlands (Borch et al., 2010; Reddy and D'Angelo, 1997). Redox potential gradients that occur both at large and micro-scales in wetlands can be attributed to the hydrological fluctuations, the presence of terminal electron acceptors (such as nitrates or sulfates) and oxygen diffusion in the root zone (Reddy and D'Angelo, 1997). Biogeochemical cycles of many major elements such as carbon, nitrogen, iron, manganese, and sulfur are mainly governed in wetlands by redox-dependent microbial processes and can occur simultaneously (Faulwetter et al., 2009). For instance, mineralization of organic matter can occur in both aerobic and anaerobic conditions (Truu et al., 2009). Nitrogen cycle is closely linked to redox processes of carbon and results from the combination if nitrification – denitrification, or anaerobic oxidation of ammonium (Anammox) (Borch et al., 2010). Oxygen, NO_3^-, MnO_2, $Fe(OH)_3$, SO_4^{2-} and CO_2 are all involved in microbial respiratory and are thus called terminal electron acceptors (TEA). Microbial degradation processes require the flow of electrons from organic matter, or contaminants (electron donors) to one or several TEA(s). Thus, bacterial degradation is highly dependent on the prevailing terminal electron accepting processes (TEAPs) (McClain et al., 2003).

Pesticide degradation, retention and distribution dynamics in wetlands are controlled by variables, such as pH and redox potential, which influence pesticide sorption/desorption and possible degradation. Knowledge on the dynamics of pesticide retention and degradation in wetland compartments is essential to understand pesticide sink and source functioning of redox-dynamic environments. However, all these processes are interrelated and their distinction remains a challenge that can be tackled by combining different experimental scales and approaches.

4. Combining scales and analytical methods to evaluate pesticides in the environment

Scaling issues are fundamental to all ecological investigations (Haila, 2002). While large-scale experiments mainly provide information on ecological patterns and overall functioning of ecosystems, fine-scale approaches enable to understand individual molecular processes that are responsible of the large-scale response. Multi-scale investigations thus serve a valuable role in biogeochemical studies because they enable to gain mechanistic knowledge on the underlying processes that regulate larger-scale responses of hydrosystems towards pesticide contamination. To gain insights on pesticide degradation processes, several analytical techniques can be combined, including the analysis of the i) parent compound, ii) degradation products, iii) enantiomers, in the case of chiral pesticides, and iv) compound-specific isotope analysis (CSIA). Since the analysis of parent compounds is not sufficient to distinguish transformation from dilution or sorption, it should be coupled with another method (Fenner et al., 2013). Evidences of biodegradation may be provided by using degradation products quantification, enantiomeric analysis or CSIA methods. These emerging analytical tools allow to gain mechanistic insights into pesticide transformation processes (Borch et al., 2010) and can be used to evaluate pesticide transformation in the field.

The analysis of degradation products is one of the mostly used method to assess the occurrence of pesticide degradation in the environment. However, pesticide degradation pathways are currently poorly understood and most of the degradation products of pesticides are still unknown. Moreover, degradation products are often more difficult to analyse because they are often unstable compounds (Andreu and Pico, 2004). For these reasons, the analysis of degradation products has to be coupled to other emerging techniques to characterise pesticide degradation.

Enantiomeric analyses of chiral compounds rely on the enrichment of the remaining fraction of non-degraded pesticides in one or another enantiomer. A shift, compared to the initial enantiomeric signature of the active substance, can indicate a preferential degradation of one enantiomer (Büser et al., 2000). For instance, Liu et al. (2006) reported a greater degradation of *S*-metolachlor compared to the *R*-enantiomer in soils (Figure I-7). However, enantiomer analysis to assess biodegradation fails if both enantiomers are equally degraded by the same enzyme of if *R*- and *S*- preferential enzymes are simultaneously present in the environment (Milosevic et al.,

2013). Enantiomer analyses have been successfully applied to evaluate the degradation of chiral pesticides such as phenoxyacid herbicides (Milosevic et al., 2013), metolachlor (Büser et al., 2000), or organochlorine pesticides (Yang et al., 2010).

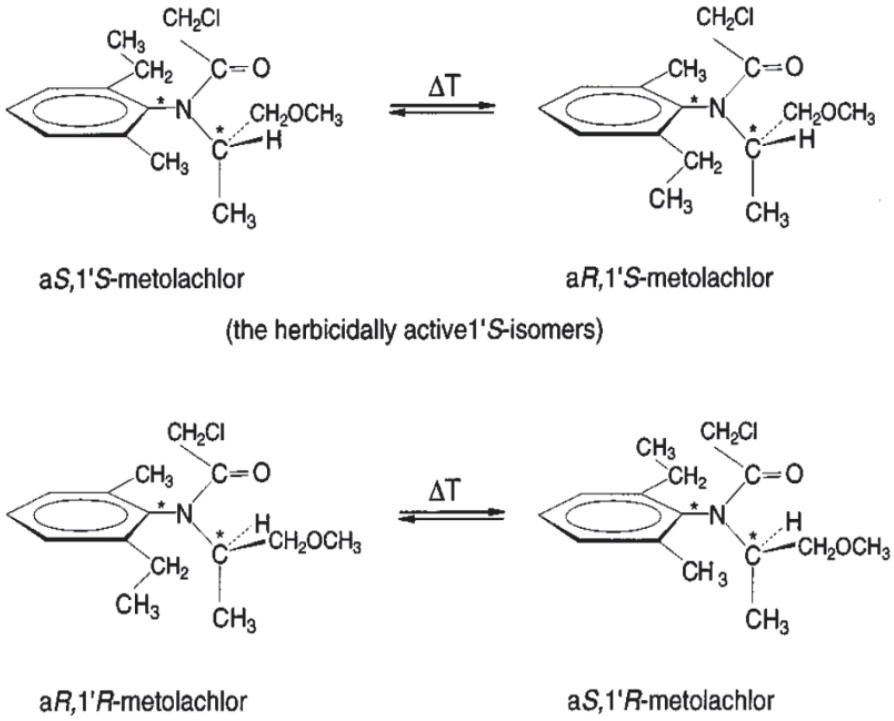

Figure I-7. Structures of the four stereoisomers of metolachlor (asterisks denote the chiral elements); ΔT relates to the rapid interconversion during thermal equilibration (Büser et al., 2000).

For chiral molecules whose degradation occurs without enantioselectivity or for achiral molecules, compound-specific stable isotope analyses (CSIA) provide a valuable tool for the assessment of contaminant transport and fate in the environment. CSIA relies on the enrichment of the heavy isotope of an element in the remaining fraction of a compound during the degradation process (Thullner et al., 2012). Consequently, the isotopic composition of the contaminants can provide insights about key degradation pathways occurring *in situ*, and in some cases enable measuring the extent of biodegradation (Elsner, 2010). During the last decade, CSIA has been increasingly applied to study several groups of contaminants, most notably chlorinated ethenes (e.g. Imfeld et al., 2008). Recently, CSIA methods have been developed for pesticides: lindane (Badea et al., 2009), isoproturon (Penning et al., 2010), atrazine (Meyer et al., 2008) and chloroacetanilide herbicides (Elsayed et al., 2013).

Bridging the gap between results and interpretation from fields and laboratory experiments is one of the major challenges in pesticide research, which is partly tackled in the present thesis. Thus, lab-defined benchmark studies to develop and test novel tools are necessary to gain mechanistic knowledge that could thus be taken back to the field. Since field observations are often related to complex and interrelated mechanisms that remain poorly understood, the overall goal of combining different scales and tools is to understand the transport and transformation mechanisms of individual pesticides (Figure I-8).

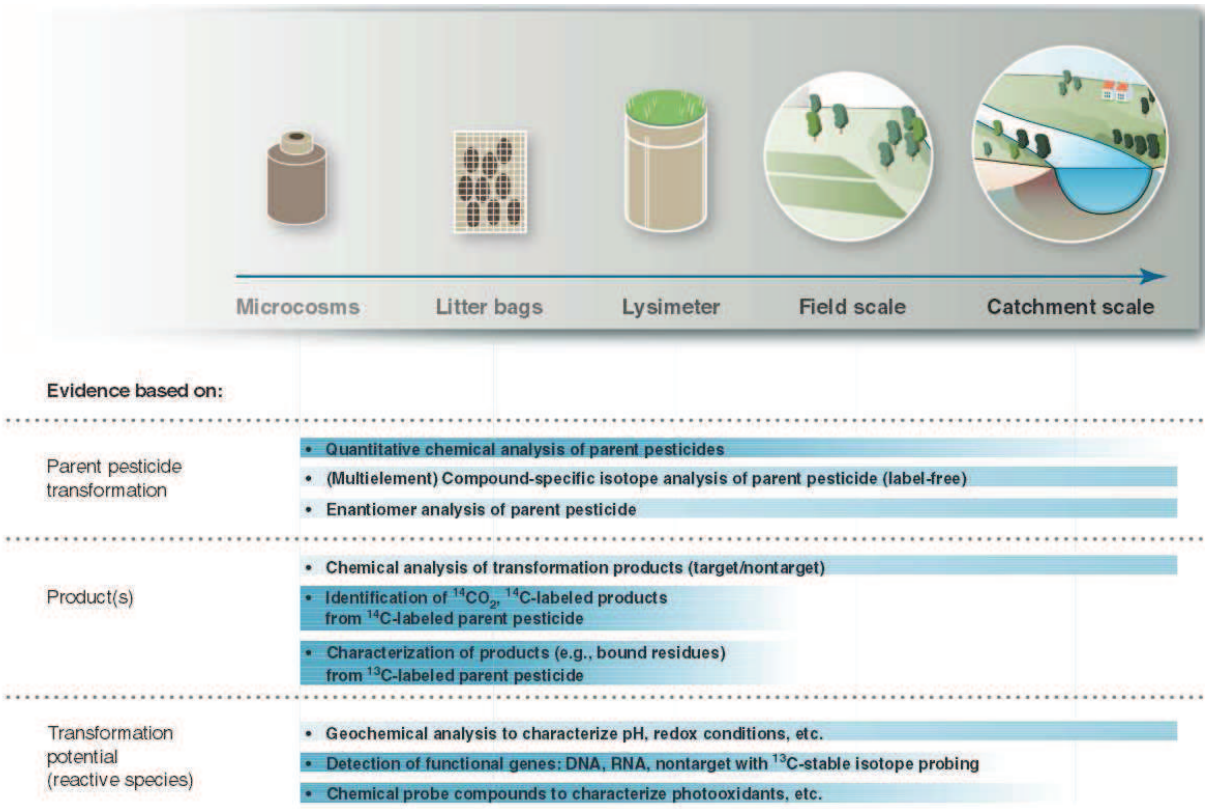

Figure I-8. Available analytical approaches to identify pesticide transformation in natural environments (Fenner et al., 2013).

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Chapter II. Aim & Objectives

1. Research focus

The previous literature review gaves rise to scientific gaps and challenges that need to be addressed concerning the transport and degradation processes of emerging agrochemicals in wetland systems. This PhD thesis attempts to provide some evidences to partially answer these remaining issues.

Even if considerable efforts have been made since the last decades to open the black box that represent wetlands' processes and functioning, there is a need to improve knowledge on the *in situ* mechanisms that affect the transport and degradation of emerging agrochemical like pesticides in relation with hydrological and biogeochemical conditions. The dearth of knowledge partly lies in the difficulty to link molecular processes of pesticide degradation observed in the lab to field observations. This is mostly due to the complex interplay of environmental factors that often cloud data interpretation. One of the major challenges in pesticide studies, and in a larger extent in emerging contaminants investigations, is therefore to predict their fate in the environment by providing mechanistic clues of observed degradation and transport processes observed in the field in order to improve management practices in agricultural areas.

Analytical tools currently available including biomolecular techniques, isotopic, enantiomer tools and analyses of the parent compounds and their degradation products cannot be applied at every scale due to their sensitivity (Fenner et al., 2013) (see Chapter I, Figure I-8). Thus, the understanding of pesticide fate in wetlands implies the combination of different approaches and the integration of multiple experimental scales. Such a comprehensive approach may help predicting processes of pesticide dissipation in redox-dynamic environments, such as wetland.

Several questions arise, highlighting the major gaps of knowledge to the understanding of the fate of pesticides in wetlands in relation to wetlands functioning:

- How do the wetland hydro-biogeochemical functioning influence pesticide transport and transformation over space and time?

- How wetland compartments (i.e. sediment, water, suspended solids, vegetation, (micro)organisms) are involved in pesticide retention and biodegradation and how do these processes evolve in relation to the wetland lifespan?
- How do redox gradients induced by hydrological conditions and biological activity affect the transport and biodegradation of pesticides in wetland systems?
- How available analytical approaches to identify pesticide transformation in natural environments can be efficiently combined to evaluate pesticide transport and biodegradation in wetland systems?

2. Research objectives

The overall aim of this thesis is to evaluate the transport and the biodegradation of pesticides in wetland systems in connection with the wetland functioning. In a downscaling approach, three different wetland systems receiving agricultural runoff, representing three scales were investigated and compared to meet the following objectives:

- a stormwater wetland (320 m^2) was investigated *(Chapter III)* to:

i) assess the ability of stormwater wetland systems to dissipate pesticides in runoff from a vineyard catchment during an entire investigation period;

ii) evaluate the relationships between the pesticide dissipation and changes of both hydrological and biogeochemical conditions (section 1);

iii) decipher the dissipation processes by quantifying pesticide retention in wetlands compartments and degradation processes based on a mass balance approach, and link pesticide dissipation to the wetland lifespan (section 2);

iv) evaluate the temporal variability of the transport and dissipation of a model compound, glyphosate and its main degradation product AMPA during three consecutive periods of pesticides application in relation to the development of the wetland (section 3).

- subsurface flow constructed wetlands (7 m^2) were investigated *(Chapter IV)* to:

i) evaluate and compare the transport and biodegradation of *S*-metolachlor (commercial product Mercantor Gold®) and artificial tracers under varying hydrological conditions and redox gradients;

ii) evaluate the *in-situ* biodegradation of *S*-metolachlor while testing emerging analytical approaches for evaluating pesticides in wetland systems, including the enantiomer analysis and the compound-specific isotope analysis (CSIA).

- lab-scale wetland columns (0.02 m^2) were investigated *(Chapter V) to:*

i) compare the transport and the dissipation of three herbicides of the chloroacetanilide family, i.e. acetochlor, alachlor and metolachlor in relation to biogeochemical conditions (section 1);

ii) Evaluate the *in-situ* biodegradation of the three chloroacetanilide herbicides, while testing emerging analytical approaches, such as enantiomer analysis (Section 1) and CSIA (section 2).

3. Graphical outline of the thesis

A synoptic overview of the PhD thesis is presented in the graphical abstract (Figure II-1).

Wetlands are complex ecosystems in which a wide range of pesticide processes are embedded and thus occur simultaneously. Distinguishing the different ongoing dissipation processes remains difficult in field studies and requires benchmark studies at smaller scale. Reciprocally, to understand the overall function of wetland system with respect to pesticides, field experiments are required.

Therefore, a multi-scale approach has been applied in my PhD thesis in order to gain mechanistic knowledge at small-scale (simplified systems), enabling a better understand larger scale observations. In a downscaling approach, three different wetland systems receiving agricultural runoff were investigated: i.e. a stormwater wetland (field scale, 320 m^2, Chapter III), subsurface flow constructed wetlands (SSFCWs) (mesocosm scale, 7 m^2, Chapter IV) and lab-scale wetlands (0.02 m^2, Chapter V). Several approaches were combined to evaluate pesticide transport and degradation processes. Pesticide mass budgets were established, while biogeochemical conditions were evaluated in each wetland. The evaluation of pesticides at the different scales has involved the comprehensive use of different analytical approaches, i.e. the analyses of degradation products, enantiomers and stable isotopes to quantify and characterize degradation processes.

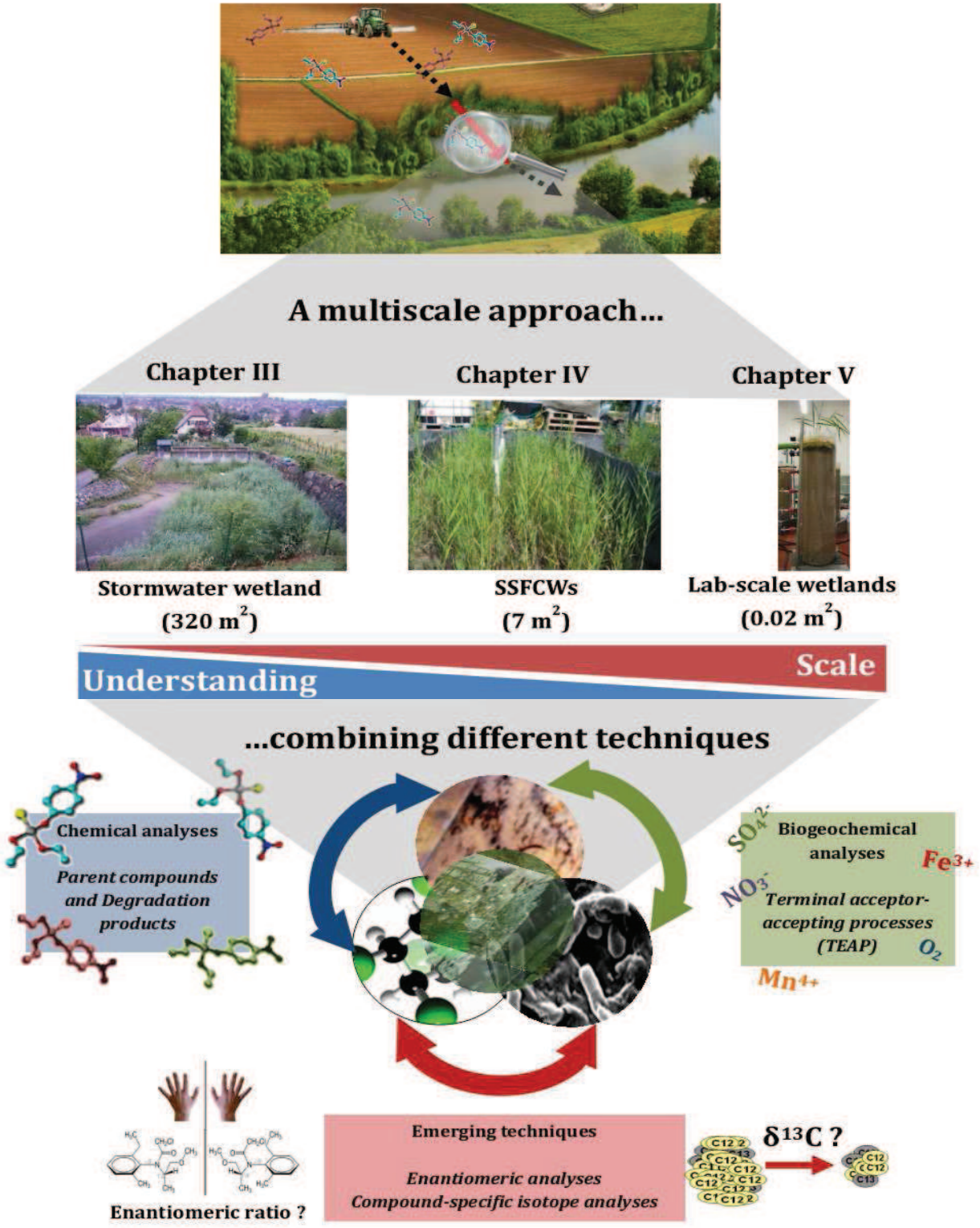

Figure II-1. Graphical outline of the PhD thesis

4. Thesis layout

This PhD thesis incorporates 3 refereed publications, 2 submitted publications and 1 publication in prep., for which I am the main author. Two additional refereed publications for which I am a co-author are provided in the Appendices (chapter VII). The first two experiments (Chapter III, sections 1 and 2) have been subjected to peered-reviewed publications in international journals. Chapters III to V present these publications as they were published, publications in prep. and a brief overview of the context placed before each of these chapters to guide the reader.

Chapter III contains 3 sections that correspond to 2 refereed publications and 1 submitted publication, related to the transport and degradation of pesticides in a stormwater wetland (Rouffach, Alsace, France). A first section (section 1; Science of the Total Environment, 2011) aims at understanding the transport and degradation of a mixture of 20 pesticides and degradation products during an agricultural period, in relation with prevailing biogeochemical conditions. Section 1 provides an overall overview of the ability of stormwater wetlands (320 m^2) to dissipate pesticides in runoff from a vineyard catchment. In a second section (section 2, submitted to Environmental Science and Technology, 2014), we evaluate the partitioning of a mixture of 12 pesticides between the dissolved phase and the suspended solids and the influence of partitioning on pesticide transport and dissipation in the stormwater wetland. A second objective was to assess, using mass balance approach, the distribution of the pesticides among the wetland compartments to understand its sink and source properties. A third section (section 3; Chemosphere, 2013) evaluates more specifically the potential of degradation of the stormwater wetland. For this purpose, we chose the most widely used herbicide worldwide, glyphosate, and its main degradation product, aminomethylphosphonic acid (AMPA) as model compounds to investigate the potential of degradation of stormwater wetlands. In the latter study, we compared the transport and degradation of Glyphosate and AMPA during three successive agricultural season of glyphosate use.

Following the downscaling approach, Chapter IV focuses on mesocosm subsurface flow constructed wetlands (SSFCWs) (7 m^2), to evaluate the transport and degradation of a second model compound, the chloroacetanilide herbicide *S*-metolachlor, chosen for its widespread use and its chiral properties. This chapter (publication in prep.) aimed to compare the influence of the hydrological conditions on the transport of S-metolachlor using enantiomeric and isotope

approaches and to ascertain the possible use of dye tracers as low-cost and environmentally-friendly surrogates of pesticides in transport and dissipation studies.

Chapter V further scales down the previous mesocosm experiment in a lab-scale column experiment. In this chapter, the transport and dissipation of three chloroacetanilide herbicides i.e. acetochlor, alachlor and metolachlor in the vadose zone was investigated. Section 1 (submitted to "Environmental Pollution", 2014) compares the transport and dissipation processes of the three herbicides in upflow lab-scale wetlands (0.02 m^2), with a particular emphasize on metolachlor degradation using the enantiomer analysis. Section 2 deals with the use of compound-specific isotope analyses as emerging tools to track chloroacetanilide biodegradation.

Chapter VI provides a general conclusion of the PhD thesis, drawing together the findings of chapters III, IV & V. A summary briefly recalls the current gaps of knowledge in the understanding of the fate of emerging organic contaminants such as pesticides in wetland systems, and presents the major findings and novel perspectives that this thesis contributed to bring in the context of these gaps. The implications of these findings, and the further research associated with them are also discussed, and a final conclusion closes this chapter.

To conclude, Chapter VII provides the appendices and the supplementary information from the different experiments, not otherwise presented in the attending papers. This chapter also provides refereed publications that are not presented in the present thesis.

Chapter III. Stormwater wetlands to investigate wetland functioning toward pesticide contamination

Overview

This chapter includes two peer-reviewed publications (Section 1, Science of the Total Environment; and section 3, Chemosphere) and a submitted publication (Section 2, Environmental Science and Technology). This chapter provides overall information on the transport, dissipation, partitioning and retention of a mixture of pesticides in a stormwater wetland (320 m^2). The influence of the hydrological conditions and the wetland development (including the development of the vegetation) on pesticide fate were specifically emphasized (Sections 1 and 2). In addition, the degradation potential of the wetland was investigated using glyphosate and its main degradation product, the aminomethyl phosphonic acid (Section 3).

Section 1. Removal of a pesticide mixture in a stormwater wetland collecting runoff from a vineyard catchment

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1. Abstract

Wetlands can collect contaminated runoff from agricultural catchments and retain dissolved and particle-laden pesticides. However, knowledge about the capacity and functioning of wetland systems with respect to the removal of pesticides is very limited. Here we show that stormwater wetlands can efficiently remove pesticides in runoff from vineyard catchments during the period of pesticide application, although flow and hydrochemical conditions of the wetland largely vary over time. During the entire agricultural season, the inflowing load of nine fungicides, six herbicides, one insecticide and four degradation products was 8.039 g whereas the outflowing load was 2.181 g. Removal rates of dissolved loads by the wetland ranged from 39% (simazine) to 100% (cymoxanil, gluphosinate, kresoxim methyl and terbuthylazine). Dimethomorph, diuron, glyphosate, metalaxyl and tetraconazole were more efficiently removed in spring than in summer. More than 88% of the input mass of suspended solids was retained, underscoring the capability of the wetland to trap pesticide-laden particles via sedimentation. Only the insecticide flufenoxuron was frequently detected in the wetland sediments. Our results demonstrate that stormwater wetlands can efficiently remove pesticide mixtures in agricultural runoff during critical periods of pesticide application, although fluctuations in the runoff regime and hydrochemical characteristics can affect the removal rates of individual pesticides.

2. Introduction

Runoff and associated erosion represent a primary mode of mobilization and transfer of pesticides from agricultural land to aquatic ecosystems (Leu et al., 2004; Brady et al., 2006). The contamination of water bodies by pesticides can induce significant threat to drinking water resources and aquatic ecosystems (Probst et al., 2005; Tesfamichael and Kaluarachchi, 2004). Wetland systems can be hydrologically connected to an upstream agricultural catchment and collect contaminated runoff. Natural and artificial wetlands have intrinsic physical, chemical and

biological retention and degradative processes useful for treating various recalcitrant organic substances (Imfeld et al., 2009), including pesticides (Gregoire et al., 2009). Several studies have shown the potential of constructed wetlands as a management practice targeting removal of pesticides from agricultural runoff (Rose et al., 2006; Moore et al., 2009; Budd et al., 2009; Schulz and Peall, 2001). Among constructed wetlands, stormwater wetlands are engineered worldwide with the primary objective to temporarily store urban or agricultural runoff (Gregoire et al., 2009). These wetland systems represent a potential tool for better management practice of contaminated stormwater (Reichenberger et al., 2007). However, quantitative knowledge about the capacity and functioning of stormwater wetlands with respect to the removal of pesticides in runoff is currently very limited.

The mechanisms responsible for the removal of pesticides in constructed wetlands include sorption, photolysis, hydrolysis and biodegradation, which largely depend on the physico-chemical properties of the molecules (Stangroom et al., 2000). Partitioning of pesticides between water, dissolved organic carbon (DOC) and suspended solids also play a fundamental role in the fate of hydrophobic chemicals in wetlands (Luo et al., 2009). In constructed wetlands collecting pesticide runoff, various studies highlighted that intermittent flow conditions and hydrochemical characteristics are key variables that control removal mechanisms, such as sedimentation or degradation (Gregoire et al., 2009; Budd et al., 2009; Kadlec and Wallace, 2008; Hijosa-Valsero et al., 2010). For instance, transient changes of redox conditions in wetland environment can affect the fate and removal of various types of organic contaminants (Imfeld et al., 2009; Borch et al., 2010), and reflect also sedimentation rates, macrophyte development or flow conditions. Hence, the removal of pesticides in runoff by stormwater wetlands is suspected to largely vary, along with changes in runoff regime and hydrochemical characteristics. However, comprehensive field studies on the removal of runoff-associated pesticides in constructed wetlands based on mass balance data and in relation to fluctuation of hydrochemical characteristics and flowconditions are rare. Furthermore, very little information is available on the environmental fate and retention in wetland systems of EU priority pesticides such as diuron and simazine, widely-used herbicides such as glyphosate and its degradation product AMPA, and commonly used fungicides such as dimethomorph or metalaxyl. While the primary function of stormwater wetlands is to temporarily store runoff generated by storm rainfall events, we hypothesized that these systems can additionally contribute to the mitigation of both dissolved and particle-laden pesticides in agricultural runoff. Therefore, the main objective of the present study was to assess the ability of a stormwater wetland to remove

pesticides in runoff from a vineyard catchment during an entire period of pesticide application. In particular, we evaluated the relationships between pesticide removal and changes of both hydrochemical characteristics and flow conditions.

3. Material and methods

3.1. Description of the vineyard catchment and the stormwater wetland

The studied wetland is located at the outlet of a 42.7 ha vineyard catchment in Rouffach (Alsace, France; 47°57′9 N, 07°17′3 E). The characteristics of the catchment and agricultural practices have already been described (Grégoire et al., 2010). Application of pesticides typically takes place from mid-April (bud breaking of grapevine) until August (grapevine maturity). Nine fungicides, six herbicides, one insecticide and four degradation products were selected for the present study because of their widespread use as well as their high frequency of application and detection revealed in previous studies (Grégoire et al., 2010). The studied compounds belong to 12 different chemical groups and largely differ with respect to their physico-chemical properties (see Table VII-1 in the Appendices). Rainfall–runoff events do not generate permanent streams in the catchment and statistically occur every week through the year. Runoff converges at the outlet of the catchment and is collected by the stormwater wetland, which is sized for a 100-year return period of rainfall.

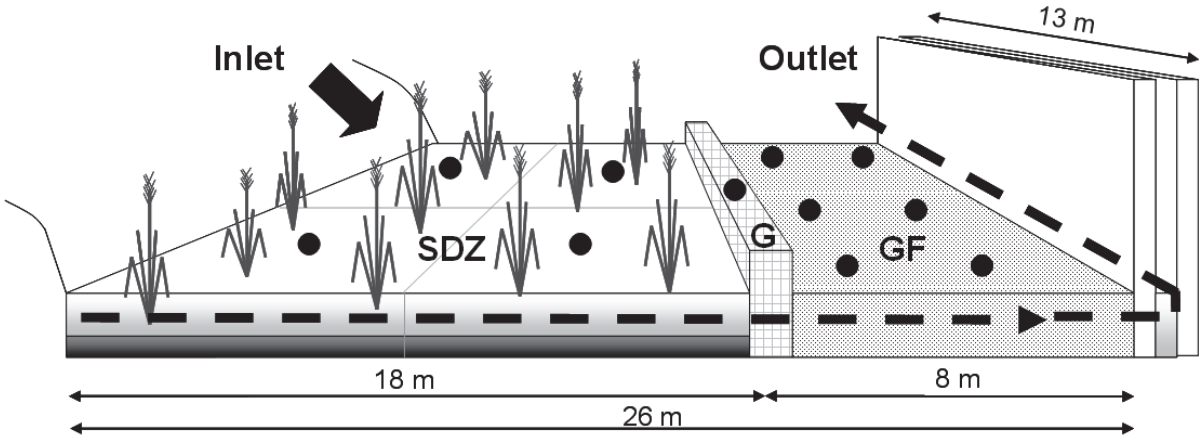

Figure III-1. Schematic of the storm water wetland (Rouffach, Alsace, France) and sampling locations (●) in the sediment deposition zone (SDZ), the gabion barrier (G) and the gravel filter (GF).

The stormwater wetland has a surface area of 319 m^2 and a total volume of 1500 m^3 and was initially constructed to control flooding in the downstream urban area (Figure III-1). The wetland is composed of two main zones in series. The first zone is a sediment deposition pond (234 m^2) that collects suspended solids. The water storage capacity of the sediment deposition zone was 40 m^3. Hence, runoff water mixes with water stored during quiescent period. Water depth in the sediment deposition zone varied from 0.05 to 0.5 m from April to September. Physico-chemical characteristics of wetland sediments were (%): clay 44, fine silt 33, coarse silt 10, fine sand 5, coarse sand 8; organic carbon 14.8; SiO_2 50, Al_2O_3 9.5, MgO 2.2, CaO 11.6, Fe_2O_3 4.1, MnO 0.1, Na_2O 0.7, K_2O 2.5 and pH 8.1 (in water) (n = 5). A gabion barrier is used to enhance the dispersion of water ahead of the gravel filter. The second zone is a 13 m long, 8 m wide and 0.6 m deep gravel filter (saturated hydraulic conductivity, $K=10^{-3}$ m s^{-1}) that increases the hydraulic retention time in the wetland, and thus the capacity of contaminant removal.

Detailed characteristics and hydraulic functioning of the wetland and gravel filter have been studied previously (Wanko et al., 2009) and detailed hydrological characteristics of the wetland that correspond to the investigation period are provided in the Supplementary information (Table VII-2 in the Appendices). Due to the clay liner on the wetland bed ($Ks < 10^{-10}$ m s^{-1}) and based on the water balance, water losses by vertical subsurface infiltration between the sediment/gravel and the clay liner were negligible. The bottom slope of the stormwater wetland was 2.8%. The vegetation cover in the sediment deposition zone, mainly formed of *Phragmites australis*, *Schoenoplectus lacustris* and *Typha latifolia*, was < 1% of the area in April, 5% in May, 25% in June, 60% in July, 70% in August and 85% in September. *Phragmites australis* ranged between 70% and 80% of the total vegetation cover through the investigation period. The vegetation in the gravel filter, mainly formed by *Lolium perenne* and *P. austalis*, varied respectively from 20 to 30% and from 5 to 15% of the area throughout the investigation period. A detailed survey of the vegetation in the stormwater wetland was performed in June and results are provided in Table VII-3 in the Appendices. Algae, mainly *Chara vulgaris*, appeared in the sediment deposition zone since August and covered more than 70% of the area in September.

3.2. Sampling procedure

Daily rainfall and evapotranspiration were measured at a weather station located on the catchment (Meteo France, station no. 68287003). Samples were collected from the inlet, the

sediment deposition zone, the gravel filter, and the outlet of the wetland (Figure III-1) from April 01 through September 29 2009, corresponding to the period of pesticide application. Runoff discharges were continuously monitored by measurements of water depth using bubbler flow modules (Hydrologic, Sainte-Foy, Québec, Canada) combined with a Venturi channel at the inlet and a V-notch weir at the outlet. Water samples were collected every 6 m^3 at the inlet of the wetland using a 4010 Hydrologic automatic sampler (Hydrologic, Sainte-Foy, Québec, Canada) and at the outlet using a 6712FR ISCO Teledyne automatic sampler (Lincoln, Nebraska, US). The detailed procedure of sample collection and storage ensuring reliable pesticide measurements was previously tested and discussed (Domange and Grégoire, 2006). Briefly, water samples (100 mL) were collected in glass jars, stored in the dark at 4°C until collection after each runoff event, and placed on ice during transportation to the laboratory. A series of discrete water samples taken over a runoff event were combined in a single composite sample. Suspended solids were obtained from continuously operating samplers consisting of 2 mm and 50 µm sieves in series and installed at the inlet and outlet of the wetland. The samplers were emptied every week throughout the investigation period. In order to ensure representative and reliable measurements, pesticide concentrations in suspended-solids were measured only when the mass of collected material reached 20 g or more.

In parallel, 10 sampling campaigns were performed every two weeks during quiescent period (i.e. in the period between two runoff events) on day 21 (April 21, 2009), 35, 49, 63, 76, 91, 111, 128, 141 and, after harvesting grapevine, on day 182 (September 29, 2009) to collect water and sediment samples within the wetland. At each sampling campaign, grid-cell sampling was performed in the sediment deposition zone by dividing the zone in four equal rectangular cells (9×6 m) (Figure III-1). Four water samples (collected from 0 to 10 cm depth from the water surface) and four surface sediment grab samples (collected from 0 to 5 cm depth from the sediment surface) were separately collected at the center of each cell. Pore water samples were also collected in the gabion barrier from a PE well and in the gravel filter from six PE wells (Figure III-1). To ensure representative sampling, the wells were purged using a pump to replace the equivalent of one volume of the tube. Dissolved oxygen, pH, conductivity, redox potential and temperature were directly measured in the field using WTW multi 350i portable sensors (WTW, Weilheim, Germany). Water samples were dispensed into 100 ml glass and plastic vials for pesticide analysis (headspace free) and 1 L acid washed HDPE bottles (10% HCl and rinsed with distilled water) for hydrochemical analysis. Water and sediment samples were placed on ice and directly transported to the laboratory for chemical analysis. A chemical

analysis of water samples was performed within 2 days of collection. Sediment samples were kept at −20 °C until chemical analysis, for a maximum of 30 days.

3.3. Analysis of water and sediment samples

Eighteen hydrochemical parameters (TIC, DIC, NPOC, DOC, TKN, PO_4^{2-}, P_{tot}, NO_3^-, NO_2^-, NH_4^+, Mn^{2+}, Fe^{2+}, Fe_{tot}, SO_4^{2-}, Mg^{2+}, Na^+, Cl^-, and K^+) were determined by FR EN ISO standards and laboratory procedures. Pesticide analysis was performed according to the NF XPT 90–210 French standards at the Pasteur Institute of Lille (France), which is a service of pesticide residues analysis accredited by the French National Accreditation Authority (COFRAC). For international quality control purposes, the COFRAC calibration certificate is recognized by other European calibration services (EA - European Cooperation for Accreditation). Briefly, water samples were filtered through 1 µm glass fiber filters, solid–liquid extracted before analyzing the subsequent extracts. The 16 pesticides and four degradation products were quantified using liquid chromatography coupled to tandem mass spectrometry (LC–MS–MS). Quantification of glyphosate, AMPA and gluphosinate was performed after derivatization with fluorenemethoxycarbonyle (FMOC). Limits of pesticide quantification in water samples ranged from 0.02 to 0.1 µg L^{-1}. Quantification of pesticide residues in sediment samples was performed by LC–MS–MS measurements following ultrasonic and methanol extraction. Limits of quantification ranged from 2 to 10 µg kg^{-1}. Extraction efficiencies of pesticides from water and sediment samples were estimated for each sample set by spiking with surrogates. Surrogate recovery for water samples ranged from 70 to 89% and those of sediment from 68 to 85%. Further quality control was achieved by using a blank for each set of samples. Detection and quantification limits, relative standard deviation (RSD) and recovery efficiencies for each studied pesticide are provided for both water and sediment samples in the Table VII-4 in the Appendices.

3.4. Data analysis

Dissolved pesticide concentrations found at the inlet and outlet of the wetland were compared using the paired nonparametric Wilcoxon Signed Rank test. Correlations between hydrological variables and pesticide metrics were tested by the rank-based Spearman's test. Hydrochemical data were subjected to principal component analysis (PCA), which were

performed on the basis of the correlation matrix. In turn, the numerical data matrices were converted using the program R (R: Copyright 2005, The R Foundation for Statistical Computing, Version 2.1.1). Principal component analysis (PCA) is an ordination method that allows summarizing large data sets and exploring the spatial and temporal trends in the data (Imfeld et al., 2009).

Reduction of pesticide concentration, R_C (%), was calculated for each runoff event as the reduction of mean concentrations at the outlet relatively to the mean concentrations at the inlet of the wetland. A nondetect (n.d.) was treated as zero. The R_C (%) in a given period was the average of all runoff event R_C (%) values for this period. Pesticide event loads at the inlet and the outlet of the wetland were obtained by multiplying the mean pesticide concentrations by the corresponding runoff volume. Removal rates of pesticide load R_L (%) were calculated for each runoff event as the reduction of the load at the outlet relatively to the load at the inlet of the wetland using Eq. (1).

$$R_L(\%) = \left[1 - \frac{M_{out}}{M_{in}}\right] \times 100 = \left[1 - \frac{C_{out}\, V_{out}}{C_{in}\, V_{in}}\right] \times 100$$

where M_{in} and M_{out} are the influent and effluent pesticide loadings, V_{in} and V_{out} are the influent and effluent volumes, and C_{in} and C_{out} the inlet and outlet mean concentrations, respectively. Load (mg) at the inlet or outlet of the wetland was calculated from the integral sum of all event loads in a given period (i.e. between 2 sampling campaigns or in a season).

4. Results

4.1. Hydrological and hydrochemical characteristics of the wetland

Detailed climatic and hydraulic data from April 01 through September 29 is provided in Figure VII-1 and in Table VII-2 in the Appendices. Rainfall amount, duration, mean and maximal intensities, as well as the duration of the period between two rainfall events did not significantly differ between spring and summer (p>0.37). Rainfall on the vineyard catchment amounted to 251 mm between April 06 and September 29, and the direct rainfall input on the wetland was 153 m^3. Water loss resulting from evaporation was 99 m^3. Thirty runoff events ranging from 0.3 to 141.8 m^3 occurred during the investigation period, generating a total volume of 730 m^3. The mean quiescent period between two runoff events ranged from 2.4 h to 27 days during the investigation period and did not significantly differ between spring and summer (p>0.61). The

budget of water volumes inflowing and outflowing the wetland was balanced when direct rainfall and evapotranspiration volumes were included. Flow rates at the wetland inlet ranged from 0 to 158.7 m^3 h^{-1} (mean ± SE: 6.3 ± 9.6 m^3 h^{-1}) during the investigation period. Inlet flow rates in spring (2.1 ± 2.7 m^3 h^{-1}) and summer (12.2 ± 11.8 m^3 h^{-1}) did not significantly differ ($p>0.09$), although larger and more variable flow rates were observed in summer. In contrast, outlet flow rates significantly differed between spring (0.3±0.8 m^3 h^{-1}) and summer (0.2 ± 1.0 m^3 h^{-1}) ($p<0.001$), which strongly suggests that larger vegetation cover in summer reduced the flow rate. During the investigation period, the hydraulic retention time (HRT) of the wetland ranged between 6.7 and 14 h (mean ± SE: 10.8 ± 2.6 h) for runoff events exceeding 40 m^3, whereas smaller runoff events could be stored in the wetland. The duration of runoff events ranged between 0.78 and 15 h. However, only one runoff event lasts longer than 12 h and likely completely flushed the stormwater wetland.

The PCA ordination plot (Figure III-2) shows for each of the 10 sampling campaigns the replicate samples collected from the sediment deposition zone and the gravel filter as well as the hydrochemical variables. Symbols in the plot lying close together display similar hydrochemical patterns. The principal component analysis of hydrochemical data revealed that hydrochemical conditions changed in the wetland over time. Water samples collected from the first (April 21) to the fifth sampling campaigns (June 15) clustered together and were clearly separated from those collected from the sixth (June 30) to the tenth sampling campaign (September 29), which indicates distinct hydrochemical profiles between the two periods corresponding to spring and summer. On the variables plot (Figure III-2), scores of PC1 correlated positively to cations (Ca^{2+}, Mg^{2+}, and Na^{+}), anions (Cl^{-}, NO_2^{-}, and NO_3^{-}), redox potential, as well as organic (DOC and NPOC) and inorganic carbon (TIC and DIC). In addition, they correlated negatively to temperature, showing that these hydrochemical variables considerably changed in the wetland between spring and summer. Samples corresponding to the tenth sampling campaign (September 29) were associated with higher concentrations of ferrous iron, manganese and ammonium, indicating the prevalence of reducing conditions in the wetland. Detailed hydrochemical data are provided in the Appendices (Table VII-5). Mean water temperature and pH across all sampling points and campaigns was 19.0 ± 4.3°C and 7.6 ± 0.3, respectively. In spring, oxic conditions prevailed in the wetland, as inferred from mean values of redox potential larger than 50 mV, concentrations of ferrous iron lower than 1 mg L^{-1}, and concentrations of dissolved oxygen higher than 2.9 mg L^{-1} in the sediment deposition zone. In summer, lower concentrations of dissolved oxygen and negative values of redox potential indicated the prevalence of an anoxic

milieu. In spring, Fe^{2+} concentrations were one order of magnitude lower than those of total iron, suggesting the prevalence of the ferric form. In contrast, larger Fe^{2+} concentrations (up to 6.0 mg L^{-1}) attested the occurrence of anoxic conditions in summer. The analysis of both hydrological and hydrochemical data revealed that conditions in the wetland differed between spring and summer. Therefore, pesticide removal by the wetland in spring and summer is compared.

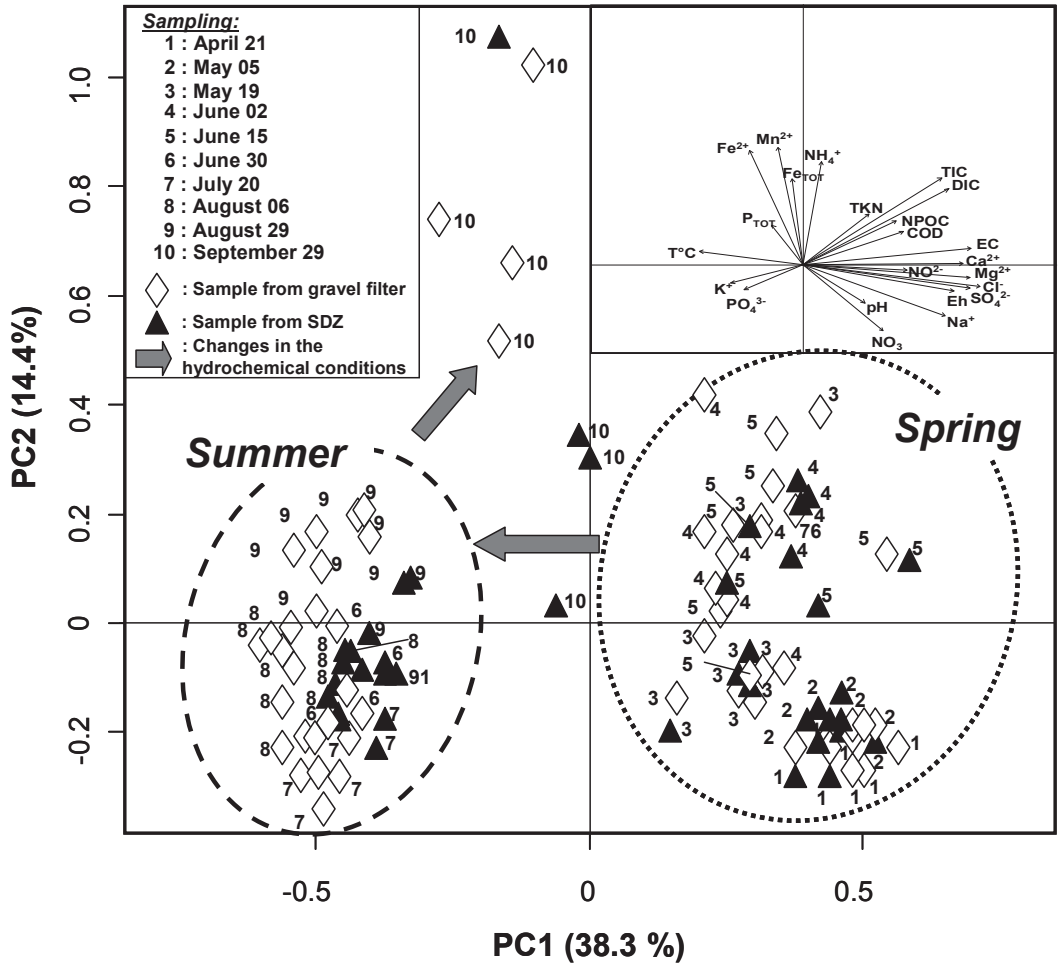

Figure III- 2. PCA ordination plots of hydrogeochemical characteristics of water samples collected in the storm water wetland (Rouffach, Alsace, France) from April 06, 2009 (1st sampling campaign) to September 29, 2009 (10th sampling campaign). Values on the axes indicate the % of the total variation explanation by the corresponding axis (PC 1, principal component axis 1; PC 2, principal component axis 2). The first and second principal components accounted for 52.7 % of the variance in the data set. Objects are labelled according to the section of the wetland they were collected from (▲, sediment deposition zone; ◊, gabion barrier and gravel filter) and numbered according to the sampling campaign: 1 (April 06, 2009) to 10 (September 29, 2009). Description vectors correspond to: T°C, temperature; P_{tot}, total phosphorus; Fe^{2+}, ferrous iron; Mn^{2+}, manganese; Fe_{tot}, total iron; NH_4^+, ammonium; TKN, Total Kjeldahl Nitrogen; TIC, total inorganic carbon; DIC, dissolved inorganic carbon; NPOC, non-purgeable organic carbon; DOC, dissolved organic carbon; EC, electric conductivity; Ca^{2+}, calcium; NO^{2-}, nitrite; Mg^{2+}, magnesium; Cl^-, chlorine; SO_4^{2-}, sulfate; Eh, redox potential; Na^+, sodium; NO_3, nitrate; PO_4^{3-}, orthophosphate; K^+, potassium.

4.2. Occurrence and concentration reduction of pesticides in the wetland

Detailed data of pesticide concentrations in water, in suspended solids and wetland sediments as well as reduction of pesticides based on inlet and outlet concentrations are provided in the Appendices (Table VII-6). Mean concentrations of dissolved pesticides generally decreased between the inlet, the sediment deposition zone, the gravel filter and the outlet of the wetland (Figure III-3A and B). Temporal variation of pesticide concentrations in runoff reflects both timing of pesticide applications in the catchment and changes in rainfall–runoff patterns over time, as previously shown (Grégoire et al., 2010). Degradation products of diuron (DCPU, DCPMU and 1,3-dichloroaniline) were systematically below the detection limit, suggesting that diuron was not subject to aerobic degradation or that degradation products were readily degraded in the wetland. In spring, reduction in mean concentrations from inlet to outlet ranged from 71 (AMPA) to 100% (cymoxanil, dimethomorph, gluphosinate, kresoxim methyl, terbuthylazine and tetraconazole). In summer, concentration reductions were lower compared to those observed in spring, and ranged from 0 (tetraconazole) to 100% (azoxystrobin, cyprodinil, isoxaben, kresoxim methyl and terbuthylazine). Concentrations from inlet to outlet significantly differed for cymoxanil, diuron, glyphosate, AMPA, isoxaben, metalaxyl, simazine, terbuthylazin and tetraconazol in spring and for glyphosate in summer (p<0.05). Pesticide concentrations in water from the sediment deposition zone and the gravel filter were smaller in spring compared to those measured in summer, although concentrations found in the inflowing runoff were similar. Altogether, the results indicate lower efficacy of the wetland in reducing pesticide concentrations in summer. Patterns of pesticide concentrations associated with suspended solids and the wetland sediments also differed between spring and summer (Figure III-3C). Flufenoxuron, dimethomorph, and cyprodinil concentrations associated with suspended solids in inlet samples increased over time and then decreased. However, mean concentrations of pesticides and degradation products in the wetland sediments were close to or below the detection limits, except for flufenoxuron. The results indicate no significant transfer of dissolved or particle-laden pesticides from the water column to the bed sediments, and thus no accumulation or persistence of pesticides in the wetland sediments.

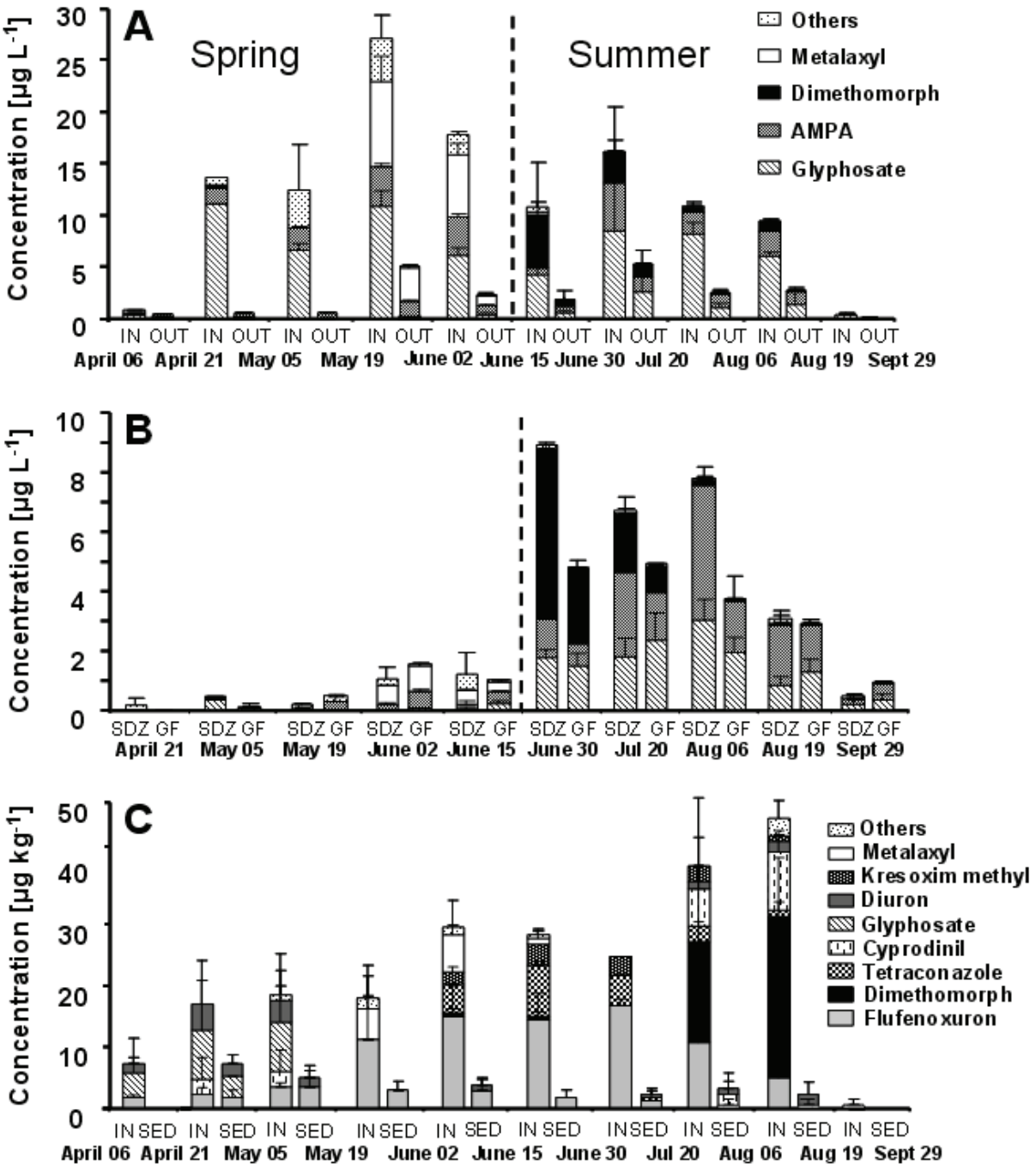

Figure III-3 Mean concentrations of pesticides (A) in the inflowing runoff (IN) and the outlet (OUT), (B) within the sediment deposition zone (SDZ) and the gravel filter (GF), and (C) associated with inflowing suspended solids (IN) and sediment of the stormwater wetland (Rouffach, Alsace, France). Error bars show the standard deviation.

4.3. Removal of dissolved pesticides by the wetland

During the investigation period, the load of the 20 pesticides and degradation products entering the wetland was 8.039 g whereas 2.181 g passed through the wetland (Table III-1). Inflowing load in summer (6.819 g, i.e. 85% of the total dissolved load) was larger compared to that of spring (1.219 g, i.e. 15% of the total dissolved load). This reflects both the seasonal change of runoff regime as underscored in paragraph 4.1 and pesticide applications in the vineyard catchment (31% of the total applications occurred in spring and 69% in summer, data not shown). Glyphosate, AMPA, dimethomorph and the other compounds accounted for respectively, 51.7, 20.4, 21.1, and 6.8% of the total inflowing load.

Table III- 1 Load estimates (mg) of dissolved pesticides and load reduction, RL (%) by the storm water wetland (Rouffach, Alsace, France) in spring (April 06 to June 15, 2009), in summer (June 15 to September 29, 2009) and during the wine growing season (April 6 to September 29, 2009). Degradation products are shown in italics.

Compound	Spring			Summer			Wine growing season		
	Inlet	Outlet	R_L [%]	Inlet	Outlet	R_L [%]	Inlet	Outlet	R_L [%]
Azoxystrobin	0	0	**n.a.**	18.1	1.3	**93**	18.1	1.3	**93**
Cymoxanil	12.3	0	**100**	0	0	**n.a.**	12.3	0	**100**
Cyprodinil	0	0	**n.a.**	18.6	4.3	**77**	18.6	4.3	**77**
Carbendazim	0	0	**n.a.**	0	0	**n.a.**	0	0	**n.a.**
Dimethomorph	3.4	0	**100**	1693	409	**76**	1696	409	**76**
Diuron	26.5	7.4	**72**	13.2	5.7	**57**	39.7	13	**67**
DCPU	0	0	**n.a.**	0	0	**n.a.**	0	0	**n.a.**
DCPMU	0	0	**n.a.**	0	0	**n.a.**	0	0	**n.a.**
3.4-dichloroaniline	0	0	**n.a.**	0	0	**n.a.**	0	0	**n.a.**
Flufenoxuron	0	0	**n.a.**	0	0	**n.a.**	0	0	**n.a.**
Gluphosinate	93.0	0	**100**	0	0	**n.a.**	93.0	0	**100**
Glyphosate	585	56.3	**90**	3571	811	**77**	4156	867	**79**
AMPA	217	196	**10**	1421	585	**59**	1637	781	**52**
Isoxaben	17.0	6.1	**64**	7.4	0	**100**	24.5	6.1	**75**
Kresoxim methyl	1.0	0	**100**	15.7	0	**100**	16.7	0	**100**
Metalaxyl	237	65.1	**73**	35.5	15.6	**56**	272	80.8	**70**
Pyrimethanil	0	0	**n.a.**	12.6	5.3	**57**	12.6	5.3	**57**
Simazine	13.0	8.4	**36**	2.1	0.8	**60**	15.1	9.2	**39**
Terbuthylazine	10.4	0	**100**	3.8	0	**100**	14.2	0	**100**
Tetraconazole	3.8	0	**100**	7.3	3.6	**50**	11.0	3.6	**67**
Total pesticides	1219	339	**72**	6819	1842	**73**	8039	2181	**73**

n.a. not applicable

According to the removal rates calculated from the difference between loads at the outlet and the inlet of the wetland during the entire period of investigation, pesticides can be classified as (i) efficiently retained (removal rates between 80 and 100%; i.e. azoxystrobin cymoxanil, gluphosinate, kresoxim methyl and terbuthylazine); (ii) moderately retained (removal rates between 50 and 80%; i.e. cyprodinil, dimethomorph, diuron, glyphosate, AMPA, isoxaben, metalaxyl, pyrimethanil and tetraconazole); and (iii) poorly retained (removal rates lower than 50%; i.e. simazine). Summing seasonal loads of all compounds, very similar removal rateswere found for spring and summer (i.e. 72 and 73%, respectively), indicating that seasonal changes of pesticide loadings did not affect the removal capacity of the wetland. This was supported by the absence of significant correlation between pesticide loadings in runoff and pesticide removal rates (p>0.1) throughout the investigation period, suggesting no threshold at which pesticide removal would decrease at larger loads. However, removal rates of individual compounds largely varied between spring and summer (Table III-1).

4.4. Retention of particle-laden pesticides

The role of sedimentation in pesticide removal was evaluated based on analysis of total suspended solids (TSS) and dissolved organic carbon (DOC). Detailed concentration data of TSS and DOC, as well as loads of pesticide associated with suspended-solids entering the wetland are provided in the SI (Tables VII-5 and VII-7 in the Appendices, respectively). The pesticide load associated with suspended solids in inflowing runoff was 198 mg for the entire investigation period, which represents 2.4% of the total load. The trifling contribution of the solid load to the total load of pesticides is due to low fractions (<1%) of glyphosate, AMPA and dimethomorph associated with suspended solids, while these compounds accounted for 93% of the total dissolved pesticide load. Nevertheless, partition coefficient Kd in inflowing runoff ranged between −4.22 (glyphosate) and 1.07 (diuron), reflecting large variations of the partitioning patterns among pesticides and seasons (see Table VII-7 in the Appendices for detailed values of Kd). Pesticide concentrations in suspended solids at the outlet of the wetland could not be obtained on a runoff-event basis because the amount of material collected in the sieve of the suspended solid samplers was too low (<5 g of sediment) to enable reliable measurements. Therefore, rates of pesticide removal attributable to retention by the wetland of pesticides associated with suspended solids could not be calculated using a mass balance approach. Nevertheless, average sedimentation rates estimated from discharge measurements and TSS

values were 2.7 kg day^{-1} (99% of the input mass) in spring and 7.0 kg day^{-1} (88% of the input mass) in summer, indicating that the wetland can act as a sink for particle-laden wetland in summer a larger concentration reduction (>90%) for individual pesticides of a mixture of atrazine, *S*-metolachlor and fipronil than those observed in our study, although no pesticide load removal estimates was provided. The same authors emphasized that factors such as wetland size, sediment characteristics, type and density of vegetation and hydrochemical conditions that prevailed at a particular stage of the wetland lifespan can largely influence the ability to mitigate pesticide mixtures.

In stormwater wetlands, removal processes of dissolved and particle-laden pesticides such as sedimentation, photolysis, hydrolysis and degradation are intimately linked with both the prevailing hydrochemistry and the rapid changes of runoff regime. Moreover, their respective contribution strongly depends on the properties of the molecules. Smaller log K_{ow} pesticides (with log K_{ow} < 3) result in loading being predominantly associated with runoff and wetland water, lower partitioning to suspended solids or DOC, and a potentially faster degradation in the dissolved phase owing to higher availability of molecules in abiotic or biotic transformation processes. For less hydrophobic pesticides included in this study (e.i. azoxystrobin, cymoxanil, carbendazim, dimethomorph, diuron, gluphosinate, glyphosate, AMPA, metalaxyl, pyrimethanil, and simazine) an important hydrochemical characteristic in constructed wetlands is their pH. Azoxystrobin, 3.4-dichloroaniline, and simazine were expected to dissipate through aqueous photolysis that prevailed in the wetland during the entire investigation period, given that their half-life was lower than 6 days (refer to Table VII-1 in the Appendices for compound properties). Cymoxanil can be degraded by aqueous hydrolysis at pH 7 which is supported by the complete removal of cymoxanil by the wetland. In contrast, degradation of carbendazim, dimethomorph, diuron, glyphosate and pyrimethanil via photolysis or hydrolysis was not a dominant removal process (half life > 40 days). Nevertheless, mean quiescent period (±SD) between runoff events were 5.1 ± 7.3 and 5.2 ± 5.7 days for spring and summer respectively, indicating sufficient time for both biotic and abiotic degradation reactions to occur in the wetland. In spring, runoff events were lower than 40 m^3 and thus could be stored in the wetland and treated until the next runoff event. Moreover, average inlet flow rates were smaller in spring compared to those observed in summer, although the difference was not statistically significant, and outlet flow rate were significantly larger in spring. Small runoff volumes entering the wetland, low flow rates and longer quiescent periods can increase the contact time between runoff water and wetland pesticides. Since the pore size of the filter paper

used for separating TSS from DOC was 0.45 µm, only finer particles were included in the DOC mass balance analysis. Mass balance of DOC between the inlet and the outlet showed that the output mass (12.9 kg) exceeded by 34% the input mass. This indicates that pesticide removal cannot be attributed to the retention of the DOC-bound fraction in the wetland. Additionally, re-suspension of particles from the wetland bed to the water column during higher flow regime and plant material, as well as sediment re-suspension by the aquatic fauna and proliferation of algae likely reduced the removal of pesticides associated with TSS and DOC by the wetland.

5. Discussion

Pesticides in runoff from agricultural catchments typically occur in mixtures. Therefore studies on pesticide mixtures are necessary to understand how mitigation capacities in wetland systems develop over time and can be used for reducing impacts on receiving aquatic ecosystems. Lizotte et al. (2009) observed in a 700 m long backwater compartments. In contrast, larger runoff volumes and inlet flow rates, such as those observed in summer, are expected to limit the occurrence of removal processes. Nevertheless, larger vegetation cover in summer compared to that observed in spring can largely enhance pesticide removal efficiencies by increasing both sorption sites (e.g. Rogers and Stringfellow, 2009) and contact time, thus compensating shorter times of contact and degradative reactions during high flow conditions. Furthermore, incomplete flushing of the wetland during low to moderate flow conditions (<40 m^3) can also cause longer retention of stable and less sorptive substances. For instance, Lange et al. (in press) used uranine (DT50-photolysis = 11 days) as a reference to mimic photolysis, and sulforhodamine B (Log Kow = −2.02) as one to mimic moderate sorption of contaminants in various wetland systems, including our stormwater wetland. Their study simulated a 37.5 m^3 runoff event and indicated favourable conditions for photocatalytic decay (removal of uranine by 57%) and high sorption capacities (removal of sulforhodamine B by 82%) in our stormwater wetland. In contrast, shorter circuiting and contact time with sediment and vegetation under high flow or flood conditions is expected to decrease removal of dissolved contaminant via sorption and degradation processes, as previously described (Lange et al., in press; Holland et al., 2004).

Besides sorption, larger plant cover and density can also directly affect the removal of pesticides in wetlands. Under anaerobic conditions (prevalent in summer), it is likely that the elimination of chlorinated pesticides (i.e. simazine and terbuthylazine) via reductive dechlorination was also favored by the occurrence of biofilm, sediment, root complexes as well

as potential sources of electron donors provided by roots and organic matter in the wetland (Matamoros et al., 2007; Braeckevelt et al., 2007). Besides, plant uptake cannot be excluded for compounds with a log K_{ow} ranging between 1 and 3 (Cedergreen et al., 2005). However, due to large variations of the vegetal biomass and type in our wetland on both spatial and temporal scales, the contribution of vegetation and vegetal material to pesticide removal could not be quantified in the present study. Though the comprehensive sampling highlighted major hydrochemical changes in the wetland during quiescent period between runoff events, transient changes during runoff events may also occur. Intermittent flow regime in stormwater wetland is presumed to enhance the mixing of anaerobic zones in sediments with the adjacent aerobic and anoxic micro-sites in the rhizosphere, leading to temporal variations of hydrochemical conditions (Allen et al., 2002; Caselles-Osorio and García, 2007).

Oxic conditions that prevailed in spring can be related to higher removal of dimethomorph, diuron, glyphosate, metalaxyl and tetraconazole, whereas higher temperatures and anaerobic conditions in summer can be related to the removal of AMPA, isoxaben and simazine. Seasonal changes of the duration and frequency of rainfall–runoff events, vegetal covering and ecotypes, as well as hydrochemical and climate conditions very likely determined the dominant microbial populations present in the wetland (Truu et al., 2009), as well as the metabolic pathway that pesticides and their degradation products took (Matamoros et al., 2007).

In summer, higher plant density slowed water flows and allowed for particle settling to occur, and may have increased degradation rates by favoring oxidative transformation pathways in the rhizosphere (Burke and Wadzuk, 2009; Faulwetter et al., 2009). Glyphosate and AMPA that accounted for 72.1% of the contaminant load entering the wetland are major compounds in our study. Biodegradation of glyphosate in the environment takes place under both aerobic and anaerobic conditions, although biodegradation under anaerobic conditions is normally less than under aerobic conditions. Biodegradation of AMPA is generally slower than that of glyphosate possibly because of AMPA transient capacity to be strongly sorbed through the phosphonate group and thus protected against further biodegradation (Borggaard and Gimsing, 2008). Among the compounds studied, glyphosate and AMPA are strongly sorbed by soil minerals, and have been previously observed to rapidly adsorb to wetland sediments, before being gradually removed within 5 to 15 days (Tsui and Chu, 2008). This is in agreement with our results showing no accumulation of glyphosate and AMPA in the wetland sediments and efficient degradation of glyphosate into AMPA in the dissolved phase. This was underscored by a larger

AMPA to glyphosate ratio at the outlet (3.5) compared to that found at the inlet (0.4) in spring. In summer, AMPA to glyphosate ratio at the outlet was 0.9, which indicates a more effective removal of AMPA in the dissolved phase.

Since glyphosate and AMPA were not detected in sediments and the occurrence of abiotic degradation mechanisms is unlikely for these compounds, the results indicate that glyphosate was microbially degraded into AMPA, which in turn was gradually degraded in the water column of the wetland. Though variable-charge minerals, such as aluminum or iron oxides, can adsorb large amounts of glyphosate and AMPA, competitive adsorption with phosphorus may occur, explaining the absence of significant sorption onto wetland sediments (Borggaard and Gimsing, 2008). Ratios of dissolved inorganic phosphorus to glyphosate (µmol/µmol) ranging between 20 and 21,040 indicate that competitive adsorption can hinder the partitioning of glyphosate and AMPA into the wetland sediment. Several studies have shown that a large portion of the removal of hydrophobic chemicals with log K_{ow} values > 3 in aquatic environments is due to the sedimentation of pesticide-laden solids (Rose et al., 2006; Luo et al., 2009; Matamoros et al., 2008; Matamoros and Bayona, 2006). However, concentrations in the wetland sediments of flufenoxuron, cyprodinil, isoxaben, kresoxim methyl, tetraconazole and terbuthylazine could not be detected or were one order of magnitude lower than concentrations at the wetland inlet. Although aqueous photolysis of isoxaben and flufenoxuron cannot be excluded (DT_{50}=6 days, at pH=7), significant degradation of hydrophobic compounds in the wetland is not expected due to reduced bioavailability. Therefore, a large fraction of these contaminants passed through the stormwater wetland in association with suspended particles. Transport of pesticides-laden sediment through the wetland under high flow regime has been previously suggested to decrease the removal of hydrophobic pesticides by affecting the degree of bottom scouring and re-suspension of settled solids (Budd et al., 2009). However, no significant correlation was found between runoff volumes and removal rates of dissolved pesticides (i.e. DOC-laden pesticides and pesticides in the aqueous phase) observed in our study, suggesting no threshold at which removal of dissolved pesticides would be reduced at greater runoff inflow. Nevertheless, positive correlations between runoff volumes and both TSS and DOC loads at the inlet (p<0.001) indicate larger particle mass transport through the wetland during large flow events. This is also underscored by moderate load removal of cyprodinil and isoxaben, suggesting that hydrophobic compounds associated with DOC, which are taken into account in the mass balance of dissolved pesticides, were transferred through the wetland.

It also has to be noted that pesticide concentrations in fall (from October 01 to December 30, 2009) ranged from not detected to 0.85 ± 0.42 µg L^{-1} (Glyphosate) at the inlet, and from not detected to 0.57 ± 0.13 µg L^{-1} (AMPA) at the outlet. No significant release of pesticides with log K_{ow} value and no release of hydrophobic pesticides could be observed during fall which indicates that the most of the pesticides mass (>99.6%) entered the wetland and passed from April to September, which correspond to the period of pesticide application (see also Table VII-8 in the Appendices).

6. Conclusion

Our results provide quantitative field data of pesticide mixtures in runoff and stormwater wetlands, in both the particulate and dissolved phases, that often fail to completely evaluate the potential of best management practices (BMPs) for agricultural stormwater. To the best of our knowledge, this paper is the first investigation that reports detailed concentrations and mass balances of pesticides mixtures in a stormwater wetland collecting agricultural runoff during an entire agricultural season.

The results for pesticides and some of their degradation products in this study indicate that stormwater wetlands collecting agricultural runoff have good capacities for retaining, at various flow conditions and loadings, mixtures of pesticides with different physico-chemical properties. Seasonal removal rates of dissolved loads by the wetland ranged from below 60% (simazine, AMPA and pyrimethanil) to 100% (cymoxanil, gluphosinate, kresosxim methyl and terbuthylazine). Our findings also underscore the crucial role of vegetation characteristics for retaining pesticides and of dissolved organic carbon for transporting hydrophobic pesticides in stormwater wetlands.

Accompanied with careful guidance and planning, stormwater wetlands have the potential to serve as a tool for urban and agricultural stormwater management practices, thus contributing to the improvement of water quality for receiving aquatic ecosystems. However, the use of stormwater wetlands as a management practice targeting pesticide mitigation should not be utilized as a unique solution to treat pesticide runoff, but should rather integrate in the design of holistic approaches to stormwater management. The present study demonstrates that the runoff regime works in concert with hydrochemical characteristics to mitigate pesticide in runoff, which should be included into design considerations of stormwater wetlands. However,

further knowledge about hydrological and biogeochemical processes that alter stormwater wetlands during their lifespan is necessary to improve removal of pesticides in runoff.

7. Acknowledgements

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Overview

The previous study showed that stormwater wetlands can efficiently dissipate pesticides from runoff and that dissipation largely varied according to the physico-chemical characteristics of pesticide molecules and intrinsic biogeochemical conditions. For information, a second study aiming at characterizing the influence of the environmental parameters that affect macroinvertebrate communities in the stormwater wetland was carried out during the same investigation period (see "Seasonal changes of macroinvertebrates communities in a stormwater wetland collecting pesticide runoff from a vineyard catchment (Alsace, France)" and published in the Archives of Environmental Contamination and Toxicology (2012) in the Appendices).

A complementary study (see "Removal of dissolved pesticide mixture by a stormwater wetland receiving runoff from a vineyard catchment: an inter-annual comparison" published in International Journal of Analytical Chemistry (2011) in the Appendices) showed that the dissipation efficiency of the stormwater wetland increased between 2009 and 2010, due to a larger vegetation cover, highlighting the apparent essential role of the vegetation in pesticide dissipation.

These previous studies, aiming at understanding the overall system functioning, did not enable to decipher pesticide degradation from retention processes as pesticides were not quantified in the wetland compartments. In the following study (section 2), the partitioning, transport, storage and degradation of 12 pesticides were systematically quantified in all the compartments of the stormwater wetland during an entire agricultural season. Pesticide mass budgets were in particular useful to evaluate the dynamics of pesticide sink and source properties, while distinghuishing degradation from storage properties of the various wetland compartments.

Section 2. Pesticide Mass Budget in a Stormwater Wetland

Elodie Maillard and Gwenaël Imfeld* (submitted to Environmental Science and Technology, * corresponding author)

1. Abstract

Wetlands are reactive landscape zones providing important ecosystem services, including the improvement of water quality. Field studies distinguishing pesticide degradation from retention and release to evaluate the sink and source functions of wetland systems are scarce. Here we quantified the partitioning, retention and degradation of 12 pesticides in the aqueous phase, total suspended solids (TSS), bed sediments, and living organisms in a wetland receiving agricultural runoff. The pesticide mass budget showed that i) dissolved pesticides accounted for 95% of the total load entering the wetland, ii) pesticide partitioning between the dissolved phase and TSS varied according to the molecules, and 85% of the AMPA and dithiocarbamates loads were stored in bed sediments < 250 µm in spring and late summer, and iii) pesticide dissipation was not influenced by the hydrological regime or the incoming pesticide loads but varied according to the molecules and the biogeochemical conditions. The wetland vegetation enhanced pesticide degradation during the vegetative phase and pesticide release during plants senescence. Dithiocarbamates were degraded under oxic conditions in spring, whereas glyphosate and AMPA degradation occurred under reducing conditions in summer. We anticipate our results to be a starting point for considering pesticide sink and source functions of redox-dynamic environments.

2. Introduction

Wetlands are terrestrial-aquatic interface ecosystems in the landscape characterized by dynamic hydrological, biogeochemical and biota gradients. The co-existence of microenvironments sustains the service functions of wetland ecosystems, including water quality improvement. In agricultural catchments, natural or constructed wetlands can receive pesticide-contaminated runoff. Stormwater wetlands primarily control floods and may act as pesticide sinks through both retention and degradation processes (Imfeld et al., 2013; Maillard et al., 2011a; Maillard et al., 2011b). Although several studies have demonstrated the efficiency

of wetland systems to dissipate runoff-related pesticides (Blankenberg et al., 2006; Lizotte et al., 2009; Moore et al., 2002; Schulz et al., 2003), quantitative information on the fate of pesticides in wetlands and their potential release remains scarce. Clearly, pesticide partitioning, retention and degradation processes should be thoroughly evaluated to better understand the sink and source functions of wetland systems receiving pesticide-contaminated water.

Pesticide partitioning between the aqueous phase, the dissolved organic matter (DOM) and the total suspended solids (TSS) is governed by several processes (Birch et al., 2012; Delgado-Moreno et al., 2010; Vignati et al., 2009; Zgheib et al., 2010). Pesticide partitioning is mainly controlled by their physico-chemical characteristics and the nature of runoff-related particles, including their size, shape, polarity or charge distribution (Spark and Swift, 2002). In wetland systems, pesticide partitioning onto solids affects their retention and degradation (Reid *et al.*, 2000). Sorption of hydrophobic pesticides enhances their retention in wetlands through TSS settling (Budd *et al.*, 2009; Moore *et al.*, 2009) while reducing their bioavailability and biodegradation (Rodriguez-Liebana *et al.*, 2011). Sorption is a kinetic-driven process that varies from complete reversibility to total irreversibility depending on the nature of the bond and the contact time of the pesticide-particle interactions, namely the ageing(Gevao *et al.*, 2000). Hence, the transport and partitioning of dissolved and solid-bound pesticides in wetlands involve simultaneous pesticide dissipation processes and dynamic exchanges between the wetland compartments.

Pesticide dissipation includes degradative processes (i.e. degradation by photolysis, hydrolysis, biodegradation) and non-degradative processes (i.e. volatilization and sorption). Degradation and retention should be distinguished to evaluate pesticide behavior with respect to the wetland functioning. Pesticide retention consists in the temporal immobilization of pesticides by sorption, which depends on pesticide partitioning into runoff, hydrological conditions and wetland biogeochemistry (Budd *et al.*, 2011; Maillard *et al.*, 2011a). Pesticide distribution is the spatial allocation of the pesticide pools among the wetland compartments (e.g. sediment beds, vegetation, suspended-solids, and animal species), which depends on the nature of wetland compartments and the physico-chemical properties of the molecule. Pesticide degradation, retention and distribution dynamics in wetlands are controlled by variables, such as the pH and the redox potential, influencing pesticide sorption/desorption. Therefore, the evaluation of pesticide sink and source functions of redox-dynamic environments requires quantitative knowledge on the dynamics of pesticide retention and degradation in the different wetland compartments.

This study aimed at evaluating the partitioning, the retention and the degradation of 12 widely used herbicides and fungicides in a stormwater wetland system collecting contaminated runoff from a vineyard catchment. The stormwater wetland provides a 'natural laboratory' for studying pesticide behavior in redox- and hydrologically dynamic environments. We established a pesticide mass budget over the winegrowing season to quantify i) the transport of the dissolved and solid-bound pesticides in runoff, and ii) the distribution of pesticides among the wetland compartments (i.e. the aqueous phase, TSS, bed sediment, vegetation, algae and invertebrates) with respect to the hydrological and biogeochemical conditions. To the best of our knowledge, this represents the first field study providing detailed quantitative information on the dynamics of pesticide dissipation in a wetland system.

3. Material and methods

3.1. Description of the stormwater wetland.

The studied stormwater wetland is located at the outlet of a 42.7 ha vineyard catchment in Rouffach (Alsace, France, 47°57'9 N, 07°17'3 E). Characteristics of the catchment and the stormwater wetland have been described previously (Maillard *et al.*, 2011a). Briefly, the wetland has a surface area of 319 m^2 and is composed of a forebay (215 m^2) and a gravel filter to increase the hydraulic retention time (HRT) in the wetland, and thus pesticide dissipation (Figure III-4). *Phragmites australis* (Cav.) Trin. Ex Steud (90%), *Juncus effusus* (Lin.) (5%) and *Typha latifolia* (Lin.) (5%) covered from 80 to 100% of the forebay area. Bed sediment characteristics are provided in Table VII-9 in the Appendices. Daily rainfall and evapotranspiration were measured at a weather station located on the catchment (Météo France, station no. 68287003).

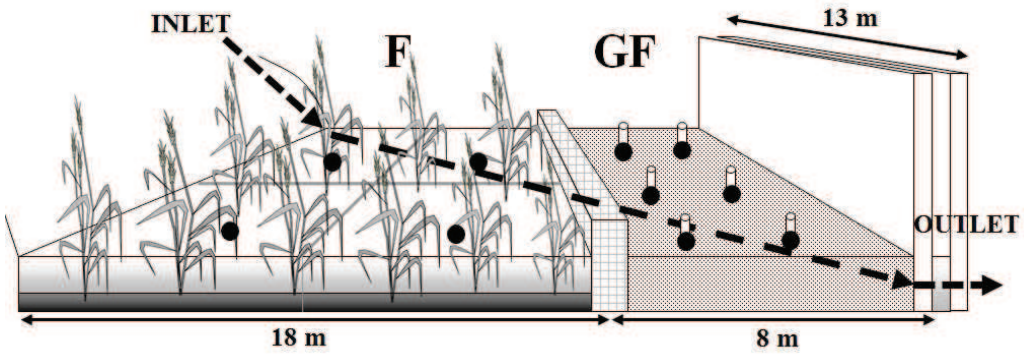

Figure III-4 Layout of the stormwater wetland (Rouffach, Alsace, France) and sampling locations (●) in the forebay (F) and in the gravel filter (GF). The dotted line shows the water direction flow.

3.2. Sampling procedure.

Runoff discharges and volumes were continuously monitored between March 23rd and September 28th, 2011, i.e. during 189 days using bubbler flow modules (Hydrologic, Sainte-Foy, Québec, Canada) combined with a Venturi channel at the inlet and a V-notch weir at the outlet of the stormwater wetland. The hydrological budget of the wetland was balanced when direct rainfall and evapotranspiration volumes were included.

300 mL water samples were collected every 3 m^3 in glass jars using a 4010 Hydrologic automatic sampler (Hydrologic, Sainte-Foy, Québec, Canada) at the wetland inlet and a 6712FR ISCO Teledyne automatic sampler (Lincoln, Nebraska, US) at the outlet. 300 mL water samples were collected every 2 hours at the center of the wetland forebay using an Avalanche ISCO automatic sampler (Lincoln, Nebraska, US). Discrete flow- and time-proportional water samples taken over a week were combined with single composite samples prior to analysis. Samples were stored in the dark at 4 °C, transported on ice to the laboratory and filtered within 6 hours after collection.

The inlet, outlet and forebay samples were filtered through 0.7 µm glass fiber filters to separate TSS from the dissolved fraction (<0.7 µm). The <0.7 µm dissolved fraction was filtered through 0.22 µm glass fiber filters to obtain the <0.22 µm dissolved fraction. Pesticides were quantified in the TSS (>0.7 µm) and the dissolved fractions (<0.7 and <0.22 µm).

The sampling campaigns were carried out monthly in the stormwater wetland from March 23rd to September 07th, 2011, on the days 0, 28, 56, 84, 111, 140 and 168 to quantify pesticides in the different wetland compartments, i.e. the aqueous phase, TSS, bed sediments, vegetation, algae and invertebrates. A grid-cell sampling was conducted by dividing the forebay area in four equal rectangular cells (9 m × 6 m). Samples were collected at the center of each cell (Figure III-4), and the four sub-samples were pooled to obtain a single composite sample for each wetland compartment.

Water samples were collected from the wetland forebay (2.5 L per cell) and from the 6 piezometers in the gravel filter (2 L per piezometer). Water samples from the forebay and the gravel filter were filtered to obtain the TSS > 0.7 µm and the dissolved fractions < 0.7 µm and < 0.22 µm. Grab bed sediments were collected from the top 10 cm of each cell using a coring tube. Composite sediment samples were wet sieved sequentially to quantify pesticides in the fractions > 1000 µm (coarse), 250-1000 µm (medium), and 50-250 µm (fine). The fraction < 50 µm was accounted in the TSS fraction. Composite samples of *Phragmites australis* were separated in

aerial and root sub-samples. Pelagic algae were collected by pumping and sieving (> 50 µm) 20 L of forebay water (4 L per cell). Benthic invertebrates (>1 g) were collected with a Surber-type net (500 cm^2, mesh size 250 µm; 2 surber nets per cell representing 0.4 m^2). Invertebrates mainly consisted in *Chironomidae, Oligochaete* and mollusks (Martin *et al.*, 2012).

A portion of the collected composite samples was used to estimate the mass of the corresponding wetland compartment, including the three granulometric fractions of bed sediment, plant roots and aerial parts, algae and invertebrates, and another portion was kept at −20 °C for pesticide analysis.

3.3. Chemical analyses.

Dissolved oxygen, pH, conductivity, redox potential and temperature were directly measured in the field using WTW multi 350i portable sensors (WTW, Weilheim, Germany) at the center of the 4 cells of the forebay and in the 6 piezometers of the gravel filter. Hydrochemical characteristics (TIC, DIC, NPOC, DOC, TKN, PO_4^{3-}, P_{tot}, NO_3^-, NO_2^-, NH_4^+, Mn^{2+}, Fe^{2+}, Fe_{tot}, SO_4^{2-}, Mg^{2+}, Na^+, Cl^- and K^+) were determined in water samples using FR EN ISO standards and laboratory procedures.

Ten fungicides - i.e. cyazofamid, cyprodinil, difenoconazole, dithiocarbamates (metiram-zinc and mancozeb), fludioxonil, kresoxim methyl, metalaxyl, pyrimethanil, spiroxamine, tetraconazole, and 1 herbicide, glyphosate, and its main degradation product aminomethyl phosphonic acid (AMPA) were selected because of their widespread use as well as their high frequency of application on the catchment (see Table VII-10 in the Appendices for detailed characteristics of the molecules). Pesticide analyses were performed according to NF XPT 90–210 standards and procedure. Fungicides and herbicides were extracted from water and quantified using online solid phase extraction - liquid chromatography coupled to tandem mass spectrometry (SPE LC–MS–MS). The quantification of glyphosate and AMPA was performed after derivatization with fluorenemethoxycarbonyle (FMOC). Quantification of pesticide residues in bed sediment, TSS, vegetation and invertebrate samples was performed by LC–MS–MS measurements following ultrasonic and methanol extraction. Detection and quantification limits, and analytical uncertainties of pesticides for the different environmental matrixes are provided in Table VII-10 in the Appendices.

3.4. Data analysis.

Weekly mass budgets were used to evaluate pesticide dissipation in the wetland and monthly mass budgets were used to evaluate pesticides distribution among wetland compartments. A non-quantified concentration (n.d.) was treated as zero, assuming that no pesticide was present when the concentration was below the quantification limit. Weekly loads of dissolved and solid-bound pesticides at the inlet, outlet and in the forebay were estimated by multiplying pesticide concentrations by the corresponding water volumes and TSS loads.

The pesticide load in each wetland compartment was estimated monthly by multiplying the pesticide concentration in each wetland compartment by the compartment mass. Pesticide loads in bed sediments were estimated based on the pesticide concentrations and the mass of each sediment fraction. The mass of each sediment fraction was calculated based on the sediment fraction density and the sediment volume in the wetland (top 10 cm). For the vegetation, biomasses of roots and aerial parts were estimated based on the plant cover density (plant stems/m^2). The invertebrate biomass retrieved in the surber area (0.4 m^2) was extrapolated to the forebay area. The algal biomass was estimated based on the total water volume in the wetland at the collection time. The uncertainty surrounding the mass estimates of water and TSS at the inlet, outlet, in the forebay and in the gravel filter was 5%, that for plant aerial parts, plant roots, and the bed sediments fractions was 10%, and that for algae and invertebrates was 5%.

The monthly mass budgets of total pesticides were calculated according to eq 1:

$$\sum_{i=1}^{4} \text{Load IN}_i - \sum_{i=1}^{4} \text{Load OUT}_i - \sum \text{Load STORED}_{compartments} = \text{Load DEGRADED} \quad (1)$$

where inflowing (*Load IN*) and outflowing (*Load OUT*) pesticide loads are the sum of weekly pesticide loads at the wetland inlet and outlet respectively for a given month, and *Load* $STORED_{compartments}$ denotes the total pesticide load stored in the wetland on a monthly basis. *Load DEGRADED* > 0 indicates pesticide degradation in the wetland, whereas *Load DEGRADED* < 0 stands for pesticide accumulation since the previous month. The detailed calculations of pesticide loads stored in the different wetland compartments are provided in the Appendices

(eq S1, p.200). Temporal changes in the pesticide stocks (i.e. pesticide retention or release) accounting for all wetland compartments were calculated according to eq 2:

$$\Delta \text{ Load STORED} = \text{Load STORED}_{\text{month}_{m+1}} - \text{Load STORED}_{\text{month}_{m}} \quad (2)$$

Δ Load STORED < 0 indicates pesticide degradation or release by the wetland, and Δ Load STORED > 0 indicates pesticide storage in the wetland compartments (see Figure VII-2 in the Appendices).

Hydrological, pesticides data and hydrochemical data were compared using the paired non-parametric Wilcoxon signed rank and the Spearman rank correlation tests, with the *p*-value set at 0.05 (R software, Version 3.0.1).

4. Results and discussion

4.1. Pesticide partitioning in runoff.

The runoff volume entering the wetland during the investigation period was 1,944 m^3. The input of TSS was 2.3 tons and that of dissolved organic carbon (DOC) was 36.1 kg. 95% of the total pesticide load detected at the inlet of the wetland was found in the dissolved phase of runoff (< 0.7 µm). The total dissolved pesticide load in runoff entering the wetland was 56.3 g (< 0.7 µm) and 58.9 g (< 0.22 µm), whereas the load of solid-bound pesticides accounted for only 3.0 g. The low amount of solid-bound pesticides reflects the impact of grass cover on the vineyard plots, which may significantly reduce erosion and solid transport. Maximum rainfall intensities significantly correlated on a weekly basis with both TSS and the total pesticide loads (i.e dissolved and solid-bound loads) entering the wetland ($p < 0.05$). However, the rainfall intensity did not impact the export of solid-bound pesticides in runoff, as indicated by the absence of correlation between the rainfall intensities and the solid-bound pesticide loads.

No significant difference could be observed between loads of dissolved pesticides in the fractions < 0.7 and < 0.22 µm at the inlet and at the outlet of the wetland, indicating predominant transport of pesticides in the fraction < 0.22 µm, in association with fine organic material or metal oxides in the aqueous phase. This is in agreement with previous observations showing the predominant transport of pesticides in association with colloids < 0.45 µm (Gooddy *et al.*, 2007).

Four groups of pesticides could be distinguished according to their partitioning in runoff water (Figure III-5). Dithiocarbamates, cyprodinil and difenoconazole were exclusively associated with TSS and were not found in the dissolved phase (< 0.7 µm). Cyazofamid was found in filtrates < 0.7 µm but never in those < 0.22 µm, indicating its transport on dissolved organic or mineral materials ranged from 0.22 µm to 0.7 µm. Kresoxim methyl, pyrimethanil, metalaxyl and tetraconazole were mostly detected in the dissolved phases, underscoring their predominant occurrence in the aqueous phase, possibly in association with colloids < 0.22 µm. Glyphosate, AMPA, fludioxonil and spiroxamine were found in the three fractions, and mainly in the dissolved phases (Figure III-5).

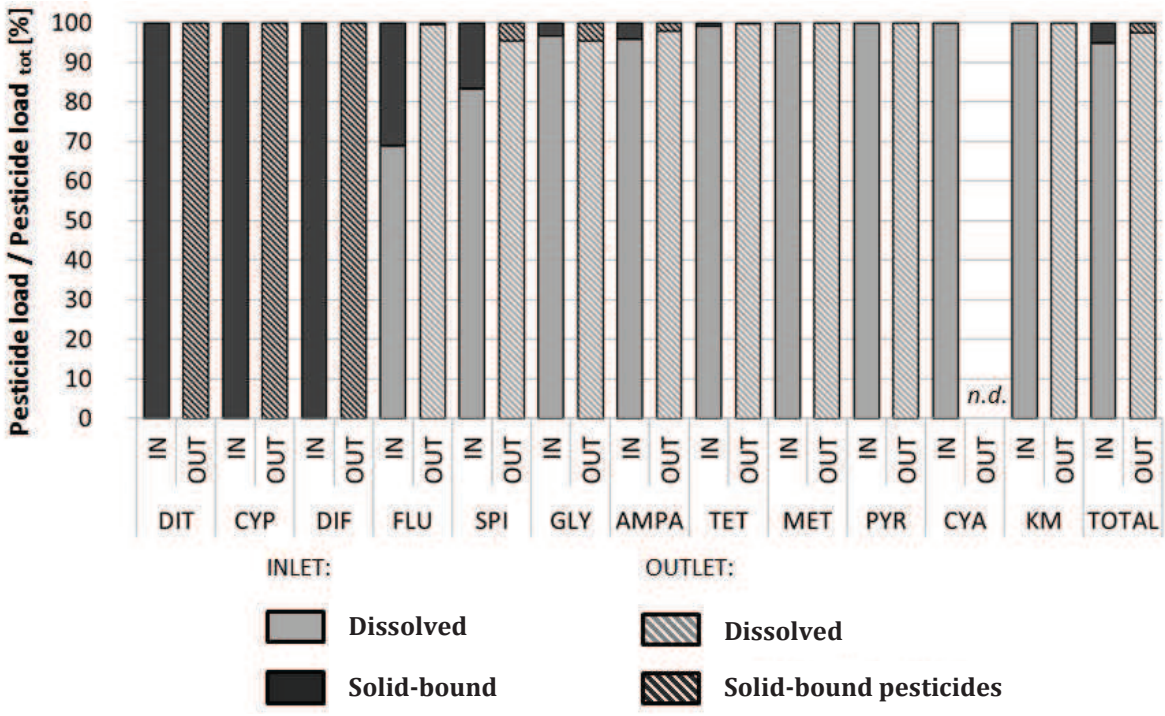

Figure III-5 Relative loads of solid-bound (> 0.7 µm) and dissolved (< 0.7 µm) pesticides at the inlet and the outlet of the stormwater wetland (Rouffach, Alsace, France). n.d. = not detected. DIT: dithiocarbamates, CYP: cyprodinil, DIF: difenoconazole, FLU: fludioxonil, SPI: spiroxamine, GLY: glyphosate, AMPA: Aminomethyl phosphonic acid, TET: tetraconazole, MET: metalaxyl, PYR: pyrimethanil, CYA: cyazofamid, KM: kresoxim-methyl.

Glyphosate and AMPA accounted for the largest portion of the total pesticide loads entering the wetland. Glyphosate represented 86.3% of the total dissolved load of pesticides and 56.7% of the total solid-bound load, whereas AMPA represented 9.5% of the total dissolved load and 7.7% of the solid-bound load (Figure III-6). Glyphosate, AMPA, metalaxyl and pyrimethanil accounted for the largest part of dissolved pesticides (<0.7 µm), whereas glyphosate, AMPA,

dithiocarbamates, spiroxamine and fludioxonil contributed to the pesticide load in TSS. Our results underline that both dissolved and solid-bound pesticides should be considered when evaluating pesticide transport and dissipation in wetland systems.

The octanol-water partition coefficient (log K_{ow}) and the distribution coefficient (K_d) are commonly used to estimate pesticide sorption and environmental risks (Weber *et al.*, 2004). In our study, no significant relationship could be found between phase partitioning of pesticides and the lab-defined log K_{ow} values ($p > 0.05$). Despite the relatively low log K_{ow} values for dithiocarbamates (1.3 for mancozeb, and 1.76 for metiram-zinc), dithiocarbamates were exclusively associated with TSS. In contrast to the above, the hydrophobic cyazofamid and kresoxim methyl were only measured in the dissolved phases (Figure III-5, Table VII-10 in the Appendices). The average K_d values observed at the inlet of the stormwater wetland for glyphosate were 4 orders of magnitude lower (0.07 L kg^{-1}) compared to those retrieved from the literature (ranging from 19 to 547 L kg^{-1})(Farenhorst *et al.*, 2009). Hence, lab-defined sorption properties might be limited for predicting pesticide partitioning under field conditions(Oliver *et al.*, 2012) because sorption equilibrium is probably not be reached in runoff, in particular during fast-flow events(Bondarenko *et al.*, 2006; Lafrance and Caron, 2012; Oliver *et al.*, 2012).

For most pesticides, no significant correlation could be observed between concentrations of TSS or DOC and pesticides at the wetland inlet, outlet and the forebay. Although dissolved organic matter in runoff may increase the apparent pesticide solubility and reduce sorption onto TSS (Elkhattabi et al., 2007; Warren et al., 2003) or soil (2011), pesticides may interact with only a portion of DOC in runoff and preferentially sorb to the wetland bed sediments and vegetation. Moreover, the protection against erosion provided by grass covers in the vineyard and the occurrence of moderate rainfall events possibly decreased the mobilization of DOC-associated pesticides from soil. However, DOC concentrations positively correlated with glyphosate and AMPA in spring ($p < 0.005$), in agreement with previous observations showing that 40% of the amount of glyphosate in a sandy soil was associated with humic and fulvic acids (2009).

The partitioning of pesticides between the aqueous phase, DOC and TSS observed in runoff may in turn impact pesticide dissipation in wetland systems receiving agricultural runoff.

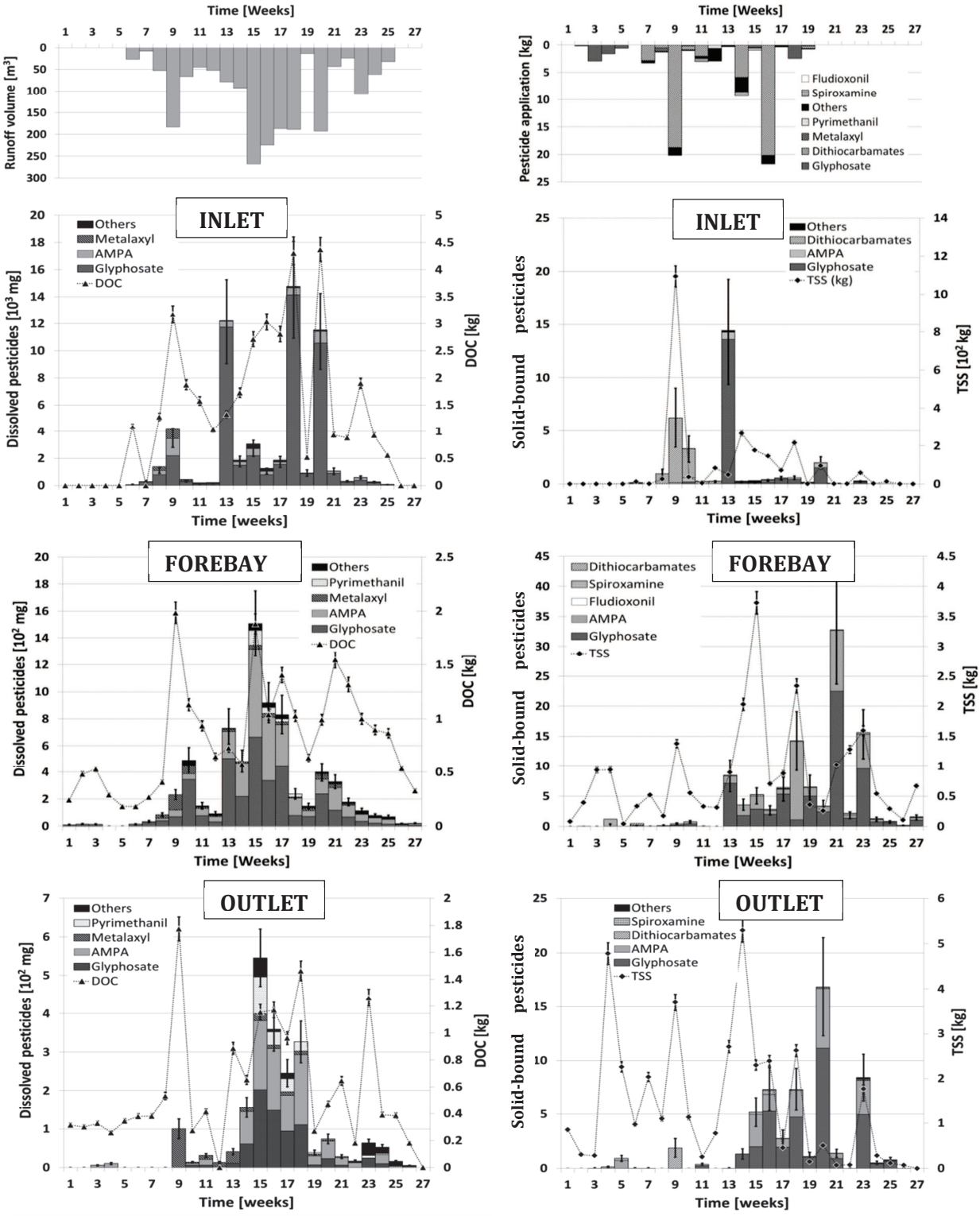

Figure III-6 Loads of dissolved (<0.7 µm; on the left) and solid-bound (>0.7 µm; on the right) pesticides at the inlet, in the forebay and at the outlet of the stormwater wetland (Rouffach, Alsace, France). Error bars correspond to the analytical uncertainty. For total pesticide loads, errors were calculated via error propagation, incorporating analytical uncertainties of individual pesticide concentration measurements as well as the uncertainty of water volume measurements.

4.2. Pesticide dissipation by the wetland.

The dissipation rate of total pesticide loads by the wetland was 96.3% for the entire investigation period, although the weekly dissipation rates ranged from negative to 100%. 2.1 g of dissolved pesticides and 0.06 g of solid-bound pesticides were released by the wetland (average daily flux of pesticides: 11.3 mg day^{-1}). The dissipation rate of total solid-bound pesticides (>0.7 µm) by the wetland was 98%, ranging from 75.5% for pyrimethanil to 99.8% for dithiocarbamates (Figure VII-3 in the Appendices). This underscores the high capacity of the wetland to trap solid-bound pesticides through settling processes, although certain variation could be observed depending on the molecules. The weekly dissipation rates of dissolved pesticides (< 0.7 µm) averaged 96.2 ± 8.2%, but ranged from negative values for fludioxonil (-1266%) to 100% for cyazofamid. Fludioxonil entered the wetland in late summer (after day 147) and overall larger fludioxonil loads were released by the wetland (59.3 mg) compared to those entering (4.3 mg). This may be due to fludioxonil desorption and release from the bed sediments.

No significant correlation could be observed on a weekly basis between the dissipation of total pesticide loads and the average quiescent period (time between two runoff events) or the average hydraulic retention time (HRT) in the wetland. This indicates that the hydrological conditions did not significantly affect the dissipation of both dissolved and solid-bound pesticides in the wetland, although they may influence the dynamics of pesticide distribution among the wetland compartments.

4.3. Pesticide dynamics in the wetland.

The pesticide mass budget enabled to distinguish three main phases of the wetland functioning with respect to pesticide distribution, degradation and retention: spring (March 23rd to May 18th), summer (May 18th to August 10th) and late summer (August 10th to September 7th) (see Figure III-7 and III-8).

4.3.1 Spring: degradation of stored pesticides during the vegetative phase.

The period from March 23rd (day 0) to May 18th (day 56) was characterized by scarce runoff events (86.9 m^3 i.e. 4.5% of the total volume entering the wetland during the investigation period). The weekly rainfall intensity was low and ranged from 0.2 to 1 mm 6 min^{-1}. The average

quiescent period between 2 runoff events was 13.5 ± 9.6 days and the average HRT was 6.7 ± 3.0 days. 80% of the forebay area was covered by vegetation, with densities varying from 35 to 150 stems m^{-2} between day 0 and day 56. The biomass of the aerial plant parts increased from 0.5 to 3.7 kg m^{-2} and that of roots from 1.6 to 8.2 kg m^{-2} between day 0 (March 23rd) and day 56 (May 18th), reflecting the vegetative phase of *Phragmites* in spring. Oxic conditions prevailed in the wetland forebay, as indicated by the average values of oxygen concentration (3.9 ± 4.1 mg L^{-1}), redox potential (50 mV ± 160 mV) and the relatively high concentrations of nitrates (4.4 ± 4.2 mg L^{-1}) and sulfates (204.5 ± 25.7 mg L^{-1}). Nitrate and sulfate were released by the wetland in spring (Table VII-3 in the Appendices). Nitrate release may occur by nitrification or resulting from vegetation decay (Bragazza *et al.*, 2007), while sulfate release by the wetland emphasizes the occurrence of sulfite and sulfide oxidation (Gonzalias *et al.*, 2007).

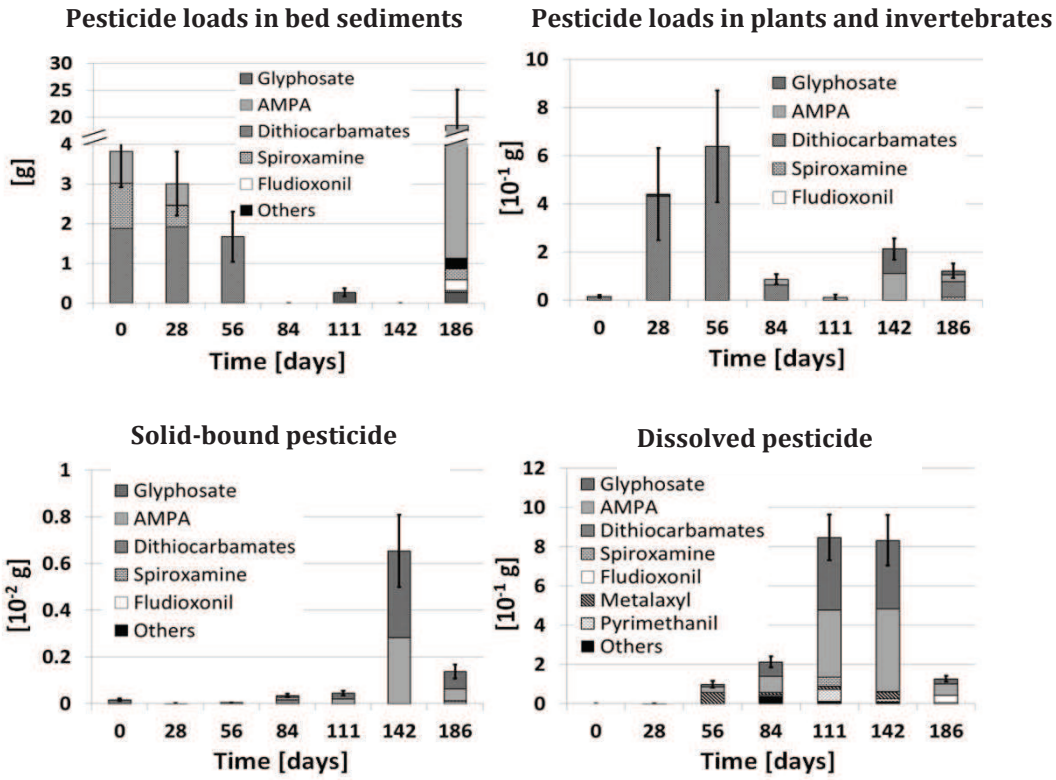

Figure III-7 Monthly pesticide mass budget (g) in bed sediments, plants and invertebrates, suspended solids and in the dissolved phase of the stormwater wetland (Rouffach, Alsace, France). The error bars correspond to the analytical uncertainty. For pesticide loads, errors were calculated via error propagation, incorporating the analytical uncertainties of individual pesticide concentration measurements of and the uncertainty of compartmental (bio)mass measurements.

The total load of runoff-related pesticides entering the wetland was 1.8 g from day 0 to day 56, i.e. 1.1 g of glyphosate, 0.4 g of AMPA, 0.3 g of metalaxyl, 0.1 g of dithiocarbamates and 2.2 mg of spiroxamine, which corresponds to only 3.1% of the total load entering the wetland during the investigation period (Figure III-6). Dissolved pesticides in the fraction < 0.7 µm accounted for 94.3% of the total incoming load. Metalaxyl and spiroxamine were only quantified in the dissolved phase, whereas dithiocarbamates were exclusively associated with TSS and represented 88% of the total solid-bound load.

On day 0, the total pesticide amount in the wetland was 3.8 g, and 91.6% of which was stored in fine bed sediments (50–250 µm). Dithiocarbamates (1.9 g), spiroxamine (1.1 g) and AMPA (0.8 g) mainly contributed to the total pesticide load. Since no pesticides were used in the catchment before day 0, pesticides initially found in the wetland sediments originated from the previous winegrowing period, persisting from one period to the other due to lower microbial degradation activity in winter (Huang *et al.*, 2013). Hence, the wetland may release solid-bound pesticides during storm events and act as a pesticide source in winter (Rose *et al.*, 2008).

From day 0 to day 56, the amount of total pesticides stored in the wetland decreased from 3.8 g to 2.4 g. Dithiocarbamates > spiroxamine > AMPA > glyphosate mainly contributed to the total pesticide load. Dithiocarbamates were taken up by plants, as indicated by a decrease of the dithiocarbamates load in fine bed sediments (from 1.6 g to 1.3 g), and a corresponding increase in the vegetation (from 7.1 mg to 508.7 mg in roots, and from 9.1 mg to 128.0 mg in the aerial parts). 2.3 mg of dithiocarbamates were detected in algae on day 56, accounting for less than 0.1% of the total stored pesticide load. Dithiocarbamates are organosulfur compounds applied on the vineyard catchment as a Cu-based fungicide, which may form stable complexes with transition metals such as Cu(II) or Fe(II). Metal-dithiocarbamates complexes may reduce the dithiocarbamates oxidation at environmentally relevant pH values (Weissmahr and Sedlak, 2000) and passively get diffused across the membrane of living organisms (Phinney and Bruland, 1994). The formation of trace metal-dithiocarbamates complexes may explain the persistence of dithiocarbamates in bed sediments from one agricultural season to the other and the larger plant uptake.

Only 15.1 mg of AMPA was released by the wetland during this period (maximum AMPA concentration: 0.2 µg L^{-1}) while no glyphosate or AMPA entered the wetland. This indicates the AMPA desorption from the bed sediments or plant roots (Figure III-6). Hence, the wetland

principally acted as an AMPA source in spring. Loads of spiroxamine and AMPA decreased in bed sediments between the day 0 and the day 56 (from 1.1 g to n.d. for spiroxamine and from 0.8 to 0.03 g for AMPA) (Figure VII-2B in the Appendices). This underscores the degradation of spiroxamine and AMPA by the wetland under aerobic conditions (Figures III-7 and III-8), possibly favored by the release of oxygen and root exudates into the rhizosphere supporting microbial degradation activity (García *et al.*, 2010), (Borggaard and Gimsing, 2008; Sukul *et al.*, 2010).

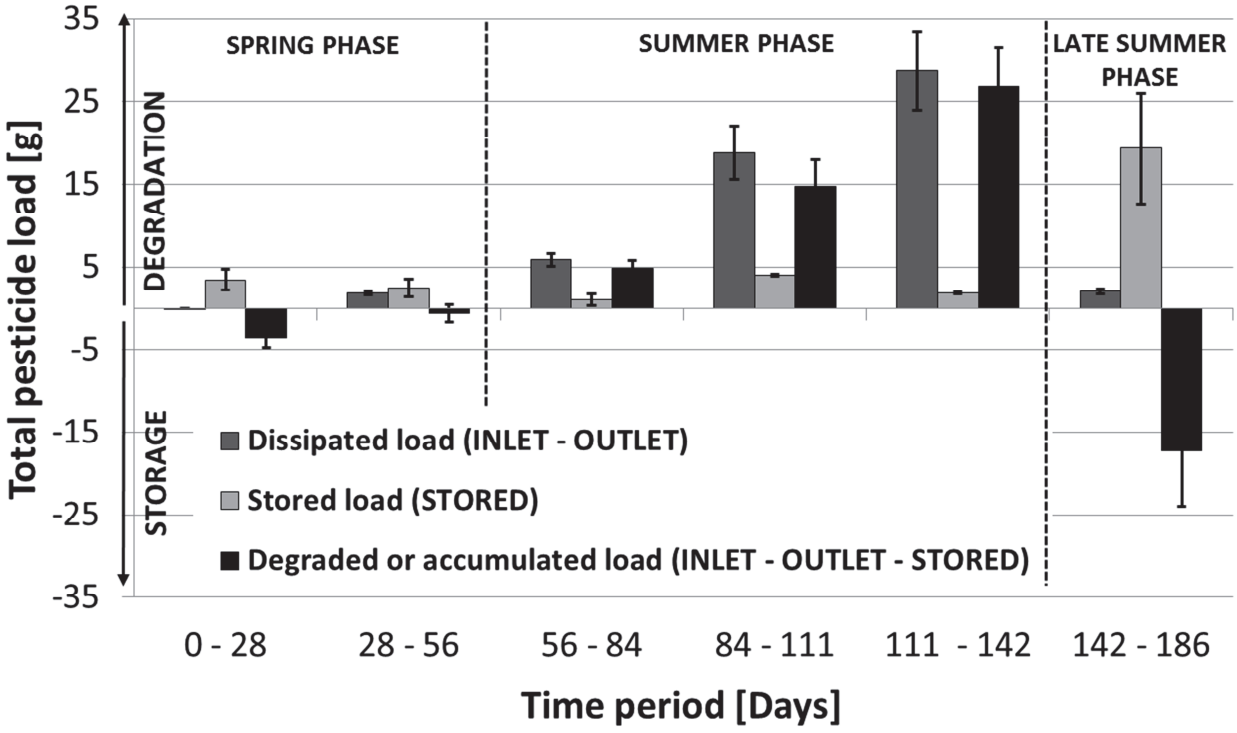

Figure III. 8 Monthly pesticide mass budget emphasizing pesticide storage vs. degradation in the stormwater wetland (Rouffach, Alsace, France). The dissipated pesticide load (INLET load – OUTLET load) refer to the load stored (STORED load) or degraded (INLET load – OUTLET load – STORED load > 0) in the wetland, whereas the accumulated load (INLET load – OUTLET load – STORED load < 0) is the load accumulated in the wetland from one period to the other. The error bars correspond to the analytical uncertainty and, errors were calculated via error propagation, incorporating analytical uncertainties of individual pesticide concentration measurements and the uncertainty of compartmental (bio)mass measurements.

Due to the dense vegetation cover and the relatively high photodegradation half-life times ($DT50_{photolysis}$ > 10 days), significant pesticide photodegradation is not expected, with the exception of cyazofamid ($DT50_{photolysis}$ = 0.1 days) (Table VII-10 in the Appendices). Hydrolysis is expected to be negligible in the wetland conditions, except for dithiocarbamates (DT50 = 1.3

days, pH = 7, 20°C). However, the formation of metal-dithiocarbamates complexes precludes the dithiocarbamates hydrolysis (Weissmahr and Sedlak, 2000). Pesticide loss by volatilization can be neglected in the mass budget for pesticides with low vapor pressure (<0.18 mPa), and estimates of total mass losses by volatilization for pyrimethanil, metalaxyl and spiroxamine (vapor pressure <3.5 mPa, non-dimensional Henry constant <10-7) are in the range of the analytical error (< 1% of the total mass budget).

Although pesticides were degraded in the wetland in spring, they also accumulated considering that the amount of pesticides found in the wetland on day 56 (2.4 g) exceeded the one entering the wetland (1.8 g) (Figure III-8). Stored pesticides can be partly released from the wetland bed sediments but can also transit from the bed sediments to the vegetation, revealing that the wetland mostly acted as a pesticide sink during the vegetative phase.

4.3.2 Summer: high pesticide input and high degradation by the wetland.

From May 18th (day 56) to August 10th (day 140), 1,589.6 m^3 of runoff water entered the wetland, which corresponds to 81.8% of the total entering volume. The maximum rainfall intensity on a weekly basis was in average 5 times higher than in spring. The average quiescent period was 6.4 ± 2.3 days, indicating more frequent runoff events in summer than in spring. Larger plant density and higher evapotranspiration rates decreased the outflow discharge resulting in longer HRT in summer (14.7 ± 8.4 days). The vegetation cover was 80% with an average density of 175 stems m^{-2}, i.e. 4 times higher than in spring. Common reed evapotranspiration was 19.3 mm d^{-1} from day 56 to day 140, in agreement with previous observations (Borin *et al.*, 2011). Anoxic conditions prevailed in summer, as indicated by dissolved oxygen concentrations averaging 0.3 ± 0.3 mg L^{-1} and redox potential values ranging from -20 to -120 mV). Nitrate and sulfate mass budgets indicated nitrate (-69 ± 42%) and sulfate reduction in the wetland from the end of June (day 91).

52.5 g of dissolved and 2.9 g of solid-bound pesticides entered the wetland, which represented 93% of the total input load during the investigation period. Glyphosate (46.0 g; 87.6%), AMPA (4.5 g; 8.6%), metalaxyl (961 mg; 1.8%), pyrimethanil (366.9 mg; 0.7%), and spiroxamine (251.3 mg; 0.5%) mainly contributed to the dissolved pesticides load, which accounted for 97.5% of the total pesticide load. Pesticides mostly contributing to the solid-bound load were glyphosate (1.7

g; 58.7%), dithiocarbamates (921.8 mg; 32.1%) and AMPA (224.0 mg; 7.8%). The total pesticide load released by the wetland in summer was 1.9 g, corresponding to 3.4% of the total inflowing load. The total pesticide loads in the wetland were 0.3, 1.2 and 1.1 g between day 56 and 84, day 84 and 111 and day 111 and 140, respectively, and accounted for less than 6% of the total load entering the wetland. This indicates fast pesticide degradation in the wetland, and that pesticide retention was not the predominant dissipation process in summer (Figure III-8).

Even though larger pesticides loads entered the wetland in summer, stored pesticides loads were one order of magnitude lower than those found in spring. The largest pesticide loads were found in the dissolved phase of the wetland forebay (203.0 mg on day 84 and 765.4 mg on day 140 g), which indicates limited pesticide storage in the sediments and the vegetation, likely due to regular mixing of the forebay water during runoff events. Other relevant pools of pesticides in the wetland were fine bed sediments (270.7 mg on day 111) and plant roots (from 55.8 mg on day 84 to 177.5 mg on day 140). Pesticides loads in algae and invertebrates accounted respectively for 10.4 (31.3 mg) and 0.13% (0.4 mg) of the total stored pesticide load on day 84. Algae were no more observed in the wetland after day 84.

The AMPA fraction (%AMPA), calculated as a percentage of total loads of glyphosate and AMPA, ranged from 11.1 to 62.5% in the wetland forebay and from 47.1 to 100% at the wetland outlet, which indicates that glyphosate was degraded into AMPA in the wetland under anoxic conditions (Figure III-6). The dissipation rate of glyphosate for the investigation period was 98.5%, whereas that of AMPA was 83.0%. This highlights that AMPA was more persistent than glyphosate under anoxic conditions, whereas it was rapidly degraded under oxic conditions (Borggaard and Gimsing, 2008; Imfeld *et al.*, 2013). Plant roots accumulated glyphosate (101.0 mg or 0.3 mg m^{-2} wetland) and AMPA (76.5 mg or 0.2 mg m^{-2} wetland) between July 12th and August 10th, underscoring sorption and/or plant uptake. Between day 56 (May 18th) and day 142 (August 10th), dithiocarbamates did not accumulate in the wetland, although change in stored loads indicates a gradual decrease of dithiocarbamates degradation over time (Figure VII-2C in the Appendices).

Overall, the wetland acted as a pesticide sink in summer. Degradation was the main dissipation process resulting in low pesticide accumulation despite large incoming pesticide loads. The occurrence of anoxic conditions in the wetland was compatible with pesticide degradation, as shown for glyphosate and AMPA, whereas dithiocarbamates degradation seemed to be less efficient under anaerobic conditions.

4.3.3 Late summer: pesticide degradation and accumulation in the mature wetland.

From August 10th (day 142) to September 7th (day 168), 235.4 m^3 of runoff entered the wetland, corresponding to 13.8% of the total volume entering the wetland during the investigation period. The average quiescent period was similar to that in summer (6.5 ± 2.2 days), whereas the average HRT (26.9 ± 8.1 days) was 2 times longer than in summer due to lower inflowing water volumes (59 ± 36 m^3) compared to those in summer (133 ± 83 m^3). Although the vegetation cover was denser (200 stems m^{-2}), the plant root biomass was lower than in summer (-32%), and the evapotranspiration decreased between day 142 and 168, emphasizing the vegetation senescence in late summer. Anoxic conditions still prevailed in the wetland forebay (dissolved oxygen averaged 0.2 ± 0.1 mg L^{-1}), but were less reductive compared to those in summer, as indicated by lower mass depletion for nitrate (37%) and sulfate (28 ± 53%) in the wetland.

2.2 g of dissolved pesticides and 38.8 mg of solid-bound pesticides entered the wetland, corresponding to only 3.8% of the total inflowing pesticide load during the investigation period (Figure III-7). Most contributive molecules were glyphosate (1.7 g), AMPA (0.5 g), tetraconazole (17.1 mg), spiroxamine (12.7 mg) and metalaxyl (10.7 mg), whereas difenoconazole, fludioxonil and cyprodinil were detected but could not be quantified. Pesticides were not used in the vineyard catchment after day 132 (August 2nd).

In late summer, the main compartments for pesticide storage were fine bed sediments (18.4 g of pesticides) > plant roots (122.6 mg) > dissolved phase (105.4 mg) > coarse bed sediments (46.1 mg) > TSS of the forebay (12.1 mg). The pesticide load in the wetland was mainly composed of AMPA (17.4 g), glyphosate (321.2 mg), fludioxonil (306.5 mg) and spiroxamine (284.1 mg), and was lower than that found in the dissolved phase, TSS and vegetal biomass in summer (Figure III-7).

The accumulation of AMPA in fine bed sediments reflects the settling of solid-bound pesticides from the water column and sorption of dissolved AMPA due to longer HRT in late summer (Figures III-7 and III-8). These results are in agreement with previous studies underscoring that AMPA was more sorptive than glyphosate (2011) and primarily sorbs to metal (hydro)oxides of clay materials and humic substances (Vereecken, 2005). In particular, AMPA was found to be more persistent than glyphosate in soils (Kjær *et al.*, 2005; Mamy *et al.*,

2008) due to the formation of non-extractable residues stabilizing AMPA and thus decreasing its bioavailability (Al-Rajab and Schiavon, 2010). Lower pesticide degradation by the wetland in late summer compared to summer is due to the larger portion of fine bed sediments. The larger clay content of fine bed sediment (+22% from day 0 to day 168) increased the specific surface area, which resulted in larger loads of pesticides associated with clay metals (hydr)oxides and stronger sorption of AMPA compared to glyphosate (Figure III-7 and Figure VII-2 in the Appendices). Plant decay may also contribute to pesticide accumulation in bed sediments by increasing both the content of organic matter and the diversity of carbonaceous sorbents.

In late summer, the wetland mostly acted as a pesticide sink characterized by moderate pesticide degradation and accumulation in fine bed sediments. The occurrence of a persistent pool of AMPA in the wetland sediments which can be released in winter has to be carefully considered in the management of wetland systems receiving pesticide runoff.

4.4. Environmental implications for wetlands receiving pesticide fluxes.

In this study, we evaluated the partitioning, the degradation and the distribution of pesticides in a wetland system based on a detailed pesticide mass budget. The results provide a rational basis for understanding the pesticide sink functions provided by wetlands as an ecosystem service to improve water quality.

The sink function of wetlands regarding pollutants is a long-standing ecological statement that has been explored in many studies, whereas the concept of wetlands acting as pollutant sources is more recent and raises fundamental issues on the metabolism of organic pollutants in wetland systems. The sink-source dynamics of wetland systems with respect to pesticides appears crucial to understand pesticide retention and degradation processes. In this study, we show that wetland systems mainly act as pesticide sinks from spring to summer. The pesticide retention and degradation by the wetland, and the distribution dynamics of pesticides among the wetland compartments are intimately linked to the vegetation life cycle.

Our results demonstrate that stormwater wetlands can remove efficiently both dissolved and solid-bound pesticides, even when the pesticide loads are predominately transported in the dissolved phase. Hydrophobic molecules were efficiently retained by the wetland whereas more

hydrophilic molecules, such as AMPA or fludioxonil, were fairly transported and less retained in spring and late summer. Plant roots and fine sediments (50 and 250 μm) were the main wetland compartments contributing to the retention of glyphosate, AMPA and dithiocarbamates. Pesticides did not accumulate in the vegetation except during the vegetative stage. Wetland vegetation enhanced pesticide degradation in the rhizosphere, and pesticide degradation was linked to the development of vegetation in the wetland forebay. Pesticide degradation by the wetland was maximal in summer when the vegetation was mature, under anoxic conditions, and when large pesticide loads entered the wetland. In spring and late summer, the wetland mostly accumulated pesticides in fine wetland bed sediments. AMPA accumulation in fine sediments in late summer raise critical issues concerning the ecotoxicological risk posed by the release of emerging and poorly described degradation products from wetland systems, and the management of wetland sediments.

Overall, this study is a first attempt to quantify pesticide pools in wetland compartments under field conditions and to distinguish storage from degradation in redox-dynamic environments, such as wetlands. We anticipate our study to be a starting point for investigating the pesticide sink and source functions of wetland systems.

5. Acknowledgements

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The second section evaluated the storage and the degradation dynamics of several widely used pesticides in wetland systems. In particular, degradation was shown to be a major dissipation process in summer, during the period of pesticide application, whereas storage only occurred in early spring and late summer. We focused in the following study on the transport and the dissipation of the most widely used herbicide glyphosate and its main degradation product AMPA. The consideration of degradation products in general and in particular of AMPA in both surface and ground-water is a current challenge that requires detailed field studies.

Section 3. Transport and attenuation of dissolved glyphosate and AMPA in a stormwater wetland

1. Abstract

Glyphosate is an herbicide used widely and increasingly since the early 1990s in production of many crops and in urban areas. However, knowledge on the transport of glyphosate and its degradation to aminomethylphosphonic acid (AMPA) in ecosystems receiving urban or agricultural runoff is lacking. Here we show that transport and attenuation of runoff-associated glyphosate and AMPA in a stormwater wetland differ and largely vary over time. Dissolved concentrations and loads of glyphosate and AMPA in a wetland receiving runoff from a vineyard catchment were assessed during three consecutive seasons of glyphosate use (March to June 2009, 2010 and 2011). The load removal of glyphosate and AMPA by the wetland gradually varied yearly from 75% to 99%. However, glyphosate and AMPA were not detected in the wetland sediment, which emphasises that sorption on the wetland vegetation, which increased over time, and biodegradation were prevailing attenuation processes. The relative load of AMPA as a percentage of total glyphosate increased in the wetland and ranged from 0% to 100%, which indicates the variability of glyphosate degradation via the AMPA pathway. Our results demonstrate that transport and degradation of glyphosate in stormwater wetlands can largely change over time, mainly depending on the characteristics of the runoff event and the wetland vegetation. We anticipate our results to be a starting point for considering degradation products of runoff-associated pesticides during their transfer in wetlands, in particular when using stormwater wetlands as a management practice targeting pesticide attenuation.

2. Introduction

Glyphosate [N-(phosphonomethyl)glycine] is a broad spectrum, post-emergence herbicide used worldwide to control weeds in agricultural, silvicultural and urban areas. Glyphosate is particularly used in the production of soybeans, corn, potatoes, and cotton that have been genetically modified to tolerate glyphosate (Bonny, 2008). The worldwide use of glyphosate requires paying special attention to its transport by runoff from land to aquatic ecosystems. Several studies reported that glyphosate and its main degradation product aminomethylphosphonic acid (AMPA) are frequently detected in runoff, surface and ground water, with relative concentrations varying according to the farming practices and/or the

hydrological conditions (Battaglin et al., 2005; Grégoire et al., 2010; Hao et al., 2011; Maillard et al., 2011; Coupe et al., 2012). However, information on the transport of glyphosate and its degradation to AMPA in ecosystems that receive contaminated runoff is scarce. Glyphosate is widely used throughout the growing season in intensive and perennial cultures, such as that of vine, and significant runoff loss of glyphosate and AMPA from vineyard catchments was observed previously (Shipitalo et al., 2008; Grégoire et al., 2010; Maillard et al., 2011).

During rainfall-runoff events, buffer zones such as wetland systems can intercept and partly retain runoff-related pesticides, thus limiting their transport to aquatic ecosystems (Grégoire et al., 2009). Stormwater wetlands are engineered worldwide to temporally store urban or agricultural runoff, and recent studies have underscored their potential as a management practice targeting pesticide attenuation and water quality improvement (Page et al., 2010; Budd et al., 2011; Maillard et al., 2011). In previous studies, we evaluated the transport of pesticide mixtures in a stormwater wetland that collects runoff from a vineyard catchment (Maillard et al., 2011; Martin et al., 2012). However, the transport and degradation of glyphosate in temporally or permanently flooded ecosystems remain poorly described, and has not yet been quantitatively evaluated in wetland systems that receive contaminated runoff. Such quantitative evaluation is required to better understand the functioning and the role of wetland systems thatreceive pesticide runoff. In this study, the transport and attenuation of glyphosate and AMPA in a stormwater wetland that collect runoff from a vineyard catchment was evaluated during three successive periods of glyphosate use. To the best of our knowledge, this represents the first thorough field study on the transport and degradation of glyphosate in wetlands receiving contaminated runoff.

3. Material and methods

3.1. Chemicals

Glyphosate (see Figure VII-4 in the Appendices) is the active compound of most used herbicide preparations worldwide, such as Roundup. The chemical characteristics of glyphosate are strongly dependent on pH due to the four ionisable hydrogen atoms on its functional groups (pKa values 2.0, 2.6, 5.6 and 10.6). Pure glyphosate is a crystalline solid with very high water solubility (12 g L^{-1} at 20°C) (Franz et al., 1997), very low vapour pressure (5.7×10^{-8} Pa at 25°C) (Battaglin et al., 2005), and its partition coefficient (K_d) values range from 19 to 547 L kg^{-1}, depending on the surface characteristics of the solid, the pH of the solution and the

concentration of di- and trivalent cations (Farenhorst et al., 2009). Less is known about the environmental fate of AMPA (Figure VII-4 in the Appendices). The water solubility of AMPA is 5.8 g L^{-1} and its soil half-life range between 76 and 240 d, which is longer than that of glyphosate (1 - 174 d) (Farenhorst et al., 2009).

3.2. Description of the vineyard catchment

The 42.7 ha vineyard catchment is located in Rouffach, Alsace, France (47°57'9'' N, 07°17'3'' E) and was described previously (Grégoire et al., 2010). The study was carried out between March 23 and June 30, 2009, 2010 and 2011 because glyphosate use mainly proceeds in spring, from the end of March (bud-breaking of grapevine) to June (fruit-setting of grapevine). The use of glyphosate was estimated based on yearly surveys addressed to the vine-growers (surveys covered at least 80% of the vineyard area). The detailed use of glyphosate in commercial preparations is provided in Table VII-12 in the Appendices. The mean precipitation from March 23 to June 30 is 204 ± 70 mm (1998–2011). Rainfall-runoff events do not generate permanent stream in the catchment and statistically occur every week. During rainfall-runoff events, contaminated runoff converges at the outlet of the catchment where it is collected by the stormwater wetland. Surface runoff constitutes the main route of pesticide entry in the wetland.

3.3. Description of the stormwater wetland

The wetland was constructed in 2002 to control flooding into the urban area. The stormwater wetland, described previously in Maillard et al. (2011), has a surface area of 319 m^2 and a total volume of 1500 m^3 (a scheme of the wetland is provided in Figure VII-5 in the Appendices). It is composed of a naturally planted forebay (215 m^2). The mean hydraulic retention time was 11.0 ± 8.3 h during the periods of investigation. The water storage capacity of the wetland forebay was 50 m^3. Water depth in the forebay varied from 0.1 to 0.5 m during the investigation periods, depending on the runoff volume entering. A secondary small inflow also contributed to the volume entering the wetland from March to May. The budget of water volumes entering and outflowing the wetland was balanced when direct rainfall and evapotranspiration volumes were included (data not shown). Due to the clayey wetland bed (permeability (Ks) < 10^{-10} m s^{-1}) and based on the water balance, water losses by vertical infiltration were negligible. The chemical composition of wetland sediment was (mean ± SD%; *n* = 5): organic carbon 15.0 ± 0.9, SiO_2 49.6 ± 0.5, Al_2O_3 10.4 ± 1.1, MgO 2.2 ± 0.1, CaO 11.6 ± 1.1,

Fe_2O_3 4.5 ± 0.5, MnO 0.1 ± 0.0, Na_2O 0.6 ± 0.1, K_2O 2.4 ± 0.2 and P_2O_5 0.4 ± 0.1. The sediment texture was (%): clay 44, fine silt 33, coarse silt 10, fine sand 5, and coarse sand 8. The pH value was 8.1. Sediments were removed from the wetland forebay on February 2008. Glyphosate and AMPA were analysed in the wetland sediment in 2009 and 2011, as described previously (Maillard et al., 2011). In 2009, the vegetation cover in the wetland forebay, mainly formed of Phragmites australis, Juncus effusus and Typha latifolia, was <1% of the area in March and April, 10% in May, and 50% in June. In 2010 and 2011, the same plant species were present and the vegetation covered 100% of the forebay area from April to June. *Phragmites australis* (Cav.) represented 90% of the total vegetation cover through the investigation period. No algal growth was observed.

3.4. Runoff discharge measurement and sampling procedure

Runoff discharges entering and outflowing the wetland were continuously monitored from March 23 to June 30, 2009, 2010 and 2011. The water depth was measured using bubbler flow modules (Hydrologic, Sainte-Foy, Canada) combined with a Venturi channel at the wetland inlet and a V-notch weir at the outlet. Flow proportional water samples were collected at the inlet using a 4010 Hydrologic automatic sampler (Sainte-Foy, Québec, Canada), and at the outlet using a 6712FR ISCO Teledyne automatic sampler (Lincoln, Nebraska, US). The detailed procedure of sample collection and storage ensuring reliable pesticide measurements was tested and described previously (Domange and Grégoire, 2006). Briefly, water samples (300 mL) were collected in jars, stored in the dark at 4°C after each runoff event, and placed on ice during transportation to the laboratory for chemical analysis. The series of discrete flow proportional water samples taken over a runoff event were combined in a single composite sample prior to analysis.

3.5. Chemical analysis

Conductivity, pH, dissolved oxygen and redox potential were directly measured in the field using WTW multi 350i portable sensors (WTW, Weilheim, Germany). Concentrations of dissolved organic carbon (DOC), total suspended solids, total phosphorus and PO_4^{3-} were determined by FR EN ISO standards and laboratory procedures. Glyphosate and AMPA were analysed according to the NF XPT 90-210 at the Pasteur Institute of Lille (France), which is

accredited by the French National Accreditation Authority, and recognised by the European Cooperation for Accreditation. Water samples were filtered through 1 µm glass fiber filters and solid-phase extracted. Glyphosate and AMPA were extracted from sediment samples by ultrasonic and methanol extraction. Quantification of glyphosate and AMPA was performed after derivatisation with fluorenemethoxycarbonyl (Le Bot et al., 2002). Both compounds had a quantification limit of 0.10 µg L^{-1} and 10 µg kg^{-1} in water and sediment samples, respectively. Extraction efficiencies of pesticides were obtained for each water sample set by spiking with surrogates. Relative standard deviation was 16% for both compounds. Recovery efficiency was 86% for glyphosate and 81% for AMPA. Further quality control was achieved by using a blank for each set of samples.

3.6. Data analysis and calculation

Hydrological and hydrochemical variables were compared using the paired nonparametric Wilcoxon signed rank and the Spearman rank correlation tests. When glyphosate and AMPA concentrations were lower than the quantification limit, the concentrations were set to zero for calculating the occurrence and loading. For quantifying the transport of the total glyphosate loadings in the wetland, AMPA, as a glyphosate-derived compound, was expressed on a glyphosate mass equivalent. The mass equivalent load of glyphosate (MEL_{gly}) was calculated according to the following equation:

$$MEL_{gly} = Load\ (glyphosate) + \left\{Load\ (AMPA)\left[\frac{MW_{gly}}{MW_{AMPA}}\right]\right\} \quad (1)$$

where MW_{gly} = molecular weight of glyphosate (0.16907 kg mol^{-1}), and MW_{AMPA} = molecular weight of AMPA (0.11104 kg mol^{-1}). For quantification of the total seasonal glyphosate load as a percentage of the seasonal applied amount of glyphosate on the vineyard catchment, a seasonal export coefficient of glyphosate (SEC_{gly}) was calculated according to the following equation:

$$SEC_{gly} = \frac{MEL_{gly}\ (mass\ season^{-1})}{Glyphosate\ application\ (mass\ season^{-1})}\ 100 \quad (2)$$

The relationship between AMPA and glyphosate was evaluated by calculating the %AMPA as a percentage of total loads of glyphosate and AMPA according to the following equation:

$$\%AMPA = \frac{[AMPA]}{([glyphosate]+[AMPA])} 100 \quad (3)$$

where [AMPA] and [glyphosate] are their respective molar loadings in water. A %AMPA equal to zero indicates either that both AMPA and glyphosate were below the quantification limit or that only AMPA was above it.

4. Results

4.1. Hydrological characteristics and glyphosate export

Climatic and hydrological characteristics from March 23 to June 30, 2009, 2010 and 2011 are summarised in Table III-2 and Figure III-9. Detailed information on the hydrological and hydrochemical conditions at the wetland is provided in Tables VII-13 to VII-15 in the Appendices. Comparison of climatic characteristics revealed that temperature, solar radiation and evapotranspiration values were significantly lower in 2009 compared to those in 2010 and 2011 ($p \leq 0.05$). 19, 33 and 24 rainfall-runoff events, which were separated by at least 2 h, occurred in 2009, 2010, and 2011, respectively. The rainfall amount, duration, intensity, the dry period between two rainfall-runoff events (i.e. quiescent period), and the runoff volumes entering into the wetland did not significantly differ among years (Table III-2). The quiescent period ranged from 2 h to 28 d. The total runoff volume that entered into the wetland during the investigation period was lower in 2009 (408 m^3) compared to that in 2010 (609 m^3) and in 2011 (645 m^3). Runoff events that generated volumes lower than 50 m^3 accounted for more than 80%, indicating that small and moderate runoff events prevailed. Altogether, the analysis of climatic and hydrological conditions revealed that conditions and rainfall-runoff patterns globally were similar in 2009, 2010 and 2011, although monthly variation occurred.

Yearly patterns of glyphosate use are provided in Figure III-9. Most glyphosate is applied in late March and April. There were small applications in May (2010) and two in June (2011). Runoff events generating volume larger than 50 m^3 mainly occurred in May and June and influenced the seasonal pattern of both concentrations and apportionments of both glyphosate and AMPA in runoff entering the wetland (Figure III-9). In contrast, MEL_{gly} that entered the wetland in March and April 2009 and 2011 was lower than 70 mg, likely due to the occurrence of less intense rainfall-runoff events. The SEC_{gly} was 0.07 in 2009, 0.2 in 2010 and 0.06% in 2011, which indicates relatively low MEL_{gly} export. Although 3–5 times less glyphosate was used

in 2010, MEL_{gly} export was larger compared to 2009 and 2011. This can be explained by more frequent and intense rainfall-runoff events following the applications and lower quiescent period (see Table III-2 and Figure III-9).

Table III- 2 Hydrology, hydrochemistry and glyphosate at the stormwater wetland (Rouffach, Haut-Rhin, France) from March 23 to June 30, 2009, 2010 and 2011.Values are provided as the mean and ranges

			2009		2010		2011	
Hydrology	Rainfall	[mm]	140		144		130	
	Runoff coefficient	[%]	0.82 (0.05-2.6)		0.80 (0.01-1.98)		1.18 (0.11-2.39)	
	Inflowing runoff volume	[m^3]	408		609		645	
	Number of runoff events	[-]	19		33		24	
	Quiescent period	[day]	11 (0.1-28)		7.4 (0.15-11)		10 (0.06-28)	
	Glyphosate use	[kg]	3.883		1.303		5.371	
	SEC_{gly}	[%]	0.07		0.2		0.06	
Hydrochemistry	Temperature	[°C]	16.6 (4.9-24)		11 (3.5-16)		14 (8.5-18)	
	pH	[-]	7.3-8.1		6.2-8.1		7.4-8.1	
	Redox potential	[mV]	153 (39-260)		-50 (-216-142)		249 (106-334)	
	Dissolved oxygen	[mg L^{-1}]	6.9 (1.5-13)		9.7 (3.6-12)		2.9 (0.5-8.5)	
	Total suspended solids	[mg L^{-1}]	15 (0.6-97)		20 (7.2-34)		30 (4.9-96)	
	Dissolved organic carbon	[mg L^{-1}]	14 (3.0-22)		5.2 (1.3-9.5)		7.2 (4.6-10)	
	Total phosphorus	[mg L^{-1}]	0.11 (n.d.-0.31)		0.05 (n.d.-0.39)		0.48 (0.38-0.84)	
	Orthophosphorus	[mg L^{-1}]	0.41 (n.d.-1.86)		0.26 (n.d.-1.23)		n.d.	
	Vegetation cover	[%]	<1 - 25		100		100	
			Inlet	**Outlet**	**Inlet**	**Outlet**	**Inlet**	**Outlet**
Glyphosate and AMPA	Glyphosate concentration	[µg L^{-1}]	3.6 (0.2-11.0)	0.1 (n.d.-0.7)	30 (0.1-110)	0.2 (n.d.-1.7)	26 (n.d.-150)	0.1 (n.d.-0.8)
	AMPA concentration	[µg L^{-1}]	1.1 (0.1-2.3)	0.4 (n.d.-0.7)	5.7 (n.d.-19)	0.3 (n.d.-0.9)	3.1 (n.d.-7.0)	0.1 (n.d.-1.1)
	MEL_{gly}	[g]	2.37	0.61	14	1.49	21	0.27
	%AMPA	[%]	35 (11-50)	79 (0-100)	27 (0-92)	73 (0-100)	34 (5-100)	36 (0-100)
	MEL_{gly} removal	[%]	75		89		99	
	Glyphosate load removal	[%]	92		95		100	
	AMPA load removal	[%]	30		76		95	

Note: n.d. = not detected ranges.

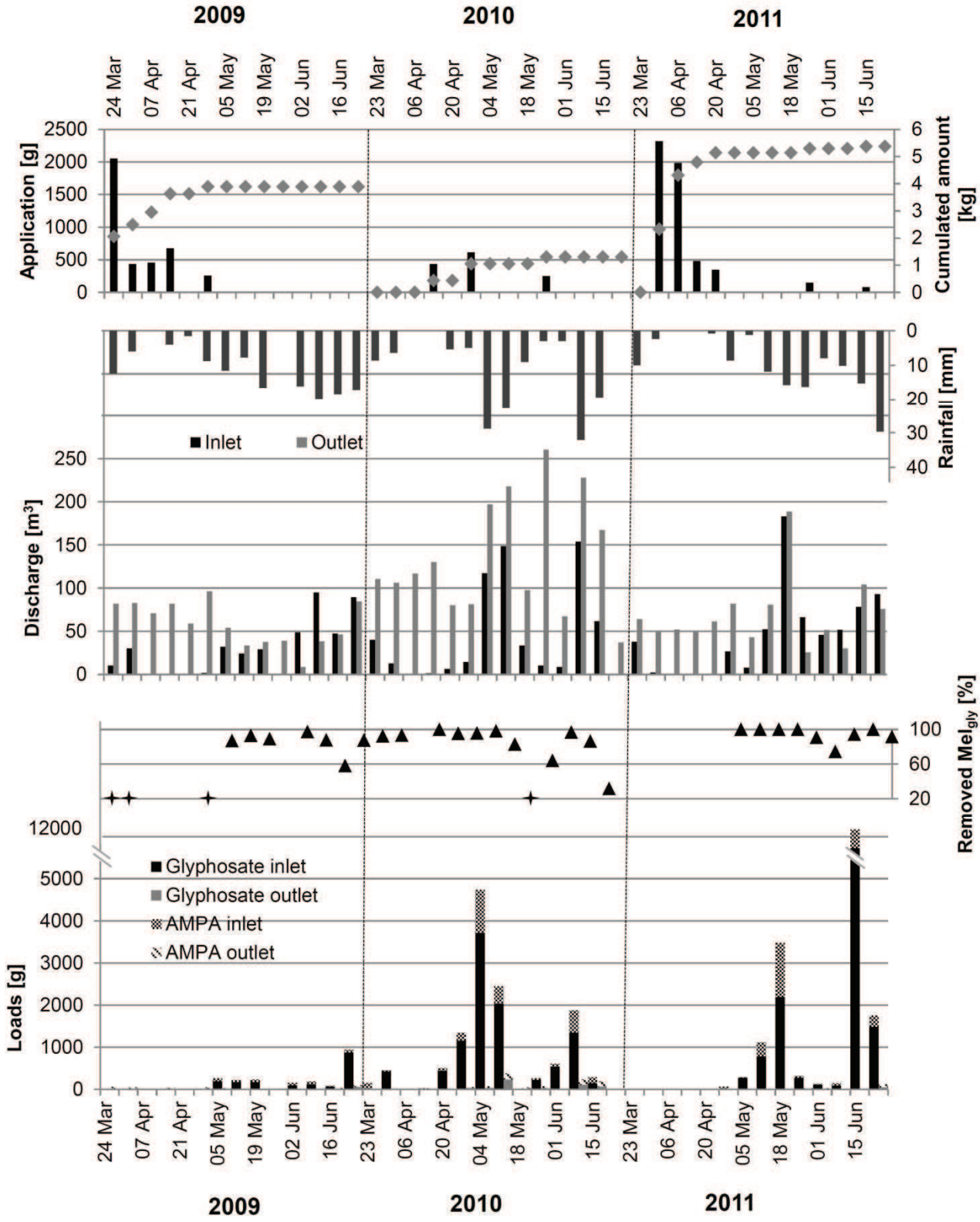

Figure III- 9. Temporal changes of glyphosate use, hydrological condition in the vineyard catchment (Rouffach, France) and total mass equivalent loads of glyphosate (MEL_{gly}) at the stormwater wetland from March to June 2009, 2010 and 2011. Stars represent negative removed MEL_{gly}.

4.2. Occurrence and concentration of glyphosate and AMPA in the wetland

Concentrations and loadings of glyphosate and AMPA in the wetland are summarised in Table 1 and in Figure III-9. 98% of water samples (n = 46) collected at the inlet of the wetland through the three investigation periods had glyphosate and AMPA concentrations above the quantification limits. In contrast, only 52% and 83% of water samples (n = 64) collected at the outlet of the wetland had quantifiable concentrations of glyphosate and AMPA, respectively, which indicates that transport through the wetland reduced the occurrence of glyphosate and AMPA. Glyphosate concentrations entering the wetland ranged from 0.1 to 150 µg L^{-1}. Mean inlet concentration (mean ± SD µg L^{-1}) was 3.6 ± 3.6 in 2009, 30 ± 30 in 2010 and 26 ± 48 in 2011, whereas that of AMPA was 1.1 ± 0.7 in 2009, 5.7 ± 4.9 in 2010 and 3.1 ± 2.6 in 2011. The mean concentration of glyphosate in 2009, 2010 and 2011 decreased by 36, 150 and 263 times from the inlet to the outlet of the wetland, respectively, whereas that of AMPA only decreased by 3, 19, 31 times, respectively. This indicates that concentration reduction by the wetland increased over year, although attenuation of glyphosate always was larger than that of AMPA on the seasonal time scale (Table III-2). Concentrations of glyphosate and AMPA in the wetland sediments were below the detection limits in 2009 (Maillard et al., 2011) and 2011, which indicate no significant transfer of dissolved pesticides from the water column to the bed sediments or degradation of glyphosate and AMPA bond to sediment during the study period.

4.3. Transport and attenuation of MEL_{gly} in the wetland

In order to quantify the transfer and attenuation of glyphosate and AMPA in the wetland, the MEL_{gly} (see Eq. (1)) was evaluated at the wetland inlet and outlet (Figure III-9). Results of the correlation tests between hydrological characteristics and the MEL_{gly} are provided in Table SM-5. The total MEL_{gly} entering the wetland in 2009, 2010 and 2011 was 37.26 g, and that outflowing was 2.29 g, which corresponds to an overall MEL_{gly} removal efficiency of 94%. The seasonal MEL_{gly} removal efficiency increased over time (75% in 2009, 90% in 2010, and 99% in 2011). Interestingly, the MEL_{gly} entering the wetland also increased over time (2.38 g in 2009, 14.10 g in 2010 and 20.79 g in 2011), proportionally to the MEL_{gly} removed by the wetland (1.78 in 2009, 12.61 in 2010 and 20.52 g in 2011). This underscores the absence of threshold at which MEL_{gly} removal by the wetland would decrease at larger loading, which is further

supported by a positive correlation between the inlet discharge, runoff-associated MEL_{gly} and MEL_{gly} removal by the wetland on the seasonal time scale ($p < 0.001$). Hence, the stormwater wetland very likely was not saturated by large input of glyphosate and AMPA during the study period, which may be due to the relatively low runoff coefficient at the study site. Change in the MEL_{gly} removal on the seasonal time scale is shown in Figure III-9. On a weekly basis, the MEL_{gly} removal efficiencies generally ranged between 80% and 100% (Figure III-9), indicating that the wetland maintained its capacity to attenuate varying runoff-associated MEL_{gly} through the investigation period. When no storm event occurred and the wetland still was releasing water from previous storms, the weekly MEL_{gly} exported by the wetland ranged from 18 to 60 mg (Figure III-9). In these cases, the outflowing MEL_{gly} was larger than that at the inlet, thus yielding negative MEL_{gly} removal by the wetland.

4.4. Transport and attenuation of AMPA in the wetland

From March 23 to June 30, 2009, 2010 and 2011, the total load of AMPA entering and outflowing the wetland was 5.558 and 1.047 g, respectively. This corresponds to a total removal efficiency of 81%, and underscores that possible degradation of glyphosate to AMPA did not result in larger amount of AMPA at the outlet compared to the inlet during the study period. The seasonal AMPA removal efficiency (28% in 2009, 76% in 2010, and 95% in 2011) and the amount of AMPA removed by the wetland (0.188 g in 2009, 2.007 g in 2010 and 2.386 g in 2011) both increased over time. Globally, AMPA removal was lower than that of MEL_{gly} and glyphosate. The accumulation of AMPA following glyphosate degradation in the wetland was evaluated based on the relative proportion of AMPA as a percentage of total glyphosate and AMPA loadings (%AMPA, see Eq. (3)) (Figure III-10). The %AMPA generally exceeded 60% at the outlet, whereas AMPA rarely prevailed at the inlet. The mean %AMPA through the investigation periods was 32 ± 23% at the inlet and 63 ± 40% at the outlet, which clearly emphasises that the AMPA fraction increased during transport through the wetland. However, %AMPA ranged from 0% to 100% both at the inlet and the outlet of the wetland (Figure III-10), which underlines the temporal variability of the AMPA portion in the MEL_{gly}.

5. Discussion

Several attenuation processes may simultaneously and synergistically control the transfer of dissolved glyphosate and AMPA in wetlands. Under the physico-chemical conditions

observed in the studied wetland (pH of water and sediment ranging between 7.5 and 8.1), attenuation processes such as volatilization, aqueous hydrolysis and photolysis of glyphosate and AMPA very likely were insignificant (Battaglin et al., 2005; Farenhorst et al., 2009). Therefore, the transfer and attenuation of glyphosate and AMPA in the wetland is expected to mostly vary according to their partitioning between the aqueous and solid phases, and the biodegradation activity. The partitioning and biodegradation of glyphosate and AMPA are themselves controlled by the runoff characteristics, the apportionment of runoff-related glyphosate, the extent of sediment sorption, as well as climatic and hydrochemical variables. In particular, the gradual increase of MEL_{gly} removal over time and the increase of %AMPA in the wetland suggest an initial fast attenuation of glyphosate entering the wetland driven by sorption to the wetland sediment and the temporal development of the vegetation, followed by a slower attenuation phase controlled by biodegradation.

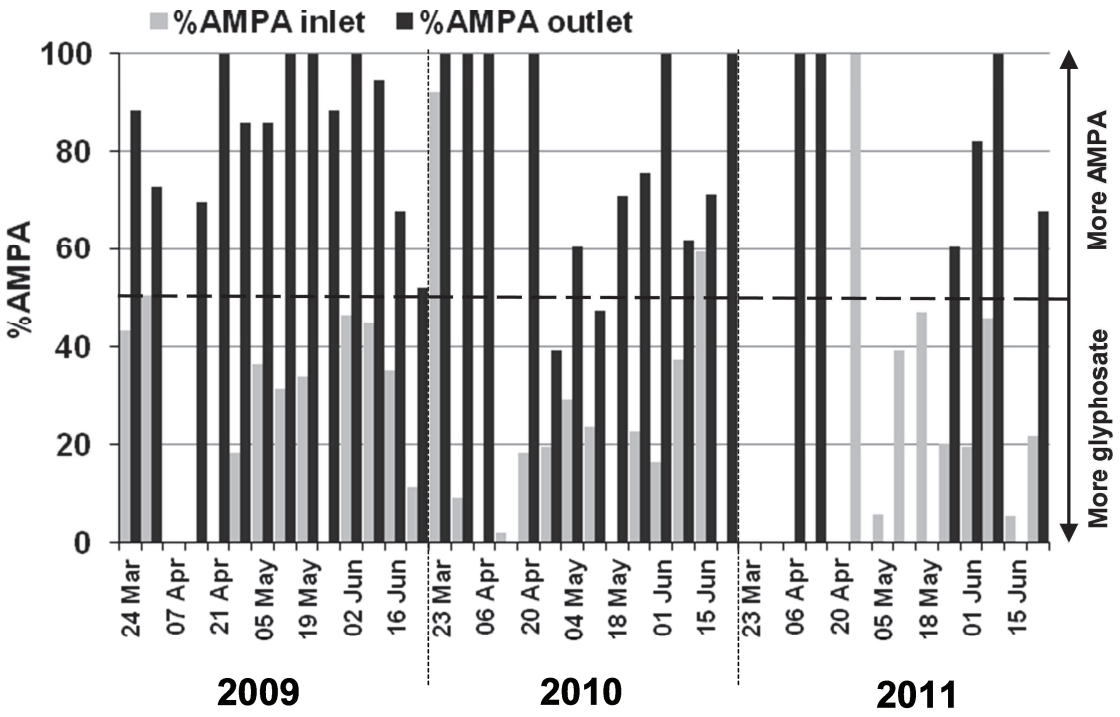

Figure III- 10. Temporal changes of the relative proportion of AMPA to the total loads of glyphosate and AMPA (%AMPA) at the stormwater wetland from March to June 2009, 2010 and 2011.

Iron and aluminium oxides represented 9.5% and 4.1% of the chemical composition of the wetland clayey sediment, respectively. Therefore, sorption of anionic forms of glyphosate and AMPA to sediment oxides and hydroxides, via both chelation with polyvalent cations in

interlayer of variable charge minerals and bonding to humic substances-metallic complexes, likely also contributed to the observed attenuation (Borggaard and Gimsing, 2008). However, glyphosate and AMPA concentrations in the wetland sediments generally were below the detection level and did not accumulate. This is in agreement with previous studies, insofar glyphosate can be gradually degraded in a few days in wetland sediments (Tsui and Chu, 2008).

The gradual increase of MEL_{gly} removal correlated with the larger cover of wetland vegetation (from <1% in March 2009 to 100% in June 2011), which suggests that vegetation also contributed to glyphosate and AMPA attenuation. Cattail and common reed dominated in the wetland, and are characterized by large leave and stem surface areas, which may enable sorption pseudo-equilibrium of glyphosate and AMPA to be rapidly reached (Rogers and Stringfellow, 2009). During runoff events, higher water level and subsequent submersion of vegetal surfaces is expected to proportionally enhance sorption of glyphosate and AMPA to the wetland vegetation, and thus MEL_{gly} removal. In addition, foliar uptake of glyphosate and AMPA sorbed to plant material, which is a well-known and initially targeted translocation pathway (Wang and Liu, 2007), cannot be excluded. However, owing to large spatial and temporal variations in the vegetal biomass and species in the studied wetland, the contribution of vegetation in glyphosate and AMPA attenuation could not be quantified. Wetland plants can also actively transport oxygen, nutrients and diverse carbon sources into the rhizosphere, thereby promoting metabolic and co-metabolic microbial degradation of various groups of pollutants under a broad range of redox conditions (Imfeld et al., 2009; García et al., 2010). A gradual adaptation of wetland microorganisms for the use of various phosphorus sources, including glyphosate and AMPA may explain the gradual increase of seasonal MEL_{gly} removal. Glyphosate degrading bacteria are commonly found in the environment (Huang et al., 2005), and their activity in the studied stormwater wetland has been demonstrated recently in enrichment culture experiments (Bois et al., 2011). The latter study revealed glyphosate biodegradation ranging from 40% to 84%, with degradation rates ranging from 0.17 to 0.35 mg L^{-1} h^{-1} (determined in 200 µL LB cultures with 1 mg L^{-1} bacteria after 96 h incubation at 28 °C with initial glyphosate concentration of 40 mg L^{-1}), which emphasises the potential for fast and significant glyphosate degradation in the wetland. Since the quiescent period (i.e. time between two runoff events) apparently increased when the MEL_{gly} removal decreased ($p < 0.001$, see Table VII-16 in the Appendices), regular and transient runoff passing through the wetland did not seem to result in lower MEL_{gly} removal. Biodegradation of glyphosate (i.e. the activity of the C–P lyase) can be enhanced under phosphate starvation, since microorganisms use glyphosate

as an alternative phosphorus source (Borggaard and Gimsing, 2008). In contrast, phosphate content can decrease the sorption of glyphosate and AMPA due to competition for similar binding sites between the phosphonate group and phosphate (de Jonge et al., 2001). Although orthophosphate concentrations in the wetland did not significantly correlate with MEL_{gly} removal (p = 0.11), molar concentrations of dissolved inorganic phosphorus systematically were 2–6 orders of magnitude larger than those of glyphosate and AMPA (Table III-2), and concentration of P_2O_5 in sediment reached 4 g kg^{-1}. This indicates that an effect of phosphate starvation was very unlikely, whereas competitive adsorption with phosphate may have limited partitioning of glyphosate and AMPA to the wetland sediment, thereby enhancing their bioavailability in the aqueous phase.

In addition, high sorption of glyphosate to metals associated with dissolved humic substances may have lowered its sorption to the wetland sediments and favoured its gradual release during runoff events (Piccolo et al., 1996; Delgado-Moreno et al., 2010). Biodegradation of AMPA generally is slower than that of glyphosate, possibly due to its capacity to sorb through the phosphonate group (Borggaard and Gimsing, 2008). Thus, sorption through the phosphonate group can result in lower desorption efficiencies and bioavailability. AMPA is produced by a cleavage of the C–N bond of glyphosate (Borggaard and Gimsing, 2008; Bergstrom et al., 2011), and is further cleaved by the enzyme C–P lyase to produce inorganic phosphate and methylamine, which is ultimately mineralized to CO_2 and NH_3 (Metcalf and White, 2004). In this study, an increase in the %AMPA between the inlet and the outlet of the wetland indicates that the AMPA pathway occurred in the wetland. However, the co-occurrence in the wetland of the second glyphosate degradation pathway, i.e. the sarcosine pathway, cannot be excluded, although sarcosine was not evaluated in this study due to its ubiquity in the environment (Borggaard and Gimsing, 2008). The %AMPA also reflects temporal changes in the glyphosate degradation efficiencies in the wetland. As glyphosate degradation occurred, the amount of dissolved glyphosate available for transport through the wetland decreases, whereas the amount of AMPA relatively increases. Consequently, AMPA may accumulate in the wetland when its degradation efficiency is significantly lower than that of glyphosate.

6. Conclusions

This study is the first to quantitatively evaluate the transport and attenuation of dissolved glyphosate and AMPA in a stormwater wetland receiving runoff from a vineyard

catchment with respect to the hydrological and hydrochemical conditions. The results indicate that the transport of dissolved glyphosate and AMPA through the wetland differed and largely varied both on seasonal and yearly time scales. Attenuation of glyphosate and AMPA loadings by the wetland generally was larger than 80% and gradually increased over time, which correlated with larger vegetation cover, and possibly with gradual adaptation of glyphosate-degrading microorganisms. However, the fraction of AMPA generally was larger at the wetland outlet, which emphasises the persistence of AMPA and varying efficiencies of glyphosate degradation. Therefore, the transfer of degradation products of runoff-associated pesticides through wetland systems, and in particular those used as a management practice targeting pesticide attenuation, should be carefully considered. Further quantitative field studies evaluating sorption of glyphosate and AMPA to the vegetation and adaptation of degrading microorganisms are necessary for better understanding the functioning of wetlands intercepting pesticide runoff.

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Chapter III have focused on pesticide dissipation in a field-scale wetland with a special attention paid to pesticide partitioning, retention and degradation quantification. However, field-scale studies have limitations. The complex array of interactions found in natural ecosystems complicates the understanding of pesticide fate in wetlands. In addition, field-scale ecosystem experiments are difficult to replicate, and the transposition of field observations to another wetland may be tricky. To obtain more universal and transposable information on pesticide transport and degradation processes, there is a need to change the scale of investigation. In chapter IV, we studied the transport and the degradation of a model compound, the herbicide *S*-metolachlor, in sub-surface flow constructed wetlands. *S*-metolachlor was selected because of its widespread use worldwide in a variety of crops including sunflower, beetroots and maize and because this molecule is a good model compound for modern and emerging chiral pesticides, consisting of a mixture of enantiomers, which are non-superimposable mirror images of each other.

Chapter IV. Mesocosm-scale wetland experiment to decipher pesticide retention and biodegradation

Overview

The previous chapter showed that pesticide dissipation can largely vary according to the hydrological conditions and biogeochemical development of wetlands. Depending on the prevailing redox conditions -that are mainly influenced by the hydrological patterns-, pesticide degradation pathways can largely vary, leading to the occurrence of different degradation products and possibly different prevailing degradation pathways. Therefore, chapter IV aims to investigate the influence of hydrological regime on the dissipation, and more precisely on the biodegradation of a model chiral pesticide, coupling hydrochemical, parent compounds and degradation products analyses as well as emerging techniques, i.e. enantiomer and isotope analyses.

Section 1. Impact of hydrology on *S*-metolachlor biodegradation in subsurface flow constructed wetlands

1. Abstract

The transport and the degradation of the widely used chiral herbicide *S*-metolachlor (*S*-MET) was evaluated in constructed wetlands operated under different hydrological regimes (batch flow vs. continuous flow) using several analytical approaches, including the quantification of *S*-metolachlor and its degradation products, as well as enantiomer and compound-specific isotope analyses (CSIA). In parallel, uranine (UR) and sulforhodamine B (SRB) were used as environmentally harmless surrogates to trace the transport of *S*-metolachlor. Conditions in the batch flow wetland alternated from oxic to anoxic (Eh from -440 to 320 mV) and remained anoxic in the continuous flow wetland (Eh from -560 to -190 mV). Dissipation rates of *S*-MET, UR and SRB were higher (> 93%) in the batch flow system and CSIA confirmed the occurrence of *in-situ* biodegradation of *S*-metolachlor under oxic conditions ($\Delta\delta^{13}C_{inlet\text{-}outlet}$ = 1.2‰). Metolachlor oxanilic acid (OXA) prevailed in the batch flow wetland, whereas metolachlor ethanesulfonic acid (ESA) prevailed in the continuous flow system, indicating distinct degradation pathways in each wetland. No significant enantiomer fractionation of *S*-metolachlor occurred in the wetlands, suggesting the absence of enantioselective biodegradation. Our study demonstrates the usefulness of combining complementary analytical approaches to evaluate the transport and preferential degradation processes of chiral micropolluants in redox-dynamic environments, such as wetlands.

2. Introduction

Wetlands are dynamic interface ecosystems that can receive contaminated runoff or groundwater and provide ecological services including the improvement of water quality. Hydrology is the primary driver of wetlands ecological functioning and biogeochemical cycles (Cole and Brooks, 2000; Hunt et al., 1999). Wetland soils saturation induces prevailing anaerobic conditions in which the plant-mediated transfer of oxygen in bed sediments creates redox gradients, especially in the wetland rhizosphere (Dong et al., 2011). Fluctuations in the water

table generate steep temporal gradients of oxidation-reduction conditions in the vadose zone (Alewell et al., 2008; Haberer et al., 2012; Hernandez and Mitsch, 2007; Rezanezhad et al., 2014). Gradients of redox conditions directly influence the prevailing degradation pathways and the biogeochemical cycles by controlling electron donors and acceptors concentrations and availability (Borch et al., 2010; Burgin et al., 2011). The biogeochemical behavior of not redox-sensitive compounds, such as organic contaminants in wetlands, can be indirectly coupled to these redox reactions (Borch et al., 2010). Pesticide degradation can thus be significantly impacted by biogeochemical processes at biogeochemically active interface ecosystems, such as wetlands. However, knowledge on transformation processes that modern chiral pesticides and their emerging degradation products undergo in the environment is currently limited.

S-metolachlor is one of the widely used chloroacetanilide herbicide for pre-emergence control of annual grasses and some broad-leaved weeds in a variety of crops, including maize, sugar beet and sunflowers (Pereira et al., 2009). Due to its prevalent use and relatively high solubility, *S*-metolachlor and its main degradation products ethanesulfonic acid (ESA) and oxanilic acid (OXA) are frequently detected in surface and groundwater (Bian et al., 2009; Boyd, 2000), raising critical issues for the sustainability of aquatic ecosystems and human health (Baran and Gourcy, 2013; Reemtsma et al., 2013; Steele et al., 2008). *S*-metolachlor is a chiral molecule which consists in the mixture of four stereoisomers (two pairs of enantiomers i.e. the *R*-enantiomer and the *S*-enantiomer and two pairs of diastereoisomers) due to the presence of an asymmetrically substituted carbon and a chiral axis (Buser et al., 2000; Ma et al., 2006). Metolachlor is currently commercialized as *S*-metolachlor which consists of a mixture of 80-100% of the herbicidally-active *S*-enantiomers and 0-20% of the *R*-enantiomers. While enantiomers are not affected by abiotic transport and transformation processes, such as sorption, hydrolysis, volatilization or photolysis, biological processes, such as plant uptake and microbial degradation, may be enantioselective (Wong et al., 2002). However, factors driving the environmental transport and the degradation of *S*-metolachlor are poorly understood and can be investigated by coupling different analytical approaches.

Enantiomer analysis of *S*-metolachlor may be indicative of the occurrence of biodegradation, since a previous study reported a shorter half-life of the *S*-enantiomer compared to *rac*-metolachlor in the environment (Ma et al., 2006). However, enantiomer analysis is inappropriate to evaluate *S*-metolachlor biodegradation if enzymes capable of degrading both enantiomers are present (Milosevic et al., 2013). In this case, the use of compound-specific stable

isotope analysis (CSIA) may be useful to evaluate the degradation of organic pollutants (Thullner et al., 2012). CSIA relies on the enrichment of the heavy isotope of an element in the unreacted fraction of a compound during the degradation process (Milosevic et al., 2013). Consequently, the isotopic composition of the contaminants enables the detection of *in situ* biodegradation of organic compounds (Elsner, 2010), including chloroacetanilide herbicides (Elsayed et al., 2013). In parallel, the coupled study of pesticides and environmentally harmless and low-cost fluorescent dye tracers, such as uranine (UR) and sulforhodamine B (SRB), enable for a thorough understanding of the fate of micropollutants in wetland systems (Durst et al., 2013; Kunkel and Radke, 2011; Lange et al., 2011; Passeport et al., 2010) Contrary to pesticide analyses that are often complicated and expensive methods, the analysis of dye tracer allows a high-resolution sampling and to for the comparative investigation of the pesticide transport, retention and degradation (Gjettermann et al., 2011). UR is sensitive to photodegradation, mobile and low to moderately sorptive, whereas SRB is more stable to photolysis and subject to sorption (Kasnavia et al., 1999; Sabatini, 2000).

In this study, we used subsurface flow constructed wetlands (SSFCWs) as model ecosystems to investigate the transport and the degradation of *S*-metolachlor in redox-dynamic ecosystems. The objectives were i) to evaluate the influence of the hydrological conditions (batch vs. continuous flow conditions) on the transport and degradation of *S*-metolachlor based on metolachlor, degradation products, enantiomer and isotope analyses, and ii) to evaluate the use of artificial dye tracers as possible surrogates for understanding the environmental transport and dissipation of pesticides . To the best of our knowledge, this is the first integrative study on the environmental transport and degradation of *S*-metolachlor based on the combination of several analytical approaches.

3. Material and methods

3.1. Chemicals

The physico-chemical properties of *S*-metolachlor (S-MET), uranine (UR), sulforhodamine B (SRB) and Bromide (Br) are provided in Table VII-17 in the Appendices. Br was obtained as sodium bromide from Merck (Darmstadt, Germany), UR and SRB were supplied by Sicomet (Flörsheim, Germany) and Chroma (Münster, Germany). The commercial product Mercantor Gold® (containing 960 g L^{-1} of *S*-metolachlor and 87.4 ± 1.1% of the *S*-enantiomer) from Syngenta St Sauveur, France) was used to prepare the *S*-metolachlor solution. Because of

Mercantor Gold® extremely low solubility in water, a *S*-metolachlor stock solution (30 g L^{-1}) was prepared in methanol (Chromasolv® Plus, analytical grade purity > 99.9%; Sigma Aldrich corp., St Quentin Fallavier, France). Then, 10 mL of the *S*-metolachlor solution in methanol was added to 1L of ultrapure water and stirred overnight to allow methanol evaporation. This solution containing 300 mg of *S*-metolachlor was added to tap water in the inlet tanks and supplied to each of the two SSFCWs for 2 weeks.

3.2. Subsurface-flow constructed wetlands

Two subsurface-flow constructed wetlands (SSFCWs) (48°4'54"N, 7°21'20"E, Colmar, Alsace, France) were investigated between May 24th (day 0) and August 16th 2012 (day 84). The 7.2 m^2 SSFCWs were 4 m long, 1.80 m wide and 0.52 m deep and were filled with gravel (grain size 4-8 mm) at the bottom to enhance water drainage and sand (grain size 0-4 mm) (Figure IV-1). The thicknesses of the gravel and the sand layers were 0.12 m and 0.40 m respectively, with volumes of gravel and sand in each wetland of respectively 0.92 and 2.93 m^3. The water volume needed to saturate each SSFCW was 600 L, corresponding to a porosity of 15.6% of the mineral matrix. The SSFCWs were planted three months prior the beginning of the experiment with *Phragmites australis* (Cav.) Trin. ex Steud. (20 plants m^{-2}), *Phalaris arundinacea* (3 plants m^{-2}) and *Glyceria maxima* (2 plants m^{-2}). A survey of the vegetation growth and cover was carried out weekly and data are provided in Table VII-18 in the Appendices. Three piezometers were deployed at regular 1 m intervals in the wetlands for the monitoring of water level and water sampling (Figure IV-1). The outlets of the two SSFCWs converged towards a main pipe and a man hole, where devices for flow measurement were deployed (Figure IV-1). Meteorological data, i.e. hourly precipitation, wind speed, global radiation and evapotranspiration (ETP Pennman) were measured at a weather station located at the study site, and are provided in Table VII-18 in the Appendices.

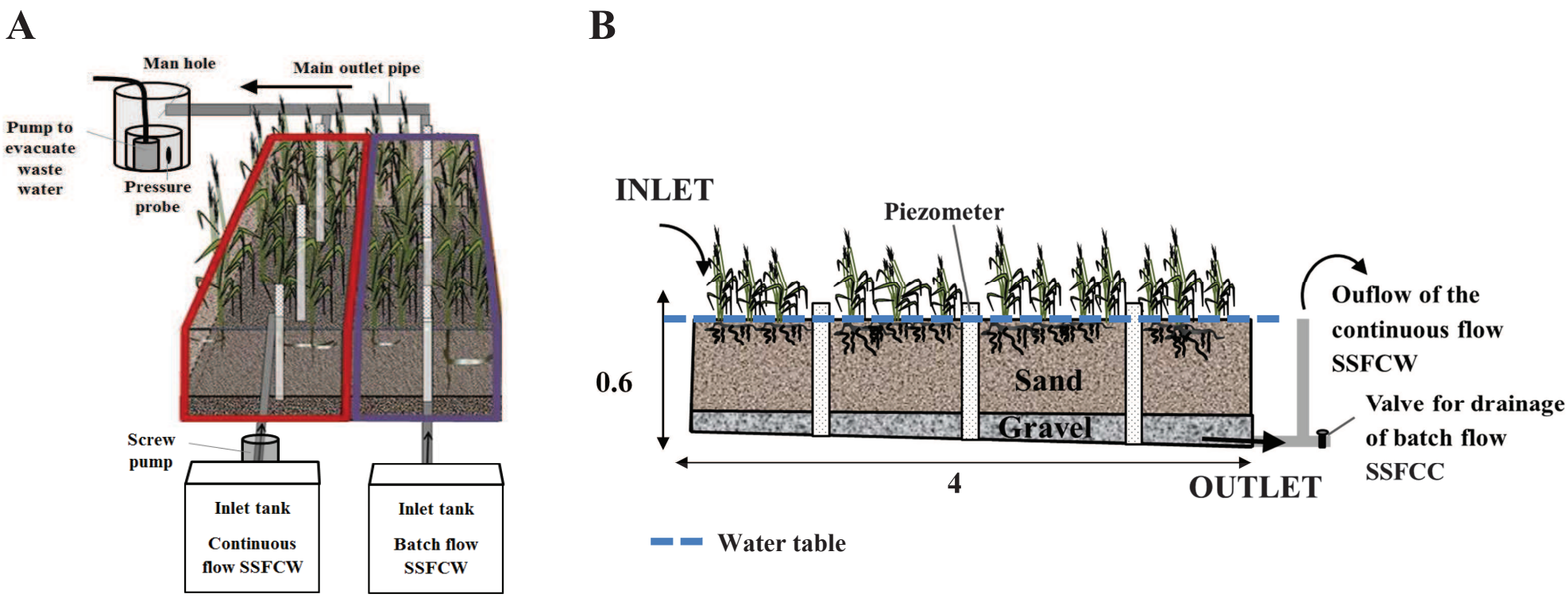

Figure IV- 1. Layout of A. the overall experimental design; B. a subsurface flow constructed wetland (SSFCW).

3.3. System operations and hydrological regimes

The detailed sequences of *S*-metolachlor and tracers injection are provided in Table IV-1. In the batch-flow SSFCW, four batch operations of a duration of 14 days each, and separated by a dry period of 7 days were carried out during the investigation period. Contaminated water was supplied to the SSFCW, sampled 7- and 14- days after the water supply, and totally drained after 14 days. The SSFCW was then dried for 7 days and filled again. Holding HDPE tanks of 1 m^3 of tap water served as reservoirs to control the flux of inlet contaminated water. Inlet solutions were prepared by adding 300 mg of *S*-metolachlor to a tap water volume that varied from one batch operation to another, based on the rain volume that remained in the SSFCW prior to saturation. Inlet tap water volumes for the four batch operations ranged from 100 L (final concentration of *S*-metolachlor: 3 mg L^{-1}) to 600 L (final concentration of *S*-metolachlor: 500 µg L^{-1}).

Table IV- 1. Experimental sequence of dye tracers and S-metolachlor injections in the continuous and the batch-flow SSFCWs.

Date	Days	Week	Continuous flow SSFCW	Batch-flow SSFCW
24/5 - 31/5	0 - 7	1	**1st step injection**	**Batch load**
31/5 - 7/6	7 - 14	2		
7/6 - 14/6	14 - 21	3	flushing with non-contaminated tap water	dry period
14/6 - 21/6	21 - 28	4		**Batch load**
21/6 - 28/6	28 - 35	5		
28/6 - 5/7	35 - 42	6		dry period
5/7 - 12/7	42 - 49	7	**2nd step injection**	**Batch load**
12/7 - 19/7	49 - 56	8		
19/7 - 26/7	56 - 63	9	flushing with non-contaminated tap water	dry period
26/7 - 2/8	63 - 70	10		**Batch load**
2/8 - 9/8	70 - 77	11		
9/8 - 16/8	77 - 84	12		dry period

For the continuous flow SSFCW, two 14 days phases of continuous *S*-metolachlor and tracers injection were carried out during the investigation period. The first injection phase was carried out from May 24th to June 7th, 2012 (i.e. from day 0 to day 14) and the second injection phase from July 5th to July 19th, 2012 (i.e. from day 42 to day 84). After each injection phase, the SSFCW was continuously supplied for 4 weeks with uncontaminated tap water (i.e. from day 14

to day 42 following the first step injection and from day 56 to day 84 after the second step injection). During the first injection step (day 0 to day 42), the inlet flow rate ranged from 5.1 to 7.6 L h^{-1}, with a mean value of 5.9 ± 1.3 L h^{-1}. The second step injection was carried out with variable flow conditions to enhance fluctuating conditions. The flow rate ranged from 0 to 15.7 L h^{-1} and averaged 4.2 ± 3.3 L h^{-1}. Continuous loading was accomplished by using a screw pump (Seepex, Bottrop, Germany). Mercantor Gold® contaminated water was prepared with tap water to yield a final concentration of 178 µg L^{-1} *S*-metolachlor in the 2 m^3 HDPE tank.

3.4. Flow measurement and sampling procedure

Continuous flow measurement at the outlet of the two SSFCWs was carried out using a pressure probe with Mikromec® logger (Technetics, Freiburg, Germany) in the man hole and the outflow rate from the batch-flow wetland was measured during the water drainage. The continuous injection in the continuous flow SSFCW was shortly interrupted to exclusively measured effluents from the SSFCW batch flow system.

Samplings were carried out weekly from May 24th (day 0) to August 16th (day 84). 2L-samples were collected weekly at the inlet and outlet of the SSFCWs and in the three piezometers for *S*-metolachlor (1 L), hydrochemical (0.8 L) and dye tracers (0.2 L) analyses. The three piezometer samples from the batch flow SSFCW were pooled to obtain a single composite sample, while piezometer samples from the continuous flow SSFCW were analysed separately. Outlet tracer samples from the continuous flow SSFCW were collected using an autosampler from the outlet T-pipe every hour up to 3.5 days after tracers' injection and after beginning of flushing with tap water. When the concentration plateau was expected to have established, the sampling intervals was decreased to two hours. During the drainage of the batch flow SSFCW, outlet samples were collected every 10 minutes and then pooled in a composite sample for hydrochemical and *S*-metolachlor analyses. In parallel, dye tracers were continuously quantified using a flow-through filter fluorometer placed in the outlet T-pipe during drainage.

At the end of the experiment (on day 84), sand and plants samples were collected to quantify *S*-metolachlor sorption and plant uptake. Sand samples were collected in the SSFCWs at three different depths (0-10 cm; 20-30 cm and 40-50 cm) and at two locations in each SSFCW, 0.5 m from the inlet and 0.5 m from the outlet. Two entire plants of each species were sampled

in each SSFCW and aerial parts were separated from the roots. Sand, root and aerial plant samples were stored at -20 °C until analysis.

3.5. *S*-metolachlor and degradation products analysis

3.5.1 Extraction

The extraction of sand and plant samples was carried out on 5 g of dried sample with 4 mL of ACN/pure water (v:v 60/40), shaked for 1 min (vortex), incubated for 30 min at 115°C, shaked for 1 min, and centrifuged during 10 min at 3500 rpm. The supernatant was then collected and a second extraction was carried out, using the same protocol. 0.1% H_3PO_4 were added to the sample for the second extraction. The 8 mL-sample was then filtered using a 0.2 µm PTFE filter and evaporated. 50 mL of pure water were added to the sample and were extracted by solid-phase extraction (SPE).

SPE of water, plant and sand samples was carried out with using SolEx C18 cartridges (Dionex®, CA, USA) packed with 100 mg bonded silica. AutoTrace 280 SPE system was used for simultaneous extraction of 6 samples. The extraction cartridges were washed with 5 ml of ethyl acetate, followed by 5 mL of methylene chloride and sequentially conditioned by 10 mL of methanol and 10 mL of deionised water. Cartridges were then loaded with the samples and dried with nitrogen for 10 min. Elution of *S*-metolachlor and its degradation products was performed by 3 mL followed by 2 mL of ethyl acetate and methylene chloride respectively. The extracts were concentrated under nitrogen flux to 1 droplet, and 2 mL of methylene chloride were added.

3.5.2 Quantification of S-metolachlor and degradation products

S-metolachlor was quantified using a Focus-ITQ 700 model GC-MS/MS apparatus from Thermo Scientific (Les Ulis, France) and Xcalibur (version 2.0.7) for data acquisition. *S*-metolachlor separation was conducted on a 30 m x 0.25 mm ID, 0.25 µm film thickness OPTIMA 5MS (5% phenyl - 95% dimethylpolysiloxane) fused-silica capillary column (Macherey Nagel GmbH, Düren, Germany), with helium as a carrier gas, at a flow rate of 1 mL min^{-1}. The oven was held at 50°C for 2 min, ramped at 30°C min^{-1} to 150°C, then up to 250°C at 5°C min^{-1} and finally

ramped at 30°C min^{-1} to 300°C and held for 5 min. A volume of 3 µL of sample was injected on a split/splitless injector (pulsed splitless at 2.5 mL min^{-1} for 1 min) using an AI/AS 3000 autosampler (Thermo Fisher Scientific, Les Ulis, France). The injector temperature and transfer line were set at 280°C and 300°C. The mass spectrometer was operated in the electron ionization mode (EI, 70 eV). The ion source temperature was maintained at 210°C. For GC-MS/MS analysis, 10 µL of the internal standard Metolachlor-d_6 to yield a final concentration 100 µg L^{-1} was added to 190 µL of water samples. The detection limit of *S*-metolachlor was 0.7 µg L^{-1} and the quantification limit was 2 µg L^{-1}. Retention times, selected ions used for identification and recoveries are detailed in Table VII-19 in the Appendices.

Ethane sulfonic (ESA) and oxanilic acids (OXA) degradation products of *S*-metolachlor (ESA and OXA) were analysed using a TSQ Quantum ACCESS LC/MS equipped with a Thermo Scientific Accela autosampler with a temperature-controlled sample tray (15°C) (Les Ulis, France). Xcalibur (version 2.1.0) was used for data acquisition. Injection volume was 20 µL. The mobile phase consisted of 0.1% formic acid/high-purity water (A) and 0.1% formic acid/acetonitrile (B). The gradient program started with 35% B held for 5 min, then B increased from 35% to 95% for 16 min, 95% B was held for 5 min, then B decreased from 95% to 35% for 1 min and held at 35% for 5 min. The flow rate was 0.3 mL min^{-1}. The analytical column was a EC 150/3 Nucleodur Polar Tec (particle size 3µm, length 150 mm, internal diameter 3 mm) and a precolumn EC 4/3 Polar Tec, 30 mm (Macherey Nagel, France). Column oven temperature was set at 60°C to achieve better separation and peak shapes. The mass spectrometer (MS) was a Thermo TSQ Quantum triple quadrupole mass spectrometer (Les Ulis, France) operated using a heated electrospray ionization (HESI) source. The mass spectra were recorded in the negative ion mode (spray voltage: 3500 V) for the 6 degradation products and in the positive mode (spray voltage: 4250 V) for the internal standard Alachlor-d_{13}. The vaporiser temperature was 300°C, sheath gas N_2 pressure 10 (arbitrary units), auxiliary gas pressure 20 (arbitrary units), ion sweep gas pressure 0 and the ion transfer capillary temperature 300°C. The best sensitivity in multiple reaction monitoring operation was achieved through the acquisition of selected reaction monitoring (SRM) transitions with MRM mode. For identification of the studied compounds, two SRM transitions and a correct ratio between the abundances of the two optimized SRM transitions (SRM1/SRM2) were used along with retention time matching. Limits of detection were 0.06 and 0.04 µg L^{-1} and limits of quantification were 0.10 and 0.06 µg L^{-1} for MOXA and MESA, respectively. Information about SRM transitions and analytical uncertainties are provided in Table VII-19 in the Appendices.

3.5.3 Enantiomer analysis of *S*-metolachlor

The enantiomer analysis of *S*-metolachlor was carried out with a Trace GC 2000 series GC-MS apparatus (Thermo Scientific, Les Ulis, France) using a 30 m × 0.25 mm ID, 0.25 µm film 20% tert-butyldimethylsilyl-beta-cyclodextrin dissolved in 15% phenyl-, 85% methylpolysiloxane column (BGB Analytik, Boeckten, Switzerland) with helium as a carrier gas at a flow rate of 1.0 mL min^{-1}. The column was held at 50°C for 3 min, ramped at 15°C min^{-1} to 150°C, and finally ramped at 0.5°C min^{-1} to 190°C and held for 5 min. A volume of 3 µL of sample was injected on a split/splitless injector (pulsed splitless flow for 1 min). The injector, the transfer line and ion source temperatures were maintained at 250°C, 250°C and 230°C, respectively. The mass spectrometer was operated in the electron ionization mode (EI+, 70 eV). Quantification was based on the daughter ions 162 and 166 and quantification on the parent ions 238 and 242 for respectively *S*-metolachlor and the internal standard metolachlor-d_6. The stereoisomer elution was *aS1'S, aS1'R, aR1'S, and aR1'R.*

3.6. Carbon isotope analysis

The carbon isotope composition analysis of *S*-metolachlor by CSIA was previously developed and described in Elsayed et al. (2013). Briefly, *S*-metolachlor carbon isotope composition was analysed using a GC–C–IRMS system consisting of a gas chromatograph (Agilent 6890) coupled via a GC/C III interface to an isotope ratio mass spectrometer (Finnigan MAT 252, Thermo Fisher Scientific). The oxidation furnace of the GC/C III interface containing (Pt, Ni, CuO) was set to a temperature of 980°C. A BPX5 column (60 m x 0.32 mm, 0.5 µm film thickness, SGE, Ringwood, Australia) was used for chromatographic separation, with helium as the carrier gas at a flow rate of 2.0 mL min^{-1}. The column was held at 50°C for 5 min, heated at a rate of 20°C min^{-1} to 150°C, then up to 250°C at 5°C min^{-1}, then heated at 20°C min^{-1} to 300°C and held for 1 min, and finally heated at 20°C min^{-1} to 320°C, where it was held for 5 min. Samples (4 µL volume) were injected into a split/splitless injector operated in splitless mode and held at 280°C. A *S*-metolachlor standard with known isotopic composition was measured every nine injections to control the quality of the measurements. Reference carbon isotope composition values of *S*-metolachlor standards were obtained using an elemental analyser-isotopic ratio mass spectrometer (EA–IRMS, eurovector, Milan, Italy) coupled via a conflo III (open split, Thermo Fisher Scientific, Bremen, Germany) to a MAT 253 isotope ratio mass spectrometer

(Thermo Fisher Scientific). The reproducibility of triplicate measurements was ≤ 0.2 ‰ (1σ). The $\delta^{13}C$ values were calibrated using a two-point calibration against the V-PDB standard.

3.7. Tracer analyses

Bromide was used as a conservative tracer to assess the hydraulic properties of the SSFCWs. The analysis of bromide was carried out on filtered samples (< 0.45 µm) using ionic chromatography (IC, Dionex-DX 500) with an accuracy of 8%. UR and SRB were quantified by fluorescence spectrometry (Perkin Elmer LS 50 B) according to the methods described in Leibundgut et al. (2009). Detection limits in wetland samples were 0.05 µg L^{-1} (UR) and 0.1 µg L^{-1} (SRB) and accuracy was 1.6% for UR and 3.6% for SRB measurements. Fluorescence spectrometry was used for all the samples, except the outlet samples collected during the drainage of the batch flow SSFCW that were analysed online using a flow-through filter fluorometer which was placed in the outlet T-pipe during drainage.

3.8. Hydrochemical analyses

Electrical conductivity, pH, dissolved oxygen concentrations and redox potential were measured weekly at the inlet, outlet and in the 3 piezometers of each SSFCW using a Hanna Instrument multi-parameters probe HI9828 (Tanneries, France). Between the samplings, these physico-chemical parameters were continuously monitored at the outlet of the continuous flow SSFCW and in the second piezometer of the batch-loaded SSFCW.

Concentrations of Fe^{2+}, Fe^{3+}, Cl^-, NO_3^-,NO_2^-, NH_4^+, PO_4^{3-}, SO_4^{2-}, Mn^{2+}, total organic carbon (TOC) and dissolved organic carbon (DOC) were determined by FR EN ISO standards and laboratory procedures.

3.9. Data analysis

S-metolachlor, degradation products and tracers loads were calculated by multiplying their concentrations between consecutive sampling dates and the corresponding weekly volume measured at the inlet and the outlet of the wetlands. The loads dissipation (%) of *S*-metolachlor

and dye tracers were calculated using a mass balance approach, as the relative decrease of the outlet load ($Load_{OUT}$) to the inlet load ($Load_{IN}$) for each SSFCW.

For quantifying the transport of the total *S*-metolachlor loadings in the wetlands, OXA and ESA, as *S*-metolachlor-derived compounds, were expressed on a *S*-metolachlor mass equivalent. This calculation enables to quantify the total mass of parent compound and degradation products that exit the system, compared to the injected mass. The mass equivalent load of *S*-metolachlor ($MEL_{S\text{-}metolachlor}$) was calculated according to the following equation:

$$MEL_{S-metolachlor} = Load_{S-metolachlor} + \left\{Load_{OXA}\left[\frac{MW_{S-metolachlor}}{MW_{OXA}}\right]\right\} + \left\{Load_{ESA}\left[\frac{MW_{S-metolachlor}}{MW_{ESA}}\right]\right\} \quad (1)$$

where $MW_{S\text{-}metolachlor}$ = molecular weight of S-metolachlor (283.79 g mol^{-1}), MW_{OXA} = molecular weight of OXA (279.33 g mol^{-1}) and MW_{ESA} = molecular weight of ESA (329.1 g mol^{-1}).
Hydrological, pesticide and hydrochemical data were compared using the paired non-parametric Wilcoxon signed rank test, with *p*-values set at 0.05 (R software, Version 3.0.1).

The enantiomer fraction (EF) was used to indicate the relative amounts of the pairs of metolachlor enantiomers (Harner et al., 2000), as defined in eq. 2:

$$EF = \frac{S-enantiomers}{S-enantiomers+R-enantiomers} = \frac{aS1'S+aR1'S}{aS1'S+aR1'S+aS1'R+aR1'R} \quad (2)$$

where *S*-enantiomers stand for the peak areas of *aS*1'*S* and *aR*1'*S*, and the *R*-enantiomers for the peak areas of *aS*1'*S* and *aR*1'*S*. Pure enantiomers have EFs of 0 or 1, while racemic mixtures have an EF of 0.5.

The carbon isotope ratios were reported in δ notation in parts per thousands (‰) relative to the international carbon isotope standard Vienna Pee Dee Belemnite (V-PDB), according to the following equation:

$$\delta^{13}C_{sample} = \frac{(R_{sample} - R_{standard})}{R_{standard}} \quad (3)$$

where R_{sample} and $R_{standard}$ are the ratios $^{13}C/^{12}C$ of sample and standard.
Changes in the carbon isotope composition between the inlet and the outlet of the SSFCWs ($\Delta\delta^{13}C$) were calculated as the difference between $\delta^{13}C_{IN}$ and $\delta^{13}C_{OUT}$.

4. Results and discussion

4.1. Water balance and biogeochemical development of the wetlands

The details on the hydrological balances are provided in Table VII-20 (Appendices) and detailed hydrochemical data are shown in Table VII-21 (Appendices) in the Supplementary Material.

In this study, bromide (Br) was used as a conservative tracer to determine the hydraulic characteristics of the continuous flow SSFCW during the first step injection (from May 24 to July 5 2012). The breakthrough curves of Br, UR and SRB showed that Br reached the average plateau concentration of 35 mg L^{-1} after 129 h (5.4 days), followed by UR and SRB that reached plateau concentrations after 221 and 223h. The mass recovery of bromide was 75.6% during the first step injection. The incomplete recovery may be attributed to plant uptake that was reported in previous studies (Kung, 1990; Xu et al., 2004). For instance, Kung (1990) showed that at least 53% of the injected bromide mass was absorbed by potato plants. In another study, Xu et al. (2004) showed bromide uptake by reed grass at Cl^-/Br^- ratios lower than 2, which was the case in our study.

The biogeochemical conditions largely differed between the two wetlands, as inferred from the redox potential values and the dissolved oxygen concentrations (Figure IV-2). The continuous flow SSFCW was permanently flooded and dissolved oxygen concentrations decreased from 4.2 mg L^{-1} at the beginning of the investigation period to 0 mg L^{-1} after day 7 (May 31th). Oxic and anoxic conditions alternated in the batch flow SSFCW, and dissolved oxygen concentrations averaged 3.1 ± 1.8 mg L^{-1} during the investigation period, although dissolved oxygen concentrations were systematically < 0.02 mg L^{-1} at the end of each of the four batches. Redox conditions varied from -190 to -560 mV (-460 ± 90 mV) in the continuous flow system and from 320 to -440 mV (50 ± 230 mV) in the batch flow system. Electrical conductivity was significantly lower in the continuous flow SSFCW (values ranged from 570 to 1130 µS cm^{-1}) than

in the batch flow system (values ranged from 680 to 1360) ($p < 0.005$). pH values ranged from 6.8 to 7.5 in the continuous flow SSFCW, which significantly differed from values from 7.2 to 7.8 found in the batch flow SSFCW ($p < 0.05$). In anoxic conditions, the release of dissolved organic carbon by the roots and associated microbial activity may lead to a gradual decrease of available terminal-electron acceptors and to a decrease of pH values reflecting respiration processes (Borne et al., 2014).

In the continuous flow SSFCW, NO_3^- concentrations were significantly lower at the outlflow (8.03 ± 8.41 mg L^{-1}) compared to the inflow (20.86 ± 10.62 mg L^{-1}), indicating nitrate reduction and denitrification ($p < 0.05$). Mn^{2+} was released from the continuous-flow wetland, with inlet and outlet concentrations ranging from 3.06 ± 3.85 mg L^{-1} and from 48.13 ± 67.8 mg L^{-1}, suggesting manganese reduction processes. In contrast, no significant differences in the concentrations of terminal-electron acceptors between the inlet and the outlet of the batch flow SSFCW could be observed.

The wetland vegetation was well developed (maximum density: 145 plants m^{-2}) in the continuous flow wetland whereas plant growth decreased in the batch flow bed (maximum density: 64 plant m^{-2}), possibly owing to the toxic stress caused by *S*-metolachlor and tracers, as well as the release of sulfide during microbially-mediated sulfatoreduction (Table VII-18 in the Appendices). Liu et al. (2012) previously demonstrated the toxic effect of rac- and *S*-metolachlor on rice and maize roots, with roots growth greatly affected by *S*-metolachlor.

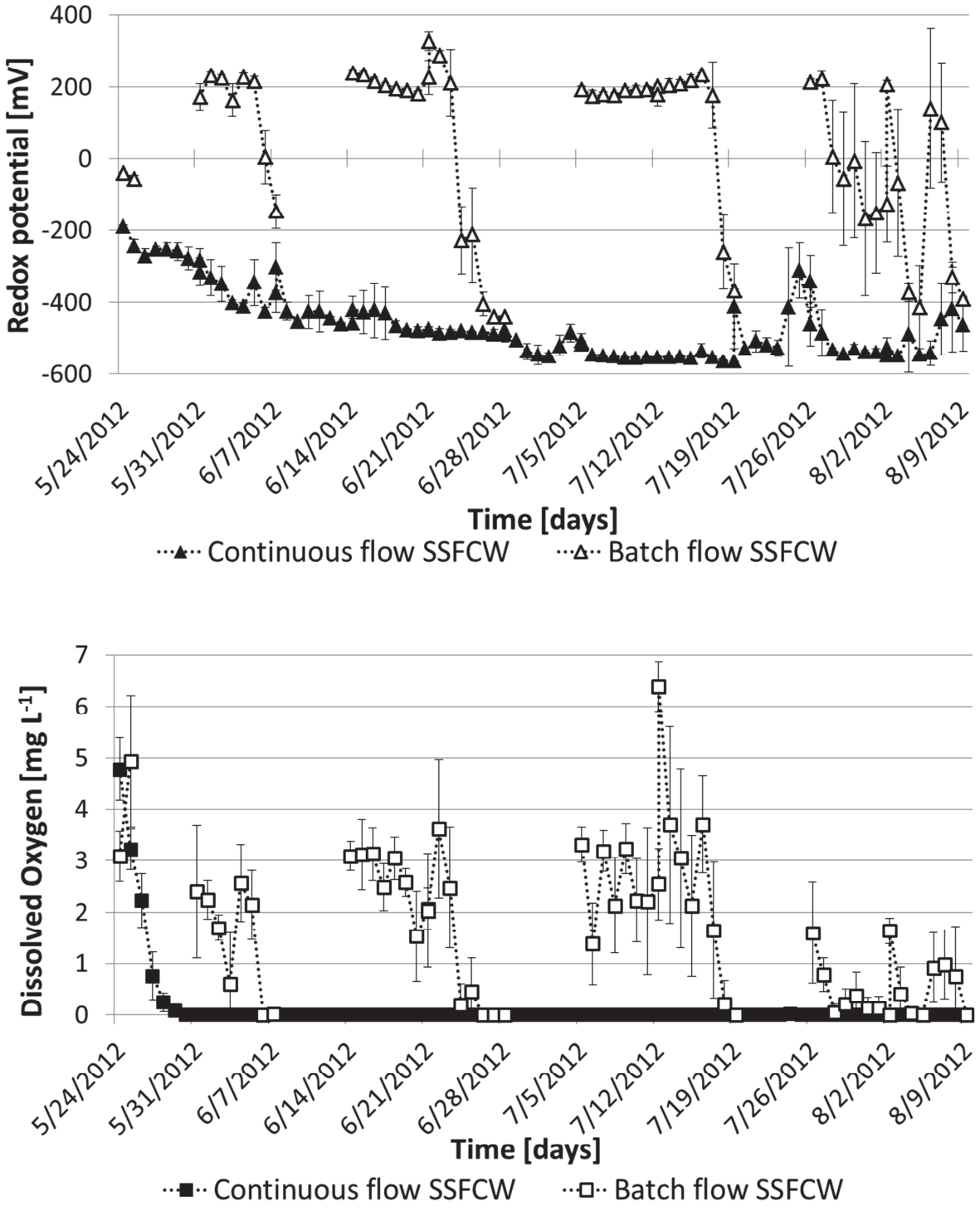

Figure IV- 2. Temporal changes in the redox potential values and the dissolved oxygen concentrations in the continuous flow and the batch-flow SSFCWs. Error bars represent the standard deviations of daily measurements.

4.2. Influence of the hydrological regimes on the dissipation of *S*-metolachlor and dye tracers.

Larger dissipations of *S*-metolachlor, UR and SRB were observed for in the batch flow system compared to the continuous flow (Figure IV-3). *S*-metolachlor dissipation rates ranged from 93.3 to 96.3% in the batch flow system, and were close to those of UR (96.3 ± 1.2%) and SRB (95.1 ± 3.2%). Compared to UR and *S*-metolachlor, SRB showed a highest dissipation rate during the first batch, suggesting SRB sorption on the sand matrix and/or plant roots (Durst et al., 2013). Contrary to *S*-metolachlor and UR that mainly interact with organic matter (Baran and Gourcy, 2013; Kasnavia et al., 1999), the sorption of SRB depends on the mineral composition of the matrix and is likely to occur on both mineral and organic matrixes due to the presence of two electronegatively charged sulfonic acid groups versus the carboxylic group (Kasnavia et al., 1999; Leibundgut et al., 2009; Sabatini, 2000). The slight decrease of SRB dissipation over time may be attributed to the sorption/desorption equilibrium that was possibly reached after the first batch operation, since SRB degradation is unlikely to occur. Photodegradation of uranine (DT50 hydrolysis = 0.5 days, see Table VII-17) likely occurred when at the beginning of each batch loading a shallow water column formed at the wetland surface (2 – 5 cm of water ponding, corresponding to 20% of the total volume injected in the SSFCW) and stayed for 7 days, until infiltration (Wang et al., 2008).

Sorption on sand and plant material as well as plant uptake were not the main dissipation pathways in both wetland systems. *S*-metolachlor concentrations in the sand and the vegetation in the continuous flow system were always under the quantification limit, indicating that sorption of *S*-metolachlor was not a significant mass-dissipation process. In the batch flow system, 62.5 mg of *S*-metolachlor remained stored in the sand matrix of the wetland (i.e. 5% of the total supplied amount, i.e. 1200 mg), especially in the surface layer 0 – 20 cm (24 µg kg^{-1} i.e. 60.1 mg). This indicates that sorption on sand did not significantly contribute to the apparent dissipation of *S*-metolachlor, and degradation was therefore the major dissipation process. *S*-metolachlor concentrations in roots of *Phalaris arundinacea* reached 414.4 µg kg^{-1}, corresponding to a load of only 39 µg, and an average concentration of 145 µg kg^{-1} of *S*-metolachlor was detected in the roots of *Phragmites australis*, corresponding to a total *S*-metolachlor load of 1.7 mg stored in the root biomass at the end of the experiment. *S*-metolachlor concentrations ranging from 2 to 27 µg kg^{-1} were measured in the aerial part of the vegetation, emphasizing the occurrence of plant uptake of *S*-metolachlor. Our results are in

agreement with previous observation showing a low sorption of *S*-metolachlor, mainly correlated with the organic matter content of soils (Si et al., 2009; Weber et al., 2007) and a low plant uptake that was shown to account for less that 10% of the total metolachlor load (Moore et al., 2001).

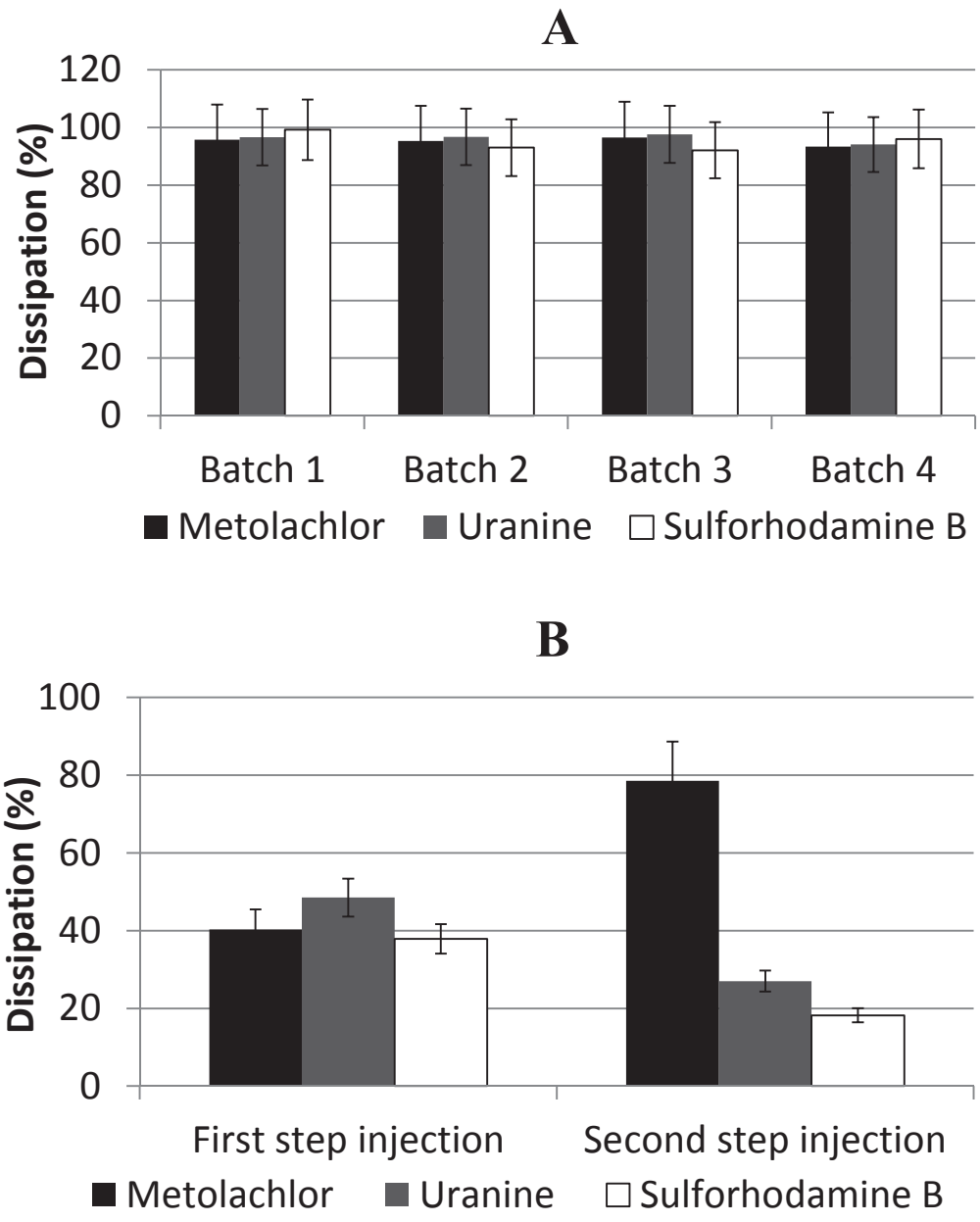

Figure IV- 3. Load dissipation of S-metolachlor, uranine and sulforhodamine B in the batch-flow (A) and the continuous flow (B) SSFCWs. Error bars represent the propagated error incorporating the analytical uncertainties of individual chemical concentration measurements as well as the uncertainty of water volume measurements.

In the continuous flow SSFCW, dissipation of UR, SRB and *S*-metolachlor was lower than in the batch flow system, ranging from 37.9% for SRB to 48.5% for UR (Figure IV-3). The shorter hydraulic residence time of in the continuous flow SSFCW might have lowered the pollutant mass degradation. Since no supernatant was observed during the investigation period, photodegradation of UR is expected to be limited. During the second step injection, the dissipation of UR and SRB decreased to 27 and 18% whereas that of *S*-metolachlor increased and reached 78%. These changes in the dissipation are likely linked with the gradual establishment of anoxic conditions, which may have favored the degradation rate of *S*-metolachlor, while decreasing that of UR and SRB.

Our results emphasize that SRB and UR were suitable surrogates for the transport of *S*-metolachlor in the batch flow system that presented longer hydraulic retention time and alternating oxic and anoxic conditions. However, in the continuous flow system, SRB and UR behaved differently from *S*-metolachlor, due to different degradation patterns for *S*-metolachlor, and UR and SRB under anoxic conditions. In a previous study, Durst et al. (2013) showed that UR can serve as a reference tracer for sustained upward vertical-flow transport of the pesticide isoproturon. However, the fungicide metalaxyl (an acetamide pesticide) had a transport that greatly differed from both SRB and UR, mainly because metalaxyl was highly dissipated (60%) compared to the dye tracers. Hence, UR and SRB are relevant surrogate for better understanding the transport of pesticides subject to photodegradation and/or sorption, but are of limited interest for pesticides whose degradation is the main dissipation pathway.

Many studies reported a greater dissipation of organic contaminants including pesticides under oxic conditions (Ávila et al., 2013). Anaerobic degradation of chloroacetanilide herbicides was previously reported to be an important degradation pathway (Konopka, 1994), and the co-existence of oxic and anoxic conditions in the batch flow system may favor the *S*-metolachlor dissipation. To evaluate *S*-metolachlor biodegradation in the wetlands, the mass budget analysis of *S*-metolachlor was completed by an analysis of metolachlor degradation products, as well as enantiomer and CSIA analyses.

4.3. *S*-metolachlor biodegradation in the wetlands

Different patterns of *S*-metolachlor oxanilic acid (OXA) and *S*-metolachlor ethanesulfonic acid (ESA) degradation products were observed in the two systems (Figure IV-4).

4.3.1 Continuous-flow wetland

In the continuous flow wetland, where dissipation was lower, *S*-metolachlor ESA was the prevailing degradation product detected at the outlet (Figure 4), in agreement with previous findings underlining that ESA degradates often prevailed in the environment (Bian et al., 2009). The fractions of ESA were always higher than that of OXA and ranged from 0 to 95.6% of the *S*-metolachlor load. The molar equivalent load of ESA (MEL_{ESA}) ranged from 0 to 66.3 mg whereas the MEL_{OXA} ranged from 0 to 20.0 mg. In particular, the highest amounts of ESA were measured during the period of tap water flushing, highlighting the high mobility of metolachlor ESA released from the wetland.

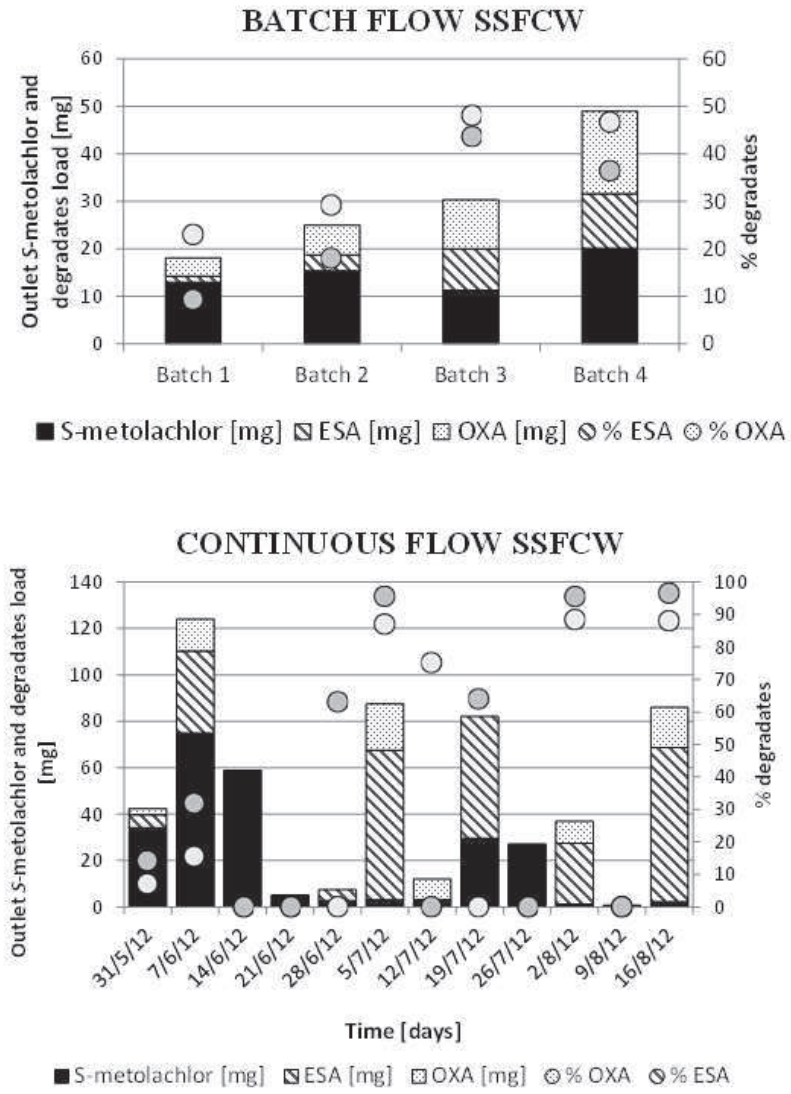

Figure IV- 4. Mass equivalent load of S-metolachlor (MELS-metolachlor) and % of degradates at the outlet of the SSFCWs.

The biodegradation of chloroacetanilide herbicides is putatively mediated co-metabolically by a glutathione-S-transferase (GST) reaction catalysed by plants and microorganisms. In the GST pathway, chloroacetanilides are conjugated with the glutathione and transformed to a chloroacetanilide–cysteine conjugate, which is further transformed either to sulfinylacetic acid that can be oxidized to an alchocol then to OXA or to a thiol, oxidized to form an ESA degradate (Aga and Thurman, 2001; Graham et al., 1999). *S*-metolachlor degradation in the continuous flow system can be partly attributed to the presence of reduced forms of sulfur, such as sulfites and sulfides that can abiotically react through a nucleophilic attack on the 2-chloro group of acetanilide herbicides leading to a dechlorinated thiosulfonic acid degradate

(Stamper and Tuovinen, 1998). Stamper and Tuovinen (1998) reported the co-existence of microbially-mediated chloroacetanilides degradation and nucleophilic attack on the 2-chloro group of acetanilide herbicides due to the presence of reactive sulfite or sulfide species. The reaction follows a S_N2 nucleophilic substitution in which the chlorine is replaced by the reactive sulfite specie (Bian et al., 2009; Stamper and Tuovinen, 1998). Even if sulphatoreduction was not significant in our study, this reaction may have occurred in microenvironment, as indicated by redox values < 400 mV.

Insignificant carbon isotope enrichments ($\Delta\delta^{13}C$ < 0.5‰) could be observed, underscoring that the amount of biodegraded *S*-metolachlor or the initial degradation step did not yielded signification carbon isotope fractionation. In contrast, a significant enantiomer fractionation (EF = 0.88 at the inlet and EF = 0.91 at the outlet of the wetland) could be observed during the second injection period, on July 12th, indicating the preferential degradation of the *R*-enantiomer. As no clear enrichment of one or the other enantiomer was otherwise observed, the presence of enzymes capable to degrade both enantiomers, leading to an apparent absence of enantioselective degradation, cannot be excluded (Milosevic et al., 2013).

4.3.2 Batch-flow wetland

In the batch flow SSFCW, gradually increasing concentrations and loads of ESA and OXA could be observed over time. 1.5 and 3.8 mg of ESA and OXA were respectively retrieved from the wetland effluent during the first batch operation, and 13.4 and 17.3 mg found after the fourth batch *S*-metolachlor. Dissipation rates for *S*-metolachlor varied from 93.3% (batch 4) to 96.5% (batch 3) and did not significantly change over time. However, the dissipation rate for $MEL_{s\text{-metolachlor}}$ decreased over time from 93.5% (batch 1) to 83.6% (batch 4). This underscores that *S*-metolachlor biodegradation rates were constant over time, while those of ESA and OXA degradation decreased over time. The occurrence of *in-situ* biodegradation during the fourth batch was also confirmed by the CSIA of *S*-metolachlor, as indicated by an insignificant change in the carbon isotope composition of metolachlor between the wetland inlet and outlet during the first batch ($\Delta\delta^{13}C_{Inlet\text{-}Outlet}$ = 0.6‰) to a small but significant fraction for the fourth batch ($\Delta\delta^{13}C_{Inlet\text{-}Outlet}$ = 1.2‰) (Figure IV-5). In a previous study, Elsayed et al. (2013) showed that metolachlor was more persistent under nitrate reducing conditions than alachlor and acetochlor in a lab-scale wetland experiment. The alternation of oxic and anoxic conditions in the present study seems to significantly increase the *S*-metolachlor degradation, as previously observed in top soils (Ma et al., 2006; White et al., 2010).

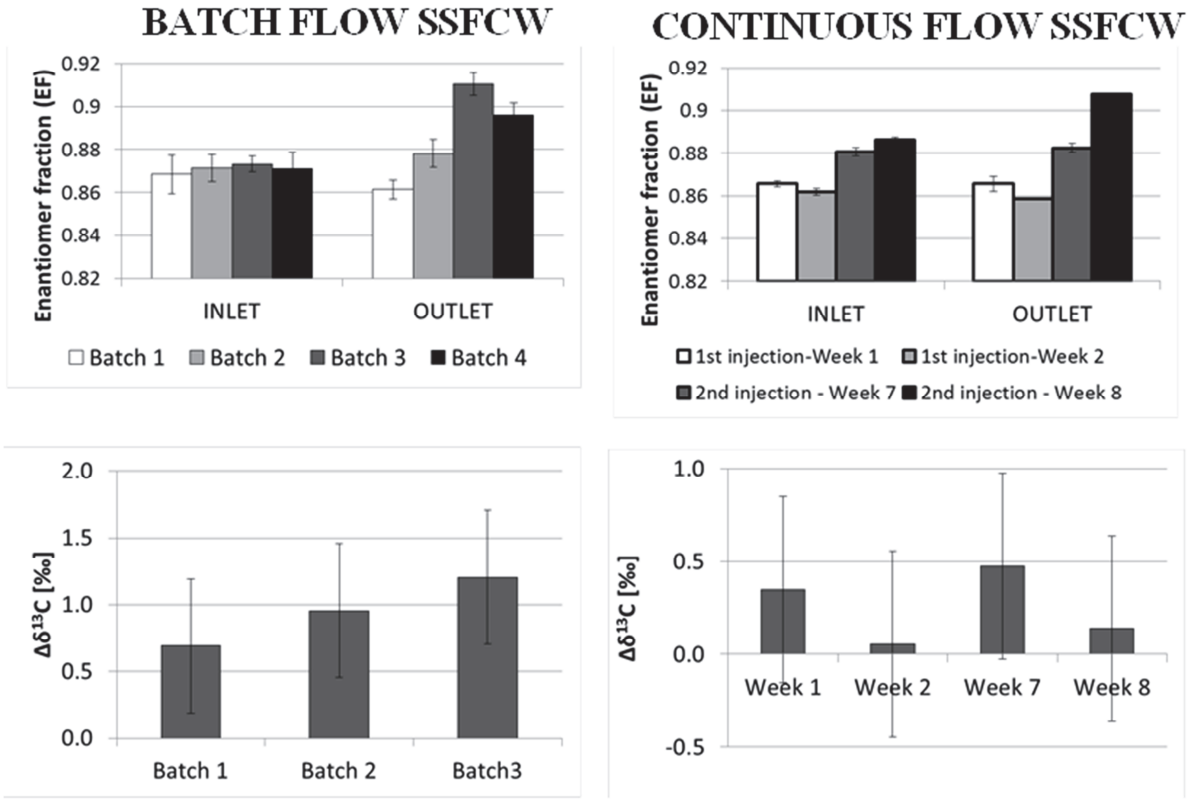

Figure IV- 5. Enantiomer fractions (on the top) and carbon stable isotope compositions measured in the batch flow SSFCW (on the left) and in the continuous flow SSFCW (on the right).

OXA was the prevailing degradation product in the batch flow SSFCW, suggesting that the OXA pathway may prevail under oxic conditions. Despite high *S*-metolachlor dissipation rates in the batch flow wetland (up to 93%), the relative fraction of ESA and OXA never exceeded 50% (Figure 4), indicating that ESA and OXA were rapidly degraded or that other pathways of *S*-metolachlor biodegradation co-existed in the wetland. This is in agreement with previous observations by Baran and Gourcy (2013) showing a greater degradation of OXA and ESA compared to *S*-metolachlor in soils. (Hladik et al., 2008; Hladik et al., 2005) reported a wide range of possible neutral degradates of metolachlor and revealed that their total concentration was detected in the Chesapeake Bay exceeding that of the parent compounds by a factor 20 to 30.

The enantiomer analysis of *S*-metolachlor emphasized the preferential degradation of the *R*-enantiomer in the batch-flow wetland during the third batch operation (EF = 0.87 at the inlet and 0.91 at the outlet of the wetland) (Figure IV-5). This result contrasts with previous

studies that showed a preferential degradation of the *S*-enantiomer compared to the *R*-enantiomer (Ma et al., 2006) or that the degradation of *S*-metolachlor was not enantioselective in soils (Klein et al., 2006). Alternating oxic and anoxic conditions in the batch flow system may have favored the development of different microbial populations depending on the redox conditions that may have preferentially degraded in alternance one or the other enantiomer (Milosevic et al., 2013).

5. Conclusions

The impact of the hydrological regime on the transport and the degradation of the widely used chiral herbicide *S*-metolachlor was evaluated in constructed wetlands using different analytical approaches, including the quantification of *S*-metolachlor and its degradation products, as well as enantiomer and compound-specific isotope analyses (CSIA). In parallel, uranine (UR) and sulforhodamine B (SRB) were jointly used as environmentally harmless surrogates to trace the transport of *S*-metolachlor. Our results showed comparable behavior of S-metolachlor, UR and SRB in the batch flow system (> 93% dissipation), whereas dissipation processes varied according to the molecules under anoxic conditions prevailing in the continuous flow SSFCW (from 18% dissipation for SRB to 78% dissipation for *S*-metolachlor).

The occurrence of both ESA and OXA degradates during biodegradation observed both under aerobic and anaerobic conditions underlines their relevance for groundwater and ecotoxicological risk assessment. The combination of different analytical approaches enabled gathering multiple lines of evidence for *in-situ* biodegradation of *S*-metolachlor under the various biogeochemical conditions provided by the wetlands. While CSIA of *S*-metolachlor and the degradation products analyses may help to distinguish biodegradation pathways, enantiomer analysis of *S*-metolachlor yielded contrasted results. Enantiomer fractionation may be insufficient to evaluate *in-situ* biodegradation when degradation rates and mass dissipation are low.

We anticipate our study to be a starting point to couple the quantification of parent compounds and degradates with enantiomer and CSIA analyses to evaluate and predict the fate of widely used and emerging chiral pesticides in receptor aquatic environments with respect to redox conditions.

6. Acknowledgements

The authors are members of REALISE, the Network of Laboratories in Engineering and Science for the Environment in the Alsace Region (France; http://realise.u-strasbg.fr), from which support is gratefully acknowledged. This research has been funded by the Research Program EC2CO (CNRS-INSU) and the PhytoRET project (C.21) of the European INTERREG IV program Upper Rhine. Elodie Maillard was supported by a fellowship of the Alsace Region. Omniea F. Elsayed was supported by a fellowship of the European Union under the 7th Framework Programme (Marie Curie Initial Training Network CSI: Environment, Contract Number PITN-GA-2010-264329). The authors thank Emil Blattmann, Carole Lutz, Sophie Gangloff, Marie-Pierre Ottermatte, Eric Pernin, René Boutin, Benoit Guyot, Yannis Risacher for their analytical support and Georges Reeb (Atelier REEB, Alsace) and Nicolas Thévenin (RITTMO, Colmar) for their support in the design of the experimental setup.

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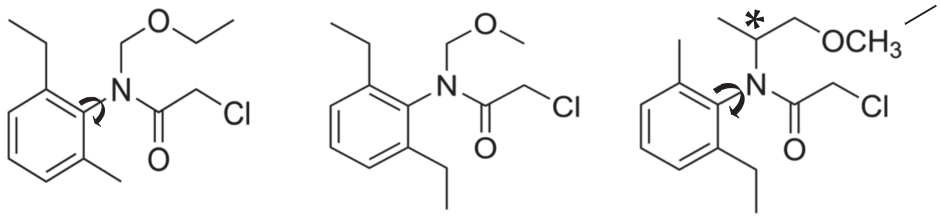
This study was a first attempt to combine enantiomer and isotope techniques to investigate the transport and degradation of the widely used chloroacetanilide herbicide *S*-metolachlor. The results showed that biodegradation of the commercial mixture of *S*-metolachlor mainly occurred under oxic-anoxic alterning system, i.e. batch flow system, compared to the anoxic system, i.e. continuous flow system. Mesocosm studies are hybrid studies between complex field scale investigations and perfectly controlled laboratory investigation.

A last experiment was therefore carried out in lab-scale wetlands under controlled conditions, with active substances to avoid the effects of adjuvants to decrease the level of study complexity. In this last series of experiments, the transport and the degradation of *rac*-metolachlor was compared to 2 other chloroacetanilide herbicides, i.e. alachlor and acetochlor. The aim was to compare, using enantiomeric and isotopic techniques, the transport and biodegradation of the three chloroacetanilides and to understand the influence of the chemical structure of the pesticides on these processes.

Chapter V. Lab-scale wetland experiment to trace *in-situ* degradation of pesticides

Overview

Chapter V includes 2 sections. The first section aims at understanding the transport and biodegradation of three herbicides of the chloroacetanilide family (Figure V-1) along a redox gradient, from oxic to nitrate-reducing conditions. The objectives were to assess the influence of i) the biogeochemical conditions on the transport and attenuation of the chloroacetanilide herbicides, ii) the chemical structure on pesticide biodegradability. The study described in the first sectionwas submitted to Environmental Pollution. The second section contains a peer-reviewed publication for which I am the second author (Chemosphere, 2013). In this section, a compound-specific isotope analysis method was developed by Omniea Fawzy Elsayed (PhD student at LHyGeS) and successfully applied on chloroacetanilide herbicides to track biodegradation processes in wetland systems.



Acetochlor Alachlor Metolachlor

Figure V- 1. Chemical structure of chloroacetanilide herbicides

Section 1. Transport and biodegradation of three chloroacetanilide herbicides in lab-scale wetlands: A comparative study

Elodie Maillard, Omniea Fawzy Elsayed, Maurice Millet, Gwenaël Imfeld* (submitted to Environmental Pollution, * corresponding author).

1. Abstract

The transport and biodegradation of the chloroacetanilide herbicides *rac*-metolachlor, alachlor and acetochlor in vertical upflow lab-scale wetlands were evaluated during 112 days using i) hydrogeochemical analyses, ii) quantitative and enantiomer analysis of parent compounds, and iii) analysis of degradation products. An oxic zone at the bottom could be distinguished from a nitrate-reducing zone at the rhizospheric zone in all wetlands. Mass dissipation averaged 61 ± 14%, 52 ± 12% and 29 ± 19% for acetochlor, alachlor and metolachlor, respectively, and mainly occurred in the rhizospheric zone. Degradation of the chloroacetanilide herbicides into ethane sulfonic acid (ESA) and oxanilic acid (OA) prevailed after day 70. Enantiomer fractions (EF) for *rac*-metolachlor were 0.494 ± 0.006 in the oxic zone and 0.480 ± 0.005 in the rhizospheric zone, indicating a significant enantioselective biodegradation. In this study, the use of complementary analytical approaches provided valuable information to identify and compare the degradation of structurally-similar pesticides in redox-dynamic environments.

2. Introduction

Chloroacetanilides herbicides are used worldwide for the pre-emergence control of annual grasses and broad-leaved weeds in a variety of crops, including maize, sugar beet and sunflowers (Pereira et al., 2009). Chloroacetanilide herbicides and their degradation products, including ionic ethane sulfonic acids (ESA) and oxanilic acids (OXA) derivatives, are frequently detected in both ground- and surface waters (Baran and Gourcy, 2013; Gadagbui et al., 2010; Heberle et al., 2000; Hladik et al., 2008; Hladik et al., 2005). Due to their prevalent use and their relatively high solubility, chloroacetanilide herbicides and their degradation products can be easily transported to non-target water bodies during runoff (Bian et al., 2009; Boyd, 2000),

raising critical issues regarding the sustainability of aquatic ecosystems and human health (Baran and Gourcy, 2013; Reemtsma et al., 2013; Steele et al., 2008). However, the transport and the fate of chloroacetanilides from agricultural source areas and potential sink zones of the landscape, such as wetlands and surface-groundwater interfaces, remain poorly understood.

Among chloroacetanilide herbicides, acetochlor, alachlor and metolachlor share the same molecular core of 2-chloroacetanilide and differ in their alkoxy moieties (Liu et al., 2001; Saha et al., 2012). Structural variations of the moieties may result in differences in terms of environmental transport, toxicity and transformation of chloroacetanilide herbicides (Barbash, 2007; Saha et al., 2012). Metolachlor is a chiral compound consisting of 4 stereoisomers (including two pairs of enantiomers, i.e. *S*- and *R*-enantiomers) (Ma et al., 2006). Alachlor and racemic metolachlor (*rac*-metolachor; with an enantiomeric ratio close to 50:50) were extensively used in the mid-1990s and then respectively replaced by acetochlor and *S*-metolachlor, which is isomerically enriched with the herbicidally active *aR*,1'*S* and *aS*,1'*S* isomers (Müller et al., 2000; Xu et al., 2010). Although abiotic degradation and transport processes are not affected by the spatial configuration of enantiomers, chiral pesticides can undergo enantioselective biological processes (Wong et al., 2002). However, the influence of the molecular structure of chloroacetanilide herbicides and the spatial configuration of metolachlor isomers on their environmental transport and biodegradation is rarely investigated. In particular, knowledge on processes governing the transport and biodegradation of chloroacetanilide herbicides in wetlands, and similar groundwater/surface water systems, is currently lacking.

Wetlands are reactive zones of the landscape that can intercept contaminated surface or groundwater and provide important ecosystem services, including pesticide dissipation. Wetland systems have intrinsic biological, chemical and physical processes that contribute to pesticide dissipation (Imfeld et al., 2013; Maillard et al., 2011; Reichenberger et al., 2007). The transport of contaminants and their predominant transformation pathways are related to the biogeochemical processes that occur in wetlands (Borch et al., 2010). Microbially-mediated degradation is the major pathway contributing to the dissipation of chloroacetanilide herbicides in wetland systems (Gadagbui et al., 2010), whereas abiotic transformations, such as photolysis or hydrolysis, have been shown to play a minor role (Zhang et al., 2011).

In a previous study, we demonstrated the occurrence of *in-situ* biodegradation of chloroacetanilides in lab-scale wetlands using compound-specific isotope analysis (Elsayed et al.,

2013). In the present study, we compared the transport and biodegradation of metolachlor, alachlor and acetochlor in the lab-scale wetlands using i) hydrochemical analyses to characterize the biogeochemical dynamics, ii) quantitative and enantiomer analyses of the parent compounds, and iii) analysis of ethane sulfonic (ESA) and oxanilic acids (OXA) degradation products. The vertical-flow wetlands were designed to study the upward discharge of contaminated water into environments at groundwater/surface-water interfaces. To the best of our knowledge, this is the first quantitative and comparative study on the transport of chloroacetanilides herbicides in wetland systems.

3. Material and methods

3.1. Chemicals

Analytical standards of acetochlor [2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl) acetamide], alachlor [2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl) acetamide] and metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide] (Pestanal®, analytical grade purities >97%; Sigma Aldrich, St Quentin Fallavier, France). Physico-chemical properties of the chloroacetanilide compounds are provided in Table VII-22 in the Appendices. Alachlor-d_{13} (2,6-diethylphenyl d_{13}) and metolachlor-d_6 (propyl d_6) (Dr. Ehrenstorfer GmbH, Augsburg, Germany) were used as internal standards . Chromasolv® Plus methylene chloride, methanol and ethyl acetate of analytical grade purity ≥ 99.9% (Sigma Aldrich, St Quentin Fallavier, France) were used for the extraction.

3.2. Lab-scale wetlands

The wetlands consisted of four borosilicate glass columns (inner diameter: 15 cm, height: 65 cm), filled with 5 cm of gravel (Ø 1 – 2 mm) and 52 cm of sand (Ø 0.40 – 0.63 mm) and planted with Phragmites australis (Cav.) (Figure V-1). Physical properties and chemical characteristics of the sand and gravel have been described elsewhere (Durst et al., 2013) (Table VII-23 in the Appendices). The wetlands were kept in an air conditioned room at 20 ± 0.5 °C, and exposed daily to light from a LED lamp (Greenpower LED lamp, Philips®, Eindhoven, The Netherlands) for 8 hours. All tubing and stoppers in contact with the wetlands matrix was made of Viton® (Rotilabo®, Carl-Roth, Karlsruhe, Germany). Five sampling ports were located at 15, 25, 35, 45 and 55 cm from the inlet point in each column. Dissolved oxygen concentrations were

monitored using non-invasive oxygen sensors (Presens®, Germany) facing each of the five sampling points that were mounted inside the columns (Figure V-2).

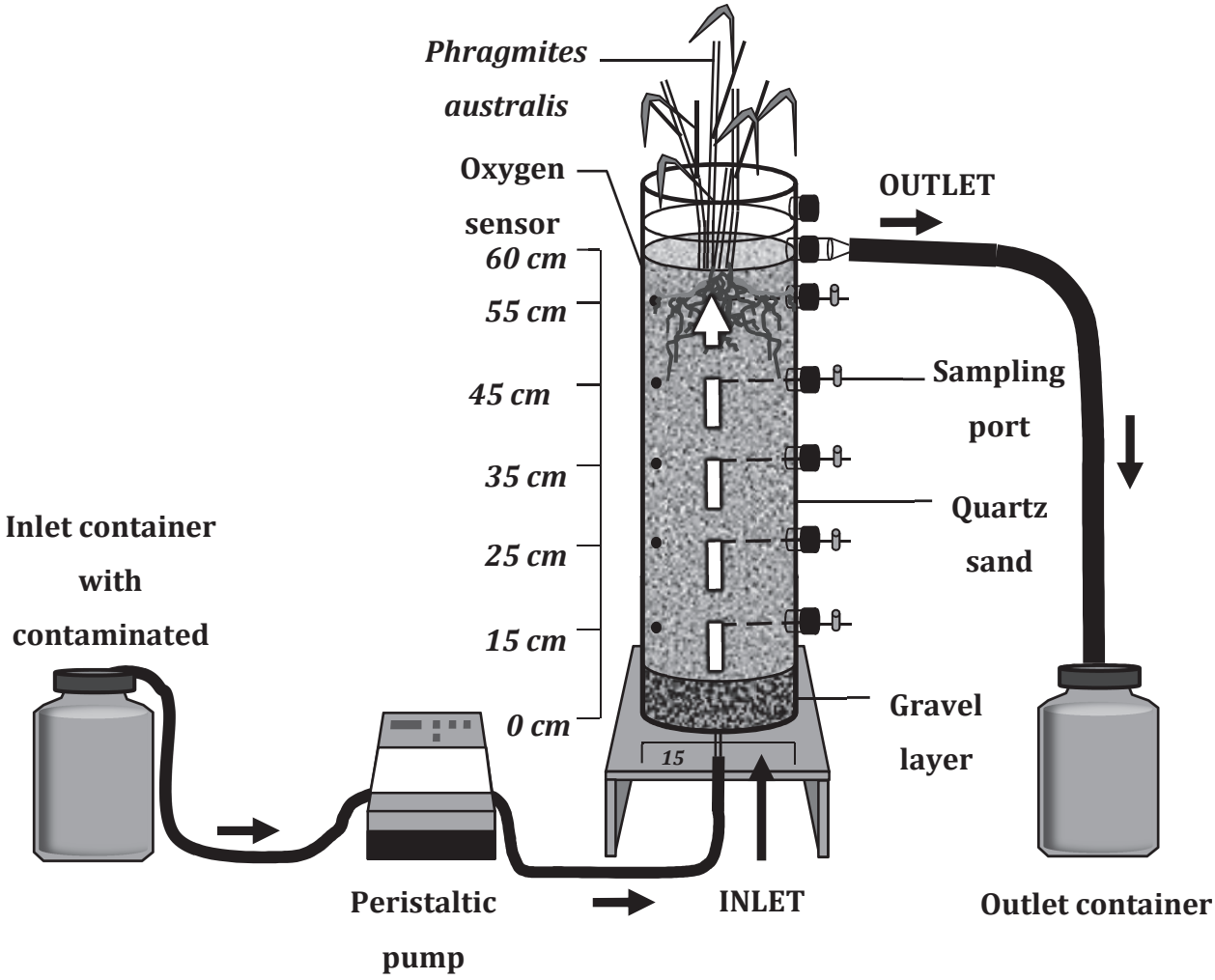


Figure V-2 Layout of the experimental set-up for one lab-scale wetland.

Water used to supply the wetlands was taken monthly from a vegetated ditch (Alteckendorf, France) that collects chloroacetanilide contaminated agricultural runoff from sugar beet and corn catchments. Inlet water composition can be found in the Appendices, Table VII-24. The water was continuously pumped to the wetlands inlets at a flow rate of 0.33 mL min^{-1} using a high precision pump (ISMATEC® IPC model ISM936D, Zürich, Switzerland). The nominal residence time was 9.3 days. The lab-scale wetlands were covered with reflective foil to prevent algal growth and photolytic decay of chloroacetanilides.

3.3. Experimental setting and sampling procedure

The lab-scale wetland experiment was carried out over 202 days. The injection of chloroacetanilides in wetlands was preceded by an inoculation period of 104 days, during which the wetlands were supplied with vegetated ditch water without addition of herbicides. Metolachlor, acetochlor and alachlor were dissolved in ultrapure water and stored at -20 °C until they were added to the inlet water. Three columns were supplied each with one of the target herbicides metolachlor, acetochlor and alachlor with an initial concentration of 1.8 µM for metolachlor and 1.9 µM for acetochlor and alachlor. A fourth column was supplied with vegetated ditch water without addition of herbicides. The supplied water was renewed and herbicides were added biweekly, after each sampling campaign. No significant change in chloroacetanilides concentrations was observed in the inlet tanks during two weeks period between sampling campaigns (nonparametric Wilcoxon test for paired samples, $p > 0.05$).

Sampling campaigns were carried out biweekly at 0, 14, 28, 42, 56, 70, 84 and 98 and 112 days where day 0 represents the first day of chloroacetanilide injection. At each sampling campaign, water samples were retrieved with glass syringe from the previous and the freshly prepared tank with spiked herbicides, the wetland inlet, outlet, and from the five sampling ports for hydrochemical (30 mL), as well as chloroacetanilide, degradation products and enantiomeric analyses (20 mL).

3.4. Hydrogeochemical analysis

Hydrogeochemical parameters were analysed every two weeks at the inflow, outflow and at the 5 sampling ports of the lab-scale wetlands to characterize the biogeochemical development of the wetlands according to contaminant transport. Concentrations of Fe^{2+}, Cl^{-}, NO^{3-}, PO_4^{3-}, SO_4^{2-}, Mn^{2+}, Al^{3+}, Si^{2+}, Mg^{2+}, Ca^{2+}, Na^{+} and K^{+} were determined by FR EN ISO standards and laboratory procedures. Concentrations of total organic carbon (TOC), dissolved organic carbon (DOC) were only measured at the inlet and outlet of the wetlands. Electrical conductivity, pH and dissolved oxygen were directly measured in wetland inlet and outlet water using WTW multi 350i sensors (WTW, Weilheim, Germany). Dissolved oxygen concentrations inside the wetlands were measured using oxygen sensor spots (Presens, Germany) (Figure V-2).

3.5. Analysis of chloroacetanilide herbicides and degradation products

The solid-phase extraction procedure and quantification of chloroacetanilide herbicides were carried out as previously described (Elsayed et al., 2013). The detailed protocols for the extraction and quantification of the chloroacetanilides in water, sand and plant, and the ethane sulfonic (ESA) and oxanilic acids (OXA) degradation products of metolachlor (MESA and MOXA), alachlor (AlESA and AlOXA) and acetochlor (AcESA and AcOXA) are provided in the Appendices (Table VII-25).

Briefly, solid-phase extraction was carried out using an AutoTrace 280 SPE system and SolEx C18 cartridges (Dionex®, CA, USA) packed with 100 mg bonded silica. Chloroacetanilide herbicides were quantified using a Focus-ITQ 700 model GC-MS/MS apparatus (Thermo Scientific, Les Ulis, France) on a 30 m x 0.25 mm ID, 0.25 µm film thickness OPTIMA 5MS (5% phenyl - 95% dimethylpolysiloxane) fused-silica capillary column (Macherey Nagel GmbH, Düren, Germany), with helium as a carrier gas, at a flow rate of 1 mL min^{-1}. Detection limits were 1.7, 0.7 and 0.7 µg L^{-1} and quantification limits were 5, 2, 2 µg L^{-1} for acetochlor, alachlor and metolachlor, respectively.

ESA and OXA degradation products were analysed using a TSQ Quantum ACCESS LC/MS (Thermo Scientific, Les Ulis, France) equipped with a column was a EC 150/3 Nucleodur Polar Tec (particle size 3µm, length 150 mm, internal diameter 3 mm) and a precolumn EC 4/3 Polar Tec, 30 mm (Macherey Nagel, France). The mobile phase consisted of 0.1% formic acid/high-purity water and 0.1% formic acid/acetonitrile. Column oven temperature was set at 60°C to achieve better separation and peak shapes. The mass spectrometer (MS) was a Thermo TSQ Quantum triple quadrupole mass spectrometer (Les Ulis, France) operated using a heated electrospray ionization (HESI) source. Limits of detection were 0.06, 0.02, 0.02, 0.10, 0.06, 0.04 µg L^{-1} and limits of quantification were 0.10, 0.02, 0.04, 0.16, 0.10, 0.06 µg L^{-1} for AcOXA, AcESA, AlOXA, AlESA, MOXA and MESA, respectively.

3.6. Enantiomer analysis of metolachlor

Metolachlor enantiomer analysis was carried out with a Trace GC 2000 series GC-MS apparatus (Thermo Scientific, Les Ulis, France) using a 30 m × 0.25 mm ID, 0.25 µm film 20%

tert-butyldimethylsilyl-β-cyclodextrin dissolved in 15% phenyl-, 85% methylpolysiloxane column (BGB Analytik, Boeckten, Switzerland) with helium as a carrier gas at a flow rate of 1.0 mL min^{-1}. The column was held at 50°C for 3 min, ramped at 15°C min^{-1} to 150°C, and finally ramped at 0.5°C min^{-1} to 190°C and held for 5 min. A volume of 3 µL of sample was injected on a split/splitless injector (pulsed splitless flow for 1 min). The injector, the transfer line and ion source temperatures were maintained at 250°C, 250°C and 230°C, respectively. The mass spectrometer was operated in the electron ionization mode (EI+, 70 eV). The quantification was based on the parent ions 238 and 242 and the daughter ions 162 and 166 for respectively metolachlor and metolachlor-d_6. The stereoisomer elution was *aS*1'*S*; *aS*1'*R*; *aR*1'*S*; *aR*1'*R* (see Figure VII-6 in the Appendices).

3.7. Data analysis

Daily chloroacetanilide loadings inflowing and outflowing the wetlands were estimated by interpolating and multiplying chloroacetanilide concentrations between consecutive sampling dates and the corresponding flow rate. Loads of chloroacetanilides at a given point between two sampling campaigns were calculated from the integral sum of all the daily load estimates. Mass removal of chloroacetanilides [%] was calculated as the relative decrease of outlet mass ($mass_{out}$) to the inlet mass ($mass_{in}$) for each lab-scale wetland on the same time period. The remaining fractions of chloroacetanilides (C_x/C_{In}) are the ratios of concentrations measured at each sampling points along the flow path (C_x) and at the inlet (C_{In}).
Hydrochemical, chloroacetanilides and enantiomeric data were compared using the paired non-parametric Wilcoxon signed rank and the Spearman rank correlation tests, with p-value set at 0.05 (R software, Version 3.0.1).

The enantiomer fraction (EF) was used to indicate the relative amounts of the pair of *rac*-metolachlor enantiomers (Harner et al., 2000), as defined in eq. 1:

$$EF = \frac{S-enantiomers}{S-enantiomers+R-enantiomers} = \frac{aS1'S+aR1'S}{aS1'S+aR1'S+aS1'R+aR1'R} \quad (1)$$

where *S*-enantiomers stand for the peak areas of *αS*1'*S* and *αR*1'*S*, and the *R*-enantiomers for the peak areas of *αS*1'*S* and *αR*1'*S*. Pure enantiomers have EFs of 0 or 1, while racemic mixtures have an EF of 0.5.

The diastereoisomer ratio was used to indicate the relative amounts of the pair of metolachlor diastereoisomers (that are not enantiomers), as defined in eq. 2:

$$DR = \frac{aS1'S + aR1'R}{aR1'S + aS1'R} \quad (2)$$

Contrary to EF, the value of DR of a chiral compound is not necessarily equal to 1 for racemic compounds but can largely vary (from 1:1 to up than 2:1) depending on batches and conditions during the synthesis (Büser et al., 2000).

4. Results and discussion

4.1. Biogeochemical development of the lab-scale wetlands

A redox gradient was observed along the flow path in the four lab-scale wetlands, with oxic conditions prevailing at the bottom of the wetlands (mean dissolved oxygen concentration: 212 ± 24 µM between 0 and 25 cm), and anoxic conditions observed at the top (31 ± 42 µM between 45 and 60 cm), separated by a transition zone (152 ± 64 µM between 25 and 45 cm) (Table V-1). Dissolved oxygen concentrations were significantly different between the 3 zones in the three wetlands receiving chloroacetanilides ($p < 0.001$). Oxygen concentrations measured in the anoxic zones (45 – 60 cm) of the alachlor and acetochlor wetlands were lower (3 ± 10 µM) than those of the metolachlor and control wetlands (58 ± 44 µM) ($p < 0.001$) (Figure V-1). pH values ranged from 7.3 to 8.3 and were similar in the wetland ($p < 0.05$). Electrical conductivity values were similar in the acetochlor (679 ± 32 µS cm^{-1}) and alachlor (682 ± 33 µS cm^{-1}) wetlands and between the metolachlor (674 ± 99 µS cm^{-1}) and the control (720 ± 35 µS cm^{-1}) wetlands ($p < 0.05$).

Nitrate reducing conditions occurred in the four wetlands, especially in those receiving alachlor and acetochlor. Nitrate concentrations significantly decreased in the four wetlands throughout the experiment ($p < 0.05$). The average nitrate mass dissipation rates were 79 ± 11%, 74 ± 15%, 55 ± 12% and 34 ± 19% in the wetlands receiving acetochlor, alachlor and metolachlor and the control wetland, respectively. Nitrate concentrations in the four wetlands were significantly lower in the oxic zone (0 – 25 cm) compared to the anoxic zone (45 – 60 cm) (Table V-1). This is in line with previous observations based on a similar experimental set-up, showing that higher nitrate removal rate occurred in the upper part of the upflow wetlands (Tan

et al., 2004). Manganese and ferrous iron concentrations were very low in inlet water and did not significantly vary between inlets and outlets of the wetlands, ranging respectively from 0.1 to 0.3 µM and from 1 to 3 µM. Sulphate reducing conditions were observed for the wetland receiving acetochlor (reduction of 11%, $p < 0.001$), indicating the prevalence of stronger anoxic conditions in this wetland.

Table V- 1 Concentrations of redox sensitive species (Mean ± SD) in the oxic zone (0 – 25 cm), the transition zone (25-35 cm) and the anoxic zone (45-60 cm) of the 4 lab-scale wetlands.

		Redox sensitive species concentrations [µM] mean ± SD				
		Oxygen	**NO_3**	**Fe**	**Mn**	**SO_4**
Acetochlor	**Oxic zone**	215 ± 16	399 ± 110	1.6 ± 2	0 ± 0	489 ± 51
	Transition zone	142 ± 74	373 ± 56	0.9 ± 0.7	0.1 ± 0	476 ± 72
	Anoxic zone	2 ± 5	69 ± 13	3.9 ± 4.8	0.6 ± 0.4	367 ± 92
Metolachlor	**Oxic zone**	204 ± 19	419 ± 66	3 ± 4	0.1 ± 0.1	447 ± 99
	Transition zone	146 ± 25	385 ± 64	1.6 ± 2.1	0.2 ± 0.2	486 ± 32
	Anoxic zone	55 ± 5	165 ± 78	1.7 ± 1.5	0.6 ± 0.4	472 ± 28
Control	**Oxic zone**	228 ± 19	421 ± 53	2.2 ± 3.5	0.1 ± 0	426 ± 108
	Transition zone	217 ± 22	436 ± 58	0.9 ± 0.7	0.1 ± 0.1	492 ± 29
	Anoxic zone	62 ± 39	274 ± 143	2.1 ± 2.5	0.1 ± 0	378 ± 125
Alachlor	**Oxic zone**	194 ± 28	378 ± 119	1.9 ± 1.7	0.1 ± 0	457 ± 84
	Transition zone	101 ± 59	352 ± 76	1.9 ± 2.8	0.1 ± 0	472 ± 120
	Anoxic zone	4 ± 13	151 ± 86	2.3 ± 3	0.2 ± 0.2	394 ± 105

Dissolved organic carbon (DOC) concentrations were significantly higher at the outlets (21.3 ± 14.8 mg C L^{-1}) of the wetlands compared to the inlets (10.9 ± 11.5 mg C L^{-1}) ($p < 0.05$). Total organic carbon (TOC) concentrations were 10.3 ± 11.9 mg C L^{-1} at the inlets and 22.3 ± 16.4 mg C L^{-1} at the outlets. DOC was released from the wetlands, due to bacterial decomposition of plant detritus into dissolved organic carbon such as humic acids and/or the release of DOC by plant roots (Pinney et al., 2000). However, chloroacetanilide concentrations at the wetland outlets were not correlated with those of DOC or TOC ($p > 0.05$). This suggests that DOC-related transport of chloroacetanilides played a minor role, possibly due to low amounts of humic acid and lower availability of sorption site in higher molecular weight DOC (Ding et al., 2002).

Oxygen, nitrate and sulphate data show the establishment of a redox gradient in the rhizosphere zones of the wetlands, which may in turn affect the biodegradation of chloroacetanilide herbicides in the wetlands.

4.2. Chloroacetanilide dissipation in the wetlands

The average remaining fractions of chloroacetanilides (C_x/C_{In}) along the wetlands' flowpath varied from 0.4 to 1 for alachlor, from 0.4 to 1.2 for acetochlor and from 0.6 to 1 for metolachlor (Figure V-3). Remaining fractions >1 obtained at the wetland outflow and in the oxic zone of the acetochlor wetland indicate that acetochlor accumulated in the wetland, due to preferential flow paths and root channeling in the upper part of the wetland (Bundt et al., 2001). The average mass dissipation rates during the experiment were 61 ± 14%, 52 ± 12% and 29 ± 19% for acetochlor, alachlor and metolachlor, respectively (Figure V-4). Dissipation varied over time and ranged from 56 to 90%, from 32 to 64% and from 8 to 70% for acetochlor, alachlor and metolachlor, respectively. The chloroacetanilides loads significantly differed between the oxic zone and the outlet, indicating that dissipation mainly occurred in the anoxic zone corresponding to the rhizosphere ($p < 0.05$).

Larger dissipations were observed between day 0 and day 28, reaching 90% for acetochlor, 70% for metolachlor and 62% for alachlor, whereas after day 28, chloroacetanilides dissipation rates stabilized and averaged 54 ± 7%, 52 ± 8% and 21 ± 7% for acetochlor, alachlor and metolachlor, respectively. The dissipation rates for the three chloroacetanilides did not significantly change from day 28 to day 112 ($p > 0.05$).

Since the organic matter content was low in the quartz sand matrix (0.15 ± 0.05%), the larger dissipation rates between day 0 and day 28 are attributed to the sorption of chloroacetanilides on the organic matter from plant roots and plant decay and most probably to dilution (Figure V-4) (Liu et al., 2000; Si et al., 2009). Chloroacetanilide concentrations in the sand and plant roots collected 2 months after the end of the chloroacetanilide injection were systematically below the quantification limits, except in the aerial part of the wetland that received *rac*-metolachlor (0.34 µg). This indicates that sorption and plant uptake were not relevant processes for chloroacetanilide dissipation since no chloroacetanilide release was observed after the injection period. Chloroacetanilide uptake by plants is expected to be limited. Since alachlor, acetochlor and metolachlor are relatively hydrophobic ($\log K_{ow} > 3$), their diffusion through the lipid barrier of roots epidermis is expected to be limited (Moore et al., 2001; Schröder and Collins, 2002; Verkleij et al., 2009). Volatilization was not expected to occur since Henry constants of metolachlor, alachlor and acetochlor are $>10^{-3}$ Pa m^3 mol^{-1} (Table VII-22 in the Appendices). Photolysis was avoided by a reflective foil that covered the wetlands and

protected them from light decay. Hence, biodegradation is expected to be the major dissipation pathway of chloroacetanilides in the wetlands.

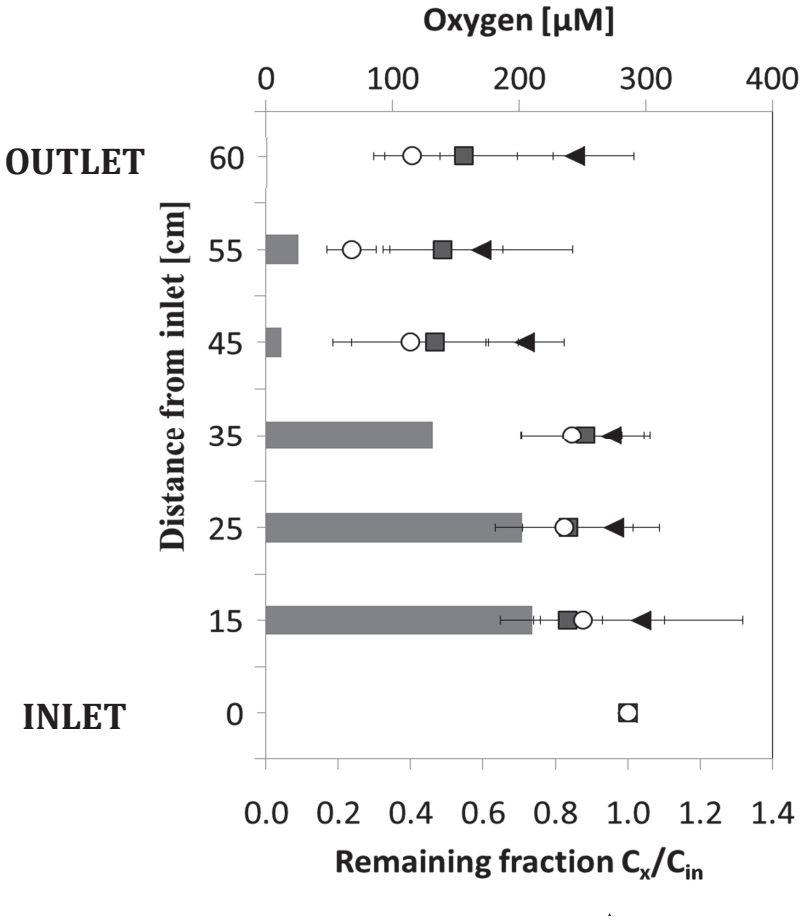


Figure V- 3 Mean remaining fractions and oxygen concentrations along the flow path, between day 0 and day 112. Error bars indicate the standard deviation of chloroacetanilide measurements.

Degradation of chloroacetanilides may occur under both aerobic (Dictor et al., 2008) and anaerobic conditions (Bian et al., 2009). In our study, chloroacetanilide dissipation mainly occurred under nitrate and sulphate reducing conditions. Alachlor degradation has been observed to be larger under anaerobic conditions (Graham et al. 2000), and fast chloroacetanilides degradation may occur both biotically and abiotically in the presence of sulphate reducing conditions and large amounts of sulfide (Cai et al., 2007). During abiotic processes, organic and inorganic sulfides can react through a nucleophilic attack on the 2-chloro group of acetanilide herbicides (Stamper and Tuovinen, 1998). In parallel, microbially-mediated reductive dechlorination can occur during sulphate reduction (Cai et al., 2007; Stamper et al., 1997). The reaction follows a S_N2 nucleophilic substitution in which the chlorine is replaced by

the reactive sulfite specie (Bian et al., 2009; Stamper and Tuovinen, 1998). This may explain the larger degradation of acetochlor under sulphate reducing condition prevailing in the wetland compared to that of alachlor and metolachlor.

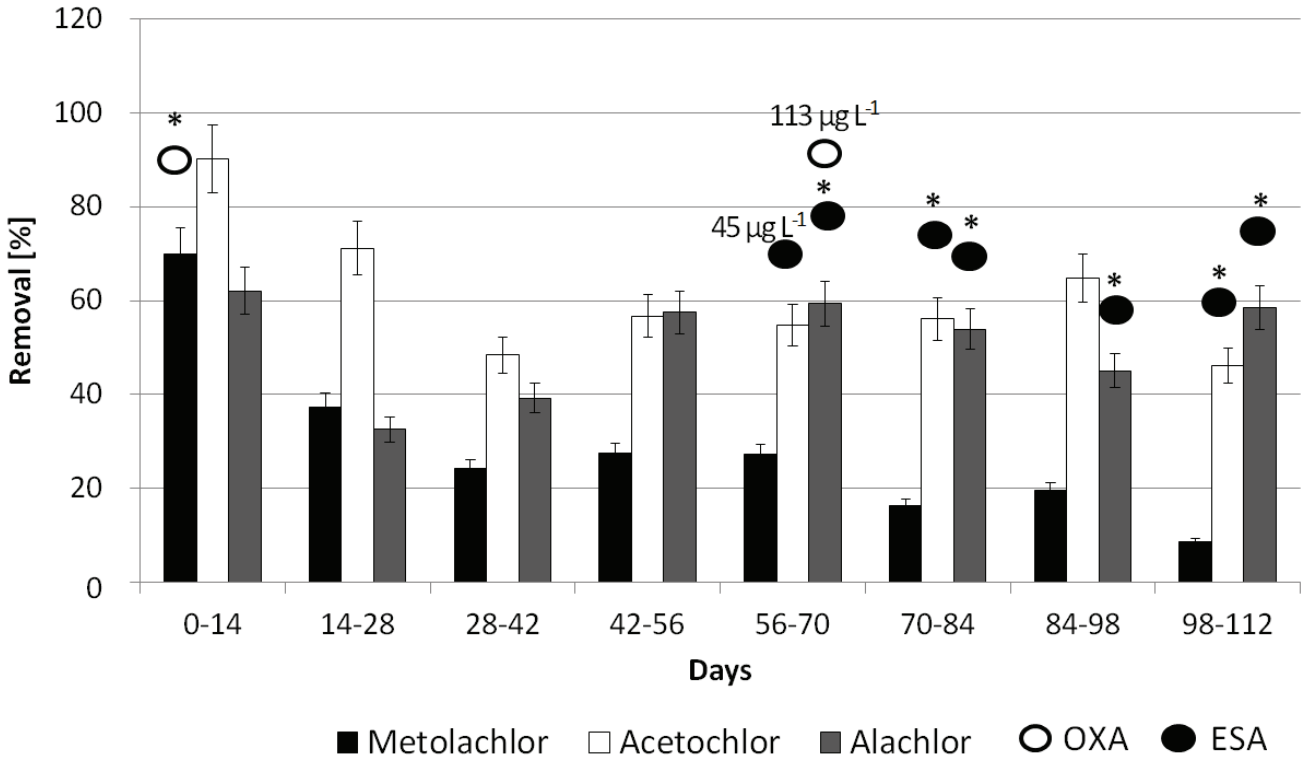


Figure V- 4 Load removal of metolachlor, alachlor and acetochlor between two sampling campaigns, from day 0 to day 112. Circles placed above the bars represent the presence of the respective oxanilic acid (OXA) (empty circles), or ethanesulfonic acid (ESA) (solid circles) degradates of the corresponding chloroacetanilide herbicide. The concentration of degradates is mentioned above their respective circles. * is indicates a concentration below the limit of quantification. Error bars correspond to the analytical uncertainty.

Chloroacetanilide biodegradation is expected to mainly result from cometabolic processes. Larger cometabolic biodegradation in response to increased carbon sources were reported for many pesticides such as atrazine (Abdelhafid et al., 2000), simazine (Cox et al., 2001), or fenitrothion and dimethoate (Sánchez et al., 2004), and acetochlor (Cai et al., 2007). Release of root exudates in the rhizosphere may enhance microbial activity and xenobiotics degradation under reducing conditions (Briceño et al., 2007). The transformation of chloroacetanilide herbicides is putatively mediated co-metabolically by a glutathione-S-

transferase (GST) reaction catalysed by plants and microorganisms. In the GST pathway, chloroacetanilides are conjugated with the glutathione and transformed to a chloroacetanilide–cysteine conjugate, which is further transformed either to sulfinylacetic acid that can be oxidized to an alchocol then to OXA or to a thiol, which is further oxidized to form an ESA degradate (Aga and Thurman, 2001; Graham et al., 1999).

The persistence of metolachlor in comparison to alachlor and acetochlor was also observed in anoxic soil (Konopka, 1994), in oxic water (Graham et al., 1999), and in aerobic microbial cultures (Zhang et al., 2011). Firstly, the toxicity of metolachlor to bacterial communities of the lab-scale wetland may decrease its biodegradation compared to that of alachlor and acetochlor. For instance, metolachlor can inhibit the growth and impair the respiratory activity of bacteria (*B. stearothermophilus*) (Pereira et al. 2009). Secondly, the substitution of an amide alkoxymethyl side chain, present in alachlor and acetochlor with a bulkier alkoxyethyl side chain in metolachlor, may lead to greater steric hindrance around the carbon chlorine bond and lower degradation rates (Graham et al., 1999; Zhang et al., 2011). Hence, the persistence of metolachlor in the wetland is possibly due to the larger moiety of the molecule that limits the substitution reaction through the alcohol or thiol intermediate, leading to the formation of metolachlor ESA and OXA.

4.3. ESA and OXA degradation products in the wetlands

Chloroacetanilide herbicides appear to be degraded primarily by microbial activity in the wetlands, resulting in the formation of ESA and OXA degradation products, which are the most frequently detected degradation products in both surface and ground-water (Aga et al., 1999; Scribner et al., 2000) (Figure V-4). Ethane sulfonic acid degradates of acetochlor and alachlor were predominantly found in the lab-scale wetlands. AlESA was detected at the outlet of the lab-scale wetland on days 70, 84, 98 and 112, i.e. during the second half of the experiment but was always under the quantification limit (< 0.16 µg L^{-1}). AcESA was detected at the outlet of the wetland on days 70, 84 and 112 (Figure V-4) and could only be quantified on day 70 (concentration of 45 µg L^{-1} corresponding to a total mass of 289 µg of AcESA released between day 56 and day 70). Acetochlor and alachlor ESA and OXA were almost systematically detected after day 70. MESA and MOXA degradation products were rarely detected, underscoring the less degradation of metolachlor compared to acetochlor and alachlor. MOXA was detected on day 14, when conditions were slightly more oxic. AlOXA was detected on day 70. Concentration of AlOXA

(113 µg L^{-1} i.e. 701.3 µg) was the highest concentration of degradation product measured during the investigation period.

Our results show that chloroacetanilide degradation occurs via the OXA and ESA pathways under dominantly suboxic conditions The degradation of chloroacetanilide herbicides to the ESA degradates results in the removal of a chlorine atom and the addition of a sulfonic acid functional group to the molecule, whereas degradation to OXA involves the substitution of chlorine with a carboxyl group. The predominance of the ESA degradate compared to OXA, in particular for acetochlor, is possibly linked to the presence of large sulfite amount produced during the sulphatoreduction, thereby leading to a nucleophilic substitution of the chlorine of chloroacetanilide by sulfite (Bian et al., 2009; Stamper and Tuovinen, 1998). The latter observation is also in agreement with previous findings underscoring that ESA degradation products prevailed in the environment (Bian et al. 2009). This reaction may also have occurred in the wetland for alachlor, but to a lesser extent, due to the absence of significant sulphatoreduction. Graham et al. (2000) showed that AlOXA was the more prominent degradate of alachlor in aquatic systems, which is consistent with the higher concentrations of AlOXA observed on day 70. The absence of OXA and ESA degradates before day 70 can be explained by higher degradation rates of ESA and OXA or by the occurrence of alternative degradation pathways under anoxic conditions, which did not involve OXA and ESA as intermediate products. Neutral degradation products may have been produced in anoxic conditions that prevailed between day 0 and day 70 in the rhizosphere (Hladik et al. 2005). The slight increase of dissolved oxygen on day 70 and after may have led to changes in microbial communities and degrading activities, leading to the prominent degradation of chloroacetanilide herbicides *via* ESA and OXA degradates.

Since the degradation rates of ESA and OXA by microorganisms in water may be slow, they are likely to be persistent, in particular in groundwater (Hladik et al., 2005; Steele et al., 2008). Moreover, increased polarity of the degradation products, increases their water solubility and their leaching to groundwater (Potter and Carpenter, 1995; Thurman et al., 1996). ESA and OXA degradation products of chloroacetanilides can reach groundwater in concentrations above 0.1 µg L^{-1}, and display comparable or higher risk than the parent compounds and are thus relevant in ecotoxicology and groundwater quality contexts (Steele et al., 2008).

4.4. Metolachlor enantiomer fractionation

The average enantiomer fraction (EF) values for metolachlor was lower in the rhizosphere zone of the wetland (EF = 0.480 ± 0.005, mean ± SD) compared to that found in the oxic zone (EF=0.494 ± 0.006) (Figure V-5). The slight enrichment in the *R*-enantiomer and the preferential degradation of the *S*-enantiomer underscores the occurrence of an enantioselective biodegradation of metolachlor in the rhizosphere, as confirmed by the mass-balance analysis and the correlation between the metolachlor concentrations and the EF values (p=0.00618 rho=0.39). In contrast, diastereoisomeric ratios (DR) did not vary between the inlet and the outlet and between the oxic and the anoxic zone, suggesting that metolachlor biodegradation in the wetland was not diastereoisomer-selective ($p > 0.05$).

Although the enantiomer analysis of metolachlor is a valuable tool to assess the relative contribution of racemic- *versus* *S*-metolachlor in the environment (Büser et al., 2000), enantiomer analysis can also be used to identify biodegradation in redox-dynamic environments, such as wetlands. Our results are in agreement with previous observations underscoring that *S*-metolachlor was more easily degraded than *rac*-metolachlor (Ma et al., 2006), but contrast with those emphasizing the absence of enantioselectivity during that metolachlor degradation (Klein et al., 2006).

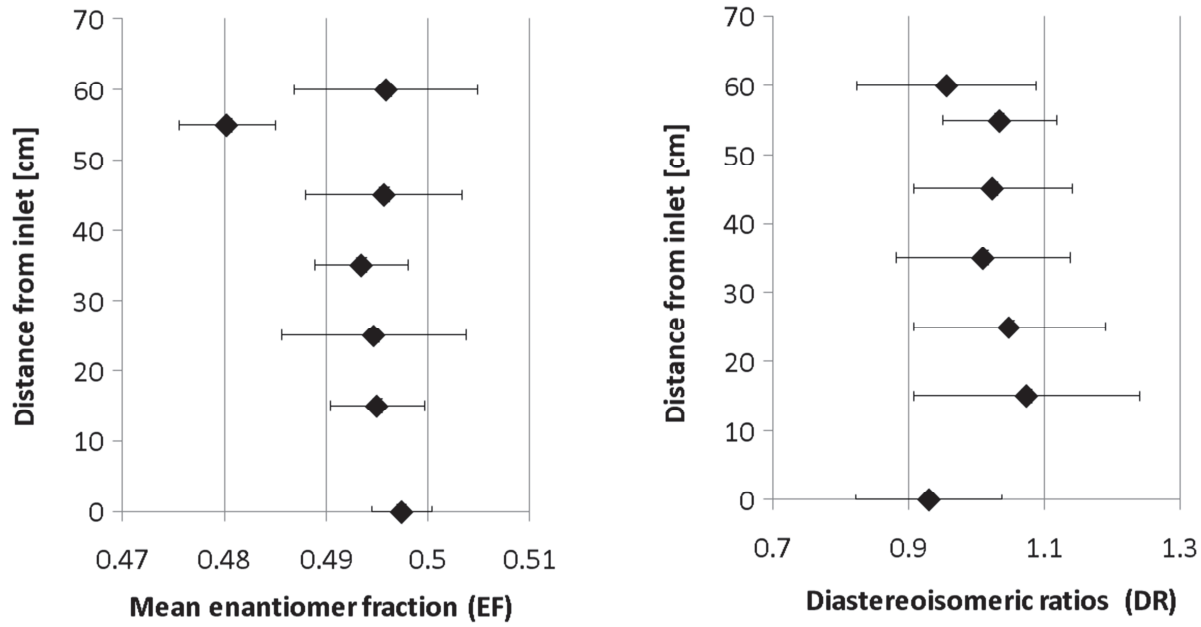


Figure V- 5 Mean enantiomer fraction (EF) and diastereoisomeric ratios (DR) of metolachlor along the flow path, between day 0 and day 112. Error bars represent the standard deviation of analytical measurements.

Redox conditions in the field may largely vary and several degradation pathways involving each different microorganisms, which preferentially degrade the *R*- or the *S*-enantiomers, may co-exist. Concomitant enantioselective degradation of *R*- and *S*-enantiomers may thus result in non-detectable enantioselective degradation in the bulk sample. In our wetland experiment, the occurrence of anoxic conditions in the rhizosphere zone likely favored the predominance of a degradation pathway, involving the preferential degradation of the *S*-metolachlor. Some microorganisms show enantioselective microbial degradation of metolachlor (Ma et al., 2006), resulting in preferential degradation of an enantiomer, either due to preferential microbial uptake or enzyme activity. Hypothetically, both the transporter protein and the enzyme might be enantiospecific during metolachlor degradation, as previously shown with phenoxy acid herbicides (Müller et al., 2006), and selective enrichment of either enantiomer is therefore indicative of the occurrence of biodegradation, even in the absence of mass balances.

In addition to enantioselective microbial degradation, the enantioselective uptake of metolachlor by the vegetation cannot be excluded in our study. The preferential absorption of the *RS*-isomer of the insecticide cycloxaprid compared to that of the *SR*-cycloxaprid has been recently shown (Zhang et al. 2013). In addition, varying enantiomeric ratios in plant tissues

were observed for chlordane (White et al., 2002). However, preferential plant uptake of the *S*-metolachlor is expected to be limited in our case since the mass balance clearly indicates that metolachlor was mostly degraded in the aqueous phase of the wetland (Figure V-4).

The results also suggest that enantioselective degradations may affect the environmental fate of metolachlor and should therefore be considered when the environmental behavior of these compounds is assessed. The potential eco-toxicity of the *S*- compared to the *R*-metolachlor is still controversial. While the *R*-metolachlor was shown to be more toxic for various organisms such as earthworms (Xu et al., 2010) or aquatic invertebrates (Liu et al., 2006), *S*-metolachlor had higher adverse effects than racemic metolachlor on crop roots (Liu et al., 2012). This indicates that both enantiomers may be toxic in the environment, depending on the targeted organism.

5. Conclusions

The transport and biodegradation of structurally similar pesticides in the environment are rarely investigated. Our results indicated moderate mass removal of alachlor and acetochlor in lab-scale wetlands, in contrast to lower removal of metolachlor which can be explained by structural differences among the chloroacetanilides. Chloroacetanilides were better degraded in the rhizosphere zone under nitrate reducing conditions.

The occurrence of ESA and OXA degradation products during biodegradation of chloroacetanilide in the wetlands underline the relevance of their monitoring in groundwater quality and ecotoxicology contexts, since they can reach groundwater and persist for long periods. This study also underscores the usefulness of enantiomer analyses of metolachlor as a possible indicator to evaluate anaerobic biodegradation in redox-dynamic environments, such as wetlands. However, in case of small degradation rates, enantiomeric fractionation may be insufficient to evaluate *in-situ* biodegradation. We anticipate our study to be a starting point to couple the quantification of the parent compounds and their degradation products with enantiomer analysis to evaluate and predict the fate of widely used and emerging chiral pesticides in receptor aquatic environments.

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Enantiomeric analyses are relevant to characterize the biodegradation of chiral pesticides. However, stable isotope analyses are powerful techniques, useful to characterize the biodegradation of non-chiral pesticides. In the following section, we tracked chloroacetanilide carbon isotope fractionation as a clue of biodegradation processes.

Section 2. Using compound-specific isotope analysis to assess the degradation of chloroacetanilide herbicides in lab-scale wetlands

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1. Abstract

Compound-specific isotope analysis (CSIA) is a promising tool to study the environmental fate of a wide range of contaminants including pesticides. In this study, a novel CSIA method was developed to analyse the stable carbon isotope signatures of widely used chloroacetanilide herbicides. The developed method was applied in combination with herbicide concentration and hydrochemical analyses to investigate in situ biodegradation of metolachlor, acetochlor and alachlor during their transport in lab-scale wetlands. Two distinct redox zones were identified in the wetlands. Oxic conditions prevailed close to the inlet of the four wetlands (oxygen concentration of 212 ± 24 μM), and anoxic conditions (oxygen concentrations of 28 ± 41 μM) prevailed towards the outlet, where dissipation of herbicides mainly occurred. Removal of acetochlor and alachlor from inlet to outlet of wetlands was 56% and 51%, whereas metolachlor was more persistent (23% of load dissipation). CSIA of chloroacetanilides at the inlet and outlet of the wetlands revealed carbon isotope fractionation of alachlor (ε_{bulk} = -2.0 ± 0.3‰) and acetochlor (ε_{bulk} = -3.4 ± 0.5‰), indicating that biodegradation contributes to the dissipation of both herbicides. This study is a first step towards the application of CSIA to evaluate the transport and degradation of chloroacetanilide herbicides in the environment.

2. Introduction

Chloroacetanilide herbicides are used to control annual grasses and broad-leaved weeds on a variety of crops including maize, sugar beet and sunflower. Metolachlor-S and acetochlor are among the ten most commonly used herbicides in the European Union and the United States (EPA, 2011). Alachlor and racemic metolachlor were commonly used in the 1990s (EPA, 1999), until the introduction of metolachlor enriched in the active S isomer in 1996, and banning the use of alachlor in 2006 in the European Union. The extensive use of chloroacetanilide herbicides is reflected in their frequent detection in ground and surface waters, and the concomitant

detection of their ionic and neutral degradation products (Steele et al., 2008; Hladik et al., 2005). However, the processes governing transport and biodegradation of chloroacetanilides from agricultural land, passing through reactive zones of the landscape, such as wetlands or ground-surface water interfaces, before they reach other ecosystems, remain poorly understood.

Wetland systems can intercept upward flow of pesticide-contaminated water from shallow aquifers during groundwater discharge (Alewell et al., 2008), and influence key ecosystem services, such as water quality improvement. Several biotic (e.g. biotransformation, plant uptake) and abiotic (e.g. adsorption, volatilisation, and photolysis) processes control the transport of organic contaminants in wetlands (Imfeld et al., 2009). Transport of pesticides in wetlands has been mostly evaluated based on assessment of pesticide concentrations and removal efficiencies (Stehle et al., 2011). However, such approach provides no distinction between destructive and non-destructive contaminant attenuation processes, which may lead to inaccurate estimations of the remediation potential of wetland systems. Few studies focused on degradation processes in wetlands through examining the relation between functional genes and observed degradation (Bers et al., 2012), or using microcosms (Runes et al., 2001) and mineralization experiments to assess degradation kinetics and pathways (Gebremariam and Beutel, 2010). While these studies provide information about the potential of pesticide biodegradation in complex environmental systems, they offer no direct evidence of in situ biodegradation processes.

Compound-specific stable isotope analysis (CSIA) provides a valuable tool for the assessment of contaminant transport and fate in the environment (Thullner et al., 2012). CSIA relies on the enrichment of the heavy isotope of an element in the unreacted fraction of a compound during the degradation process. This isotope enrichment occurs due to slight differences in activation energies required to break bonds involving heavy vs. light isotopes (Hofstetter and Berg, 2011). Consequently, the isotopic composition of the contaminants can provide insights about key degradation pathways occurring in situ, and in some cases enables measuring the extent of biodegradation (Elsner, 2010). During the last decade, CSIA has been increasingly applied to study several groups of contaminants, most notably chlorinated ethenes (Imfeld et al., 2008), petroleum hydrocarbons (Richnow et al., 2003), and alkanes (Bouchard et al., 2008). Recently, CSIA methods have been developed for a handful of pesticides: lindane (Badea et al., 2009), isoproturon (Penning et al., 2010), atrazine (Meyer et al., 2008), 2,6-dichlorobenzamide (BAM) (Reinnicke et al., 2012), a metabolite of dichlobenil and phenoxy-acid

herbicides (Maier et al., 2013). However, CSIA methods have not yet been reported for the evaluation of pesticide biodegradation in complex and dynamic environmental systems.

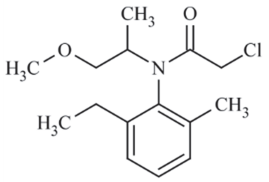
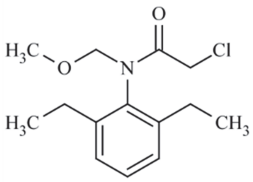
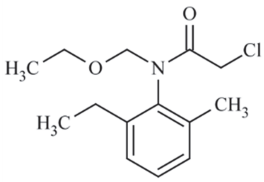
The aim of this study was to explore the applicability of CSIA as a tool to assess the biodegradation of chloroacetanilide herbicides in wetland systems. A gas chromatography combustion isotope ratio mass spectrometry (GC–C–IRMS) method was developed for stable carbon isotope analysis of chloroacetanilide herbicides in environmental aqueous samples. The novel method was applied to assess the in situ biodegradation of metolachlor, acetochlor and alachlor during transport in vertical-flow lab-scale wetlands, designed to study the upward discharge of pesticide-contaminated water into environments at groundwater/surface-water interfaces. In addition, hydrogeochemical development of the systems was monitored to determine prevailing biogeochemical processes.

3. Materials and methods

3.1. Chemicals

Physico-chemical properties of metolachlor, alachlor and acetochlor are listed in Table V-2.

Table V- 2. Physicochemical properties of metolachlor, alachlor and acetochlor.

	Metolachlor	**Alachlor**	**Acetochlor**
Chemical structure			
Molecular formula	$C_{15}H_{22}ClNO_2$	$C_{14}H_{20}ClNO_2$	$C_{14}H_{20}ClNO_2$
Molar mass [g mol^{-1}]	283.8	269.8	269.8
***DT50$_{photolysis}$* [days]**[a]	stable	0.5	stable
***DT50$_{hydrolysis}$* [days]**[a]	stable	0.5	stable
***DT50$_{soil}$* [days]**[a]	90	14	14
***K$_{oc}$* [mL g^{-1}]**[a]	200	124	156
Log *K$_{ow}$*[a]	3.4	3.1	4.1
H$_{cc}$20°C	4.13×10^{-07}	1.31×10^{-06}	8.64×10^{-09}

[a] Source: Pesticide Properties DataBase online (http://sitem.herts.ac.uk/aeru/projects/ppdb/index.htm.).

Chloroacetanilides (metolachlor (racemic), alachlor, acetochlor; Pestanal®, analytical grade purity: 97.2, 96.8 and 99.2 respectively) and solvents (dichloromethane and ethyl acetate; HPLC grade purity >99.9%) were purchased from Sigma–Aldrich (St. Louis, USA). Alachlor-d_{13} and metolachlor-d_6 were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Stock and standard solutions of chloroacetanilides were prepared in dichloromethane and were stored at -20°C.

3.2. Chloroacetanilide extraction from water samples and quantification

Solid-phase extraction (SPE) of 10 mL lab-scale wetland water samples was carried out using SolEx C18 cartridges (Dionex®, CA, USA) packed with 100 mg irregular silica particles. The extraction procedure was adapted from USA EPA method 525.2 using an AutoTrace 280 SPE system (Dionex®, CA, USA). The procedure of chloroacetanilide extraction and quantification is described in Supplementary Material.

3.3. Carbon isotope analysis

The carbon isotope composition of alachlor, acetochlor and metolachlor was analysed using a GC–C–IRMS system consisting of a gas chromatograph (Agilent 6890) coupled via a GC/C III interface to an isotope ratio mass spectrometer (Finnigan MAT 252, Thermo Fisher Scientific). The oxidation furnace of the GC/C III interface containing (Pt, Ni, CuO) was set to a temperature of 980°C. A BPX5 column (60 m × 0.32 mm, 0.5 µm film thickness, SGE, Ringwood, Australia) was used for chromatographic separation, with helium as the carrier gas at a flow rate of 2.0 mL min^{-1}. The column was held at 50 °C for 5 min, heated at a rate of 20 °C min^{-1} to 150 °C, then up to 250 °C at 5 °C min^{-1}, then heated at 20 °C min^{-1} to 300 °C and held for 1 min, and finally heated at 20 °C min^{-1} to 320 °C, where it was held for 5 min. Samples (4 µL volume) were injected into a split/splitless injector operated in splitless mode and held at 280 °C. A chloroacetanilide standard with known isotopic composition was measured every nine injections to control the quality of the measurements.

Reference carbon isotope composition values of standards of alachlor, acetochlor and metolachlor were obtained using an elemental analyser-isotopic ratio mass spectrometer (EA–IRMS, eurovector, Milan, Italy) coupled via a conflo III (open split, Thermo Fisher Scientific,

Bremen, Germany) to a MAT 253 isotope ratio mass spectrometer (Thermo Fisher Scientific). The reproducibility of triplicate measurements was 60.2‰ (1σ). The $\delta^{13}C$ values were calibrated using a two-point calibration against the V-PDB standard.

3.4. Lab-scale wetlands characteristics and set-up

The wetlands consisted of four borosilicate glass columns (inner diameter: 15 cm, height: 65 cm), filled with 5 cm of gravel (Ø 1– 2 mm) and 52 cm of sand (Ø 0.40–0.63 mm) and planted with *Phragmites australis* (Cav.) (Figure V-1). Physical properties and chemical characteristics of the sand and gravel have been described elsewhere (Durst et al., 2013). The wetlands were kept in an air conditioned room at 20 °C ± 0.5 °C, and exposed daily to light from a LED lamp (Greenpower LED lamp, Philips®, Eindhoven, The Netherlands) for 8 h. All tubing and stoppers in contact with the wetlands matrix was made of Viton® (Rotilabo®, Carl-Roth, Karlsruhe, Germany). Five sampling ports were located at 15, 25, 35, 45 and 55 cm from the inlet point in each column. Dissolved oxygen concentrations were monitored using non-invasive oxygen sensors (Presens®, Germany) facing each of the five sampling points that were mounted inside the columns (Figure V-1). Water used to supply the wetlands was taken monthly from a vegetated ditch (Alteckendorf, France) that collects chloroacetanilide contaminated agricultural runoff from sugar beet and corn catchments. Inlet water composition can be found in the Appendices, Table VII-26. The water was continuously pumped to the wetlands inlet at a flow rate of 0.33 mL min^{-1} using a high precision pump (ISMATEC® IPC model ISM936D, Zürich, Switzerland). Nominal residence time was 9.3 d. Lab-scale wetlands were covered with reflective foil to prevent algal growth and photolytic decay of chloroacetanilides.

The lab-scale wetland experiment was carried out over 202 d. The injection of chloroacetanilides in wetlands was preceded by an inoculation period of 104 d, during which the wetlands were supplied with vegetated ditch water without addition of herbicides. Metolachlor, acetochlor and alachlor were dissolved in ultrapure water and stored at -20 °C until they were added to the inlet water. Three columns were supplied each with one of the target herbicides metolachlor, acetochlor and alachlor with an initial concentration of 1.8 µM for metolachlor and 1.9 µM for acetochlor and alachlor. A fourth column was supplied with vegetated ditch water without addition of herbicide. The supplied water was renewed and herbicides were added biweekly, after each sampling campaign. No significant change in chloroacetanilide concentrations was observed in the inlet tanks during the two weeks between the sampling campaigns (nonparametric Wilcoxon test for paired samples, $p > 0.05$).

3.5. Pore water sampling

Sampling campaigns were carried out biweekly at 0, 14, 28, 42, 56, 70, 84 and 98 d, where day 0 represents the first day of chloroacetanilide injection. At each sampling campaign and for each lab-scale wetland, water samples were retrieved with a glass syringe from the wetland inlet, outlet and from the five sampling ports for hydrochemical (30 mL), herbicide concentration and CSIA analyses (30 mL).

3.6. Hydrochemical analysis

Conductivity, pH and dissolved oxygen concentration were directly measured in the wetland inlets and outlets water using WTW multi 350i sensors (WTW, Weilheim, Germany). Concentrations of dissolved organic carbon, major ions, total phosphorous, total sulphur and metals were determined by FR EN ISO standards and laboratory procedures.

3.7. Data analysis

Inlet hydrochemistry, chloroacetanilide concentrations and stable carbon isotope inlet values were calculated as the average values of freshly spiked water and the same inlet water after two weeks, at the time of the sampling. The nonparametric Wilcoxon test for paired samples was applied at a significance level of 0.05 to compare inlet and outlet hydrochemical parameters using the program R (R: Copyright 2005, The R Foundation for Statistical Computing, Version 2.15.1).

Daily chloroacetanilide loadings inflowing and outflowing the wetlands were estimated by interpolating and multiplying chloroacetanilide concentrations between consecutive sampling dates and the corresponding daily flow rate. Loads of chloroacetanilides at a given point between two sampling campaigns was calculated from the integral sum of all the daily load estimates. Mass removal of chloroacetanilides (%) was calculated as the relative decrease of outlet mass ($mass_{out}$) to the inlet mass ($mass_{in}$) for each lab-scale wetland on the same time period.

3.8. Carbon isotope notation and calculation

The carbon isotope ratios were reported in d notation in parts per thousand (‰) relative to the international carbon isotope standard Vienna Pee Dee Belemnite (V-PDB), according to the following equation:

$$\delta\,^{13}C_{sample} = \frac{(R_{sample} - R_{standard})}{R_{standard}} \quad (1)$$

where R_{sample} and $R_{standard}$ are the ratios $^{13}C/^{12}C$ of sample and standard.

Bulk isotope enrichment factors (ε_{bulk}) were calculated from logarithmic linearization of the Rayleigh equation:

$$\ln\left(\frac{\delta^{13}C_x + 1000}{\delta^{13}C_{in} + 1000}\right) = \frac{\varepsilon_{bulk}}{1000} \times \ln\left(\frac{C_x}{C_{in}}\right) \quad (2)$$

where $\delta^{13}C_{in}$ and $\delta^{13}C_x$ are the measured carbon isotope ratios of the substrate at inlet and at a given sampling point, and C_{in} and C_x are the concentrations of the substrate in inlet and at a given sampling point, respectively.

In order to compare observed carbon isotope fractionation with fractionation reported in literature, the apparent kinetic isotope effect (AKIE) values were calculated using Eq. (3), according to Elsner et al. (2005):

$$AKIE \approx 1 = 1 + z \times \left(\frac{n}{x}\right) \times \frac{\varepsilon_{bulk}}{1000} \quad (3)$$

where *n* is the number of atoms of a given element, *x* is the number of indistinguishable reactive positions, and *z* is the number of positions in intramolecular competition.

4. Results and discussion

4.1. CSIA method development

The analytical methods were evaluated for possible isotope artefacts since the chloroacetanilide SPE extraction and concentration procedure, and further isotope composition analysis may alter the measured $\delta^{13}C$ values. The isotopic composition of the three compounds obtained by GC–C–IRMS and EA–IRMS were compared to assess the accuracy of the GC–C–IRMS method. GC–C– IRMS and EA–IRMS methods showed a good agreement indicating a good performance of the GC–IRMS method (Table V-3). The shift in $\delta^{13}C$ values was 60.3 ± 0.3‰ for alachlor and acetochlor and 0.8 ± 0.2‰ for metolachlor, which was considered to be acceptable given the high reproducibility of the results obtained. The independence of $\delta^{13}C$ values of the amount of compound injected in the GC–C–IRMS (i.e. the linear range), was confirmed for a range of signal amplitudes from 120 to 7000 mV, from 100 to 5600 mV and from 150 to 8000 mV for metolachlor, alachlor and acetochlor, respectively (Figure VII-7 in the Appendices). Only signals within this range of amplitudes were used for determination of chloroacetanilide isotope values in lab-scale wetlands.

Table V- 3 Comparison between EA-IRMS, GC-C-IRMS δ13C [‰] (mean ± standard deviation) values vs. VPDB for standards and GC-C-IRMS values for solid phase extracted standards.

	$\delta^{13}C$ [‰]			$\Delta\delta^{13}C$ [‰]	
Compound	**EA-IRMS (n = 3)**	**GC-C-IRMS (n = 15)**	**GC-C-IRMS extracted (n = 9)**	**EA-IRMS vs. GC-C-IRMS**	**extracted vs. non extracted**
Metolachlor	-30.2 ± 0.06	-31.0 ± 0.22	-30.7 ± 0.26	0.8 ± 0.2	0.3 ± 0.3
Alachlor	-33.8 ± 0.05	-33.9 ± 0.19	-33.9 ± 0.33	0.1 ± 0.2	0.2 ± 0.2
Acetochlor	-29.8 ± 0.21	-29.5 ± 0.34	-30.1 ± 0.16	0.3 ± 0.3	0.6 ± 0.2

Finally, the effect of the SPE extraction procedure on measured $\delta^{13}C$ values was evaluated. For this purpose, standards of each of metolachlor, alachlor and acetochlor were spiked into vegetated ditch water (i.e. water supplied to the lab-scale wetlands) to give concentrations in the range of 0.9–18.5 µM. The prepared spiked water was extracted using the

same SPE procedure as that used for the lab-scale wetlands samples. Differences between average $\delta^{13}C$ values measured for standards extracted from lab-scale wetlands water and averages of non-extracted standards were 60.6 ± 0.2‰ (n = 9) (Table V-3). This indicates the absence of significant fractionation effects for the SPE method used in this study, and thus the suitability of the method for CSIA of chloroacetanilides in lab-scale wetlands samples.

4.2. Lab-scale wetlands experiment

4.2.1 Transport and attenuation of chloroacetanilide herbicides

Hydrochemical parameters were monitored to evaluate the evolution of prevailing hydrogeochemical conditions in the wetlands throughout the experiment (Table VII-26 in the Appendices). pH values ranged from 7.3 to 8.3 for the four wetlands, and did not significantly change between inlet and outlet (p > 0.05). A spatial gradient of oxygen concentrations was observed in the four lab-scale wetlands with oxic conditions (212 ± 24 µM) prevailing at the bottom of the wetlands between 15 and 25 cm, and anoxic conditions between 45 and 55 cm from inlet points. Oxygen concentrations at the top of the wetlands were lower for acetochlor and alachlor wetlands (2 ± 10 µM) than for metolachlor and control wetlands (52 ± 44 µM). Nitrate inlet concentration averaged 484 ± 38 µM and decreased significantly inside the four wetlands (p < 0.05). Nitrate dissipation observed in the four wetlands indicates that nitrate reducing conditions prevailed in anoxic zones towards wetland outlet. Higher nitrate dissipation occurred in wetlands supplied with acetochlor and alachlor (78% and 73% decrease in average NO_3^- concentrations respectively) compared to metolachlor and control wetlands (47% and 25% respectively), following the same trend observed for oxygen profiles. Sulphate concentrations did not change significantly between the inlet and outlet (537 ± 24 µM) throughout the experiment except for a small (9%) but significant (p = 0.02) decrease in the outlet of the wetland supplied with acetochlor, which indicates the prevalence of stronger reducing conditions in this wetland. Other redox sensitive species, such as manganese and ferrous iron, remained low (<4 µM) over the course of the experiment, and are thus expected to play only a minor role in biodegradation processes in the systems.

The dissipation of chloroacetanilide herbicides in the wetlands was evaluated using a mass-balance approach. Removal of acetochlor and alachlor loads from inlet to outlet from day 24 to day 98 averaged 56 ± 6% and 53 ± 11% respectively, whereas metolachlor was more persistent with an average mass removal of 23 ± 5% (Table VII-27 in the Appendices). Higher

removal rates (62–90% for the three herbicides) observed during the early stages of the experiment gradually decreased and stabilised in the period between day 14 to day 28. This could be explained by chloroacetanilide sorption to sand, glass or plant roots in the wetlands that occurred in the initial phase of chloroacetanilide injection. During this initial phase of the experiment it is likely that equilibrium was established between sorbed and dissolved chloroacetanilide, therefore, effects of sorption on chloroacetanilide removal at later stages of the experiment are expected to be minimal. In a previous study, more than 95% of sorption of metolachlor, alachlor and acetochlor on soil was found to occur within 4 h (Liu et al., 2000).

Other abiotic processes alone are unlikely to cause the observed removal of acetochlor and alachlor. Photolysis was avoided by the reflective foil used to protect the systems from light. Volatilization of chloroacetanilides from the water phase is unlikely since their volatilization rate is very low at 20 °C (see Table V-2). Although biotic removal through plant uptake cannot be excluded, chloroacetanilides have relatively high octanol/water coefficient (Log K_{ow} 3.1–4.1) and are thus expected to be retained by the lipids in root epidermis hindering further uptake by plant cells (Trapp, 1995). In a previous study, metolachlor found in plant samples accounted for maximally 10% of the total measured metolachlor mass in constructed wetlands (Moore et al., 2001). Therefore, microbial degradation is considered to be the main process affecting chloroacetanilide herbicides attenuation in the lab-scale wetlands, as previously suggested in field experiments (Stamper and Tuovinen, 1998; Fenner et al., 2013).

Degradation of chloroacetanilides was previously observed under both aerobic and anaerobic conditions (Graham et al., 2000). In our study, attenuation of chloroacetanilides occurred essentially in oxygen depleted zones in the upper parts of the wetlands (Figure VII-8 in the Appendices), suggesting enhanced degradation of chloroacetanilides under anoxic conditions. Biodegradation in the oxygen depleted zones in the upper part of the wetlands may be associated with the root zones where enhanced microbial activity and density is expected (Imfeld et al., 2009), as previously demonstrated for metolachlor (Anderson et al., 1994). Enhanced degradation of chloroacetanilides in predominantly anoxic zones comes in accordance with previous investigation in a mesocosm scale field experiment, where removal of alachlor was larger under anaerobic conditions than under aerobic conditions (Graham et al., 2000). It should be noted that oxygen measurements were taken along the periphery of the column, and thus did not account for micro-redox gradients which could be present in the lab-scale wetlands (Alewell et al., 2008), therefore aerobic degradation may also have contributed to chloroacetanilide removal in the wetlands.

The persistence of metolachlor in comparison to other chloroacetanilides observed in our experiment was previously demonstrated in anoxic soil (Konopka, 1994), in oxic water (Graham et al., 1999), and in aerobic microbial cultures (Zhang et al., 2011). The persistence of metolachlor in the wetlands can be explained by the substitution of an amide alkoxymethyl side chain, present in alachlor and acetochlor with a bulkier alkoxyethyl side chain in metolachlor, which may lead to greater steric hindrance around the carbon chlorine bond and lower degradation rates (Zhang et al., 2011).

In order to evaluate the occurrence of in situ degradation in the wetlands and to obtain insights into mechanisms involved in the initial transformation step of chloroacetanilides, CSIA was applied to analyse the isotope composition of chloroacetanilides in labscale wetland samples.

4.2.2 Chloroacetanilide carbon isotope fractionation

The developed CSIA method was used to evaluate in situ biodegradation of chloroacetanilides in the lab-scale wetlands. Samples from inlets, outlets and two points inside the wetlands at 25 and 55 cm from the inlet of the wetlands were collected for carbon isotope analysis at days 42, 70 and 98 (Figure V-5). CSIA of chloroacetanilides at the wetland inlet and outlet revealed similar average carbon isotope enrichment for alachlor ($\Delta\delta^{13}C$ of 2.5 ± 0.33‰) and acetochlor ($\Delta\delta^{13}C$ of 2.6 ± 0.02‰). In contrast, enrichment in carbon isotope composition of metolachlor from the wetland inlet to the outlet was small ($\Delta\delta^{13}C \leq 0.7$‰). Isotope composition values at 25 and 55 cm from the inlet indicate that carbon isotope fractionation occurred in anoxic zones towards the outlet of the wetlands and correlated with the mass dissipation of the herbicides (Figure V-5). The correlation between changes in the carbon isotope composition of chloroacetanilide and their mass removal confirms the occurrence of biodegradation in the wetlands.

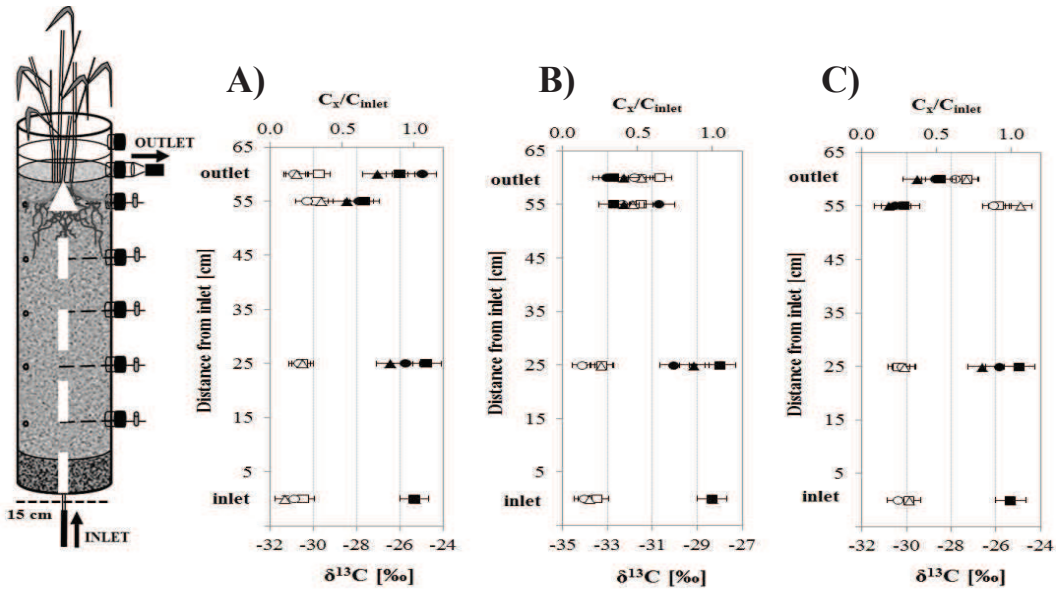


Figure V- 6 Carbon isotope composition represented in $\delta^{13}C$ notation (open symbols) and ratio of concentration at a given point (x) to inlet concentration (filled symbols) of metolachlor A) alachlor B) and acetochlor C) inside lab-scale wetlands on day 42 (squares), day 70 (circles) and day 98 (triangles). Arrows indicate the direction of water flow. Error bars indicate uncertainty of $\delta^{13}C$ (± 0.5‰), and propagated error of C_x/C_{inlet} based on standard deviations of concentrations.

Estimates of enrichment factors for alachlor and acetochlor were calculated according to the Rayleigh model (Eq. (2)) for closed systems taking inlet values as initial values of $d^{13}C$. Enrichment factors were not calculated for metolachlor because variations in carbon isotopic composition of metolachlor were too small (60.8‰) to be considered significant given the uncertainty of the method. Separate enrichment factors (ε_{bulk}) were calculated by linear regression for days 42, 70 and 98 to examine the evolution of prevailing degradation pathways through time. Enrichment factors ranged from -1.5 ± 0.9‰ to -2.1 ± 0.4‰ for alachlor and -3.2 ± 1.2‰ to -3.6 ± 1.1‰ for acetochlor and were not significantly different taking into account the uncertainty of the calculations (Table V-4). Average enrichment factors calculated using all points from the three sampling dates were ε_{bulk} = -2.0 ± 0.3‰ for alachlor and ε_{bulk} =-3.4 ± 0.5‰ for acetochlor (Figure VII-9 in the Appendices). A potential source of bias contributing to the observed fractionation is the possibility of preferential flow paths in the wetlands. This phenomenon might have led to the mixing of water containing chloroacetanilides coming from different flow paths with different travel times and extent of chloroacetanilide degradation upon reaching of the outlet (Thullner et al., 2012). This mixing effect is evident in the wetland supplied with acetochlor (Figure V-5C), where concentrations were higher at the outlet than at the sampling port at 55 cm. However, this effect did not change significantly the enrichment

factor calculated for acetochlor (ε_{bulk} = -3.2 ± 0.2‰ without the outlet value, and -3.4 ± 0.5‰ including the outlet value).

Table V- 4 Bulk enrichment factors and AKIE values calculated for alachlor and acetochlor for sampling campaigns at days 42, 70 and 98 and for data from the three campaigns combined. For AKIE calculations n = 14, z = 1 and x = 1.

	ε_{bulk}[a] [‰]		AKIE[b]	
day	**Alachlor**	**Acetochlor**	**Alachlor**	**Acetochlor**
42	-2.1 ± 0.4	-3.5 ± 0.4	1.031 ± 0.006	1.052 ± 0.006
70	-1.5 ± 0.9	-3.2 ± 1.2	1.022 ± 0.013	1.046 ± 0.018
98	-2.1 ± 0.2	-3.6 ± 1.1	1.030 ± 0.003	1.053 ± 0.017
all	-2.0 ± 0.3	- 3.4 ± 0.5	1.028 ± 0.004	1.051 ± 0.007

[a] Standard errors for ε_{bulk} were calculated *via* linear regression analysis.
[b] Errors for AKIE values were calculated *via* error propagation based on ± 1 standard deviation of the ε_{bulk} values.

Since this is the first report for chloroacetanilide isotope fractionation, no values of enrichment factors are available for comparison. In order to further interpret the observed fractionation and provide indication of prevailing degradation mechanisms, we calculated AKIE values for alachlor and acetochlor degradation in lab-scale wetlands. AKIE values have the advantage of being associated with isotope effect of the specific bond cleavage, and therefore can be used for comparison of isotope fractionation between different compounds. Different degradation pathways for chloroacetanilide herbicides have been reported including thiolytic (glutathione-dependent) dechlorination (Graham et al., 1999), hydrolytic dechlorination, N-atom dealkylation (Sanyal and Kulshrestha, 2002) and reductive dechlorination (Helbling et al., 2010). Glutathione-dependant dechlorination and N-atom dealkylation have been mainly observed in aerobic systems. No studies were found in the literature on chloroacetanilide degradation pathways under anaerobic conditions.

For AKIE calculations, the number of carbon atoms the in reactive position does not vary for different possible reactions, where in all cases *x* and *z* are equal to one. AKIE values ranged from 1.022 to 1.031 for alachlor, and from 1.046 to 1.053 for acetochlor (Table V-4). Acetochlor AKIE values fit in the range of experimentally derived AKIEs from the literature for S_N2 type nucleophilic substitution reactions involving C–N, C–Cl or C–O bonds (1.03–1.09), whereas they are higher than typical reported values of SN1 type substitution reactions (1.00–1.03), reductive

dechlorination (1.027–1.033), and oxidative N-dealkylation reactions (up to 1.019) (Skarpeli-Liati et al., 2012), suggesting hydrolytic or thiolytic S_N2 type nucleophilic substitution to be the first degradation step for acetochlor in our systems. On the other hand, alachlor AKIE values overlap with reported ranges for both nucleophilic substitution and reductive dechlorination reactions. It should be noted that other rate limiting steps preceding the initial degradation step such as transport across cell membrane and substrate binding to enzyme reactive site could lower observed fractionation thereby leading to lower AKIEs (Nijenhuis et al., 2005).

Additional insights on mechanisms of bond cleavage could be obtained using multi-element CSIA of nitrogen, hydrogen or chlorine (Vogt et al., 2008). Unfortunately, the utility of nitrogen and hydrogen isotope analysis is reduced by current detection limits of CSIA. Given the small contribution of nitrogen and hydrogen to the molar mass of a chloroacetanilide molecule and the amount required of each element for analysis (30 and 42 ng on column for hydrogen and nitrogen, respectively), inlet concentrations in the range of 50 to 100 µM would have been needed for CSIA in our experiment. This limitation currently reduces the potential of multi-element CSIA analyses of pesticides in the range of environmental concentrations. Chlorine isotope analysis, on the other hand, would require concentrations in the same range as carbon isotope analysis, but chlorine isotopic analytical methods still need to be developed (Elsner et al., 2012). In addition to CSIA, degradation product analysis can provide important complementary information to help unravel degradation pathways. In the case of chloroacetanilide herbicides, this is not a straightforward task due to the large number of potential degradation products, and the lack of information on degradation pathways. Recent developments in high resolution mass spectrometry methods may help develop such investigations in the near future (Fenner et al., 2013).

Our results highlight the applicability of CSIA to study the fate of relatively large organic compounds (containing 14–15 carbon atoms) in the environment. Based on calculated average enrichment factors, we expect differences in carbon isotopic composition ($\Delta\delta^{13}C$) of 1.4‰ and 2.4‰ for alachlor and acetochlor respectively at 50% degradation, and 4.6‰ and 7.8‰ respectively at 90% degradation. Given the fact that such differences are detectable within current analytical limitations, the suitability of CSIA to study the fate of larger organic contaminants in the environment is demonstrated. This encourages further development of such methods for a large variety of micropollutants, and most notably pharmaceuticals and pesticides.

5. Conclusion

CSIA is an emerging tool in the study of pesticides transport and transformation in dynamic and complex ecosystems such as wetlands. In this study, moderate mass removal for acetochlor and alachlor of 56% and 51% respectively was observed under denitrifying conditions, in contrast to lower removal for metolachlor of 23%. With a novel CSIA method, a pronounced carbon stable isotope fractionation was measured, indicating in situ biodegradation of alachlor (ε_{bulk} = -2.0 ± 0.3) and acetochlor (ε_{bulk} = -3.4 ± 0.5) in the lab-scale wetlands. Our results demonstrate the potential of CSIA, combined with traditional approaches, in evaluating the transport of chloroacetanilide herbicides in biogeochemically dynamic environments such as wetlands. Further laboratory investigations aiming at providing reference enrichment factors and analysis of degradation products under different hydrogeochemical conditions are needed to allow the evaluation of chloroacetanilide transport and degradation in the environment.

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Chapter VI. General conclusions and perspectives

1. Summary and conclusions

The functioning of surface and subsurface ecosystems, such as soils, wetlands, and aquifers is intimately linked to the earth's biogeochemical cycles and human activities. Understanding the environmental transport and transformation of pollutants from toxic and diffuse sources represents a major challenge in the 21st century, and have become of utmost importance for the preservation of soil and water resources. A significant fraction of industrial or agricultural pollutants used in urban or agricultural areas can be mobilised from their sources and transported overland *via* runoff in freely dissolved form, and in less bioavailable colloidal or organic matter-bound forms. Pollutants may also infiltrate into soil and sediments, and eventually reach the aquifer. Surface and sub-surface pollutant fluxes may be intercepted and transformed in transitional reactive zones of the landscape, such as wetlands or ground-surface water interfaces, before they reach other ecosystems. These reactive zones of the landscape are 'biogeochemical hotspots' that bear dynamic interfaces between water, soil/sediment and microorganisms, and develop over space and time combinations of electron acceptors and donors reflecting microbial diversity and biogeochemical activity (Lee et al., 2009; McClain et al., 2003). These special features confer to wetlands the status of 'bioreactors' that sustain their service functions, including the improvement of water quality and make them being privileged ecosystems to thoroughly study the transport and degradation of pollutants.

In this context, the fundamental questions that bother me is: what is the hydrological and biogeochemical functioning of these 'hotspots' with respect to pollutant transport and transformation? The transport and biotransformation of widely used and emerging pollutants in biogeochemical hotspots remains poorly understood: despite significant research effort has been dedicated to characterize the transport and degradation of pesticides in the environment, it still remains difficult to predict pesticide mobility and transformation under specific field conditions, from source to sink ecosystems (Bloomfield et al., 2006; Fenner et al., 2013). To tackle this issue, I have combined in my PhD thesis complementary analytical approaches and embedded scales to evaluate the reactive transport of pesticides in wetland systems, used as

'laboratories' for environmental reactions affecting the fate of organic pollutants in the environment. The following pages include a short summary of my thesis and an overview of some of short- and longer-term research perspectives.

The specific aim of my thesis was to gain knowledge on underlying mechanisms governing pesticide transport and degradation, using wetlands as model 'natural laboratories' of redox-dynamic ecosystems. Distinguishing the different ongoing dissipation processes in wetlands remains complex due to embedded processes and requires benchmark studies at smaller scale. Therefore, a multi-scale approach has been applied in this work in order to gain mechanistic knowledge at small-scale (simplified systems), enabling a better understanding of larger scale observations. For this purpose, the investigation of three wetland scales, i.e. a stormwater wetland (320 m^2), two subsurface flow constructed wetlands (7 m^2) and four lab-scale wetlands (0.02 m^2) provided valuable information on retention and degradation processes of a large range of pesticides using several complementary analytical techniques.

My results demonstrate that stormwater wetlands are relevant for dissipating dissolved and solid-bound pesticides (Chapter III, section 1, 2 and 3). However, the large variability of dissipation rates was attributed to fluctuations in the runoff regime (Chapter III, section 1), the intrinsic physico-chemical characteristics of pesticide molecules (Chapter III, sections 1 and 3), and the seasonal changes (Chapter III, section 1, 2 and 3). Fluctuation in the hydrological conditions resulted in drastic changes of redox conditions, which in turn impacted the dissipation of individual pesticides in the wetlands. The development of the wetland vegetation was shown to significantly contribute to pesticide dissipation by promoting pesticide biodegradation in the rhizosphere during the vegetative phase (Chapter III, sections 2 and 3). However, the wetland vegetation also contributed to pesticide release during plants senescence (Chapter III, section 2).

Degradation was shown to be the major dissipation process in the studied stormwater wetland and was largely correlated to the vegetation cycle (Chapter III, sections 2 and 3). Compared to degradation, pesticide storage in the wetland compartments accounted for a smaller part of the mass budget and was mainly observed in early spring and late summer. Fine bed sediments < 250 µm and plant roots were the most relevant pools of pesticides. Seasonal changes (Chapter III) and hydrological conditions (Chapter III, Chapter IV) were shown to significantly affect the dissipation of pesticides in wetlands, by primarily affecting the redox conditions in such systems. Redox conditions were found in the different studies as the major

factor that influenced pesticide degradation in wetlands (Chapter IV and chapter V, sections 1 and 2). Depending on the prevailing redox conditions, degradation pathways can largely vary, leading to the occurrence of different degradation products and possibly different prevailing degradation pathways (Chapter IV).

Enantiomer and compound-specific isotope analyses were shown to be valuable and complementary tools to assess biodegradation of chiral pesticides. This combined analytical approach, used for the first time in the context of wetlands treating organic pollutants, revealed that the slight variability in the spatial configuration of chloroacetanilide herbicides significantly affect their degradation in wetlands, the molecules with larger steric hindrance being less degraded than similar molecules from the same family (Chapter V, sections 1 and 2). It is expected that similar conclusions can be drawn for other classes of pesticides and organic micropollutants sharing a common chemical structure but differing with respect to their functional groups.

The major implications of this work are discussed in detail in the following sections.

1.1. Retention *versus* Degradation of pesticides in wetland systems

Pesticide dissipation in wetland systems involves both degradative processes (i.e. photodegradation, hydrolysis, biodegradation) and transport processes (i.e. volatilization, retention by sorption processes). Although several studies have demonstrated the efficiency of wetland systems to dissipate runoff-related pesticides (Blankenberg et al., 2006; Lizotte et al., 2009; Moore et al., 2002; Schulz et al., 2003), the distinction between retention and degradation processes in wetlands is often lacking. In the present work, special attention has been paid to quantify the retention and the degradation processes based on pesticide mass budgets and combining novel analytical approaches for tracking *in-situ* biodegradation in redox-dynamic environments.

The retention of pesticides in the wetlands was mainly found to be attributed to the settling of pesticide-laden TSS which contributed to the accumulation of hydrophobic pesticides in the wetland sediments (Chapter III, sections 1, 2 and 3) and to pesticide sorption on plant material (Chapter III, section 2 and Chapter IV). The retention of suspended solids by the

stormwater wetland was 88% in 2009 (Chapter III, section 1) whereas 98% of the solid-bound pesticide load was retained in 2011 (Chapter III, section 2). The increasing retention of solid-bound pesticides over time can be attributed to the development of the vegetation in the stormwater wetland forebay which act as a physical barrier and enhances solids settling (Kröger et al., 2009; Rose et al., 2008). Dissipation of solid-bound pesticides ranged from 75% for pyrimethanil to 99.8% for dithiocarbamates. While the high retention of compounds such as fludioxonil, cyprodinil and difenoconazole was mainly attributed to their hydrophobicity (log K_{ow} > 4), molecules like dithiocarbamates were less expected to be exclusively found associated to total suspended solids (log K_{ow} = 1.33). However, these compounds were found to be prone to form complexes with metals such as Cu(II) and Fe(II), favoring their sorption on metal hydroxides associated with clay minerals (Weissmahr and Sedlak, 2000) (Chapter III, section 2). At the landscape scale, the efficiency of wetlands for trapping solid-bound pesticides also represents an opportunity to temporally retain pesticides fluxes and pesticide degradation in the wetland bed sediments.

Dissolved pesticides accounted for more than 95% of the total pesticide load entering the wetland during the agricultural period and were especially found in the < 0.22 µm fraction, suggesting their possible sorption on dissolved organic carbon. The stormwater wetland efficiently removed both dissolved (72.8% dissipation in 2009 and 96.2% in 2011) and solid-bound pesticides, although pesticide loads were predominantly transported in the dissolved phase. Retention processes *via* sorption on the wetland compartments is expected to be a major dissipation process in wetlands, especially for hydrophobic molecules (Budd et al., 2011). The mass budgets carried out in 2011 in the studied stormwater wetland (Chapter III, section 2) showed that fine bed sediments (50-250 µm) and plant roots were major wetland compartments involved in the retention of glyphosate, AMPA and dithiocarbamates. Retention processes were especially observed in early spring, resulting from an accumulation of pesticides since the previous agricultural season and in late summer. The accumulation of AMPA in fine sediments in late summer raises critical issues concerning ecotoxicological risk and sediment management in wetlands, especially for emerging and poorly described degradation products.

Although pesticide uptake by plants cannot be excluded, wetland vegetation might especially sustain pesticides degradation by the rhizosphere microorganisms. The same observations were made in the subsurface flow constructed wetlands, in which the amount of *S*-metolachlor found in the sand and the vegetation represented less than 6% of the total mass injected during the investigation period (Chapter IV). Chloroacetanilide herbicides were

detected in the sand matrixes of the lab-scale wetlands (Chapter V, section 1), but could not be quantified. However, plant uptake and translocation to the aerial parts of the plant was observed with a concentration that reached 90.2 µg L^{-1} in the plant tissue. While no massive pesticide accumulation occurred in bed sediments and plant materials in the present work, this raises however concerns for the long-term exposure of wetland systems, both constructed and natural, to pollutant sources (Huerta Buitrago et al., 2013). From a management viewpoint, the assessment of the environmental hazard and ecotoxicological risk associated to the contamination of bed sediments and wetland vegetation is fundamental to anticipate pollutants release by the wetlands and to carefully plan yearly or seasonal sediment excavation and/or plant cutting.

Pesticide degradation was shown to be the main dissipation process in the three experimental setups of wetlands presented in this thesis (Chapter III, sections 1, 2 and 3; Chapter IV; Chapter V, sections 1 and 2). Pesticide degradation was mainly linked to the development of vegetation in the wetland, which support the development of microbial biomass and the major part of the biogeochemical reactions. Degradation processes were evaluated using different approaches including the calculation of mass budgets (Chapter III, sections 1, 2, 3), the analysis of degradation products (Chapter III, section 3; Chapter IV, Chapter V, section 1), and combined enantiomer and isotope analyses (Chapter IV, Chapter V, sections 1 and 2).

The stormwater wetland was shown to efficiently dissipate pesticides through degradation processes estimated based on a detailed mass budget encompassing the main wetland compartments (Chapter III, section 3). The analysis of pesticide degradation products constitutes a direct approach to investigate degradation processes (Boxall et al., 2004; Fenner et al., 2013). The combined analysis of glyphosate and its main degradation product AMPA showed that the studied stormwater wetland efficiently degraded glyphosate during three consecutive agricultural periods i.e. in 2009, 2010 and 2011 (Chapter III, section 2). Glyphosate load dissipation increased from 75 to 99% between 2009 and 2011, along with the development of the vegetation. However, hydrological conditions and patterns were the main drivers that controlled the extent of biodegradation in this study. Longer hydraulic residence times may favour degradation processes in wetlands (Kröger et al., 2009) with proportion of AMPA related to glyphosate varying from 0 to 100%, depending on hydrological conditions. Similarly, hydrological conditions were shown to be key parameters controlling *S*-metolachlor degradation in subsurface flow constructed wetlands (Chapter IV). Metolachlor ethanesulfonic acid (ESA) prevailed in the continuous flow wetland, whereas metolachlor oxanilic acid (OXA) prevailed in the batch flow system, indicating distinct degradation pathways in each wetland, influenced by

redox conditions (anoxic conditions in the continuous flow wetland and alternation of oxic and anoxic conditions in the batch flow wetland) (Chapter IV).

The dissipation rates of *S*-metolachlor was higher (> 93%) in the batch flow system, which was further confirmed by CSIA, underlining the occurrence of *in-situ* biodegradation of *S*-metolachlor under oxic conditions ($\Delta\delta^{13}C_{inlet\text{-}outlet}$ = 1.2‰). However, no significant enantiomer fractionation of *S*-metolachlor occurred in the wetlands, suggesting the absence of enantioselective biodegradation (Chapter IV). Mass dissipation of chloroacetanilides averaged 61 ± 14%, 52 ± 12% and 29 ± 19% for acetochlor, alachlor and metolachlor, respectively, and mainly occurred in the rhizospheric zone (Chapter V, section 1). Degradation of the chloroacetanilide herbicides into ethane sulfonic acid (ESA) and oxanilic acid (OXA) prevailed in the rhizospheric zone after day 70. Enantiomer fractions (EF) for metolachlor were significantly different in the rhizospheric zone (0.480 ± 0.005) compared to the oxic zone (0.494 ± 0.006), indicating enantioselective biodegradation with a preferential degradation of the *S*-enantiomer.

Overall, the present work enabled to distinguish retention from degradation processes in the three investigated systems, using different approaches and tools. In particular, biodegradation was the most significant degradation processes in the studied wetland systems. The hydrological conditions directly impacted the biogeochemistry of the wetlands, which in turn controlled the fate of pesticides in all the experimental scales investigated here.

1.2. Relevance of a multi-scale approach to investigate the fate of pesticides in wetlands

Wetlands are complex and dynamic ecosystems characterized by important spatial and temporal variability of their hydrological, biota and biogeochemical conditions (e.g. redox conditions) (Brinson, 1993). Wetlands complexity is an important asset as the co-existence of micro-environments sustains the service functions of wetland ecosystems, including water quality improvement. However, in the same time, this constitutes a major issue to the understanding of the underlying mechanisms that regulate wetlands ecosystem services (Viglizzo et al., 2004). When receiving contaminants such as pesticides, the ecosystem responses and contaminants behavior are expected to depend on both the micro- and macro-scale variations of the environmental conditions. Understanding pesticides fate in wetlands thus remains closely linked to scales considerations and complementary informations can be

retrieved from investigations involving multiple scales. In this thesis, a downscaling approach was applied to gain mechanistic knowledge on the underlying chemical and molecular processes that regulate pesticide dissipation in wetland systems.

To understand interactions between the different levels and scales, the analogy can be done with the widely used biological concept of bottom-up and top-down regulations. The bottom-up approach focuses on the individual elements (e.g. microbial or chemical reactions) and their interactions to investigate the system's behavior, whereas the top-down approach considers the system as a whole, holistically, and uses macroscopic behavior of the system as variables to understand the system dynamics at the macroscopic scale, largely based on experimental observations. In wetlands, interactions can occur between scales, forming a complex system with multiple spatial and temporal scales and feedback loops, which becomes extremely difficult for interpreting experimental results. Wetlands functioning is regulated at scales of many orders of magnitude in space and time, with space spanning from the molecular scale (10^{-10} m) to the ecosystem scale (10^{4} m^2), and time from nanoseconds (10^{-9} s) to years (10^{8} s) (Qu et al., 2011). Reactions at molecular scale, such as microbial processes or chemical transformation processes occur at short time scales, while wetland scale responses and their dynamics are observed at longer time scale variations (Qu et al., 2011).

Several large-scale investigations have demonstrated that wetland systems can efficiently dissipate runoff-related pesticides under different environmental conditions (Blankenberg et al., 2006; Lizotte et al., 2009; Moore et al., 2002; Schulz et al., 2003). The large-scale studies carried out in this thesis in a stormwater wetland (Chapter III) focused on the pesticide dissipation dynamics (section 1 and section 3) related to the sink and the source properties of wetlands receiving contaminated runoff (section 2). We have shown that the stormwater wetland efficiently removed both solid-bound and dissolved pesticides over the three consecutive years of investigation and the increasing dissipation over time was directly related to the development of the vegetation cover in the wetland. The relatively high-resolution and simultaneous sampling of all environmental compartments enabled to identify the major pools of stored pesticides in the wetland (section 2) and the sink-source properties of the wetland over time, with respect to the hydrological and biogeochemical conditions. Fine bed sediments and plant roots were relevant storage compartments for pesticides (especially AMPA) in spring and late summer, indicating that a potential pesticide release can occur during these periods.

The sink function of wetlands regarding pollutants is a long-standing ecological statement that has been explored in many studies, whereas wetlands acting as pollutant sources constitute a more recent concept. The sink-source dynamic functioning of wetlands with respect to pesticides appears crucial to understand pesticide retention and degradation processes. In this study, we showed that wetland systems mainly act as pesticide sinks from spring to summer. Pesticide retention and degradation were closely linked to the vegetation life cycle, and the distribution dynamics of pesticides among wetland compartments were intimately linked to the wetland development. Large-scale experiments were shown to be valuable when information on ecological patterns and overall functioning of wetlands with respect to pesticides are sought. In particular, this work constituted a first attempt to quantify pesticide pools in wetland compartments under field conditions in order to distinguish storage from degradation in redox-dynamic environments, such as wetlands (section 2). Even though large-scale experiments enable to distinguish retention from degradation processes when coupled to a high-resolution sampling, the f molecular processes that govern pesticide transport and degradation cannot be distinguished at this scale. Therefore, more 'reductionistic' approaches conducted at meso- or micro-scales are required to gain more mechanistic knowledge on the intrinsic processes that control pesticide fate in wetlands (Li et al., 2004).

Meso- and micro-scale wetland studies enable generally shorter investigation periods than large-scale investigations. The use of mesocosms in wetland science has been common over the last decades, in particular to study the fate of contaminants or biogeochemical cycles (Ahn and Mitsch, 2002). Model systems such as microcosms or mesocosms have useful features i.e. tractability, generality and realism (Srivastava et al., 2004). One of the major challenges is to perform small-scale experiments that are reproducible and which are not completely artificial (that can be representative of a natural system). For instance, classical tests that are carried out by providers before pesticide commercialization (e.g. photodegradation tests using xenon lamps) are usually unsuitable to really predict the fate of these compounds in more complex field conditions, when hydro-biogeochemical dynamic patternss intervene. In this thesis, a mesocosm experiment was carried out to decipher the influence of hydrological and redox conditions on the transport and degradation of a model chiral pesticide, i.e. *S*-metolachlor (Chapter IV).

In the mesocom study, *S*-metolachlor was preferentially degraded under both suboxic to anoxic conditions, leading to the preferential formation of the OXA degradation product in

suboxic conditions and the preferential formation of ESA in anoxic conditions. This mesocosm-scale experiment enabled to understand the influence of redox conditions on the degradation of *S*-metolachlor, showing that metolachlor ESA and OXA were also released in suboxic to anoxic conditions and not only under oxic conditions. A complementary mesocosm experiment was carried out in lab-scale wetlands to compare the transport and degradation of three chloroacetanilide herbicides i.e. acetochlor, alachlor and metolachlor (Chapter V). In the later study, the degradation of the three compounds was shown to occur in the rhizospheric zone, under nitrate reducing conditions. However, compared to acetochlor and alachlor, metolachlor was more persistent, underscoring that the spatial chemical structure of pesticides is essential for controlling their biodegradability in the environment. In fact, molecules that possess greater steric hindrances during the enzyme-molecule interactions may be less degraded. Findings that arose from these two experiments could probably not be concluded from actual size wetlands, due to their heterogeneity. A further step will be to go back to the field and try to link laboratory and field observations, for example by investigating preferential degradation pathways that occur in the field according to hydro-biogeochemical patterns.

Overall, this thesis also illustrates that scaling is fundamental for ecological investigations (Haila, 2002), and is essential to understand the transport and transformation of pesticides and other micro-pollutants, in particular for developing reactive transport models involving key elemental processes of pesticide fate and applying them in the complex field conditions. However, a detailed evaluation of pesticide reactive transport at the different scales implies the use of a comprehensive approach, including several complementary analytical approaches.

1.3. Combining approaches for investigating the fate of micropollutants in wetland systems: enantiomer and stable isotope analyses

The investigation of pesticide fate in wetlands requires the combination of several disciplines including hydrology, biology, ecology, chemistry, physics and microbiology to assess i) the influence of the different environmental conditions, ii) the interactions between pesticide molecules and the wetland compartments (i.e. the water, sediments, vegetation and algae, (micro-)organisms), and iii) the abiotic and biotic processes that govern pesticide transformation. The development of an approach relying on different but complementary

methods is necessary to have a holistic and integrative understanding of environmental factors governing the transport and degradation of contaminants in wetlands. In this thesis, hydrochemical analyses, quantification of the parent compounds and of the degradation products as well as enantiomer and compound-specific isotope analyses were combined to quantify and characterize degradation processes.

Hydrochemical analyses provide valuable insights on the prevailing redox conditions in wetland systems. Fluctuations in the water table and the development of biological activity can generate steep temporal gradients of oxidation-reduction conditions in terrestrial/aquatic interfaces (Alewell et al., 2008; Haberer et al., 2012; Hernandez and Mitsch, 2007; Rezanezhad et al., 2014). Gradients of redox conditions directly promote pathways and rates of microbially-mediated biogeochemical cycles and contaminant degradation by controlling electron donors and acceptors concentrations and availability (Borch et al., 2010; Burgin et al., 2011). Even though the analysis of hydrochemical parameters can be applied to both large and small scales, better insights can result from smaller size experiments due to reduced heterogeneity.

In this thesis, the biodegradation of chloroacetanilide herbicides was shown to occur under preferential nitrate reducing conditions, as inferred by the mass budget of the parent compounds and CSIA (Chapter V). Enhanced degradation of chloroacetanilides in predominantly anoxic zones comes in accordance with previous investigation in a mesocosm scale field experiment, where removal of alachlor was larger under anaerobic conditions than under aerobic conditions (Graham et al., 2000). Under highly reducing conditions (Eh -190 to >400 mV), an important mass removal of *S*-metolachlor was observed (Chapter IV), concomitantly with a larger production of the ESA degradate although no significant changes in the isotope composition of the *S*-metolachlor could be observed. This suggests that the removal of *S*-metolachlor under highly reducing conditions can be mostly abiotic and/or that biotic processes are limited. Several studies previously reported the occurrence of abiotic degradation of chloroacetanilides by reactive sulfite or sulfide species, that react through a nucleophilic attack on the 2-chloro group of acetanilide herbicides (Stamper and Tuovinen, 1998).

To conclude, hydrochemical analyses provided important information about the influence of environmental conditions, in particular the redox conditions, on the degradation of pesticides in redox-dynamic systems. However, even if this approach was also applied in a larger-scale system (Chapter III), the role of prevailing conditions on the transport of the

pesticide mixture could only be indirectly inferred, and the larger heterogeneity of the system hampered drawing straightforward conclusions and causal relationships.

In combination with the quantification of the parent compounds, the analysis of degradation products enables to identify the occurrence of transformation processes (Fenner et al., 2013). However, the analysis of degradation products may have limitations when used to quantify degradation processes. In particular, degradation of the commercialized molecules (source substances) results in the formation of degradation products that are mostly unknown. The identification of degradation products and of the main degradation pathways that cause their formation is thus an important challenge to understand pesticide fate from source (fields) to sink (non-target receptor) areas. For instance, more than 20 neutral and 6 ionic degradation products for chloroacetanilide herbicides are currently reported in the literature (Hladik et al., 2008; Hladik et al., 2005). However, except for ionic compounds (OXA and ESA degradates), no analytical standard is currently available for the neutral compounds, so their identification is limited (Boxall et al., 2004). ESA and OXA degradates were reported as the main degradation products of the chloroacetanilide herbicides (Baran and Gourcy, 2013). In our column experiment, degradation and more precisely biodegradation was identified thanks to both the chloroacetanilide mass budgets and CSIA. However, ESA and OXA were rarely detected at the outlet of the lab-scale wetlands, suggesting that these degradation products were probably not the major routes of chloroacetanilide degradation into nitrate reducing conditions and/or that they were rapidly degraded in the wetland. The sole analysis of the degradation products is thus insufficient when complete degradation pathways are unknown and require to be coupled to other techniques, such as compound-specific analyses.

Compound-specific stable isotope analysis (CSIA) measurements provided valuable insights on *in-situ* biodegradation in this thesis (Chapter IV and chapter V) as previously described. The isotopic composition of contaminants can provide insights about key degradation pathways occurring *in-situ*, and in some cases enables measuring the extent of biodegradation (Elsner, 2010). During the last decade, CSIA has been increasingly applied to study several groups of contaminants, including pesticides, such as lindane (Badea et al., 2011), isoproturon (Penning et al., 2010), atrazine (Meyer et al., 2008) and phenoxy-acid herbicides (Maier et al., 2013). However, CSIA methods have not yet been reported for the evaluation of pesticide biodegradation in environmental systems. In this thesis, the CSIA method developed by Elsayed et al. (2013) was successfully applied on chloroacetanilide herbicides. Further characterization of the prevailing degradation pathways in wetlands could include the combined isotope

measurement of more than one element, for example by combining the analysis of carbon isotopes to the hydrogen and chlorine signature. The combination of CSIA methods with enantiomeric analyses for chiral pesticides such as metolachlor is another promising approach. Badea et al. (2011) developed an enantiomer-specific stable carbon isotope analysis (ESIA) that provided valuable indications for the biodegradation of α-hexachlorocyclohexane. In this thesis, a method was developed to analyse the possible enantioselective degradation of metolachlor in the studied wetlands (Chapter IV and Chapter V) and ESIA of metolachlor in wetland is the logical next step of the present study.

Enantiomeric analyses showed significant shifts in the lab-scale wetland that received metolachlor, suggesting a preferential degradation of the *S*-enantiomer in the rhizospheric zone under nitrate reducing conditions (Chapter V). However, no significant shifts could be distinguished in the SSFCWs, which underscores that the degradation was not enantioselective or that enzymes that preferentially degrade respectively *R*- and *S*-enantiomers were both present in the systems (Milosevic et al., 2013). Previous studies reported contrasted information. Klein et al. (2006) observed a non-enantioselective degradation of metolachlor in soils, whereas Ma et al. (2006) observed a preferential degradation of the *S*-enantiomer. A further step, would be to test in a microcosm experimental set-up the influence of specific redox conditions on the enantioselective degradation of metolachlor, as it may largely control degradation pathways and thus influence the extent of enantioselective degradation. Hence, both enantiomer and isotope fractionation (or enrichment) factors could be retrieved in benchmark experiments and further used to evaluate, and possibly quantify, the *in-situ* biodegradation under more complex, field-relevant situations.

To conclude, the combination of different analytical tools in this thesis represents a valuable approach to assess the transport and transformation processes of pesticides in wetland systems. The integration of the different datasets gathered when such a comprehensive approach is applied to evaluate transformation of pesticides and other micropollutants in the environment opens novel research perspectives which are discussed in the following section.

2. Implications and further research

This thesis demonstrated the relevance of combining multiple scales and analytical approaches to gain knowledge on fundamental mechanisms that govern the responses of wetland ecosystems regarding pesticide contamination. This study is a first attempt i) to

quantify the pesticide pools in wetland compartments under field conditions in order to distinguish storage from degradation in redox-dynamic environments, such as wetlands, and ii) to investigate sink and source functions of wetlands with respect to the behavior of organic pollutants. In addition, this constituted a starting point to couple the quantification of the parent compounds and their degradation products with enantiomer and isotope analyses to evaluate and predict the fate of widely used and emerging chiral contaminants in receptor aquatic environments.

The extraordinary diversity and complexity of biogeochemical hotspots in the landscape, such as wetlands and hyporheic zone, makes this a rich topic with many avenues of investigation; four of them are briefly described below.

Pesticide sorption under field-relevant conditions. There is a need to better understand sorption processes and their implications concerning the bioavailability of pesticides, in particular when pesticide dissipation is targeted. In-depth knowledge about the influence of the nature of soils particles requires further investigation. Pesticide sorption can typically occur on the mineral matter, but in a larger extent on the organic fraction of soils and sediments. The nature of organic matter (size of the particles, aromaticity, and polarity) can largely affect pesticide sorption and thus impact their transport and potential of degradation. A next study step could include sequential extractions of organic matter by different filtrations (e.g. ultra-filtration) and pesticide analyses on each fraction of organic matter. For this purpose, techniques such as Specific UltraViolet Absorption (SUVA) to link pesticide sorption with organic matter aromaticity have proved their usefulness. This would help to understanding the interactions between pollutants and mineral and/or organic matter, which is crucial to model and predict their accumulation, biodegradation, mobility and toxicity in wetland systems and thus to guide best management practices.

Biotransformation of chiral pesticides. Achieving a molecular-level characterisation of the yet unknown processes and microbial players that control degradation of chiral pollutants in the environment remain challenging. Today, 30% of modern pesticides are chiral molecules (i.e. existing as 2 (or more) species – enantiomers – that are non-superimposable mirror images of each other), which may exhibit enantioselective transformation and/or toxicity. The environmental fate, transport and transformation of each enantiomer remain poorly known. Therefore, the comprehensive exploration of chiral pesticides and their degradation products from land (sources) to aquatic ecosystems (sinks) could be pioneered based on a combination of

cutting-edge techniques in analytical chemistry, isotopic geochemistry, microbial molecular ecology, and biogeochemical reactive transport modelling. The application of stable isotope analysis, and in particular enantiomer-specific isotope analysis (ESIA), to pesticides and pharmaceuticals is currently very limited. Novel methods for sample collection, preparation and isolation for quantitative, enantiomer and isotopic analysis of chiral pesticides and their degradation products could be developed based on benchmark, microcosm experiments of bacterial pesticide degradation. Further, microbial cultures obtained from environmental material, and in which pollutant degradation is observed, can be supplied with unlabelled (^{12}C), and following microbial development and characterisation, with labelled (^{13}C) pesticides to access active degraders by the stable isotope probing approach (SIP), before direct sequencing of DNA fractions by classical and high-throughput techniques, such as Illumina Hi-Seq and Roche 454 (MID pair) pyrosequencing. The direct perspective of this research would be to develop bioindicator-based tools for assessing ecosystem functioning and recovery following exposure to pesticides.

Applying and improving wetland ecotechnology for treating pesticide effluents. Constructed wetlands (CWs e.g. subsurface flow constructed wetlands) were primarily designed for the removal of conventional wastewater quality parameters (nitrogen, phosphorus, biological oxygen demand, total suspended solids). These engineered systems have also proved to efficiently remove organic contaminants including pesticides (Matamoros et al., 2007) and pharmaceuticals (Ávila et al., 2013; Verlicchi and Zambello, 2014). However, processes governing the removal of emerging contaminants in constructed wetlands are poorly understood and require further investigations. In particular, in-depth knowledge on the transport and transformation processes of emerging contaminants in wetlands and on the environmental parameters that mainly affect these processes are crucial to optimize the effectiveness of CWs. For instance, the use of artificial dye tracers may be useful to evaluate the potential of new decentralized constructed wetland designed to collect urban or agricultural effluents to attenuate micropollutants, and thus could efficiently serve the 'toolbox' of the wetland practitioners. The implementation of CWs is a valuable and low-cost alternative of conventional wastewater treatment plants (WWTPs) that requires further research to optimize the removal of the most critical compounds and to better assess the ecotoxicological risk for aquatic environments (Verlicchi and Zambello, 2014).

Biogeochemical modelling. Increasing details and resolution with which geochemical processes and microbial activity can now be characterised creates new challenges for the

quantitative reactive modelling of the biogeochemical functioning of environmental systems. Biogeochemical modelling has great potential for studying the impact of individual processes on the overall performance of environmental systems, such as the ability to degrade organic contaminants, and the system of interactions between the microbiome and the environment. For instance, reactive transport models (RTMs) have emerged as an essential diagnostic tool for the quantitative analysis of the biogeochemical functioning of complex subsurface environments. In the future, these models will be used to describe and predict the feedback between microbes and organic or inorganic pollutants in dynamic environments.

Overall, gaining knowledge on the fate of emerging contaminants is needed to help designing and managing treatment wetlands receiving organic contaminants and to give insights on ecotoxicological impact of organic contaminants.

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Chapter VII. Appendices

Table VII- 1 Type, physico-chemical properties and toxicity of the studied compounds

Compound	Chemical group	Type	Log K_{ow} [a]	Henry constant [a]	DT50 (aerobic-anaerobic soil) [b]	Aqueous photolysis DT50 (pH = 7) [a]	Aqueous hydrolysis DT50 at (20°C, pH = 7)	EC50 [a, c]
			[-]	Pa m^3 mol^{-1} (25°C)	[day]	[day]	[day]	[ppm]
Azoxystrobin	Strobilurin	Fungicide	2.5	7.40×10^{-09}	112 -119	8.7	stable	0.23
Cymoxanil	Cyanoacetamide oxime	Fungicide	0.67	3.80×10^{-05}	n.a.	1.7	1.1	27
Cyprodinil	Anilinopyrimidine	Fungicide	4	6.60×10^{-03}	126-183	13.5	stable	0.033
Carbendazim	Benzimidazole	Fungicide	1.48	3.60×10^{-03}	n.a.	stable	350	0.15
Dimethomorph	Morpholine	Fungicide	2.68	2.04×10^{-05}	n.a.-26	97	70	> 10.6
Diuron	Phenylurea	Herbicide	2.87	2.00×10^{-06}	372-925	43	Stable	5.7
DCPU	Unclassified	Metabolite	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
DCPMU	Unclassified	Metabolite	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
3.4-dichloroaniline	Unclassified	Metabolite	2.69	1.48	n.a.	0.25	n.a.	0.44
Flufenoxuron	Benzoylurea	Insecticide	4.01	2.64×10^{-06}	n.a.	6	267	0.000043
Gluphosinate	Organophosphate	Herbicide	-3.96	n.a.	n.a.	n.a.	n.a.	n.a.
Glyphosate	Phosphonoglycine	Herbicide	-3.2	2.10×10^{-07}	96-22	69	Stable	40
AMPA	Unclassified	Metabolite	-1.63	0.16	n.a.	n.a.	n.a.	n.a.
Isoxaben	Benzamide	Herbicide	3.94	1.96×10^{-04}	205- n.a.	6	Stable	> 1.3
Kresoxim methyl	Strobilurin	Fungicide	3.40	3.60×10^{-07}	2-1	28	34	0.186
Metalaxyl	Phenylamide	Fungicide	1.65	1.60×10^{-05}	n.a.	Stable	106	28
Pyrimethanil	Anilinopyrimidine	Fungicide	2.84	3.60×10^{-03}	n.a.	Stable	Stable	2.9
Simazine	Triazine	Herbicide	2.3	5.60×10^{-05}	110-71	1.9	96	1.1
Terbuthylazine	Triazine	Herbicide	3.4	4.18×10^{-03}	n.a.	Stable	Stable	21.2
Tetraconazole	Triazole	Fungicide	3.56	3.60×10^{-04}	n.a.	217	Stable	3.0

[a] Obtained from the PPDB (2007, 2008, 2009). The Pesticide Properties DataBase (PPDB) developed by the Agriculture & Environment Research Unit (AERU) at the University of Hertfordshire, from the database that originally accompanied the EMA (Environmental Management for Agriculture) software (also developed by AERU), with additional input from the EU-funded FOOTPRINT project (FP6-SSP-022704). http://www.herts.ac.uk/aeru/footprint.

[b] Obtained from the PAN (PAN, 2006) pesticide data base

[c] Daphnia magna test, 48 h obtained from PPDB data base

Table VII- 2 Climatic and hydrological conditions in the vineyard catchment (Rouffach, Haut-Rhin, France) and the stormwater wetland in spring (April 06 to June 15, 2009) and in summer (June 15 to September 29, 2009). Values are provided as the median and ranges.

		Spring (April 06 to June 15, 2009)	**Summer** (June 16 to September 29, 2009)
Temperature	[°C day^{-1}]	16.1 (9.6 - 26.7)	19.1 (12.5 - 27.9)
Solar radiation	[joules cm^{-2} day^{-1}]	1978 (471 - 2909)	1720 (431 - 2897)
Evaporation	[mm]	280.5	390.4
Rainfall	[mm]	88.8	162.2
Number of rainfall events	[-]	32	45
Rainfall amount	[mm]	1.7 (0.2 - 15.4)	1 (0.2 - 24)
Rainfall duration	[hs]	1.9 (0.1 - 16.7)	1.1 (0.1 - 13.9)
Mean rainfall intensity	[mm h^{-1}]	1.4 (0.2 - 6.7)	2 (0.1 - 21)
Maximum rainfall intensity	[mm h^{-1}]	4 (2 - 38)	2 (2 - 46)
Number of runoff events	[-]	13	17
Discharge	[m^3]	4 (0.4 - 85)	8.4 (0.3 - 141.8)
Quiescent period	[day]	4 (0.1 - 27)	3.6 (0.3 - 22.4)
Runoff coefficient	[%]	0.2 (0.05 - 1.3)	0.6 (0.02 - 1.5)
Inlet flow rate	[m^3 h^{-1}]	0.28 (0 - 23.5)	2.5 (0 - 158.7)
Outlet flow rate	[m^3 h^{-1}]	0.28 (0 - 4.1)	0.002 (0 - 11)

Table VII- 3 Main vegetation species and vegetation cover (%) in the gravel filter and the sediment deposition zone of the stormwater wetland (Rouffach, Haut-Rhin, France). Detailed surveys of the vegetal species were carried out on June 2 and June 30, 2009.

Family	Genus	Species	June 2 2009		June 30 2009	
			Sediment deposition zone	Gravel filter	Sediment deposition zone	Gravel filter
			[%] of the total area	[%] of the total area	[%] of the total area	[%] of the total area
Apiaceae	*Daucus*	*carota*	0	2	0	4
Apiaceae	*Torilis*	*japonica*	<1	3	<1	3
Asteraceae	*Erigeron*	*canadensis*	<1	2	<1	2
Asteraceae	*Matricaria*	*chamomilla*	1	2	1	3
Asteraceae	*Sonchus*	*arvensis*	<1	2	<1	2
Brassicaceae	*Isatis*	*tinctoria*	0	3	0	3
Brassicaceae	*Thlaspi*	*arvense*	0	2	0	2
Charophyceae	*Chara*	*vulgaris*	<1	0	3	0
Cyperaceae	*Schoenoplectus*	*lacustris*	5	0	8	0
Dipsacaceae	*Dipsacus*	*sylvester*	0	1	0	1
Equisetaceae	*Equisetum*	*arvense*	<1	2	<1	2
Fabaceae	*Medicago*	*sativa*	<1	<1	<1	1
Plantaginaceae	*Plantago*	*major*	0	<1	0	1
Poaceae	*Lolium*	*perenne*	<1	25	<1	26
Poaceae	*Phragmites*	*australis*	20	9	35	11
Renonculaceae	*Ranunculus*	*sceleratus*	2	0	3	0
Rhamnaceae	*Frangula*	*alnus*	0	2	0	2
Rubiaceae	*Galium*	*mollugo*	<1	2	<1	2
Typhaceae	*Typha*	*latifolia*	1	0	1	0
Total cover in [%] of the total area			**29**	**57**	**51**	**65**

Table VII- 4 Detection and quantification limits, as well as relative standard deviation (RSD) and recovery efficiency for the investigated pesticide in both water and sediment samples. Values are provided as the median and ranges.

Compound	Water samples				Sediment samples			
	LOD [µg L^{-1}]	LOQ [µg L^{-1}]	RSD [%]	Recovery [%]	LOD [µg kg^{-1}]	LOQ [µg kg^{-1}]	RSD [%]	Recovery [%]
Azoxystrobin	0.02	0.05	15	85	1	2	35	71
Cymoxanil	0.02	0.05	35	70	1	2	33	70
Cyprodinil	0.01	0.02	20	89	2	5	35	81
Carbendazim	0.02	0.05	25	80	1	2	38	75
Dimethomorph	0.02	0.05	30	76	1	2	40	72
Diuron	0.01	0.02	25	85	1	2	38	69
DCPU	0.02	0.05	26	82	1	2	36	72
DCPMU	0.02	0.05	25	79	1	2	38	73
3.4-dichloroaniline	0.03	0.10	23	75	3	10	41	76
Flufenoxuron	0.02	0.05	24	81	1	2	36	75
Gluphosinate	0.03	0.10	25	85	3	10	34	72
Glyphosate	0.03	0.10	16	81	3	10	26	76
AMPA	0.03	0.10	16	86	3	10	28	72
Isoxaben	0.02	0.05	15	89	1	2	26	71
Kresoxim methyl	0.03	0.10	23	81	1	2	28	70
Metalaxyl	0.02	0.05	17	86	1	2	36	72
Pyrimethanil	0.02	0.05	22	89	1	2	32	81
Simazine	0.01	0.02	22	85	1	2	29	82
Terbuthylazine	0.01	0.02	20	90	1	2	31	80
Tetraconazole	0.02	0.05	25	86	1	2	29	71

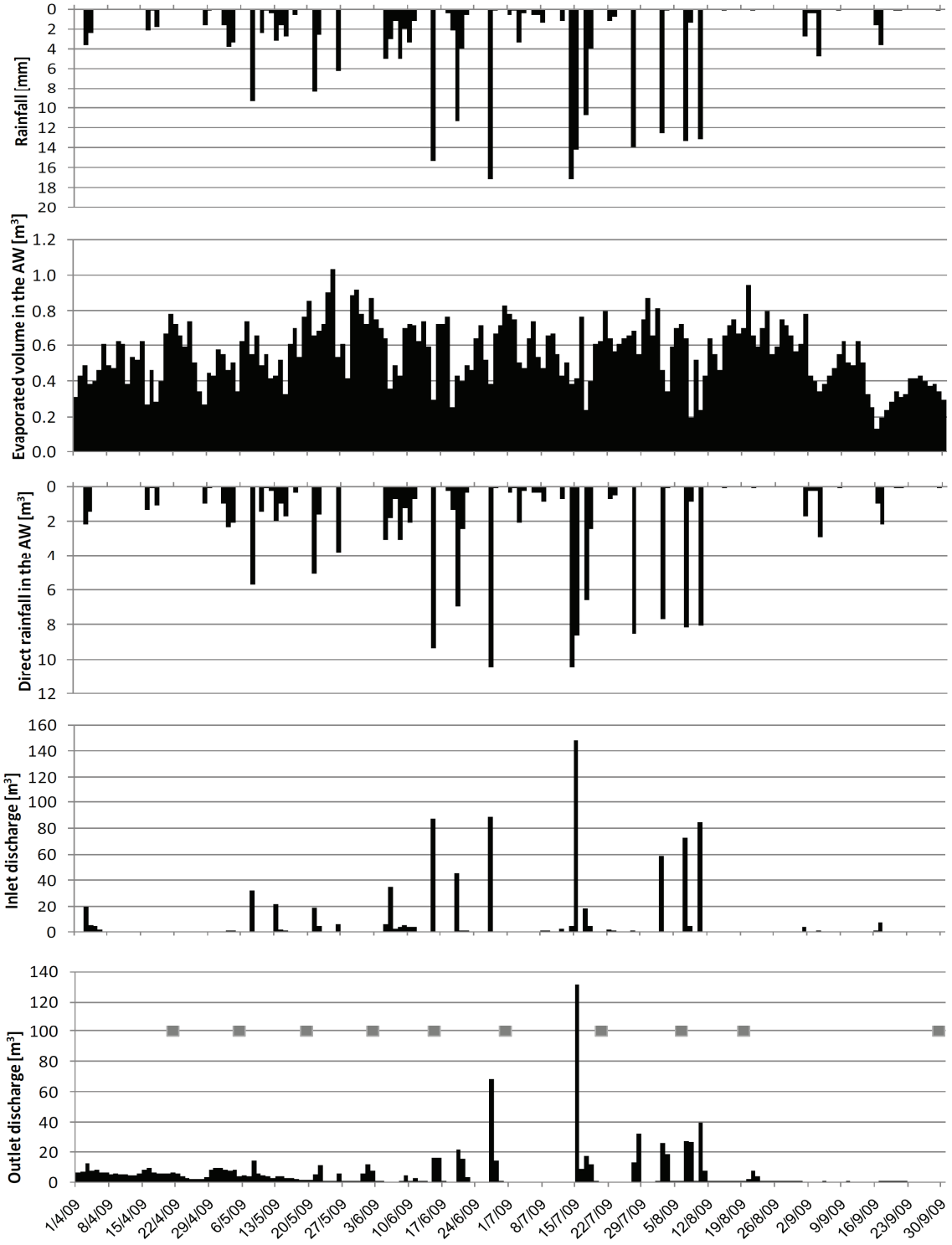


Figure VII- 1 Daily rainfall [mm] in the catchment area, evaporated volume (m^3), direct rainfall in the wetland [mm], and daily discharges (m^3) at the inlet and outlet of the stormwater wetland (Rouffach, Haut-Rhin, France) during the investigation period (April 06 to September 29, 2009) that corresponded to the wine growing season and the period of pesticide. Grey squares indicate water and sediment sampling in the wetland.

Table VII- 5 Selected hydrochemical variables in the storm water wetland (Rouffach. Haut-Rhin, France) in spring (April 06 to June 15, 2009) and in summer (June 15 to September 29, 2009). Hydrochemical values are provided as the mean as well as minimum and maximum values (in parentheses) over n measurements of samples collected in the inlet, the sediment deposition zone (SDZ), the gravel filter (GF) and the outlet during the 10 sampling campaigns.

		Spring				Summer			
Parameter	**Unit**	**Inlet** (n = 6)	**SDZ** (n = 26)	**GF** (n = 30)	**Outlet** (n = 13)	**Inlet** (n = 9)	**SDZ** (n =24)	**GF** (n = 28)	**Outlet** (n = 7)
Temperature	[°C]	n.a	21.4 (15.5-26.5)	17.5 (14.0-21.0)	n.a	n.a	23.3 (18.6-28.9)	21.4 (17.2-25.2)	n.a
Redox potential	[mV]	n.a.	204 (44-297)	86 (-187-330)	n.a.	n.a.	-81 (-228-188)	-328 (-457-89)	n.a.
Dissolved oxygen	[mg L^{-1}]	n.a.	9.2 (2.9-13.8)	0.8 (0.2-3.2)	n.a.	n.a.	3.7 (0.2-8.8)	0.2 (n.d.-0.9)	n.a.
Electric conductivity	[µS cm^{-1}]	263 (207-405)	908 (117-1036)	965 (904-1041)	1021 (835-1605)	175 (24-288)	376 (269-522)	418 (270-1473)	488 (132-901)
pH	[-]	7.8 (7.6-7.9)	7.8 (7.5-8.1)	7.4 (7.3-7.6)	8.0 (7.7-8.2)	7.4 (6.7-7.9)	7.5 (7.0-8.0)	7.4 (6.8-7.7)	7.7 (7.3-8.1)
Total suspended solids	[mg L^{-1}]	1106 (248-4695)	20.7 (4.8-59.8)	6.3 (0.6-14.5)	17.5 (1.0-96.8)	1170 (170-3702)	43.0 (3.2-266)	33.6 (2.2-69.6)	30.0 (0.7-66.8)
Total volatile suspended solids	[mg L^{-1}]	180 (79-543)	6.9 (0.8-29.2)	2.6 (0.7-8.0)	10.6 (1.0-63.0)	160 (36-445)	19.2 (0.4-116)	8.3 (n.d.-20.4)	10.5 (1.0-18.2)
Total organic carbon (NPOC)	[mg L^{-1}]	24.6 (14.1-30.9)	19.8 (13.3-65.6)	20.0 (12.4-73.5)	14.2 (8.7-20.2)	18.0 (7.6-31.8)	13.7 (6.7-34.9)	6.9 (5.5-8.4)	12.1 (7.5-22.5)
Dissolved organic carbon	[mg L^{-1}]	24.8 (13.2-31.7)	19.8 (13.5-62.5)	19.8 (12.2-72.0)	15.1 (8.8-22.8)	11.8 (7.4-23.7)	12.1 (7.2-27.4)	6.7 (5.5-8.7)	11.2 (7.4-21.9)
Total inorganic carbon	[mg L^{-1}]	45.9 (28.0-73.4)	63.8 (55.6-68.2)	66.6 (44.6-79.4)	65.6 (51.1-90.6)	37.3 (26.7-55.2)	47.9 (29.5-92.2)	47.3 (34.4-96.7)	46.4 (25.8-62.4)
Dissolved inorganic carbon	[mg L^{-1}]	34.5 (23.7-61.3)	61.0 (45.2-67.7)	64.5 (56.5-75.8)	62.5 (51.1-90.6)	23.1 (14.4-42.7)	44.1 (25.3-71.4)	45.1 (26.7-96.1)	43.1 (22.3-61.2)
Ortho-phosphorus	[mg L^{-1}]	0.09 (n.d.-0.17)	0.06 (0.06-0.06)	0.13 (0.08-0.21)	0.18 (0.05-0.34)	0.19 (0.12.-0.28)	0.14 (0.01-0.30)	0.21 (0.04-0.50)	0.14 (0.06-0.36)
Total phosphorus	[mg L^{-1}]	n.a.	0.40 (0.14-1.88)	0.48 (0.12-2.33)	0.18 (0.05-0.34)	2.7 (n.d.-7.0)	0.74 (0.11-2.4)	0.63 (0.18-1.4)	0.55 (0.06-0.36)
Chloride	[mg L^{-1}]	2.2 (1.4-3.0)	30.9 (26.9-36.3)	30.5 (26.1-38.0)	28.0 (6.0-38.0)	4.5 (2.0-5.9)	2.2 (1.4-4.1)	2.6 (1.0-6.8)	9.3 (1.4-28.5)
Total Kjeldahl Nitrogen	[mg L^{-1}]	n.a.	4.6 (1.1-11.6)	3.8 (1.1-8.5)	3.5 (1.7-7.5)	5.7 (2.4-15.3)	2.5 (1.0-5.4)	2.1 (1.0-3.8)	2.8 (1.9-5.7)
Nitrate	[mg L^{-1}]	2.3 (0.72-3.7)	8.8 (2.4-18.5)	6.7 (0.86-25.5)	9.1 (1.6-19.6)	7.0 (1.8-15.4)	2.8 (0.2-11.5)	4.28 (0.30-19.2)	5.5 (0.85-16.4)
Nitrite	[mg L^{-1}]	0.85 (0.18-2.2)	0.26 (0.11-0.75)	0.31 (0.02-0.88)	0.15 (0.01-0.53)	0.15 (0.01-0.32)	0.03 (n.d.-0.10)	0.04 (n.d.-0.15)	0.03 (n.d.-0.06)
Ammonium	[mg L^{-1}]	0.35 (0.12-0.52)	0.37 (0.10-0.97)	0.14 (0.05-0.81)	0.10 (0.06-0.24)	0.15 (n.d.-0.36)	0.19 (0.01-1.4)	0.27 (0.01-0.94)	0.16 (0.01-0.28)
Total iron	[mg L^{-1}]	31.0 (7.8-101)	1.7 (1.1-2.3)	4.4 (1.2-6.0)	5.5 (1.6-11.4)	19.1 (0.93-65.7)	1.6 (0.12-5.4)	1.7 (1.0-3.0)	1.5 (0.45-4.1)
Iron(II)	[mg L^{-1}]	0.42 (0.11-0.85)	0.15 (0.02-0.35)	0.19 (0.01-0.97)	0.07 (0.02-0.27)	0.48 (0.10-1.3)	0.68 (0.15-6.0)	0.87 (0.09-3.3)	0.43 (0.02-1.8)
Manganese(II)	[mg L^{-1}]	0.85 (0.26-2.1)	0.24 (0.24-0.24)	1.65 (0.41-3.47)	1.4 (0.37-2.7)	0.70 (0.22-1.2)	0.72 (0.38-1.7)	1.33 (0.20-5.5)	2.6 (0.8-6.9)
Sulfate	[mg L^{-1}]	132 (13.7-252)	241 (159-325)	232 (145-309)	206.3 (118-314)	64.6 (22.7-96.4)	29 (5.1-63.6)	22 (1.9-62.0)	111 (5.9-538)
Copper(II)	[µg L^{-1}]	n.d.	n.d.	n.d.	n.d.	236 (84-326)	n.d.	n.d.	9.4 (0.7-13.3)
Sodium	[mg L^{-1}]	4.3 (1.5-8.1)	9.8 (5.0-14.6)	10.3 (2.0-19.2)	9.1 (1.7-13.9)	3.5 (0.67-9.5)	3.7 (0.94-9.1)	3.7 (1.3-13.4)	6.1 (1.4-15.4)
Potassium	[mg L^{-1}]	8.8 (4.1-15.6)	5.2 (2.8-14.6)	4.2 (1.4-9.1)	5.3 (0.7-14.9)	9.9 (2.1-19.3)	5.3 (1.1-9.4)	6.1 (3.5-10.0)	5.7 (1.2-11.4)
Calcium	[mg L^{-1}]	98.0 (33.6-168)	125 (96-164)	129 (74.1-175)	120 (60.6-170.8)	64.8 (15.8-193)	64.5 (46.2-85.9)	60.6 (41.9-105)	61.8 (42.5-96.0)
Magnesium	[mg L^{-1}]	14.9 (3.2-45.1)	35.2 (27.7-50.4)	35.3 (15.5-50.2)	33.6 (22.9-45.0)	6.7 (0.63-27.2)	12.4 (5.8-29.1)	13.6 (8.7-21.9)	17.8 (6.2-31.8)

Table VII- 6 Mean concentrations and ranges of dissolved and particle-bound pesticides in the inlet, the sediment deposition zone (SDZ), the gravel filter (GF) and the outlet of the storm water wetland (Rouffach, Haut-Rhin, France) in spring (April 06 to June 15, 2009) and in summer (June 15 to September 29, 2009). Reduction in mean concentrations from inlet to outlet is given in percent (R_C %). n.d.: not detected.

Compound	Dissolved pesticides [µg L^{-1}]					Particle-bound pesticides [µg kg^{-1}]		Dissolved pesticides [µg L^{-1}]					Particle-bound pesticides [µg kg^{-1}]	
	Inlet (n = 10)	SDZ (n = 20)	GF (n = 10)	Outlet (n = 17)	R_C [%]	Inlet (n = 7)	SDZ (n = 16)	Inlet (n = 18)	SDZ (n = 20)	GF (n = 10)	Outlet (n = 9)	R_C [%]	Inlet (n = 9)	SDZ (n = 20)
Azoxystrobin	n.d.	n.d.	n.d.	n.d.	n.a.	n.d.	n.d	0.02 (n.d.-0.12)	0.01 (n.d.-0.07)	n.d.	< 0.02 (n.d.-0.02)	100	< LOQ (n.d.-2.0)	n.d
Cymoxanil	0.13 (n.d.-0.90)	0.03 (n.d.-0.60)	n.d.	n.d. [b]	100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.	n.d.	n.d.
Cyprodinil	n.d.	n.d.	n.d.	n.d.	n.a.	0.71 (n.d.-5.0)	n.d.	0.02 (n.d.-0.14)	0.01 (n.d.-0.04)	0.01 (n.d.-0.03)	n.d.	100	30.8 (n.d.-145)	< LOQ (n.d.-7.0)
Carbendazim	n.d.	0.01 (n.d.-0.11)	n.d.	n.d.	n.a.	n.d.	n.d.	n.d.	0.04 (n.d.-0.20)	n.d.	n.d.	n.a.	< LOQ (n.d.-2.0)	n.d.
Dimethomorph	0.03 (n.d.-0.18)	n.d.	n.d.	n.d.	100	< LOQ (n.d.-1.0)	n.d.	2.22 (n.d.-10.0)	1.62 (n.d.-5.80)	0.69 (n.d.-2.70)	0.50 (n.d.-2.20)	78	11.3 (n.d.-33)	n.d.
Diuron	0.16 (0.11-0.32)	0.04 (n.d.-0.08)	0.01 (n.d.-0.05)	0.02 [a] (n.d.-0.04)	90	< LOQ (n.d.-7.0)	< LOQ (n.d.-4.0)	0.02 (n.d.-0.16)	0.01 (n.d.-0.03)	0.01 (n.d.-0.03)	0.01 (n.d.-0.04)	46	2.1 (n.d.-3.5)	< LOQ (n.d.-4.0)
DCPU	n.d.	n.d.	n.d.	n.d.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.	n.d.	n.d.
DCPMU	n.d.	n.d.	n.d.	n.d.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.	< LOQ (n.d.-2.0)	n.d.
3.4-dichloroaniline	n.d.	n.d.	n.d.	n.d.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.	n.d.	n.d.
Flufenoxuron	n.d.	0.04 (n.d.-0.59)	n.d.	n.d.	n.a.	8.3 (1.5-18.5)	2.75 (n.d.-6.0)	n.d.	n.d.	n.d.	n.d.	n.a.	6.1 (3.5-16.0)	< LOQ (n.d.-3.0)
Gluphosinate	0.85 (n.d.-6.30)	0.11 (n.d.-1.40)	n.d.	n.d.	100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.	n.d.	n.d.
Glyphosate	4.13 (0.30-11.0)	0.10 (n.d.-0.40)	0.07 (n.d.-0.30)	0.08 [a] (n.d.-0.3)	98	< LOQ (n.d.-16.0)	< LOQ (n.d.-6.0)	5.95 (0.20-15.0)	1.61 (n.d.-3.90)	1.49 (0.20-3.0)	1.28 [b] (0.30-3.90)	79	11.8 (n.d.-45.0)	n.d
AMPA	1.37 (0.20-2.30)	0.08 (n.d.-0.30)	0.27 (n.d.-0.60)	0.39 [a] (n.d.-0.70)	71	n.d.	n.d.	2.53 (n.d.-21.0)	2.05 (n.d.-4.80)	1.35 (0.50-2.30)	1.09 (0.50-1.80)	57	5.8 (n.d.-21.0)	n.d
Isoxaben	0.11 (n.d.-0.23)	0.02 (n.d.-0.10)	0.04 (n.d.-0.18)	0.01 [a] (n.d.-0.15)	89	< LOQ (n.d.-2.0)	n.d.	0.01 (n.d.-0.08)	n.d.	n.d.	n.d.	100	< LOQ (n.d.-1.0)	n.d.
Kresoxim methyl	0.01 (n.d.-0.05)	0.02 (n.d.-0.10)	n.d.	n.d.	100	1.0 (n.d.-4.0)	n.d.	0.02 (n.d.-0.40)	n.d.	n.d.	n.d.	100	< LOQ (n.d.-3.0)	n.d.
Metalaxyl	1.43 (n.d.-5.80)	0.21 (n.d.-0.64)	0.23 (n.d.-0.92)	0.25 [b] (n.d.-1.20)	83	1.7 (n.d.-10.0)	n.d.	0.05 (n.d.-0.35)	n.d.	n.d.	0.04 (n.d.-0.37)	10	n.d.	n.d.
Pyrimethanil	n.d.	n.d.	n.d.	n.d.	n.a.	n.d.	n.d.	0.02 (n.d.-0.08)	0.01 (n.d.-0.06)	0.01 (n.d.-0.05)	0.01 (n.d.-0.05)	65	1.5 (n.d.-4.5)	n.d.

(End of table)

Compound	Spring							Summer						
	Dissolved pesticides [µg L^{-1}]					Particle-bound pesticides [µg kg^{-1}]		Dissolved pesticides [µg L^{-1}]					Particle-bound pesticides [µg kg^{-1}]	
	Inlet (n = 10)	SDZ (n = 20)	GF (n = 10)	Outlet (n = 17)	R_C [%]	Inlet (n = 7)	SDZ (n = 16)	Inlet (n = 18)	SDZ (n = 20)	GF (n = 10)	Outlet (n = 9)	R_C [%]	Inlet (n = 9)	SDZ (n = 20)
Simazine	0.08 (0.04-0.18)	n.d.	0.02 (n.d.-0.03)	0.02 [a] (n.d.-0.03)	80	n.d.	n.d.	< 0.02 (n.d.-0.02)	n.d.	n.d.	n.d.	n.a.	n.d.	n.d.
Terbuthylazine	0.06 (0.06-0.07)	n.d.	n.d.	n.d. [b]	100	n.d.	n.d	< 0.02 (n.d.-0.04)	n.d.	n.d.	n.d.	100	n.d.	n.d.
Tetraconazole	0.02 (n.d.-0.09)	0.01 (n.d.-0.07)	n.d.	n.d. [a]	100	2.4 (n.d.-9.5)	n.d.	0.01 (n.d.-0.07)	n.d.	n.d.	0.01 (n.d.-0.03)	0	1.1 (n.d.-3.0)	< LOQ (n.d.-2.0)

Table VII- 7 Load estimates [mg] of dissolved pesticides and suspended-solid associated pesticides entering the storm water wetland in spring (April 06 to June 15, 2009), in summer (June 15 to September 29, 2009) and during the entire investigation period (April 6 to September 29, 2009). Solid to dissolved load ratio [%] are also provided. Degradation products are shown in italics.

Compound	Spring			Summer			Wine growing season		
	Dissolved load	**Solid load**	**LogK_d**	**Dissolved load**	**Solid load**	**LogK_d**	**Dissolved load**	**Solid load**	**LogK_d**
Azoxystrobin	0	0	**n.a.**	18.1	0.03	**-2.73**	18.1	0.03	**-2.73**
Cymoxanil	12.3	0	**n.a.**	0	0	**0**	12.3	0	**0**
Cyprodinil	0.00	0.01	**n.a.**	18.6	11.27	**-0.22**	18.6	11.28	**-0.22**
Carbendazim	0	0	**n.a.**	0	0	**n.a.**	0	0	**n.a.**
Dimethomorph	3.4	0.01	**-2.62**	1693	11.97	**-2.15**	1696	12.0	**-2.15**
Diuron	26.5	0.03	**-2.93**	13.2	156	**1.07**	39.7	155	**0.59**
DCPU	0	0	**n.a.**	0	0	**n.a.**	0	0	**n.a.**
DCPMU	0	0	**n.a.**	0	0.34	**n.a.**	0	0.34	**n.a.**
3.4-dichloroaniline	0	0	**n.a.**	0	0	**n.a.**	1.3	0	**0**
Flufenoxuron	0	1.24	**n.a.**	0	3.96	**n.a.**	0	5.19	**n.a.**
Gluphosinate	93	0	**n.a.**	0	0	**n.a.**	93	0	**0**
Glyphosate	585	0.04	**-4.22**	3571	6.66	**-2.73**	4156	6.69	**-2.79**
AMPA	217	0.00	**n.a.**	1421	3.77	**-2.58**	1637	3.77	**-2.64**
Isoxaben	17	0.33	**-1.71**	7.4	0.02	**-2.69**	24.5	0.35	**-1.85**
Kresoxim methyl	1	0.08	**1.10**	15.7	0.41	**-1.59**	16.7	0.49	**-1.54**
Metalaxyl	237	0.15	**0.06**	35.5	0	**n.a.**	272.5	0.15	**-3.26**
Pyrimethanil	0	0	**n.a.**	12.6	1.28	**-0.99**	12.6	1.28	**-0.99**
Simazine	13	0	**n.a.**	2.1	0.00	**n.a.**	15.1	0	**0**
Terbuthylazine	10.4	0	**n.a.**	3.8	0	**n.a.**	14.2	0	**0**
Tetraconazole	3.8	0.18	**-1.31**	7.3	0.73	**-1.00**	11	0.92	**-1.08**
Total	1219	2.07	**-2.77**	6819	196	**2.9**	8039	198	**-1.61**

Table VII- 8 Mean concentrations and ranges and loads estimates of dissolved pesticides at the inlet and the outlet of the stormwater wetland (Rouffach, Haut-Rhin, France) during fall.

Compound	Concentration range[a]				Load[b]	
	Inlet (n^c=4) [µg L^{-1}]	SE	Outlet (n^c=11) [µg L^{-1}]	SE	Inlet (n^c=2) [mg]	Outlet (n^c=2) [mg]
Azoxystrobin	n.d.	-	n.d.	-	0	0
Cymoxanil	n.d.	-	n.d.	-	0	0
Cyprodinil	n.d.	-	n.d.	-	0	0
Carbendazime	n.d.	-	n.d.	-	0	0
Dimethomorph	0.09 (n.d. - 0.13)	0.06	n.d.	-	3.12	0
Diuron	n.d.	-	n.d.	-	0	0
DCPU	n.d.	-	n.d.	-	0	0
DCPMU	n.d.	-	n.d.	-	0	0
3,4 DCA	0.03 (n.d. - 0.10)	0.05	n.d.	-	1.40	0
Flufenoxuron	n.d.	-	n.d.	-	0	0
Gluphosinate	n.d.	-	n.d.	-	0	0
Glyphosate	0.85 (0.40 - 1.30)	0.42	0.12 (n.d. - 0.40)	0.12	12.2	1.80
AMPA	0.58 (0.50 - 0.60)	0.05	0.57 (0.40 - 0.80)	0.13	14.9	7.20
Isoxaben	n.d.	-	n.d.	-	0	0
Kresoxim methyl	n.d.	-	n.d.	-	0	0
Metalaxyl	n.d.	-	n.d.	-	0	0
Pyrimethanil	n.d.	-	n.d.	-	0	0
Simazine	n.d.	-	n.d.	-	0	0
Terbuthylazine	n.d.	-	n.d.	-	0	0
Tertraconazole	n.d.	-	n.d.	-	0	0

[a] Between October 01 to December 30, 2009

[b] Calculated between October 13 and November 13, 2009 (dissolved loads were not calculated for the whole period (October 01 to December 30, 2009) due to intermittent freezing events starting from November 13 limiting the continuous monitoring of runoff discharge)

[c] Number of analysed samples

Table VII- 9 Physico-chemical characteristics of the bed sediments. Analytical uncertainty is 5% for the major elements.

			Day 0 (March 23rd)			Day 84 (June 15th)			Day 168 (September 7th)		
			50 µm – 250 µm	250 µm - 1mm	>1mm	50µm – 250 µm	250 µm - 1mm	>1mm	50 µm - 250 µm	250 µm - 1mm	>1mm
Physico-chemical composition	OC	[%]	16.67	14.95	13.88	13.47	15.18	15.32	15.90	15.01	15.51
	SiO_2	[%]	49.47	49.62	48.62	49.90	49.60	49.10	50.20	50.30	49.50
	Al_2O_3	[%]	10.29	11.64	11.82	12.48	11.95	12.65	11.17	12.43	11.66
	MgO	[%]	2.04	2.24	2.23	2.15	2.03	1.97	1.93	2.09	1.98
	CaO	[%]	12.90	11.80	14.20	11.55	11.63	11.76	12.28	10.59	12.66
	Fe_2O_3	[%]	4.53	5.15	5.27	5.08	4.76	5.07	4.43	4.78	4.69
	MnO	[%]	0.08	0.09	0.08	0.09	0.08	0.08	0.07	0.07	0.08
	TiO_2	[%]	0.57	0.61	0.60	0.58	0.52	0.56	0.55	0.57	0.54
	Na_2O	[%]	0.61	0.45	0.45	0.40	0.40	-	0.60	0.40	0.40
	K_2O	[%]	2.10	2.36	2.39	2.55	2.55	2.72	2.32	2.70	2.50
	P_2O_5	[%]	0.39	0.36	0.32	0.22	0.21	0.21	0.25	0.21	0.21
	pH	[-]		7.64			n.a.			n.a.	
Texture	Clay	[%]		16			22			38	
	Silt	[%]		25			26			44	
	Sand	[%]		59			52			18	

Table VII- 10. Physico-chemical properties of pesticides, and analytical limits and uncertainties for quantification in water, bed sediments and total suspended solids, and vegetation and invertebrates. Cyazofamid (CYA), cyprodinil (CYP), difenoconazole (DIF), dithiocarbamates (DIT), fludioxonil (FLU), glyphosate (GLY), AMPA, kresoxim methyl (KM), metalaxyl (MET), pyrimethanil (PYR), spiroxamine (SPI) and tetraconazole (TET).

	Unit	CYA	CYP	DIF	DIT	FLU	GLY	AMPA	KM	MET	PYR	SPI	TET
Water solubility (20°C)	[mg L^{-1}]	0.114	13	15	2 - 6.2	1.8	10500	5.8	2.0	26000	121	405	156.6
Log K_{ow}	[-]	3.2	4	4.36	1.33	4.12	-3.2	-1.63	3.40	1.65	2.84	2.89	3.56
DT50 aerobic-anaerobic soil	[day]	10 - n.a.	126 - 183	130 - n.a.	0.1	164 - n.a.	96 - 22	n.a.	2 - 1	n.a.	n.a.	25	n.a.
Aqueous photolysis DT50 (pH=7)	[day]	0.1	13.5	stable	stable	10	69	n.a.	28	stable	stable	50.5	217
Aqueous hydrolysis DT50 (20°C, pH=7)	[day]	25	stable	stable	1.3	stable	stable	n.a.	34	106	stable	stable	stable
EC50	[ppm]	0.19	0.033	0.77	0.073	0.4	40	n.a.	0.186	28	2.9	6.1	3.0
Water													
Detection limit	[µg L^{-1}]	0.03	0.01	0.02	0.02	0.02	0.03	0.03	0.03	0.01	0.02	0.02	0.02
Quantification limit	[µg L^{-1}]	0.1	0.02	0.05	0.05	0.05	0.1	0.1	0.1	0.02	0.05	0.05	0.05
Analytical uncertainty	[%]	24	27	25	21	23	26	24	23	25	26	23	25
Bed sediments and TSS													
Detection limit	[µg kg^{-1}_{dry}]	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
Quantification limit	[µg kg^{-1}_{dry}]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Analytical uncertainty	[%]	33	32	26	45	32	36	37	28	27	30	35	28
Vegetation and invertebrates													
Detection limit	[µg kg^{-1}_{wet}]	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
Quantification limit	[µg kg^{-1}_{wet}]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Analytical uncertainty	[%]	30	21	25	43	23	33	31	25	25	26	25	27

Source: Pesticide Properties Data Base (PPDB) (http://sitem.herts.ac.uk/aeru/projects/ppdb/index.htm)

Eq S1. Detailed calculations of monthly dissipated pesticides and pesticides stored in the wetland compartments are provided in the following equation:

$$\begin{aligned}\text{DEGRADED PESTICIDE LOAD} &= \sum_{i=1}^{4}\left[V_{IN_i}\left(C_{IN\ DISS_i} + (C_{IN\ TSS_i} \times M_{IN\ TSS_i})\right)\right] \\ &- \sum_{i=1}^{4}\left[V_{OUT_i}\left(C_{OUT\ DISS_i} + (C_{OUT\ TSS_i} \times M_{OUT\ TSS_i})\right)\right] \\ &- \left[\sum_{j=1}^{7}(C_j \times Mass_j) + V_{FOREBAY}(C_{FOREBAY\ DISS} + (C_{FOREBAY\ TSS} \times M_{FOREBAY\ TSS})) \right. \\ &\left. + V_{FILTER}(C_{FILTER\ DISS} + (C_{FOREBAY\ TSS} \times M_{FILTER\ TSS}))\right] \end{aligned} \quad (S1)$$

where $V_{IN\ i}$ denotes the inlet water volume and $V_{OUT\ i}$ the outlet water volume, $C_{IN\ DISS\ i}$, $C_{OUT\ DISS\ i}$ and $C_{IN\ TSS\ i}$, $C_{OUT\ TSS\ i}$ are pesticide concentrations measured in the week i, in the dissolved phase (<0.7 µm) and in TSS at the inlet and outlet respectively, and $M_{IN\ TSS\ i}$ and $M_{OUT\ TSS\ i}$ stands for the TSS loads measured at the wetland inlet and the outlet respectively. C_j represents pesticide concentrations and $Mass_j$ the (bio)mass of the wetland compartments j, i.e. algae, *Phragmites australis* roots, *Phragmites australis* aerial parts, invertebrates, bed sediments 50-250 µm, bed sediments 250 µm–1000 µm and bed sediments >1000 µm. Pesticide loads stored in the forebay and the gravel filter water were calculated as the sum of the dissolved and the solid-bound loads where $V_{FOREBAY}$ and V_{FILTER} are the water volumes stored in the forebay and the gravel filter respectively; $C_{FOREBAY\ DISS}$, $C_{FILTER\ DISS}$ and $C_{FOREBAY\ TSS}$, $C_{FILTER\ TSS}$ are the pesticide concentrations measured monthly, and $M_{FOREBAY\ TSS}$ and $M_{FILTER\ TSS}$ represent the TSS loads measured in the forebay and in the gravel filter respectively.

Table VII- 11. Hydrochemistry at the inlet, the outlet and in the forebay of the stormwater wetland (Rouffach, Alsace, France) during the periods 0-56, 56-142 and 142-168 days. Analytical uncertainties were 5% for major ions, metals and carbon concentrations. Precision was ± 0.5% for conductivity and dissolved oxygen measurements, ± 0.004 for pH and ± 10 mV for redox potential.

		Day 0 - Day 56			Day 56 - Day 142			Day 142 - Day 168		
Parameter	**Unit**	**INLET**	**FOREBAY**	**OUTLET**	**INLET**	**FOREBAY**	**OUTLET**	**INLET**	**FOREBAY**	**OUTLET**
D.O.	ppm	n.a.	3.86 ± 4.09	n.a.	n.a.	0.28 ± 0.27	n.a.	n.a.	0.21 ± 0.19	n.a.
Redox	mV	n.a.	47 ± 160	n.a.	n.a.	-44 ± 35	n.a.	n.a.	n.a.	n.a.
pH	-	n.a.	7.34 ± 0.29	n.a.	n.a.	7.08 ± 0.26	n.a.	n.a.	7.03 ± 0.16	n.a.
E.C.	$\mu S\ cm^{-1}$	n.a.	925 ± 130	n.a.	n.a.	513 ± 106	n.a.	n.a.	448 ± 44	n.a.
Temp.	°C	n.a.	11.93 ± 2.54	n.a.	n.a.	16.93 ± 1.75	n.a.	n.a.	15.475 ± 0.10	n.a.
TOC	ppm	44.34 ± 18.21	7.22 ± 3.24	6.16 ± 1.79	26.72 ± 9.33	14.54 ± 13.86	10.3 ± 6.32	21.4 ± 9.93	12.43 ± 0.86	12.88 ± 7.11
DOC	ppm	32.82 ± 11.81	6.6 ± 2.85	5.78 ± 1.39	21.62 ± 8.61	11.8 ± 3.74	8.78 ± 1.01	22.89 ± 9.86	12.47 ± 0.92	12.67 ± 7.00
Al^{3+}	ppm	0.08 ± 0.05	0.05 ± 0.02	0.04 ± 0.03	0.17 ± 0.11	0.04 ± 0.03	0.03 ± 0.01	0.07 ± 0.04	0.03 ± 0.02	0.05 ± 0.08
Fe^{3+}	ppm	0.10 ± 0.09	0.04 ± 0.04	0.01 ± 0.02	0.21 ± 0.14	0.15 ± 0.13	0.09 ± 0.06	0.15 ± 0.05	0.2 ± 0.1	0.54 ± 0.98
Fe^{2+}	ppm	0.80 ± 0.94	0.04 ± 0.03	0.04 ± 0.06	0.51 ± 0.81	0.17 ± 0.17	0.21 ± 0.16	0.09 ± 0.07	0.07 ± 0.06	0.04 ± 0.03
Cu^{2+}	ppb	23.14 ± 13.17	7.32 ± 2.29	7.04 ± 2.72	36.59 ± 19.62	8.48 ± 3.38	5.84 ± 3.41	34.03 ± 13.17	4.91 ± 2.06	3.79 ± 3.64
SO_4^{2-}	ppm	77.92 ± 103.06	204.56 ± 25.72	209.29 ± 22.14	8.68 ± 3.07	48.79 ± 66.08	36.20 ± 46.62	5.58 ± 2.85	4.73 ± 3.49	4.17 ± 2.6
NO_2^-	ppm	0.36 ± 0.21	0.19 ± 0.04	0.11 ± 0.09	0.1 ± 0.11	0.09 ± 0.13	0.09 ± 0.14	0.04 ± 0.08	0.02 ± 0.05	0.04 ± 0.07
NO_3^-	ppm	0.99 ± 0.39	4.44 ± 4.17	1.57 ± 1.55	0.92 ± 0.77	0.41 ± 0.51	0.96 ± 0.15	0.3 ± 0.61	0.36 ± 0.33	0.46 ± 0.56
NH_4^+	ppm	0.16 ± 0.09	0.06 ± 0.09	n.d.	0.09 ± 0.06	0.09 ± 0.15	0.04 ± 0.06	0.03 ± 0.05	0.11 ± 0.25	n.d.
PO_4^{3-}	ppm P	0.43 ± 0.03	0.45 ± 0.03	0.42 ± 0.04	0.64 ± 0.12	0.55 ± 0.17	0.55 ± 0.17	0.53 ± 0.21	0.38 ± 0.04	0.36 ± 0.03

Average Value ± standard deviation

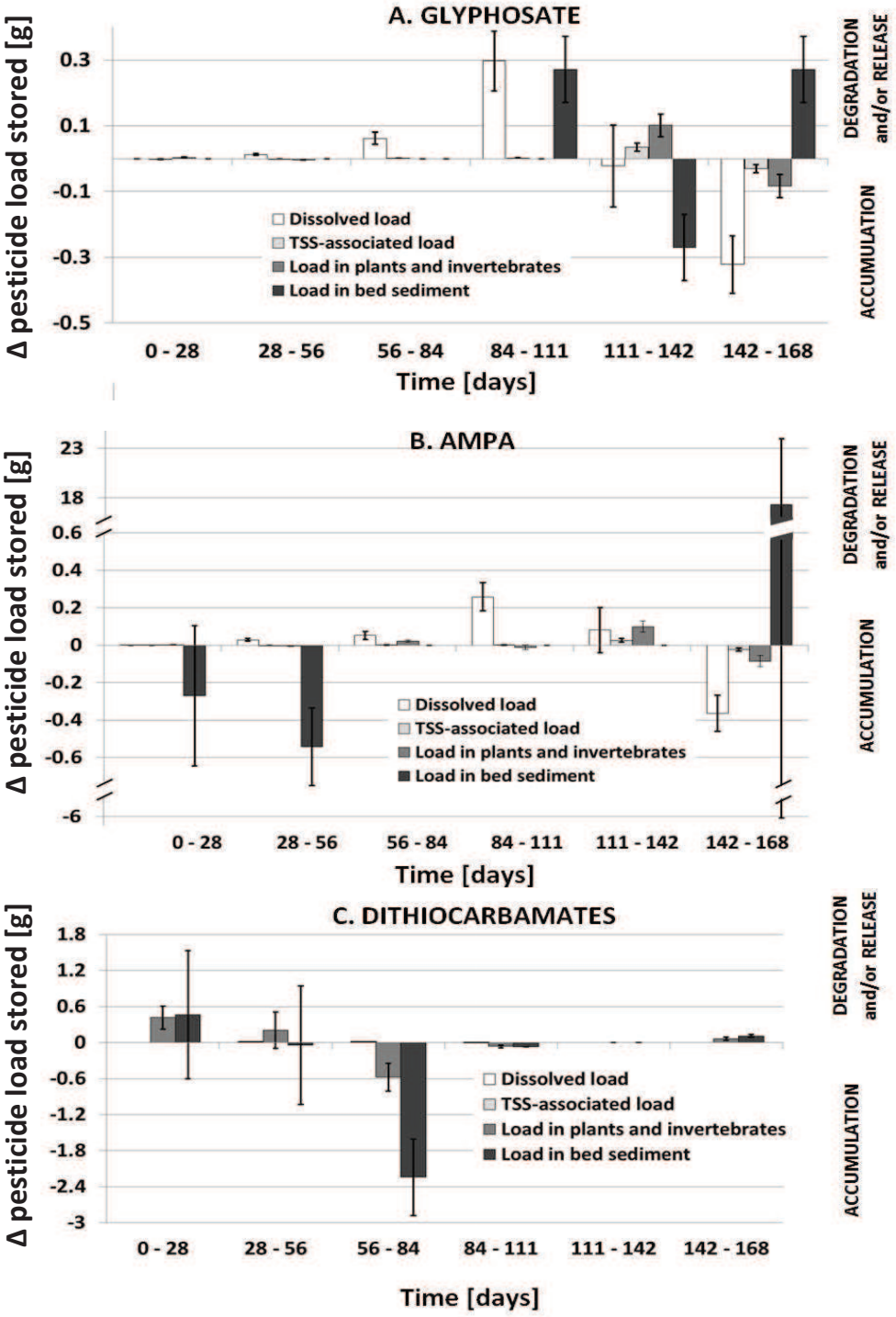


Figure VII- 2. Temporal changes in the pesticide loads stored in the stormwater wetland (Rouffach, Alsace, France) for glyphosate (A), AMPA (B) and dithiocarbamates (C). Days 0, 28, 56, 84, 111, 142 and 168 correspond to the monthly sampling campaigns (i.e. March 23th, April 20th, May 18th, June 15th, July 12th, August 10th and September 7th, 2011).

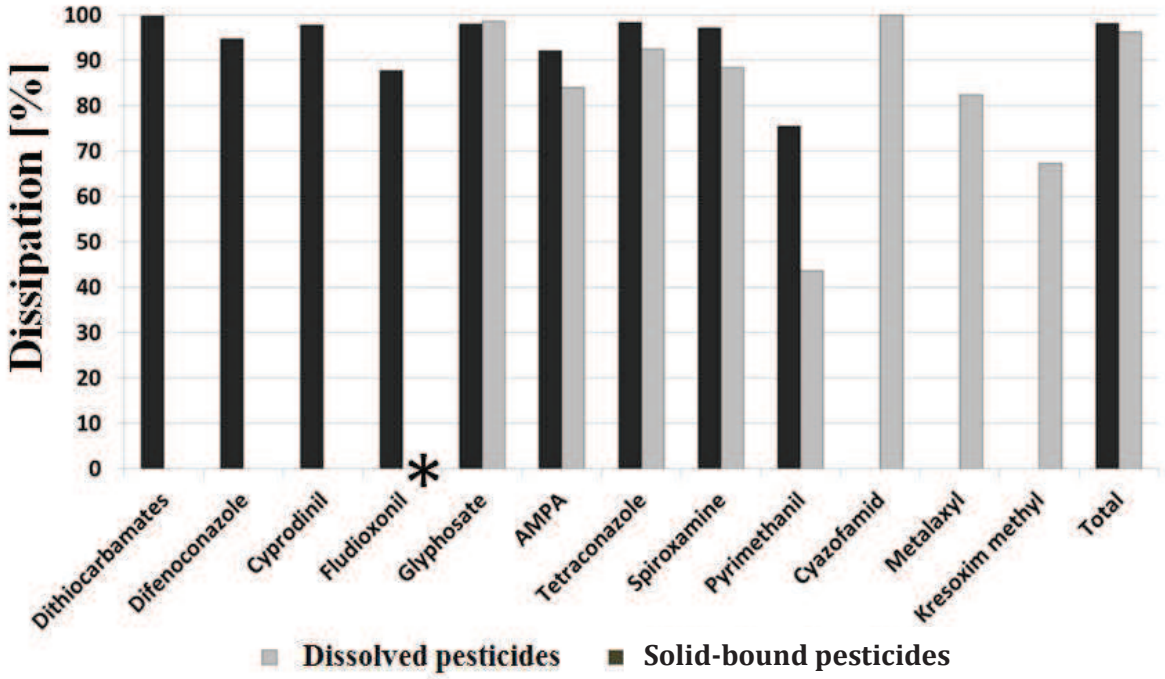


Figure VII- 3. Dissipation rates of dissolved (< 0.7 µm) and solid-bound pesticides (> 0.7 µm) in the stormwater wetland (Rouffach, Alsace, France) from March 23rd to September 7th, 2011.

** Fludioxonil dissipation in the dissolved phase was negative (- 1267%).*

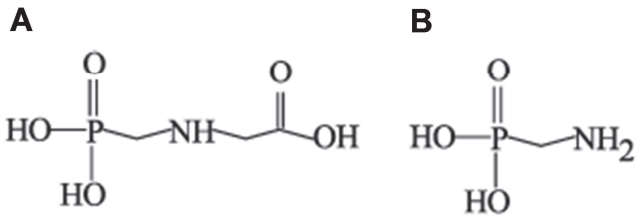


Figure VII- 4 Chemical structures of glyphosate (A) and aminomethyl phosphonic acid (AMPA) (B).

Table VII- 12 Glyphosate commercial preparations and amounts of glyphosate used at the vineyard catchment (Rouffach, Alsace, France) from March 23 to June 30 2009, 2010 and 2011. Values are given in grams of glyphosate.

Commercial formulation	2009	2010	2011
Agave	0	0	2175
Amega max	0	363	0
Catamaran	0	250	0
Glifax	0	0	158
Glyfos	0	0	1954
Prologue	0	0	349
Roundup	715	170	191
Roundup flash	1112	0	540
Touchdown S4	2053	518	0
Total	**3881**	**1303**	**5370**

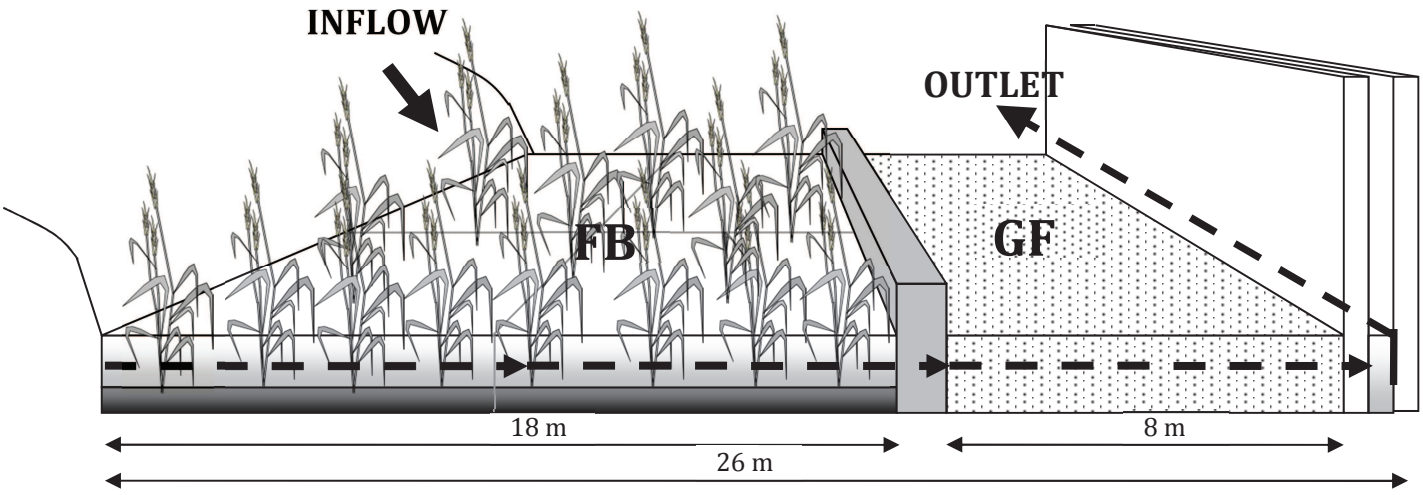


Figure VII- 5 Cross-section of the stormwater wetland (Rouffach, Alsace, France). The dotted line indicates the direction of water flow. FB = forebay, GF = gravel filter.

Table VII- 13 Climatic and hydrological characteristics at the wetland (Rouffach, Alsace, France). Values are provided as the median and ranges from March 23 to June 30 2009, 2010 and 2011.

	Unit	2009				2010				2011			
		April	May	June	April-June	April	May	June	April-June	April	May	June	April-June
Temperature	[°C day^{-1}]	13.6 (8.6 - 17.2)	16.9 (10.9 - 26.7)	18.2 (13.4 - 24.1)	16.1 (8.6 - 26.7)	11.3 (5.1 - 20.9)	11.9 (6.8 – 24)	18.9 (12.2 - 24.5)	14 (5.1 - 24.5)	15.2 (8 - 18.7)	17.4 (0 - 23.3)	18.3 (10.7 - 26.7)	16.7 (8 - 26.7)
Solar radiation	[joules/cm²/day]	1743 (471 – 2409)	2043 (779 - 2909)	2270 (431 - 2897)	1918 (431 - 2909)	1637 (427 - 2531)	1282 (267 - 2896)	2258 (423 - 3000)	1612 (267 - 3000)	2043 (1159 - 2399)	2269 (637 - 2886)	1832 (436 - 2954)	2000 (436 - 2954)
Evaporation	[mm]	101	131	122	353	99	86	126	311	114	137	115	366
Rainfall	[mm]	12	46.6	72.2	130.8	13	69.1	57.1	139.2	3.8	62.7	50.9	117.4
Number of rainfall events	[-]	8	20	14	42	9	15	15	39	3	9	19	31
Number of runoff events	[-]	2	7	8	17	5	13	11	29	1	9	11	21
Rainfall duration	[hs]	1.5 (1.0 - 4.1)	3.9 (0.3 - 13.6)	4.9 (1.9 - 16.7)	3.6 (0.3 - 16.7)	1.8 (0.4 - 5)	8.3 (2.6 - 30.9)	2.7 (0.4 - 18.7)	4.5 (0.4 - 30.9)	0.5 (0.4 - 0.6)	1.1 (0.3 - 10.4)	1.8 (0.1 - 7.4)	1.4 (0.1 - 10.4)
Quiescent period	[day]	4.6 (0.88 - 8.36)	4.1 (0.26 - 28)	2.9 (0.1 - 10.6)	4.1 (0.1 - 28)	3.8 (0.42 - 11.2)	1.1 (0.18 - 7.9)	0.9 (0.15 - 7.4)	1.1 (0.15 - 11.2)	5.4 -	2.3 (0.1 - 28)	1.4 (0.06 - 6)	1.8 (0.06 - 28)
Mean rainfall intensity	[mm h^{-1}]	1.9 (0.4 - 2.6)	0.6 (0.4 - 6.7)	0.9 (0.3 - 9.1)	0.9 (0.3 - 9.1)	1.9 (0.3 - 4.5)	0.4 (0.2 - 1.4)	1 (0.4 - 18)	0.8 (0.2 - 18)	1.5 (1.3 - 4)	4.0 (0.4 - 9.3)	1.0 (0.3 - 14)	1.3 (0.3 - 9.3)
Maximum rainfall intensity	[mm h^{-1}]	4 (2 - 12)	8 (2 - 38)	6 (4 - 30)	6 (2 - 38)	7 (4 - 10)	4 (2 - 5)	6 (2 - 48)	4 (2 - 48)	2 (2 - 6)	6 (2 - 20)	4 (2 - 60)	4 (2 - 60)
Rainfall amount	[mm]	2.3 (1.6 - 3.6)	3 (1.4 - 8.8)	6.2 (1.8 - 17.2)	3.2 (1.4 - 17.2)	2.5 (1.6 - 3.4)	3 (1.4 - 20.8)	2.5 (1.4 - 13.7)	2.6 (1.4 - 20.8)	0.8 (0.6 - 2)	2.8 (0.4 - 7.9)	1.8 (0.4 - 19.4)	2 (0.4 - 19.4)
Runoff coefficient	[%]	1.1 (0.8 - 2.6)	0.3 (0.05 - 2.6)	0.9 (0.11 - 1.3)	0.8 (0.05 - 2.6)	0.6 (0.04 - 0.86)	0.92 (0.17 - 1.98)	0.7 (0.01 - 1.76)	0.78 (0.01 - 1.98)	0.29 -	1.15 (0.11 - 2.39)	1.37 (0.22 - 2.16)	1.19 (0.11 - 2.39)

Table VII- 14 Hydrochemical characteristics at the inlet of the stormwater wetland (Rouffach, Alsace, France) from March 23 to June 30, 2009, 2010 and 2011. Values are provided as the mean and ranges. n.d. = non-detected, below the detection limit; n.a. = not available.

		2009				2010				2011			
	Unit	April	May	June	April-June	April	May	June	April-June	April	May	June	April-June
TSS	[mg L^{-1}]	344	2028 (249-4694)	1025 (240-3702)	1386 (240-4694)	253 (205-323)	296 (62-574)	1929 (134-5639)	891 (62-5639)	n.a.	1752 (112-5972)	11512 (110-2866)	1419 (110-5972)
TOC	[mg L^{-1}]	14.1	28.721 (10.0-31.8)	25.0 (10.0-31.8)	24.0 (10.0-31.8)	28.2	13.2 (5.7-30.3)	44.8 (24.7-97.1)	26.8 (5.7-97.1)	n.a.	44.5 (24.6-60.5)	29.0 (24.6-34.3)	35.9 (24.6-60.5)
DOC	[mg L^{-1}]	13.2	25.6 (21.5-30.6)	22.1 (8.8-31.7)	22.3 (8.8-31.7)	n.a.	9.0 (4.6-24.6)	20.7 (9.2-34.3)	14.0 (4.6-34.3)	n.a.	27.7 (17.3-41.2)	23.8 (17.0-35.1)	25.2 (17.0-41.2)
PO_4^{3-}	[mg L^{-1}]	n.a.	0.03 (n.d.-0.06)	0.06 (n.d.-0.14)	0.05 (n.d.-0.14)	0.08	0.05 (n.d.-0.14)	0.08 (n.d.-0.13)	0.07 (n.d.-0.14)	n.a.	0.15 (0.13-0.18)	0.20 (0.14-0.24)	0.17 (0.13-0.24)
P total	[mg L^{-1}]	1.8	n.a.	2.8 (0.2-7.0)	2.6 (0.2-7.0)	1.0 (0.6-1.3)	0.8 (0.4-1.4)	1.8 (n.d.-3.3)	1.2 (n.d.-3.3)	n.a.	n.d	n.d.	n.d.
NH_4^+	[mg L^{-1}]	0.41	0.26 (n.d.-0.52)	0.15 (n.d.-0.36)	0.20 (n.d.-0.52)	n.d.	0.16 (n.d.-0.48)	0.07 (n.d.-0.40)	0.11 (n.d.-0.48)	n.a.	0.15 (0.10-0.26)	0.13 (0.10-0.15)	0.14 (0.10-0.26)
NO_3^-	[mg L^{-1}]	2.76	0.87 (0.72-1.03)	5.35 (n.d.-9.82)	4.20 (n.d.-9.82)	0.48	0.26 (n.d.-0.58)	0.22 (n.d.-0.78)	0.26 (n.d.-0.78)	n.a.	1.23 (0.64-1.92)	1.33 (0.86-2.47)	1.28 (0.64-2.47)
NO_2^-	[mg L^{-1}]	0.20	1.17 (0.18-2.17)	0.05 (n.d.-0.32)	0.29 (n.d.-1.17)	n.d.	n.d.	n.d.	n.d.	n.a.	0.36 (0.14-0.55)	0.14 (0.10-0.19)	0.25 (0.10-0.55)
SO_4^{2-}	[mg L^{-1}]	n.a.	132.7 (13.7-251.7)	29.0 (8.8-44.0)	63.6 (8.8-251.7)	n.a.	13.3 (5.6-32.0)	12.9 (7.8-17.8)	13.1 (5.6-32.0)	n.a.	15.9 (12.2-24.9)	9.1 (7.1-10.8)	12.1 (7.1-24.9)
Cl^-	[mg L^{-1}]	n.a.	1.42 (1.96-7.31)	4.77 (1.42-7.31)	4.21	6.56	3.29 (n.d.-9.64)	0.91 (n.d.-1.95)	2.51 (n.d.-9.64)	n.a.	2.79 (1.65-3.94)	2.36 (1.84-3.83)	2.55 (1.65-3.94)
Fe^{2+}	[mg L^{-1}]	0.24	0.67 (0.49-0.85)	0.48 (n.d.-1.28)	0.49 (n.d.-1.28)	0.0732	0.09 (0.03-0.30)	0.24 (0.03-0.80)	0.15 (0.03-0.80)	n.a.	1.4 (0.1-2.6)	0.16 (0.11-0.30)	0.78 (0.1-0.30)
Fe total	[mg L^{-1}]	9.362	63.8 (26.1-101.5)	23.1 (3.4-65.7)	35.5 (3.4-101.5)	0.2 (0.1-0.3)	0.2 (0.1-0.4)	0.2 (0.1-0.4)	0.2 (0.1-0.4)	n.a.	1.4 (0.3-3.1)	0.3 (0.2-0.4)	0.8 (0.2-3.1)
Cu^{2+}	[mg L^{-1}]	n.d.	0.1 (n.d.-0.3)	0.1 (n.d.-0.3)	0.1 (n.d.-0.3)	41	21.1 (8.0-44.0)	26.8 (9.0-56.0)	24.5 (8.0-56.0)	n.a.	23.5 (13.2-38.1)	31.5 (26.0-43.5)	28.0 (13.2-43.5)
Na^+	[mg L^{-1}]	8.08	4.6 (3.4-5.2)	3.0 (n.d.-9.5)	4.0 (n.d.-9.5)	4.2 (2.5-6.9)	3.4 (1.6-6.9)	3.2 (1.9-5.0)	3.5 (1.6-6.9)	n.a.	3.9 (2.3-6.0)	3.8 (2.5-5.3)	3.8 (2.3-6.0)
K^+	[mg L^{-1}]	4.11	11.3 (4.6-18.8)	10.5 (2.1-19.3)	10.2 (2.1-19.3)	11.2 (2.7-28.5)	9.8 (3.0-28.6)	1.9 (1.2-2.4)	7.3 (1.2-28.6)	n.a.	5.0 (3.5-7.8)	3.9 (3.0-4.9)	4.4 (3.0-7.8)
Ca^{2+}	[mg L^{-1}]	110.7	158.2 (97.5-253.0)	79.9 (14.4-193.5)	108.5 (14.4-253.0)	47.9 (23.7-62.9)	36.3 (22.3-62.9)	35.3 (23.8-50.6)	38.3 (22.3-62.9)	n.a.	49.4 (39.1-65.0)	38.7 (35.7-51.5)	43.5 (35.7-65.0)
Mg^{2+}	[mg L^{-1}]	45.1	12.8 (4.7-22.4)	8.8 (1.2-27.2)	13.2 (1.2-45.1)	2.9 (1.4-4.2)	1.7 (0.9-3.7)	1.8 (1.2-2.8)	2.0 (0.9-4.2)	n.a.	2.3 (1.0-4.9)	1.4 (1.1-1.7)	1.8 (1.0-4.9)
Mn^{2+}	[mg L^{-1}]	n.d.	1.1 (0.3-2.1)	0.4 (n.d.-1.2)	0.6 (n.d.-2.1)	0.1 (n.d.-0.4)	0.1 (n.d.-0.5)	0.2 (n.d.-0.6)	0.15 (n.d.-0.6)	n.a.	0.3 (0.2-0.4)	0.1 (n.d.-0.2)	0.2 (n.d.-0.4)
Si^{2+}	[mg L^{-1}]	n.a.	n.a.	n.a.	n.a.	4.393	2.2 (1.3-4.4)	2.4 (1.9-3.5)	2.4 (1.3-4.4)	n.a.	1.9 (1.5-2.5)	1.8 (1.2-2.5)	1.9 (1.2-2.5)

Table VII- 15 Hydrochemical characteristics at the outlet of the stormwater wetland (Rouffach, Alsace, France) from March 23 to June 30 2009, 2010 and 2011. Values are provided as the mean and ranges. n.d. = non detected, below the detection limit; n.a. = not available.

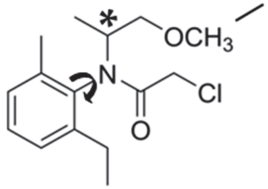
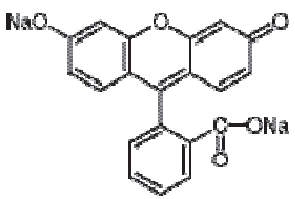
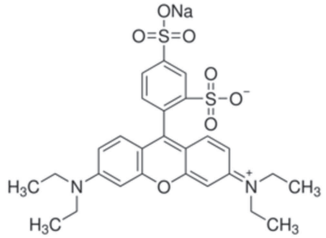
		2009				2010				2011			
	Unit	April	May	June	April-June	April	May	June	April-June	April	May	June	April-June
Redox	[mV]	253 (247-260)	126 (5-212)	201 (147-283)	178 (5-283)	5 (-69-93)	-157 (-218- -52)	-37 (-201-94)	-80 (-218-94)	248 (181-334)	208 (107-307)	287 (286-306)	251 (107-334)
D.O.	[mg L^{-1}]	7.8 (7.2-8.4)	6.9 (6.3-8.6)	6.3 (4.7-7.3)	6.8 (4.7-8.6)	10.2 (9.4-11.3)	9.2 (7.4-10.9)	10.8 (9.2-13.3)	10.0 (7.4-13.3)	8.5	1.9 (1.2-2.5)	1.5 (0.5-2.1)	2.4 (0.5-8.5)
Conductivity	[µS cm^{-1}]	1504 (1402-1605)	909 (835-1022)	947 (877-1052)	1006 (835-1605)	959 (867-1069)	904 (389-1076)	626 (1-1019)	819 (1-1076)	965 (891-1021)	947 (824-1019)	567 (424-692)	807 (424-1021)
pH	[-]	8.1-8.1	8.0-8.2	7.7-8.1	7.7-8.2	6.2-7.9	7.3-7.9	7.5-8.1	6.2-8.1	7.4-7.8	7.6-7.7	7.6-8.1	7.4-8.1
TSS	[mg L^{-1}]	2.7 (n.d.-7.0)	6.5 (3.4-14.0)	1681.2 (2.2-7568.0)	656.7 (n.d.-7568.0)	23.8 (7.2-41.1)	40.0 (3.5-203.8)	17.7 (8.8-36.6)	28.7 (3.5-203.8)	36.2 (5.4-96.4)	23.1 (11.9-47.2)	34.1 (4.9-69.8)	31.3 (4.9-96.4)
TOC	[mg L^{-1}]	19.0 (8.7-15.2)	14.3 (13.0-16.6)	19.4 (16.5-22.5)	15.2 (8.7-22.5)	5.3 (4.5-6.4)	4.6 (4.4-5.2)	7.7 (4.4-10.6)	5.7 (4.4-10.6)	6.1 (4.7-7.6)	7.9 (5.6-10.0)	13.5 (8.1-29.1)	9.2 (4.7-29.1)
DOC	[mg L^{-1}]	12.1 (8.8-17.4)	14.4 (12.8-16.1)	19.8 (16.5-22.8)	15.8 (8.8-22.8)	5.5 (4.0-9.5)	4.7 (3.8-5.8)	6.6 (4.3-8.6)	5.5 (3.8-9.5)	5.8 (5.2-6.3)	7.4 (4.6-9.4)	8.9 (8.0-10.6)	7.3 (4.6-10.6)
PO_4^{3-}	[mg L^{-1}]	0.07 (0.03-0.10)	0.02 (n.d.-0.08)	n.d.	0.03 (n.d.-0.10)	0.01 (n.d.-0.02)	n.d.	0.03 (n.d.-0.13)	0.01 (n.d.-0.13)	0.13 (0.12-0.16)	0.14 (0.13-0.31)	0.20 (0.15-0.27)	0.31 (0.12-0.27)
P total	[mg L^{-1}]	1.3 (0.6-2.0)	0.14 (n.d.-0.43)	0.34 (n.d.-0.60)	0.5 (n.d.-2.0)	0.03 (n.d.-0.17)	0.28 (n.d.-0.88)	0.47 (n.d.-1.83)	0.28 (n.d.-1.83)	n.d.	n.d.	n.d.	n.d.
NH_4^+	[mg L^{-1}]	0.09 (0.06-0.10)	0.05 (n.d.-0.11)	0.08 (n.d.-0.26)	0.07 (n.d.-0.26)	0.03 (n.d.-0.13)	0.04 (n.d.-0.27)	n.d.	0.02 (n.d.-0.27)	n.d.	n.d.	n.d.	n.d.
NO_3^-	[mg L^{-1}]	16.87 (13.21-19.60)	3.41 (n.d.-8.89)	4.74 (1.59-13.64)	7.7 (n.d.-19.6)	0.53 (0.48-0.64)	0.03 (n.d.-0.16)	0.02 (n.d.-0.13)	0.13 (n.d.-0.64)	1.06 (0.57-1.64)	0.67 (0.45-0.81)	0.53 (n.d.-1.13)	0.76 (n.d.-1.64)
NO_2^-	[mg L^{-1}]	0.09 (0.03-0.18)	0.1 (n.d.-0.24)	0.1 (n.d.-0.53)	0.10 (n.d.-0.53)	n.d.	0.004 (n.d.-0.04)	n.d.	0.002 (n.d.-0.04)	0.33 (n.d.-1.13)	0.16 (0.12-0.21)	0.12 (n.d.-0.48)	0.20 (n.d.-1.13)
SO_4^{2-}	[mg L^{-1}]	201.4 (175.0-240.0)	223.1 (139.9-314.4)	267.6 (155.7-538.7)	230.2 (139.9-538.7)	206.9 (183.0-226.6)	156.3 (52.4-251.7)	80.3 (n.d.-198.7)	145.6 (n.d.-226.5)	215.9 (188.2-231.8)	195.0 (152.3-237.2)	52.8 (20.8-69.7)	154.6 (20.8-237.2)
Cl^-	[mg L^{-1}]	30.9 (25.8-38.0)	27.4 (24.4-31.6)	30.1 (28.5-34.5)	29.3 (24.4-38.0)	26.0 (24.4-31.7)	16.2 (5.8-23.4)	16.8 (5.9-25.6)	19.1 (5.8-31.7)	26.5 (23.8-27.7)	20.5 (13.8-26.4)	6.6 (5.3-8.6)	17.9 (5.3-27.7)
Fe^{2+}	[mg L^{-1}]	0.02 (n.d.-0.04)	0.02 (n.d.-0.06)	0.09 (n.d.-0.27)	0.05 (n.d.-0.27)	n.d.	0.02 (n.d.-0.08)	0.08 (n.d.-0.28)	0.03 (n.d.-0.28)	0.02 (n.d.-0.03)	0.2 (n.d.-0.5)	0.3 (0.3-0.4)	0.2 (n.d.-0.5)
Fe total	[mg L^{-1}]	2.3 (n.d.-11.4)	n.d.	0.9 (n.d.-3.6)	1.0 (n.d.-11.4)	0.1 (0.1-0.1)	0.1 (0.03-0.42)	0.1 (0.02-0.18)	0.09 (0.02-0.42)	0.02 (n.d.-0.03)	0.22 (n.d.-0.68)	0.3 (0.1-0.5)	0.19 (n.d.-0.68)
Cu^{2+}	[mg L^{-1}]	n.d.	n.d.	n.d.	n.d.	n.a.	8.8 (5.0-16.0)	7.5 (7.0-8.0)	8.3 (5.0-16.0)	5.8 (3.2-9.3)	8.1 (3.7-10.6)	4.4 (2.4-7.5)	6.10 (2.4-10.6)
Na^+	[mg L^{-1}]	11.0 (6.0-13.7)	9.8 (1.6-14.3)	6.1 (n.d.-9.4)	8.8 (n.d.-14.3)	10.7 (10.1-11.2)	9.4 (5.7-11.4)	7.6 (5.3-12.0)	9.04 (5.3-12.0)	12.1 (10.8-12.7)	11.1 (9.2-12.6)	5.8 (3.9-7.1)	9.7 (3.9-12.7)
K^+	[mg L^{-1}]	3.7 (0.7-6.5)	5.6 (0.9-12.8)	6.8 (1.2-14.9)	5.4 (0.7-14.9)	4.3 (3.3-5.0)	2.9 (2.0-4.9)	1.0 (0.5-2.4)	2.5 (0.5-5.0)	2.7 (1.9-3.3)	2.6 (2.0-4.5)	1.8 (1.2-2.3)	2.4 (1.2-4.5)
Ca^{2+}	[mg L^{-1}]	115.6 (100.3-130.1)	152.3 (111.4-207.2)	119.3 (91.2-147.2)	128.4 (91.2-207.2)	122.6 (107.0-137.0)	121.1 (53.5-145.0)	99.4 (71.5-149.0)	113.9 (53.5-149.0)	133.2 (125.3-139.5)	133.0 (108.9-146.0)	87.5 (63.8-104.1)	117.9 (63.8-146.0)
Mg^{2+}	[mg L^{-1}]	41.8 (34.0-45.0)	28.6 (25.2-32.4)	32.3 (25.4-37.5)	34.1 (25.2-45.0)	40.3 (34.5-47.1)	39.5 (12.7-50.3)	25.1 (11.0-41.7)	34.6 (11.0-50.3)	49.8 (42.5-54.3)	50.6 (38.4-57.6)	27.1 (16.0-34.4)	42.5 (16.0-57.6)
Mn^{2+}	[mg L^{-1}]	n.d.	0.1 (n.d.-0.4)	1.4 (n.d.-2.7)	0.6 (n.d.-2.7)	0.3 (0.02-0.9)	1.1 (0.03-1.76)	0.5 (0.003-1.1)	0.72 (0.003-1.76)	0.3 (0.2-0.7)	0.9 (0.7-1.2)	0.4 (0.2-0.8)	0.6 (0.2-1.2)
Si^{2+}	[mg L^{-1}]	n.a.	n.a.	n.a.	n.a.	n.a.	3.2 (2.5-3.6)	4.0 (3.0-4.8)	3.5 (2.5-4.8)	3.8 (3.5-4.2)	3.9 (3.7-4.1)	4.0 (3.0-4.5)	3.9 (3.0-4.5)

Table VII- 16. Correlation (Spearman Rank Sum test) between hydrological characteristics (i.e. inlet discharge and the quiescent period) and glyphosate transfer (i.e. mass equivalent load of glyphosate entering the wetland (MELgly in); fraction of AMPA entering (AMPA in) and outflowing (AMPA out) the wetland; removal of MELgly by the wetland) through the stormwater wetland (Rouffach, Alsace, France) from March 23 to June 30 2009, 2010 and 2011.

	Glyphosate	Rho[1]	p-value
Inlet discharge	$MEL_{gly\ in}$	0.76	< 0.001
	$\%AMPA_{in}$	0.67	0.263
	$\%AMPA_{out}$	-0.23	0.085
	MEL_{gly} removal	0.68	< 0.001
Quiescent period	$MEL_{gly\ in}$	-0.62	< 0.001
	$\%AMPA_{in}$	-0.35	0.024
	$\%AMPA_{out}$	0.18	0.263
	MEL_{gly} removal	-0.61	< 0.001

[1]Spearman Rank Sum coefficient

Table VII- 17 Physico-chemical properties and molecular structures of the injected molecules S-metolachlor (S-MET), uranine (UR), sulforhodamine (SRB) and bromide (Br).

Property	Unit	S-MET	UR	SRB	BR
Molecular formula	[-]	$C_{15}H_{22}ClNO_2$[a]	$C_{20}H_{10}O_5Na_2$[c]	$C_{27}H_{30}O_7N_2S_2Na_2$[c]	Br
Molecular weight	g mol^{-1}	283.79[a]	376.15[c]	604.67[c]	102.89[d]
Aqueous solubility	[g L^{-1}]	0.48[a]	25[d]	70[d]	850
DT50 photolysis	[days]	stable[a]	0.5[c]	34[d]	stable[d]
DT50 hydrolysis	[days]	stable[a]	stable[c]	stable[c]	stable[d]
Organic carbon - water partitioning K_{oc}	L kg^{-1}	226[b]	0.62[e]	147 498[e]	-
Octanol - water partitioning (Log K_{ow})	[-]	3.4[a]	0.67[f]	-	-
Molecular structure	[-]				

[a]*Source: Pesticide properties database online (http://sitem.herts.ac.uk/aeru/projects/ppdb/index.htm)*

[b]*Source: European Commission, 2004 (ec.europa.eu/food/plant/protection/evaluation/newactive/s_metolachlor.pdf)*

[c]*Source: Gaspar, 1987*

[d]*Source: Leibundgut et al., 2009*

[e]*Source: based on K_d values from Sabatini, 2000*

[f]*Source: Merck Millipore (http://www.merckmillipore.de)*

Table VII- 18. Meteorological and vegetation data.

Dates	Precipitation	Mean wind speed	Mean global radiation	Mean ETP Penman	Continuous flow SSFCW		Batch flow SSFCW	
					Plant height	Plant density	Plant height	Plant density
[days]	[mm]	[km h^{-1}]	[Joules cm^{-2}]	[mm]	[cm]	plants m^{-2}	[cm]	plants m^{-2}
05/24 - 05/31	2.21	2.1 (1.4 - 3.4)	2558 (2075 - 2958)	5.9 (4.3 - 7.7)	40 (8 - 95)	45	45 (7 - 77)	50
05/31 - 06/07	4.17	1.9 (1.6 - 2.3)	1678 (644 - 2884)	3.8 (2.3 - 4.8)	55 (37 - 96)	48	60 (40 - 90)	60
06/07 - 06/14	37.93	1.9 (1.1 - 2.5)	1723 (812 - 2548)	3.5 (1.2 - 5.4)	50 (5 - 105)	53	52 (5 - 93)	64
06/14 - 06/21	6.19	1.6 (1.3 - 2)	1891 (1403 - 2367)	3.9 (2.9 - 5.3)	62 (7 - 108.5)	65	47 (11 - 92)	55
06/21 - 06/28	7.01	1.6 (1.1 - 2.4)	2446 (1712 - 3123)	4.5 (3.5 - 5.9)	67 (9 - 111)	80	47 (11 - 90)	50
06/28 - 07/05	39.35	1.1 (0.7 - 1.9)	1738 (466 - 2638)	3.8 (1.3 - 6.6)	71 (8 - 110)	110	47 (9 - 92)	50
07/05 - 07/12	15.88	1.5 (1.1 - 2.1)	2134 (1913 - 2852)	4.3 (3.8 - 4.9)	75 (8 - 112)	130	44 (17 - 94)	42
07/12 - 07/19	6.41	1.5 (1.2 - 2)	1794 (928 - 2551)	3.8 (2.4 - 4.9)	76 (17 - 116)	142	47 (12 - 94)	36
07/19 - 07/26	8.99	1.1 (0.2 - 1.6)	2235 (951 - 2898)	4.1 (2.3 - 5.4)	77 (23 - 111)	145	64 (13 - 122)	38
07/26 - 08/02	13.55	1 (0.9 - 1.2)	2121 (1339 - 2660)	4.5 (3.7 - 5.6)	77 (23 - 112)	143	50 (13 - 123)	32
08/02 - 08/09	10.95	1.2 (0.8 - 1.6)	1851 (809 - 2565)	3.9 (2.7 - 4.7)	78 (22 - 112)	145	50 (12 - 122)	35
08/09 - 08/16	12.44	1.1 (0.8 - 1.4)	2164 (1765 - 2548)	4.2 (3.3 - 5.2)	78 (24 - 109)	143	*n.a.*	*n.a.*

Table VII- 19. Analytical data of the GC-MS/MS quantification of S-metolachlor and the LC-MS quantification of its degradation products metolachlor ESA and metolachlor OXA.

		Molecules	Quantification	Identification	Retention time [min]	Recovery [%]	SD [%]
GC-MS/MS			daughter ion 1 m/z	daughter ion 2 m/z			
	Parent compound	Metolachlor	162	133	16.78	96	8
	Internal standard	Metolachlor-d_6	134	-	16.71	-	-
LC-MS			Transition SRM1	Transition SRM2			
	Degradation products	Metolachlor OXA	206	172	11.36	3.28	3.97
		Metolachlor ESA	121	192	9.9	0.58	0.42
	Internal standard	Alachlor-d_{13}	251	175	18.3	-	-

Table VII- 20 Water balances of the two subsurface flow constructed wetlands.

		Continuous flow SSFCW				Batch flow SSFCW			
Date	Precipitation		Inflow	ETP	Outflow	Residual volume	Inflow	ETP	Outflow
	[L]		[L]	[L]	[L]	[L]	[L]	[L]	[L]
24/5 - 31/5	16	First step injection	923	499	441	Batch 1			
31/5 - 7/6	30		826	223	638	0	600	254	175
7/6 - 14/6	273		837	129	1069				
14/6 - 21/6	45		865	189	746	Batch 2			
21/6 - 28/6	51		1221	523	750	490	110	319	164
28/6 - 5/7	283		1243	245	1824				
5/7 - 12/7	114	Second step injection	194	93	394	Batch 3			
12/7 - 19/7	46		994	114	1044	495	105	379	195
19/7 - 26/7	65		1300	168	1496				
26/7 - 2/8	98		400	141	403	Batch 4			
2/8 - 9/8	79		474	375	186	250	350	381.968	234
9/8 - 16/8	90		773	202	720				

Table VII- 21 Detailed hydrochemical data from the inlet, outlet and piezometers of the two subsurface flow constructed wetlands.

		Batch-flow SSFCW			Continuous flow SSFCW		
Parameter	**Unit**	**Inlet**	**Piezometers**	**Outlet**	**Inlet**	**Piezometers**	**Outlet**
FeII	[mg L^{-1}]	0.04 ± 0.04	0.02 ± 0.03	0.04 ± 0.05	0.01 ± 0.02	0.01 ± 0.02	0.01 ± 0.02
FeIII	[mg L^{-1}]	0.03 ± 0.03	0.14 ± 0.42	0.03 ± 0.24	0.01 ± 0.01	0.06 ± 0.23	0.03 ± 0.05
COT	[mg L^{-1}]	24.58 ± 11.99	7.46 ± 7.59	11.99 ± 11.55	4.77 ± 3	3.09 ± 3.27	4.74 ± 5.62
CIT	[mg L^{-1}]	25.78 ± 14.77	42.61 ± 16.35	14.77 ± 49.87	27.27 ± 13.91	35.72 ± 8.95	39.13 ± 12
COD	[mg L^{-1}]	23.63 ± 11.48	23.09 ± 60.36	11.48 ± 16.9	4.78 ± 2.75	6.72 ± 15.19	5.6 ± 8.35
CID	[mg L^{-1}]	22.98 ± 16.12	39.81 ± 13.91	16.12 ± 39.21	26.66 ± 14.04	31.49 ± 8.58	34.82 ± 10.68
Cl	[mg L^{-1}]	215.75 ± 161.56	166.75 ± 123.11	161.56 ± 91.77	136.09 ± 44.61	163.6 ± 57.79	152.57 ± 55.75
SO4	[mg L^{-1}]	39.25 ± 2.99	43.9 ± 6.68	2.99 ± 79	39.25 ± 3.08	42.52 ± 12.75	43.09 ± 16.14
Pt	[mg L^{-1}]	0.25 ± 0.28	0.14 ± 0.2	0.28 ± 0.15	0.18 ± 0.27	0.11 ± 0.22	0.2 ± 0.33
NH4	[mg N L^{-1}]	0.15 ± 0.18	0.05 ± 0.12	0.18 ± 0.08	0.21 ± 0.35	0.11 ± 0.16	0.08 ± 0.14
NO2	[mg N L^{-1}]	0.94 ± 1.87	0.09 ± 0.13	1.87 ± 0	0.2 ± 0.18	0.12 ± 0.18	0.08 ± 0.11
NO3	[mg N L^{-1}]	11.85 ± 13.72	7.03 ± 7.96	13.72 ± 4.96	20.86 ± 10.62	12.25 ± 10.88	8.03 ± 8.41
PO4	[mg P L^{-1}]	0.22 ± 0.21	0.29 ± 0.12	0.21 ± 0.38	0.35 ± 0.21	0.34 ± 0.19	0.31 ± 0.14
Mn	[mg L^{-1}]	4.83 ± 3.9	34.38 ± 33.42	3.9 ± 344.48	3.06 ± 3.85	68.42 ± 92.22	48.31 ± 67.77
DO	[mg L^{-1}]	*n.a.*	3.15 ± 1.79	*n.a.*	*n.a.*	1 ± 1.33	0.12 + 0.63
Redox	[mV]	*n.a.*	130.59 ± 73.19	*n.a.*	*n.a.*	109.18 ± 88.11	-467.74 + 92.25
pH	[-]	*n.a.*	7.35 ± 0.19	*n.a.*	*n.a.*	7.41 ± 0.19	7.13 + 0.18
Conductivity	[µS cm^{-1}]	*n.a.*	1102.79 ± 208.31	*n.a.*	*n.a.*	872.54 ± 102.34	733.21 + 124.43
Temperature	°C	*n.a.*	20.06 ± 1.52	*n.a.*	*n.a.*	20.39 ± 1.68	19.28 + 1.33

** Mean ± SD*

Analysis of chloroacetanilide herbicides and degradation products

1. Preparation and extraction from sand and plant samples

The same protocol was applied for the extraction of sand and plant samples. Briefly, 5 g of sample (sand or plant) were extracted with 4 mL of ACN/pure water (v:v 60/40), shaked for 1 min (vortex), incubated for 30 min at 115°C, shaked for 1 min, centrifuged during 10 min at 3500 rpm. The supernatant was then collected and a second extraction was carried out, using the same protocol. 0.1% H_3PO_4 were added to the sample for the second extraction. The 8 mL-sample was then filtered using a 0.2 µm PTFE filter and evaporated. 50 mL of pure water were added to the sample and were extracted by solid-phase extraction (see below).

2. Solid phase extraction

Solid-phase extraction was carried out with 10 mL water samples using SolEx C18 cartridges (Dionex®, CA, USA) packed with 100 mg bonded silica. AutoTrace 280 SPE system was used for simultaneous extraction of 6 samples. The extraction cartridges were washed with 5 ml of ethyl acetate, followed by 5 mL of methylene chloride and sequentially conditioned by 10 mL of methanol and 10 mL of deionised water. Cartridges were then loaded with the samples and dried with nitrogen for 10 min. Elution of the chloroacetanilide herbicides and their degradation products was performed by 3 mL followed by 2 mL of ethyl acetate and methylene chloride respectively. Finally, the extract was concentrated under nitrogen flux to 1 droplet, and 2 mL of methylene chloride were added.

2. Analysis of alachlor, metolachlor and acetochlor

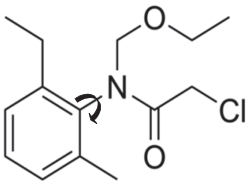
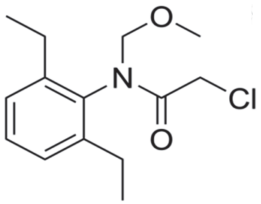
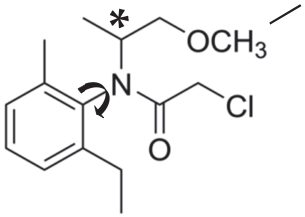
Chloroacetanilide herbicides were quantified using a Focus-ITQ 700 model GC-MS/MS apparatus from Thermo Scientific (Les Ulis, France) and Xcalibur (version 2.0.7) for data acquisition. Metolachlor, acetochlor and alachlor separations were conducted on a, 30 m x 0.25 mm ID, 0.25 µm film thickness OPTIMA 5MS (5% phenyl - 95% dimethylpolysiloxane) fused-silica capillary column (Macherey Nagel GmbH, Düren, Germany), with helium as a carrier gas, at a flow rate of 1 mL min-1. The oven was held at 50°C for 2 min, ramped at 30°C min-1 to 150°C, then up to 250°C at 5°C min-1 and finally ramped at 30°C min-1 to 300°C and held for 5 min. A volume of 3 µL of sample was injected on a split/splitless injector (pulsed splitless at 2.5 mL min^{-1} for 1 min) using an AI/AS 3000 autosampler (Thermo Fisher Scientific, Les Ulis, France). The injector

temperature and transfer line were set at 280°C and 300°C. The mass spectrometer was operated in the electron ionization mode (EI, 70 eV). The ion source temperature was maintained at 210°C. For GC-MS/MS analysis, 10 µL of internal standards (final concentration 100 µg L-1) were added to 190 µL of water samples. Detection limits were 1.7, 0.7 and 0.7 µg L^{-1} and quantification limits were 5, 2, 2 µg L^{-1} for acetochlor, alachlor and metolachlor, respectively. Retention times, selected ions used for identification and recovery are detailed in Table VII-20.

3. Analysis of ESA and OXA degradation products

Ethane sulfonic (ESA) and oxanilic acids (OXA) degradation products of metolachlor (MESA and MOXA), alachlor (AlESA and AlOXA) and acetochlor (AcESA and AcOXA) were analysed using a TSQ Quantum ACCESS LC/MS equipped with a Thermo Scientific Accela autosampler with a temperature-controlled sample tray (15°C) (Les Ulis, France). Xcalibur (version 2.1.0) was used for data acquisition. Injection volume was 20 µL. The mobile phase consisted of 0.1% formic acid/high-purity water (A) and 0.1% formic acid/acetonitrile (B). The gradient program started with 35% B held for 5 min, then B increased from 35% to 95% for 16 min, 95% B was held for 5 min, then B decreased from 95% to 35% for 1 min and held at 35% for 5 min. The flow rate was 0.3 mL min-1. The analytical column was a EC 150/3 Nucleodur Polar Tec (particle size 3µm, length 150 mm, internal diameter 3 mm) and a precolumn EC 4/3 Polar Tec, 30 mm (Macherey Nagel, France). Column oven temperature was set at 60°C to achieve better separation and peak shapes. The mass spectrometer (MS) was a Thermo TSQ Quantum triple quadrupole mass spectrometer (Les Ulis, France) operated using a heated electrospray ionization (HESI) source. The mass spectra were recorded in the negative ion mode (spray voltage: 3500 V) for the 6 degradation products and in the positive mode (spray voltage: 4250 V) for the internal standard Alachlor-d13. The vaporiser temperature was 300°C, sheath gas N2 pressure 10 (arbitrary units), auxiliary gas pressure 20 (arbitrary units), ion sweep gas pressure 0 and the ion transfer capillary temperature 300°C. The best sensitivity in multiple reaction monitoring operation was achieved through the acquisition of selected reaction monitoring (SRM) transitions with MRM mode. For identification of the studied compounds, two SRM transitions and a correct ratio between the abundances of the two optimized SRM transitions (SRM1/SRM2) were used along with retention time matching. Limits of detection were 0.06, 0.02, 0.02, 0.10, 0.06, 0.04 µg L-1 and limits of quantification were 0.10, 0.02, 0.04, 0.16, 0.10, 0.06 µg L-1 for AcOXA, AcESA, AlOXA, AlESA, MOXA and MESA, respectively. Information about SRM transitions and analytical uncertainties are provided in Table VII-20.

Table VII- 22 Physico-chemical characteristics of the chloroacetanilide herbicides.

Properties	Unit	Acetochlor	Alachlor	Metolachlor
Formula	-	$C_{14}H_{20}ClNO_2$	$C_{14}H_{20}ClNO_2$	$C_{15}H_{22}ClNO_2$
Molecular weight	**g mol^{-1}**	269.77	269.77	283.8
Molecular structure	-			
Solubility in water (20°C)	**mg l^{-1}**	282	240	530
Henry constant (25°C)	**Pa m^3 mol^{-1}**	2.1 x 10^{-03}	3.20 x 10^{-03}	2.40 x 10^{-03}
log $_{Kow}$ (pH 7, 20°C)	-	4.14	3.09	3.4
Koc	**ml g^{-1}**	156	124	120
DT50 soil (aerobic)	**days**	14	14	90
DT50 (aerobic) (20°C, field)	**days**	12.1	14	21
DT50 photolysis (pH 7)	**days**	stable	0.5	stable
DT50 hydrolysis (pH 7, 20°C)	**days**	stable	30 (PAN)	stable
DT50 sediments	**days**	19.7	2	365
DT50 water	**days**	40.5	-	88

Source: Pesticide properties database online
(http://sitem.herts.ac.uk/aeru/projects/ppdb/index.htm)

Table VII- 23 Physicochemical properties of the filling materials used for the lab-scale wetlands.

	Parameters	Units	Fine gravel	Medium sand
Physical properties	Grain size	[mm]	1 - 2	0.4 - 0.63
	Porosity	[vol %]	41.9 ± 0.1	43.9 ± 0.8
	Bulk density	[g cm^{-3}]	0.69 ± 0.02	0.72 ± 0.01
	Hydraulic conductivity	[m s^{-1}]	1.3 10^{-3} ± 3.8 10^{-5}	1.04 10^{-3} ± 1.5 10^{-4}
Chemical composition	Organic carbon		0.15 ± 0.05	0.31 ± 0.08
	SiO_2		91.2	98.5
	Al_2O_3		4.2	1.0
	MgO		0.1	-
	CaO		0.2	-
	Fe_2O_3	[m%]	0.5	0.1
	MnO		-	-
	TiO_2		-	-
	Na_2O		0.4	-
	K_2O		2.5	0.4
	P_2O_5		0.1	-

Table VII- 24 Chemical composition of the inlet water supplied to the four lab-scale weltands.

Parameter	Unit	Acetochlor	Alachlor	Control	Metolachlor
pH	-			7.8 ± 0.1	
EC	µS cm^{-1}			792 ± 90	
Cl^-	mmol L^{-1}	3.02 ± 1.09	2.90 ± 1.22	2.98 ± 1.13	3.01 ± 1.12
SO_4^{2-}	mmol L^{-1}	0.55 ± 0.03	0.54 ± 0.03	0.54 ± 0.045	0.55 ± 0.03
NO_3^-	mmol L^{-1}	0.48 ± 0.03	0.47 ± 0.05	0.48 ± 0.04	0.49 ± 0.04
Si	mmol L^{-1}	0.22 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.22 ± 0.01
Al	µmol L^{-1}	1.22 ± 0.73	1.21 ± 0.72	1.34 ± 0.82	1.73 ± 1.24
Mg	mmol L^{-1}	0.97 ± 0.04	0.97 ± 0.04	0.97 ± 0.05	0.97 ± 0.05
Ca	mmol L^{-1}	3.23 ± 0.48	3.23 ± 0.33	3.24 ± 0.40	3.27 ± 0.50
Fe	µmol L^{-1}	1.91 ± 2.44	2.49 ± 2.05	2.04 ± 2.69	2.48 ± 2.00
Mn	µmol L^{-1}	0.15 ± 0.09	0.16 ± 0.10	0.16 ± 0.09	0.16 ± 0.09
Na	mmol L^{-1}	0.90 ± 0.07	0.93 ± 0.07	0.91 ± 0.07	0.92 ± 0.05
K	mmol L^{-1}	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
P	µmol L^{-1}	0.35 ± 0.10	0.84 ± 1.14	0.38 ± 0.13	0.41 ± 0.16

Mean ± SD

Table VII- 25 Analytical data of the GC-MS/MS quantification of metolachlor, alachlor and acetochlor and the LC-MS quantification of their degradation products metolachlor ESA, metolachlor OXA, alachlor ESA, alachlor OXA, acetochlor ESA, acetochlor OXA.

			Quantification	Identification	Retention time (min)	Recovery (%)	SD (%)
			daughter ion 1 (m/z)	daughter ion 2 (m/z)			
GC-MS/MS	Chloroacetanilide herbicides	Acetochlor	146	131	15.3	43	8
		Alachlor	188	160	15.47	82	8
		Metolachlor	162	133	16.78	96	8
	Internal standard	Alachlor-d_{13}	172		15.3	-	-
		Metolachlor-d_6	134		16.71	-	-
			Transition SRM1	Transition SRM2			
LC-MS	Degradation products	Acetochlor OXA	146	144	10	0.6	0.35
		Acetochlor ESA	144	162	10.5	10.69	3.82
		Alachlor OXA	160	158	9.7	1.32	1.16
		Alachlor ESA	160	176	10.1	5.11	6.46
		Metolachlor OXA	206	172	11.36	3.28	3.97
		Metolachlor ESA	121	192	9.9	0.58	0.42
	Internal standard	Alachlor-d_{13}	251	175	18.3	-	-

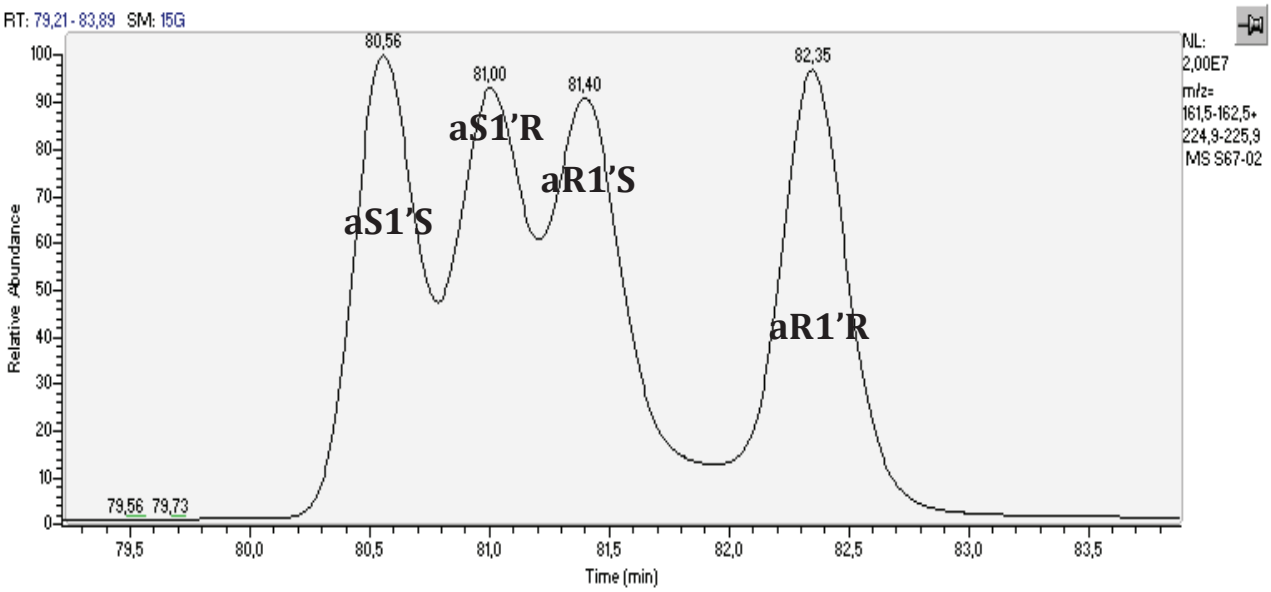


Figure VII- 6. Metolachlor peaks obtained with chiral GC-MS analysis.

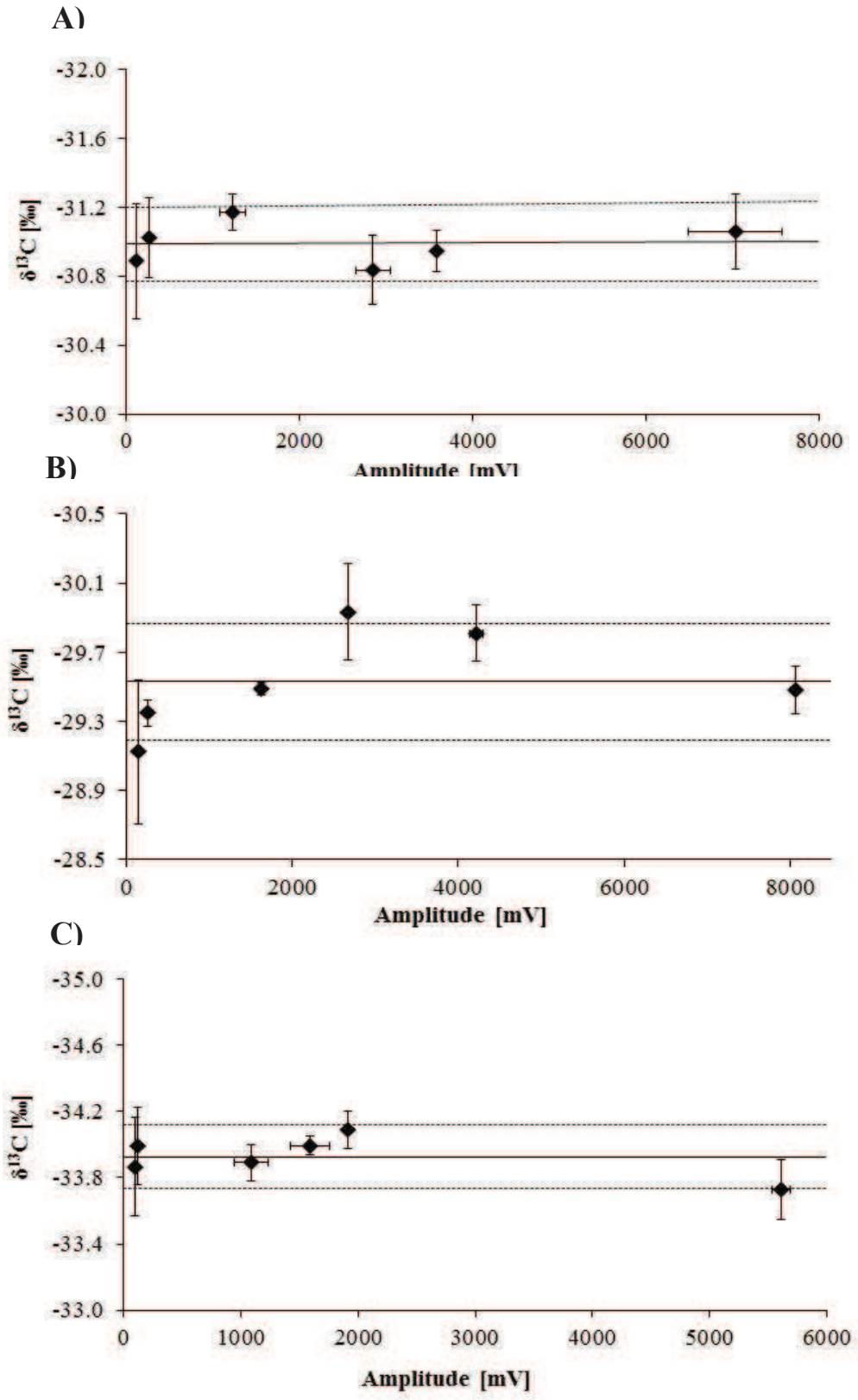


Figure VII- 7 Linearity test for (A) metolachlor, (B) acetochlor and (C) alachlor. Signal amplitudes represented here correspond to concentrations of 92.7, 37.1, 27.8, 3.7 and 2.8 µM for alachlor and acetochlor, and 88.1, 35.2, 26.4, 17.6 and 3.5 µM for metolachlor. Solid lines represent the means of all measurements, dotted lines represent one standard deviation (2σ) of all measurements. Error bars represent one standard deviation (2σ) of triplicate measurements.

GC-C-IRMS linearity test for chloroacetanilides

The range in which the measured $\delta^{13}C$ values are independent of the amount of compound injected in the GC-C-IRMS, referred to as the linear range, was determined for each of the compounds by monitoring the $\delta^{13}C$ values for different amounts of compound injected. A series of standards were dissolved in DCM to concentrations of 92.7, 37.1, 27.8, 3.7 and 2.8 µM for alachlor and acetochlor, and 88.1, 35.2, 26.4, 17.6 and 3.5 µM for metolachlor (corresponding to 2 to 63 ng of carbon injected on column), and measured by GC-C-IRMS. The corresponding range of signal amplitudes was 120 to 7000 mV, 100 to 5600 mV and 150 to 8000 mV for metolachlor, alachlor and acetochlor respectively. The tested signal range had an acceptable variance in isotopic values compared to the averaged isotopic composition. Despite a lower reproducibility for lower amplitude signals, the obtained values were always within 0.5‰ of the averaged $\delta^{13}C$ value for the three compounds (Figure VII-7). This indicates the reliability of the $\delta^{13}C$ values measured within the tested concentration ranges. All measured lab-scale wetland samples had signal amplitudes within the linear range.

Table VII- 26. Average values (mean ± standard deviation) for hydrochemical parameters of inlets and outlets of lab-scale wetlands at days 0, 14, 28, 42, 56, 70, 84 and 98. Ranges are indicated between brackets

Parameter	Inlet	Outlet metolachlor	Outlet alachlor	Outlet acetochlor	Outlet control
pH	7.7 ± 0.1 (7.7 - 8.1)	7.7 ± 0.2 (7.4 - 7.9)	7.7 ± 0.2 (7.5 - 8.1)	7.8 ± 0.2 (7.4 - 8.3)	7.8 ± 0.3 (7.3 - 8.1)
EC	776 ± 85 (627 - 902)	708 ± 26 (677 - 754)	678 ± 33 (630 - 715)	675 ± 32 (630 - 707)	713 ± 31 (679 - 749)
DOC	3.8 ± 1.3 (2.6 - 5.2)	21.8 ± 8.1 (9.6 - 30.6)	58.6 ± 63.3 (4.4 - 172.2)	43.5 ± 79.6 (5.3 - 239.2)	27.7 ± 21.4 (7.2 - 64.1)
Nitrate [µM]	484 ± 38 (389 - 534)	256 ± 63 (170 - 345)	132 ± 91 (37 - 301)	107 ± 63 (26 - 186)	364 ± 106 (247 - 488)
Ferrous iron [µM]	2 ± 2 (0 - 8)	1 ± 1 (0 - 3)	2 ± 1 (0 - 4)	3 ± 5 (0 - 13)	1 ± 1 (0 - 3)
Manganese [µM]	< 1	< 1	< 1	< 1	< 1
Sulphate [µM]	539 ± 36 (433 - 591)	555 ± 62 (429 - 610)	522 ± 34 (476 - 581)	488 ± 37 (441 - 553)	570 ± 54 (481 - 656)
Phosphate [µM]	< 1	≤ 1	≤ 1	< 1	≤ 1
Chloride [mM]	3.0 ± 1.1 (2.0 - 5.2)	3.0 ± 2.0 (1.7 - 7.5)	2.9 ± 1.2 (2.1 - 5.7)	3.0 ± 1.5 (1.8 - 6.3)	3.0 ± 2.0 (1.9 - 7.6)
Phosphorus [µM]	0 ± 0 (0 - 1)	1 ± 0 (0 - 1)	1 ± 0 (0 - 1)	2 ± 4 (0 - 8)	0 ± 0 (0 - 1)
Sulphur [µM]	549 ± 19 (506 - 576)	572 ± 41 (498 - 605)	539 ± 40 (490 - 605)	476 ± 46 (418 - 528)	568 ± 22 (534 - 597)
Sodium [µM]	909 ± 65 (761 - 1.040)	915 ± 152 (630 - 1.070)	940 ± 132 (665 - 1.060)	957 ± 105 (743 - 1.040)	941 ± 165 (583 - 1.080)
Magnesium [µM]	959 ± 34 (909 - 1.040)	1,010 ± 160 (691 - 1.120)	966 ± 104 (744 - 1.060)	946 ± 100 (765 - 1.070)	989 ± 124 (728 - 1.090)
Potassium [µM]	24 ± 3 (18 - 33)	40 ± 37 (14 - 113)	30 ± 14 (20 - 59)	77 ± 57 (32 - 198)	24 ± 9 (15 - 37)
Calcium [mM]	3.2 ± 0.4 (2.2 - 3.6)	2.2 ± 0.3 (1.8 - 2.5)	2.3 ± 0.3 (1.9 - 2.7)	2.4 ± 0.4 (2.0 - 2.9)	2.3 ± 0.3 (1.8 - 2.7)

Table VII- 27 Average mass removal [%] of metolachlor, alachlor and acetochlor between inlets and outlets of lab-scale wetlands between day 28 and 98, and during the three periods of isotope investigation.

Time period [days]	Mass removal [%]		
	Metolachlor	**Alachlor**	**Acetochlor**
28 - 42	24	39	48
56 - 70	27	59	55
84 - 98	20	45	65
28 - 98 (n = 5)	23 ± 5	51 ± 9	56 ± 6

Table VII- 28 Mean and standard deviations of stable carbon isotope (δ13C) triplicate measurements for two weeks old inlets (two weeks inlet), freshly spiked inlets (fresh inlet) and outlets at days 42, 70 and 98. Propagated errors for average inlet values are indicated in brackets.

		day 42	day 70	day 98
Metolachlor	two weeks inlet	-30.5 ± 0.10	-30.6 ± 0.27	-31.2 ± 0.18
	fresh inlet	-30.3 ± 0.05	-31.1 ± 0.14	-31.3 ± 0.07
	average inlet	-30.4 (0.06)	-30.9 (0.15)	-31.3 (0.09)
	Outlet	-29.7 ± 0.26	-30.8 ± 0.08	-30.8 ± 0.10
Alachlor	two weeks inlet	-33.3 ± 0.10	-33.9 ± 0.36	-33.8 ± 0.06
	fresh inlet	-33.6 ± 0.23	-34.1 ± 0.17	-33.8 ± 0.14
	average inlet	-33.5 (0.13)	-34.0 (0.20)	-33.8 (0.08)
	Outlet	-30.6 ± 0.03	-31.8 ± 0.33	-31.5 ± 0.26
Acetochlor	two weeks inlet	-29.3 ± 0.08	-29.8 ± 0.11	-29.3 ± 0.18
	fresh inlet	-29.4 ± 0.22	-29.9 ± 0.18	-29.4 ± 0.26
	average inlet	-29.4 (0.12)	-29.8 (0.11)	-29.4 (0.16)
	Outlet	-26.8 ± 0.12	-27.2 ± 0.23	-26.8 ± 0.12

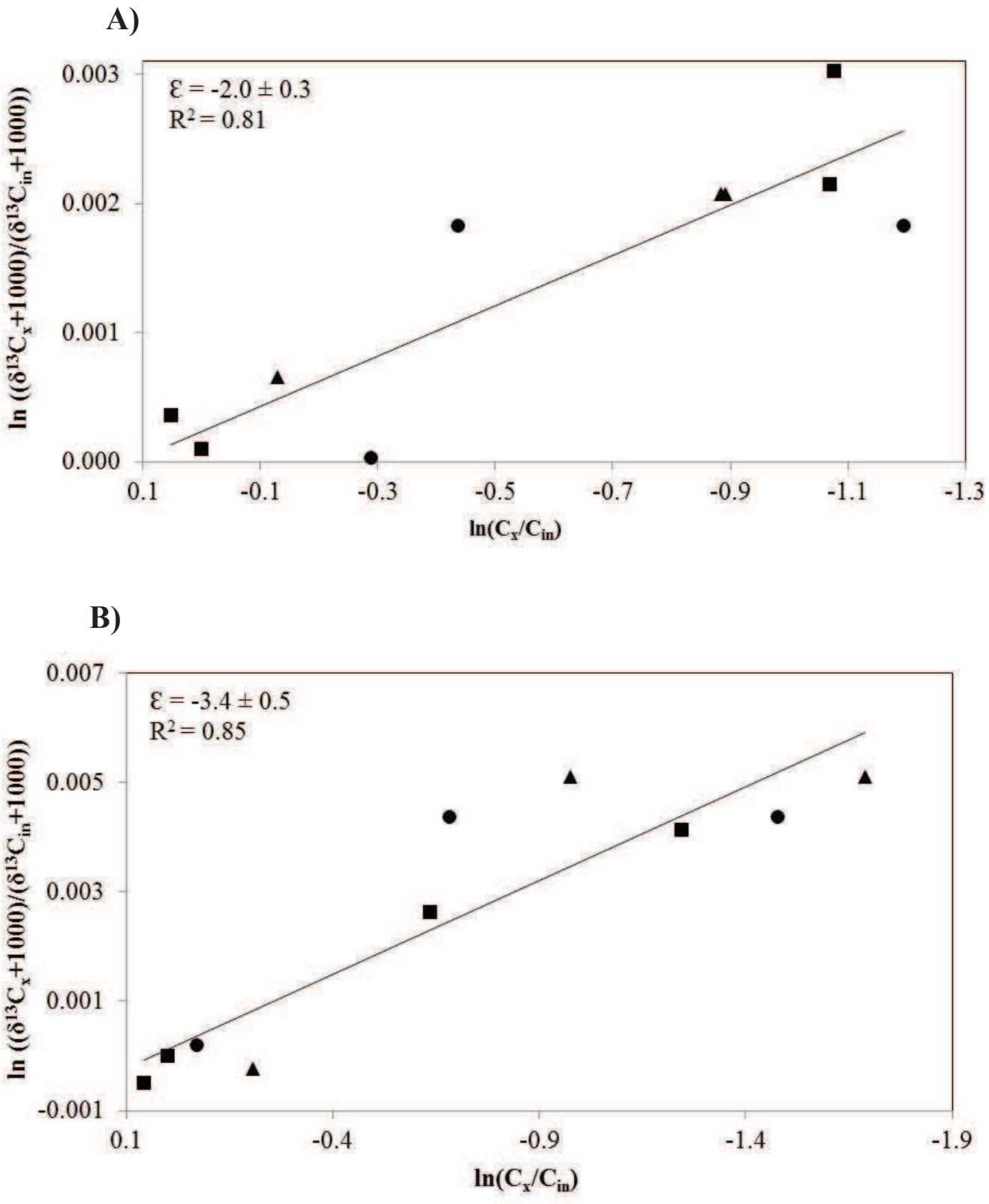


Figure VII- 8 Linearized Rayleigh plots for alachlor A) and acetochlor B). Data from day 42 (squares), day 70 (circles) and day 98 (triangles) are shown.

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Removal of dissolved pesticide mixtures by a stormwater wetland receiving runoff from a vineyard catchment: an inter-annual comparison

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Removal of dissolved pesticide mixtures by a stormwater wetland receiving runoff from a vineyard catchment: an inter-annual comparison

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Wetlands can collect contaminated runoff from agricultural catchments and have intrinsic physical, chemical and biological retention and removal processes useful for mitigating pesticides. However, knowledge about the ability of stormwater wetlands to mitigate pesticide mixtures in runoff is currently very limited. We show here that stormwater wetlands that primarily serve for flood protection can also be effective tools for reducing concentrations and removing loads of runoff-related pesticides and some of their degradation products into downstream aquatic ecosystems. Dissolved concentrations and loads of seven fungicides, six herbicides and four degradation products in runoff from a vineyard catchment were continuously recorded at the inlet and the outlet of the stormwater wetland during two successive periods of pesticide application (April to June). Reduction of pesticide concentrations by the wetland ranged from 50% (simazine) to 100% (azoxystrobin, cymoxanil, cyprodinil, gluphosinate, terbuthylazine and tetraconazole). Removal rates of dissolved load ranged from 26% for aminomethylphosphonic acid (AMPA) to 100% (azoxystrobin, cymoxanil, cyprodinil, diuron, 1-(3,4-dichlorophenyl)-3-methylurea (DCPMU), gluphosinate, kresoxym methyl, terbuthylazine and tetraconazole). More than 77% of the input mass of total suspended solids was retained, underscoring the capability of the wetland to trap pesticide-laden particles via sedimentation. Inter-annual change in the removal of AMPA, isoxaben, kresoxim methyl and simazine was mainly linked to the larger vegetal cover in 2010. Our results demonstrate that stormwater wetlands can remove pesticide mixtures in agricultural runoff, although removal of individual pesticides can vary over time, depending on the characteristics of runoff events and the vegetation cover.

Keywords: load; glyphosate remediation; wetland; sorption; Phragmites; pollution; best management practice

1. Introduction

Pesticide contamination raises critical issues regarding the sustainability of water bodies, inducing significant threat to drinking water resources and aquatic ecosystems [1,2]. Indeed, surface runoff is a major process of contaminants transfer [3–5], as a significant portion (0.1 to 5%) of pesticides applied to agricultural fields can move from agricultural areas into aquatic ecosystems during rainfall-runoff events [6,7]. In the catchment area,

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buffer zones such as wetlands can intercept and partly retain runoff-related contaminants, thus limiting the contamination of water bodies [8,9].

Wetlands capitalize intrinsic physical, chemical and biological retention as well as degradative processes useful for treating various organic chemicals [10], including pesticides [9]. Several studies have proven the capacity of natural and constructed wetlands to mitigate runoff-related pesticides [11–15]. Among constructed wetlands, stormwater wetlands are engineered worldwide with the primary objective to temporarily store urban or agricultural runoff [9]. Stormwater wetlands also represent a potential tool for better management practices of contaminated stormwater, including the removal of pesticide mixtures in agricultural runoff [16]. Indeed, their removal in wetlands largely depends on factors such as physicochemical properties of active molecules, hydrological conditions (e.g. frequency and intensity of rainfall-runoff events), composition and loads of the pesticide mixtures as well as vegetation characteristics [8,17]. The removal of runoff-related pesticides by stormwater wetlands is suspected to be controlled mainly by hydrological factors, intrinsic wetland characteristics such as vegetation cover as well as biotic and abiotic removal processes [16]. However, quantitative knowledge about the capacity of stormwater wetlands to reduce concentrations and remove loads of runoff-related pesticides is currently very limited. Comprehensive field studies that evaluate the mass loading removal of pesticide mixtures by wetland systems are rare. Moreover, very little information on the environmental fate and retention in wetland systems of EU-priority herbicides (e.g. diuron and simazine), widely used herbicides and their degradation products (e.g. glyphosate and AMPA), as well as common fungicides (e.g. dimethomorph and metalaxyl), is currently available.

Herbicides and fungicides are applied on the Alsatian vineyards (France), mainly from April to June (spring). Both timing of pesticide application and rainfall-runoff characteristics have been showed to determine the concentration and mass loading of runoff-related pesticides at the outlet of a representative vineyard catchment [18]. Stormwater wetlands of the Alsatian region primarily serve for flood protection and may also collect pesticide-contaminated runoff from agricultural catchments. Therefore, we hypothesize that stormwater wetlands can play a pivotal role in mitigating runoff-related pesticides. In a previous study, we assessed over an entire period of pesticide application (April to September) the ability of a stormwater wetland receiving runoff from a vineyard catchment to remove a mixture of pesticides [19]. However, to the best of our knowledge, the inter-annual variability in the removal of pesticide mixtures by a stormwater wetland has not been reported yet. The present study aims at assessing and comparing the ability of a stormwater wetland to remove pesticide mixtures in runoff from a vineyard catchment during two periods of pesticide application (April to June).

2. Material and methods

2.1 *Description of the vineyard catchment*

The present study was performed from April 01 to June 30, 2009 and similarly in 2010 at a stormwater wetland located at the outlet of a 42.7 ha vineyard catchment in Rouffach (Alsace, France; 47°57′9 N, 07°17′3 E). The characteristics of the catchment area and agricultural practices have been previously described [18]. Briefly, application of pesticides proceeds mainly from the middle of April (bud breaking of grapevine) until the end of June (fruit setting). Seven fungicides, six herbicides and four degradation products were

selected for the present study because of their widespread use as well as high frequency of application and detection revealed in previous studies [18]. The selected compounds belong to 11 different chemical groups and are listed in Table 1. Rainfall-runoff events do not generate permanent stream in the catchment and statistically occur every week through the year. The average annual precipitation is 609 ± 121 mm (period from 1998 to 2009). Runoff is collected at the outlet of the vineyard catchment by a stormwater wetland and represents the main entry route of pesticides into the wetland [18].

2.2 *Characteristics of the stormwater wetland*

The stormwater wetland has a surface area of 319 m^2 and a total volume of 1500 m^3 (Figure 1). The hydraulic characteristics and functioning of the stormwater wetland were previously described [20,21]. The wetland is composed of a sediment deposition forebay (215 m^2) that collects suspended solids in runoff and a gravel filter (13 m long, 8 m wide and 0.6 m deep). Water depth in the forebay varied from 0.05 to 0.4 m from April to June, depending on the runoff volume entering. In addition, the water storage capacity of the sediment deposition zone was 50 m^3. The hydraulic retention time (HRT) of the wetland was found to be between 10 and 12 hours during the investigation periods. The chemical composition of wetland sediments was (%): organic carbon 14.8, SiO_2 50, Al_2O_3 9.5, MgO 2.2, CaO 11.6, Fe_2O_3 4.1, MnO 0.1, Na_2O 0.7, and K_2O 2.5 ($n = 5$). The sediment texture was (%): clay 44, fine silt 33, coarse silt 10, fine sand 5, and coarse sand 8 ($n = 5$). The pH value was 8.1 through the experiment. Owing to the clay liner on the wetland bed ($Ks < 10^{-10}$ m s^{-1}) and based on the water balance, water losses by vertical infiltration were negligible. Sediments and vegetation in the forebay area were removed in February 2009. Sediment removal occurs every four years and is part of the local maintenance scheme of the stormwater detention systems. In 2009, the vegetation cover in the sediment deposition zone, mainly formed of *Phragmites australis*, *Juncus effusus* and *Typha latifolia*, was $<1\%$ of the area in April, 5% in May, and 25% in June. In 2010, the same plant species were present and the vegetation covered 100% of the forebay area from April to June. *Phragmites australis* represented 90% of the total vegetation cover through the investigation period. No algal proliferation was observed during the April to June investigation periods. A detailed survey of the vegetation in the stormwater wetland was performed in June 2009. The results are provided in Table S1 of the supplementary information.

2.3 *Water flow measurement and sampling procedure*

Runoff discharges entering and outflowing the wetland were continuously monitored by measuring water depth using bubbler flow modules (Hydrologic, Sainte-Foy, Québec, Canada) combined with a Venturi channel at the inlet and a V-notch weir at the outlet. Water samples were collected every 6 m^3 at the inlet using a 4010 Hydrologic automatic sampler (Sainte-Foy, Québec, Canada) and at the outlet of the wetland using a 6712FR ISCO Teledyne automatic sampler (Lincoln, Nebraska, US). Because the transfer of pesticides in stormwater wetland is suspected to occur mostly in the dissolved phase and owing to difficulties in collecting, analysing and estimating the fraction of particle-laden pesticides [22–25], the present study focuses mainly on dissolved pesticides. The detailed procedure of sample collection and storage ensuring reliable pesticide measurements was previously tested and described [18,26]. Briefly, water samples (300 mL) were collected

Table 1. Type, physicochemical properties and toxicity of the pesticides and degradation products. Degradation products are shown in italics.

Compound	Chemical group	Type	Formula	Limit of quantification [$\mu g\,L^{-1}$]	Log K_{ow}[1] [–]	Henry constant[1] $Pa\,m^3\,mol^{-1}$ (25°C)	DT_{50}[2] (aerobic–anaerobic soil) [day]	Aqueous photolysis DT_{50} (pH = 7)[1] [day]	Aqueous hydrolysis DT_{50} (20°C, pH = 7)[1] [day]	EC50[1,3] [ppm]
Azoxystrobin	Strobilurin	Fungicide	$C_{22}H_{17}N_30_5$	0.05	2.5	7.40×10^{-09}	112–119	8.7	stable	0.23
Cymoxanil	Cyanoacetamide oxime	Fungicide	$C_7H_{10}N_4O_3$	0.05	0.67	3.80×10^{-05}	n.a.	1.7	1.1	27
Cyprodinil	Anilinopyrimidine	Fungicide	$C_{14}H_{15}N_3$	0.02	4	6.60×10^{-03}	126–183	13.5	stable	0.03
Dimethomorph	Morpholine	Fungicide	$C_{21}H_{22}ClNO4$	0.05	2.68	2.04×10^{-05}	n.a.–26	97	70	>10.6
Diuron	Phenilurea	Herbicide	$C_9H_{10}Cl_2N_2O$	0.02	2.87	2.00×10^{-06}	372–925	43	stable	5.7
DCPU	Unclassified	*Degradation product (Diuron)*	$C_7H_6Cl_2N_2O$	0.05	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
DCPMU	Unclassified	*Degradation product (Diuron)*	$C_8H_8Cl_2N_2O$	0.05	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
3,4–dichloroaniline	Unclassified	*Degradation product (Diuron)*	$C_6H_5Cl_2N$	0.10	2.69	1.48	n.a.	0.25	n.a.	0.44
Gluphosinate	Organophosphate	Herbicide	$C_5H_{18}N_3O_4P$	0.10	−3.96	n.a.	n.a.	n.a.	n.a.	n.a.
Glyphosate	Phosphonoglycine	Herbicide	$C_3H_8NO_5P$	0.10	−3.2	2.10×10^{-07}	22–96	69	stable	40
AMPA	Unclassified	*Degradation product (Glyphosate)*	$C_7H_{10}N_2O_4$	0.10	−1.63	0.16	n.a.	n.a.	n.a.	n.a.
Isoxaben	Benzamide	Herbicide	$C_{18}H_{24}N_2O_4$	0.05	3.94	1.96×10^{-04}	205–n.a.	6	stable	>1.3
Kresoxym methyl	Strobilurin	Fungicide	$C_{18}H_{19}NO_4$	0.10	3.40	3.60×10^{-07}	2–1	28	34	0.186
Metalaxyl	Phenylamide	Fungicide	$C_{15}H_{21}NO_4$	0.05	1.65	1.60×10^{-05}	n.a.	stable	106	28
Simazine	Triazine	Herbicide	$C_7H_{12}ClN_5$	0.02	2.3	5.60×10^{-05}	71–110	1.9	96	1.1
Terbutylazine	Triazine	Herbicide	$C_9H_{16}ClN_5$	0.02	3.4	4.18×10^{-03}	n.a.	stable	stable	21.2
Tetraconazole	Triazole	Fungicide	$C_{13}H_{11}Cl_2F_4N_3O$	0.05	3.56	3.60×10^{-04}	n.a.	217	stable	3.0

Notes: [1]Obtained from the PPDB (2007, 2008, 2009). The Pesticide Properties DataBase (PPDB) developed by the Agriculture & Environment Research Unit (AERU) at the University of Hertfordshire, from the database that originally accompanied the EMA (Environmental Management for Agriculture) software (also developed by AERU), with additional input from the EU–funded FOOTPRINT project (FP6–SSP–022704). http://www.herts.ac.uk/aeru/footprint.

[2]Obtained from the PAN (PAN, 2006) pesticide database.

[3]Daphnia magna test, 48 h obtained from PPDB database.

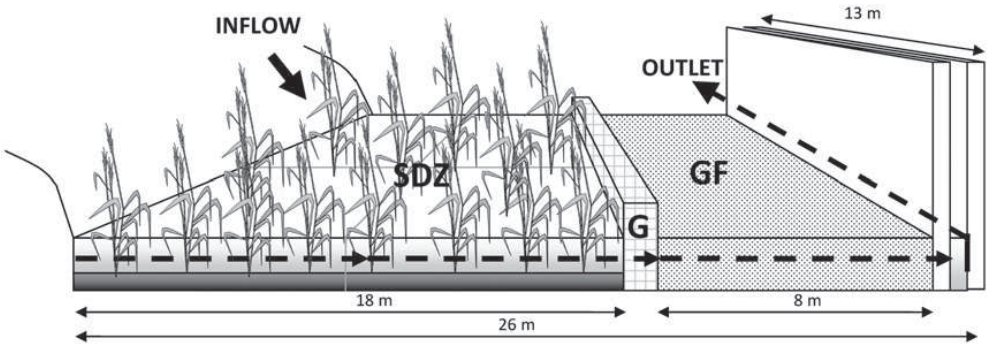

Figure 1. Cross-section of the stormwater wetland (Rouffach, Alsace, France) and position of the sediment deposition forebay (SDF), the gabion barrier (G) and the gravel filter (GF). The dotted line indicates the direction of water flow.

in glass jars, stored in the dark at 4°C after each runoff event, and placed on ice during transportation to the laboratory for chemical analysis. A series of discrete flow proportional water samples taken over a runoff event were combined in a single composite sample prior to analysis.

2.4 *Hydrochemical and pesticide analyses*

Conductivity, pH, and redox potential were directly measured in the field using WTW multi 350i portable sensors (WTW, Weilheim, Germany). Concentrations of dissolved organic carbon (DOC), total suspended solids (TSS), total Kjeldahl nitrogen (TKN), NH_4^+, NO_3^-, NO_2^-, total phosphorus and PO_4^{3-} were determined by FR EN ISO standards and laboratory procedures. Water samples collected for pesticide measurements were dispensed into 100 mL vials and kept at –20°C for a maximum of 30 days until pesticide analysis. Pesticide analysis was performed according to the NF XPT 90–210 French standards at the Pasteur Institute of Lille (France), which is a service of pesticide residues analysis accredited by the French National Accreditation Authority (COFRAC). The COFRAC calibration certificate is recognized by other European calibration services (EA – European Cooperation for Accreditation). Water samples were filtered through 1 μm glass fibre filters, solid-phase extracted before analyzing the subsequent extracts. The 13 herbicides and fungicides (azoxystrobin, cymoxanil, cyprodinil, dimethomorph, diuron, gluphosinate, glyphosate, isoxaben, kresoxim methyl, metalaxyl, simazine, terbuthylazine, tetraconazole) and the four degradation products (3,4-dichlorophenyl urea (DCPU), DCPMU, 3,4-dichloroaniline and AMPA) were quantified using liquid chromatography coupled to tandem mass spectrometry (LC-MS-MS). Quantification of glyphosate, AMPA and gluphosinate was performed after derivatization with fluorenemethoxycarbonyle (FMOC). Limits of pesticide quantification ranged from 0.02 to 0.10 $\mu g\,L^{-1}$ and are provided in Table 1. Extraction efficiencies of pesticides were estimated for each water sample set by spiking with surrogates. Surrogate recovery ranged from 70 to 89%. Further quality control was achieved by using a blank for each set of samples. Detection and quantification limits, relative standard deviation (RSD) and recovery efficiencies for each pesticide are provided for water samples in the Table S2 of the Supplementary Information.

2.5 *Data analysis*

Dissolved pesticide concentrations and values of hydrochemical parameters at the inlet and outlet of the wetland were compared using the paired nonparametric Wilcoxon Signed Rank test. Reduction of pesticide concentration (R_C (%)) was calculated as the relative decrease of mean concentration at the outlet with respect to that at the inlet. A nondetect (n.d.) was treated as zero, assuming that no pesticides were present when the analysis revealed that the concentrations were below the detection limits. The R_C (%) in a given period was the average of all runoff event R_C (%) values. Pesticide event mass loadings at the inlet and the outlet of the wetland were obtained multiplying mean pesticide concentrations by the corresponding water volume of the runoff event. Removal rate of pesticide mass loading R_L (%) was calculated as the relative decrease of mass loading at the outlet with respect to that at the inlet for each runoff event using Equation (1).

$$R_L\ (\%) = \left[1 - \frac{M_{\text{out}}}{M_{\text{in}}}\right] \times 100 = \left[1 - \frac{C_{\text{out}} V_{\text{out}}}{C_{\text{in}} V_{\text{in}}}\right] \times 100 \quad (1)$$

where M_{in} and M_{out} are the influent and effluent pesticide mass loadings in the dissolved phase (mg), V_{in} and V_{out} are the influent and effluent volumes (m^3), and C_{in} and C_{out} the inlet and outlet mean concentrations ($\mu g\, L^{-1}$), respectively. Mass loadings at the inlet and the outlet of the wetland were calculated from the sum of all event loads during a given investigation period.

3. Results

3.1 *Climatic and hydrological characteristics*

Climatic and hydraulic data from April 1 to June 30 2009 and 2010 are provided in Table 2, in Figure 2 and monthly data are provided in Table S3 in the Supplementary Information SI. From April 01 through June 30, 23 runoff events occurred in 2009, and 30 were accounted for in 2010. Rainfall amount, duration, mean and maximal intensities as well as the duration of the period between two rainfall events did not significantly differ between 2009 and 2010 sampling periods (Table 2). However, mean solar radiation significantly differed between 2009 ($1903 \pm 617\, J\, cm^{-2}\, d^{-1}$) and 2010 ($1699 \pm 731\, J\, cm^{-2}\, d^{-1}$) sampling periods ($p < 0.05$). The total volume of runoff that entered the wetland during the investigation period was 398 m^3 in 2009 and 568 m^3 in 2010. The volume of individual runoff event ranged from 0.16 m^3 to 88.3 m^3 in 2009 and from 0.07 m^3 to 140 m^3 in 2010. In 2009 and 2010, respectively 91.3% and 89.6% of runoff volumes were lower than 50 m^3. The absence of significant difference in runoff coefficients between 2009 and 2010 ($p > 0.53$) underscores that land use and soil management practices on the vineyard catchment area were similar in 2009 and 2010 (data not shown). The mean quiescent period between two runoff events ranged from 4 hours to 27 days in 2009 and from 6 hours to 11 days in 2010, and did not significantly vary between the two years ($p > 0.75$). Month-to month comparison of climatic characteristics (i.e. temperature, solar radiation, rainfall and evapotranspiration) revealed no significant differences between April 2009 and 2010, as well as between June 2009 and 2010. However, temperature, solar radiation and evapotranspiration were significantly lower in May 2009 compared to those of May 2010 ($p < 0.001$) (Table S3). The budget of water volumes inflowing and outflowing the wetland was balanced when direct rainfall and evapotranspiration volumes were included (Table S3). Altogether, the analysis of climatic and

Table 2. Climatic and hydrological conditions and wetland hydrochemical characteristics. Values are provided as the mean and ranges at the inlet and the outlet of the stormwater wetland (Rouffach, Haut–Rhin, France) from 1 April to 30 June 2009 and 2010.

	Parameter	Unit	2009			2010		p^1
Climatic data	Solar radiation	[J cm^{-2} day^{-1}]	1903 (431–2909)			1699 (267–3000)		***
	Temperature	[°C day^{-1}]	16.1 (8.6–26.7)			14 (5.1–24.5)		***
	Rainfall	[mm]	130.8			139.2		n.s.
	Quiescent period[3]	[day]	3.5 (0.2–27)			2.4 (0.3–11)		n.s.
	Inflowing runoff volume	[m^3]	398			568		n.s.
			Inlet	Outlet	p^2	Inlet	Outlet	p^2
Water chemistry	pH	–	7.7 (7.0–7.9)	8.0 (7.7–8.2)	***	7.7 (7.4–8.5)	7.7 (7.3–8.0)	n.s.
	Electric conductivity	[µS cm^{-1}]	245 (175–405)	1006 (835–1605)	***	222 (125–442)	831 (389–1070)	***
	Redox potential	[mV]	151 (3.7–275)	178 (5.3–283)	n.s.	−45 (–210–118)	−76 (–217–94)	n.s.
	Total suspended solids	[mg L^{-1}]	1386 (237–4695)	126 (1.0–2008)	***	922 (61.7–5639)	31.0 (3.5–204)	***
		[kg]	405	90.5		554	19.2	
	Dissolved organic carbon	[mg L^{-1}]	22.7 (8.7–31.7)	14.8 (7.7–21.9)	*	14.0 (4.6–5639)	5.5 (2.1–9.5)	***
		[kg]	6.5	9.9		6.0	3.0	
	Ammonium	[mg L^{-1}]	0.20 (n.d.–0.52)	0.05 (n.d.–0.26)	**	0.12 (n.d.–0.48)	0.05 (n.d.–0.35)	*
	Nitrate	[mg L^{-1}]	4.19 (n.d.–9.82)	8.09 (n.d.–19.60)	n.s.	0.09 (n.d.–0.78)	0.06 (n.d.–0.64)	n.s.
	Nitrite	[mg L^{-1}]	0.29 (n.d.–2.17)	0.10 (n.d.–0.53)	n.s.	n.d.	n.d.	–
	Total phosphorus	[mg L^{-1}]	2.59 (0.24–7.00)	0.57 (n.d.–2.00)	*	1.33 (0.42–3.27)	0.31 (n.d.–1.83)	***
	Orthophosphorus	[mg L^{-1}]	0.18 (0.10–0.42)	0.10 (n.d.–0.34)	n.s.	0.20 (n.d.–0.43)	0.04 (n.d.–0.39)	**

Notes: [1]Comparison between periods of pesticide application using the Wilcoxon Signed Rank test: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; n.s. = not significant.
[2]Comparison between inlet and outlet concentrations using the Wilcoxon Signed Rank test: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; n.s. = not significant.
[3]Time between two runoff events entering the wetland.

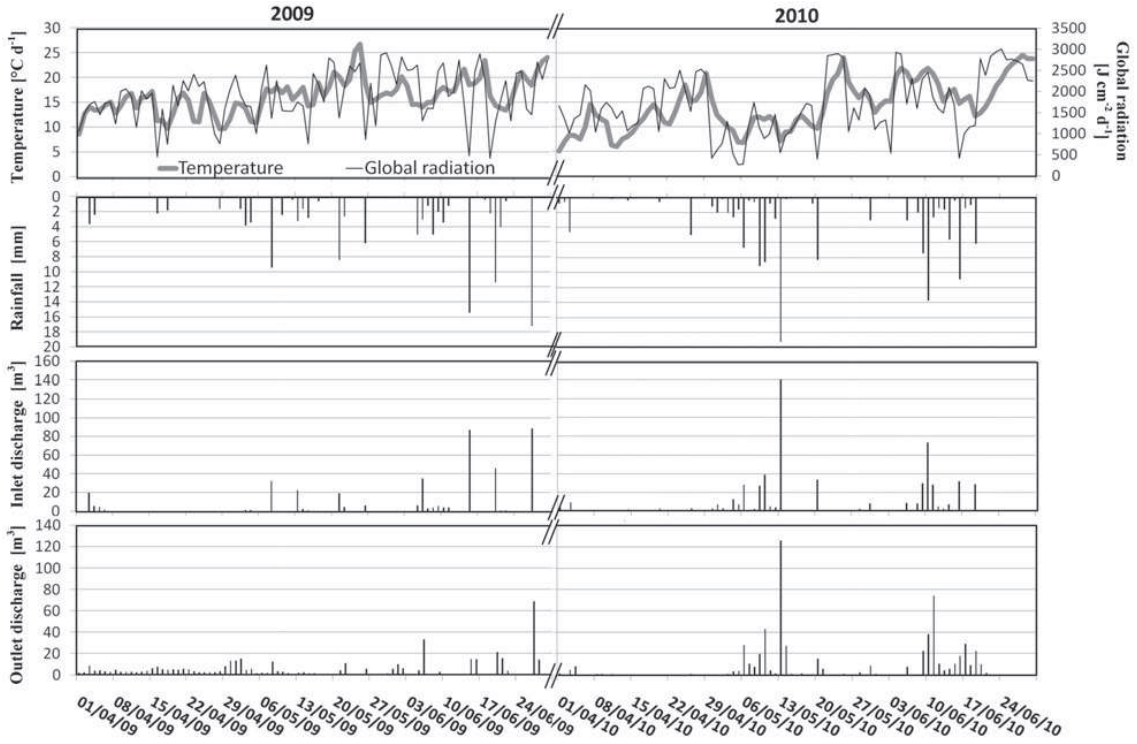

Figure 2. Temporal changes of climatic and hydrological conditions in the vineyard catchment (Rouffach, Haut-Rhin, France) from April 1 to June 30 2009 and 2010.

hydraulic data revealed that rainfall and runoff patterns globally were similar between the April to June 2009 and 2010 periods.

3.2 *Hydrochemistry*

Detailed hydrochemical data are provided in Table 2. Comparison of hydrochemical conditions between the inlet and the outlet of the wetland revealed no significant changes in either redox potential values, concentrations of nitrogen compounds (except ammonium) or orthophosphorus in 2009. Similarly, in 2010, comparison of pH, redox potential and nitrate concentrations found at the inlet and outlet saw no significant difference ($p > 0.05$). In contrast, concentrations of suspended solids, dissolved organic carbon, total phosphorus and values of electric conductivity significantly differed between the inlet and the outlet of the wetland in 2009 and 2010 ($p < 0.05$). Comparison of hydrochemical conditions at the wetland inlet between 2009 and 2010 sampling periods revealed no significant changes of pH, electric conductivity, total suspended solids, total phosphorus and orthophosphorus. However, dissolved organic carbon (DOC), ammonium, nitrite, nitrate concentrations and redox values significantly changed between 2009 and 2010 sampling periods ($p < 0.05$).

The calculation of sedimentation rates from discharge measurements and total suspended solids (TSS) values revealed that the wetland retained 78% ($3.5\,\mathrm{kg\,d^{-1}}$) of the input mass in 2009 and 97% ($5.9\,\mathrm{kg\,d^{-1}}$) in 2010. This clearly indicates that the wetland acts as a sink for particle-laden pesticides. Since the pore size of the filter paper used to separate TSS from dissolved organic carbon (DOC) was 1.2 µm, only finer particles were

included in the DOC. Therefore, suspended solids were defined as particles larger than 1.2 μm, collected after filtration. The calculation of DOC loads at the inlet and the outlet from April to June 2009 revealed that the output mass (9.9 kg) exceeded the input mass by 34%. In contrast, from April to June 2010, 50% of the input mass of DOC (6.0 kg) was removed by the wetland (Table 2).

3.3 *Removal of dissolved pesticides by the wetland*

3.3.1 *Occurrence and reduction of pesticide concentrations*

Detailed data of dissolved pesticide concentrations as well as reduction of pesticides based on the inlet and outlet concentrations are provided in Table 3. In 2009, mean inlet concentrations of glyphosate, AMPA, dimetomorph and metalaxyl were respectively, 4.15, 1.21, 1.21 and 1.15 $\mu g\,L^{-1}$ whereas mean concentrations of the other target compounds were systematically below 1.00 $\mu g\,L^{-1}$. Mean pesticide concentrations at the outlet were 3 (AMPA) to 34 (glyphosate) times lower compared with those at the inlet and never exceeded 0.50 $\mu g\,L^{-1}$. Concentrations of azoxystrobin, cymoxanil, metalaxyl, tetraconazole, glyphosate, AMPA, isoxaben, simazine, and terbutylazine significantly differed from inlet to outlet ($p < 0.05$) (Table 3). Reduction in mean concentrations ranged from 66% (AMPA) to 100% (azoxystrobin, cymoxanil, cyprodinil, DCPMU, gluphosinate, kresoxym methyl, terbutylazine and tetraconazole).

In 2010, mean concentrations at the inlet were below 1.00 $\mu g\,L^{-1}$, except for those of glyphosate (30.38 $\mu g\,L^{-1}$) and AMPA (5.67 $\mu g\,L^{-1}$). In addition, mean concentrations at the wetland outlet never exceeded 0.50 $\mu g\,L^{-1}$. Finally, degradation products of diuron (DCPU, DCPMU and 3,4-dichloroaniline) were systematically below the detection limit, suggesting that diuron was not subject to aerobic degradation or that degradation products were readily degraded in the wetland [27]. Concentrations from inlet to outlet significantly differed for dimetomorph, glyphosate, AMPA, and isoxaben ($p < 0.001$) (Table 3). Reductions in mean concentrations from inlet to outlet were higher than 80% for all the molecules except for simazine (i.e. reduction in mean concentration of 50%), and even reached 100% for diuron and terbutylazine.

3.3.2 *Removal of pesticide loads by the wetland*

Detailed data of dissolved pesticide mass loadings and load removal for individual pesticides are provided in Table 4. In 2009, the total mass loading of all the 15 detected pesticides and degradation products entering the wetland from April to June was 3.304 g whereas only 0.636 g were observed to pass through the wetland (Table 4). Mass loading of glyphosate, dimethomorph, AMPA, and metalaxyl accounted respectively for 44.1, 27.7, 10.7 and 9.4% of the total load entering the wetland. In 2010, the total mass loading of all the eight detected pesticides and degradation products entering the wetland from April to June was 13.183 g when only 1.088 g was found to pass through the wetland. Glyphosate and AMPA accounted, respectively, for 75.7 and 18.8% of the total load entering the wetland.

The overall load removal rate calculated from the total mass loading of pesticides at the outlet relatively to that at the inlet of the wetland was 81% in 2009 and 92% in 2010 (Table 4). Despite the larger pesticide load entering the wetland in 2010 compared to that in 2009, the removal ability of the wetland was not affected. However, removal rates of

Table 3. Mean concentrations and ranges of dissolved pesticides at the inlet and the outlet of the stormwater wetland (Rouffach, Haut–Rhin, France) from 1 April to 30 June 2009 and 2010. Reduction in mean concentrations from inlet to outlet are given in percent (R_C %).

	2009				2010			
	Inlet ($n = 13$)	Outlet ($n = 19$)		R_C	Inlet ($n = 19$)	Outlet ($n = 22$)		R_C
Compound	[µg L^{-1}]	[µg L^{-1}]	p^1	[%]	[µg L^{-1}]	[µg L^{-1}]	p^1	[%]
Azoxystrobin	0.02 (n.d.–0.12)	n.d.	*	100	n.d.	n.d.	–	–
Cymoxanil	0.10 (n.d.–0.90)	n.d.	*	100	n.d.	n.d.	–	–
Cyprodinil	0.02 (n.d.–0.14)	n.d.	n.s.	100	n.d.	n.d.	–	–
Dimethomorph	1.21 (n.d.–10.00)	0.09 (n.d.–1.70)	n.s.	98	0.26 (n.d.–1.80)	0.03 (n.d.–0.24)	***	88
Diuron	0.14 (n.d.–0.32)	0.02 (n.d.–0.04)	n.s.	86	<0.01 (n.d.–0.02)	n.d.	n.s.	100
DCPU	n.d.	n.d.	–	–	n.d.	n.d.	–	–
DCPMU	<0.01 (n.d.–0.05)	n.d.	n.s.	100	n.d.	n.d.	–	–
3,4-dichloroaniline	n.d.	n.d.	–	–	n.d.	n.d.	–	–
Gluphosinate	0.65 (n.d.–6.30)	n.d.	n.s.	100	n.d.	n.d.	–	–
Glyphosate	4.15 (0.30–11.00)	0.12 (n.d.–0.70)	***	97	30.38 (n.d.–110)	0.29 (n.d.–1.70)	***	99
AMPA	1.21 (0.20–2.30)	0.41 (n.d.–0.70)	***	66	5.67 (n.d.–19.00)	0.34 (n.d.–0.90)	***	94
Isoxaben	0.10 (n.d.–0.23)	0.01 (n.d.–0.15)	***	90	0.17 (n.d.–2.10)	<0.01 (n.d.–0.07)	***	97
Kresoxim methyl	0.03 (n.d.–0.40)	n.d.	n.s.	100	0.07 (n.d.–1.20)	<0.01 (n.d.–0.17)	n.s.	89
Metalaxyl	1.15 (n.d.–5.80)	0.24 (n.d.–1.20)	*	79	0.81 (n.d.–7.70)	0.14 (n.d.–2.00)	n.s.	83
Simazine	0.07 (n.d.–0.18)	0.02 (n.d.–0.03)	***	71	<0.01 (n.d.–0.03)	<0.01 (n.d.–0.02)	n.s.	50
Terbuthylazine	0.05 (n.d.–0.07)	n.d.	***	100	<0.01 (n.d.–0.02)	n.d.	n.s.	100
Tetraconazole	0.02 (n.d.–0.09)	n.d.	*	100	n.d.	n.d.	–	–

Note: [1]Comparison of inlet and outlet concentrations using the Wilcoxon Signed Rank test: *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; n.s. = not significant.

Table 4. Loads of dissolved pesticides [mg] and load removal rates, R_L (%) by the stormwater wetland (Rouffach, Alsace, France) from 1 April to 30 June 2009 and 2010. Degradation products are shown in italics.

	2009			2010		
Compound	Inlet [mg]	Outlet [mg]	R_L [%]	Inlet [mg]	Outlet [mg]	R_L [%]
Azoxystrobin	15.1	0	100	0	0	–
Cymoxanil	12.3	0	100	0	0	–
Cyprodinil	10.4	0	100	0	0	–
Dimethomorph	916	144	84	268	31.2	88
Diuron	38.4	9.1	76	<0.1	0	n.a[1]
DCPU	0	0	–	0	0	–
DCPMU	1.3	0	100	0	0	–
3,4-dichloroaniline	0	0	–	0	0	–
Gluphosinate	108	0	100	0	0	–
Glyphosate	1458	128	91	9983	439	96
AMPA	352	259	26	2480	467	81
Isoxaben	25.8	6.1	76	53.7	7.2	87
Kresoxim methyl	16.7	0	100	57.1	13.0	77
Metalaxyl	311	80.8	74	341	130	62
Simazine	15.6	9.2	41	<0.1	0.8	n.a[1]
Terbuthylazine	14.6	0	100	0	0	–
Tetraconazole	8.4	0	100	0	0	–
Total	**3304**	**636**	**81**	**13,183**	**1088**	**92**

Note: [1]n.a. = not assessed owing to low inlet load (<0.1 mg).

pesticide loads largely differed according to the individual compounds. In 2009, azoxystrobin, cymoxanil, cyprodinil, dimethomorph, DCPMU, gluphosinate, glyphosate, kresoxim methyl, terbutylazine and tetraconazole were efficiently removed (removal rates ranging from 80 to 100%), diuron, isoxaben and metalaxyl were moderately removed (removal rates ranging from 50 to 80%), and AMPA and simazine were poorly removed (removal rates lower than 50%) (Table 4). In 2010, dimethomorph, glyphosate, AMPA and isoxaben were efficiently removed (removal rates ranging from 80 to 100%) and kresoxim methyl and metalaxyl were moderately removed (removal rates ranging from 50 to 80%).

4. Discussion

4.1 *Influence of the hydrological conditions on pesticide removal*

In stormwater wetlands that primarily serve for flood protection rather than for the treatment of runoff-related contaminants, the period between two runoff events (quiescent period) and the runoff volume entering the wetland are two hydrological factors that can affect the efficiency of pesticide removal [28,21]. Longer quiescent periods and small runoff volumes entering the wetland can enhance degradative processes by increasing the contact time between runoff water and wetland compartments [21]. In contrast, shorter quiescent periods and larger runoff volumes are expected to limit the occurrence of degradative processes. For instance, half-life of cymoxanil, cyprodinil, 3,4-dichloroaniline, isoxaben

and simazine upon aqueous photolysis or hydrolysis are lower than 15 days (Table 1) which suggests that quiescent periods between two runoff events (i.e. from 0.2 to 27 days in 2009, and from 0.3 to 11 days in 2010) were sufficient to remove partly these compounds in the wetland. However, this is valid for low runoff volumes ($<50\,m^3$) that result in shallow water depths ($<0.2\,m$) in the wetland and can thus be partly treated while stored during the quiescent period. As in 2009 and 2010, respectively 91.3% and 89.6% of runoff events were lower than $50\,m^3$, most of the contaminated runoff events could be stored and treated partly by the wetland. In contrast, larger runoff events observed during the investigation periods resulted in larger water depth in the wetland thus potentially limiting the occurrence of removal processes. Furthermore, incomplete flushing of the wetland during low or moderate runoff events ($<50\,m^3$) can also cause longer retention of stable and less-sorptive substances. In contrast, shorter water pathway and contact time with sediment and vegetation under high flow conditions is expected to decrease removal of dissolved contaminant by sorption and degradation processes, as previously described [21,28].

Lange *et al*. [21] characterized the hydraulic properties of the stormwater wetland using sodium bromide as a non-reactive tracer. Their study simulated a $37.5\,m^3$ runoff event and indicated a mean hydraulic detention time in the stormwater wetland of 58.2 h, which suggests a high retention capacity for runoff water and associated contaminants. In parallel, uranine (DT_{50}-photolysis = 11 days) was used as a reference to mimic photolysis, and sulforhodamine B ($\mathrm{Log}\,K_{ow} = -2.02$) as one to mimic moderate sorption of contaminants. This study underscored favourable conditions for photocatalytic decay (removal of uranine by 57%) and high sorption capacities (removal of sulforhodamine B by 82%) in the stormwater wetland. Hence, processes such as sedimentation, photolysis, hydrolysis and degradation involved in the removal of both dissolved and particle-laden pesticides in stormwater wetlands depend on flow conditions, water depth, and properties of the contaminant molecules. However, as described below, the removal of pesticide mixtures is also intimately linked to hydrochemical and vegetation characteristics that prevail in the wetland.

4.2 *Removal of hydrophilic pesticides by the wetland*

Pesticides with $\mathrm{Log}\,K_{ow} < 3$ (i.e. azoxystrobin, cymoxanil, dimethomorph, diuron, gluphosinate, glyphosate, AMPA, metalaxyl, simazine) result in loads being predominantly associated with runoff and wetland water, lower partitioning to suspended solids and DOC, and a potentially faster degradation in the dissolved phase owing to the higher availability of molecules in abiotic and biotic transformation processes. Since the pH values at the inlet and the outlet of the wetland ranged from 7.0 to 8.5, partial degradation of cymoxanil ($DT_{50} < 2$ days at pH 7) by aqueous hydrolysis, and of 3.4-dichloroaniline and simazine by aqueous photolysis ($DT_{50} < 6$ days at pH 7) was likely to occur (refer to Table 1 for compound properties). In contrast, degradation of dimethomorph, diuron, and glyphosate by photolysis or hydrolysis was likely not a dominant removal process in our wetland since their DT_{50} is larger than 40 days. Under more reducing conditions that prevailed in the wetland in 2010, the elimination of chlorinated pesticides (i.e. simazine, terbuthylazine) via reductive dechlorination may be favoured and sustained by the occurrence of biofilm, sediment, root complexes as well as potential sources of electron donors provided by plant roots and organic matter [30,31]. However, larger

vegetation cover may also have increased degradation rates by enhancing oxidative transformation pathways at the rhizosphere level which benefited from oxygen transfer through plants and release by roots [32,33].

Among the compounds studied, glyphosate and AMPA are strongly sorbed by soil minerals, and have been previously observed to rapidly adsorb to wetland sediments, before being gradually removed within 5 to 15 days [29]. Biodegradation of AMPA is generally slower than that of glyphosate possibly owing to the transient capacity of AMPA to be strongly sorbed through the phosphonate group which protects it against further biodegradation [25]. This is in agreement with our results showing efficient degradation of glyphosate into AMPA in the dissolved phase, as underscored by a higher AMPA to glyphosate mass ratios at the outlet (i.e. 2.02 in 2009, and 1.06 in 2010) compared to that found at the inlet (i.e. 0.24 in 2009, and 0.25 in 2010). AMPA also suffers from a lower load removal compared to that of glyphosate. However, understanding factors influencing the degradation of glyphosate into AMPA in biogeochemical dynamic environments such as wetlands requires further investigation.

4.3 *Removal of hydrophobic pesticides by the wetland*

Several studies have shown that the removal of hydrophobic chemicals with $\mathrm{Log}K_{\mathrm{ow}}$ values >3 in aquatic environments is due mainly to the sedimentation of pesticide-laden solids [11,34–36]. Hence, cyprodinil, isoxaben, kresoxim methyl, tetraconazole and terbuthylazine ($\mathrm{Log}\,K_{\mathrm{ow}} > 3$) can efficiently be trapped in the wetland. Higher plant density in 2010 likely slowed water flows and allowed for particle settling to occur, as indicated by larger TSS removal in 2010 compared to that in 2009. Although aqueous photolysis of isoxaben cannot be excluded ($\mathrm{DT}_{50} = 6$ days at $\mathrm{pH} = 7$), fast degradation of hydrophobic compounds in the wetland is not expected to happen owing to reduced bioavailability. Larger output load of DOC compared to input indicates that pesticide removal in 2009 cannot be attributed to the retention of the DOC-bound fraction in the wetland. This suggests that a large fraction of hydrophobic pesticides passed through the stormwater wetland in association with DOC in 2009. For example, Delgado-Moreno *et al*. [37] showed that DOC ($38 \pm 2\,\mathrm{mg\,L^{-1}}$) increases the distribution of pyrethroids from the sediment to the solution phase and plays an important role in mobilizing pyrethroids in runoff and surface streams. Since hydrological and hydrochemical conditions were similar in 2009 and 2010, the higher retention rate of particles by the wetland calculated in 2010 can be attributed mainly to the larger vegetation cover [38]. However, under higher flow regime than that observed in the present study, transport of pesticides-laden sediment through the wetland may decrease the removal of hydrophobic pesticides by affecting the degree of bottom scouring and re-suspension of settled solids [14]. Under such conditions, direct transport of runoff particles and re-suspension of particles from the wetland bed to the water column may increase when the vegetation cover is less dense, resulting in a lower removal rate of pesticides associated with TSS and DOC.

4.4 *Inter-annual variation of pesticide removal by the wetland*

Although inter-annual variations of climatic and flow conditions were not significant, reduction in mean concentrations and load removal rates by the wetland globally were larger in 2010 compared to those in 2009. Therefore, these inter-annual changes in

pesticide removal by the wetland seemed to be related to inter-annual differences in the vegetation cover observed in the wetland forebay. In 2009, the vegetation covered less than 1% of the forebay area in April and vegetation cover was 25% in June. In contrast, the vegetation cover was 100% from April to June 2010.

For compounds with a $\mathrm{Log}K_{\mathrm{ow}}$ ranging between 1 and 3 (which are still considered to be able to move through the lipid layer of plants) plant uptake cannot be excluded [39]. Plants found in the wetland such as cattail or common reed have a large internal pore space and potential surface area allowing moderate to highly hydrophobic pesticides such as cyprodinil, isoxaben, terbuthylazine and tetraconazole (with $\mathrm{Log}\,K_{\mathrm{ow}} > 3$) to rapidly absorb [40]. Another study showed that metalaxyl and simazine could be taken up by bulrush (*Typha latifolia*) and accumulated in their leaves after 3 and 1 day, respectively [41]. Rogers *et al.* [40] have shown that sorption of chlorpyrifos ($\mathrm{Log}\,K_{\mathrm{ow}} = 4.96$) to plant stems was more than 10 times higher than to sediments and reached a pseudo-equilibrium in less than 8 hours. They also indicated that partitioning coefficients were increasing as the perimeter of the plant stem and pore space also increased. It can thus be inferred that surface sorption to aquatic vegetation of the stormwater wetland likely increased the residence time of hydrophobic compounds. However, owing to large spatial and temporal variations in the vegetal biomass and type in our wetland, the specific contribution of vegetation and vegetal material in removing pesticides could not be quantified in the present study.

Inter-annual variation of the vegetation cover and the extent of water level fluctuation during and after a runoff event also can affect volatilization and photolysis of pesticides by changing the exposure of plant material to ambient air and direct sunlight. For instance, larger vegetation cover in the wetland in 2010 can reduce photocatalytic reactions in the water column, which may explain the lower simazine removal in 2010 compared to 2009. However, owing to larger airflow and surface exposure, pesticide volatilization from the surface of plants has been reported to be higher than that of the surface of soil for pesticides with a Henry constant (K_{h}) $> 2.65 \times 10^{-5}$ [42,43]. Volatilization from air-exposed plant surfaces following a runoff event may explain the large load removal of compounds such as terbutylazine, tetraconazole, and cyprodinil ($K_{\mathrm{h}} > 3.0 \times 10^{-4}$, see Table 1 for the Henry constant values). Larger load removal of isoxaben ($K_{\mathrm{h}} = 1.96 \times 10^{-4}$) in 2010 compared to that in 2009 can be attributed to volatilization combined with photodegradation. Volatilization and photodegradation are expected to simultaneously occur owing to direct contact of light and air with the surface of plant material when water level gradually decreased in the wetland after a runoff event. Though volatilization can be an effective removal pathway for relatively volatile pesticides in wetlands, pesticides can be transported over long distances in the atmosphere, and further contaminate terrestrial or aquatic ecosystems via deposition after a rainfall.

Hence, degradation, sorption and volatilization might have been involved simultaneously or alternatively in the removal of pesticides in the stormwater wetland. The occurrence and the respective contribution of these processes are mainly dependent on hydrological conditions (i.e. runoff volumes and quiescent periods between runoff events), climatic conditions (i.e. temperature and solar radiation) and vegetation characteristics. Entering of runoff water in the wetland followed by submersion of plant surfaces and, when water level decreases, air and sun exposure and degradation of remaining compounds sorbed to plant material and sediments, is a cyclic process that may help reducing the transfer of pesticide mixtures into downstream aquatic ecosystems. However, the behaviour of stormwater wetlands receiving pesticides during high flow conditions

or floods and the role of wetland vegetation in the removal of pesticides deserves further investigations.

5. Conclusion

This study was designed to determine the ability of a stormwater wetland to reduce pesticide mixtures in runoff during periods of pesticide application on a vineyard catchment. Quantitative field study on the dissolved pesticide load often fail to evaluate the potential of best management practices (BMP) for the mitigation of mixtures of runoff-related pesticides and very rarely consider the temporal variability in contaminant removal. Our results provide detailed dissolved concentrations and loads of pesticide mixtures and show that stormwater wetlands that primarily serve for flood protection can also be effective tools for reducing concentrations and removing loads of a wide range of runoff-related pesticides and some of their degradation products into downstream aquatic ecosystems. Calculation of the mass loadings permits to determine more accurately than mean pesticide concentrations the efficiency of buffering systems in removing pesticides during critical periods of application. The wetland vegetation, by increasing the hydraulic residence time and thus the possibility of microbial degradation, sorption to sediments and uptake by wetland macrophytes, has a pivotal role in retaining moderately hydrophobic compounds. Although the use of stormwater wetlands as a management practice targeting pesticide mitigation should not be conceived as a unique solution to treat pesticide runoff, in many cases where other best management practices are not available, the introduction and maintenance of a large vegetation cover in stormwater detention systems can help reducing the transfer of contaminants from land into water bodies.

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Seasonal Changes of Macroinvertebrate Communities in a Stormwater Wetland Collecting Pesticide Runoff From a Vineyard Catchment (Alsace, France)

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Abstract Agricultural land use may influence macroinvertebrate communities by way of pesticide contamination associated with agricultural runoff. However, information about the relation between runoff-related pesticides and communities of benthic macroinvertebrates in stormwater wetland that receive agricultural runoff does not currently exist. Here we show changes in macroinvertebrates communities of a stormwater wetland that collects pesticide-contaminated runoff from a vineyard catchment. Sixteen runoff-associated pesticides, including the insecticide flufenoxuron, were continuously quantified at the inlet of the stormwater wetland from April to September (period of pesticide application). In parallel, benthic macroinvertebrate communities, pesticide concentrations, and physicochemical parameters in the wetland were assessed twice a month. Twenty-eight contaminated runoffs ranging from 1.1 to 114 m^3 entered the wetland during the study period. Flufenoxuron concentrations in runoff-suspended solids ranged from 1.5 to 18.5 μg kg^{-1} and reached 6 μg kg^{-1} in the wetland sediments. However, flufenoxuron could not be detected in water. The density, diversity, and abundance of macroinvertebrates largely varied over time. Redundancy and formal concept analyses showed that concentrations of flufenoxuron, vegetation cover, and flow conditions significantly determine the community structures of stormwater wetland macroinvertebrates. This study shows that flow conditions, vegetation cover, and runoff-related pesticides jointly affect communities of benthic macroinvertebrates in stormwater wetlands.

Runoff represents a prevailing process of mobilization and transfer of pesticides from agricultural land into aquatic ecosystems (Liess and Schulz 1999; Schriever and Liess 2007). Agricultural surface runoff can transport large amounts of pesticides and cause, in receiving ecosystems, adverse ecotoxicological effects on nontarget organisms, such as macroinvertebrates (Liess and Schulz 1999; Liess and von der Ohe 2005; Thiere and Schulz 2004). In particular, sediments in ecosystems receiving contaminated runoff can be a sink for pesticides associated with suspended solids (Fiedler and Rösler 1993). However, the relation between pesticides in agricultural runoff and the seasonal dynamics of benthic macroinvertebrate communities has rarely been assessed in the field (Jergentz et al. 2004; Liess and Schulz 1999; Thiere and Schulz 2004).

Stormwater wetlands are engineered worldwide with the primary objective to collect and temporarily store runoff (Gregoire et al. 2009). Stormwater wetlands can be hydrologically connected to an upstream agricultural catchment and receive runoff-related pesticides (Maillard et al. 2011). Previous studies have shown the effect on freshwater macroinvertebrate communities of habitat degradation (Stepenuck et al. 2008), organic pollution (Neumann et al. 2002; Whitehurst 1991) and flow conditions (Gallardo et al. 2008). Various studies have attempted to relate transient insecticide peaks from agricultural runoff with changes of the macroinvertebrate communities in rural streams or rivers (Berenzen et al. 2005; Thiere and Schulz 2004). However, the effect of mixtures of

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herbicides, fungicides, and insecticides on macroinvertebrates has rarely been investigated in wetlands receiving contaminated runoff (Liess and von der Ohe 2005). Pesticides have been reported to affect wetland macroinvertebrates at various levels of their biological organization (Berenzen et al. 2005; Probst et al. 2005). Schulz et al. (2002) evaluated the effect of azinphopmethyl, an organophosphate insecticide, on macroinvertebrate communities in a wetland system and reported shifts in the density of several macroinvertebrate populations. However, the density, diversity, and dynamics of benthic macroinvertebrates in stormwater wetlands that received agricultural runoff has not been so far described nor related to environmental variables, such as vegetation density, flow condition, and concentrations of runoff-related pesticides.

Herbicides, fungicides, and insecticides are frequently applied on Alsatian vineyards (France) from April to August. Recent studies have reported that both timing of pesticide application and rainfall-runoff characteristics determines concentrations and loads of runoff-related pesticides at the outlet of a representative vineyard catchment (Gregoire et al. 2010; Maillard et al. 2011). These studies also showed that the recently introduced insecticide flufenoxuron (Cascade; 1-[-4(2-chloro-α-α-α-trifluoro-*p*-tolyloxyl)-2-fluorophenyl]-3-(2,6-difluorobenzoyl) urea is a major compound applied on the catchment. Flufenoxuron is a benzoylurea pesticide that acts as an insect-growth regulator and chitin-synthesis inhibitor to control immature stages of insects and phytophagous mites on fruits and vegetables (Mommaerts et al. 2006). Although flufenoxuron has not been detected in runoff water, it is suspected to be mainly associated with suspended solids in runoff. Hence, flufenoxuron may contaminate sediments in aquatic ecosystems, such as stormwater wetlands that receive contaminated runoff, and affect nontarget organisms, such as macroinvertebrates.

The aim of the present study was to assess, during an entire period of pesticide application (April to September 2009), the communities of benthic macroinvertebrates in a stormwater wetland that receives contaminated runoff from a vineyard catchment area. We hypothesised that macroinvertebrates are mostly affected by runoff-related pesticides, in particular the insecticide flufenoxuron, rather than by flow conditions, water chemistry, and vegetation in the wetland. Redundancy analysis was used to evaluate the relation between changes in the macroinvertebrate communities and characteristics of the stormwater wetland (i.e., flow conditions, water chemistry, vegetation, and pesticide concentrations in both water and sediments). In addition, the data were explored using formal concept analysis (Galois lattices) to retrieve sets of environmental characteristics associated with specific groups of macroinvertebrates, which were then interpreted with respect to the functioning of the stormwater wetland.

Material and methods

Study Area and Pesticide Application

The present study was performed between April and September 2009 at a stormwater wetland located at the outlet of a 42.7-ha vineyard catchment in Rouffach (Alsace, France; 47°57′9 N, 07°17′3 E). The characteristics of the catchment area and agricultural practices have been previously described (Gregoire et al. 2010). Briefly, application of pesticides mainly proceeds from middle of April (bud-breaking of grapevine) until the end of September (harvesting). Nine fungicides, 6 herbicides, 1 insecticide (i.e., flufenoxuron), and 4 degradation products (*N*-(3.4-dichlorophenyl)-*N*-(methyl)-urea (DCPMU), *N*-(3.4 dichlorophenyl)-urea [DCPU] and 3.4-dichloroaniline, which are degradation products of diuron, and aminomethylphosphonic acid [AMPA], a degradation product of glyphosate) were selected for the present study because of their widespread use as well as high frequency of application and detection as shown in previous studies (Gregoire et al. 2010; Maillard et al. 2011). The studied compounds belong to 12 different chemical groups listed in the Table 1. Rainfall-runoff events do not generate permanent stream in the catchment and statistically occur every week. The mean annual precipitation is 609 ± 121 mm (period from 1998 to 2009). Runoff converges at the outlet of the catchment where it is collected by a stormwater wetland and represents the main route of pesticide entry into the wetland.

Stormwater Wetland

The stormwater wetland has a surface area of 319 m^2 and a total volume of 1500 m^3 and was constructed in 2002 to control flood in the downstream urban area (Fig. 1). The wetland is composed of a forebay (215 m^2), which collects suspended solids in runoff, and a 13 m long, 8 m wide, and 0.6 m deep gravel filter. Sediments are removed from the forebay every 3 years and were removed on November 2008, 5 months before the beginning of the present study. The average hydraulic retention time of the wetland was 10.8 ± 2.6 h during the investigation period. The water-storage capacity of the sediment deposition zone was 40 m^3. Water depth was homogeneous within the forebay but varied from 0.05 to 0.5 m from April to September. The granulometry of the wetland sediments was: clay 44%, fine silt 33%, coarse silt 10%, fine sand 5%, and coarse sand 8%. The chemical composition of the sediment was as follows: organic carbon 14.8 %, SiO_2 50%, Al_2O_3 9.5%, MgO 2.2%, CaO 11.6%, Fe_2O_3 4.1%, MnO 0.1%, Na_2O 0.7%, and K_2O 2.5% ($n = 5$ [pH = 8.1]). A detailed survey of the vegetation in the stormwater wetland was performed in June, and the results are listed in Supplemental

Table 1 Mean concentrations and ranges of pesticides in inlet runoff in the water column and in sediments of the stormwater wetland (Rouffach, Haut-Rhin, France)

Compound	Chemical group	Type	Log K_{oc} (mg L^{-1})	EC_{50}/NOEC (*D. magna*)[e] (µg L^{-1})	Mode of action	Spring (April 6 to June 15)			Summer (June 15 to September 29)		
						Inlet runoff ($n = 10$)[a] (µg L^{-1})	Water column of the wetland ($n = 20$)[a] (µg L^{-1})	Wetland sediment ($n = 20$) [a] (µg kg^{-1})	Inlet runoff ($n = 18$)[a] (µg L^{-1})	Water column of the wetland ($n = 20$)[a] (µg L^{-1})	Wetland sediment ($n = 20$) [a] (µg kg^{-1})
Azoxystrobin	Strobilurin	Fungicide	482	230/44	Respiration inhibitor	ND	ND	ND	0.02 (ND–0.12)	0.01 (ND–0.07)	ND
Cymoxanil	Cyanoacetamide oxime	Fungicide	43.6	27000/67	Foliar with protective and curative activity	0.13 (ND–0.90)	0.03 (ND–0.60)	ND	ND	ND	ND
Cyprodinil	Anilinopyrimidine	Fungicide	1706	33/8.8	Protein inhibitor	ND	ND	ND	0.02 (ND–0.14)	0.01 (ND–0.04)	<LOQ (ND–7.0)
Carbendazim	Benzimidazole	Fungicide	225	150/1.5	Inhibition of mitosis and cell division	ND	0.01 (ND–0.11)	ND	ND	0.04 (ND–0.20)	ND
Dimethomorph	Morpholine	Fungicide	348	>10600/5	Lipid synthesis inhibitor.	0.03 (ND–0.18)	ND	ND	2.22 (ND–10.0)	1.62 (ND–5.80)	ND
Diuron	Phenylurea	Herbicide	1067	5700/96	Photosynthesis inhibitor	0.16 (0.11–0.32)	0.04 (ND–0.08)	<LOQ (ND–4.0)	0.02 (ND–0.16)	0.01 (ND–0.03)	<LOQ (ND–4.0)
DCPU	Unclassified	*Metabolite*	NA	NA/NA	NA	ND	ND	ND	ND	ND	ND
DCPMU	Unclassified	*Metabolite*	NA	NA/NA	NA	ND	ND	ND	ND	ND	ND
3.4-dichloro-aniline	Unclassified	*Metabolite*	2.69	120/5	NA	ND	ND	ND	ND	ND	ND
Flufenoxuron	Benzoylurea	Insecticide	486092	0.043/ 0.07	Inhibitor of chitin biosynthesis	ND	ND	2.75 (ND–6.0)	ND	ND	<LOQ (ND–3.0)
Gluphosinate	Organophosphate	Herbicide	NA	NA/NA	Glutamine synthetase inhibitor	0.85 (ND–6.30)	0.11 (ND–1.40)	ND	ND	ND	ND
Glyphosate	Phosphonoglycine	Herbicide	21699	40000/30000	Inhibition of lycopene cyclase	4.13 (0.30–11.0	0.10 (ND–0.40)	<LOQ (ND–6.0)	5.95 (0.20–15.0)	1.61 (ND–3.90)	ND
AMPA	Unclassified	*Metabolite*	8027	40000/NA	NA	1.37 (0.20–2.30)	0.08 (ND–0.30)	ND	2.53 (ND–21.0)	2.05 (ND–4.80)	ND
Isoxaben	Benzamide	Herbicide	354	>1300/690	Disrupts root and stem development in germinating seeds	0.11 (ND–0.23)	0.02 (ND–0.10)	ND	0.01 (ND–0.08)	ND	ND
Kresoxim methyl	Strobilurin	Fungicide	308	186/32	Block electron transfer and respiration of the fungi	0.01 (ND–0.05)	0.02 (ND–0.10)	ND	0.02 (ND–0.40)	ND	ND
Metalaxyl	Phenylamide	Fungicide	500	28000/NA	Suppress sporangial formation, mycelial growth	1.43 (ND–5.80)	0.21 (ND–0.64)	ND	0.05 (ND–0.35)	ND	ND
Pyrimethanil	Anilinopyrimidine	Fungicide	301	2900/940		ND	ND		0.02 (ND–0.08)	0.01 (ND–0.06)	ND
Simazine	Triazine	Herbicide	130	1100/2500	Inhibits photosynthesis (photosystem II)	0.08 (0.04–0.18)	ND		<0.02 (ND–0.02)	ND	ND
Terbuthylazine	Triazine	Herbicide	231	21200/19	NA	0.06 (0.06–0.07)	ND		<0.02 (ND–0.04)	ND	ND
Tetraconazole	Triazole	Fungicide	1152	3000/190	NA	0.02 (ND–0.09)	0.01 (ND–0.07)		0.01 (ND–0.07)	ND	<LOQ (ND–2.0)

Degradation products are indicated in italics

ND not detected, *NA* not available, *<LOQ* lower than the detection limit, EC_{50} half maximal effective concentration, *NOEC* no observed effect concentration

[a] Mean and range values are given integrating

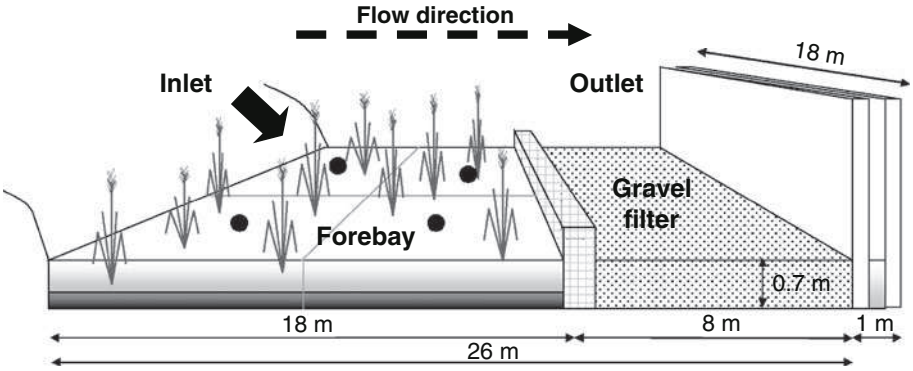

Fig. 1 Schematic of the storm water wetland (Rouffach, Alsace, France). The four invertebrate, water, and sediment sampling locations in the sediment-deposition zone of the wetland forebay are indicated by *black circles*. *Black arrows* indicate water direction within the wetland

Table S1. Vegetation cover in the sediment-deposition zone was estimated as the vegetated cover in the forebay relative to the total surface. The vegetation cover mainly was formed of *Phragmites australis*, *Schoenoplectus lacustris*, and *Typha latifolia*. The vegetation cover represented <1% of the area in April, 5% in May, 25% in June, 60% in July, 70% in August, and 85% in September. *P. australis* ranged between 70 and 80% of the total vegetation cover throughout the investigation period.

Sampling Procedure and Identification of Macroinvertebrates

Water samples for hydrochemical and pesticide analyses were collected at the inlet and in the sediment-deposition zone of the wetland from April 6 through September 29, 2009. Runoff discharges were continuously monitored by water-depth measurements using bubbler flow modules (Hydrologic; Sainte-Foy, Québec, Canada) combined to a Venturi channel. Water samples were collected every 6 m^3 at the inlet of the wetland using a 6712FR ISCO Teledyne automatic sampler (ISCO, Lincoln, NE, USA). Water samples (100 mL) were collected in jars and were stored in the dark at 4°C. The water samples systematically were collected after each runoff event and placed on ice during transportation to the laboratory. For the detailed procedure of water sampling, refer to Domange and Gregoire (2006) and Gregoire et al. (2010). Data regarding gross-solids sampling, handling, and analysis in runoff water have been limited and often inconsistent. In this study, suspended solids were obtained from continuously operating samplers consisting of 2-mm and 50-µm sieves intercepting runoff and installed in series at the inlet of the wetland. The samplers were emptied twice a month.

Ten sampling campaigns were performed on April 21, May 5, May 19, June 2, June 15, June 30, July 20, August 6, August 19, and September 29, 2009. At each sampling campaign, four replicate samples of macroinvertebrates, water, and sediment were taken from the forebay. Grid-cell sampling was performed by dividing the forebay area in four equal rectangular cells (9 × 6 m), and samples were collected at the centre of each cell (Fig. 1). Benthic macroinvertebrates were collected between the plants using a Surber-type net (area 400 cm^2, mesh size 250 µm). Macroinvertebrate samples were preserved in the field in 10% formaldehyde. Macroinvertebrates were sorted in plastic tubs, preserved in 70% ethanol, and identified at the order level. *Chironomidae* and *Oligochatea*, which were the most represented orders, were mounted between slides. *Chironomidae* were cleared with Amann's lactophenol, and *Oligochaetea* were covered with Canada balsam (Carl Roth, Karlsruhe, Germany). *Chironomidae* and *Oligochaetea* were identified at the genus level using a stereomicroscope. Coinciding with the sampling of macroinvertebrates, four replicate grab samples of water (collected at 0- to 10-cm depth from the water surface) and surface sediment (collected from 0- to 5-cm depth from the sediment surface) also were taken for pesticide and chemical measurements. Dissolved oxygen, pH, conductivity, redox potential, and temperature in water were directly measured in the field using WTW Multi 350i portable sensors (WTW, Weilheim, Germany). Water samples were dispensed in parallel into 100-mL glass-and-plastic vials (headspace-free) for pesticide analysis. Plastic vials were used to limit sorption of glyphosate and aminomethylphosphonic acid from the pesticide extraction solutions (i.e., methanol) onto glassware surfaces (Giannopolitis and Kati 2008). One litre of pre-acid washed (10% HCl, distilled water-rinsed) high-density polyethylene bottles for hydrochemical analysis. Water and sediment samples were placed on ice and transported directly to the laboratory for chemical analysis. Pesticide and chemical analysis of water samples was performed within 24 h of collection. Sediment samples were kept at −20°C until chemical analysis, which occurred within 1 month of collection.

Hydrochemical and Pesticide Analysis

Twenty-one hydrochemical parameters (total suspended solids [TSS], total volatile suspended solids, total inorganic

carbon, dissolved inorganic carbon, nonpurgeable organic carbon [NPOC], dissolved organic carbon, total Kjeldahl nitrogen [TKN], PO_4^{2-}, P_{tot}, NO_3^-, NO_2^-, NH_4^+, Mn^{2+}, Fe^{2+}, Fe_{tot}, SO_4^{2-}, Mg^{2+}, Ca^{2+}, Na^+, Cl^-, and K^+) were determined by ISO standards (French) and laboratory procedures. Pesticide analysis was performed according to the NF XPT 90-210 French standards at the Pasteur Institute of Lille (France), which is a service of pesticide residues analysis accredited by the French National Accreditation Authority (COFRAC). The COFRAC calibration certificate is recognized by other European calibration services (European Cooperation for Accreditation). Briefly, water samples were filtered through 1-μm glass-fiber filters before analyzing the subsequent extracts. The 16 pesticides (azoxystrobin, cymoxanil, cyprodinil, carbendazim, dimethomorph, diuron, flufenoxuron, gluphosinate, glyphosate, isoxaben, kresoxim methyl, metalaxyl, pyrimethanil, simazine, terbuthylazine, tetraconazol) and the 4 degradation products (DCPU, DCPMU, 3.4-dichloroaniline, and AMPA) were quantified using liquid chromatography coupled to tandem mass spectrometry (LC-MS-MS). Quantification of glyphosate, AMPA, and gluphosinate was performed after derivatization with fluorenemethoxycarbonyle. Limits of pesticide quantification in water samples ranged from 0.02 to 0.1 μg L^{-1} (refer to Supplemental Table S2 for detailed detection and quantification limits of pesticides). Pesticide residues in sediment samples were extracted by ultrasonic extraction with methanol followed by LC-MS-MS measurements. Limits of quantification ranged from 2 to 10 μg kg^{-1}. Extraction efficiencies of pesticides from water and sediment samples were estimated for each sample set by spiking with surrogates. Surrogate recovery for water samples ranged from 70 to 89% and those of sediment from 68 to 85% (refer to Supplemental Table S2). Further quality control was achieved by using a blank for each set of samples.

Data Analysis

Pesticide loads at the inlet of the wetland that correspond to a single runoff event were obtained by multiplying mean pesticide concentrations by the corresponding runoff volume. Loads at the inlet of the wetland were calculated from the integral sum of all event loads between two sampling campaigns. The diversity of macroinvertebrates for each sampling campaign was estimated using the Shannon index (H′) (Shannon and Weaver 1949). Ordination methods were performed using R software (R Development Core Team 2010, version 2.12.1). Before statistical analysis, data of macroinvertebrate abundances were transformed with the Hellinger transformation to satisfy the assumption of the statistical tests and avoid numerical hindrance associated with long gradients (Legendre and Gallagher 2001). First, a hierarchical cluster analysis was performed on the macroinvertebrate matrix using the method of Ward's minimum variance. The optimal number of clusters was then determined using the criterion of average silhouette of the data (Rousseauw 1987). Second, redundancy analysis (RDA) was used to test and quantify the relative importance of environmental variables on the variation of structure of benthic macroinvertebrate. Tested variables were pesticide concentrations in water and sediments, pesticide loads entering the wetland between two sampling campaigns, hydrochemical variables, runoff volumes entering the wetland between two sampling campaigns, time between two runoff events, and vegetation cover of the wetland. Taxa representing <5% of the total individuals present throughout the investigation period (i.e., *Erpobdella* sp., *Aulodrilus* sp., *Clirellio* sp., *Haplotaxis* sp., *Moldaviensis* sp., *Enchytraeidae* sp., *Camptocladius* sp., *Omisus* sp., *Paracricotopus* sp., *Polypedilum* sp., *Tanypus* sp., *Virgotanytarsus* sp., *Psychodidae* sp., *Boyeria* sp., *Ischnura* sp., *Radix* sp., and *Stagnicola* sp.) were discarded from the statistical analysis to avoid inadequate estimates of the site positions in the plot. Only environmental variables with a significance level $p < 0.001$ were considered significant and were included in the final model. The significance of the constrained ordination process was tested with the Monte Carlo permutation test (1000 permutations).

To complement the redundancy analysis, a formal concept analysis (Barbut and Monjardet 1970; Davey and Priesley 1990; Ganter and Wille 1999) was performed to extract sets of environmental variables that explain variations in the macroinvertebrate community structures. Detailed information on the principle and use of formal concept analysis is listed in the Supplementary Information. The formal concept analysis previously was used to cluster objects and attributes from binary data sets and to analyze the relation between clusters of objects and attributes (Bertaux et al. 2009; Messai et al. 2005). In the present study, the original data were discretized before the formal concept analysis. The most significant variables retained in the final redundancy model (i.e., concentrations of metalaxyl in water and of flufenoxuron in the wetland sediment, vegetation cover, duration of the time between events, rainfall volume) were selected for the formal concept analysis. The values were converted into quartiles (with the first quartile representing the lowest values), and quartiles with the same value were merged. The resulting data were then transformed into a disjunctive binary table. A Galois lattice was built from this table using Conexp software. The lattice contained 96 concepts, i.e., couples gathering one set of environmental data values and one set of macroinvertebrates communities from which 17 key concepts explaining the temporal variation of macroinvertebrates assemblages were retrieved.

Results

Hydrological and Hydrochemical Conditions

Hydrological and hydrochemical characteristics of the stormwater wetlands are listed in Table 2. The analysis of both hydrological and hydrochemical data consistently showed that conditions in the wetland largely differed during the investigation period, in particular between spring (April 01 to June 15) and summer (June 15 to September 29). The minimal threshold for considering a runoff event in the present study was 1 m^3. Twenty-eight rainfall-runoff events ranging from 1.1 to 114 m^3 occurred during the investigation period, generating a total runoff volume of 730 m^3. From April 1 to June 15, the mean runoff volume was 16.5 ± 9.5 m^3, whereas it was 31.4 ± 31.9 m^3 from June 15 to September 29, indicating larger and more variable inflows in summer compared with spring. Mean water temperature and pH value across all sampling points in the wetland and campaigns were 19.0 ± 4.3°C and 7.6 ± 0.3°C, respectively. In spring, oxic conditions prevailed in the wetland, as inferred from the mean values of redox potential >50 mV, concentrations of ferrous iron <1 mg L^{-1}, and concentrations of dissolved

Table 2 Hydrological characteristics and water chemistry of the stormwater wetland (Rouffach, Alsace, France)

Parameter	Spring (April 06 to June 15) ($n = 26$)[a]	Summer (June 15 to September 29) ($n = 24$)[a]
Inlet runoff volume (m^3)	16.5 (7.0–30.2)	31.4 (1.1–114)
No. of runoff events	10	18
Quiescent period (days) (between 2 runoff events)	5.1 ± 7.3	5.2 ± 5.7
Inlet flow rate (m^3 h^{-1})	2.1 ± 2.7	12.2 ± 11.8
Outlet flow rate (m^3 h^{-1})	0.3 ± 0.8	0.2 ± 1.0
Total load of dissolved pesticides entering the wetland (g)	1.219	6.819
Vegetation cover (%)	15 (0–50)	70 (50–90)
Temperature (°C)	21.4 (15.5–26.5)	23.3 (18.6–28.9)
Redox potential (mV)	204 (44–297)	–81 (–228–188)
Dissolved oxygen (mg L^{-1})	9.2 (2.9–13.8)	3.7 (0.2–8.8)
Electric conductivity (μS cm^{-1})	908 (117–1036)	376 (269–522)
pH (–)	7.8 (7.5–8.1)	7.5 (7.0–8.0)
Total suspended solids (mg L^{-1})	20.7 (4.8–59.8)	43.0 (3.2–266)
Total volatile suspended solids (mg L^{-1})	6.9 (0.8–29.2)	19.2 (0.4–116)
NPOC (mg L^{-1})	19.8 (13.3–65.6)	13.7 (6.7–34.9)
Total inorganic carbon (mg L^{-1})	63.8 (55.6–68.2)	47.9 (29.5–92.2)
Dissolved inorganic carbon (mg L^{-1})	61.0 (45.2–67.7)	44.1 (25.3–71.4)
Dissolved organic carbon (mg L^{-1})	19.8 (13.5–62.5)	12.1 (7.2–27.4)
Orthophosphorus (mg L^{-1})	0.06 (0.06–0.06)	0.14 (0.01–0.30)
Total phosphorus (mg L^{-1})	0.40 (0.14–1.88)	0.74 (0.11–2.4)
Chloride (mg L^{-1})	30.9 (26.9–36.3)	2.2 (1.4–4.1)
TKN (mg L^{-1})	4.6 (1.1–11.6)	2.5 (1.0–5.4)
Nitrate (mg L^{-1})	8.8 (2.4–18.5)	2.8 (0.2–11.5)
Nitrite (mg L^{-1})	0.26 (0.11–0.75)	0.03 (n.d.–0.10)
Ammonium (mg L^{-1})	0.37 (0.10–0.97)	0.19 (0.01–1.4)
Total iron (mg L^{-1})	1.7 (1.1–2.3)	1.6 (0.12–5.4)
Iron(II) (mg L^{-1})	0.15 (0.02–0.35)	0.68 (0.15–6.0)
Manganese(II) (mg L^{-1})	0.24 (0.24–0.24)	0.72 (0.38–1.7)
Sulfate (mg L^{-1})	241 (159–325)	29 (5.1–63.6)
Sodium (mg L^{-1})	9.8 (5.0–14.6)	3.7 (0.94–9.1)
Potassium (mg L^{-1})	5.2 (2.8–14.6)	5.3 (1.1–9.4)
Calcium (mg L^{-1})	125 (96–164)	64.5 (46.2–85.9)
Magnesium (mg L^{-1})	35.2 (27.7–50.4)	12.4 (5.8–29.1)

[a] Mean and range values are given integrating during the corresponding season

oxygen >2.9 mg L^{-1} in water from the sediment-deposition zone. In summer, lower concentrations of dissolved oxygen and negative values of redox potential in the wetland indicated the prevalence of an anoxic milieu.

Dissolved and Particle-Associated Pesticides in the Wetland

Measurements of pesticide concentrations and loads performed during runoff and in the stormwater wetland are presented in Table 1 and Figure 2. The total dissolved load of pesticides that entered into the wetland in summer (6.819 g, i.e., 85% of total dissolved load) was larger compared with that in spring (1.219 g, i.e. 15% of the total dissolved load) (Fig. 2b). The variation of pesticide loadings that entered the wetland reflects both the seasonal change of runoff volumes (see Fig. 2a) and timing of pesticide applications in the vineyard catchment (31% of the total applications occurred in spring, and 69% occurred in summer [data not shown]), in agreement with previous studies on the catchment area (Gregoire et al. 2010). Pesticide concentrations in water from the wetland were smaller in spring compared with those measured in summer, although dissolved concentrations found in runoff entering the wetland were similar in spring and summer (Table 1). Dissolved concentrations of flufenoxuron were systematically lower than the detection limit during the investigation period. Patterns of pesticide concentrations associated with suspended solids and the wetland sediments also differed between spring and summer (Fig. 2c). Flufenoxuron, dimethomorph, and cyprodinil concentrations associated with suspended solids at the inlet increased over time and then decreased from July 20 onward. However, mean concentrations of pesticides and degradation products in the wetland sediments, except for flufenoxuron, were close to or lower than the detection limits. Concentrations of flufenoxuron in suspended solids ranged from lower than the limit of quantitation (LOQ) to 15 μg kg^{-1} in

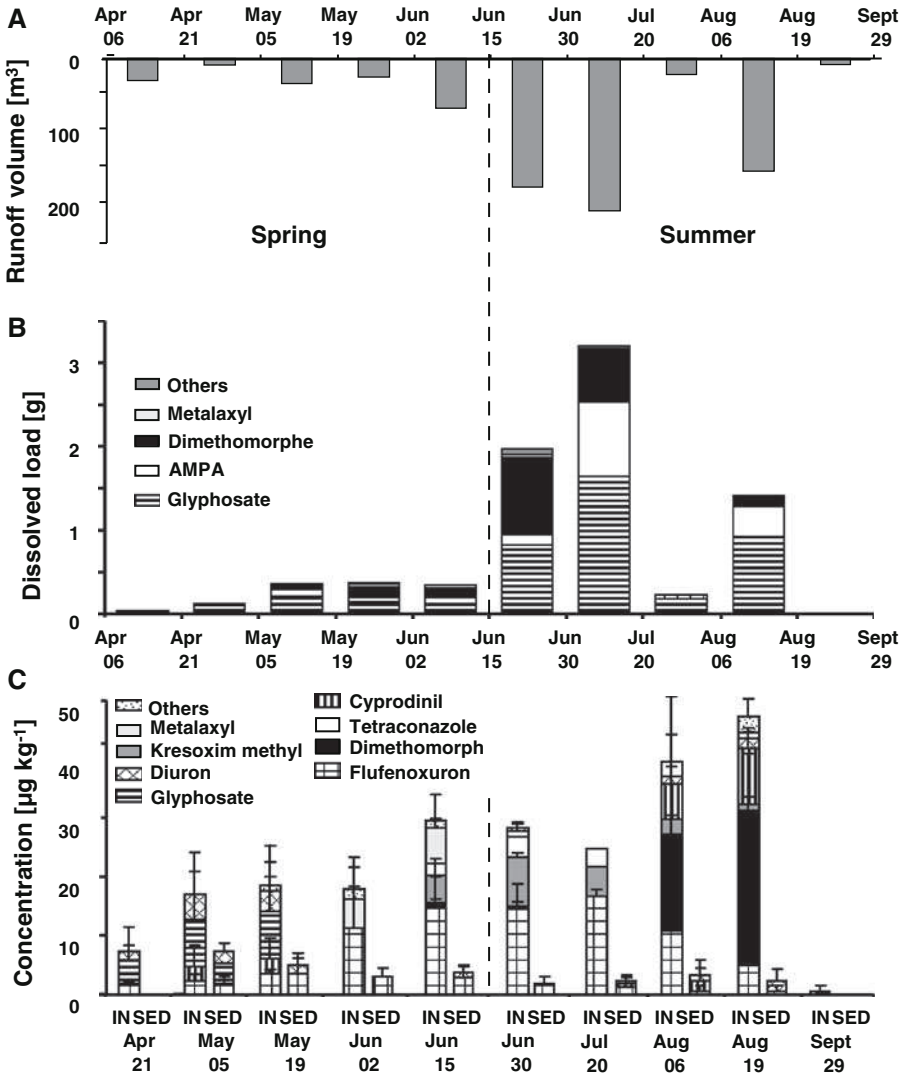

Fig. 2 Temporal changes of pesticide inputs into the stormwater wetland (Rouffach, Alsace, France). **a** Runoff volume entering in the storm basin between two sampling campaigns. **b** Loads of dissolved pesticides in runoff between two sampling campaigns. **c** Mean concentrations of pesticides associated with inflowing suspended solids (IN) and wetland sediments (SED). *Error bars* are ±1 SD

spring and lower than LOQ to 16.75 μg kg^{-1} in summer, whereas concentrations in wetland sediments ranged from lower than LOQ to 3.50 μg kg^{-1} in spring and lower than LOQ to 1.75 μg kg^{-1} in summer (Fig. 2c).

Abundance and Distribution of Macroinvertebrate Taxa

Changes of the abundance and distribution of macroinvertebrate taxa during the investigation period are summarized in Fig. 3. In total, 40 different macroinvertebrate taxa, representing 14,489 enumerated and identified individuals, were found in the stormwater wetland. Twenty-five core taxa represented >5% of the total identified taxa. The average number of individuals per m^2 during the 10 sampling campaigns was 837 ± 343 (mean ± SDs; n = 40). The generic richness ranged from 12 (on September 29) to 23 (on April 21 and May 19) (Fig. 3a). Maximum and minimum values of the Shannon diversity index were observed on April 21 ($H' = 1.53 \pm 0.25$; $n = 4$) and on June 15 ($H' = 0.6 \pm 0.27$; $n = 4$), respectively. Diptera and Oligochaeta orders dominated throughout the investigation period representing, respectively, 61.0 ± 15.5% and 33.8 ± 14.3% of the total abundance of macroinvertebrates (Fig. 3b). The other orders were as follows: Nematoda (2.6 ± 3.6%), Gastropoda (1.5 ± 1.0%), Ephemeroptera (0.8 ± 1.0%), Coleoptera (0.2 ± 0.6%), Odonata (0.14 ± 0.3%), and Hirunidea (0.02 ± 0.05%). The genera *Chironomus* and *Tubifex* dominated during the investigation

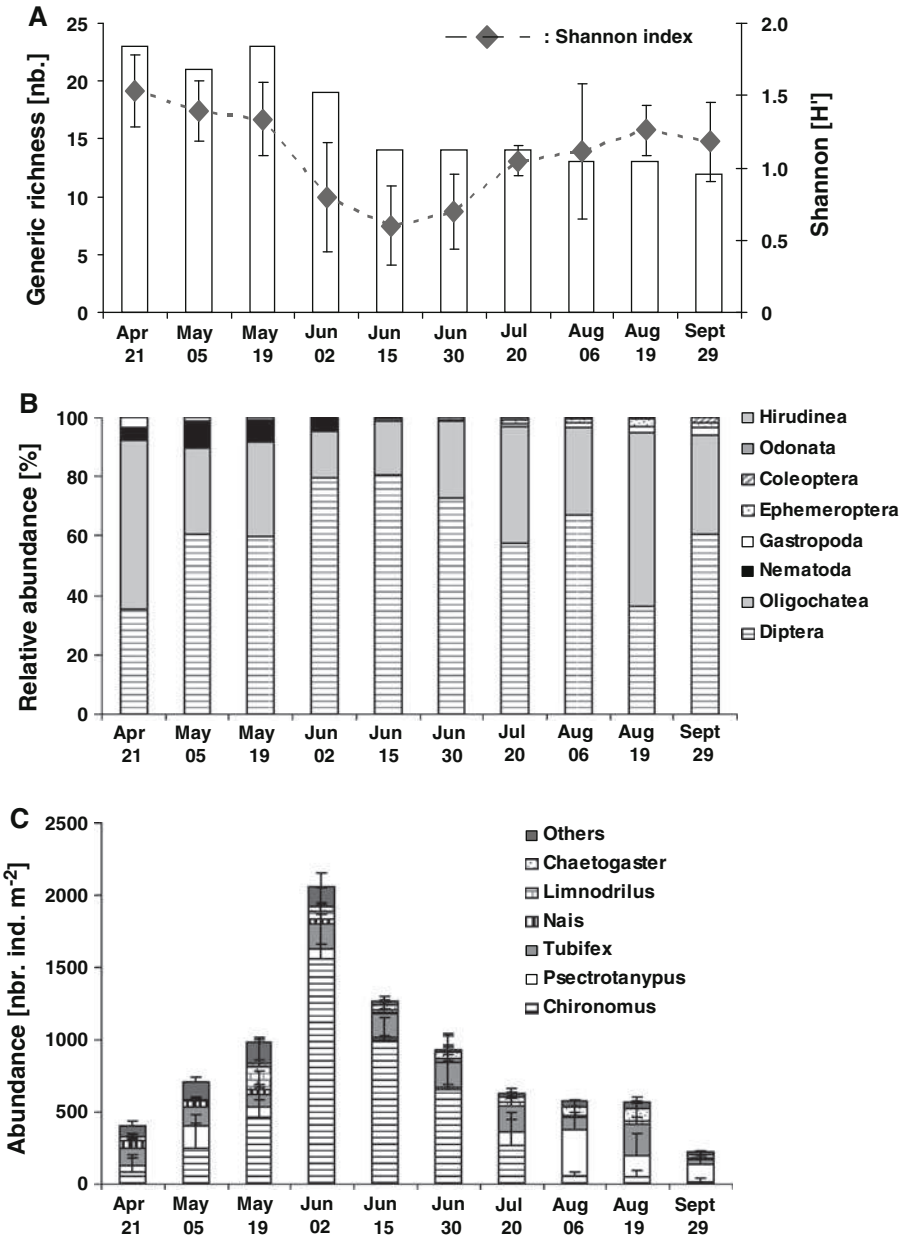

Fig. 3 Temporal changes in the macroinvertebrate assemblages in the stormwater wetland (Rouffach, Alsace, France). **a** Richness and Shannon index at the generic level. **b** Relative abundance at the order level. **c** Abundance at the generic level during the investigation period. Data are mean ± 1 SD

period and represented 61.7 ± 32.9% and 58.0 ± 13.3%, respectively, of the Diptera and Oligochaeta (Fig. 3c). The abundance of macroinvertebrate was 404 ± 310 individuals/m^2 ($n = 4$) at the beginning of the investigation period (April 21), was maximal on June 02 (2063 ± 525 individuals/m^2; $n = 4$), and then progressively decreased over time (228 ± 98 individuals/m^2 on September 29; $n = 4$) (Fig. 3c). Changes in the abundance of *Chironomus* sp. mainly contributed to the overall changes of the macroinvertebrate abundance. The orders Odonata, which are mainly predators of macroinvertebrate larvae, and Ephemeroptera were found in the stormwater wetland from June 02 to September 29.

Dynamics of Macroinvertebrate Communities

RDA analysis allowed evaluation of the relation between wetland macroinvertebrate assemblages, flow conditions, water chemistry, and pesticides and showed distinctive patterns (Fig. 4). The ordination triplot shows 7 macroinvertebrate taxa, 12 significant environmental variables associated with changes in the macroinvertebrate communities, and 4 replicate samples of benthic macroinvertebrate assemblages for each of the 10 sampling campaigns. Symbols in the plot lying close together display similar patterns of macroinvertebrate community. The symbols mostly clustered according to sampling date, indicating that macroinvertebrate communities changed during time. The first two axes explain 51.7% of the total inertia and clearly separate assemblages belonging to sampling campaigns 1 (April 21) to 6 (June 30) from assemblages belonging to sampling campaigns 7 (July 20) to 10 (September 29). The slight variability among the 4 replicate samples collected during a sampling campaign results from heterogeneity among and within habitats, indicating the importance of habitat differences in terms of plant density and structure (Sahuquillo et al. 2007). On the initial 57 environmental variables included in the RDA model, only the 12 most significant variables ($p < 0.001$) were retained in the final model (i.e., flufenoxuron in sediments, metalaxyl in water, rainfall volumes, vegetation cover, quiescent time between two runoff events, Mn^{2+}, Na^{+}, Mg^{2+}, electric conductivity, Cl^{-}, SO_4^{2-}, and redox potential). The non-selected variables were redundant or did not increase the significance of the model. The twelve selected variables explained 66.7% ($p < 0.005$) of the total variance in macroinvertebrate structures, whereas 33.3% remained unexplained. The model with solely metalaxyl in water and flufenoxuron in sediments as constraining variables explained 23.1% ($p < 0.001$) of the total community variance.

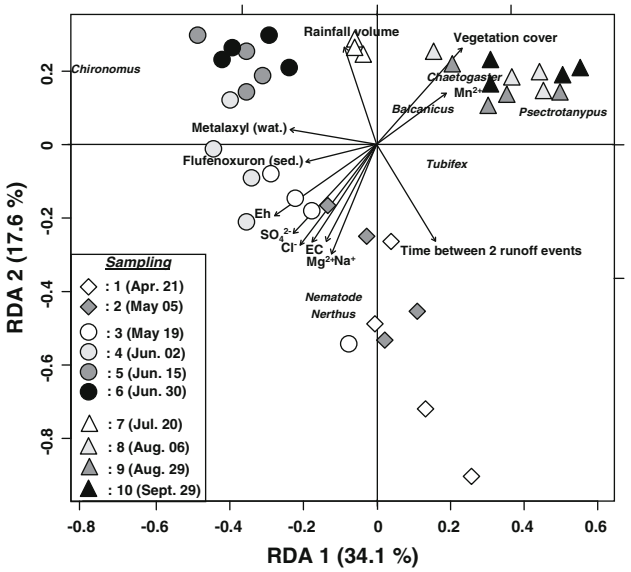

Fig. 4 RDA of 40 macroinvertebrate taxa and 12 environmental variables (showing significant correlation to the community data; Monte Carlo permutation test, $p < 0.001$). Each *symbol* represents a replicate of macroinvertebrate assemblage (of four replicates per sampling date). Most discriminating macroinvertebrate taxa (*Chaetogaster, Psectrotanypus, Tubifex, Nematode, Nerthus*, and *Chironomus*) are indicated in *italics*. The value % on the axes indicates the percentage of total variation that they explain. The variance decomposition analyses of RDA on the macroinvertebrate community patterns showed that the selected environmental variables explained 66.7% of the variance. Sum of all canonical eigenvalues 0.180; total inertia 0.269; Monte Carlo overall permutation test $F = 4.52$, $p = 0.005$. Eh = redox potential, EC = electric conductivity

The hierarchical cluster analysis, combined with a determination of the optimal number of clusters, allowed to clearly separate three main clusters of macroinvertebrate assemblages during the investigation period. In the first cluster, assemblages from April 21 to May 5 were characterized by *Nerthus* (Oligochaeta) and nematode taxa. Macroinvertebrate assemblages were mainly associated with larger values of redox potential and concentrations of dissolved ions (Na^{+}, Mg^{2+}, Cl^{-}, and SO_4^{2-}). From May 19 to June 30, rainfall and runoff events and related pesticide concentrations in both the dissolved and the solid phases were larger (Figs. 2, 3). The genus *Chironomus* (Diptera) was dominant in the communities. Greater concentrations of flufenoxuron and metalaxyl detected in the wetland from May 5 to June 30 correlated with changes in the macroinvertebrate assemblages. This change was emphasized by the shift from the first to the second cluster on the ordination triplot. The third cluster corresponded to macroinvertebrate assemblages collected from July 20 to September 29. During this period, changes of the macroinvertebrate community structures correlated with larger vegetation cover, lower pesticide concentrations, and reducing conditions, as inferred from lower redox potential values and larger Mn^{2+} concentrations in the wetland. The

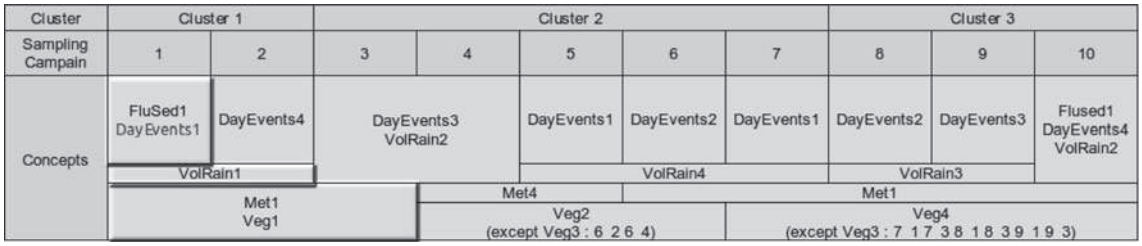

Fig. 5 Concepts extracted from the Galois lattice. The lattice was built on the most significant environmental variables selected in the RDA. Variables are denoted by a name and a number representing a quartile, e.g., FluSed1 (flufenoxuron in sediment, first quartile), DayEvents2 (quiescent period between two runoff events, second quartile), VolRain3 (rainfall volume, third quartile), Met4 (metalaxyl in water, fourth quartile), and Veg4 (vegetal cover, fourth quartile). There are only two quartiles for metalaxyl: The first one represents 75% of the values (concentration was lower than the LOQ). Each sampling is denoted by a number from 1 to 10. Each cell of the table, which is to be read from *bottom to top*, represents a concept, e.g., the cell in the bottom left corner represents the concept {Veg1, Met1} prevailing in sampling campaigns 1 to 3. The cell on the previous one represents the concept {Veg1, Met1, VolRain1}, which prevailed in sampling campaigns 1 and 2

genera *Chaetogaster* (Oligochaeta), *Balcanicus* (Ephemeroptera), and *Tubifex* (Oligochaeta) were preponderant in the communities.

Seventeen concepts were retrieved from the formal concept analysis (Fig. 5). Each concept consists of a set of sampling campaigns and their environmental characteristics. For example, the concept ({Veg2, MET4, Flused4}; {4_3, 4_4, 5_2, 5_3, 5_4}) is read as follows: Veg2, MET4, and Flused4 represent, respectively, the second quartile of vegetal cover, the fourth quartile of metalaxyl, and the fourth quartile of flufenoxuron values; 4_3, 4_4, represents replicate macroinvertebrate samples collected in the wetland during the fourth sampling campaign (June 02); and 5_2, 5_3, 5_4 replicates macroinvertebrate samples collected during the fifth sampling campaign (June 15). This concept indicates that theses particular macroinvertebrate assemblages are associated with an intermediate vegetal cover and high concentrations of flufenoxuron and metalaxyl. The formal concept analysis showed that the three clusters of macroinvertebrate assemblages, which were highlighted by the hierarchical cluster analysis, are characterized by different sets of environmental data. Cluster 1 (April 21 to May 5) is characterized by MET1, Veg1, VolRain1, DayEvents4; cluster 2 (May 19 to June 30) is characterized mainly by MET4, Veg2, VolRain2 or 4; and cluster 3 (July 20 to September 29) is characterized by MET1, Veg3 or 4, VolRain3 (Fig. 5).

Discussion

The aim of the present investigation was to evaluate, in a stormwater wetland, changes of macroinvertebrate communities in relation to runoff-related mixtures of pesticides, water chemistry, vegetation cover, and hydrological conditions. Although most studies focus on one target compound or compounds belonging to the same chemical group, we specifically evaluated the relation between different runoff-related pesticides, including the insecticide flufenoxuron, and benthic macroinvertebrate communities under field conditions and during an entire season of pesticide application. However, it was not possible to find an appropriate uncontaminated control site to specifically address the effect of pesticides. Indeed, other stormwater wetlands largely differed with respect to hydrological parameters or characteristics of the agricultural catchment. This renders comparison between stormwater wetlands difficult. To the best of our knowledge, most regional stormwater wetlands are hydrologically connected to agricultural catchment and thus receive runoff-related pesticides, with contamination patterns and regimes depending on the characteristics of the catchment. Therefore, we evaluated, within the same stormwater wetland, temporal changes of macroinvertebrates with respect to varying levels of runoff contamination. Because no published data on macroinvertebrate in stormwater wetlands exist so far, the results obtained in the present study only could be compared with data obtained in wetland systems, agricultural streams, or ponds that receive contaminated runoff.

Pesticide-Related Runoff

In our experimental design, surface runoff from the vineyard catchment represents the main entry route of dissolved and particle-associated pesticides in the studied stormwater wetland. Larger rainfall-runoff events induced larger loads of pesticide mixture entering into the stormwater wetland. Among the 20 different pesticides and degradation products examined in the present study, flufenoxuron and metalaxyl were the only contaminants that significantly correlated with changes in the macroinvertebrate community.

Generally, high concentrations of particle-associated flufenoxuron entering the wetland were found in runoff

entering the wetland. The largest flufenoxuron concentration reached 16.75 μg kg^{-1}. Flufenoxuron concentrations were on the same order of magnitude than those of other insecticides, such as azinphos-methyl, chlorpyrifos, or endosulfan, found in river suspended particles during runoff (Jergentz et al. 2004; Thiere and Schulz 2004). Flufenoxuron was detected throughout the investigation period in both runoff suspended particles and wetland sediments. In contrast, concentrations of the other pesticides in wetland sediments were low, which indicates the absence of pesticide accumulation in the wetland. High concentrations and persistence of flufenoxuron in wetland sediments can affect macroinvertebrate populations. Indeed, flufenoxuron is extremely toxic to *Daphnia magna* (48-h EC_{50} 0.04 μg L^{-1}) (Pesticide Safety Directorate 1995) at concentrations lower than the detection limit of flufenoxuron reached in the present study (detection limit 0.1 μg L^{-1}). Hence, flufenoxuron possibly was bioavailable and directly altered macroinvertebrate communities, although it could not be detected in the water column of the wetland. Moreover, flufenoxuron sorbed to the wetland sediment may slowly degrade and progressively release into the water column *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)alanine, its degradation product, and/or other unknown products. Nevertheless, *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)alanine has been found to be much less toxic to *D. magna* (48-h EC_{50} 852 mg L^{-1}) than flufenoxuron. Dissolved metalaxyl concentrations in the wetland (up to 5.8 μg L^{-1}) were four orders of magnitude lower than the reported 48-h EC_{50} (28 mg L^{-1}). Although redundancy analysis showed that greater metalaxyl concentrations between May 19 and June 15 correlated with changes in the macroinvertebrate assemblages, it is unlikely that metalaxyl affected the macroinvertebrates and directly caused those changes.

Impact of Hydrology and Hydrochemistry

In addition to pesticides, hydrologic factors, such as runoff frequency and flow characteristics, as well as hydrochemical parameters, such as turbidity, also can influence the diversity and structure of benthic macroinvertebrate communities. For instance, contaminated runoff events simultaneously can generate chemical stress due to pesticides and hydrological stress in the stormwater wetland. In the present study, the cumulated discharge volume between two sampling campaigns and the period between the last runoff event and the sampling were used as indicators of hydrologic stress. Both the redundancy and formal concept analyses emphasized that hydrological conditions significantly determined changes in macroinvertebrate communities. Hydrodynamic stress in stormwater wetlands is intermittent and results in a sudden increase of water flow and suspended particles during runoff events. This stress partly can explain short-term changes in macroinvertebrate abundance and diversity. In particular, recurrent runoff events and high turbidity level may favor species in stormwater wetland that have a high recovery potential and/or a short generation time. Nevertheless, the concentration of suspended solids during runoff (TSS ranged from 3.2 to 266 mg L^{-1} during the investigation period) could not be identified as a causal factor that significantly influenced the abundance of benthic macroinvertebrates in the stormwater wetland. In contrast, TSS values $\leq$173 mg L^{-1} in the Murrumbidgee River have been shown to impact negatively *Ephemeroptera*, *Trichoptera*, and *Odonata*, whereas chironomid species remained unaffected (Hogg and Norris 1991). A high level of turbidity in the stormwater wetland only was observed during a runoff event and occurred during a few hours. This may explains why no response of turbidity-sensitive species could be observed in the stormwater wetland.

Distribution of Macroinvertebrate Taxa and Analysis of Community Dynamics

Chironomus sp. (Diptera), *Tubifex* sp. (Oligochaeta), *Psectrotanypus* sp. (Diptera), *Chaetogaster* sp. (Oligochaeta), and *Limnodrillus* sp. (Oligochaeta) dominated the communities and mainly contributed to the community shifts that occurred in the stormwater wetland. Within the Diptera, the response to pollution depends on the genera. A relative increase of some genera in a community typically occurs in poorly functioning systems that collect anthropogenic inputs. For instance, the genus *Chironomus* is known as a sensitive indicator of various insecticide contaminations in water and sediments (Ibrahim et al. 1998). Tubificid worms, including the genera *Tubifex* and *Limnodrillus*, are widely distributed freshwater macroinvertebrates in Europe. Tubificids mix and process large volumes of sediments for feeding and are thus exposed to sediment-associated contaminants. Tubifex and several other oligochaete species have proven suitable for the evaluation of acute and chronic toxicity, bioaccumulation, as well as benthic in situ indicators (Chapman and Wang 2001; Smutna et al. 2008).

The redundancy analysis (Fig. 4) showed that metalaxyl and flufenoxuron concentrations were significantly associated with the first shift of the community structures that occurred between the second (May 5) and fifth (June 15) sampling campaigns. However, flow conditions, hydrochemical parameters, and vegetation cover also were associated with the observed changes. Hence, the occurrence of pesticides could not be identified as the main factor of changes in the macroinvertebrate communities. During this period, metalaxyl concentrations in runoff increased from lower then LOQ to 5.8 μg L^{-1} and that of flufenoxuron in the wetland sediments increased from lower then LOQ to 6.0 μg kg^{-1}. The formal concept

analysis underscored also that macroinvertebrate assemblages during this period were associated with intermediate vegetation cover and high pesticide concentrations. These changes corresponded to a decrease in generic richness and diversity, as inferred from the Shannon index. In addition, a decrease in the relative abundance of sensitive taxa after pesticide runoff was observed, which suggests that change in the macroinvertebrate community partly was induced by pesticides in runoff. The previsible non-effect concentration of flufenoxuron is 0.591 μg a.s. $kg_{wet\ sediment}^{-1}$ (Directive 98/8/EC). The average flufenoxuron concentrations observed in the wetland sediments were greater than this indicative value, which underscores a possible effect of flufenoxuron on macroinvertebrate communities during the investigation period. Nevertheless, the effects observed for some taxa simply may reflect the effect on an individual pesticide. In contrast, for other taxa the simultaneous occurrence of several pesticides may cause a larger impact than each individual pesticide. In the latter scenario, the design employed here cannot determine whether these larger impacts were due to additive or synergistic effects among runoff-related pesticides.

The second shift in macroinvertebrate community structures was observed between June 15 and August 6. This shift corresponded to large rainfall-runoff events and pesticide loads (Fig. 2). Formal concept analysis clearly indicates that macroinvertebrate assemblages during this period were associated with low concentrations of metalaxyl and large rainfall volumes. Correspondingly, the vegetation cover was larger and the outlet flow rate lower in summer. This indicates that vegetation cover decreased the flow rate in the wetland and thus the hydrological stress. Larger vegetation cover, lower flow rates, and low pesticide concentrations might have largely contributed in structuring macroinvertebrate communities in the stormwater wetland during this period. This is in agreement with previous studies showing that colonization of macroinvertebrate communities is structured by wetland vegetation (de Szalay and Resh 2000). However, this second community shift also may be explained by a delayed response and thus reflect the maximum effect of flufenoxuron-contaminated runoff several weeks after they were first detected in the stormwater wetland. Latency in the observed effects can be explained by the inhibition of chitin synthesis by flufenoxuron, which affects arthropod molting and metamorphosis (Mommaerts et al. 2006).

In addition, TU (Toxic Unit) > 0.001 (calculated for the pesticide mixture using the EC_{50} as toxicological end point) also can be used as a risk threshold for aquatic invertebrates, as indicated by the European Guidance Document on Risk Assessment (i.e., 1000 is the currently applied application factor for aquatic invertebrates). This information can be related to the observed community shifts. The validity of these thresholds has been reported in previous studies (Liess and Von der Ohe 2005; Schafer et al. 2007). TU values for aquatic invertebrates based on pesticide concentrations in the water column of the stormwater wetland were maximal on May 5 (TU $= 0.009$) and also exceeded the risk threshold on June 30 (TU $= 0.002$) and July 20 (TU $= 0.002$). TU values were <0.001 on the other sampling dates. Hence, TU values larger than the risk threshold correlate with observed shifts in the macroinvertebrate communities of the stormwater wetland.

Nevertheless, natural temporal variability within macroinvertebrate communities in wetland may also contribute masking the effect of ecological changes after disturbances. This may limit usefulness of wetland invertebrate as ecological indicators of hydrological and chemical disturbance in wetlands (Miller et al. 2008). Within-year changes of macroinvertebrate communities certainly obscure informative patterns from community associations with environmental gradients. In the present study, the collection of macroinvertebrate samples every 2 weeks and a thorough and simultaneous characterization of key environmental variables enabled relation of observed patterns to some environmental variables. Better understanding of temporal changes in macroinvertebrate community structure and composition is required for future invertebrate-based monitoring in systems, such as stormwater wetlands.

In conclusion, the present study described the dynamics of macroinvertebrates at the community and genus levels in a stormwater wetland that collected pesticide-contaminated runoff. The study was conducted during an entire period of pesticide application (April to September). Our results also emphasize that the combination of RDA with formal concept analysis is a powerful tool to help interpret the response of the community to environmental variables in field-based studies. The RDA enabled evaluation of the relations between macroinvertebrate assemblages and environmental conditions while identifying the most relevant environmental variables. Formal concept analysis identified precise associations between the most significant variables and macroinvertebrate communities, thus complementing the results provided by RDA. Overall, this study is the first to link changes of the macroinvertebrate community structure with hydrological and hydrochemical characteristics in a stormwater wetland connected to an agricultural catchment. It notably indicates that runoff contaminated with the insecticide fluflenoxuron is a significant factor that can affect macroinvertebrate communities. Nevertheless, flow conditions and vegetation also were associated with changes in macroinvertebrate communities. These results argue for more research to address the impact of pesticide mixtures on aquatic communities in general and on macroinvertebrates in environment receiving runoff-related pesticides in particular.

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Transport and Degradation of Pesticides in Wetland Systems : A Downscaling Approach

Résumé en français

La compréhension des mécanismes de transport et de dégradation des pesticides émergents est primordiale pour prédire leur devenir dans l'environnement. Les zones humides peuvent intercepter des eaux de ruissellement ou des souterraines contaminées par les pesticides et les traiter par le biais de processus de rétention et de dégradation, encore peu connus. Dans une approche multi-échelles, trois zones humides recevant des eaux polluées par les pesticides ont été utilisées comme des « laboratoires naturels » pour étudier le devenir de pesticides couramment utilisés. Cette thèse souligne l'influence des conditions hydrologiques et redox sur la distribution des pesticides au sein des différents compartiments des zones humides ainsi que sur leur potentiel de dégradation. Alors que les études à grande échelle fournissent des informations intégratives sur la dissipation et la rétention des pesticides en lien avec le développement de la végétation, les études à petite échelle utilisant des techniques innovantes telles que les analyses isotopiques et énantiomériques permettent l'exploration des processus moléculaires de dégradation des pesticides.

Résumé en anglais

A mechanistic understanding of transport and degradation processes of modern agricultural pesticides, including chiral pesticides, is critical for predicting their fate in the environment. In agricultural landscapes, wetlands can intercept pesticide-contaminated runoff or groundwater and improve water quality through various retention and degradation processes, which remain unknown. In a downscaling approach, three different wetlands receiving agricultural runoff were used as 'natural laboratories' to investigate the fate of widely used pesticides. Overall, our results showed that dynamics of hydrological and redox conditions largely influenced pesticide sorption mechanisms and their distribution over time within wetland compartments, thereby controlling degradation processes. While large-scale studies provide integrative information on pesticide dissipation and distribution patterns with respect to wetland functioning, small-scale investigations using novel methods such as isotope and enantiomer analyses characterize underlying molecular processes governing pesticide degradation.