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THÈSE

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FLORIAN COBERT

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PROCESSUS ET MÉCANISMES PHYSICO-CHIMIQUES ET BIOLOGIQUES RESPONSABLES DU FRACTIONNEMENT DES ISOTOPES DU CALCIUM

DIRECTEUR DE THÈSE :

M. STILLE Peter	Directeur de recherche, Université de Strasbourg

ENCADRANT PRINCIPAL :

Mme SCHMITT Anne-Désirée	Maître de conférences, Université de Franche-Comté

RAPPORTEURS :

Mme TRÉMOLIÈRES Michèle	Professeur, Université de Strasbourg
M. KYSER Kurt	Professeur, Queen's University
M. DROUET Thomas	Professeur, Université Libre de Bruxelles

ÉXAMINATEURS :

Mme POSZWA Anne	Maître de conférences, Université de Nancy
M. TIPPER Edward	Lecturer, University of St-Andrews

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Figures :

Tableaux :

Introduction générale

Le cycle du CO_2 et le changement climatique restent des sujets de grande actualité. La relation entre réchauffement climatique et gaz à effet de serre a été démontrée par de nombreuses études (Volk, 1987; Berner, 1993). Le CO_2 est l'un des principaux gaz à effet de serre ; il est donc essentiel de mieux comprendre son cycle global, afin d'améliorer nos connaissances et nos prédictions sur l'évolution des climats passés et actuels sur Terre. A l'échelle des temps géologiques, le bilan du CO_2 est contrôlé par deux processus majeurs (Volk, 1987) : (1) l'érosion des chaines de montagne (silicatées et/ou carbonatées), et (2) l'activité volcanique. L'érosion physique et chimique des continents est directement reliée au cycle du carbone, donc à celui du CO_2 atmosphérique. Cependant, c'est l'érosion des silicates de magnésium (Mg) et de calcium (Ca) qui domine ce bilan. Ceci peut être illustré par l'équation (0.1) pour l'anorthite (silicate de calcium) (Salje et al., 1993) :

$$CaAl_2Si_2O_8 \text{ (anorthite)} + 3H_2O + 2CO_2 \leftrightarrow Al_2Si_2O_5(OH)_4 \text{ (kaolinite)} + Ca^{2+} + 2HCO_3^- \quad (0.1)$$

L'équation (0.1) montre que les réactions d'altération des roches silicatées consomment deux moles de CO_2 atmosphérique et donnent des produits secondaires sous forme solide ou dissoute. Le diagramme de Goldschmidt (Figure 0.1) permet de prédire quels éléments chimiques vont se retrouver sous forme dissoute ou solide dans les eaux superficielles.

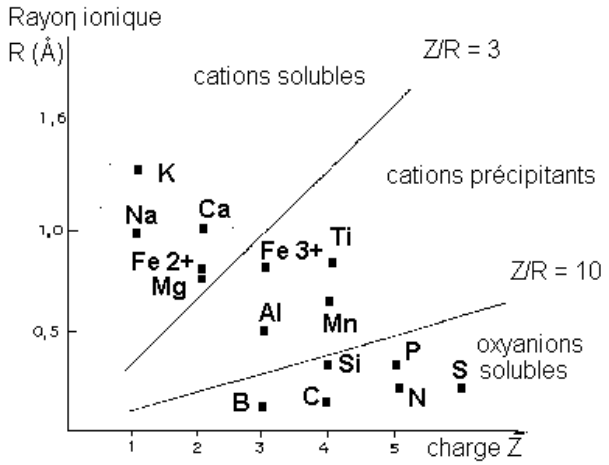

Figure 0.1: *Classification de Goldschmidt.*

Les cations obtenus suite à la réaction d'altération des roches silicatées (Équation 0.1) sont transportés sous forme dissoute ou particulaire vers les océans, où ils vont s'accumuler et précipiter sous forme de carbonates marins. Lors de la précipitation des carbonates marins une mole de CO_2 est libérée dans les océans (Équation 0.2).

$$Ca^{2+} + 2HCO_3^- \leftrightarrow CaCO_3 + H_2O + CO_2 \quad (0.2)$$

Le CO_2 ainsi libéré ira dans l'atmosphère, après un équilibrage entre le CO_2 dissous dans l'eau de mer et le CO_2 atmosphérique.

L'action couplée de l'altération des roches silicatées continentales (équation 0.1) et de la précipitation des carbonates marins (équation 0.2), peut-être considérée comme un puits du CO_2 atmosphérique à l'échelle de plusieurs centaines de milliers d'années. En effet, au cours de cette séquence, une mole de CO_2 atmosphérique est séquestrée. Ceci va contribuer à la régulation du climat sur Terre.

Il apparaît donc nécessaire de déterminer de façon qualitative et quantitative les flux de cations transportés par les fleuves vers les océans. Ceci va permettre d'estimer les taux d'altérations des roches continentales, et d'en déduire la quantité de CO_2 atmosphérique consommée. C'est dans cette optique, que de nombreuses études géochimiques se sont focalisées à déterminer le flux des éléments chimiques (Mg, Ca…) à différentes échelles spatiales (locale et globale) (p.ex Berner et al.,1983 ; Probst et al., 1990 ; Velbel, 1993 ; White and Blum, 1995 ; Gaillardet et al., 1999 ; Oliva et al., 2003 ; Wu et al., 2008 ; Noh et al., 2009). Les outils utilisés lors de ces études vont du rapport élémentaire (Ca/Na, Mg/Na…) aux isotopes radiogéniques (Sr, Nd, Pb, U). Ceci a permis d'apporter des informations sur les sources des éléments transportés et sur les processus de mélange entre différents pôles. Ainsi, Berner et al. (1983) et Velbel (1993) ont pu mettre en évidence que les cycles du Ca et du Mg sont étroitement liés au cycle du carbone, suite à l'altération des roches silicatées sur les continents et à la précipitation des roches carbonatées dans les océans. Toutes ces études, quelles que soient leur échelle d'étude, ont comme point commun qu'elles négligent les processus biotiques. Or, les plantes sont connues pour influencer les taux d'altération chimique des minéraux ainsi que les flux d'éléments entre différents réservoirs (p.ex Drever, 1994 ; Drever et Stillings, 1997 ; Moulton et al., 2000 ; Berner et al., 2004 ; Andrews et al. 2008). En effet, les plantes continentales peuvent : (1) fracturer mécaniquement les roches à l'aide de leurs racines. Les fractures ainsi formées vont entrainer une augmentation de la surface de contact entre les eaux superficielles et la roche. Ceci aura pour effet d'accélérer l'altération chimique des roches (Dexter, 1987 ; Czarnes et al., 1999) ; (2) excréter seules et/ou en symbiose avec des microorganismes (bactéries ou champignons) des protons, des acides organiques et des agents chélateurs au niveau de la rhizosphère (Darrah, 1993 ; Drever, 1994 ; Marschner, 1995 ; Hinsinger, 1998 ; Hisinger 2001 ; Turpault et al., 2005). Ainsi, les plantes vont augmenter le taux d'altération des minéraux du sol et accélérer la mobilisation

des cations d'un facteur 2 à 5 (Drever, 1994 ; Oliva et al., 1999 ; Chantigny, 2003 ; Berner et al., 2004) ; (3) retarder le lessivage des cations des solutions de sol vers les cours d'eau par le stockage et le recyclage végétal des cations (Berner et al., 2004 ; Balogh-Brunstad et al., 2008). Toutes ces observations permettent d'avancer l'hypothèse que les plantes pourraient influencer les cycles des cations à l'échelle de la rhizosphère et/ou de la parcelle et/ou du bassin versant, par le biais du prélèvement, du stockage et du recyclage des cations par la végétation.

Pour mieux comprendre le rôle de la végétation sur le cycle des éléments à l'échelle des bassins versants, les récentes études en milieu naturel utilisent de nouveaux outils, tels que les isotopes stables non conventionnels (Ca, Mg, Zn, Si, Cu…) (p.ex Schmitt et al., 2003 ; Ding et al., 2005 ; Opfergelt et al., 2006 ; Viers et al., 2007 ; Ding et al., 2008 ; Pogge von Strandmann et al., 2008 ; Tipper et al., 2008 ; Cenki-Tok et al., 2009 ; Bigalke et al., 2010a et 2010b ; Cornelis et al., 2010 ; Boulou-Bi et al., 2010 ; Holmden and Bélanger, 2010 ; Tipper et al., 2010 ; Aucourt et al., 2011 ; Juillot et al., 2011 ; Aranda et al., 2012). Leur utilisation est devenue possible grâce aux récentes avancées analytiques. Ces avancées permettent à l'heure actuelle de mesurer de façon précise et répétée de faibles variations de composition isotopique entre autres pour le Ca, Mg, Zn, Fe, SI, Cu... Parmi tous ces éléments, le Ca semble intéressant. En effet, c'est un élément majeur à la surface de la Terre impliqué dans le cycle du carbone (Équations (0.1) et (0.2)) et un macronutriment essentiel pour les organismes vivants. Ses isotopes ont montré un potentiel pour tracer :

a) le rôle de la végétation et de son recyclage sur les mécanismes d'altération au sein des petits bassins versants (p.ex Schmitt et al., 2003 ; Schmitt et Stille, 2005 ; Wiegand et al., 2005 ; Perakis et al., 2006 ; Page et al., 2008 ; Cenki-Tok et al., 2009 ; Holmden et Bélanger, 2010)
b) le transport dans les sols, les processus de dissolution/précipitation (Ewing et al., 2008), les réservoirs de Ca (échangeable vs Ca total) (Wiegand et al., 2005 ; Page et al., 2008 ; Cenki-Tok et al., 2009, Hindshaw et al., 2011 ; Farkas et al., 2011)

Ces différents processus enrichissent en général la phase résultante en isotopes légers du Ca. Cependant, les mécanismes qui conduisent à ces différents fractionnements restent encore mal connus et mal identifiés en milieu naturel, du fait qu'il y est difficile de dissocier les mécanismes biotiques des mécanismes abiotiques. Or, chacun de ces mécanismes est

essentiel à la compréhension du cycle biogéochimique global du Ca ; c'est pourquoi il est indispensable de les identifier précisément.

Les travaux de cette thèse s'inscrivent dans cette optique et ont pour but :

d'identifier et de préciser les processus biotiques et abiotiques qui contrôlent le comportement du Ca et plus spécifiquement le fractionnement des isotopes du Ca à l'interface géosphère/biosphère/hydrosphère en combinant différentes études expérimentales.

Ce travail de doctorat a été rendu possible grâce à la mise en place au LHyGeS d'un nouveau protocole automatisé pour la séparation chimique du calcium des autres éléments de la matrice et pour la mesure du rapport isotopique du calcium ($\delta^{44/40}Ca$) des différents échantillons par spectromètre de masse (Schmitt et al., 2009). Ce protocole permet d'améliorer la reproductibilité d'un facteur deux par rapport au protocole antérieur en vigueur au LHyGeS et évite les interférences (surtout le potassium) au cours de la mesure. Par conséquent des variations isotopiques plus petites peuvent être détectées.

Le présent mémoire de thèse s'organise en cinq chapitres :

- Le **Chapitre 1** dresse un état des connaissances sur le Ca et ses isotopes, sur le cycle biologique du Ca chez les plantes, ainsi que sur les isotopes stables appliqués dans le système sol/plante.

- Le **Chapitre 2** présente les modes opératoires et les méthodes de mesure utilisées au cours de ces travaux de thèse.

- Le **Chapitre 3** présente une étude en milieu contrôlé de type hydroponique qui a pour but d'identifier les processus biologiques et lithologiques qui influencent la composition isotopique du Ca au sein des plantes cultivées.

- Le **Chapitre 4** présente une étude complémentaire à l'expérimentation du chapitre 3, également de type hydroponique. Elle se différencie de la première par le fait que la solution nutritive utilisée n'est pas considérée comme un réservoir infini de Ca. Elle a pour but

d'observer l'évolution de la signature isotopique en Ca de la solution nutritive et des différents organes de végétaux cultivés au cours du temps.

- Le **Chapitre 5** présente l'influence des acides organiques et inorganiques des plantes et celle de l'activité bactérienne de la rhizosphère sur le rapport isotopique du Ca des solutions de sol et des organes de végétaux.

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Chapitre 1:

Contexte Scientifique

I. Introduction

Le calcium (Ca) est un macronutriment essentiel à la vie animale et végétale. L'étude de son cycle biogéochimique peut aider à mieux comprendre l'importance relative de l'altération des roches silicatées par rapport à celle des roches carbonatées sur la teneur en CO_2 de l'atmosphère, du fait que le cycle du Ca est intimement lié à celui du CO_2 (Berner et al., 1983 ; Velbel, 1993).

L'utilisation des isotopes du Ca pour étudier le cycle biogéochimique du Ca fût dans un premier temps très limitée, car il existe un fort fractionnement analytique lié aux mesures des rapports isotopiques par spectromètre de masse (Hirt et Epstein, 1964 ; Heumann et al., 1970) et également lié à la chimie séparative du Ca sur résine échangeuse d'ions (Heumann et Lieser, 1972 ; Heumann, 1972 ; Russell et Papanastasiou, 1978). Hirt et Epstein (1964) furent les premiers à proposer l'utilisation d'un double spike ^{43}Ca-^{46}Ca pour contrôler le fractionnement isotopique du Ca se produisant lors de l'évaporation dans les spectromètres de masse. Cependant, l'utilisation de ce double spike n'a pas permis de détecter un fractionnement naturel conséquent. Il faudra attendre Russell et al. (1978), qui, en plus d'utiliser le double spike ^{43}Ca-^{48}Ca, corrigèrent le fractionnement instrumental résultant de la mesure des rapports isotopiques par spectrométrie de masse à l'aide d'une loi exponentielle. Hart et Zindler (1988) corroborèrent l'efficacité de cette loi dans le cas de mesure des rapports isotopiques du Ca par spectromètre de masse.

Une fois ce double fractionnement identifié et éliminé, et grâce aux développements analytiques récents, il est devenu possible de mesurer le fractionnement naturel des isotopes du Ca dans différents types d'échantillons (abiotique et biotique; p.ex Russell et al., 1978 ; Skulan et al., 1997 ; Zhu et MacDougall, 1998 ; Skulan et DePaolo, 1999 ; De La Rocha et DePaolo, 2000 ; Schmitt et al., 2003 ; Wiegand et al., 2005 ; Tipper et al., 2006 et 2008 ; Cenki-Tok et al., 2009). Ainsi les études récentes de montrent que les isotopes du Ca semblent prometteurs pour étudier le cycle biogéochimique du Ca. En effet, les isotopes du Ca semblent pouvoir tracer les processus biotiques et abiotiques impliquant le Ca dans le milieu naturel et caractériser les sources de Ca des eaux de surface (Schmitt et al., 2003 ; Schmitt et Stille, 2005 ; Wiegand et al., 2005 ; Tipper et al., 2006 ; Page et al., 2007 ; Ewing et al., 2008 ; Tipper et al., 2008 ; Cenki-Tok et al., 2009 ; Holmden et Bélanger, 2010 ; Hindshaw et al., 2011).

Ce chapitre de thèse dresse un état des connaissances sur le Ca et ses isotopes, sur le cycle biologique du Ca chez les plantes, ainsi que sur les isotopes stables appliqués dans le système sol/plante.

II. Rappels isotopiques

II.1. Les isotopes naturels du Ca

II.1.1. Généralités sur les isotopes

Depuis le début du XX^{ème} siècle, les géochimistes savent que des atomes d'un même élément chimique, c'est-à-dire avec le même nombre atomique Z, peuvent compter un nombre de neutrons différents. Ces atomes d'un même élément chimique sont appelés des **isotopes** de cet élément. Par exemple, tous les isotopes naturels de l'hydrogène (Z=1) ont un proton et zéro, un ou deux neutrons (Figure I.1). Le terme isotope vient du grec « *iso topos* » qui veut dire « même lieu », ce qui représente bien le fait que tous les isotopes d'un même élément possèdent le même numéro atomique (Z).

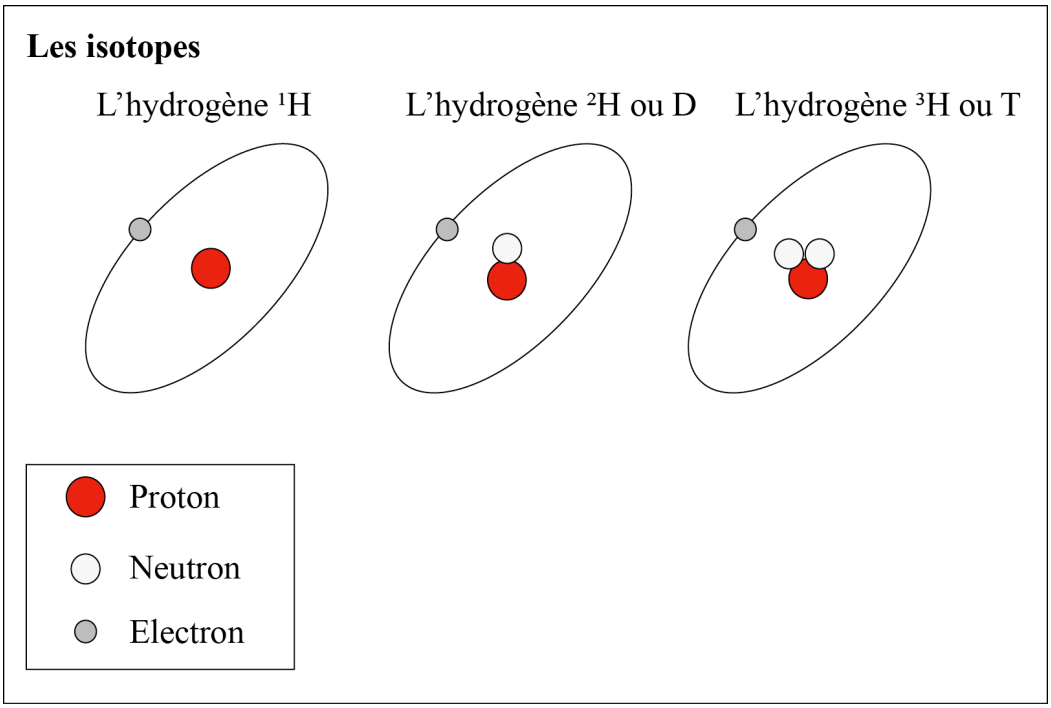

Figure I.1 *: Représentation électronique des isotopes naturels de l'H (^{1}H, ^{2}H, ^{3}H). Site internet du CEA consulté le 12 mai.2012. http://www.cea.fr/jeunes/themes/la_radioactivite/l_atome/elements_chimiques_et_isotopiques*

Les isotopes d'un même élément chimique ont des masses différentes car leur nombre de neutron diffère. Les géochimistes ont l'habitude d'appeler l'isotope d'un élément qui possède le plus de neutrons **« l'isotope lourd »**, par opposition à **« l'isotope léger »** qui lui présente le moins de neutrons. Si on prend l'exemple de l'hydrogène (Tableau I.1), les isotopes ^{2}H (D) ou ^{3}H (T) sont les isotopes lourds comparés au ^{1}H.

Tableau I.1 : *Masse isotopique des isotopes naturels de l'hydrogène (IUPAC, 1997).*

Isotopes naturels de l'hydrogène	**Masse isotopique (amu)***
^{1}H	1.00782503207 (10)
^{2}H	2.0141017778 (4)
^{3}H	3.0160492777 (25)

** Les chiffres entre parenthèse correspondent à la précision de la détermination de la masse isotopique après la décimale correspondante.*

Tous les isotopes d'un même élément présentent des propriétés chimiques identiques. En effet, ils présentent un nombre identique de protons et d'électrons. Cependant, ces isotopes peuvent posséder des propriétés physico-chimiques différentes, dues à leurs différences de masse. Cette différence de masse entraine une répartition sélective des isotopes d'un même élément, qui est aussi appelée **fractionnement isotopique** ou **discrimination isotopique**. Nous traiterons ce sujet plus loin dans le chapitre.

Dans la nature, il existe deux types d'isotopes, les isotopes stables et les isotopes radiogéniques. Ces derniers sont des éléments instables qui donnent naissance à d'autres éléments par désintégration radioactive. Sur les ~ 3000 éléments connus, plus de ~ 2700 sont des éléments instables (Hoefs, 2004). Les isotopes stables, contrairement aux isotopes radiogéniques sont des éléments qui ne subissent pas de désintégration radioactive au cours du temps. Parmi les 300 éléments chimiques stables, seuls 21 possèdent un isotope unique (Be, F, Al, Na, Co…). Tous les autres éléments présentent au moins 2 isotopes, avec, pour la grande majorité, un des isotopes qui présent une abondance > 70% par rapport aux autres.

La géochimie des isotopes stables fut longtemps limitée à l'étude des isotopes du C, H, O, N et S (Tableau I.2). Ces isotopes sont appelés par la communauté des géochimistes « **les isotopes stables traditionnels** », par opposition aux « **isotopes stables non traditionnels** ».

Tableau I.2 : *Les isotopes stables traditionnels (IUPAC, 1997).*

Éléments chimiques	**Isotopes stables (abondances relatives)**
H	^{1}H (99,9885%), ^{2}H (0,0115%)
C	^{12}C (98,93%), ^{13}C (1,07%)
N	^{14}N (99,632%), ^{15}N (0,368%)
O	^{16}O (99,757%), ^{17}O (0,038%), ^{18}O (0,205%)

Les isotopes stables non traditionnels sont par exemple les isotopes des éléments suivants : B, Li, Si, Mg, Fe, Cu, Zn, Ca et Mo (Tableau I.3). Leur utilisation en géochimie isotopique est devenue possible grâce aux récentes avancées technologiques: le développement de nouveaux spectromètres de masse de haute précision (MC-ICP-MS) ; et l'amélioration technique des mesures aux spectromètres de masse à thermo-ionisation (TIMS).

Tableau I.3: *Exemples d'isotopes stables non traditionnels (IUPAC, 1997).*

Éléments chimiques	**Isotopes stables (abondances relatives)**
Mg	^{24}Mg (78,99%), ^{25}Mg (10,00%), ^{26}Mg (11,01%)
Fe	^{54}Fe (5,845%), ^{56}Fe (91,75%), ^{57}Fe (2,12%), ^{58}Fe (0,28%)
Cu	^{63}Cu (69,17%), ^{65}Cu (30,83%)
Zn	^{64}Zn (48,63%), ^{66}Zn (27,90%), ^{67}Zn (4,10%), ^{68}Zn (18,75%), ^{70}Zn (0,62%)
Si	^{28}Si (92,22%), ^{29}Si (4,68%), ^{30}Si (3,09%)

II.1.2. Abondance des isotopes naturels du Ca

Le Ca possède six isotopes stables naturels, avec des masses allant de 40 à 48 (Tableau I.4).

Tableau I.4: *Abondance des isotopes stables du Ca (IUPAC, 1997).*

Isotopes	**^{40}Ca**	**^{42}Ca**	**^{43}Ca**	**^{44}Ca**	**^{46}Ca**	**^{48}Ca**
Abondance (%)	96,941 (156)	0,647 (23)	0,135 (10)	2,086 (110)	0,004 (3)	0,187 (21)

* *Les chiffres entre parenthèses correspondent à la précision de la détermination de l'abondance et réfèrent aux derniers chiffres de la valeur indiquée.*

Le ^{40}Ca est l'isotope stable du Ca le plus abondant, tandis que le moins abondant est le ^{46}Ca. Le Ca possède également plus d'une dizaine d'isotopes radiogéniques. Le plus connu et le plus utilisé est le ^{45}Ca, en particulier lors d'expériences de physiologie végétale, où il est utilisé comme traceur. Une partie du ^{40}Ca peut provenir de la décroissance radioactive β^- du ^{40}K. Cette décroissance peut entrainer un excès de ^{40}Ca (noté ε^{40}Ca) par rapport aux autres isotopes du Ca (Marshall et DePaolo, 1982). Cependant pour que cet excès soit significatif et quantifiable, il faut que le rapport K/Ca soit élevé au sein de l'échantillon considéré (Nägler et Villa, 2000).

II.1.3. Le fractionnement des isotopes stables

II.1.3.1. Notations utilisées en géochimie des isotopes stables

Avant d'expliquer les différents modes de fractionnement des isotopes stables, il convient d'exposer quelques conventions de notations relatives aux fractionnements isotopiques. Comme nous venons de le voir dans la partie précédente (Chapitre.1 II.1.1), c'est la différence d'abondance entre les isotopes lourds et les isotopes légers d'un même élément qui permet d'obtenir sa composition isotopique absolue. Ainsi, en faisant le rapport isotopes lourds sur isotopes légers d'un même élément, on obtient la composition isotopique absolue (R) de l'élément considéré. Pour le carbone par exemple, ce rapport (R) s'écrit :

$$R = {}^{13}C/{}^{12}C \quad (I.1)$$

Cependant ce rapport isotopique absolu R (I.1) est difficile à mesurer avec précision, et les variations naturelles des rapports isotopiques étant très faibles pour les éléments chimiques de grand nombre atomique Z, la notion de « δ » a été introduite par McKinney et al. (1950). Ces auteurs expriment à l'aide du δ, l'écart relatif entre le rapport isotopique absolu R de l'élément analysé dans un échantillon et celui mesuré dans un standard de référence, pour l'élément considéré. Avec ce nouveau terme, McKinney et al. (1950) relient de façon explicite l'abondance d'un isotope dans un échantillon et son abondance dans un matériel de référence. Ainsi, lorsque $\delta > 0$, l'échantillon contient plus d'isotopes lourds que le matériel de référence ; si $\delta < 0$, il y a moins d'isotopes lourds dans l'échantillon que dans le matériel de référence. La notation δ désigne le rapport de l'isotope lourd sur celui de l'isotope léger et ceci pour tous les systèmes isotopiques étudiés. Par exemple, dans le cas du Ca, il s'écrit (Eisenhauer et al., 2004) :

$$\delta^{44/40}Ca = \left(\frac{\left(\frac{^{44}Ca}{^{40}Ca} \right)_{Échantillon}}{\left(\frac{^{44}Ca}{^{40}Ca} \right)_{Standard}} - 1 \right) * 10^3 \quad \text{(I.2)}$$

Dans le cadre de cette thèse, tous les rapports isotopiques du Ca sont exprimés en $\delta^{44/40}Ca$ à par rapport au standard international SRM915a du NIST, obtenu à partir d'un carbonate. Certaines des premières études sur les isotopes du Ca utilisent l'eau de mer comme standard pour exprimer leur rapport isotopique du Ca. Il est donc nécessaire de convertir les données isotopiques du Ca ayant l'eau de mer comme standard, pour pouvoir les comparer directement à nos résultats utilisant le SRM915a comme standard. Le calcul permettant de convertir les données est le suivant :

$$\delta^{44/40}Ca_{SRM915a} = \delta^{44/40}Ca_{SW} + 1{,}88 \quad \text{(I.3)}$$

La valeur 1,88 correspond à la composition isotopique de l'eau de mer déterminée par Schmitt et al. (2001) par rapport au standard SRM915a du NIST.

Un autre terme utilisé par les géochimistes est l' « α ». Cet α désigne le coefficient de fractionnement élémentaire mesuré en laboratoire pour deux composés (a et b) qui sont liés par un processus naturel (par exemple entre un réactif a et un produit b au cours d'une

réaction chimique). Il est possible de définir le coefficient de fractionnement élémentaire α par la relation suivante :

$$1000\ln(\alpha) \approx (\alpha_{a\text{-}b}) * 1000 = \delta_a - \delta_b \approx \Delta_{a\text{-}b} \quad (I.4)$$

II.1.3.2. Le fractionnement isotopique ou discrimination isotopique

Comme nous l'avons vu auparavant (Chapitre.1 II.1.1), la différence de masse entre les différents isotopes d'un même élément chimique peut engendrer des différences de propriétés physico-chimiques entre les isotopes considérés. Ces différences vont conduire à un fractionnement isotopique, c'est-à-dire à un changement de la composition isotopique d'un composé au cours de certains processus. Il est possible d'expliquer le fractionnement isotopique par la théorie de la mécanique quantique à l'échelle atomique (p.ex. O'Neil, 1986 ; Hoefs, 1987 ; Kyser, 1987 ; Criss, 1999 ; Chacko et al., 2001 ; Hoefs, 2004). Ainsi, d'après la théorie de la mécanique quantique, lorsqu'un élément possède au moins deux isotopes, la probabilité d'un fractionnement isotopique est d'autant plus grande que la différence de masse est importante entre l'isotope minoritaire et l'isotope majoritaire. De ce fait, des fractionnements isotopiques ont plus de chance de se produire pour les éléments légers du tableau de Mendeleïev.

A l'échelle macroscopique, les effets de la mécanique quantique sur les propriétés physico-chimiques des isotopes d'un même élément peuvent engendrer deux types de fractionnements isotopiques : les fractionnements isotopiques à l'équilibre (chimique et physique); et les fractionnements isotopiques cinétiques (phénomènes de transport, réactions chimiques, effet de la température et effet de la biologie ; p.ex. O'Neil, 1986; Hoefs, 1987; Kyser, 1987; Vidal, 1994; Chacko et al., 2001). Nous ne détaillerons pas les différents processus qui fractionnent les isotopes stables, car ceux-ci ont déjà été décrits dans de nombreux livre sur les isotopes stables (Faure, 1986 ; Hoefs, 1987 ; Hoefs, 2004 ; Faure et Mensing, 2005).

III. Le cycle biologique du Ca

III.1. Généralités sur le calcium et sur son cycle global

Selon la classification des éléments chimiques, son numéro atomique est 20 et il appartient à la famille des alcalino-terreux. La masse atomique de cet élément est de 40,078 et sa configuration électronique la plus stable est [Ar] $4s^2$. Le degré d'oxydation du Ca est invariablement égal à +II, de ce fait, sa forme ionique est toujours de charge +2. Enfin, le Ca a un faible potentiel ionique, ce qui le rend assez facilement soluble dans l'eau.

D'un point de vue géochimique, le Ca est un élément lithophile. Ainsi, le Ca présente une forte affinité pour les phases silicatées, ce qui explique qu'il soit le 5ème élément chimique tant dans la terre silicatée (2,31%) que dans les roches de la croûte continentale (4,57%) et dans le manteau supérieur (2,40%). La quantité de Ca se trouvant dans les roches silicatées de la croûte terrestre est estimée à $6{,}4*10^{12}$ t (Figure I.2). Cependant, le principal réservoir de Ca sur Terre est constitué des roches carbonatées. Berner et al. (1983), estiment ce réservoir à $1{,}6*10^{17}$ t de Ca (Figure I.2). Les roches carbonatées et les roches silicatées sont les 2 principales sources de Ca des eaux de surface. Albaréde (2001) estime que la concentration en Ca des rivières mondiales est égale à $2*10^4$ µg/L, ce qui fait du Ca l'élément le plus abondant dans les eaux des rivières continentales. Le Ca est drainé par les eaux superficielles continentales vers les océans, essentiellement sous forme dissoute et ionisée. Le Ca est le 6ème élément présent dans les océans en terme d'abondance, sa concentration y est égale à $4{,}14*10^5$µg/L (Albaréde, 2001) et son temps de résidence y est de 1Ma. La quantité de Ca présente dans les océans est estimée à $5*10^{14}$ t, ce qui fait de ce réservoir le second réservoir de Ca sur Terre (Figure I.2).

Jusqu'à récemment, le cycle global du Ca ne prenait pas en compte la biomasse végétale terrestre, or, le Ca y est également abondant. Ceci s'explique par le fait qu'il soit un macronutriment essentiel à la vie végétale. Le Ca est le second métal le plus abondant dans les plantes continentales par gramme de matière sèche (MS) après le K. Son abondance relative moyenne dans les plantes terrestres représente 0,5% de leur MS totale (Epstein et Bloom, 2005 ; Taiz and Zaiger, 2010). Ainsi, le Ca accumulé par la biomasse végétale continentale a été estimé à $6*10^9$ t de Ca, ce qui fait de ce réservoir le 3ème réservoir de Ca sur les continents (Figure I.2).

Le Ca joue un rôle important au cours de différents processus de régulation physiologique et structurale au sein des plantes. En effet, le Ca est très important pour la stabilité et l'intégrité de différents organes des plantes (p.ex les tiges), que ce soit à l'échelle cellulaire ou intracellulaire (Marschner, 1995 ; McLauglin et Wimmer, 1999 ; Taiz et Zeiger, 2010). Le Ca est également impliqué dans de nombreux processus de régulation au sein des plantes tels que la phosphorylation des protéines nucléaires, la division cellulaire, la synthèse de parois cellulaires et de membranes cellulaires, la régulation de l'ouverture des stomates… (McLauglin et Wimmer, 1999 ; Taiz et Zaiger, 2010). Tous ces processus peuvent théoriquement influencer la signature isotopique du Ca des plantes.

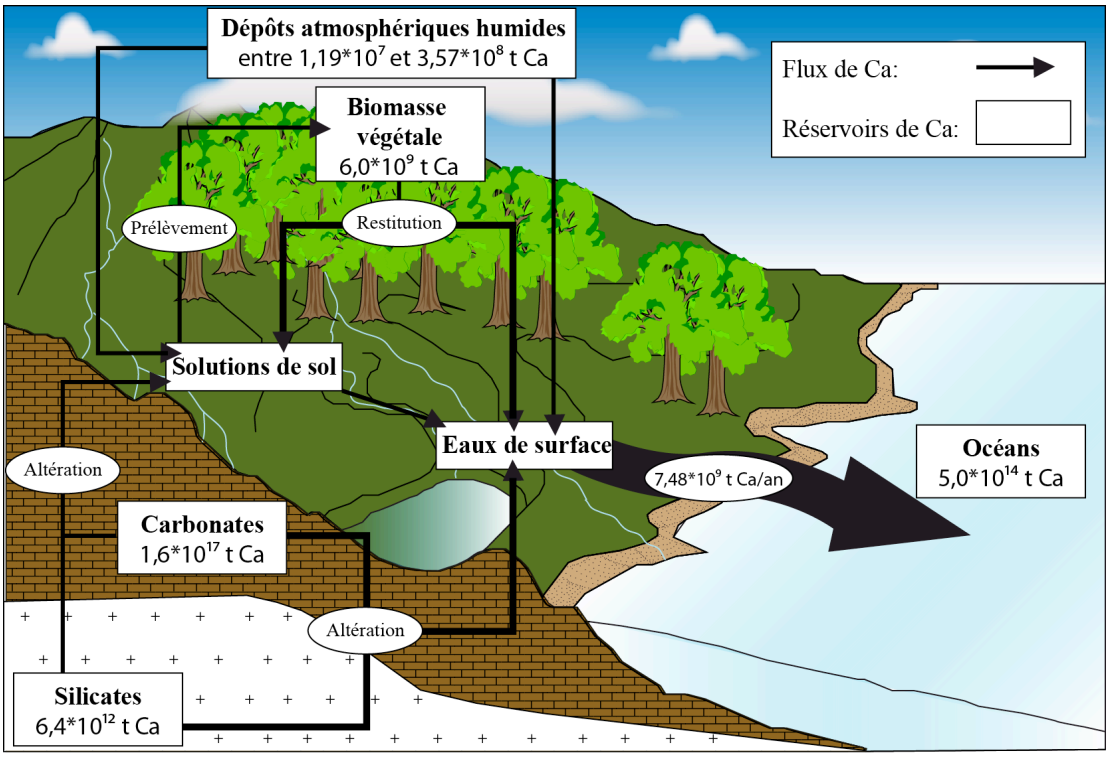

Figure I.2 *: Les différents réservoirs terrestres du Ca et les flux de Ca, des continents aux océans. Les représentations des réservoirs ne sont pas proportionnelles. Les stocks de Ca contenu dans les Silicates, les Carbonates et les Océans sont estimés à partir des travaux de Berner et al. (1983), le stock de Ca de la biomasse végétale continentale est estimé d'après Epstein et Bloom (2005) et le stock de Ca des dépôts atmosphériques humides est estimé d'après Berner et Berner (1996) et Castany (1998). Le flux de Ca des rivières mondiales est estimé d'après Albaréde (2001).*

Au cours de cette thèse, nous nous intéresserons en grande partie aux signatures isotopiques du Ca au sein des végétaux. C'est pourquoi, il semble important de faire une

synthèse (non-exhaustive) du cycle biologique du Ca. Cela permettra de mieux comprendre les processus majeurs qui sont responsables du fractionnement isotopique du Ca au sein du système plante.

III.2. La capture nutritive du Ca par les racines latérales

Le Ca capturé par les plantes se trouve essentiellement sous forme de cation divalent dans la solution de sol. Cette capture s'effectue en deux phases. La première phase est le transport du Ca de la solution de sol vers la surface des racines et la seconde phase est le transport du Ca de la surface des racines vers leur partie interne.

Le transfert du Ca de la solution de sol vers la surface des racines latérales suit un processus passif non métabolique qui est régit par le flux de masse et/ou par le potentiel électrochimique (Bangerth, 1979 ; Marschner, 1995 ; McLaughlin et Wimmer 1999 ; Taiz et Zeiger, 2010). Une fois qu'il est arrivé à la surface des racines latérales, le Ca migre vers le centre des racines. Cela correspond à la seconde phase de la capture nutritive du Ca par les racines latérales. Lors de cette phase le Ca est absorbé au niveau des cellules de l'épiderme racinaire via un gradient électrochimique et des canaux calciques, ce qui permet au Ca d'atteindre le cytoplasme des cellules de l'épiderme racinaire (White, 2000 ; Wiegand et al., 2005 ; Figure I.3).

III.3. Le transfert radial du Ca des racines latérales vers les vaisseaux xylémiques

Une fois que le Ca a pénétré à l'intérieur des racines latérales, il migre radialement à travers les tissus du cortex racinaire vers les vaisseaux conducteurs du xylème, situé au centre des racines (stèle ; Figure I.3). Au cours de ce transfert radial, le Ca peut suivre trois voies différentes (transmembranaire, symplasmique et apoplasmique ; Figure I.3), la contribution de chacune de ces voies pour acheminer le Ca jusqu'au xylème est encore inconnue (Clarkson, 1984, 1993 ; Häussling et al., 1988 ; McLaughlin et Wimmer, 1999 ; White, 2001 ; Moore et al., 2002 ; Hayter et Peterson, 2004 ; Taiz et Zeiger, 2010). La voie transmembranaire nécessite le mouvement du Ca de cellule en cellule en passant par la membrane plasmique. Pour cela, le Ca doit emprunter des pompes Ca^{2+}/ATPase pour sortir d'une cellule et des canaux calciques pour entrer dans une autre cellule (Figure I.3). D'autre part, la voie symplasmique consiste dans un premier temps à faire entrer le Ca dans les cellules par des

canaux calciques. Ensuite, le Ca se déplace de cytoplasme en cytoplasme en utilisant les plasmodesmes qui relient les différentes cellules entres elles (Figure I.3). Enfin, avec la voie apoplasmique, le Ca ne traverse aucune membrane plasmique et se déplace uniquement dans l'espace libre extracellulaire (apoplasme) (Figure I.3). Le transport apoplasmique est stoppé lorsque le Ca arrive à l'endoderme, au niveau de la bande de Caspary. La bande de Caspary est une structure hydrophobe qui force le flux de Ca empruntant la voie apoplasmique à emprunter la voie symplasmique. Pour cela, la bande de Caspary oblige le Ca à passer au travers de canaux calciques pour qu'il atteigne le cytoplasme des cellules de l'endoderme (Figure I.3). Quelque soit la voie suivie par le Ca, il empruntera toujours des pompes Ca^{2+}/ATPase pour passer de l'endoderme aux vaisseaux conducteurs du xylème (Figure I.3).

III.4. La translocation du Ca ou transfert du Ca des racines aux parties aériennes

Lorsque le Ca arrive dans les vaisseaux du xylème, il est transporté unidirectionnellement des racines vers les organes aériens, via le xylème de la racine primaire et des tiges, qui forment un conduit continu de cellules mortes (Taiz et Zeiger, 2010 ; Figure I.3). Le Ca transporté via le xylème suit le flux de sève ascendante, il est influencé par le gradient de potentiel hydrique qui résulte de l'activité des stomates (Marschner, 1995 ; Taiz et Zeiger, 2010). Ce transport longue-distance unidirectionnel est appelé translocation.

Au cours de la translocation, le Ca s'accumule principalement dans les parois pecto-cellulosiques, plus précisément au niveau des groupements $R\text{-}COO^-$ de la lamelle moyenne (Amstrong et Kirkby, 1979 ; Hanson, 1984 ; Kirkby et Pilbeam, 1984 ; Marschner, 1995 ; Bresinsky et al., 2008 ; Figure I.3). Ainsi, le Ca va s'accumuler principalement au sein des organes de plantes riches en pectine, dont le rôle est d'assurer la structure des plantes (p.ex: racine primaire et tiges). Cependant, une quantité non négligeable de Ca peut également atteindre les feuilles (Figure I.3). Il s'y accumule sous forme de cristaux d'oxalate de calcium ou sous forme libre (Bresinsky et al., 2008). Le rôle des oxalates de calcium est de protéger les plantes des herbivores (Bresinsky et al., 2008). Ils permettent également de réguler la teneur en Ca dans les végétaux pour éviter qu'elle ne soit trop élevée. Dans ce cas, les oxalates de Ca vont avoir un rôle de détoxification vis à vis du Ca (Nakata, 2003). De plus, sous certaines conditions, les oxalates de Ca formés au cours de la vie des plantes peuvent être dissous pour faire face à une carence nutritive en Ca (Nakata et al., 2003). Cependant, le Ca n'est jamais remobilisé via le phloème (Taiz et Zeiger, 2010).

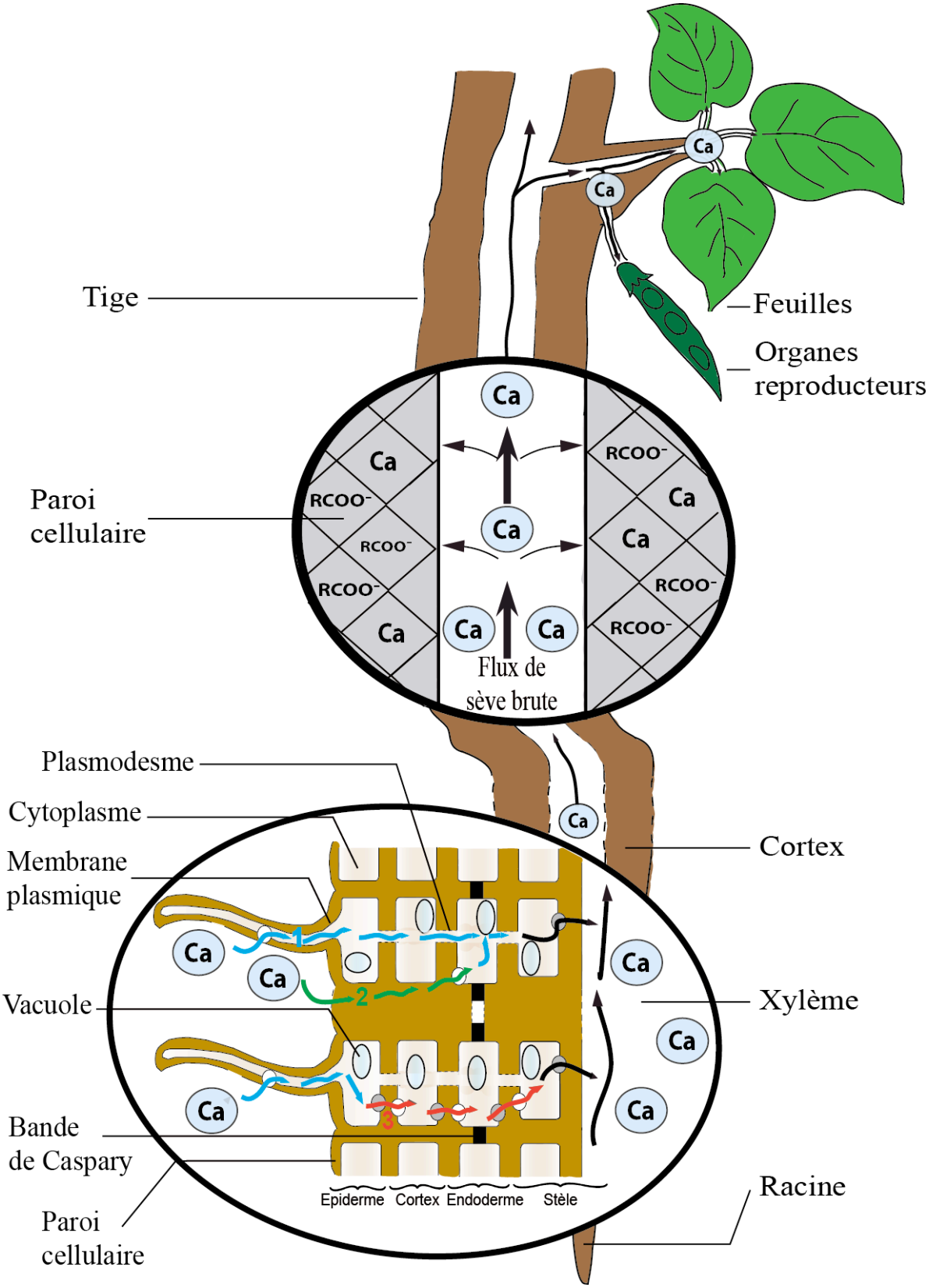

Figure I.3 : *Schéma du transfert radial du Ca dans une racine de haricot commun (1: symplasmique ; 2: apoplasmique ; 3: transmembranaire) et de sa translocation des racines aux parties aériennes, avec sa fixation sur les sites échangeurs d'ions ($RCOO^-$) des paroi-pectocellulosiques des organes de structure (p.ex: tiges et racine primaire).*

La translocation achemine également le Ca jusqu'aux organes reproducteurs des plantes (Figure I.3). Une étude récente indique que la formation des cristaux d'oxalates de calcium peut également jouer un rôle sur la régulation de la quantité de Ca transporté jusqu'aux organes reproducteurs (Nakata, 2011). En effet, la teneur en Ca dans ces organes semble être influencée par la quantité de Ca fixée dans les parois pecto-cellulosiques des cellules adjacentes aux vaisseaux conducteurs du xylème (Marschner, 1995 ; White et Broadley, 2003 ; Nakata, 2011). Toutefois, les processus contrôlant les apports de Ca jusqu'aux organes reproducteurs sont encore mal connus.

III.5. Influence de certains cations sur le cycle biologique du Ca

Certains cations comme Mg^{2+}, K^{+}, Al^{3+} et NH_4^{+} vont inhiber la capture nutritive du Ca par les racines (Bangerth, 1979 ; McLaughlin et Wimmer, 1999). Par exemple, l'un des effets de l'Al^{3+}est d'inhiber la capture nutritive du Ca en diminuant la croissance des racines (Figure I.4). L'Al^{3+} ou le Mg^{2+}, par exemple, peuvent également entrer en compétition avec le Ca^{2+} au niveau des sites échangeurs d'ions des racines, ce qui aura pour effet d'inhiber l'absorption du Ca par les racines. En effet, ces cations présentent la même affinité pour ces sites échangeurs (Ericsson et al., 1995).

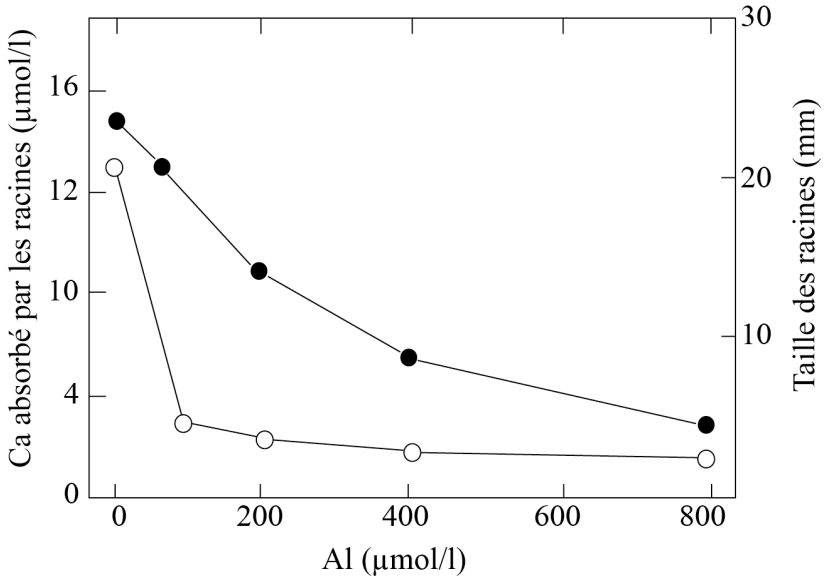

Figure I.4 *: Effet de la concentration de l'aluminium des solutions de sol sur la capture nutritive du Ca par les racines (cercle blanc) et sur la croissance des racines (cercle noir), d'après McLaughlin et Wimmer (1999).*

En revanche, d'autres éléments chimiques, comme le NO_3^- et PO_4^{2-}, peuvent favoriser la capture nutritive du Ca par les racines (Bangerth, 1979). Enfin, il peut également se produire des phénomènes de substitution du Ca par d'autres cations (H^+, Na^+, Al^{3+} ...) au niveau des groupements $R\text{-}COO^-$ au sein des végétaux, comme le suggèrent différentes études (Wallace et al., 1966 ; Huang et al., 1992 ; Rengel et Elliott, 1992 ; McLaughlin et Wimmer, 1999).

III.6. Restitution du Ca assimilé par la végétation

Le temps de résidence du Ca au sein des plantes, donc, la vitesse de restitution du Ca par les plantes est plus ou moins long en fonction du climat et du type de la végétation (Feuillus ou résineux ; Tableau I.5). Les plantes peuvent restituer le Ca sous 2 formes, soit sous forme dissoute (pluviolessivats), soit sous forme solide (litière). Ces deux formes de restitutions sont décrites dans les paragraphes qui suivent.

Lorsque la pluie atteint le feuillage des plantes, elle solubilise les dépôts atmosphériques secs se trouvant sur la surface des feuilles et elle emporte aussi les cations excrétés par les feuilles tels que le Ca^{2+} par exemple. Ainsi, le passage des pluies à travers le couvert végétal modifie leur composition chimique comparée à celle des précipitations directes, c'est-à-dire hors couvert végétal. Différents paramètres peuvent influencer cette modification des teneurs en ions des pluies, tels que la surface totale occupée par le couvert végétal et le degré de maturité d'une forêt (McLaughlin et Wimmer, 1999). Un autre paramètre peut également avoir un fort impact sur la modification de la signature chimique des pluviolessivats, il s'agit du pH. En effet, des pluies acides vont lessiver le Ca contenu dans les feuilles, plus précisément le Ca se trouvant au niveau des réserves apoplasmiques (Fink, 1991).

Tableau I.5: *Temps de résidence du Ca dans les plantes en fonction du climat et du type de végétation (Cole et Rapp, 1981).*

Type de forêt	Temps de résidence du Ca (an)
Boréale	
- Conifères	149
- Feuillus	13,8
Tempérée	
- Conifères	5,9
- Feuillus	5,9
Méditerranéenne	3,8
Moyenne	21,8

Un flux annuel moyen de Ca, restitué par la végétation via les pluviolessivats, a été estimé lors de la synthèse des données obtenues dans 12 systèmes forestiers à travers le monde (Ragsdale et al, 1992). Ce flux est d'environ 113 kg/ha/an, ce qui n'est pas négligeable. En effet, pour 30% des systèmes forestiers retenus lors de cette synthèse, le flux moyen annuel de Ca restitué par la végétation via les pluviolessivats représente environ 50% du Ca que la végétation prélève en 1 an. De plus, différentes études estiment qu'entre 4 et 20% du Ca total contenu dans les feuilles ou les aiguilles, en fonction de l'espèce végétale étudiée, est remis en circulation dans le milieu forestier par les pluviolessivats (Johnson et al., 1988 ; Potter et al., 1991). Une étude récente sur le cycle biogéochimique du Ca dans un bassin versant forestier tempéré indique également que la contribution de la végétation sur la signature géochimique des pluviolessivats ne doit pas être négligée, surtout au printemps et en été, par risque de mal estimer les différents flux de Ca au sein du bassin versant (Schmitt et Stille, 2005 ; Cenki-Tok et al., 2009).

La litière forestière restitue le Ca lors de la dégradation et de la minéralisation des racines mortes, des feuilles et des branches tombées au sol. La vitesse de dégradation et de minéralisation de ces différents organes de végétaux va dépendre du climat, mais également de la quantité et de la nature de litière produite, de l'espèce végétale et de l'âge du système forestier (Ranger et al., 2003). Sous un climat froid, la vitesse de dégradation et de minéralisation de la litière est lente et cette dernière a tendance à s'accumuler, ce qui entraine une acidification des sols. A l'inverse, sous un climat tempéré, le Ca est restitué au milieu

naturel sous sa forme ionique, entre 2 (feuillus) à 25 (résineux) fois plus rapidement que sous climat froid (estimé d'après Cole et Rapp, 1981).

Le Ca restitué par la litière ou les pluviolessivats se retrouve sous forme ionique dans les solutions de sol ou sous forme adsorbée sur différents complexes du sol (colloïdes, complexes argilo-humiques). Le devenir du Ca restitué par la végétation est multiple. Il va pouvoir être recyclé par la végétation, comme le montre une étude récente en milieu forestier boréal où jusqu'à 80% du Ca contenu dans les arbres étudiés provient du Ca restitué par la végétation (Holmden et Bélanger, 2010). S'il n'est pas recyclé par la végétation, le Ca restitué va pouvoir se retrouver sous forme dissoute dans les eaux de surface, comme le montre Zakharova et al. (2007). Cette étude indique que la contribution du Ca issu de la végétation sur la quantité de Ca présent dans les eaux de surfaces d'un bassin versant boréale n'est pas négligeable. En effet, Zakharova et al. (2007) estiment qu'entre 20 et 35% du Ca total dissous des eaux de surface proviennent de la litière forestière.

Cependant, la contribution de la végétation au cycle des cations, dont celui du Ca, est encore mal connue. Or, la contribution de la végétation terrestre sur le flux de Ca dissous des rivières semble non négligeable, que ce soit via les pluviolessivats (entre 20 et 40% ; Cenki-Tok et al., 2009 ou via la litière (entre 20 et 35% ; Zakharova et al., 2007). Il est donc nécessaire de s'intéresser au cycle biologique du Ca au sein des plantes, car c'est ce dernier qui va influencer la contribution de la végétation sur le cycle global du Ca. Pour parvenir à cette meilleure compréhension du cycle biologique du Ca, l'utilisation des isotopes stables semble tout indiquée. En effet, les isotopes stables sont sensibles aux mécanismes qui ont lieu au sein de la végétation et, depuis peu, les avancées technologiques permettent d'appliquer les isotopes stables non traditionnels, tels que ceux du Ca, aux échantillons d'origine végétale.

IV. État de l'art du fractionnement des isotopes stables non traditionnels dans le système sol/plante

La géochimie des isotopes stables s'est longtemps cantonnée aux isotopes stables traditionnels, à savoir les isotopes du C, H, O et N. Ces éléments sont les principaux constituants des végétaux terrestres (Epstein et Bloom, 2005) et ils peuvent-être impliqués dans différents processus physiologiques importants pour les plantes (Dawson et al., 2002).

Les isotopes stables traditionnels ont permis d'améliorer nos connaissances sur leurs cycles biogéochimiques dans le système sol/plante.

Par exemple, les isotopes du C apportent des informations sur des processus métaboliques qui se produisent lors de la photosynthèse comme la carboxilation, la diffusion du CO_2, la respiration (Farquhar et al., 1982 ; Brüggemann et., 2011). Les isotopes du C peuvent également apporter des informations sur d'autres processus qui ne se déroulent pas pendant la photosynthèse, à savoir le mélange des sucres lors de leur transport dans le phloème, la synthèse de composés de structure, l'excrétion racinaire et la respiration racinaire (von Caemmerer et al., 1997 ; Gessler et al., 2008). Les isotopes stables traditionnels peuvent aussi permettre de déterminer l'origine de l'H_2O et de l'N assimilés par la végétation (Gray et Thompson, 1977 ; Högberg, 1997 ; Robinson, 2001). De plus, les isotopes de l'N permettent également d'étudier les interactions entre le sol et la végétation (Högberg, 1997 ; Robinson, 2001), tout comme les isotopes du C (Brüggemann et., 2011). Les isotopes stables traditionnels C, H et O peuvent aussi permettre de reconstituer des paléoenvironnements et des paléoclimats locaux. En effet, ces isotopes se trouvant dans les cernes d'arbres peuvent nous renseigner sur l'évolution de la température et du taux d'humidité locaux au cours de la vie des arbres (Edwards et Fritz, 1986 ; De Niro et Cooper, 1990 ; Edwards, 1990 ; McCarroll et Loader, 2004 ; Battipaglia et al, 2008 ; Brüggemann et., 2011 ; Leavitt et al., 2008, 2011). Les isotopes du C et de l'oxygène peuvent également permettre de déterminer la provenance de la matière organique végétale se trouvant dans les sols, à savoir si elle provient de plantes C3, C4 ou CAM (Troughton et al., 1974 ; Sternberg et De Niro, 1983 ; Sternberg et al., 1984 ; Dzurec et al., 1985 ; Helliker et Ehleringer, 2002).

Depuis peu, les avancées techniques et analytiques permettent d'utiliser les isotopes stables non traditionnels afin d'améliorer nos connaissances sur le cycle biogéochimique des macro et micronutriments (Ca, Mg, Si, Zn, Fe et Cu) dans le système sol/plante. En effet, les isotopes stables traditionnels n'avaient pas apporté d'informations sur les cycles de ces nutriments chez les plantes.

IV.1. État de l'art du fractionnement des isotopes du Ca dans le système sol/plante

Différentes études ont tenté de démontrer le potentiel des isotopes du Ca pour étudier l'impact du système sol/plante sur le flux de Ca des eaux de rivières (Schmitt et al., 2003 ; Schmitt et Stille, 2005 ; Wiegand et al., 2005 ; Perakis et al., 2006 ; Tipper et al., 2006 ; Page et al., 2008 ; Cenki-Tok et al., 2009 ; Holmden et Bélanger, 2010 ; Hindshaw et al., 2011 ; Farkas et al., 2011). Les données de ces différentes études sont consignées dans l'Annexe 1 et représentées dans la Figure I.5. A partir de ces données, il est possible de constater que les signatures isotopiques $\delta^{44/40}$Ca sur Terre varient entre -2,20 et 2,77‰ (Figure I.5 ; Annexe 1). La plus grande variation de composition isotopique $\delta^{44/40}$Ca est observée chez les végétaux, leurs $\delta^{44/40}$Ca sont compris entre -2,20 et 1,76‰ (Figure I.5 ; Annexe 1). Ainsi, les isotopes du Ca ont le potentiel de tracer et d'identifier le rôle de la végétation et de son recyclage sur les flux de Ca des eaux de surface continentales. Ils permettent également de tracer et d'identifier la contribution des différents processus qui se produisent lors du transport du Ca dissout dans le sol (dissolution/précipitation, échange entre différent réservoirs du sol).

Les premières analyses isotopiques du Ca dans les végétaux ont été réalisées par Platzner et Degani (1990). Cependant, les données de cette étude ne peuvent pas être directement comparées aux autres travaux mesurant les isotopes du Ca dans les plantes car ces auteurs n'ont mesuré aucun échantillon commun aux autres publications (standard interne : Merck Analar $Ca(NO_3)_2$). Platzner et Degani mettent en avant que les tissus reproducteurs sont appauvris en isotopes lourds du Ca (^{44}Ca) par rapport aux autres organes de palmier-dattiers.

Schmitt et al. (2003) furent les premiers à mettre en évidence l'éventuelle contribution de la végétation sur la signature isotopique $\delta^{44/40}$Ca des eaux de surface à l'échelle d'un petit bassin versant tempéré (Aubure, Vosges, France). Au cours de cette étude, Schmitt et al. (2003) constatent que les $\delta^{44/40}$Ca des solutions de sol du bassin versant d'Aubure (1,77 ± 0,19‰) sont plus enrichis en ^{44}Ca que les deux principales sources de Ca dissout des solutions de sol, à savoir les eaux de pluie (entre 0,57 ± 0,13‰ et 1,01 ± 0,15‰) et les minéraux du sol riche en Ca (apatite ; 0,4 ± 0,10‰). Donc, la signature isotopique $\delta^{44/40}$Ca des solutions de sol ne peut pas être expliquée par la seule contribution combinée du drainage

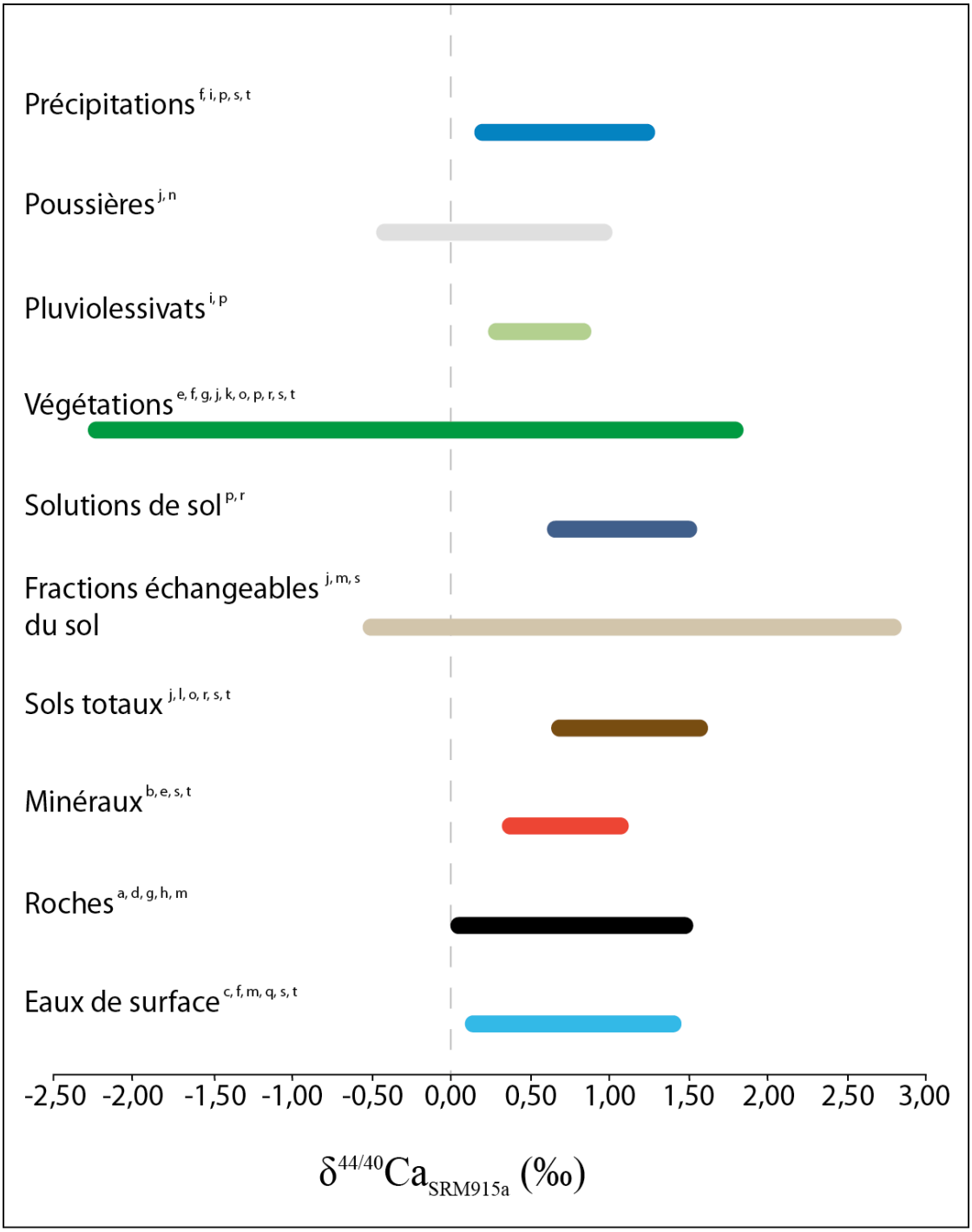

Figure I.5 *: Schéma récapitulatif des signatures isotopiques du Ca dans les différents réservoirs sur les continents. Les données sont issues de la littérature (Annexe 1).(a) Marshall et DePaolo (1982, 1989), (b) Skulan et al. (1997), (c) Zhu et MacDougall (1998), (d) Halicz et al. (1999), (e) Skulan et DePaolo (1999), (f) Schmitt et al. (2003), (g) Bullen et Bailey (2004), (h) DePaolo (2004), (i) Schmitt et al. (2005), (j) Wiengand et al. (2005), (k) Chu et al. (2006), (l) Pérakis et al. (2006), (m) Tipper et al. (2006), (n) Ewing et al. (2008), (o) Page et al. (2008), (p) Cenki-Tok et al. (2009), (q) Amini et al. (2009), (r) Holmden et Bélanger (2010), (s) Hindshow et al. (2011), (t) Farkas et al. (2011).*

des eaux de pluie dans le sol et de la dissolution des minéraux du sol du bassin versant d'Aubure, si on suppose que la dissolution congruente de l'apatite ne fractionne pas. Ainsi, Schmitt et al. (2003) avancent l'hypothèse que cet enrichissement en ^{44}Ca des solutions de sol est causé par la végétation. En effet, la signature isotopique $\delta^{44/40}Ca$ des échantillons de végétaux provenant de ce bassin versant montre que les branches (-0,58 ± 0,23‰) et les feuilles (0.25 ± 0.20‰) de hêtres sont enrichies en ^{40}Ca par rapport aux solutions de sol (1,77 ± 0,19‰). La végétation semble donc prélever préférentiellement les isotopes ^{40}Ca des solutions de sol, ce qui permet d'expliquer l'enrichissement en ^{44}Ca des solutions de sol. D'après cette étude, la végétation influencerait indirectement la signature isotopique $\delta^{44/40}Ca$ des eaux de surface par le biais des solutions de sol.

Par la suite, d'autres travaux sur les isotopes du Ca ont confirmé que la végétation s'enrichit en ^{40}Ca comparée aux solutions de sol (Wiegand et al., 2005 ; Page et al., 2008 ; Cenki-Tok et al., 2009 ; Holmden et Bélanger, 2010 ; Hindshow et al., 2011 ; Farkas et al., 2011). Ces études montrent également que ce fractionnement des isotopes du Ca entre les solutions de sol et les végétaux se produit à l'interface des racines non subérisées et des solutions de sol (Wiegand et al., 2005 ; Page et al., 2008 ; Cenki-Tok et al., 2009 ; Holmden et Bélanger, 2010). Le fractionnement moyen entre les racines et les solutions de sols $\Delta_{racines\text{-}solutions\ de\ sols}$ est, d'après les données de la littérature, égal à -1,48‰ (Annexe 1). Wiegand et al. (2005) expliquent que la capture préférentielle des isotopes ^{40}Ca par la végétation serait causée par un fractionnement cinétique des isotopes du Ca au niveau des racines. Ce fractionnement isotopique se produit lorsque les racines absorbent le Ca via un gradient électrochimique et des canaux calciques. En plus de ce fractionnement isotopique observable lors de la capture nutritive du Ca par les racines, un second fractionnement semble se produire lors du transfert longue distance du Ca des racines aux organes aériens (Wiegand et al., 2005 ; Page et al., 2008 ; Cenki-Tok et al., 2009 ; Holmden et Bélanger, 2010). Ainsi, les racines sont toujours les organes les plus enrichis en ^{40}Ca et les feuilles les plus enrichis en ^{44}Ca. Le fractionnement moyen entre les racines et les feuilles $\Delta_{feuilles\text{-}racines}$ est de 1,13‰, d'après la littérature (Annexe 1). Cette différence de $\delta^{44/40}Ca$ entre les racines et les feuilles peut être expliqué par un fractionnement cinétique qui se déroule lors de la translocation du Ca des racines aux feuilles (Wiegand et al., 2005). Ce fractionnement est causé par la fixation préférentielle des isotopes ^{40}Ca de la sève brute sur des sites échangeurs d'ions des vaisseaux

du xylème au niveau du tronc des arbres. De cette façon, la sève brute qui remonte vers la cime des arbres serait de plus en plus enrichie en ^{44}Ca.

Comme nous venons de le voir, la végétation enrichit la solution de sol en ^{44}Ca par la capture nutritive préférentielle du ^{40}Ca par les racines. Cependant, d'après la littérature, la végétation semble pouvoir influencer la signature isotopique en Ca des solutions de sol de deux autres façons, à savoir via les pluviolessivats (Schmitt et al., 2005 ; Cenki-Tok et al., 2009) et via la décomposition de la litière des sols forestiers (Wiegand et al., 2005 ; Page et al., 2008 ; Cenki-Tok et al., 2009 ; Holmden et Bélanger, 2010). En effet, Cenki-Tok et al. (2009) démontrent, à l'aide des isotopes du Ca, que la contribution des exsudats foliaires sur le $\delta^{44/40}Ca$ des pluies qui traversent la canopée n'est pas négligeable lors du printemps et de l'été. C'est au cours de ces saisons que la végétation est la plus active et que le couvert végétal est maximal. Le fractionnement moyen $\Delta_{pluviolessivats-pluies}$ pour le bassin versant d'Aubure est de -0,14‰ (Schmitt et Stille, 2005 ; Cenki-Tok et al., 2009 ; Annexe 1). Les pluviolessivats semblent être enrichis en ^{40}Ca comparés aux pluies. La décomposition de la litière des sols forestiers conduit quant à elle à la libération de ^{40}Ca dans les premiers horizons du sol (Page et al., 2008 ; Cenki-Tok et al., 2009 ; Holmden et Bélanger, 2010). En effet, les feuilles sont plus enrichies en ^{40}Ca que les solutions de sol. La décomposition de la litière des sols forestiers influence d'autant plus le $\delta^{44/40}Ca$ des sols et des solutions de sol que ceux-ci ont subi une forte perte de leur Ca échangeable via l'altération et/ou via le lessivage acide (Wiegand et al., 2005 ; Perakis et al., 2006). Enfin, la formation des minéraux secondaires entraine un fractionnement des isotopes du Ca dans les sols (Ewing et al., 2008 ; Tipper et al., 2008 ; Hindshaw et al., 2011 ; Farkas et al., 2011). Ainsi, les minéraux secondaires sont enrichis en ^{40}Ca comparés aux minéraux primaires et aux solutions de sol. L'altération des minéraux secondaires enrichit donc aussi le milieu naturel en ^{40}Ca.

IV.2. Comparaison des variations isotopiques du Ca au sein du système sol/plante à celles d'autres isotopes stables non traditionnels.

L'étude isotopique du système sol/plante à l'aide d'autres isotopes non traditionnels, tels que le Mg, Fe, Cu, Zn et Si, montre que la végétation possède toujours une signature isotopique différente de la source nutritive (Opfergelt et al., 2006 ; Weiss et al., 2005 ; Guelke and von Blanckenburg, 2007 ; Viers et al., 2007 ; Black et al., 2008 ; Ding et al., 2008 ; Moynier et al., 2009 ; Bolou-Bi et al., 2010 ; Aucour et al., 2011 ; Weinstein et al., 2011 ;

Jouvin et al., 2012). Le comportement des isotopes stables non traditionnels dont celui du Ca dans le système sol/plante est synthétisé Figure I.6. Il faut noter que pour le B, l'impact de la végétation sur son cycle est encore mal connu, du fait que, le développement d'une procédure analytique permettant de mesurer de mesurer de façon efficace les rapports isotopiques du B au sein des plantes est tout récent (Rosner et al., 2011). Enfin, les isotopes du Li ne sont pas utilisés dans le système sol/plante, car la végétation semble ne pas influencer le cycle continental du Li (Lemarchand et al., 2010).

IV.2.1. Fractionnement isotopique lors de la capture des nutriments par les racines

Pour tous les systèmes isotopiques présentés dans cette partie, il est possible d'observer un premier fractionnement entre la source nutritive et les racines, comme cela se produit également pour les isotopes du Ca (Figure I.6). Cependant, le type d'isotope (lourd ou léger) capté par les racines diffère selon les éléments, voir même selon le type de plante pour le Fe (dicotylédone : Stratégie I ou monocotylédone : Stratégie II). Ainsi, les racines prélèvent préférentiellement les isotopes légers ($\Delta_{\text{racines-source nutritive}} < 0$) du Cu, Fe (plante dicotylédone ; Stratégie I) et du Si (Ding et al., 2008 ; Guelke and von Blanckenburg, 2007 ; Jouvin et al., 2012). Ces isotopes se comportent comme les isotopes du Ca lors de leur capture nutritive par les racines (Schmitt et al., 2003 ; Wiegand et al., 2005 ; Page et al., 2008 ; Cenki-Tok et al., 2009 ; Holmden et Bélanger, 2010). A l'inverse, les études isotopiques sur le Zn, le Fe (plantes monocotylédones ; Stratégie II) et le Mg montrent que les racines prélèvent préférentiellement les isotopes lourds ($\Delta_{\text{racines-source nutritive}} > 0$; Guelke and von Blanckenburg, 2007 ; Bolou-Bi et al., 2010 ; Aucour et al., 2011 ; Jouvin et al., 2012).

Les mécanismes d'incorporation de ces différents éléments nutritifs sont différents de ceux du Ca (Wiegand et al., 2005). Le fractionnement isotopique du Si lors de sa capture nutritive au niveau des racines est dû au couplage d'une capture nutritive passive et active (Ding et al., 2008). Pour le Cu, le fractionnement isotopique semble être induit par le même mécanisme que celui qui se produit pour le Fe chez les plantes dicotylédones, à savoir par la réduction du Fe (III) en Fe (II) ou du Cu (II) en Cu (I) à la surface des racines et leur absorption via des protéines de transport spécifique (Guelke et von Blanckenburg, 2007 ; Jouvin et al., 2012). Le fractionnement isotopique du Zn lors de sa capture par les racines peut être causé par deux mécanismes : la capture du Zn à l'aide de transporteurs spécifiques

(protéines de transport) et l'adsorption-précipitation du Zn sur la surface externe des racines (Aucour et al., 2011). Le fractionnement isotopique du Mg est quant à lui induit par l'adsorption du Mg à la surface externe des racines sur des sites échangeurs d'ions (Bolou-Bi et al., 2010). Ce fractionnement suit une loi de fractionnement à l'équilibre (Black et al., 2008). Enfin, le fractionnement des isotopes du Fe au niveau des racines de plantes monocotylédones (stratégie II) est causé par l'absorption du Fe (III) complexé à des phytosiderophores via des protéines de transports spécifiques (Guelke and von Blanckenburg, 2007).

IV.2.2. Fractionnement isotopique lors de la translocation des racines aux feuilles

Une fois que les éléments nutritifs Cu, Mg, Fe, Zn et Si se retrouvent dans les plantes, ils vont tous être transportés des racines aux feuilles, c'est la translocation (Figure I.6). Le Cu, le Fe et la Si sont considérés comme des éléments immobiles, tout comme le Ca. Cela signifie que le Cu, Fe, Si et Ca ne se retrouvent pas dans le phloème. A l'inverse, le Mg et le Zn sont tous les deux des éléments mobiles.

Il est possible de regrouper les différents isotopes stables non traditionnels selon leur comportement au cours de la translocation chez les plantes. Ainsi, certains éléments présentent un enrichissement en isotopes légers au niveau des parties aériennes, comparées aux racines ($\Delta_{feuilles\text{-}racines} < 0$) comme le Mg, Zn, Cu et le Fe (plantes monocotylédones : Stratégie I ; Guelke and von Blanckenburg, 2007 ; Bolou-Bi et al., 2010 ; Aucour et al., 2011 ; Jouvin et al., 2012). A l'inverse, le Si présente quant à lui un enrichissement en isotopes lourds au niveau des parties aériennes comparées aux racines ($\Delta_{feuilles\text{-}racines} > 0$; Ding et al., 2008). Les isotopes du Ca se comportent de la même façon que le Si lors de leur translocation au sein des végétaux. La translocation du Fe chez les plantes monocotylédones (Stratégie II), quant à elle, n'induit pas de fractionnement isotopique (Guelke and von Blanckenburg, 2007).

Les mécanismes qui engendrent le fractionnement des isotopes du Cu, Fe, Zn, Mg et Si au cours de la translocation sont différents de ceux mis en avant pour le Ca. Ainsi, le fractionnement isotopique observé pour le Mg résulte de processus biologiques qui influencent différemment la composition isotopique des différents organes : formation de complexes organomagnésiens (p.ex : chlorophylle) et recyclage interne par le transport du Mg

dans la plante (Bolou-Bi et al., 2010). Jouvin et al. (2012) suggèrent que les causes du fractionnement des isotopes du Cu et du Zn au cours de la translocation sont identiques. Leur fractionnement est lié à deux processus: au transport du Cu et Zn par diffusion dans le xylème et le phloème et à des réactions d'échanges ioniques lors de leur transport dans le xylème et le phloème. Pour le Si, son fractionnement est lié à la précipitation de SiO_2 dans différents organes au cours de son transfert des racines aux feuilles (Ding et al., 2008). Ce fractionnement suit une loi de Rayleigh (Ding et al., 2005, 2008). Enfin, le fractionnement isotopique du Fe chez des plantes dicotylédones (Stratégie I) est dû au changement d'état redox du Fe lors de son transfert des racines aux feuilles (Guelke and von Blanckenburg, 2007).

IV.2.3. Le fractionnement isotopique dans les sols

L'étude du fractionnement des isotopes stables non traditionnels dans les sols montre des comportements isotopiques et des processus de fractionnement qui diffèrent en fonction des systèmes isotopiques étudiés (Skulan et al., 2002 ; Beard et Johnson, 2004 ; Ding et al., 2004 ; Fantle et DePaolo, 2004 ; Icopini et al., 2004 ; Ziegler et al., 2005 ; Tipper et al., 2006 ; Hindshaw et al., 2010 ; Bigalke et al., 2011 ; Aranda et al., 2012). Ainsi, pour le Mg et le Si, la formation de minéraux secondaires entraine un enrichissement en isotopes lourds du Mg et du Si des solutions de sol (Ding et al., 2004 ; Ziegler et al., 2005 ; Tipper et al., 2006). La même chose est observée pour les isotopes du Ca lors de la formation de phases secondaires (Tipper et al., 2006, Hindshow et al., 2010). Pour les isotopes du Fe, les études sur le sujet montrent l'inverse. La formation de phases secondaires à partir du Fe, telles que le ferrihydrite et l'hématite, entraine un enrichissement de la phase résultante en isotopes légers du Fe (Skulan et al., 2002 ; Beard et Johnson, 2004), tout comme lors de la sorption du Fe sur des particules de sol chargées (Icopini et al., 2004). Ainsi, les isotopes du Fe présents dans la phase mobile (Fe II) sont enrichis en isotopes légers, comparée à la phase immobile (Fe III). Cela signifie que c'est le degré d'oxydation du Fe qui influence le comportement de ces isotopes dans les sols (Fantle et DePaolo, 2004), tout comme pour le Cu et le Zn (Bigalke et al., 2011 ; Aranda et al., 2012).

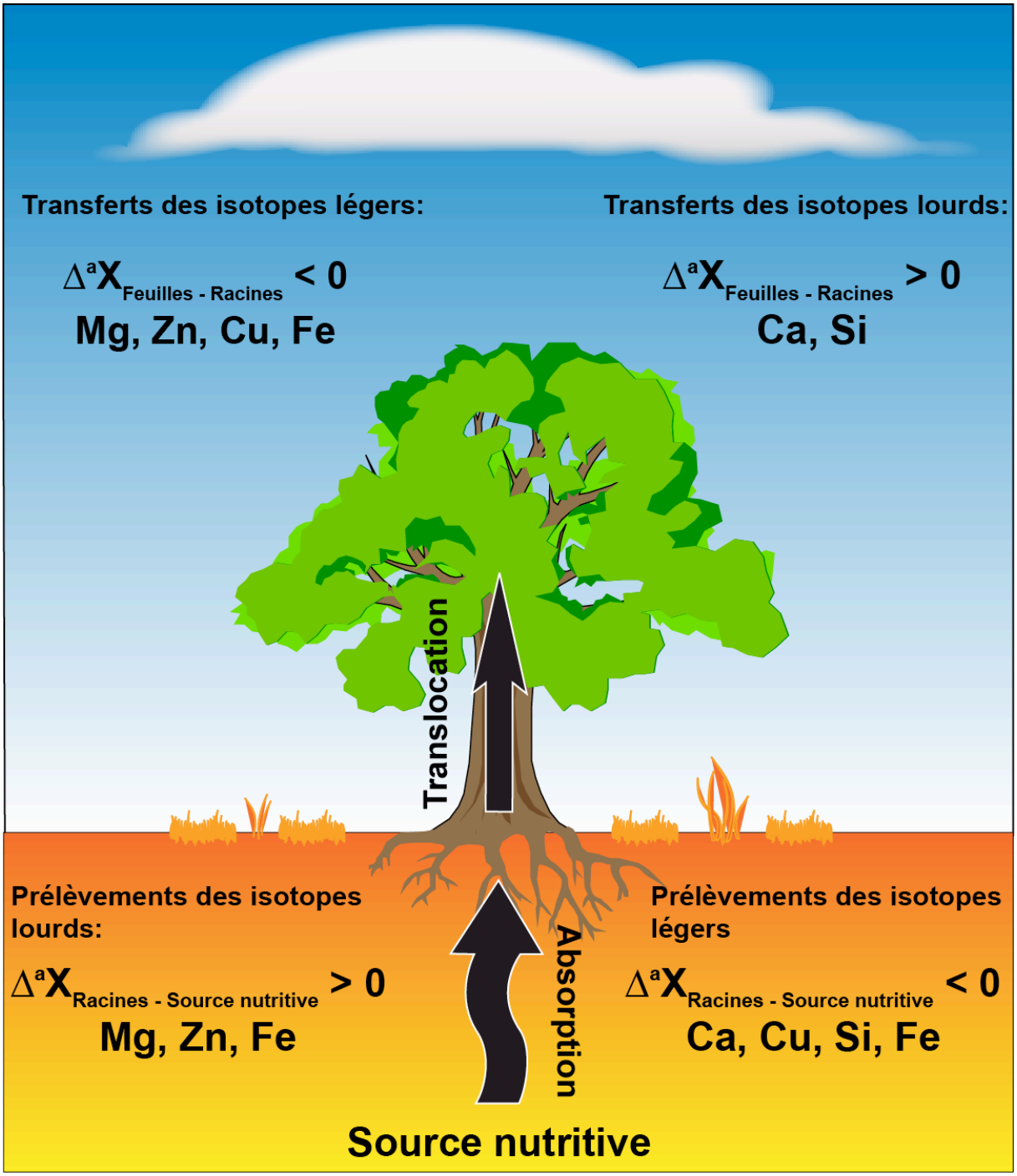

Figure I.6 : *Variations des signatures isotopiques des isotopes stables non traditionnels (^{a}X = ^{26}Mg, ^{39}Si, ^{44}Ca, ^{57}Fe, ^{65}Cu, ^{66}Zn) au sein des végétaux.* ***Mg*** *: Black et al. (2008), Bolou-Bi et al. (2010) ;* ***Si*** *:Ding et al. (2005), Opfergelt et al. (2006), Ding et al. (2008) ;* ***Ca*** *: Schmitt et al. (2003), Wiegand et al. (2005), Page et al. (2008), Cenki-Tok et al. (2009), Holmden et Bélanger (2010) ;* ***Fe*** *: Guelke and von Blanckenburg (2007) ;* ***Cu*** *: Weinstein et al., 2011, Jouvin et al. (2012) ;* ***Zn*** *: Weiss et al. (2005), Viers et al. (2007), Moynier et al. (2009), Aucour et al. (2011), Jouvin et al. (2012).*

V. Conclusion

L'influence de la végétation sur le cycle du Ca n'est donc pas négligeable. L'utilisation des isotopes du Ca a permis de mettre en évidence que les végétaux sont impliqués dans de nombreux processus qui peuvent influencer le cycle du Ca à l'échelle d'un bassin versant, *via* la capture nutritive du Ca par les racines, la translocation du Ca au sein des végétaux, les pluviolessivats et la dégradation de la litière forestière. Cependant, tous ces processus biotiques ne sont pas les seuls à influencer le cycle du Ca à l'échelle d'un bassin versant. En effet, des processus abiotiques peuvent également influencer le cycle du Ca dans le milieu naturel (p.ex formation de phase secondaire). Or, il est difficile de dissocier les processus biotiques des processus abiotiques lors d'études en milieu naturel, malgré l'utilisation de l'outil isotopique.

C'est pourquoi il est maintenant indispensable d'identifier et de préciser les processus biotiques et abiotiques qui influencent le cycle du Ca dans le système sol/plante. Pour cela, nous avons utilisé les isotopes du Ca, au cours d'études expérimentales en milieu contrôlé de type hydroponique et de type microcosme (Chapitres 3, 4 et 5).

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Chapitre 2 :

Matériels et Méthodes

I. Introduction

Au cours de cette thèse, nous avons réalisé trois expérimentations de culture de plantes en milieu contrôlé (Chapitres 3, 4, 5). Nous avons mené deux expérimentations de culture de plantes sur milieu liquide de type hydroponique (Chapitres 3 et 4) et une expérimentation de culture de plantes sur milieu solide de type microcosme (Chapitre 5). Ces trois expérimentations ont pour objectif de permettre d'identifier et de préciser les processus abiotiques et biotiques qui contrôlent le fractionnement des isotopes du Ca à l'interface sol/plante.

Dans ce chapitre, nous allons décrire les systèmes expérimentaux des cultures de plantes utilisés et présenter le protocole analytique qui nous a permis de mesurer les rapports isotopiques du Ca lors de ces trois expérimentations.

II. Systèmes de cultures de plantes en milieu contrôlé

II.1. Système de culture hydroponique

Pour pouvoir étudier le fractionnement des isotopes du Ca dans le système sol/plante, il a d'abord été nécessaire d'étudier le comportement des isotopes du Ca au sein des végétaux. Pour cela, nous avons utilisé un système expérimental de culture de plante en milieu liquide de type hydroponique (Figure II.1). Ce système expérimental permet de s'affranchir de la variation des conditions du milieu (p.ex. Tocquin et al., 2003 ; Robinson et al., 2006), tels que les apports atmosphériques et les interférences inhérentes au développement des plantes à partir d'un sol. Cette approche a notamment été retenue pour étudier l'accumulation des métaux dans les plantes, via le fractionnement des terres rares (p.ex. Ding et al., 2006), ainsi que le fractionnement isotopique du Si, Mg, Zn dans les plantes (Weiss et al., 2005 ; Opfergelt et al., 2006 ; Black et al., 2008 ; Bolou-Bi et al., 2010, Aucour et al., 2011).

Le protocole des cultures hydroponiques utilisé lors de cette thèse a été mis au point par Jaegler (2008) lors de son travail de Master 2 à l'Université de Franche-Comté au sein de l'UMR Chrono-Environnement (Besançon). Les cultures hydroponiques de plantes ayant servies pour le Chapitre 3 et 4 de cette thèse ont toutes été réalisées à l'Université de Franche-Comté au sein de l'UMR Chrono-Environnement (Besançon ; Pascale Bourgeade).

II.1.1. Dispositif de culture hydroponique :

Le dispositif de culture réalisé pour les expériences hydroponiques (Figure II.1) est adapté de Camefort (1992) et Taiz et Zeiger (2010). Il permet d'accueillir la solution nutritive et les plantes cultivées. Le nombre de bacs utilisés varie selon l'expérimentation, plus précisément selon le nombre de conditions expérimentales réalisées par expérience (Chapitres 3 et 4). Ainsi, pour l'expérimentation présentée chapitre 3, nous utilisons quatre dispositifs de cultures (Tableau II.1), tandis que pour l'expérimentation du Chapitre 4, nous utilisons uniquement deux bacs de cultures (Tableau II.1).

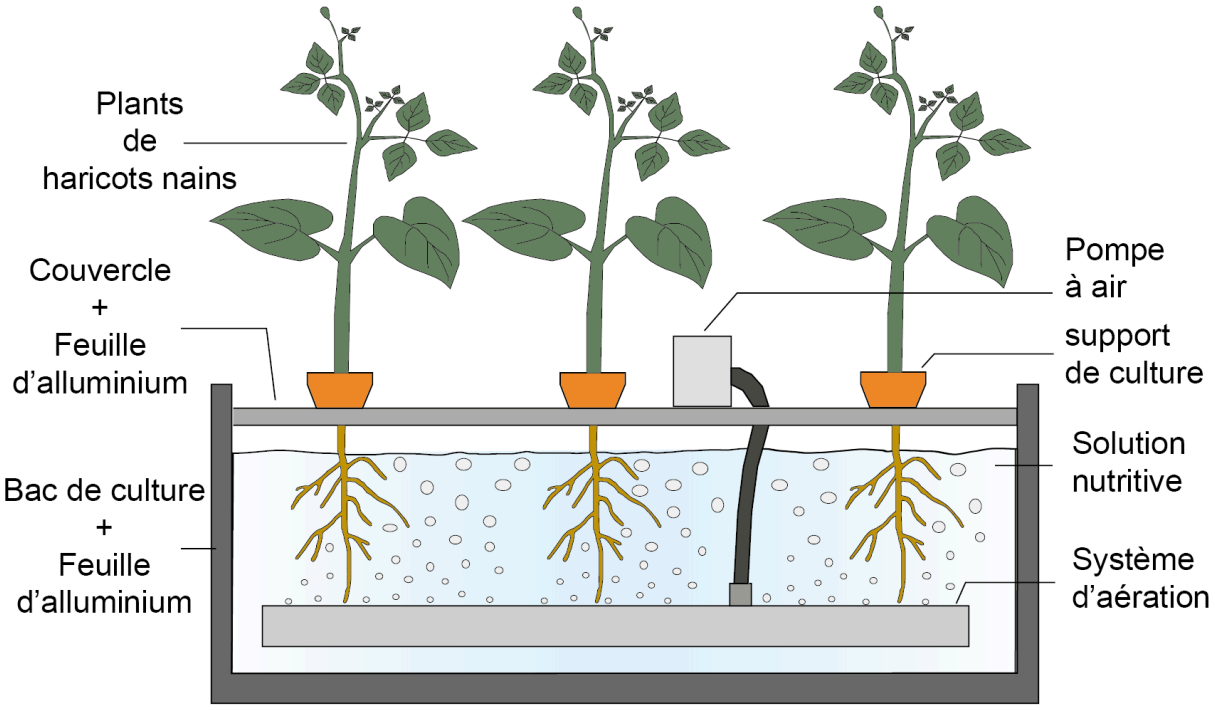

Figure II.1 : *Dispositif de culture hydroponique de plants de haricots nains (Chapitre 3 et 4) adapté de Camefort, (1992) et Taiz et Zeiger (2010). Pour chaque condition expérimentale se reporter au Tableau II.1 et aux chapitres 3 et 4.*

Le dispositif de culture est toujours constitué : d'un bac en polypropylène de dimensions 27,4 x 41 x 22 cm rempli de 15 L de solution nutritive ; d'un diffuseur d'air permettant d'homogénéiser la solution nutritive contenue dans le bac et d'un couvercle recouvert de papier aluminium pour le rendre opaque, éviter que des champignons et des

algues se développent et limiter les pertes de solution nutritive par évaporation. Le couvercle est également percé de cinq trous qui accueillent chacun un support de croissance en polypropylène avec une plante (Figure II.1).

Les solutions nutritives utilisées lors des expérimentations présentées chapitres 3 et 4 sont issues de deux solutions mères concentrées de Hoagland modifiées par Guo et al. (2002) et adaptées aux besoins de croissance des plants de haricot. La première solution nutritive reflète un sol pauvre en Ca (5 ppm Ca, « Low Ca » ; chapitres 3 et 4 ; Tableau II.1), la seconde reflète un sol riche en Ca (60 ppm Ca, « High Ca » ; chapitres 3 ; Tableau II.1). Ces deux solutions mères (Low Ca et High Ca) sont ensuite diluées à l'aide d'eau mono-distillée, afin d'obtenir les teneurs suivantes pour chaque dispositif expérimental utilisé :

- pour les macroéléments (mmol/L) : $(NH_4)_2SO_4$: 0,5 ; KH_2PO_4 : 2 ; $MgSO_4$: 0,5 ; $CaNO_3$: 1,5 (High Ca) et 0,15 (Low Ca) ; $NaNO_3$: 1,5 (High Ca) et 4,2 (Low Ca)
- Pour les microéléments (µmol/L) : Fe^{2+}-EDTA : 45 ; $MnSO_4$: 1 ; $ZnSO_4$: 1 ; $CuSO_4$: 0,1 ; H_3B_3 : 10 ; $(NH_4)Mo_7O_{24}$: 0,01

Chaque solution mère stockée est ensuite préparée à deux pH (4 et 6), afin d'observer de possibles variations causées par l'acidité des solutions nutritives (Tableau II.1). Lors de l'expérimentation présentée chapitre 3, nous utilisons les deux solutions mères (Low Ca et High Ca ; Tableau II.1) aux deux pH choisis (4 et 6 ; Tableau II.1). En revanche pour l'expérimentation du chapitre 4, nous n'utilisons qu'une seule des deux solutions mères (Low Ca ; Tableau II.1), a un seul pH (6 ; Tableau II.1). Le pH des solutions nutritives lors des expériences de culture hydroponique est quotidiennement ajusté afin de rester constant en ajoutant au maximum 200 µL de HCl 0,1 N ou de KOH 0,1 N.

Lors des expériences de culture de plantes présentées chapitre 3, les solutions nutritives sont changées régulièrement, deux fois par semaine lors des deux premières semaines d'expérimentation, puis trois fois par semaine (jusqu'à la fin de l'expérimentation). Lorsque les solutions nutritives sont remplacées, il est vérifié que la teneur en Ca des solutions nutritives n'a pas varié de plus 10% et qu'aucun fractionnement des isotopes du Ca ne se produit dans ces solutions nutritives. Cela permet de s'assurer que la quantité de Ca disponible dans les solutions nutritives peut être considérée comme infinie et homogène isotopiquement. Enfin, tous les jours, le volume de la solution nutritive est réajusté en

apportant un volume d'eau mono-distillé qui correspond aux pertes induites par l'activité des plantes cultivées (évapotranspiration).

En ce qui concerne l'expérience présentée dans le chapitre 4, deux cas de figure s'offrent à nous. Dans un premier cas, nous avons mis en place un dispositif de culture hydroponique pour lequel nous laissons la solution nutritive s'appauvrir en Ca, sans que cela n'induise des symptômes de carence chez les plantes cultivées (expérience « non limitante » ; Tableau II.1). Dans un second cas, nous laissons la solution nutritive utilisée s'appauvrir en Ca jusqu'à ce que l'appauvrissement en Ca provoque des symptômes de carence chez les plantes cultivées (expérience « limitante » Tableau II.1). Pour ces deux cas de figure, nous réajustons le volume de la solution nutritive en ajoutant de l'eau mono-distillée afin de nous assurer que nous avons toujours un volume de solution nutritive égal à 15 L. Enfin, nous réalisons un suivi (deux à trois fois par semaine) de la quantité de Ca disponible dans la solution nutritive utilisée lors de l'expérimentation « non-limitante », afin de nous assurer que la perte de Ca ne dépasse jamais 40%. Lorsque la perte de Ca dépasse 40%, nous remplaçons la solution nutritive.

Tableau II.1 : *Caractéristiques des différentes solutions nutritives utilisées lors des expériences présentées Chapitre 3 et 4.*

	Chapitre 3				Chapitre 4	
[Ca] ppm	5 ppm (Low)	60 ppm (High)	5 ppm (Low)	60 ppm (High)	5 ppm (Low)	5 ppm (Low)
pH	4	4	6	6	6	6
Conditions expérimentales	L4	H4	L6	H6	L6 « non limitante »	L6 « limitante »

Pour les deux expérimentations de cultures hydroponiques (Chapitres 3 et 4), nous avons utilisé le modèle numérique Visual MINTEQ equilibrium (Gustafsson, 2010) pour nous assurer que plus de 98% du Ca présent dans les solutions nutritives utilisées se trouve bien sous forme dissoute. Ainsi, tout fractionnement des isotopes du Ca induit par la précipitation de Ca dans la solution nutritive pourra être exclu.

II.1.2. Choix de la variété végétale

Pour les expérimentations de cultures hydroponiques (Chapitres 3 et 4), l'espèce végétale retenue est le haricot nain, *Phaseolus vulgaris* L. (« fin de Bagnol », Vilmorin). C'est un dicotylédone de la famille des Fabacées utilisé avec succès dans de nombreuses études antérieures de culture hydroponique (Zindler-Frank, 1995 ; Guo et al., 2002). Le haricot nain est majoritairement autogame, ce qui permet d'obtenir aisément des fruits sans l'aide de pollinisateurs. De plus, il présente une croissance rapide de 5 à 6 semaines.

Les plants de haricots utilisés lors de nos deux expérimentations de culture hydroponique (Chapitres 3 et 4) ont été obtenus grâce à la germination de graines de haricots nains pendant une semaine dans le noir à 25°C sur 100 g de vermiculite. Cette dernière a été au préalable lavée pendant 5 jours avec de l'eau Millipore® ultrapure (18,2 MΩ). Une fois les graines germées, nous les rinçons à l'eau Millipore® ultrapure avant de les transférer sur leur support de culture (Figure II.1).

II.1.3. Conditions de cultures

Les cultures hydroponiques (Chapitres 3 et 4), ont été réalisées au sein d'une chambre de culture de l'Université de Franche-Comté, UMR Chrono-environnement (Besançon) à 25 ± 5°C durant des cycles jour/nuit de 16/8 h avec une humidité relative de 50 ± 15% et une intensité lumineuse de 150 µmol photons m^{-2} s^{-1}. La durée des différentes expériences hydroponiques a été de 6 semaines.

II.2. Système de culture en microcosme

Pour étudier l'influence des acides organiques et inorganiques excrétés par les plantes et de l'activité des bactéries de la rhizosphère sur le rapport isotopique du Ca des solutions de sol et des organes de végétaux (Chapitre 5), nous avons utilisé un dispositif de culture de végétaux en microcosme (Figure II.2). Ce système expérimental permet de connaitre la composition minérale et organique du substrat solide, de maîtriser les solutions apportées au système, de contrôler les solutions qui percolent du système expérimental, ainsi que de s'affranchir des apports atmosphériques.

Le protocole expérimental utilisé lors de cette expérience (Chapitre 5) a été développé conjointement par le laboratoire BEF (Biogéochimie des Écosystèmes Forestiers, INRA de Nancy, Champenoux, France) et le laboratoire IAM (Interactions Arbres-Microorganismes, INRA de Nancy, Champenoux, France). Il est possible de trouver le détail de ce protocole dans Calvaruso et al. (2006). Les expérimentations en microcosme ayant servis pour l'étude présentée chapitre 5 ont été réalisées à l'INRA de Nancy (Champenoux, France).

II.2.1. Dispositif de culture en microcosme

Les expériences abiotiques et biotiques ont été réalisées à l'aide de dispositifs de culture en microcosme (Figure II.2 ; Tableau II.2 ; Chapitre 5).

Pour les expériences abiotiques, nous avons à chaque fois utilisé un seul type de colonne *in vitro* réalisé à l'aide tube en polypropylène stérile (Figure II.2.a ; Tableau II.2). Il s'agit d'une colonne dont le substrat solide est constituée d'un mélange de cristaux purs de quartz concassé (séparés en deux fractions de granulométrie de 0,5 et 1 mm et 1 et 2 mm) et de cristaux de fluoroapatites de Durango (Mexique) purs et homogènes (granulométrie comprise entre 0,5 et 1 mm ; compositions chimique : 53,55% CaO, 40,64% P_2O_5, 0,84% SiO_2, 0,41% Na_2O, 0,18% Al_2O_3, 0,08% Fe_2O_3, 0,08% MgO, 0,02% TiO_2, et 1795 ppm Ce, 1599 ppm La, 1044 ppm Nd, 671 ppm As, 502 ppm Sr, 401 ppm Y, 303 ppm Pr, 153 ppm Th, 8 ppm U ; formule structurale : $(Ca_{9,77}La_{0,03}Ce_{0,04}Sr_{0,007}Fe_{0,003}Si_{0,06})$ $(P_{1,02}O_4)_6$ $F_{2,08}$ $Cl_{0,12}$; Park et al., 2004). Les cristaux de quartz et de fluoroapatites ont été stérilisés par autoclave (20 min. à 120°C). Les colonnes abiotiques sont alimentées à l'aide d'une pompe péristaltique (débit de 0,5 mL/h), soit en eau Millipore® ultrapure, soit en acide inorganique (HNO_3) à pH 2 ou 3,5, soit en acide organique ($C_6H_8O_7$) à deux pH 2 ou 3,5 également (Figure II.2 ; Tableau II.2). Ces deux acides ont été retenus car ils sont excrétés par les organismes vivants que nous allons utiliser lors des expériences biotiques (Chapitre 5). Le pH de 3,5 a été choisi pour être représentatif des conditions trouvées dans un écosystème forestier tempéré (e.g. bassin versant du Strengbach, France ; Stille et al., 2009). Le pH de 2 est, quant à lui, utilisé pour tester l'influence d'une solution fortement acide sur le fractionnement isotopique du Ca. Enfin, la solution qui percole à travers la colonne est filtrée à l'aide d'un opercule en nylon poreux (20 µm) en sortie de dispositif, juste avant d'être collectée à l'aide d'un pilulier en polypropylène stérile de 200 mL (Figure II.2.a). Toutes les semaines, le pilulier est remplacé.

La solution collectée grâce au pilulier correspond au volume de solution qui a percolé pendant une semaine à travers la colonne.

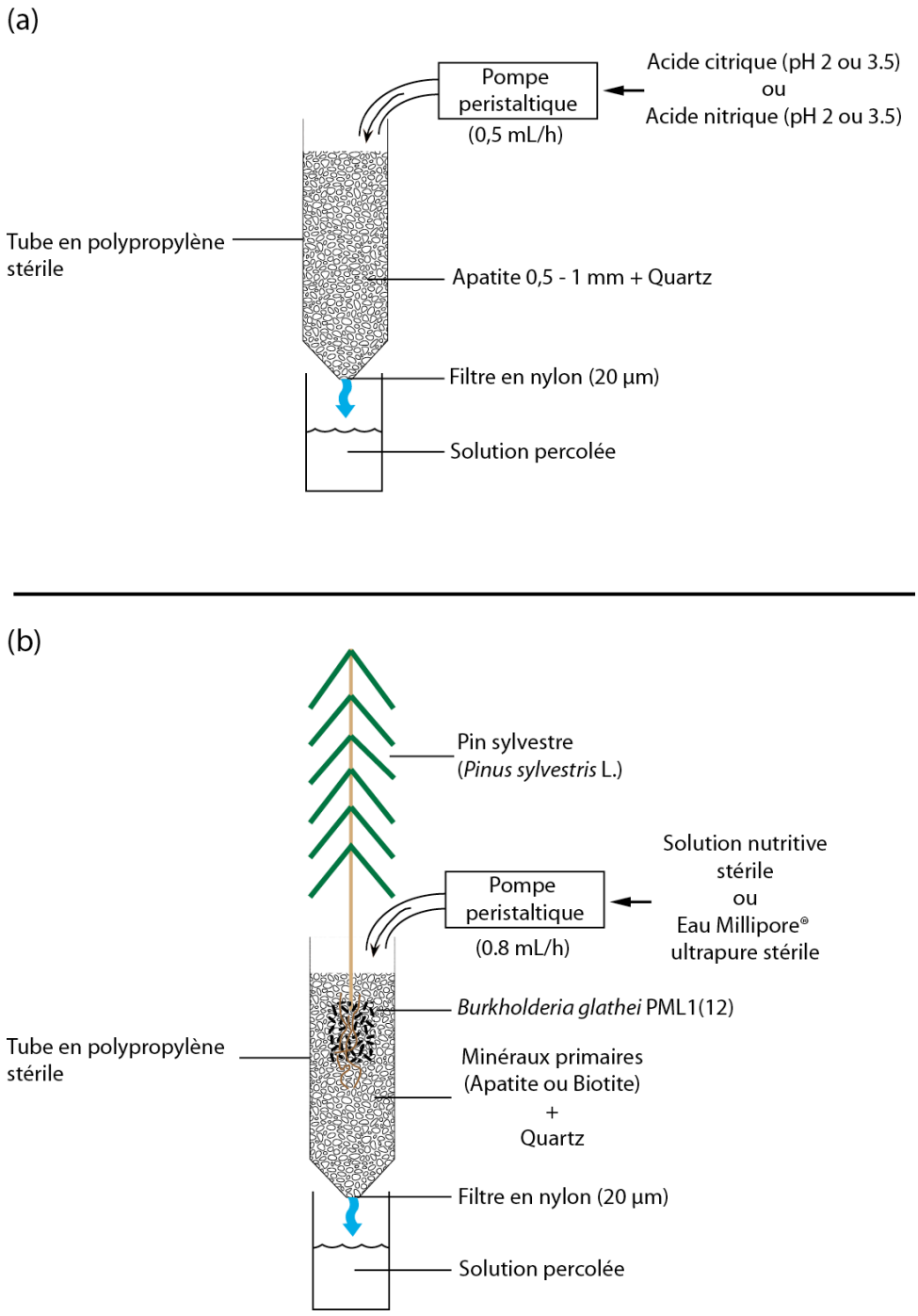

Figure II.2 : *Dispositifs de culture en microcosme (Chapitre 5). (a) Expérimentations abiotiques. (b) Expérimentations biotiques. Pour chaque condition expérimentale, se reporter au Tableau II.2 et au Chapitre 5.*

Pour les expériences biotiques, deux types de colonnes *in vitro* sont utilisées (Chapitre 5 ; Figure II.2.b ; Tableau II.2). La première sorte de colonne est identique aux colonnes utilisées lors des expérimentations abiotiques (Figure II.2.a), c'est-à-dire une colonne remplie d'un mélange de quartz pur et de fluoroapatite de Durango (granulométries identiques aux expériences abiotiques pour les deux minéraux). Le second type de colonne utilisé lors des expériences biotiques (Chapitre 5) est constitué d'un tube en polypropylène également stérile. Cependant, le substrat solide qui remplit ce second type de colonne est un mélange de quartz pur (granulométries identiques aux expériences abiotiques) et de biotites de Bancroft (Canada) pures et homogènes (Figure II.2.b ; formule structurale : $(SI_3Al)(Fe^{3+}_{0,12}Mg_{2,06}Mn_{0,02}Ti_{0,13})\ K_{0,88}Na_{0,06}O_{10}\ (OH_{0,98},F_{1,02})$; Calvaruso et al., 2006). Les mélanges de cristaux des deux sortes de colonnes sont rincés à l'eau Millipore® ultrapure et stérilisés (20 min. à 120°C). Pour chaque type de colonne utilisée lors des expériences biotiques, nous injectons dans les colonnes de l'eau Millipore® ultrapure ou une solution nutritive stérile à l'aide d'une pompe péristaltique (débit de 0,8 mL/h ; Figure II.2.b ; Tableau II.2). Deux types de solutions nutritives sont utilisées lors des expériences biotiques selon que le substrat solide soit composé d'un mélange de quartz et de biotite (solution nutritive enrichie en Ca ; Chapitre 5 ; Tableau II.2) ou d'un mélange de quartz et de fluoroapatite (solution nutritive sans Ca ; Chapitre 5 ; Tableau II.2). Cependant, ces deux solutions nutritives stériles présentent des compositions chimiques identiques à celles observées dans les sols du site atelier de Breuil-Chenu (France). Ainsi, pour les colonnes ayant un substrat solide composé de quartz et de fluoroapatites, la solution nutritive utilisée apporte tous les éléments nécessaires à la croissance des végétaux et des bactéries de la rhizosphère, à l'exception des éléments fournis par la fluoroapatite (Chapitre 5). La composition de cette solution nutritive sans Ca est la suivante : 11,2 mg/L de N, 4,2 mg/L de K, 1,0 mg/L de Mg et 1,3 mg/L de S. La composition de la solution nutritive utilisée lors des expériences sur colonnes de biotites et quartz est quant à elle la suivante : 1,5 mg/L de Na, 2,0 mg/L de P, 2,3 mg/L de Ca, 1,9 mg/L de S, 11,2 mg/L de N et 1,5 mL de Mo-Cu. Cette dernière apporte également tous les éléments nécessaires à la croissance des organismes vivants utilisés lors des expériences biotiques, qui ne seraient pas fournis par la biotite (Chapitre 5). Enfin, la solution qui percole à travers la colonne est filtrée à l'aide d'un opercule en nylon poreux (20 µm) en sortie de dispositif, juste avant d'être collectée à l'aide d'un pilulier en polypropylène stérile de 200 mL (Figure II.2.b). Toutes les semaines, le pilulier est remplacé. La solution collectée grâce au pilulier correspond au volume de solution qui a percolé pendant une semaine à travers la colonne.

II.2.2. Choix des organismes vivants

Pour réaliser les expériences biotiques en microcosme du chapitre 5 (Figure II.2.b ; Tableau II.2), nous avons utilisé comme espèce végétale des pins sylvestres (*Pinus sylvestris*) et comme bactérie de la rhizosphère la souche bactérienne *Burkholderia glathei* PML1(12). Les pins sylvestres utilisés aux cours des expériences biotiques et ont été choisis pour leur croissance relativement rapide (Chapitre 5 ; Tableau II.2) sont issus de graines germées provenant de le forêt de Haguenau (France). La procédure de germination est décrite par Koele et al. (2009). Pour s'assurer de l'absence de toute contamination des plants de pins germés à l'INRA de Nancy par des champignons de la rhizosphère, ils sont cultivés pendant 20 semaines au préalable. La souche bactérienne utilisée lors de certaines expériences biotiques avec inoculation du substrat solide (Chapitre 5 ; Chapitre II.2) est *Burkholderia glathei* PML1(12). Cette dernière a été choisie car elle présente de bonne potentialité pour altérer les minéraux (Calvaruso et al., 2006 ; Uroz et al., 2007). Elle a été isolée à partir de l'ectomycorhizosphère du couple *Scleroderma citrinum*-chêne en forêt de Breuil-Chenu (France ; Calvaruso et al., 2007).

II.2.3. Conditions des expériences en microcosme

Les différentes cultures en microcosme ont été réalisées à l'INRA de Nancy (Champenoux, France) en chambre de culture. Les conditions régnant dans la chambre de culture sont les suivantes : une photopériode de 17h, une température de nuit de 18°C, une température de jour de 25°C et un taux d'humidité bas (60%). La durée des expériences biotiques est de 14 semaines.

Les expériences abiotiques quant à elles ont duré 5 semaines et se déroulent dans une pièce (INRA de Nancy) où les apports atmosphériques en Ca sont négligeables.

Tableau II.2 : *Différentes configurations expérimentales réalisées lors de l'expérience présentée Chapitre 5.*

	Apatite + Quartz	Biotite + Quartz
Expérimentations abiotiques	HNO_3, pH 2	
	HNO_3, pH 3,5	
	$C_6H_8O_7$, pH 2	
	$C_6H_8O_7$, pH 3,5	
Expérimentations biotiques	Pins + Solution nutritive pauvre en Ca	Pins + Solution nutritive riche en Ca
	Pins + Bactérie + Solution nutritive pauvre en Ca	Pins + Bactérie + Solution nutritive riche en Ca
	Bactérie + Solution nutritive pauvre en Ca	Bactérie + Solution nutritive riche en Ca
Blancs de procédure	Solution nutritive pauvre en Ca	Solution nutritive riche en Ca
	Eau Millipore® ultrapure	Eau Millipore® ultrapure

III. Protocole analytique utilisé pour mesurer le $\delta^{44/40}$Ca des échantillons étudiés

Au cours de ce travail de doctorat, nous avons analysé des échantillons biotiques (organes de haricots, organes de pins ; respectivement Chapitres 3, 4 et Chapitre 5) qu'abiotiques (solutions nutritives et minéraux primaires ; Chapitre 3, 4 et 5) afin d'obtenir leur composition isotopique en Ca ($\delta^{44/40}$Ca ; Équation I.2). Tous les résultats géochimiques élémentaires ou isotopiques présentés dans ce manuscrit de thèse (Chapitres 3, 4 et 5) ont été obtenus au Laboratoire d'Hydrologie et de Géochimie de Strasbourg (LHyGeS, Strasbourg, France). De plus, tous les échantillons ont été préparés en salle blanche.

Dans un premier temps, il a été nécessaire de sécher les échantillons biologiques collectés (à l'exception des sèves de l'expérience du Chapitre 3) pendant 1 semaine dans une étuve à 60°C, puis de les broyer. Ensuite, nous avons mis en solution tous les échantillons d'origine biotique et les minéraux primaires. Pour cela, nous avons mis en solution environ 100 mg de chaque échantillon dans des récipients en téflon (Savilex™) à l'aide d'une attaque

acide oxydante à chaud (acides HNO_3 bidistillé / HCl bidistillé / H_2O_2 suprapur). Pour les échantillons liquides abiotiques (solutions nutritives), nous les avons seulement traités avec du H_2O_2 suprapur, afin d'éliminer toute trace de matière organique.

Une fois les échantillons dissous, nous prélevons un aliquote de ces derniers, dilué au 1/10, afin de mesurer leur teneur en Ca, Sr, Al, Mg, K, Na à l'aide d'un ICP-AES (Jobin Yvon JY 124), avec une limite de détection comprise entre 1 et 40 ppb et une précision analytique comprise entre 5 à 10%, en fonction de l'élément analysé.

Une fois la quantité de Ca présent dans chaque échantillon collecté connue, il est possible de déterminer sa composition isotopique en Ca. Pour cela, nous utilisons la procédure d'analyse isotopique mise au point au LHyGeS par Schmitt et al. (2009 ; Annexe 2) au début de mon doctorat. Cependant, ce protocole ne permet pas de mesurer les compositions isotopiques du Ca des échantillons en mode automatique et la mesure du rapport isotopique en Ca d'un échantillon dure environ 5 heures. La procédure développée consiste dans un premier temps à prélever 5 µg de Ca dans l'échantillon que l'on souhaite analyser, auquel nous rajoutons 1 µg de double spike $^{42}Ca/^{43}Ca$ avant que la chimie séparative du Ca de l'échantillon ne soit réalisée via une chromatographie ionique automatisée haute sélectivité sur Dionex® ICS-3000 à l'aide d'une résine échangeuse d'ion forte capacité (Dionex® CS16).

Après la chimie séparative du Ca, nous traitons chaque échantillon avec du HNO_3 bidistillé et du H_2O_2 suprapur, afin de supprimer toute trace d'impureté organique qui pourrait provenir de la résine échangeuse d'ion utilisée lors de la chimie séparative ou de l'échantillon lui même. Ensuite, l'échantillon est évaporé à 110°C.

Le résidu obtenu suite à l'évaporation de l'échantillon est dissous à l'aide de 1 µL de HNO_3 0,25 N. Ensuite, il est déposé sans aucun autre traitement sur un filament de Ta pur (pureté de 99,995 %), qui aura été au préalable dégazé, et oxydé sous un vide partiel. La composition isotopique du Ca de l'échantillon est ensuite mesurée par un spectromètre de masse à thermo-ionisation (TIMS, Triton Thermo-Finnigan) opérant en mode multicollection dynamique (Holmden, 2005 ; Schmitt et al., 2009). Le filament est d'abord chauffé à ~1200°C, puis le courant est ajusté de telle façon qu'on obtienne $5.0*10^{-11}$ A de $^{40}Ca^{+}$, ce qui correspond à une gamme de température du filament comprise entre 1380 à 1450°C. Nous collectons entre 130 et 200 cycles de mesure. La plupart de nos échantillons sont répliqués, ce

qui inclut l'attaque acide oxydante, la purification du Ca par chromatographie ionique et l'analyse par TIMS. Les possibles interférences isobariques aux masses 40 et 44 (correspondant au $^{41}K^{+}$ et $^{88}Sr^{+}$ qui donnent du $^{40}K^{+}$ et $^{88}Sr^{2+}$) sont toujours vérifiées. Nous ne constatons aucune interférence, et de ce fait, aucune correction n'est nécessaire. Les valeurs de $\delta^{44/40}Ca$ sont ensuite calculées en utilisant une loi de fractionnement exponentiel et la technique d'itération Newton-Raphson (Albarède et Beard, 2004). Les valeurs de $\delta^{44/40}Ca$ sont calculées hors-ligne en utilisant les optimisations de la boite à outil fournie par le logiciel commercial Matlab®.

Le blanc total de procédure du Ca varie entre 50 et 150 ng, ce qui correspond à un apport maximum de 3% de Ca. Ces valeurs de blancs impliquent qu'aucune correction n'est nécessaire sur les $\delta^{44/40}Ca$ obtenus, selon la méthode décrite dans le paragraphe précédant. La reproductibilité externe globale, obtenue à partir des données acquises lors de ce travail de thèse (Chapitres 3, 4 et 5), est de 0,12‰ (2SD, N = 170). 2SD correspond au 2σ mean (2SD/$\sqrt{}$(N)) quand N>1 et au 2σ erreur quand N=1.

Tous au long de cette thèse, nous testons la justesse de nos résultats en mesurant trois solutions de référence, communes à d'autres laboratoires (eau de mer : 1,80±0.17 ‰ (2SD, N = 34), SRM915a : 0,01±0.13 ‰ (2SD, N = 14), CaF_2 : 1,40±0.16 ‰ (2SD, N = 10) ; Figure II.3). Nos résultats sont en accord avec les valeurs précédemment publiées par Hippler et al. (2003).

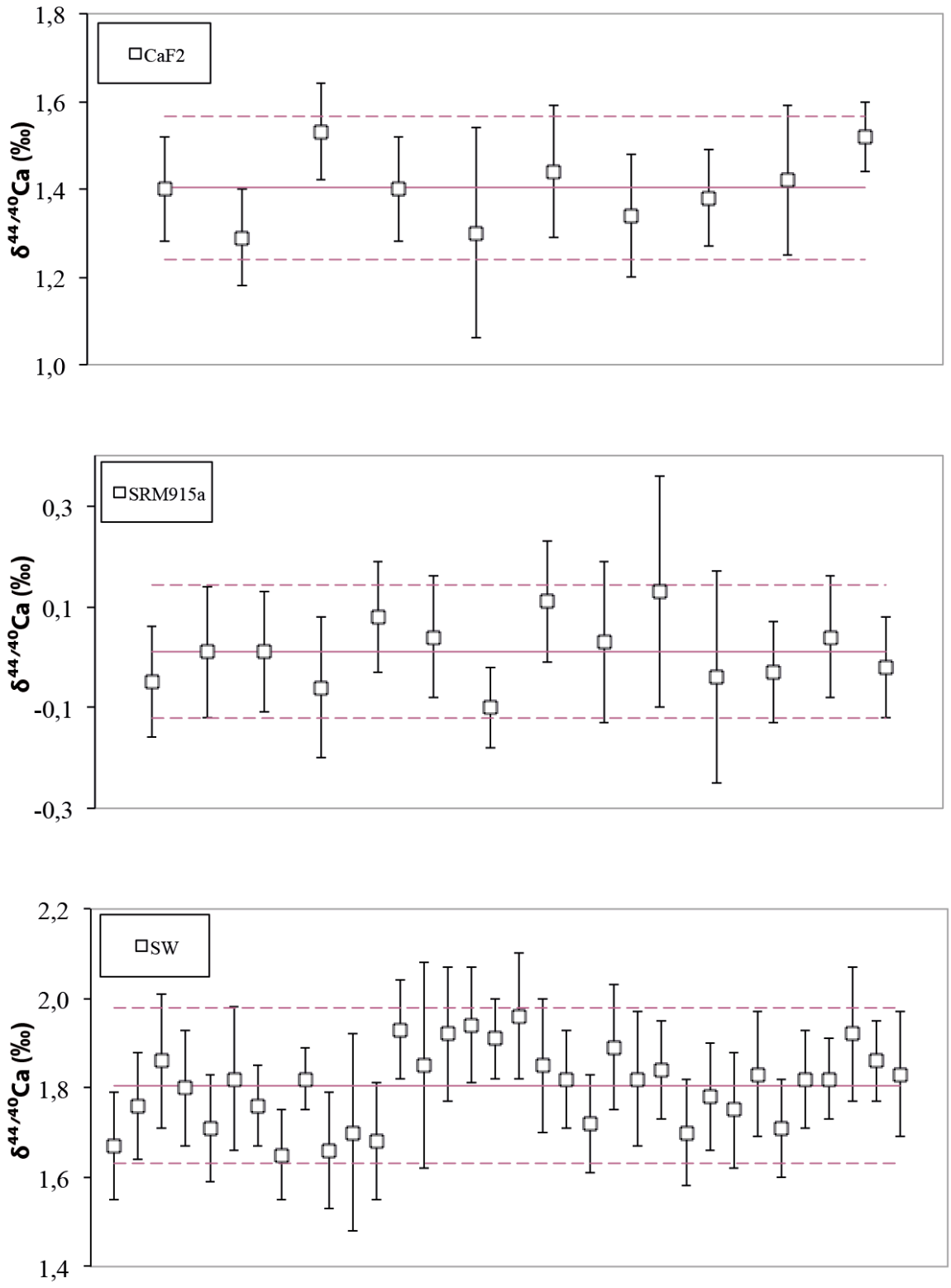

Figure II.3 : *Contrôle des standards de références au TIMS pour le NIST CaF_2, le NIST SRM915a et l'eau de mer (SW) au cours de cette thèse.*

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Chapitre 3 : Experimental identification of Ca isotopic fractionations in higher plants

Ce chapitre de thèse présente une étude en milieu contrôlé de type hydroponique qui a pour but d'identifier les processus biologiques et lithologiques qui influencent la composition isotopique du Ca au sein des plantes. Pour cela, nous avons testé l'influence de la concentration en Ca et du pH de solutions nutritives sur la signature isotopique du Ca de différents organes du haricot commun ou *Phaseolus vulgaris* L. (variété "fin de Bagnol", Vilmorin, France). Cette plante présente l'avantage d'avoir une croissance rapide, ce qui permet d'avoir en 6 semaines de culture un cycle de croissance complet du haricot. Quatre conditions expérimentales ont été retenues, elles combinent deux concentrations en Ca (5 et 60 ppm) et deux pH (4 et 6). De plus, pour chacune des expérimentations, le réservoir de Ca est considéré comme infini. Plusieurs organes ont été échantillonnés (racines latérales, racines primaires, tiges, feuilles, fleurs et gousses de haricots) à deux stades de croissance (10 jours et 6 semaines) et préparés pour les analyses isotopiques du Ca.

Les résultats de cette étude ont permis de montrer que les organes de haricot sont toujours enrichis en ^{40}Ca comparés à la solution nutritive quel que soit la condition expérimentale et d'identifier 3 niveaux de fractionnement des isotopes du Ca au sein de la plante étudiée : (1) Le premier a lieu au cours de l'adsorption préférentielle du ^{40}Ca sur les sites d'échanges cationiques de l'apoplasme des racines latérales; (2) Le second fractionnement se déroule pendant la translocation du Ca des racines primaires vers les feuilles. Durant ce trajet, le ^{40}Ca se fixe sur les acides polygalacturoniques (pectines) de la lamelle moyenne de la paroi cellulaire des vaisseaux xylémiques; (3) Le troisième et dernier fractionnement se produit au niveau des organes reproducteurs, il est aussi causé par des processus d'échange cationique avec la pectine des parois cellulaires de ces organes. Cependant, la structure des parois cellulaires des organes reproducteurs et/ou le nombre de sites d'échange cationique disponible semblent être différents de ceux de la paroi cellulaire des vaisseaux xylémiques. Ces trois mécanismes qui fractionnent les isotopes du Ca au sein de la plante semblent contrôlés par des processus d'ordre physico-chimique et conduisent à un enrichissement en isotopes légers ^{40}Ca. L'amplitude du fractionnement des isotopes du Ca au sein des organes de haricots est fortement dépendante de la composition de la solution nutritive : un faible pH (4) et une faible concentration (5ppm) n'entrainent pas d'effet sur la biomasse de haricots produite. En revanche, cela induit une amplitude de fractionnement plus faible des isotopes du Ca au sein du plant de haricot comparée à celle observée pour les autres conditions expérimentales. Pour résumer, cette étude montre que les signatures isotopiques du

Ca des plants de haricot sont contrôlées par la physico-chimie du milieu nutritif. Ce travail a fait l'objet d'une publication dans *Geochimica Cosmochimica Acta* en 2011.

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Experimental identification of Ca isotopic fractionations in higher plants

Florian Cobert [a], Anne-Désirée Schmitt [b,*], Pascale Bourgeade [b], François Labolle [c], Pierre-Marie Badot [b], François Chabaux [a], Peter Stille [a]

[a] *Laboratoire d'Hydrologie et de Géochimie de Strasbourg, Université de Strasbourg/EOST, CNRS, 1 rue Blessig, F-67000 Strasbourg, France*
[b] *Université de Franche-Comté et CNRS-UMR 6249, Chrono-environnement, 16, Route de Gray, 25030 Besançon Cedex, France*
[c] *Université de Strasbourg, Institut de Zoologie et de Biologie générale, 12, rue de l'Université, 67000 Strasbourg, France*

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Abstract

Hydroponic experiments have been performed in order to identify the co-occurring geochemical and biological processes affecting the Ca isotopic compositions within plants. To test the influence of the Ca concentration and pH of the nutritive solution on the Ca isotopic composition of the different plant organs, four experimental conditions were chosen combining two different Ca concentrations (5 and 60 ppm) and two pHs (4 and 6). The study was performed on rapid growing bean plants in order to have a complete growth cycle. Several organs (root, stem, leaf, reproductive) were sampled at two different growth stages (10 days and 6 weeks of culture) and prepared for Ca isotopic measurements.

The results allow to identify three Ca isotopic fractionation levels. The first one takes place when Ca enters the lateral roots, during Ca adsorption on cation-exchange binding sites in the apoplasm. The second one takes place when Ca is bound to the polygalacturonic acids (pectins) of the middle lamella of the xylem cell wall. Finally, the last fractionation occurs in the reproductive organs, also caused by cation-exchange processes with pectins. However, the cell wall structures of these organs and/or number of available exchange sites seem to be different to those of the xylem wall. These three physico-chemical fractionation mechanisms allow to enrich the organs in the light ^{40}Ca isotope. The amplitude of the Ca isotopic fractionation within plant organs is highly dependent on the composition of the nutritive solution: low pH (4) and Ca concentrations (5 ppm) have no effect on the biomass increase of the plants but induce smaller fractionation amplitudes compared to those obtained from other experimental conditions. Thus, Ca isotopic signatures of bean plants are controlled by the external nutritive medium. This study highlights the potential of Ca isotopes to be applied in plant physiology (to identify Ca uptake, circulation and storage mechanisms within plants) and in biogeochemistry (to identify Ca recycling, Ca content and pH evolutions in soil solutions through time).

1. INTRODUCTION

Sustainable development of forested ecosystems including understanding of nutrient element fluxes at the watershed scale require a better comprehension of the dynamics of nutritive elements at the rock–water–soil–plant interfaces and the unravelling of the multiple transfer processes occurring between these different compartments. While chemical weathering of soil minerals has intensively been studied at various scales, the role of vegetation on continental weathering processes and the contribution of plants to the dissolved loads of rivers have been less thoroughly investigated. However, plants are known to accelerate chemical weathering by mobilising metals from soils by a factor of 2–5 and to retard their release to drainage waters by recycling metals (Berner et al., 2004).

* Corresponding author. Tel.: +33 3 68 85 04 43; fax: +33 3 68 85 04 02.
E-mail address: adschmit@univ-fcomte.fr (A.-D. Schmitt).

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Recent studies have shown that plants may play a non-negligible role in the biogeochemical cycle of calcium (Ca) since roots take up Ca from the soil solution whereas leaf and needle litter and bark decomposition release Ca again to the watershed (Berner and Berner, 1996; Poszwa et al., 2000; Schmitt et al., 2003; Schmitt and Stille, 2005; Perakis et al., 2006; Cenki-Tok et al., 2009; Stille et al., 2009; Holmden and Bélanger, 2010). Ca is indeed an essential plant nutrient that plays multiple roles in plant functioning, both at cellular and intracellular scales (Marschner, 1995). This divalent cation is stable in soil solutions: it is encountered either as free metal or in hydrated form in the soils.

Former studies have highlighted the potential of Ca isotopes to better understand the role of vegetation on the Ca biogeochemical budget in temperate (Schmitt et al., 2003; Schmitt and Stille, 2005; Page et al., 2008; Cenki-Tok et al., 2009), tropical (Wiegand et al., 2005) or boreal (Bagard et al., 2010; Holmden and Bélanger, 2010) small forested watersheds. All these studies show a preferential absorption of ^{40}Ca by roots, which could be explained by kinetic Ca isotope fractionation during Ca^{2+} flux across Ca channels (i.e. specialised transport proteins) from the plasma membrane (i.e. outer boundary of a biological cell) (Wiegand et al., 2005). The intensity of this initial fractionation is for its part function of the extension of the active part of the root system, the considered tree species and/or the local soil environment (Holmden and Bélanger, 2010). Within the studied plant organs, a second fractionation takes place along the transpiration stream, with an enrichment in the heavy ^{44}Ca isotope in the order roots–stems–leaves (Platzner and Degani, 1990; Wiegand et al., 2005; Page et al., 2008; Cenki-Tok et al., 2009; Holmden and Bélanger, 2010). This suggests a chromatographic effect during translocation with ^{40}Ca precipitated in plant tissues, while ^{44}Ca remains dissolved in the translocation pathway (Wiegand et al., 2005; von Blanckenburg et al., 2010). The precise mechanisms of Ca fractionation between uptake from the nutritive solution and translocation within the plant remain, however, unclear.

Deciphering causes of isotopic fractionation in nature are difficult because, in addition to biological mechanisms, the isotopic signals are complicated by abiotic fractionations such as secondary calcium precipitations which are also enriched in ^{40}Ca compared to the surrounding waters (Gussone et al., 2003; Lemarchand et al., 2004; Tipper et al., 2006, 2008; Ewing et al., 2008; Jacobson and Holmden, 2008). Complementary studies using experimental approaches are now necessary to specify the mechanisms controlling the Ca isotopic fractionations linked to Ca absorption by plants and translocation between their different organs. Holmden and Bélanger (2010) were the first who performed a preliminary *in vivo* aspen seedling growth experiment on crushed basalt. The observed Ca isotopic fractionations were, however, small. They confirmed that roots were enriched in ^{40}Ca compared to the basalt, but the stem showed similar $\delta^{44/40}Ca$ values to roots and only the leaves were enriched in the light ^{40}Ca compared to the two other organs.

Here we present a hydroponic experiment in a way that we control the co-occurring geochemical and biological processes affecting the Ca isotopic compositions. Performing such a study on a liquid substratum allows to get rid off additional and external influences such as atmospheric depositions during the experiment (Tocquin et al., 2003; Robinson et al., 2006). The aim of the study was to determine the effect of the Ca concentration and the pH of the nutritive solution on the Ca biogeochemical cycle within plants. By trying to mimic acid, Ca depleted soils and alkaline soils enriched in Ca we tested nutrient solutions with high and low Ca concentrations and pHs and checked the influence of these parameters on Ca isotopic fractionations. This allowed to better understand biological processes that control the Ca uptake by the vegetation at the plant–soil interface and those, which fractionate Ca isotopes within the plant. With these experiments it was also possible to compare results derived from different controlled experimental conditions with field observations and to elucidate the processes controlling the Ca budget at the watershed scale.

2. HYDROPONIC EXPERIMENTAL SETUP AND ANALYSED BIOLOGICAL MATERIAL

2.1. Growth experiments

Commercial seeds of dwarf French beans, *Phaseolus vulgaris* L. (variety "fin de Bagnol", Vilmorin®, France) were chosen for the experiments. This dicotyledon belonging to the *Fabaceae* botanical family is mainly autogam that means it can produce fruits without the help of pollinators. Its growth period is equal to 5–6 weeks. It is a calcicole plant, which has been successfully used in other hydroponic studies (Zindler-Frank, 1995; Guo et al., 2002). One hundred grams of vermiculite was weighted and washed for 5 days in ultrapure Millipore® water. Bean seeds were then germinated in this vermiculite, at 25 °C, in the dark. One week later, when the hypocotyl stands up, the plantlets were rinsed with ultrapure Millipore® water and transferred to four blackened polypropylene pots containing 15 L of nutrient solution with five plants per pot (Fig. 1). The pots are closed with a top in order to minimise water losses by evaporation, in which five holes where hollowed out in order to receive the plantlets.

Two Hoagland nutrient solutions modified by Guo et al. (2002) and adapted to our experiment were prepared in order to observe biological, chemical and isotopic changes linked to the composition of the nutritive medium. The objective was to imitate the concentrations of an acid soil, impoverished in Ca (≈5 ppm Ca, "Low"), and an alkaline soil, enriched in Ca (≈60 ppm Ca, "High"). Thus, macronutrient compositions were 0.5 mM $(NH_4)_2SO_4$, 2 mM KH_2PO_4, 0.5 mM $MgSO_4$, 1.5 mM (High) and 0.15 mM (Low) $Ca(NO_3)_2$, 1.5 mM (High) and 4.2 mM (Low) $NaNO_3$, and micronutrient compositions were 45 mM Fe^{2+}–EDTA, 1 mM $MnSO_4$, 1 mM $ZnSO_4$, 0.1 mM $CuSO_4$, 10 mM H_3B_3, 0.01 mM $(NH_4)_6Mo_7O_{24}$. Nutrient stock solutions were prepared gravimetrically from analytical grade reagents; from the stock solution final nutrient solutions were prepared with mono-distilled water.

Each nutrient solution (Low and High) was prepared twice, at two different pH values (4 and 6) in order to study the possible variations caused by the acidity of the solution. Hereafter we will refer to the four experimental settings as L4 (Low Ca concentration, pH 4), L6 (Low Ca concentration, pH 6), H4 (High Ca concentration, pH 4), and H6 (High Ca concentration, pH 6). Nutrient solutions were regularly changed after plantlet transplantation (twice a week during the 2 first weeks of culture, three times later on). Samples (5 mL aliquots) were taken before and after each nutrient solution replacement for calcium analysis in order to verify that Ca depletion between two solution replacements was maintained below 10% and that no isotopic fractionation has occurred. The pH values were daily adjusted to 4 or 6 by using max. 200 µL of 0.1 N HCl or 0.1 N KOH solutions. Water lost through transpiration or evaporation was replaced daily by mono-distilled water.

Calcium only presents one oxidation stage (+II). Applying the Visual MINTEQ equilibrium model (Gustafsson, 2010) at each experimental condition we found that most Ca was in the dissolved form (>98%). Thus, Ca precipitation cannot influence the degree of Ca isotopic fractionation.

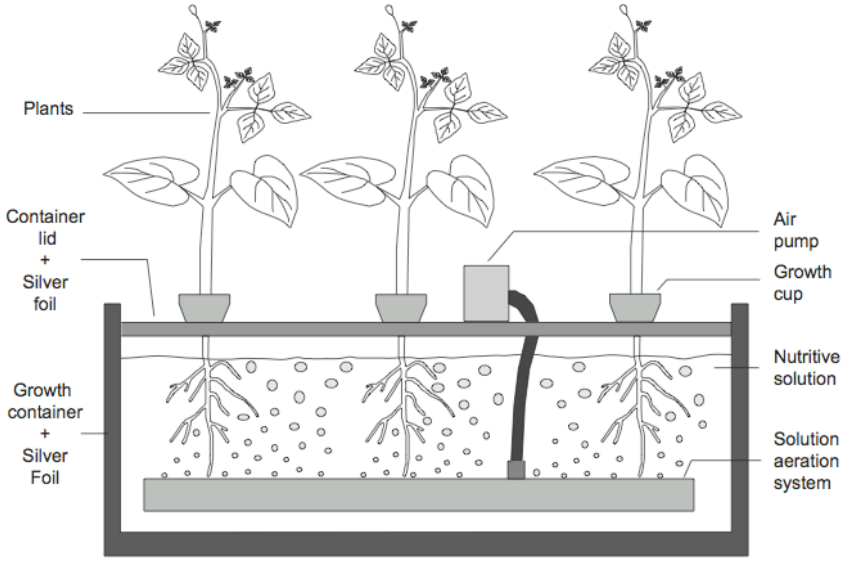

Fig. 1. Schematic of experimental protocol used for the hydroponic bean plant growth experiment. (Adapted from Camefort (1992) and Taiz and Zeiger (2010).)

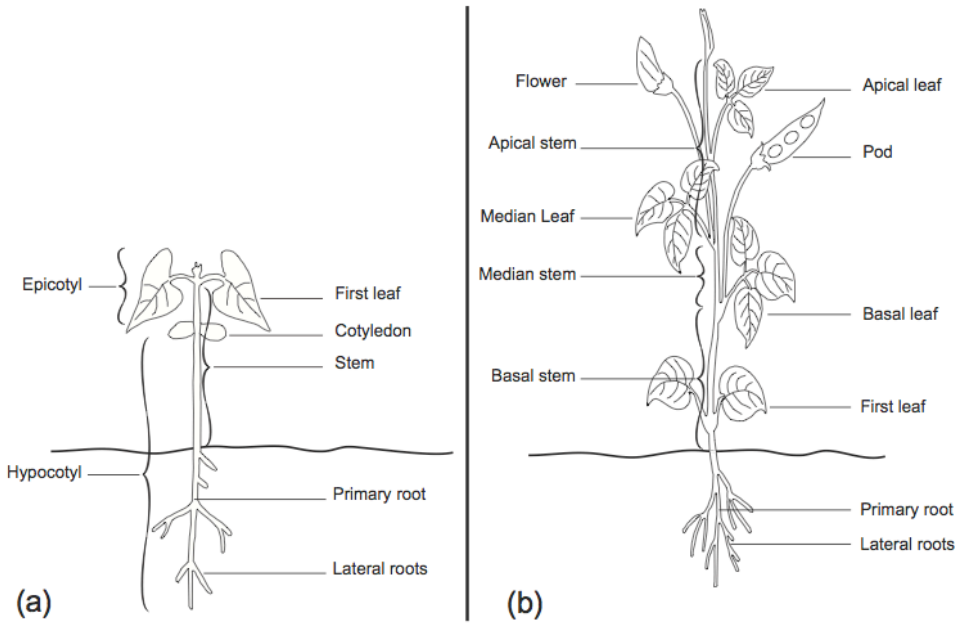

Fig. 2. Different organs of the bean plants collected and analysed after (a) 10 days and (b) 6 weeks of growth.

Table 1
Dry weights, elemental concentrations and Ca isotopic compositions of the different bean organs after 10 days of growth.

Samples		Biomass (g plant^{-1})	K (ppm)	Na (ppm)	Sr (ppm)	Mg (ppm)	Ca (ppm)	$\delta^{44/40}Ca_{SRM915a}$ (‰)	2 SD	N
Initial										
Seed	Bulk germinated seed	0.23	16,870	bdl	2.73	2107	597	n.d.	n.d.	
Cotyledon		0.20	15,180	bdl	2.56	1991	491	n.d.	n.d.	
L4										
Nutrient solution		–	72.8	91.0	0.002	11.84	5.08	0.91	0.07	3
Cotyledon	After 10 days	0.03	363	103	0.81	267	162	n.d.	n.d.	
Roots	Lateral	0.04	67,864	8878	9.16	3774	1881	0.49	0.08	2
Roots	Primary	0.08	61,068	15,064	3.91	1278	2435	0.01	0.08	2
Sap	Basal	–	408	16.98	bdl	29.01	14.54	0.29	0.14	2
Stem	Basal	0.01	101,011	3676	6.53	3044	3680	0.40	0.08	2
Stem	Apical	0.02	85,695	389	3.82	4866	3403	n.d.	n.d.	
Leaves	First	0.20	64,698	1015	1.47	5194	3551	0.44	0.08	2
Leaves	Apical[a]	0.06	56,128	121	1.42	5613	3742	n.d.	n.d.	
Whole plant[b]		0.54								
L6										
Nutrient solution		–	89.3	92.4	0.002	12.2	5.17	0.91	0.07	3
Cotyledon	After 10 days	0.03	4032	1368	20.7	7005	4867	n.d.	n.d.	
Roots	Lateral	0.09	19,906	1569	1.38	1248	713	0.40	0.16	1
Roots	Primary	0.08	13,871	1030	0.67	398	2929	0.05	0.08	2
Sap	Basal	–	2194	63.2	bdl	118	110	0.41	0.12	1
Stem	Basal	0.03	73,689	504	3.73	3218	3992	−0.15	0.15	1
Stem	Apical	0.04	56,828	267	2.14	3696	3682	n.d.	n.d.	
Leaves	First	0.28	68,009	228	4.20	10,786	11,356	0.36	0.15	1
Leaves	Apical[a]	0.09	49,012	41.8	1.89	5957	5244	n.d.	n.d.	
Whole plant[b]		0.99								
H4										
Nutrient solution		–	88.1	38.5	0.02	14.2	60.2	0.91	0.07	3
Cotyledon	After 10 days	0.03	11,120	2734	14.3	6651	6695	n.d.	n.d.	
Roots	Lateral	0.07	85,988	1882	6.14	1795	4794	0.55	0.14	1
Roots	Primary	0.09	69,595	2430	4.62	1841	6678	n.d.	n.d.	
Sap	Basal	–	436	6.71	bdl	29.4	174	n.d.	n.d.	
Stem	Basal	0.03	74,470	296	7.34	1993	13,196	−0.31	0.12	1
Stem	Apical	0.03	71,165	120	4.25	2495	9399	n.d.	n.d.	
Leaves	First	0.27	52,377	67.8	5.52	3516	17,233	n.d.	n.d.	
Leaves	Apical[a]	0.06	51,926	42.8	2.11	3116	6424	n.d.	n.d.	
Whole plant[b]		0.86								
H6										
Nutrient solution		–	91.9	36.0	0.01	13.5	60.7	0.91	0.07	3
Cotyledon	After 10 days	0.02	3646	653	31.9	7209	7077	n.d.	n.d.	
Roots	Lateral	0.09	18,333	289	1.72	1009	8834	0.49	0.08	2
Roots	Primary	0.08	13,611	343	1.10	405	8829	0.00	0.08	2
Sap	Basal	–	398	2.51	bdl	19.7	362	0.49	0.11	1
Stem	Basal	0.02	63,644	201	8.26	1574	16,458	−0.32	0.08	2
Stem	Apical	0.04	64,369	77.9	4.88	1801	11,451	n.d.	n.d.	
Leaves	First	0.25	46,929	45.5	9.47	4721	32,640	0.38	0.15	1
Leaves	Apical[a]	0.13	44,372	22.2	3.76	3037	11,777	n.d.	n.d.	
Whole plant[b]		1.16								

n.d., not determined; bdl, below detection limit.
2 SD corresponds to 2σ mean when $N > 1$ and 2σ error when $N = 1$.
The reproducibility is based on N measurements (corresponding to separate column chemistry) from aliquots of a given organ.
[a] Apical leaves after 10 days of growth = basal leaves after 6 weeks.
[b] Bulk plant without the cotyledons.

All plant cultures were performed at the UMR Chrono-environnement (Besançon) and were carried out in a culture chamber at 25 ± 5 °C during 16/8 h day/night cycles, with a light intensity of 150 µmol photons m^{-2} s^{-1} and 50 ± 15% humidity.

2.2. Studied material

Ten days after seed sowing, two plants were harvested. This stage of culture corresponds to the fall down of the cotyledons, which constitute the nutritive reserves of the

plants during their early development. Three plants were left in the polypropylene boxes, except for condition L6, where only one plant was left (in order to keep Ca concentration variations below 10%) until 6 weeks, which corresponds to the full growth period with mature bean pods but still some flowers. When harvested, plants were separated into several organs; all organs were washed in running mono-distilled water to remove the nutrient solution. The plants were separated in roots (primary and lateral), stem, leaves, flowers and pods (Fig. 2a and b). To examine whether the Ca isotopic composition changes with the age of the plant sections, bean plants were sampled for the lower, middle and upper parts of the stem and the leaves (Fig. 2b). The original seeds, and the after 10 days fallen cotyledons were also analysed (Table 1). The xylem sap was collected through capillarity from decapitated bean plants between primary roots and basal stem using a micropipette (Churr, 1998). It represents the root pressure component of xylem volume flow (Marschner, 1995). The xylem corresponds to an acidic pipeline that transports metallic ions with the ascending water flux and distributes them throughout the whole plant (Kim and Guerinot, 2007).

An aliquot of lateral roots (0.9 g) from L6 experiment after 6 weeks of growth was leached with 0.05 M HNO_3 to exchange the cations adsorbed on the root acidic groups (Bolou-Bi et al., 2010). Free Ca and Ca precipitated in oxalates were also extracted from basal leaves of H6 experiment after 6 weeks of culture following the protocol published by Zindler-Frank et al. (2001). Three grams of basal leaves were heated for 15 min at 90 °C in 30 mL ultrapure Millipore® water and frequently agitated. After centrifugation, the supernatant was used for determination of soluble oxalate. The residue was suspended again in 2 N HCl and kept for 30 min with frequent agitation. After centrifugation, the supernatant was used for determination of Ca oxalate. Plant organ samples were dried at 60 °C for 1 week in an oven and then ground to mince, homogenised and weighted.

3. ANALYTICAL METHODS

All chemical and isotopic analysis were performed under clean-room laboratory conditions at the Laboratoire d'Hydrologie et de Géochimie de Strasbourg (LHyGeS). About 100 mg of dried bean samples were digested in Savillex™ vials using a hot oxidative acid method (double-distilled HNO_3, double-distilled HCl, H_2O_2 Suprapur). Ca, Mg, K, Na, Sr and Al concentrations were measured by an ICP-AES Jobin Yvon JY 124 instrument with a detection limit between 1 and 40 ppm and accuracy of 5–10%, in function of the considered element.

Calcium isotopic compositions were determined following the procedure developed in the LHyGeS laboratory by Schmitt et al. (2009). One microgram of $^{42}Ca/^{43}Ca$ double spike was added to 5 μg of Ca before the calcium purification was performed through a high selectivity automated ion chromatography on a Dionex® ICS-3000 device with a high-capacity carboxylate-functionalized Column (Dionex® CS16). After Ca separation, samples were treated again with double-distilled HNO_3 and H_2O_2 before being totally evaporated in order to eliminate any organic impurity from the cation-exchange resin breakdown and/or from the sample itself. The residue was dissolved in 1 μL of 0.25 N HNO_3 and loaded without any further treatment on a single outgassed and in partial vacuum oxidised Ta filament (99.995% purity). Ca isotopic compositions were measured by thermal ionisation mass spectrometry (TIMS) using a Triton instrument (Thermo Scientific) operating in dynamic multicollection mode (Holmden, 2005; Schmitt et al., 2009). The filament was heated to ~1200 °C, the current was adjusted to reach a typical beam of 5.0 ± 10^{-11} A of $^{40}Ca^{+}$ corresponding to a filament temperature ranging from 1380 to 1450 °C. Between 130 and 200 cycles were collected. Most of the samples are replicated including digestion, Ca purification by ion chromatography and TIMS analyses. In order to check for possible isobaric interferences at masses 40 and 44 beams (corresponding to $^{41}K^{+}$ and $^{88}Sr^{+}$ which are proxies for $^{40}K^{+}$ and $^{88}Sr^{2+}$), were systematically checked. No interference was ever observed and, therefore, no correction was necessary. $\delta^{44/40}Ca$ values were achieved after data reduction using the exponential fractionation law and the Newton–Raphson iteration technique (Albarede and Beard, 2004). This technique converges rapidly to precise analytical results. The data reduction was performed off-line using the optimisation toolbox provided by the commercial Matlab® software. The Ca isotope values are expressed as permil deviation relative to the NIST SRM 915a standard solution: $\delta^{44/40}Ca = \{(^{44}Ca/^{40}Ca)_{sample}/(^{44}Ca/^{40}Ca)_{SRM915a} - 1\} \times 1000$ (Eisenhauer et al., 2004). Differences in Ca isotopic compositions between two reservoirs are expressed by using the Δ notation defined by $\Delta_{i-j} = \delta 44/40Ca_i - \delta 44/40Ca_j$ where i and j are the pools that are compared to each other.

The total procedure Ca blank varied between 50 and 150 ng, which corresponds to max. 3% of the amount of Ca in the samples. Therefore, blank corrections were not necessary. External reproducibility based on repeated measurements of NIST SRM 915a was 0.09‰ (2 SD, $N = 24$) and 0.12‰ (2 SD, $N = 82$) based on replicate sample measurements. The accuracy of the measurements was tested by measuring two reference solutions common to other laboratories and the results (seawater: 1.91 ± 0.10‰ (2 SD, $N = 8$), CaF_2: 1.45 ± 0.12‰ (2 SD, $N = 19$)) are in good agreement with previously published values (Hippler et al., 2003).

4. RESULTS

4.1. Biomass variation during the experiment

The bean organ dry mass, elemental concentrations and Ca isotopic compositions are summarised in Table 1 for 10 days of bean plant growth and in Table 2 for 6 weeks of growth. The weight of the initial germinated seed (including the two cotyledons, the hypocotyl, the radicle and the epicotyl; Fig. 2) was 0.23 g (Table 1). After 10 days of growth in the four different hydroponic solutions the lowest relative dry mass increase for a bean plant was observed for L4 conditions (0.80 times), whereas it was

Table 2
Dry weights, elemental concentrations and Ca isotopic compositions of the different bean organs after 6 weeks of growth.

Samples		Biomass (g plant^{-1})	K (ppm)	Na (ppm)	Sr (ppm)	Mg (ppm)	Ca (ppm)	$\delta^{44/40}Ca_{SRM915a}$ (‰)	2 SD	N
L4										
Nutrient solution		–	72.8	91.0	0.002	11.8	5.08	0.91	0.07	3
Roots	Lateral	2.17	41,058	3707	3.42	1253	1039	0.38	0.08	2
Roots	Primary	0.25	30,351	11,652	2.35	850	1120	0.11	0.08	2
Sap	Basal	–	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
Stem	Basal	0.21	26,203	2966	1.54	696	925	0.03	0.08	2
Stem	Median	1.17	34,969	727	1.43	1031	1216	0.09	0.08	2
Stem	Apical	0.04	72,265	501	1.53	3873	3013	0.08	0.08	2
Leaves	Basal	1.57	48,828	262	1.38	bdl	3948	0.22	0.08	2
Leaves	Median	0.82	46,356	135	1.44	6207	4445	0.23	0.1	1
Leaves	Apical	0.05	62,856	235	0.73	5013	2502	0.11	0.08	2
Reproductives	Flower	0.02	54,084	101	0.51	6692	3149	−0.16	0.12	1
Reproductives	Pod	1.15	35,030	44.3	0.32	3131	1148	−0.24	0.08	2
Whole plant[a]		7.45								
L6										
Nutrient solution		–	89.3	92.4	0.002	12.2	5.17	0.91	0.07	3
Roots	Leaching (lateral)	0.91	210	4.85	0.20	102	145	0.14	0.12	1
Roots	Lateral	1.59	62,122	2150	3.75	2608	2237	0.25	0.08	2
Roots	Primary	0.66	7099	4666	0.63	578	1073	−0.25	0.08	2
Sap	Basal	–	135	8.07	bdl	8.93	57.6	n.d.	n.d.	
Stem	Basal	2.41	54,014	5554	2.52	2166	1260	−0.24	0.15	1
Stem	Median	2.11	49,503	441	1.19	1658	1873	n.d.	n.d.	
Stem	Apical	0.14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
Leaves	Basal	4.83	6548	42.9	0.45	1239	1519	0.34	0.12	1
Leaves	Median	3.21	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
Leaves	Apical	1.34	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
Reproductives	Flower	0.10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
Reproductives	Pod	3.07	36,808	40.6	0.19	3003	971	−0.39	0.08	2
Whole plant[a]		19.66								
H4										
Nutrient solution		–	88.1	38.5	0.02	14.2	60.2	0.91	0.07	3
Roots	Lateral	2.18	29,166	777	1.84	596	2139	n.d.	n.d.	
Roots	Primary	0.64	12,960	9.87	2.32	1054	3147	−0.15	0.08	2
Sap	Basal	–	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
Stem	Basal	0.73	12,344	484	2.51	452	4099	−0.34	0.12	1
Stem	Median	4.87	27,287	3682	2.91	628	8918	n.d.	n.d.	
Stem	Apical	0.08	50,210	101	3.26	2639	7987	−0.22	0.08	2
Leaves	Basal	2.50	26,708	64.8	6.76	4929	23,198	0.21	0.12	1
Leaves	Median	0.97	33,063	392	2.22	715	3739	n.d.	n.d.	
Leaves	Apical	0.29	44,622	196	4.82	3877	14,815	0.19	0.08	2
Reproductives	Flower	0.09	35,773	71.5	1.22	3126	5781	−0.19	0.15	1
Reproductives	Pod	4.26	31,858	53.1	0.98	2609	3430	−0.16	0.08	2
Whole plant[a]		16.60								
H6										
Nutrient solution		–	91.9	36.0	0.015	13.5	60.7	0.91	0.07	3
Roots	Lateral	1.11	61,817	1154	5.96	1670	7758	0.19	0.08	2
Roots	Primary	0.35	30,176	3475	2.79	678	4737	−0.17	0.08	2
Sap	Basal	–	540	15.3	0.022	44.5	174	0.11	0.14	1
Stem	Basal	0.49	24,148	435	3.25	750	6595	-0.27	0.08	2
Stem	Median	1.41	37,229	172	3.35	633	5901	n.d.	n.d.	
Stem	Apical	0.06	46,793	91.6	3.34	2609	12,421	−0.14	0.12	1
Leaves	Basal	1.57	50,047	84.5	8.71	4033	28,186	0.36	0.12	1
Leaves	Oxalates (basal)	–	2328	13.6	0.91	685	6197	−0.06	0.15	1
Leaves	Free Ca (basal)	–	13,446	20.9	0.41	1551	4268	0.32	0.13	1
Leaves	Median	1.08	45,108	109	3.63	3254	14,046	n.d.	n.d.	
Leaves	Apical	0.21	37,922	73.3	2.77	2612	9828	0.08	0.12	1
Reproductives	Flower	0.10	31,852	20.5	0.93	3041	6736	−0.14	0.08	2
Reproductives	Pod	0.74	37,912	27.1	1.49	2723	5028	−0.36	0.08	2
Whole plant[a]		7.97								

n.d., not determined; bdl, below detection limit.
2 SD corresponds to 2σ mean when $N > 1$ and 2σ error when $N = 1$.
The reproducibility is based on N measurements (corresponding to a separate column chemistry) from aliquots of a given organ.
[a] Bulk plant without the cotyledons.

1.4–1.7 times higher for bean plants growing under the three other experimental conditions (Table 1). Ten days of growth correspond to the stage where the bean cotyledons fell down. As a result, a decrease of ~90% in the dry mass of cotyledons, compared to their initial mass could be observed. After 6 weeks of growth the bean plants were mature and produced eatable pods. The gain of biomass during the hydroponic experiments divided up differently into the different organs of the bean plants (Table 3). After 10 days the preponderant organs for all experimental conditions, were leaves (≈61%) and the less preponderant ones were stems (≈10%). The roots showed intermediate gains (<30% of all other organs) with an equal dividing up of the biomass into lateral and primary roots. The only exceptions were bean plants grown under L4 condition with 20% of primary roots and 10% of lateral roots. After 6 weeks of growth, the relative abundance of the organs was more variable, and reproductive organs (flowers and pods) were present and mature. The relative average proportion of leaves and roots decreased whereas it increased for stems (Table 3). It has to be noted that in the case of the L4 experiment, roots showed similar masses (≈30% of the total mass of bean plants) after 10 days and 6 weeks of experimentation. However, in contrast to the 10 days experiment, the growth of lateral roots became highly preponderant after 6 weeks (28% of the total bean plant mass) compared to primary roots (3%) (Table 3).

When considering the reproductive organs it can be observed that, in general, the percentage of flowers were very low (≈1% of the total mass). This can be related to the sampling strategy of the bean plant organs: we sampled the plant when there were mature pods, but still some flowers left. For its part the abundance of pods ranged between 10% (H6) and 25% (H4).

4.2. Elemental concentrations in plants and in the nutritive solutions

During plant growth, the different chemical elements present in the nutritive solutions have been taken up and accumulated within the different organs of the bean plants. The total amount of cations accumulated by the bean during the four different hydroponic experiments has been reported in Table 4. It is important to remind here that the only variable parameters of the four hydroponic solutions (see Section 2.1) were Ca and Na concentrations and pH; the other nutritive elements were present in similar amounts within the different experimental settings. The change of only these few parameters, however, influenced importantly the uptake of the other nutritive elements, even after 10 days of growth (Table 4).

At the beginning of the experiment, the nutrient elements were mainly concentrated in the cotyledons of the germinated seed (between 70% and 80%) (Table 4). The Mg (6%), however, seems to have been preferentially enriched in the epicotyl and the hypocotyl. After 10 days of growth, the cotyledons fall down. Compared to the initial Ca amount available in cotyledons of the germinated seed (≈100 μg), the cotyledon Ca contents increased up to 142, 146 and 200 μg for H6, L6 and H4 experiments, respectively. In contrast, for L4 experiment the cotyledon Ca content decreased (down to 3 μg), compared to 10 days (98 μg)

Table 3
Relative proportion of dry mass of organs produced during the different experiments (in %).

	Lateral roots (%)	Primary roots (%)	Σ Roots (%)[a]	Stem (%)	Leaves (%)	Flowers (%)	Pods (%)
L4_10 days	10	20	30	8	62	–	–
L4_6 weeks	28	3	31	19	33	1	15
L6_10 days	15	13	28	11	61	–	–
L6_6 weeks	8	3	11	24	48	1	16
H4_10 days	12	17	29	10	61	–	–
H4_6 weeks	13	4	17	34	23	1	25
H6_10 days	15	14	29	11	60	–	–
H6_6 weeks	16	5	21	28	40	1	10

[a] Sum of lateral and primary roots.

Table 4
Bulk plant nutritive element amount for the different experiments.

	K (mg plant^{-1})	Na (mg plant^{-1})	Sr (μg plant^{-1})	Mg (mg plant^{-1})	Ca (mg plant^{-1})
Bulk germinated seed	3.92	bdl	0.63	6.57	0.14
Cotyledons	3.03	bdl	0.51	0.40	0.10
L4_10 days	25.2	1.90	1.17	1.60	1.30
L4_6 weeks	305	13.0	13.8	4.85	15.6
L6_10 days	30.7	0.32	1.72	0.90	4.21
L6_6 weeks	n.d.	n.d.	n.d.	n.d.	n.d.
H4_10 days	33.9	0.39	2.38	0.61	6.67
H4_6 weeks	468	20.8	46.4	31.1	135
H6_10 days	24.3	0.08	3.49	0.62	12.0
H6_6 weeks	313	3.24	33.5	16.2	88.5

n.d., not determined; bdl, below detection limit.

(Table 1). The Ca accumulation in L6, H4 and H6 experiments can be explained by the continuous flow of xylem sap to cotyledons through time. In contrast, in the case of the L4 condition, the decrease of the Ca content in the cotyledon might suggest that at low Ca concentrations and pH in the nutritive solution some of the Ca became remobilised through the phloem. However, even if this remobilisation has taken place it would not be of importance, since the maximal Ca contribution of the cotyledons is 8% of the total Ca amount available for the bean plant (calculated from Table 4).

After 10 days of growth, Sr and Ca became increasingly absorbed by the bean plants in function of the following order of experimental conditions: L4, L6, H4, H6; this points to the important influence of both Ca concentrations and pH in the nutritive solution. The Ca and Sr concentrations were in general similarly fractionated between the different bean plant organs after 10 days of growth or 6 weeks (Fig. 3). Most important Sr/Ca fractionations have been observed for the lateral roots. Only the plant growing in the H6 experiment showed, compared to the nutrient solution, no Sr/Ca fractionation after 10 days of growth. Plant organs derived from the L4 experiment were the most enriched in Sr, followed by L6 and finally by H4 plant organs (Fig. 3a). After 6 weeks of growth the intensity of fractionation under the different experimental conditions became smaller, but again all the lateral roots were enriched in Sr compared to Ca and relative to the nutrient solution (Fig. 3b). With the exception of pods, flowers and leaves, L4 plant was systematically enriched in Sr compared to those from the other experimental conditions. Moreover, it can be observed that, whatever the considered experiment or growth stage, the Sr/Ca molar ratios always decreased with the exception of those of the stems from lateral roots to leaves (Fig. 3a) or to pods (Fig. 3b). The stems showed slightly higher Sr/Ca molar ratios. Flowers and pods showed Sr/Ca ratios similar to those of the initial nutritive solution.

4.3. Ca isotopic compositions

Compared to the Ca isotopic composition of the nutritive solutions, all bean organs showed an enrichment in the light ^{40}Ca isotope, which is consistent with field studies (Platzner and Degani, 1990; Schmitt et al., 2003; Wiegand et al., 2005; Page et al., 2008; Cenki-Tok et al., 2009; Holmden and Bélanger, 2010).

The observed variability of the isotopic fractionation between lateral roots that took up the nutrient elements from the solution and the nutritive solution was almost as

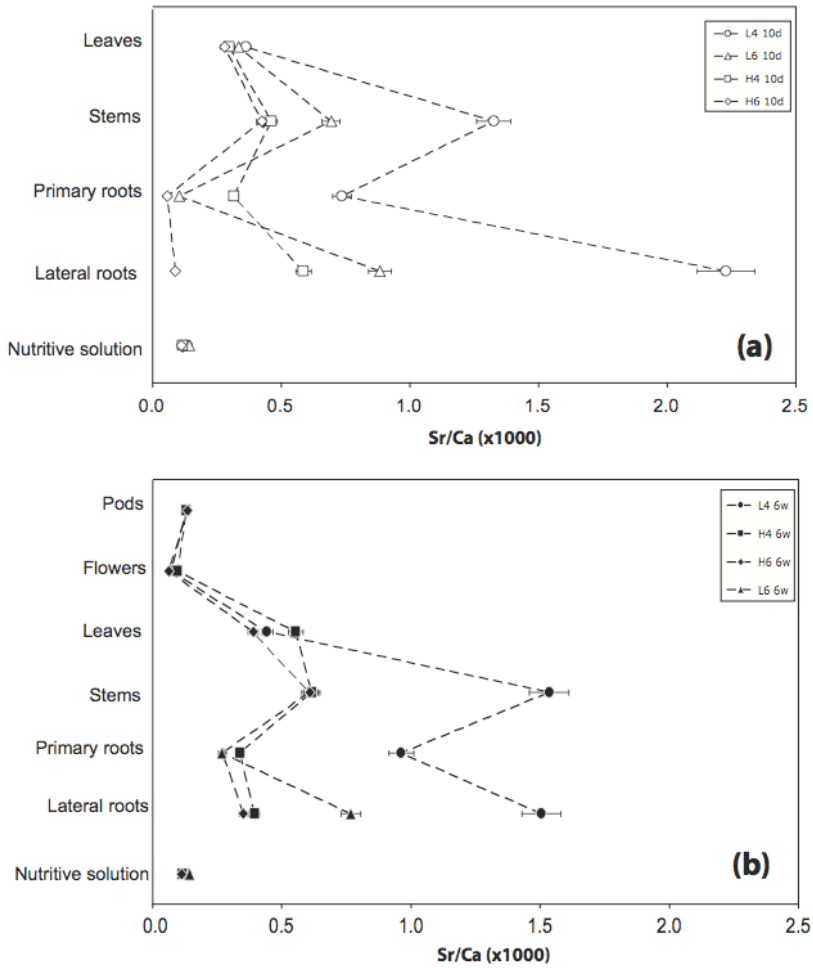

Fig. 3. Sr/Ca molar ratios in different bean organs and nutritive solutions after (a) 10 days and (b) 6 weeks of growth.

important as the global variability within a specific bean plant. The average calculated $\Delta_{\text{lateral roots–nutrient solution}}$ was equal to 0.43‰ (calculated from Table 1) and to 0.64‰ (calculated from Table 2) after 10 days and 6 weeks of growth, respectively. For L6, H4 and H6, the average calculated $\Delta_{\text{lateral root–primary root}}$ was similar after 10 days (0.45; calculated from Table 1) or 6 weeks (0.41; calculated from Table 2). In contrast, for L4 it was higher after 10 days (0.41; calculated from Table 1) than after 6 weeks (0.27; calculated from Table 2). No difference in Ca isotopic signatures could be observed between organs of different ages (basal, median and apical), neither for stem nor leaves, except for leaves from H6 condition (Fig. 4b and d).

Variable fractionations occurred in function of the considered organs, but also in function of the initial nutritive solution (Fig. 4). Indeed, the Ca isotopic fractionation of the organs from L6, H4 and H6 experiments behaved similarly (Fig. 4a and b), but slightly differently from L4 conditions (Fig. 4c and d).

For the first series of experimental conditions, lateral roots, sap and leaves were enriched in the heavy ^{44}Ca isotope whereas primary roots, stems, and reproductive organs were enriched in the light ^{40}Ca isotope, at the two studied growth stages (Fig. 4a and b). Each group of organs showed within error bars similar Ca isotopic compositions. Moreover, after 10 days of growth, lateral roots, sap and leaves were even more enriched in the heavy ^{44}Ca isotope compared to after 6 weeks (0.44 ± 0.05, 2 SE, $N = 7$ and 0.22 ± 0.07, 2 SE, $N = 8$, respectively; Fig. 4a and b). In contrast, primary roots, stems and reproductive organs had the same average $\delta^{44/40}$Ca values after 10 days of growth or 6 weeks (-0.15 ± 0.15, 2 SE, $N = 5$ and -0.23 ± 0.05, 2 SE, $N = 13$, respectively; Fig. 4a and b).

The different organs from the L4 experiment, however, behaved differently. After 10 days of growth, lateral roots, sap, basal stem and first leaves were enriched in the heavy ^{44}Ca isotope (0.41 ± 0.09, 2 SE, $N = 4$) compared to primary roots (0.01 ± 0.08, $N = 1$) (Fig. 4c). After 6 weeks, lateral roots remained the most enriched in ^{44}Ca (0.38 ± 0.08, $N = 1$), whereas reproductive organs were the most enriched in ^{40}Ca (-0.20 ± 0.08, 2 SE, $N = 2$) (Fig. 4d). Primary roots, stems and leaves showed intermediate $\delta^{44/40}$Ca values (0.12 ± 0.06, 2 SE, $N = 7$; Fig. 4d).

The lateral roots have been leached in order to get information about the isotopic composition of Ca adsorbed at the surface of lateral roots (Table 2 and Fig. 4b). After 6 weeks of culture the leachates of the lateral roots from the L6 experiment had $\delta^{44/40}$Ca values (0.14 ± 0.12) which

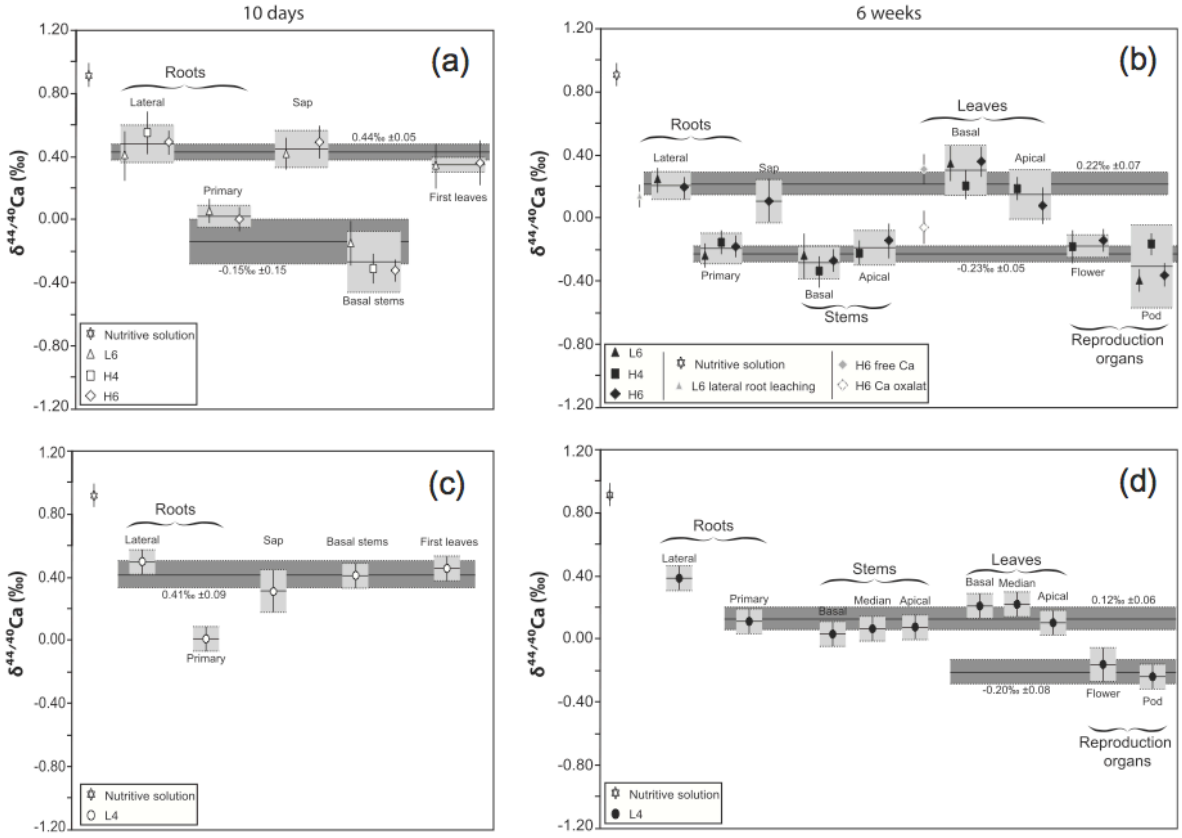

Fig. 4. Ca isotopic compositions of the different bean organs and nutritive solutions following the fluid flow pathway in the plant for L6, H4 and H6 experiments after (a) 10 days and (b) 6 weeks of growth, and for L4 experiment after (c) 10 days and (d) 6 weeks of growth. The coloured domains behind each family of organs correspond to the average value (±2 SD) of the considered organs. The colour strips behind the different families of organs correspond to the average values (± 2 SE) of organs presenting similar $\delta^{44/40}$Ca values (see text for details). NB: in (b) lateral root leachates, leaves' free Ca and oxalates have not been used for average calculations.

were within error bars similar to those of the lateral roots (0.25 ± 0.08) (Table 2 and Fig. 4b). Similarly, in order to identify Ca isotopic compositions of free Ca and Ca linked to oxalates (stock of Ca) in leaves, we measured the $\delta^{44/40}$Ca composition of these two compounds in basal leaves from the H6 experiment after 6 weeks of culture. These experiments showed within error bars similar $\delta^{44/40}$Ca for free Ca (0.32 ± 0.13‰) and whole basal leaves (0.36 ± 0.12‰). However, the Ca oxalate was comparatively enriched in the light ^{40}Ca isotope (-0.06 ± 0.15‰) (Table 2 and Fig. 4b).

5. DISCUSSION

5.1. $\delta^{44/40}$Ca fractionation mechanisms during Ca uptake by lateral roots

The results clearly demonstrate that whatever considered experiment, the Ca taken up by lateral roots is always enriched in the light ^{40}Ca isotope compared to that of the nutritive solution (see Section 4.3). This result is in accord with those obtained in field studies (Schmitt et al., 2003; Wiegand et al., 2005; Cenki-Tok et al., 2009; Holmden

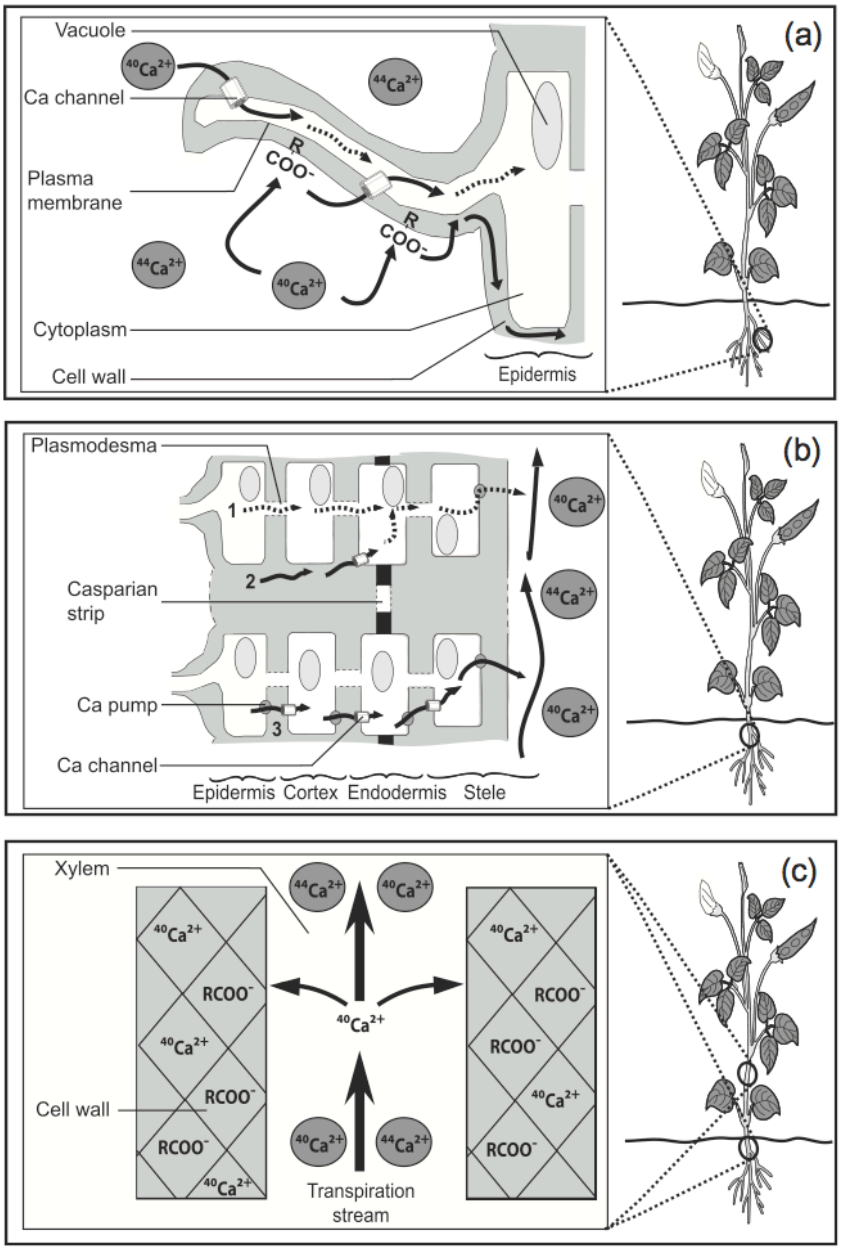

Fig. 5. Schematic of the Ca translocation within bean plants with (a) $\delta^{44/40}$Ca fractionation at the lateral roots/nutritive solution interface and (b) radial movement of Ca in the roots. 1, 2 and 3 correspond to the symplastic, apoplasmic and transmembrane pathway, respectively; (c) $\delta^{44/40}$Ca fractionation during the xylem sap transfer from roots to shoot (see text for more details). This is an interpretative schematic; the scale of the different organs has not been respected.

and Bélanger, 2010). Similarly to Ca, roots are enriched in the light Si (Opfergelt et al., 2006) and Fe isotopes (for dicotyledons; Guelke and von Blankenburg, 2007). In contrast, Mg (Black et al., 2008; Bolou-Bi et al., 2010) and Zn (Weiss et al., 2005) isotopes of roots are enriched in the heavy isotopes compared to the nutritive solution. This ^{40}Ca enrichment could be explained by kinetic isotope fractionation that favours the light isotope as was proposed by Gussone et al. (2003) for carbonate precipitation. In contrast, mass-dependent equilibrium fractionation mechanisms favour heavy isotopes on molecular sites (Schauble et al., 2006; Schauble, 2007).

During Ca uptake by lateral roots it moves from the external nutritive solution onto the cell wall of roots following a non-metabolic, passive process, driven either by the electrochemical potential or mass flow (Bangerth, 1979; Marschner, 1995; McLaughlin and Wimmer, 1999; Taiz and Zeiger, 2010). Since Ca is mainly linked to water molecules in solution, one might explain the preferential ^{40}Ca incorporation in lateral roots from the nutritive solution by different bond strength in solution and roots. The change from bonding to water molecules in solution to bonding within the lateral root structure may involve a decrease in bond strength as suggested by Mariott and Chen (2004), so that the heavy ^{44}Ca isotope preferentially remains in solution whereas the light ^{40}Ca isotope makes bonds with the lateral root structure.

Wiegand et al. (2005) explained this ^{40}Ca enrichment in roots by kinetic Ca isotopic fractionation processes, which occurred during Ca absorption by roots cells and involve electrochemical gradients and Ca channels (White, 2000). Alternatively one might suggest that Ca^{2+}–aqua complex deshydratation could be responsible for Ca isotopic fractionation during Ca uptake by the lateral roots; such a process was proposed for Ca uptake by foraminifera (Gussone et al., 2003).

The leaching experiments point to an alternative and/or complementary fractionation process. The Ca desorbed from lateral roots has indeed a $\delta^{44/40}$Ca (0.14 ± 0.12; Table 2) very similar to that of the residual lateral roots (0.25 ± 0.08; Table 2). This suggests that the Ca isotopic fractionation and ^{40}Ca enrichment could occur before entering the root cells, during Ca adsorption on R-COO^- groups of polygalacturonic acid molecules (pectins), which are present at the exterior surface of the cell wall that acts as a cation-exchange sites (Marschner, 1995). As a result, light isotopes would be adsorbed through electrostatic links on these binding sites, whereas heavy isotopes preferentially remain in the nutritive solution (Fig. 5a). This is in agreement with the study of Russell and Papanastassiou (1978), who demonstrated that when passing on cationic ion-exchange columns, the lighter isotopes are preferentially retained by the resin. Our results also show that, compared to the nutrient solution, lateral roots are more enriched in the light isotope after 6 weeks of growth than after 10 days (see Section 4.3). Thus, if the Ca-binding sites are supposed to act as a Ca exchange resin, the more Ca passes through the resin, the more it becomes enriched in the light ^{40}Ca. This is also in accord with previous experiments performed by Russell and Papanastassiou (1978). After this initial fractionation, the carboxyl-attached Ca^{2+} is replaced by other positively charged ions such as Mg^{2+} or Sr^{2+} and penetrates passively without any significant fractionation onto the apoplasm of the cortex by passing the cell wall (Fig. 5a).

5.2. $\delta^{44/40}$Ca fractionation mechanisms during Ca transfer from roots to shoot

5.2.1. Bean plants grown under L6, H4 and H6 experimental conditions

Once Ca entered the cell walls of the rhizodermic cells, it moves radially through the cortex tissues towards the root centre (stele) into the conducting xylem (Fig. 5b). To do this it follows three parallel pathways (transmembrane, symplastic and apoplastic), whose relative contribution to the delivery of Ca^{2+} to the xylem is yet unknown (Clarkson, 1984, 1993; Häussling et al., 1988; McLaughlin and Wimmer, 1999; White, 2001; Moore et al., 2002; Hayter and Peterson, 2004; Taiz and Zeiger, 2010) (Fig. 5b). The transmembrane pathway necessitates Ca movements from cell to cell through the plasma membrane using Ca^{2+} channels to get in and Ca^{2+}/ATPase pumps to get out. The symplastic pathway for its part allows the Ca to enter one time the plasma membrane using Ca^{2+} channels and then it moves from cytoplasm to cytoplasm using the plasmodesma. Finally, when following the apoplasmic pathway, Ca does not traverse any single plasma membrane. Once arrived at the endodermis, the Caspary strip, that is a hydrophobic structure that prevents the apoplastic movement, forces the Ca flux to take a symplastic pathway, so that the Ca is forced to move through Ca^{2+} channels. Whatever the followed pathway, passing from the endodermis to the conducting xylem requires Ca^{2+}/ATPase pumps. Our results show that, within error bars, similar $\delta^{44/40}$Ca values have been measured for lateral roots and xylem sap for L6, H4, H6 conditions after 10 days and 6 weeks of growth (Fig. 4). This suggests that no Ca isotopic fractionation occurs during this radial transfer. Thus we could propose either that passing Ca^{2+} channels and/or Ca^{2+}/ATPase pumps do not fractionate $\delta^{44/40}$Ca values, or produce opposite fractionation effects, that compensate each other, or that Ca mainly follows the apoplastic pathway.

Long-distance unidirectional transport occurs then in the non-living xylem elements across first the primary roots and second the stem (basal, median and apical), which are in the continuity of each other. Xylem transport is mainly driven by the gradient in water potential resulting from stomata release of water (Marschner, 1995; Taiz and Zeiger, 2010). Primary roots and stem compartments present similar $\delta^{44/40}$Ca values and are enriched in ^{40}Ca compared to basal xylem sap. Previous studies dealing with Zn isotopes in plants have proposed diffusion mechanisms to explain isotopic fractionation over short scales (<10 cm) between stem and leaves (Moynier et al., 2009). Few is known about diffusion of Ca isotopes, except for high-temperature processes (Richter et al., 2003; Watkins et al., 2011). For low-temperature surface processes, Fantle and DePaolo (2007) have shown that diffusion cannot explain Ca isotopic variations in pore fluids. Similarly, the study of Watkins

et al. (2011) suggests that Ca isotope fractionation associated with diffusion in water is limited. More studies have now to be performed to confirm these observations in highly concentrated media such as sap.

Alternatively one might suggest that the Ca isotopic fractionation is due to ion-exchange reactions with binding sites for Ca in the cell walls of the surrounding xylem tissues (Wolterbeek et al., 1984), which is essential for strengthening of the cell walls and of plant tissues. Indeed, high proportions of the total calcium in the plant tissues are located in the cell walls, and more precisely in their middle lamella where Ca is bound to the carboxyl ($R\text{-}COO^-$) groups of the polygalacturonic acids (pectins) in a more or less readily exchangeable form (Armstrong and Kirkby, 1979; Hanson, 1984; Kirkby and Pilbeam, 1984; Marschner, 1995; Bresinsky et al., 2008). As a result, the light ^{40}Ca isotope could be retained in the cell wall whereas ^{44}Ca stays in the xylem sap, which is in accord with the theoretical functioning of a cationic-exchange resin (Russell and Papanastassiou, 1978), and also with the hypothesis initially proposed by Wiegand et al. (2005) (Fig. 5c). Wiegand et al. (2005) have indeed suggested that strong ^{44}Ca enrichments occur in the xylem solution from primary roots to shoots (leaves). Although we did only collect basal xylem sap, and thus have not measured $\delta^{44/40}Ca$ value of sap collected higher in the bean organ, we can make some hypothesis. When comparing the Ca isotopic signatures of free calcium and calcium stored in the oxalates for leaves grown under H6 conditions, after 6 weeks of culture, we observe that the free calcium has a value similar to that of the bulk leaves, whereas the oxalates are enriched in the light ^{40}Ca (Table 2 and Fig. 4b). Free Ca has also a Ca isotopic composition similar to lateral roots and basal xylem sap. This might suggest that this free Ca fractionated when it entered the apoplasm of lateral roots before it attained the leaves. Moreover, based on Fig. 4a and b we observe no $\delta^{44/40}Ca$ difference between basal sap and first leaves (after 10 days of culture) or basal sap and basal or apical leaves (after 6 weeks). Only a light ^{40}Ca enrichment has been recorded for basal and apical leaves from H6 condition after 6 weeks, but both values are within error bars similar to that of the basal sap. Thus, we suggest that the Ca reservoir of the xylem sap can be considered to be Ca enriched resulting in very limited Ca isotopic variations in the xylem solution from bottom to top of the bean plant (i.e. $\delta^{44/40}Ca \sim$ constant). This, however, has to be confirmed by further studies.

Unlike Mg (Jeschke and Plate, 1991; Zong et al., 1993), but similarly to Si (Raven, 1983), Ca is essentially phloem immobile (Marschner and Richter, 1974; Bangerth, 1979; Marschner, 1995), which means that calcium-ions have to be continuously supplied to the roots and then transported to the shoots by the xylem sap, since no mineral remobilisation from leaves can occur during reproductive growth when flowers and fruits are formed. At this growth stage, root activity and nutrient uptake generally decrease, mainly as a result of decreasing carbohydrate supply to the roots (Marschner, 1995). Flowers form at nods between leaves and stem. They are enriched in the light ^{40}Ca isotope compared to leaves, but present $\delta^{44/40}Ca$ values similar to those of primary roots or stem, suggesting similar fractionation mechanisms. The ^{40}Ca enrichment during reproductive organ formation seems to be confirmed under H6 condition, where a light ^{40}Ca enrichment is observed between flowers and pods (Fig. 5b).

As a result, one might suggest that two different Ca reservoirs exist: "transfer reservoirs" enriched in the heavy ^{44}Ca, including lateral roots, sap and leaves, and "structural and/or storage reservoirs" enriched in the light ^{40}Ca, including primary roots, stem and reproductive organs.

5.2.2. Bean plants grown under L4 experimental condition

In contrast to L6, H4 and H6 experiments, the $\delta^{44/40}Ca$ variability in bean plants grown under L4 condition are rather different, suggesting that low Ca contents (≈5 ppm) and pH (4) of the nutritive solution are important factors controlling the Ca isotope fractionation in bean plants (Fig. 4c and d). The L4 experiment shows that lateral roots attain 30% of the bean organs, after 6 weeks of growth, which is very high compared to the other experimental conditions (see Section 4.1). One might suggest that this organ development could help to pump more nutrient elements in an environment that is less favourable for growth due to low pH and Ca concentration. This is in accord with the study of Miller et al. (1998) who have shown that adventitious roots of common bean present an important development of lateral roots in response to low phosphorous conditions in order to increase the phosphorous uptake from the nutrient system. Another study points to an opposite effect, with lower uptake rates linked to higher roots density, causing inter-root competition for nutrient elements (Marschner, 1995). Previous studies have reported that acid pH values are not favourable to Ca absorption (Sarkar and Wyn Jones, 1982; Fageria, 1989). Replacement of Ca by other cations from the binding sites at the exterior surface of cell walls (e.g. Al, Na or protons) has also been suggested (Wallace et al., 1966; Huang et al., 1992; Rengel, 1992; Rengel and Elliott, 1992; McLaughlin and Wimmer, 1999). Similarly, increasing sap acidity has been reported to displace Ca retained on cell wall exchange sites (McLaughlin and Wimmer, 1999). Our data are in agreement with such ion replacements. Indeed, after 6 weeks of growth the total Ca uptake per bean plant is less important than for the other conditions, whereas Na, Mg and K are the most enriched (Table 2). Our results point to possible replacement of Ca by Sr, at least for roots (lateral and primary) and stems (Fig. 3). Thus, the data suggest a progressive decrease in the number of pectate sites available for binding of Ca (Momoshima and Bondietti, 1990; Marschner, 1995). This can be explained by the pK_a of the binding sites since decreasing pH causes replacement of $R\text{-}COO^-$ groups of polygalacturonic acids (pectins) by R-COOH. The pK_a of these binding sites is equal to 3.6. This could indeed explain why, in contrast to L6, H4 and H6 experimental settings, roots and sap present similar fractionations after 10 days of culture, whereas basal stem $\delta^{44/40}Ca$ is almost not fractionated compared to the basal sap Ca isotopic composition. After 6 weeks, similar initial fractionation processes might have occurred, however, with higher $\delta^{44/40}Ca$ values for primary roots. These appear to be more enriched in the heavy ^{44}Ca isotope compared to those

derived from the other experimental conditions (Fig. 4b and d); also the fractionation is smaller between higher organs. In this case, the number of cation-exchange sites is reduced and, therefore, less fractionation occurs since R-COO$^-$ sites are replaced by R-COOH sites. This process is enhanced by the small Ca content of the nutritive solution that cannot go into competition with the protons. In the case of the H4 experimental setting, however, there are also only a few available R-COO$^-$ sites, but a lot of Ca that may replace the protons. However, reproduction organs have $\delta^{44/40}$Ca values (−0.20‰ on average) similar to those resulting from the other experiments. Leaves are high-transpiring organs that canalise the essential of the xylem sap, whereas reproductive organs are low-transpiring storage organs and their Ca isotopic compositions do not seem to be sensitive to the Ca content or pH of the nutritive solution. The Ca reaches the pods through the xylem elements. The results suggest that the cell walls of the pods consist in pectins whose exchangeable capacity remain constant and is not affected by acidity and low Ca contents. The cell wall structure of the reproductive organs could also be different from that of the primary roots and stem, which induces variable concentration of pectic substances, and thus of available cation-exchange RCOO$^-$ groups (Bateman and Basham, 1983). Consequently, one could suggest that compared to stem or primary root cell walls, reproduction organ cell walls are enriched in pectic substances and, thus, in available RCOO$^-$ acid groups (Taiz and Zeiger, 2010). One might also suggest that the pH is better controlled in these organs and, thus, is less acid. As a result, more R-COO$^-$ sites are available in these organs and the cation-exchange resin effect takes place. Thus, even if competition between Ca and other elements occurs, there should be enough available sites, so that more Ca^{2+} can be exchanged compared to what happens in the other organs. These mechanisms are nevertheless not fully understood yet and request further investigations. Consequently, three different Ca reservoirs exist: "transfer reservoirs" (lateral roots, sap and leaves) enriched in ^{44}Ca, "structural reservoirs" (primary roots and stem) in function of the Ca concentration and pH of the nutritive solution more or less enriched in ^{40}Ca and "storage reservoirs" (reproductive organs) enriched in ^{40}Ca.

5.3. Comparison with $\delta^{44/40}$Ca fractionation mechanisms in trees grown in the field

Comparing $\delta^{44/40}$Ca fractionations in bean plants grown under controlled conditions (with different nutrient supply contents) and trees grown in the field is *a priori* not easy. This for two reasons: first, the tree organ sampling strategy is very different from one study to another and the retained organs differ in size and collected positions. Second, the field Ca supply is more variable and less controlled than in the laboratory. Presently, only three studies present sufficient data on different tree samples (sugar maple (*Acer saccharum*), white ash (*Fraxious americana*), basswood (*Tilia* sp.), spruce (*Picea*), beech (*Betula* sp.), black spruce (*Picea mariana*), trembling aspen (*Populus tremula*) and jack pine (*Pinus banksiana*)) that can be used for comparison (Page et al., 2008; Cenki-Tok et al., 2009; Holmden and Bélanger, 2010). In all cases the vegetation is enriched in the light ^{40}Ca isotope compared to the Ca source and a fractionation of about 0.5‰ occurs between the soil solutions and the fine lateral roots. Therefore, similar mechanisms can be proposed to explain the Ca isotope fractionation during root uptake for plants grown under controlled conditions and plants grown up in the field. If we now compare similar organs from the bottom to the top (~2 mm diameter roots, 1 m above ground stemwood and foliage), we systematically observe, whatever the considered tree species, an enrichment in ^{44}Ca from the fine lateral roots to the foliage of 0.8–1.2‰. Based on the bean plant experiments, one might suggest that similar to the bean plants ^{40}Ca-exchange processes occur in the middle lamella of the stemwood cell walls of the trees. This is in accord with previous field studies showing that Ca moves through the transpiration stream as a part of an ion-exchange complex with adsorption and desorption along active exchange sites within the cell walls of the xylem sapwood (Ferguson and Bollard, 1976; Hanger, 1979; Momoshima and Bondietti, 1990; McLaughlin and Wimmer, 1999). During long distance migration in comparatively older and longer plant systems like trees, the $\delta^{44/40}$Ca value of the xylem sap does not remain constant from bottom to top and it gets progressively enriched in ^{44}Ca while the stemwood enriches in ^{40}Ca. Two observations support this idea. First, sap collected above ground in a beech tree is enriched in ^{44}Ca (0.16 ± 0.15‰) compared to the fine lateral roots (-0.48 ± 0.09‰) (Cenki-Tok et al., 2009). And, second, lower leaves from the same tree and the same study are enriched in ^{40}Ca (0.24 ± 0.15‰) compared to upper leaves (0.64 ± 0.21‰).

Detailed sampling of transpiration fluids and tree tissues from bottom to top of the trees should help in future studies to explain more precisely the recorded $\delta^{44/40}$Ca variability within trees. But also the age of the trees as well as the amount of available Ca in the soil should be considered in future studies because both could affect the Ca isotopic compositions of trees. Indeed, age and diameter of trees have been shown to influence the Ca-binding capacity within the stemwood, with less Ca retention in increasingly older trees, due to the diminishing number of middle lamella in the xylem cell wall (Erickson and Arima, 1974; Momoshima and Bondietti, 1990; Shortle et al., 1995).

5.4. Implications for the Ca isotopic cycle at the watershed scale

The organic matter of the fallen leaves or needles decomposes in the soil litter, which allows among others the nutrient elements such as Ca to reach the soil solutions. Thus it becomes again bioavailable and is taken up by the fine lateral roots (Likens et al., 1998). This Ca recycling by the vegetation constitutes an essential way of nutrient element supply for forests, especially in watersheds where low buffer capacities of soils hamper the neutralisation of acidic atmospheric inputs by bedrock or soil mineral weathering or by cation desorption from exchangeable sites in soils. Miller et al. (1993) and Holmden and Bélanger

(2010) show that the proportion of recycled Ca by vegetation ranges between 80% and 90%. Consequently, the quantification of the Ca recycling flux, that has long been neglected, must be taken into account to model correctly the biogeochemical behaviour of forested ecosystems.

Our results suggest that leaf litter decomposition brings important quantities of ^{40}Ca in the soil solutions; therefore, soil systems missing any important soil mineral related Ca source such as apatite or plagioclase (Aubert et al., 2001), which are comparatively enriched in the heavy ^{44}Ca isotope (Schmitt et al., 2003), become successively enriched in the light isotope with time. Previous studies suggested that physico-chemical modifications of the Ca nutrient reservoir through the lifetime of higher plants might be recorded in the trees growth rings (Åberg et al., 1989; Drouet et al., 2005). As a result, $\delta^{44/40}Ca$ records within tree rings might reflect Ca reservoir modifications or changes of the pH in soils through time (Stille et al., 2011).

6. CONCLUSION

Hydroponic cultures of bean plants have been used in order to test the influence of the Ca concentrations and pHs of the nutritive solution on the Ca isotopic composition of the different organs from roots to shoot. In doing so we tested two different Ca concentrations (5 and 60 ppm) and pHs (4 and 6). Our results show that three different fractionation steps occur, leading to ^{40}Ca enrichments. The first one takes place at the nutritive solution-lateral root interface, the latter compartment being enriched in ^{40}Ca. Moreover, this study suggests that the fractionation may take place when Ca enters the lateral roots during Ca adsorption on cation-exchange binding sites in the apoplasm. The second occurs in the cell wall of primary roots and stems also enriched in ^{40}Ca compared to xylem sap and leaves. Finally, the third is observed for storage organs (reproductive organs), which are also enriched in ^{40}Ca. In every case the fractionations are driven by physico-chemical cation-exchange processes taking place on the cell walls that are constituted of pectic acids; these acids act as ion-exchange resins. Only the third fractionation step seems to be controlled by the physiology of the plant that conditions the number of available pectic substances in the cell walls of reproduction organs. However, our study does not allow to identify fractionation processes within the cell and linked to Ca^{2+} channels or Ca^{2+}/ATPase pumps. Nevertheless it is obvious that the Ca concentration and pH of the nutritive solution affects the importance of the Ca isotopic fractionation. Indeed, with the exception of the reproduction organs, higher amplitudes of $\delta^{44/40}Ca$ values have been recorded in the case of nutritive solutions with higher Ca concentrations (60 ppm) and pH (6) values; nutritive solutions with lower concentrations and pH (5 ppm and pH 4) have significantly lower $\delta^{44/40}Ca$ values.

This study clearly emphasizes that the Ca isotope system is a powerful tool to decipher chemical interaction processes at the interface of the plant–soil system. In plant physiology, Ca isotope studies may help to understand processes allowing the uptake of Ca by roots. This tool may also allow to study the transfer mechanisms of Ca within the plant. Finally, effects of pH or Ca concentration changes in artificial nutritive solutions or soil solutions on the physiology controlled Ca isotope fractionation within plants may also be identified.

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Chapitre 4 : Calcium isotope fractionation during plant growth under limiting nutrient supply

Ce quatrième chapitre de thèse présente une étude complémentaire à l'expérimentation précédente (Chapitre 3). Elle repose également sur une expérimentation en milieu contrôlé de type hydroponique utilisant comme espèce végétale le haricot commun ou *Phaseolus vulgaris* L (Figure II.1 ; Chapitre 3). Elle se différencie de la première étude par le fait que la solution nutritive utilisée n'est pas considérée comme un réservoir infini de Ca. La solution nutritive n'étant pas régulièrement changée, la quantité de Ca disponible diminue au cours du temps. Le but de cette étude est d'observer l'évolution de la signature isotopique en Ca de la solution nutritive et des différents organes de haricot au cours du temps, afin d'en déduire les coefficients de fractionnement et de déterminer les lois qui contrôlent ces fractionnements. Pour parvenir à cela, 2 conditions expérimentales ont été mises en œuvre : (1) dans la première, la diminution de la concentration en Ca a été contrôlée et n'a pas excédé 40% de perte, de telle sorte qu'elle n'affecte pas la croissance de la plante; (2) dans la seconde expérimentation, la solution nutritive s'est constamment appauvrie en Ca au cours du temps, ce qui engendre des symptômes de déficience chez la plante.

Les résultats de cette étude montrent que l'évolution de la composition isotopique du Ca de la solution nutritive et du plant de haricot suit une loi de fractionnement des isotopes du Ca à l'équilibre avec un coefficient de fractionnement ($\alpha_{\text{plant de haricot/solution nutritive}}$) de 0,99858. Cette étude montre aussi que le $\Delta^{44/40}Ca_{\text{solution nutritive}}$ augmente linéairement avec le $\delta^{44/40}Ca_{\text{racines latérales}}$ quand le Ca devient limitant pour la croissance des végétaux. Ainsi, nous constatons qu'une faible quantité de Ca disponible pour la capture nutritive des plantes *via* les groupements carboxyliques des parois cellulaires présents sur la surface externe des racines peut entrainer un faible fractionnement entre les isotopes légers ^{40}Ca entrant dans les plants de haricots et les isotopes lourds ^{44}Ca restant dans la solution. De plus, les résultats suggèrent que, quelle que soit la concentration et le pH des solutions nutritives, les mécanismes responsables du fractionnement des isotopes du Ca lors de sa translocation sont identiques ; seule l'amplitude des fractionnements varie. Enfin, lorsque la quantité de Ca présente dans la solution devient limitante et engendre des symptômes de carence chez les plants de haricot, il apparaît que la composition isotopique des feuilles reflète celle des oxalates de Ca et non celle de la sève brute. Les résultats obtenus lors de cette étude permettent de définir de nouvelles applications potentielles des isotopes du Ca lors d'étude sur en milieu naturel : (i) identifier les mécanismes contrôlant le cycle biologique du Ca au sein des végétaux, (ii) déterminer les variations physico-chimiques du milieu nutritif au cours du temps en analysant les cernes

d'arbres, et (ii) étudier l'altération des oxalates de Ca provenant des feuilles de la litière forestière. Ce travail a fait l'objet d'une soumission dans *Geochimica Cosmochimica Acta* (le 21/03/2012).

Calcium isotope fractionation during plant growth under limiting nutrient supply

Florian Cobert [1], Anne-Désirée Schmitt [2], Pascale Bourgeade [2], François Labolle [3], Sophie Gangloff [1], Pierre-Marie Badot [2], François Chabaux [1], Peter Stille [1]

[1] Laboratoire d'Hydrologie et de Géochimie de Strasbourg; Université de Strasbourg/EOST, CNRS; 1 rue Blessig, F-67000 Strasbourg

[2] Université de Franche-Comté et CNRS-UMR 6249, Chrono-environnement, 16, route de Gray, 25030 Besançon Cedex, France

[3] Université de Strasbourg, Institut de Zoologie et de Biologie générale, 12, rue de l'Université, 67000 Strasbourg, France.

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Abstract

Hydroponic experiments have been performed on bean plants using a nutrient solution at pH 6 and with an initial Ca concentration of 5 ppm in order to test the effect of limiting Ca supply on the $\Delta^{44/40}Ca_{\text{lateral roots/nutritive solution}}$ and the $\delta^{44/40}Ca$ signature between the different organs of the bean. It results from the study that Ca uptake by roots follows an equilibrium fractionation with a fractionation factor ($\alpha_{\text{bean plant/nutritive solution}}$) of 0.99858. The study also shows that $\Delta^{44/40}Ca_{\text{nutrient solution}}$ increases linearly with $\delta^{44/40}Ca_{\text{lateral roots}}$ when the Ca becomes limitant for the growth of the plant. Thus, it appears that small amount of Ca available for cation-exchange processes with the carboxyl groups of the pectins from the cell walls of roots can induce lower fractionations between ^{40}Ca entering the bean plants and ^{44}Ca remaining in solution. Further, the study suggests that, whatever the concentration and pH of the nutritive solution, the fractionation mechanisms between roots and shoots remain the same; only the intensity of fractionation is modified. Finally, when Ca becomes limiting in the solution and

induces carency effects within the bean plants, it appears that the Ca isotopic signature of the leaves do not anymore reflect that of free Ca, but rather that of Ca oxalate crystals. The results of this study yield prospectives for new potential applications of the Ca isotope system in the field of natural environment studies: (i) identification of the mechanisms governing the Ca biological cycle within plants, (ii) determination of the physico-chemical variations of the growth medium of trees through time by analyzing tree growth rings, and (iii) study of the destruction of Ca oxalates released from leaves to soils.

Keywords:
Ca isotopes, hydroponic experiments, fractionation, plants, fractionation factor

1. Introduction

For many years the studies of nutrient element fluxes in small watersheds ignored the role of vegetation although these fluxes are significantly plant-controlled. In fact, plants exert a double action in the watershed: on the one side they accelerate the chemical alteration by factors of 2 to 5 compared to abiotic processes and mobilize the elements in soils and on the other side they delay the transfer of ions from soil solutions to rivers by (re)cycling via root uptake (Berner et al., 2004). Meanwhile there are numerous studies pointing to the important role of plants in the biogeochemical cycle of Ca (Berner and Berner, 1996; Poszwa et al., 2000; Schmitt et al., 2003; Perakis et al., 2006; Cenki-Tok et al., 2009; Stille et al., 2009; Holmden and Bélanger, 2010). Holmden and Bélanger (2010) showed in a Canadian forested watershed that up to 80% of Ca in trees originates from recycling. Calcium is an essential nutrient element and, among others, important for the building and stability of the cell walls and for the functioning of the cell membranes (Marschner, 1995; Taiz and Zeiger, 2010). In soils, it generally occurs as divalent cation or Ca hydrate. Calcium is taken up by the fine lateral roots and redistributed within the different plant organs by translocation through the xylem sap. After leaf and needle fall, it is mineralized in the litter and migrates into the soil solutions, where a part of it becomes again available for plant uptake (Likens et al., 1998; McLaughlin and Wimmer, 1999; Taiz and Zeiger, 2010).

The recent progress in Ca isotope analysis allows for an accuracy which is sufficient to identify natural Ca isotope fractionations in surface processes (~1.25‰/amu; (Russell and Papanastassiou, 1978; Holmden, 2005; Schmitt et al., 2009). Very recent studies on small watersheds in NE France (Strengbach watershed) (Schmitt et al., 2003 ; Schmitt et Stille, 2005 ; Cenki-Tok et al., 2009), on the Hawaïan Island (Wiegand et al., 2005), in the NE of the USA (Oregon coast; the Archer Creek watershed; Mt. Wachusett) (Perakis et al., 2006; Page et al., 2008; Farkas et al., 2011) or in Canada (Saskatchewan) (Holmden and Bélanger, 2010) clearly indicate that vegetation is enriched in ^{40}Ca compared to soil solutions. These studies also demonstrate that biological processes fractionate Ca isotopes within the plant along with the transfer of Ca from the roots up to the leaves (Platzner and Degani, 1990; Wiegand et al., 2005; Page et al., 2008; Cenki-Tok et al., 2009; von Blanckenburg et al., 2009; Holmden and Bélanger, 2010). Nevertheless, these studies do not allow to clearly identify the mechanisms causing these isotopic fractionations within the plant and at the plant- water- soil- rock interfaces. This is due to the fact that abiotic processes, such as e.g. precipitation of secondary minerals, might also influence the Ca isotopic composition of the

plants growth environment by enriching the new secondary mineral phases in the light ^{40}Ca isotope (Gussone et al., 2003; Lemarchand et al., 2004; Tipper et al., 2006; Ewing et al., 2008; Jacobson and Holmden, 2008; Tipper et al., 2008; Hindshaw et al., 2011).

Experiments have recently been performed in physico-chemically controlled growth environments to analyze solely the biological effects and, thus, to avoid the superposition of various isotopic fractionation effects resulting from biological and biotic processes (Cobert et al., 2011a et b). Some of them have been performed under hydroponic conditions using nutrient solutions with constant Ca isotopic composition, two Ca concentrations (5 and 60ppm) and pHs (4 and 6) (Cobert et al., 2011a). These experiments allowed to clearly identify processes causing Ca isotopic fractionation and a ^{40}Ca enrichment in the plant. Fractionation occurs during the nutrient uptake of Ca from the nutrient solution by preferential adsorption of ^{40}Ca on the lateral roots. Another fractionation controlled by ion exchange processes occurs during the transfer of Ca from roots to leaves with a preferential binding of ^{40}Ca on the pecto-cellulosic walls of the primary roots and stems. The third fractionation with ^{40}Ca enrichments occurs at the level of the reproductive organs and is probably controlled by physiological processes (Cobert et al., 2011a). The study further shows that the intensity of the Ca isotopic fractionation in the plant strongly depends on the pH and on the Ca concentration of the nutrient solution.

The experiments of the present study have also been performed under hydroponic conditions. However, in contrast to the first study, the nutrient solution has not been kept at a stable Ca concentration but became depleted with time due to the permanent Ca uptake by the plant. Two situations have been considered: in one experiment the Ca depletion has been limited and, therefore, had no effect on the growth of the plant; in the second experiment, however, the nutrient solution became completely depleted in Ca causing a deficiency symptom in the plant. The aim of the study was then to observe the evolution of the isotopic composition of Ca in the nutrient solution and in the different organs with time, to deduce fractionation coefficients and to recognize the laws controlling these fractionations. These results shall be compared with field observations and will allow to better understand the Ca cycle in the natural environment at the watershed scale.

2. Material and methods

2.1 Hydroponic bean plant growth experiment

Seeds of French beans, *Phaseolus vulgaris* L. ("fin de Bagnol", Vilmorin®, France), have been used for the growth experiments. This dicotyledon belongs to the botanical family of the Fabaceae and has previously successfully been used in hydroponic experiments (Zindler-Frank, 1995; Guo et al., 2002; Cobert et al., 2011a). It is autogame and, therefore, does not need any pollinisator to produce fruits. Its life cycle is quick; 5 to 6 weeks are necessary to obtain mature fruits in the present conditions. The bean seeds were germinated during one week in the darkness at 25°C on 100g wet vermiculite, which has been washed before during 5 days with ultrapure Millipore water. After germination, the young shoots have been transferred to polypropylene pots containing 15L of nutrient solution. The water tanks have been closed in order to minimise water losses by evaporation. The detailed procedure is given in Cobert et al. (2011a).

The Hoagland nutrient solutions used during this study have been modified according to Guo et al. (2002) and adapted to our experiments. They were prepared from micro and macro elements dissolved in mono-distilled water. They have physico-chemical characteristics similar to the nutrient solution L6 (Ca: 5ppm; pH: 6) used by Cobert et al. (2011a). It is important to note that the solutions have been prepared from two different Ca salts: Ca (NO_3) from Sigma-Aldrich® and Jeulin® with different Ca isotopic compositions, due to industrial processing and preparation (Russell et al., 1978; Schmitt et al., 2001; Hippler et al., 2003). Therefore, also the corresponding nutrient solutions are isotopically different (Tables IV.1 and IV.2). Since the results of the different experiments are constantly biased (not shown), the data obtained with the Sigma Aldrich ® Ca salt have been readapted in order to have the possibility to compare the results obtained in this study and in a previous one (Cobert et al., 2011a). Hereafter we will only refer to the bias corrected values.

Two experiments have been conducted. In the first experiment the nutrient solution with four bean plants became successively depleted in Ca. The depletion has been controlled two to three times per week by taking 5 mL out of the solution and analyzing its Ca concentration. The nutrient solution has been replaced after the Ca depletion reached 40%. In the second experiment four plants were grown and the solution has not been replaced allowing a continuous Ca depletion and finally the undernourishment of the plant. The first experiment is called "non-limited" and the second one "limited".

Table IV.1 : *Dry weights, elemental concentrations and Ca isotopic compositions of the different bean organs after 6 weeks of growth*

	Samples		Biomass (g*plant^{-1})	K (ppm)	Na (ppm)	Sr (ppm)	Mg (ppm)	Ca (ppm)	$\delta^{44/40}Ca_{SRM915a}$ (‰)	$\delta^{44/40}Ca_{SRM915a\_corr}$ (‰)	2SD	N
L6 infinite**	Roots	Lateral	1.59	62122	2150	3.75	2608	2237	0.25		0.08	2
	Roots	Primary	0.66	7099	4666	0.63	578	1073	-0.25		0.08	2
	Stem	Basal	2.41	54014	5554	2.52	2166	1260	-0.33*		0.08	2
	Stem	Median	2.11	49503	441	1.19	1658	1873	n.d.		n.d.	
	Stem	Apical	0.14	44550	295	0.91	1955	1546	-0.45*		0.08	2
	Leaves	Basal	4.83	6548	42.9	0.45	1239	1519	0.34		0.12	1
	Leaves	Median	3.21	5800	20.0	1.50	1000	600	n.d.		n.d.	
	Leaves	Apical	1.34	5223	4.45	0.03	540	220	0.25*		0.08	2
	Reproductives	Flower	0.10	13384	46.2	bdl	2015	1000	-0.33*		0.08	2
	Reproductives	Pod	3.07	36808	40.6	0.19	3003	971	-0.39		0.08	2
	whole plant		*19.66*						*-0.12*[&]			
L6 non-limited	Roots	Lateral	1.39	60776	3385	3.21	2333	2114	0.50		0.08	2
	Roots	Primary	0.43	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		n.d.	
	Stem	Basal	0.79	29344	2849	1.40	1019	1423	0.11		0.08	2
	Stem	Median	2.62	49503	441	1.19	1658	1873	n.d.		n.d.	
	Stem	Apical	0.11	72265	501	0.97	1879	2100	n.d.		n.d.	
	Leaves	Basal	1.73	48579	75.7	0.86	7643	9826	0.51		0.08	2
	Leaves	Median	1.20	45441	127	2.17	6880	7580	n.d.		n.d.	
	Leaves	Apical	0.19	62856	235	0.73	6982	2500	n.d.		n.d.	
	Reproductives	Flower	0.13	39356	91.6	bdl	5767	4890	n.d.		n.d.	
	Reproductives	Pod	2.46	44990	31.74	0.51	4290	1704	0.12		0.08	2
	whole plant		11.05						*0.37*[&]			
L6 limited	Roots	Lateral	0.07	11323	19064	1.97	1563	1839	0.97	1.38	0.08	2
	Roots	Primary	0.03	19083	25717	3.51	1153	850	0.78	1.19	0.08	2
	Stem	Basal	0.72	24661	13461	0.65	818	503	0.76	1.17	0.08	2
	Stem	Median	0.50	38000	3000	0.45	2300	823	n.d.	n.d.	n.d.	
	Stem	Apical	0.08	54381	1406	0.29	5384	1024	0.71	1.12	0.08	2
	Leaves	Basal	2.15	39663	2682	0.54	5087	2435	0.36	0.77	0.08	2
	Leaves	Median	2.00	40000	40	1.80	5200	2400	n.d.	n.d.	n.d.	
	Leaves	Apical	0.19	44672	29.1	0.45	5420	2584	0.36	0.77	0.08	2
	Reproductives	Flower	0.03	47530	bdl	0.10	6838	1970	0.38	0.79	0.08	2
	Reproductives	Pod	0.07	50496	132	bdl	4170	372	0.50	0.91	0.08	2
	whole plant		*5.84*							*0.93*[&]		

n.d.: not determined

bdl: below detection limit

2SD corresponds to $2s_{mean}$ when N>1 and $2s_{error}$ when N=1

** from Cobert et al. (2011a), except * that where measured in this study (basal stem: duplicate performed; apical stem, apical leaves and flowers measured twice here)

[&] Ca concentration weighted mean. Not measured isotopic compositions have been estimated from measured ones by supposing constant $\delta^{44/40}Ca$ values for basal-median-apical organs

The pH of the nutrient solutions used in the two experiments has been measured daily and readjusted to a pH of 6 by using max. 200 µL of 0.1N HCl or 0.1N KOH solutions. The water lost due to the beans transpiration or evaporation of the nutrient solutions has been replaced daily by monodistilled water. Using the Visual MINTEQ equilibrium model (Gustafsson, 2010) we observed that >98% of the Ca occurs in dissolved form. Therefore, Ca isotopic fractionations due to Ca precipitation in the nutrient solution can be excluded.

The growth experiments have been performed at the UMR Chrono-Environment in Besançon (France) in a culture chamber at 25±5°C with 16/8h day/night cycles. The light intensity was 150µmol photons $m^{-2}s^{-1}$ and the humidity 50±15%.

Table IV.2: Variation in the Ca concentration and isotopic composition in the solution through time

Experiment		Available Ca in the nutritive solution (in %)	pH	Ca (ppm)	$\delta^{44/40}Ca_{SRM915a}$ (‰)	$\delta^{44/40}Ca_{SRM915a\_corr}$ (‰)	2SD	N
Ca salts	Sigma Aldrich ®				0.58	0.91	0.08	2
	Jeulin ®				0.90		0.12	1
L6 infinite**	initial	100	6	5.17	0.91		0.08	2
	final	100	6	5.17	0.91		0.08	2
L6 non-limited		100	6	5.17	0.91		0.08	2
		82	6	4.24	1.18		0.15	1
		80	6	3.39	1.26		0.08	2
		70	6	2.37	1.35		0.08	2
		64	6	1.52	1.40		0.12	1
		59	6	0.90	1.40		0.12	1
L6 limited	initial	100	6	5.83	0.50	0.91	0.08	2
		77	6	3.91	0.99	1.40	0.08	2
		35	6	2.00	1.40	1.81	0.08	2
		18	6	1.02	1.68	2.09	0.08	2
	final	0	6	0.04	n.d.	2.33[&]	n.d.	n.d.

** from Cobert et al. (2011a)

[&] calculated from the equation defining the four previous $\delta^{44/40}Ca$ values, extrapolated for x=0

2.2 The studied material

The bean plants grown in the nutrient solutions of the non-limited experiment have been harvested after 6 weeks similarly to those studied previously (Cobert et al., 2011a). In contrast, the plants grown in the limited experiment were harvested after 38 days because they presented some signs of deficiency: necrosis of root tips after 28 days and necrosis of shoot apices after 30 days of growth. This caused a stop of growth: since no xylem sap was available after harvesting, one can assume that the root pressure was not sufficient to absorb any nutritive solution. Our sampling procedure was similar to that of our previous study (Cobert et al., 2011a). Lateral roots, primary roots, stems, leaves, flowers and pods have been separated and collected for analytical work. All of the organs have been carefully washed

with mono-distilled water in order to avoid eventual traces of the nutrient solution. Leaves of different ages have been collected in order to see if there are differences in isotopic fractionation. The youngest ones have been collected on the upper part and the older ones on the lower part of the stem. We also completed the Cobert et al. (2011a) dataset by analyzing apical stem and leaves and flowers from L6 experiment. Hereafter we will refer to this experiment as "infinite" because no isotopic fractionation has occurred through time in the nutritive solution.

3. Analytical methods

Chemical and isotopic analyses were performed at the Laboratoire d'Hydrologie et de Géochimie de Strasbourg (LHyGeS) under clean-room conditions. About 100 mg of dried bean samples hand-grinded to ash with an agate mortar were digested in teflon vials using a hot oxidative acid method (double distilled HNO_3, double distilled HCl, H_2O_2 suprapur). Ca, Mg, K, Na and Sr concentrations were measured by an ICP-AES Jobin Yvon JY 124 instrument with a detection limit between 1-40 ppb and accuracy of 5-10 %, in function of the considered element.

Calcium isotopic compositions were determined following the procedure developed in the LHyGeS laboratory by Schmitt et al. (2009). For more analytical details see also Cobert et al. (2011a). The Ca isotope values are expressed as permil deviation relative to the NIST SRM 915a standard solution: $\delta^{44/40}Ca = \{(^{44}Ca/^{40}Ca)_{sample}/(^{44}Ca/^{40}Ca)_{SRM915a} -1\}.1000$ (Eisenhauer et al., 2004). Differences in Ca isotopic compositions between two reservoirs (or within one given reservoir at two different times) are expressed by using the Δ notation defined by $\Delta_{i-j}=\delta^{44/40}Ca_i-\delta^{44/40}Ca_j$ where i and j are the pools (or the times) that are compared to each other. The fractionation factor alpha is defined as the ratio of two isotopes in the compound A divided by the ratio of the same isotopes in the compound B: $\alpha_{A\_B}=R_A/R_B$. The total procedure Ca blank varied between 50-150 ng, which corresponds to max. 3% of the amount of Ca in the samples. Therefore, blank corrections were not necessary. External reproducibility based on repeated measurements of NIST SRM 915a was 0.09 ‰ (2SD) and 0.12 ‰ (2SD) based on replicate sample measurements. The accuracy of the measurements was tested by measuring two reference solutions common to other laboratories and the results (seawater: 1.80±0.11 ‰ (2SD, N=7), CaF_2: 1.45±0.12 ‰ (2SD, N=19)) are in good agreement with previously published values (Hippler et al., 2003).

4. Results

4.1. Biomass variation during the experiment

The dry mass of the bean plant organs are presented in Table IV.1. The total dry biomass production for a bean plant is 1.9 times higher under non-limited (11.05g) than under limited (5.84g), conditions (Table IV.1). Moreover, the relative distribution in various organs is different for the two experiments (Table I.V3).

The preponderant organs observed for the non-limited experiment are leaves and stems with relative masses of 28% and 31%, respectively. Similar are the relative masses for pods (22%) and roots (17%). The flower masses are the lowest (1%). However, in the limited experiment only the leaves are dominant with a relative mass of 74% which is 2.5 times more than observed for the non-limited experiment. For the stems the relative mass is 22%. The mass of the other organs is low (1%). It is important to note that the relative mass of the total of roots and pods is 10 times smaller under limited than under non-limited conditions. Similarly, the shoots to roots ratio is 12 times smaller under limited than under non-limited conditions (Table IV.3).

Table IV.3: *Relative dry mass produced during the different experiments (in %)*

	Lateral roots	Primary roots	Σ Roots [1]	Stem	Leaves	Flowers	Pods
L6 infinite**	8	3	11	24	48	1	16
L6 non-limited	13	4	17	31	28	1	22
L6 limited	1	1	2	22	74	1	1

[1] *Sum of lateral and primary roots*

** *from Cobert et al. (2011a)*

4.2. Element concentrations and Ca isotopic compositions in the nutrient solutions

The elemental concentrations and the Ca isotopic compositions of the plant organs after 5-6 weeks of growth are presented in Table IV.1. The Ca isotopic composition and the Ca available for the plant in the nutritive solution during the experiments are for their part given in Table IV.2. The initial Ca concentrations of the nutrient solutions are within error bars the same for the two experiments. After 6 weeks of plant growth, however, all Ca has been taken up in the limited experiment but only 41% in the non-limited one (Table IV.2). Nevertheless, the Ca isotopic compositions of the two nutrient solutions evolved similarly and show with the depletion in Ca concentrations an enrichment in the heavy ^{44}Ca isotope. After a Ca loss of 41% for the non-limited experiment and a loss of 82% for the nutrient solution of the limited experiment the $\delta^{44/40}Ca$ is 1.40±0.08‰ and 1.68±0.08‰, respectively (Table IV.2; Fig. IV.1).

It was technically not possible to determine the $\delta^{44/40}Ca$ of the solution after it lost more than 82% of its initial Ca concentration (only 0.04 ppm Ca left).

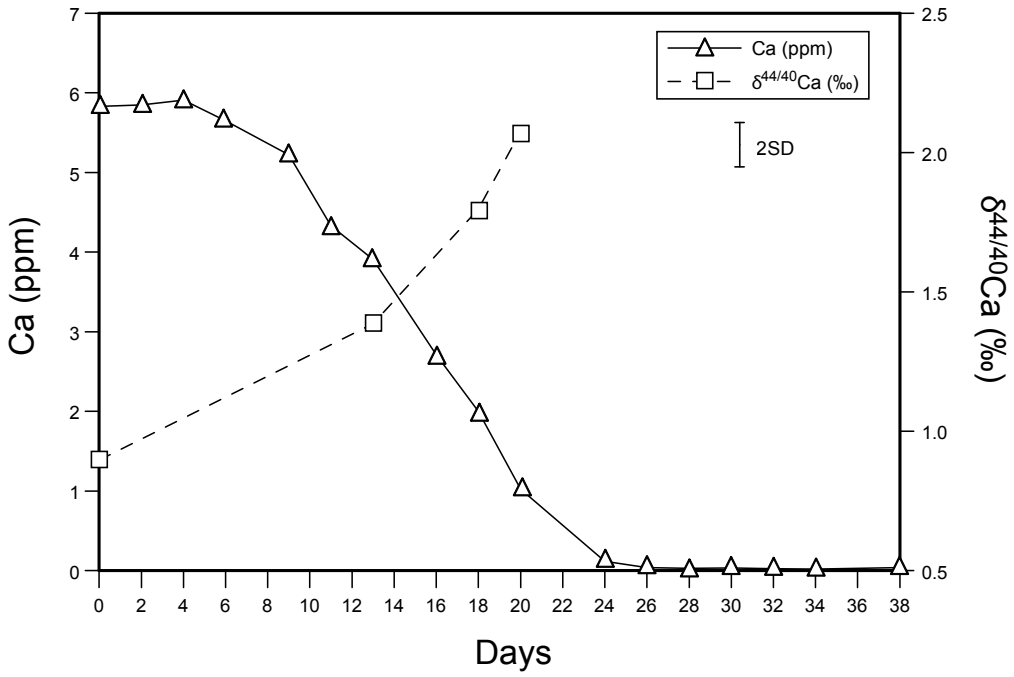

Figure IV.1 : *Variation in Ca concentration and $\delta^{44/40}Ca$ in the nutritive solution through time for the L6 limited experimental conditions*

4.3. The elemental concentrations in the bean plants

The quantity of nutrient cations absorbed by lateral roots and assimilated by the bean plants is given in Table IV.4. For the non-limited experiment one observes that with the exception of Na the quantity of each of the assimilated nutrient elements is 1.7 to 3.5 times larger than that in the limited experiment (Table IV.4). We also observe that the quantity of K, Na or Mg in the primary roots and stems of the limited experiment diminished less (17%; 70% and 25% left respectively) than the Ca (11% left) compared to the infinite experiment where no depletion is observable (calculated from Table IV.1). The molar ratios of the cations K, Mg, Sr and Ca within the beans final organs are given in Fig. IV.2 for the two different experimental conditions. Limited and non-limited experiments are characterized by specific molar ratios. K/Ca (not shown) and Mg/Ca molar ratios in the bean organs are in the limited experiment with one exception generally higher than in the non-limited experiment (Fig.

IV.2a). The Mg/Ca molar ratios of the organs grown during the limited experiment range between 1.9 and 15.4 and those of the non-limited experiment from only 1.2 to 3.5 (Fig. IV.2a). Only the lateral roots show rather similar molar Mg/Ca ratios for both, non-limited (1.5) and limited (1.2), experiments (Fig. IV.2a). Also the Sr/Ca (not shown) and Na/Ca molar ratios behave similar in both experiments (Fig. IV.2b); especially the primary roots are characterized by high Na/Ca molar ratios. The ratios are again higher for organs from the limited experiment (0.2 for pods and 17.4 for primary roots) than for those from the non-limited experiment (0.9 for primary roots).

Table IV.4 : *Bulk plant nutritive element amount for the different experiments*

	K (mg.plant^{-1})	Na (mg.plant^{-1})	Sr (µg.plant^{-1})	Mg (mg.plant^{-1})	Ca (mg.plant^{-1})
L6 infinite**	516	21.2	22.7	32.8	24.1
L6 non- limited	551	8.59	14.3	42.7	40.6
L6 limited	221	19.3	5.81	25.2	11.6

n.d.: not determined
bdl: below detection limit
*** from Cobert et al. (2011a)*

4.4. The Ca isotope ratios in the bean plants

After 6 weeks all plant organs show a slight enrichment in the light ^{40}Ca isotope compared to the initial and final nutrient solutions (Tables IV.1 and IV.2). One observes that in the case of the non-limited experiment lateral roots and basal leaves have the same Ca isotopic signature ($\delta^{44/40}$Ca: 0.51±0.01‰) as well as basal stems and pods ($\delta^{44/40}$Ca: 0.12±0.08‰) (Fig.IV.3b). Thus, the difference in fractionation between these two groups is 0.4‰. In the case of the limited experiment one observes that the lateral roots are the most enriched in ^{44}Ca ($\delta^{44/40}$Ca: 1.38±0.08‰). Primary roots, basal and apical stems have the same Ca isotopic composition ($\delta^{44/40}$Ca: 1.16±0.04‰) as well as basal and apical leaves ($\delta^{44/40}$Ca: 0.77±0.04‰) and reproductive organs ($\delta^{44/40}$Ca: 0.85±0.01‰) (Fig. IV.3c). Thus, there are significant differences in isotopic composition between the different organs of the bean plant. One also observes that Ca isotopic compositions of same organs but of different ages are similar.

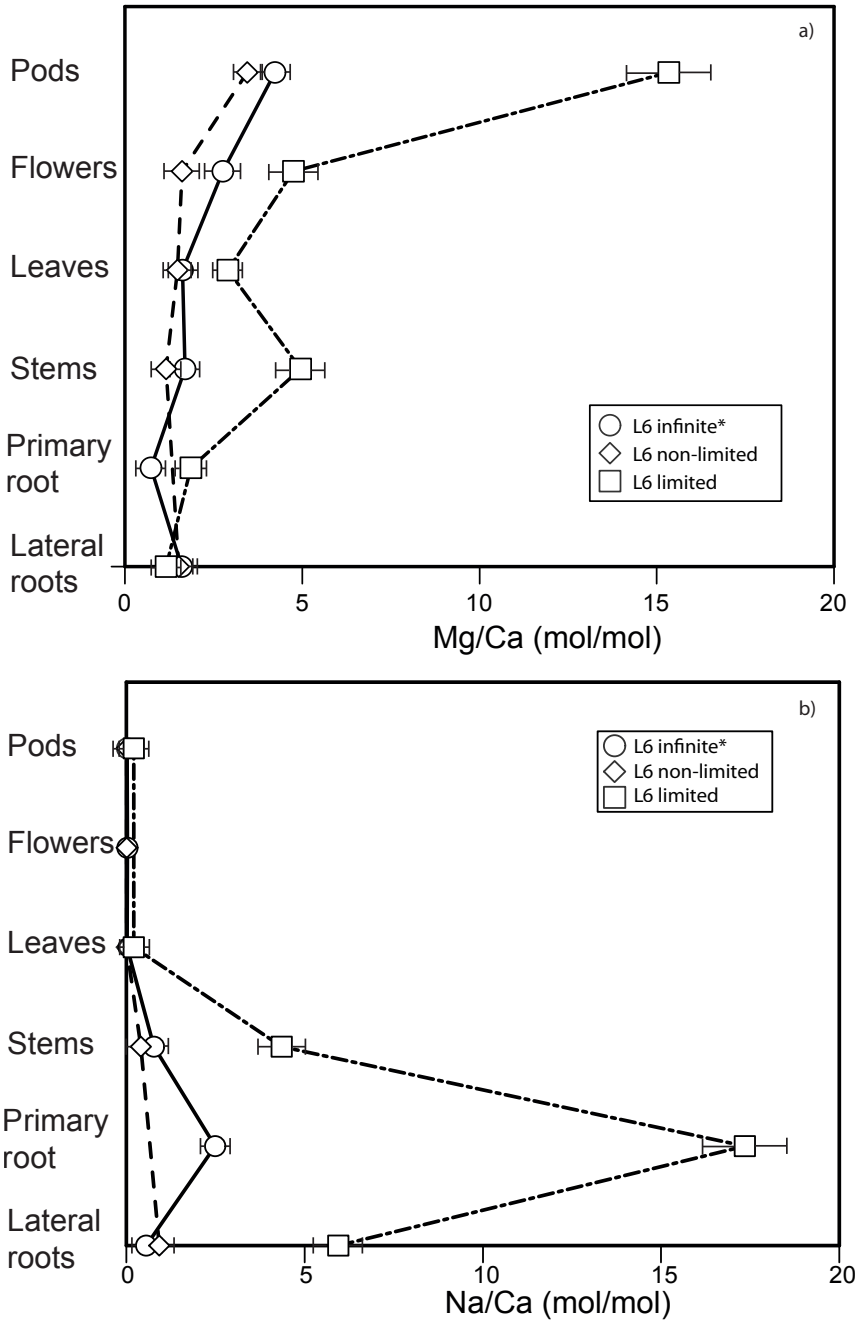

Figure IV.2 : *(a) Mg/Ca and (b) Na/Ca molar ratios in different bean organs * from Cobert et al. (2011a)*

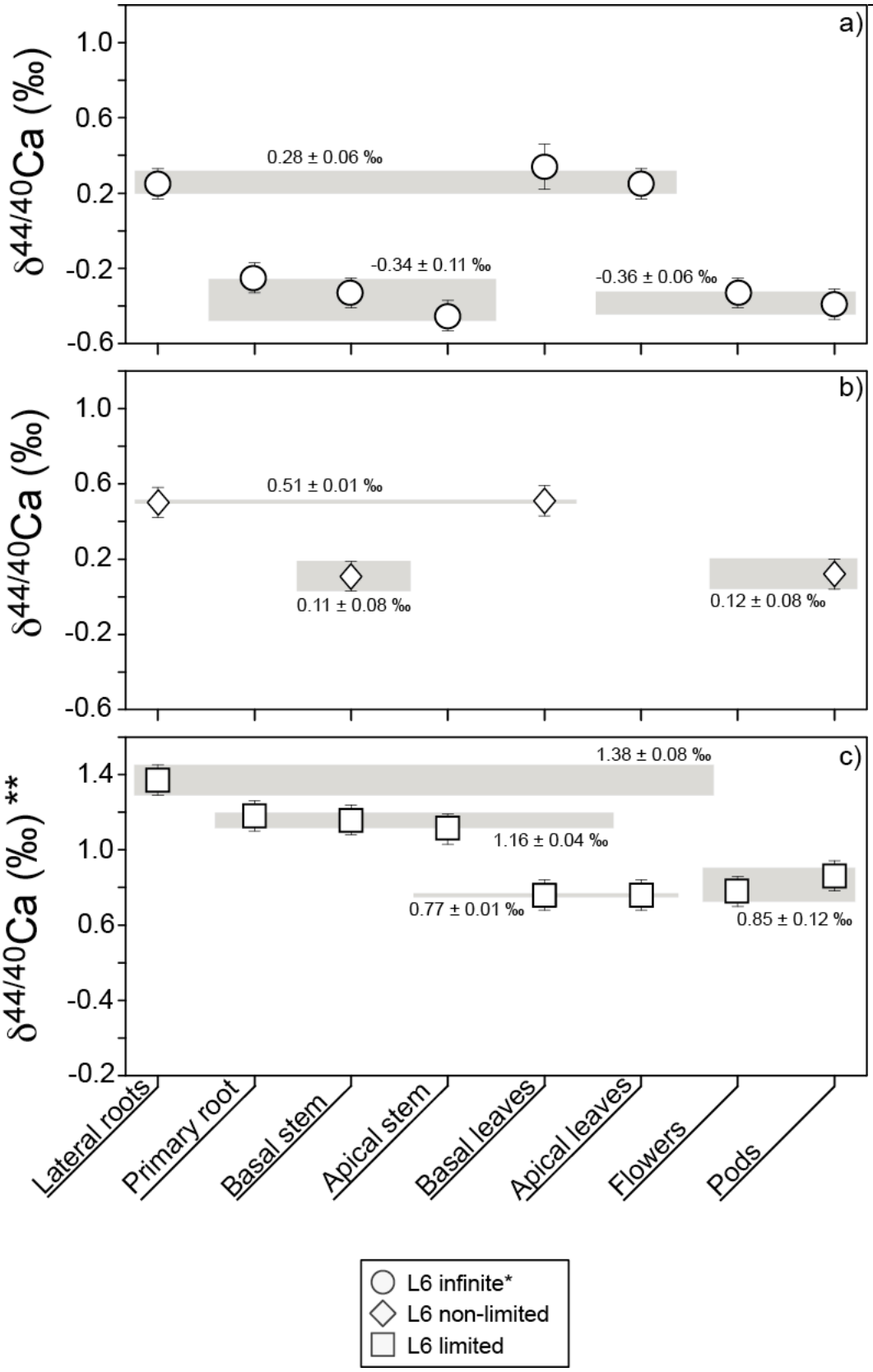

Figure IV.3 : *$\delta^{44/40}$Ca composition in various bean organs in the plant for (a) L6 infinite, (b) L6 non-limited and (c) L6 limited experimental conditions. * from Cobert et al. (2011a). ** values corrected for the bias related to the use of the two Ca salts used in the experiment.*

5. Discussion

5.1. The fractionation coefficient $\alpha_{\text{bean plant/nutrient solution}}$

The experiments show that a nutrient solution with only a limited quantity of Ca is, compared to the initial solution, enriched in the heavy ^{44}Ca with time (Table IV.2). These result are in agreement with the general observation that plants preferentially absorb the lighter ^{40}Ca isotope during their growth (Schmitt et al., 2003; Wiegand et al., 2005; Cenki-Tok et al., 2009; Holmden et Bélanger, 2010; Cobert et al., 2011a et b).

The limited experiment indicates that the exchange reactions between bean plant and nutrient solution can be explained by equilibrium fractionation (Fig. IV.4). This is in agreement with Mg isotopes (Black et al., 2008) but in contrast to Si isotopes which apparently follow the law of Rayleigh distillation (Ding et al., 2005). A fractionation factor $\alpha_{\text{bean plant/nutritive solution}}$ of 0.99858 can be derived at the end of the limited experiment which corresponds to a stage where all Ca has been consumed. The calculated final $\delta^{44/40}$Ca value for the plant is 0.91‰ which is in agreement with the whole plant mean value of 0.93‰ (Table IV.1).

For the case of the non-limited L6 experiment ($\alpha_{\text{bean plant/nutritive solution}}$: 0.99881), with a max. consumption of 40% of the Ca in initial solution, a mean $\delta^{44/40}$Ca of 0.20‰ can be calculated for the bean plant; this is in agreement with the mean value of 0.37‰ derived for the whole plant (Table IV.1). One observes a positive correlation between the amplitude of the variation of the Ca isotopic composition in the nutritive solution ($\Delta^{44/40}Ca_{solution}$) during the experiment and the weighted mean of the measured Ca isotopic compositions in the bean plants ($\delta^{44/40}Ca_{bean}$) (Fig. IV.5). This indicates that the bean plant is isotopically strongly related to the source Ca. Even for small variations in the nutrient solution, such as those observed for the non-limited experiment (~0.5‰; Table IV.2), one observes a $\delta^{44/40}$Ca enrichment of 0.5‰ between whole plants from infinite and non-limited experiments (Table IV.1).

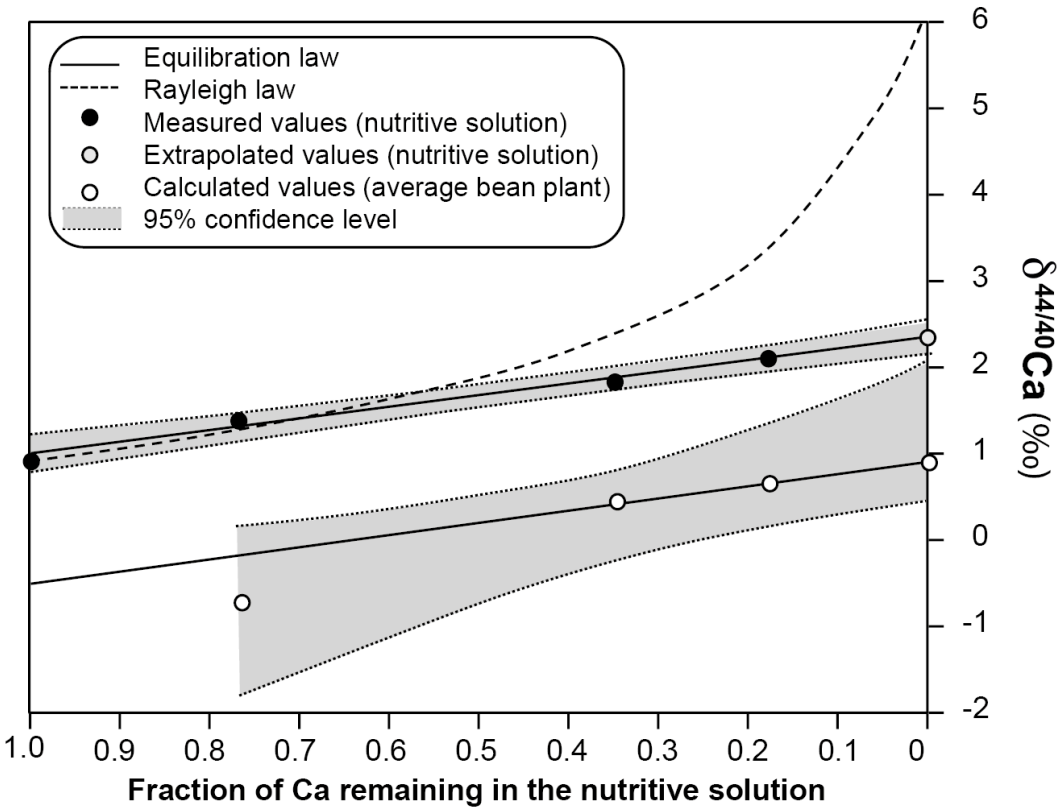

Figure IV.4 : *Rayleigh and equilibrium fractionation models for the nutritive solution and the average bean plant for L6 limited experiment (with* $\alpha_{bean\ plant/nutritive\ solution} = 0.99858$*). The 95% confidence level is plotted for the measured and extrapolated nutritive solutions and the corresponding calculated average bean plants.*

5.2. The mechanisms causing the Ca isotopic fractionation in bean plants

5.2.1. Ca uptake by the lateral roots

The Ca uptake from the nutrient solution by the lateral roots is a passive process. Similar to previous experiments and to studies in the natural environment (Schmitt et al., 2003; Wiegand et al., 2005; Cenki-Tok et al., 2009; Holmden and Bélanger, 2010; Cobert et al., 2011a), this study also confirms the preferential ^{40}Ca uptake by the lateral roots. In our previous study we proposed that the fractionation is the result of a preferential adsorption of ^{40}Ca on the carboxyl acid ($R\text{-}COO^-$) groups of the polygalacturonic acid (pectines) which are present in the cell walls of the lateral roots and which are functioning like cation exchange resins (Cobert et al., 2011a). The results also indicate that the difference between $\Delta^{44/40}Ca_{nutrient\ solution}$ and $\delta^{44/40}Ca_{lateral\ roots}$ is negligible for the limited and the non-limited experiments (0.04 and -

0.01, respectively using data from Tables 1 and 2). This suggests that for nutritive solutions with high pH (6) and limited Ca content the average fractionation between solution and lateral roots decreases when compared, with the infinite conditions of our previous study: the nutrient solution with low Ca concentration (5 ppm) and low pH (4) (L4) caused a smaller $\Delta_{\text{lateral roots/ solution}}$ variation (0.53‰) than the experiments with a pH favouring much more the root uptake (L6: 0.66‰ and H6: 0.72‰) (Cobert et al., 2011a).

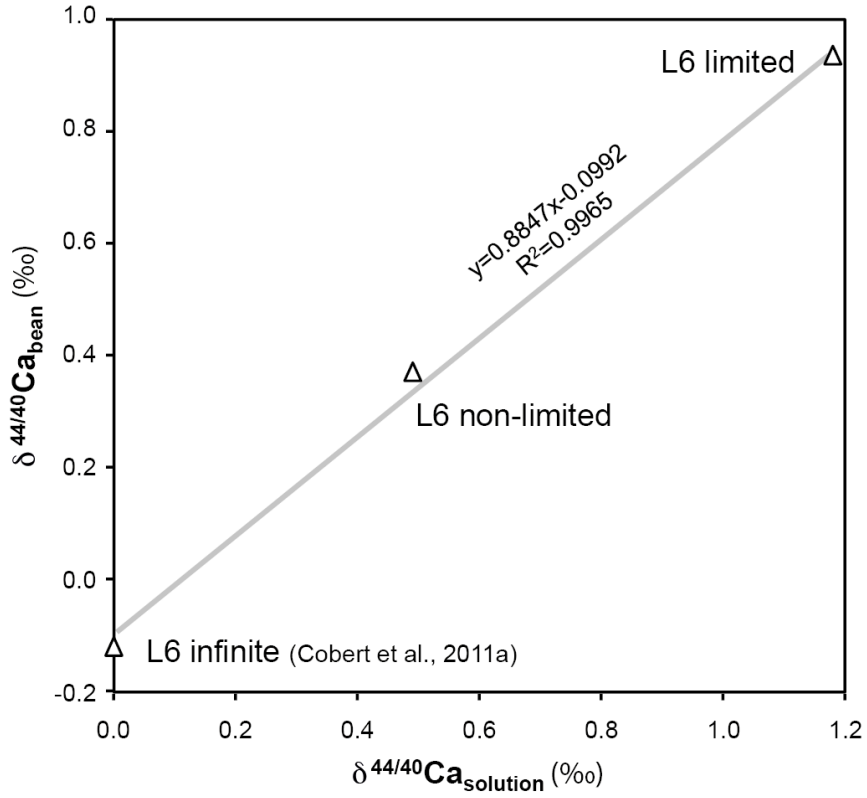

Figure IV.5 : *$\delta^{44/40}Ca_{bean}$ versus $\Delta^{44/40}Ca_{solution}$ relationships for the 3 experimental L6 conditions (infinite, non-limited and limited).*

Thus we can distinguish two environmental conditions that modify the $\Delta_{\text{lateral roots/solution}}$: (1) an environment with low pH (4) and low Ca concentration (5 ppm) which gives rise of only a few R-COO$^-$ sites available for the fixation of Ca and, therefore, allows only for small fractionations; (2) an environment with higher pH (6) and limited Ca concentration which induces many R-COO$^-$ sites accessible but only small quantity of available Ca, so that the average fractionation is close to zero.

5.2.2. The way of Ca translocation from roots to shoots

The non-limited L6 experiment of the present study indicates that the isotopic distribution in the different organs of the bean plant is similar to that of the infinite L6 experiment (Cobert et al., 2011a). Therefore, the fractionation mechanism proposed in the previous study to explain the observed fractionation might also be valid for the present study. After entering into the plant through the lateral roots the Ca migrates with the transpiration stream from the roots up to the leaves (Bresinsky et al., 2008; Taiz and Zeiger, 2010). During the transfer, the Ca isotopes become fractionated at the level of the primary roots and the stems (Cobert et al., 2011a). The Ca isotope fractionation is the result of ion exchange reactions between the Ca from the xylem sap and the polygalacturonic acids present in the cell walls of the xylem vessel. In fact, a higher part of the Ca absorbed by plants during their growth is fixed on the R-COO- groups of the polygalacturonic acids (pectines), more or less exchangeable at the level of the primary roots, stems or other organs (Arnstrong and kirkby, 1979; Hanson, 1984; Kirkby and Pilbeam, 1984; Marschner, 1995; Berinsky et al., 2008; Taiz and Zeiger, 2010). As a consequence, the light ^{40}Ca isotopes are preferentially captured by the cation exchange sites of the R-COO- of the cell walls of the xylem vessels at the level of the primary roots and stems (Cobert et al., 2011a). Such a mechanism is in accord with the recent study of Augusto et al. (2011).

For the limited experiment the isotopic distribution in the different organs is different from that of the non-limited and infinite experiments (Fig. IV.3), except for reproductive organs. Indeed, the $\Delta_{\text{lateral roots/reproductive organs}}$ is about 0.5‰, the same order as observed for the limited and the infinite experiments. In contrast, $\Delta_{\text{lateral root/(primary roots+stems)}}$ is around 0.2‰ and $\Delta_{\text{lateral roots/leaves}}$ is around 0.6‰ (Fig. IV.3).

Consequently, if we suppose that the $\delta^{44/40}Ca$ of lateral roots is representative of free Ca circulating in the xylem sap, as was suggested by Cobert et al. (2011a), then the smaller $\Delta_{\text{lateral root/(primary roots+stems)}}$ observed for the limited experiment can be explained by the small available quantity of Ca which can be fixed on the ion exchange sites present in the cell walls of the xylem vessel in lateral and primary roots of limited L6 experiment. Indeed, these two organs represent only 2% of the whole bean plant whereas they reach 11% and 17% under non limited conditions (Table IV.3). This smaller rate of absorption might cause smaller fractionation. Alternatively one might consider a substitution by other ions such as K, Mg, Na or Sr (Bangerth, 1979). This would be in agreement with the results presented in Fig. IV.2 but also with the observation that the quantity of K, Na or Mg in the primary roots and the stems

of the limited L6 experiment diminished less than Ca compared to the infinite experiment (see paragraph 4.3).

The ^{40}Ca enrichment in the leaves might be explained in different ways. It could be simply be the consequence of the limited fractionation in the absorption and conduction organs (primary roots and stems). Indeed, one might suggest that a Ca substitution by other cations could preferentially enrich the xylem sap in ^{40}Ca. As a result the free Ca would become enriched in ^{40}Ca, and thus also the leaves; this, however, only under the condition that similar to infinite experiments the $\delta^{44/40}$Ca signature of leaves would reflect that of free Ca. Nevertheless, this cannot be the case since, by analogy to chemical exchange processes in a chromatography column, it would be the ^{40}Ca (and not ^{44}Ca) that remains fixed to the pectins (Russell and Papanastassiou, 1978). In a previous study it has been suggested that the sap which reaches the leaves is strongly enriched in Ca (non-limited reservoir) and, therefore, does not allow any fractionation of Ca during fixation of ^{40}Ca on the pectins of the primary roots and stems (Cobert et al. 2011a) (Fig. IV.6a). However, a sap with limited Ca content may explain the data of the limited L6 experiment. In such a case the sap would become enriched in ^{44}Ca, whereas the absorption and conduction organs would have preferentially taken up the light ^{40}Ca isotope, which, however, is not the case (Fig. IV.6b). The ^{40}Ca enrichment in the leaves may be related to the fact that during the experiment the xylem sap becomes successively depleted in Ca because most of the Ca is fixed in the absorption and conduction organs. In this case one might suggest, similar to Augusto et al. (2011), that the Ca migration in the plant is chromatographic. According to Russell and Papanastassiou (1978), this would cause a constant enrichment of ^{40}Ca from below up to the top of the plant and, therefore, a higher $\delta^{44/40}$Ca value for the basal than for the apical organs (Fig. IV.6c); but this is, as mentioned above, not the case in our experiment. Alternatively one might suggest that quasi no free Ca is anymore circulating and, consequently, that the Ca isotopic signature of the leaves is related to the oxalate Ca which is indeed enriched in the leaf cells (Bresinski et al., 2008). The oxalate enrichment can be explained by physiological processes favouring the oxalate formation in leaves. Indeed previous studies have identified several functions of Ca oxalates in leaves: oxalates correspond to a protection against herbivorous consumption of leaves in nature, but contribute also to the regulation of tissue calcium or metal detoxification (Nakata, 2003 and references cited herein). It has recently been shown that oxalates are enriched in ^{40}Ca compared to free Ca (Cobert et al., 2011a). This order of magnitude of enrichment is in agreement with the isotopic signature of the here measured leaves. Previous studies have

shown that the amount of Ca oxalates in plants (and thus in leaves) are correlated with the amount of Ca in the growth medium (Zindler-Frank et al., 2001; Volk et al., 2002; Jauregui-Zuniga et al., 2005). The diminution of Ca in the nutrient solution would according to Nakata et al. (2003) cause a release of bound calcium for utilization by the plant. Consequently, the dissolution of Ca-oxalates would enrich the free Ca of leaves in ^{40}Ca. At this stage of knowledge we cannot choose between these two hypotheses: the ^{40}Ca enrichment in leaves might be linked either to oxalate crystals or to the dissolution of oxalate crystals.

The data of the reproductive organs are in agreement with those published previously (Cobert et al., 2011a) and suggest that their isotopic signature is not influenced by the nutrient environment. According to previous studies, cation exchange sites "lining the xylem walls and coupled with the removal of calcium from the xylem stream by adjacent cells are thought to play a role in regulating long distance calcium transport to the pod" (Marschner, 1995; White and Broadley, 2003; Nakata, 2011). Consequently, the ^{40}Ca enrichment of reproductive organs could be due to chromatographically displacement of Ca within the xylem wall (Russell and Papanastassiou, 1978; Augusto et al., 2011). Moreover Nakata (2011) has identified a "functional role for calcium oxalate formation in regulating calcium transport to the seeds". Therefore, one might also suggest that the constant ^{40}Ca enrichment in the reproductive organs, whatever the composition of the nutritive solution, reflects the $\delta^{44/40}$Ca signature of calcium oxalate crystals that are known to be enriched in ^{40}Ca (Cobert et al., 2011a). Further studies are necessary for a better understanding of the mechanisms controlling the Ca isotopic compositions of these organs.

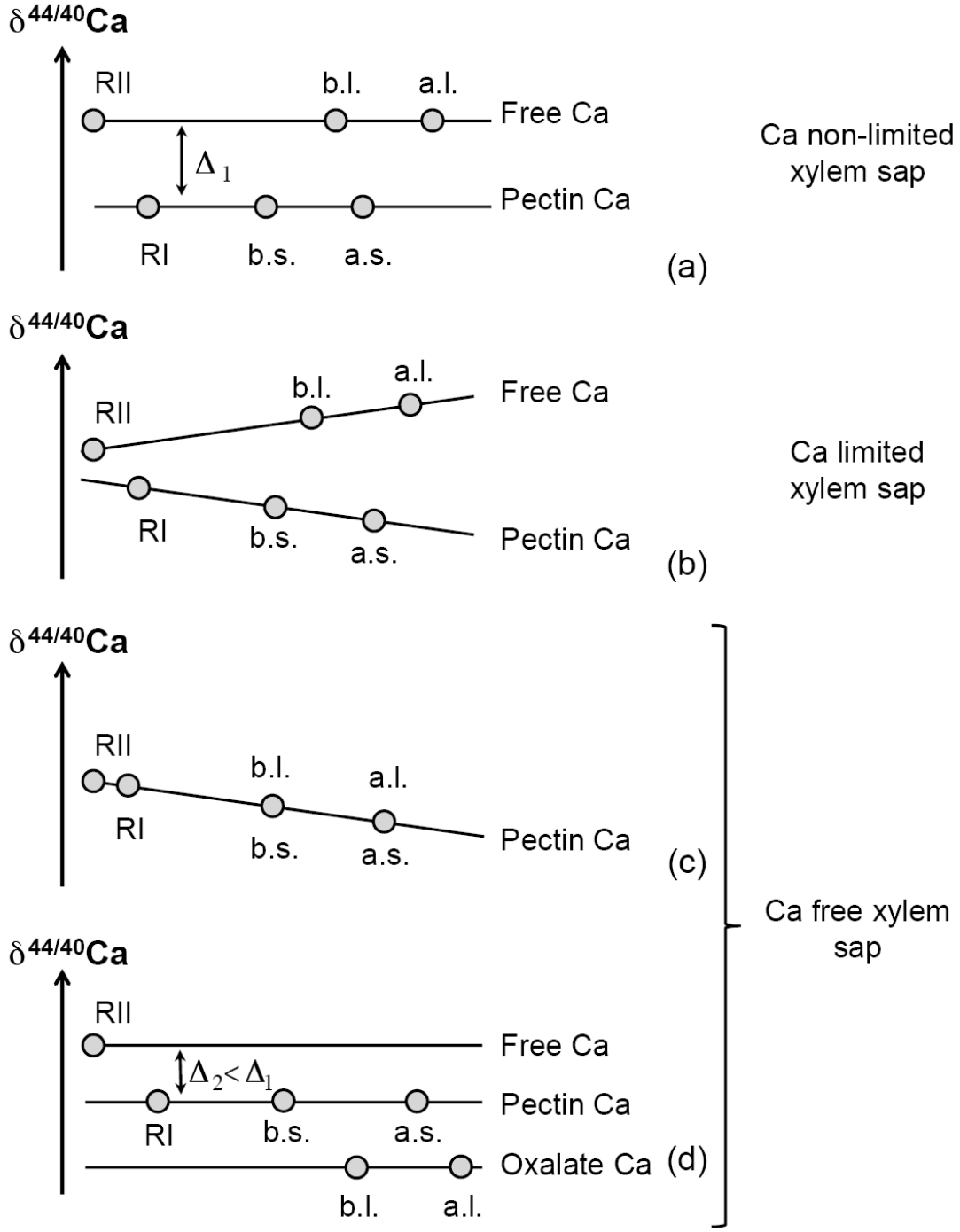

Figure IV. 6 : *$\delta^{44/40}$Ca evolution in the different bean organs considering that the xylem sap is (a) Ca non-limited, (b) Ca-limited, (c) and (d) Ca-free, with (c) the fractionation being only linked to chromatographical processes and (d) the oxalates in leaves being supposed to play a key role. RII, RI, b.l., a.l., b.s., a.s. refer to lateral roots, primary roots, basal leaves, apical leaves, basal stem and apical stem, respectively. See text for more details.*

6. Consequences for the bio-geochemical cycling of Ca at the watershed scale

From the physiological point of view the results of the present and previous studies on bean plants (Cobert et al., 2011a) might suggest that the fractionation mechanisms are similar for other plants and the vegetation in general, whatever the available Ca concentration and pH of the nutrient environment are. Only the amplitude of fractionation within the different plant organs might change. If this is true, the limited Ca availability for the plants growth would cause smaller Ca isotopic fractionations within the organs like stems enriched in polygalacturonic acids.

Taking into account present results we can suggest that at the watershed scale the weighted mean Ca isotopic signature of trees is also a function of the Ca isotopic composition of the nutrient environment and that it is always enriched in ^{40}Ca compared to this environment. Consequently, this further suggests that $\delta^{44/40}Ca$ of the trees growth rings changes if the $\delta^{44/40}Ca$ of the nutrient environment changes with time. Such a change of the nutrient environment might especially occur in ecosystems depleted in basic cations and, therefore, having only a weak buffering capacity for acid atmospheric deposits (Nakano and Tanaka, 1997; Aubert et al., 2001; Vogt et al., 2006; Chapman et al., 2008). Similarly, one would expect ^{40}Ca enrichments in the trees growth rings if enough Ca is available and ^{44}Ca enrichments if the nutrient environment is depleted in Ca. This is true for high and low pH (6 and 4, respectively) (Cobert et al., 2011a).

The results also indicate that, when the nutrient environment becomes poor in Ca, the leaves become compared to non-limited conditions enriched in ^{40}Ca. The consideration of these enrichment and depletion processes is of importance for a correct modeling of the bio-geochemical Ca cycles in function of the changes of the environmental conditions with time.

The experimental results further suggest that when Ca supply is limited, the Ca in the leaves principally occurs in form of Ca oxalate crystals. Since these crystals are only weakly soluble in the natural environment they would importantly control the speed of Ca recycling. Nevertheless, the accumulation of these salts in soils and leaf litter has seldom been observed. This probably because there are oxalotrophic bacteries which use the oxalate as source of carbon and energy. The resulting calcium carbonate precipitation, which has been observed for tropical soils, produces a stock of carbon for millions of years (Gifford, 1994 ; Cailleau et al., 2005).

7. Conclusion

The bean plant experiment has been performed to better understand the processes controlling the Ca isotopic fractionation within the bean plant organs in function of the changing Ca concentration of the nutrient solution with time. Under non-limiting and limiting conditions the isotopic compositions of nutrient solution and bean plant follow the law of equilibrium fractionation (fractionation coefficient $\alpha_{\text{bean-nutrient solution}}$=0.99858). Similarly, the nutrient solutions become enriched in ^{44}Ca with time, and the weighted mean $\delta^{44/40}$Ca values of the bean plants point also to ^{44}Ca enrichments. Our study further indicates that the mechanisms causing the Ca isotopic fractionation during root uptake or translocation of Ca from roots to uppermost organs are identical whatever the Ca concentration of the nutrient solution. Only the amplitude of the fractionation between the different organs might be changed. The results also suggest that low pH (4) with low Ca concentration experiments (Ca contents infinite) and that high pH (6) with limited Ca concentrations cause both a decrease in the intensity of fractionation at the nutrient solution/ root interface. Finally, under limiting conditions in the nutrient environment only Ca oxalates control the Ca isotopic signature of the leaves. Finally, the study clearly shows that the Ca isotope system is an important tool (1) for the identification of physico-chemical changes of the nutrient environment of an ecosystem in the past by studying tree growth rings Ca isotopic composition, (2) for a better definition of the Ca flux in the soil induced by decomposition of the soils leaf litter and (3) for modeling the transfer of nutrient elements in a forested ecosystem.

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Chapitre 5 : Biotic and abiotic experimental identification of bacterial influence on calcium isotopic signature

Cette cinquième et dernière partie de la thèse traite de l'influence des acides organiques et inorganiques des plantes et de l'activité bactérienne de la rhizosphère sur le rapport isotopique du Ca des solutions de sol et des organes de végétaux. Pour cela, deux types d'expérimentations en microcosme ont été réalisées : (1) expérimentations abiotiques de l'altération de l'apatite sous deux conditions de pH (2 ou 3,5) utilisant de l'acide citrique ou de l'acide nitrique ; (2) expérimentations biotiques sur apatite ou substratum composé de biotite pauvre en Ca, en présence de pins sylvestres, *Pinus sylvestris* L., inoculés ou non par *Burkolderia glathei* PML1(12) et en présence de *Burkolderia glathei* PML1(12) seul. Pour toutes ces expérimentations, le percolât est collecté toutes les semaines et analysé pour la concentration en Ca et son $\delta^{44/40}$Ca.

Les résultats des expérimentations abiotiques montrent que le type d'acide (organique ou minéral) utilisé pour dissoudre l'apatite n'influence pas la signature isotopique en Ca de la solution percolante. Pour les expérimentations biotiques, le principal résultat est un enrichissement de 0,22‰ en ^{44}Ca de la solution en contact avec les racines de pins et les bactéries sur le substratum composé d'apatite. Cependant, la présence de bactéries seules ou de pins seuls sur substratum composé d'apatite n'entraine pas de fractionnement de la solution qui percole dans les colonnes de microcosmes après 14 semaines d'expérimentation. Ainsi, ces résultats préliminaires suggèrent que les bactéries influencent la signature isotopique du Ca par une dissolution plus efficace de l'apatite en présence de pins. Ces résultats ont fait l'objet d'une publication dans *Rapid Communication in Mass Spectrometry* en 2011.

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Biotic and abiotic experimental identification of bacterial influence on calcium isotopic signatures†

Florian Cobert[1], Anne-Désirée Schmitt[2]*, Christophe Calvaruso[3,4,5], Marie-Pierre Turpault[4], Damien Lemarchand[1], Christelle Collignon[4,5], François Chabaux[1] and Peter Stille[1]**

[1]Laboratoire d'Hydrologie et de Géochimie de Strasbourg, Université de Strasbourg/EOST, CNRS, 1 rue Blessig, F-67000 Strasbourg, France
[2]Université de Franche-Comté et CNRS-UMR 6249, Chrono-environnement, 16, Route de Gray, 25030 Besançon Cedex, France
[3]Université du Luxembourg, Laboratoire "Radiation physics", Campus Limpersberg, Luxembourg
[4]INRA UR1138 "Biogéochimie des Ecosystèmes Forestiers", Centre INRA de Nancy, 54280 Champenoux, France
[5]INRA, UMR1136 INRA-Nancy Université "Interactions Arbres-Microorganismes", Centre INRA de Nancy, 54280 Champenoux, France

In this study, we tested experimentally the influence of plant and bacterial activities on the calcium (Ca) isotope distribution between soil solutions and plant organs. Abiotic apatite weathering experiments were performed under two different pH conditions using mineral and organic acids. Biotic experiments were performed using either apatite or Ca-enriched biotite substrates in the presence of Scots pines, inoculated or not with the rhizosphere bacterial strain *Bulkholderia glathei* PML1(12), or the *B. glathei* PML1(12) alone. For each experiment, the percolate was collected every week and analyzed for Ca concentrations and Ca isotopic ratios. No Ca isotopic fractionation was observed for the different abiotic experimental settings. This indicates that no Ca isotopic fractionation occurs during apatite dissolution, whatever the nature of the acid (mineral or organic). The main result of the biotic experiments is the 0.22 ‰ ^{44}Ca enrichment recorded for a solution in contact with Scots pines grown on the bacteria-free apatite substrate. In contrast, the presence of bacteria did not cause Ca isotopic fractionation of the solution collected after 14 weeks of the experiments. These preliminary results suggest that bacteria influence the Ca isotopic signatures by dissolving Ca from apatite more efficiently. Therefore, Ca isotopes might be suitable for detecting bacteria-mediated processes in soils.

Biogeochemical weathering processes in soils influence soil fertility and water quality and control the availability of inorganic nutrients for plants and other living organisms.[1–6] Abiotic mineral dissolution is a slow process[7] compared with biologically mediated weathering reactions by plant roots and soil microorganisms, which are essential processes controlling the availability of nutrients, especially in acidic and nutrient-poor forest soils developed in temperate and wet tropical climatic conditions.[8–11]

Possible complementary functional organizations between trees, symbiotic fungi and bacterial communities have been proposed.[12–16] These biological agents exert mechanical, i.e. physical, breakdown of rocks and minerals by roots and fungal hyphae, and biochemical actions on soil minerals, i.e. exudation of protons, and the production of organic acids and chelating molecules.[4,17] They also optimize nutrient element transfer from soil minerals to plants.[18] These processes mainly occur in the rhizosphere, which is the interface between the soil particles, soil solutions and the root system, and which is governed by intense reactions such as ion exchange, water uptake or root exudations.[17,19–22]

In the immediate vicinity of plant roots, microorganisms produce biofilms covering the minerals and thus limiting the nutrient loss by drainage.[23] Moreover, the respiration and activity of microorganisms produce CO_2 that controls the redox potential and pH of the rhizosphere.[24,25] Similarly, plant roots absorb water and ions and produce organic acids that increase the chemical weathering of soil minerals and contribute to the complexation of cations released by mineral dissolution or leaching.[25–27]

Recent studies have shown that vegetation has distinctive Ca isotopic compositions. These compositions are more enriched in the light ^{40}Ca isotope than the rock and soil minerals and soil solutions at the watershed scale.[28–34] Laboratory experiments suggest that the Ca isotopic composition of the vegetation is strongly dependent on the Ca concentration and pH of the nutrient solution.[35] Measurement

* *Correspondence to:* A.-D. Schmitt, Université de Franche-Comté et CNRS-UMR 6249, Chrono-environnement, 16, Route de Gray, 25030 Besançon Cedex, France.
E-mail: adschmit@univ-fcomte.fr

** *Correspondence to:* P. Stille, Laboratoire d'Hydrologie et de Géochimie de Strasbourg; Université de Strasbourg/EOST, CNRS; 1 rue Blessig, F-67000 Strasbourg, France.
E-mail: pstille@illite.u-strasbg.fr

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of the Ca isotope ratios could help to distinguish the respective contribution of tree roots and rhizosphere bacteria to cation release during soil weathering processes.

The aim of this study was therefore to provide an experiment to examine for the first time the influence of rhizosphere bacteria during mineral weathering on the Ca isotopic compositions of plants and percolate solutions.

EXPERIMENTAL

Microcosm growth experiments

Biological agents and chemicals

The experimental protocol was developed in a collaboration between the BEF (Biogéochimie des Ecosystèmes Forestiers, INRA, Champenoux, France) and IAM (Interactions Arbres-Microorganismes, INRA, Champenoux, France) laboratories. Details of the experimental design can be found in Calvaruso *et al.*[36] In brief, fluorapatite from Durango (Mexico) was chosen as the test mineral because of its rapid weathering rate. It has often been considered to be an important Ca source for vegetation in granitic watersheds.[28,33,37–39] Its structural formula is $(Ca_{9.77}La_{0.03}Ce_{0.04}Sr_{0.007}Fe_{0.003}Si_{0.06})(P_{1.02}O_4)_6F_{2.08}Cl_{0.12}$[40] and it contains 54.6% CaO.[11] A biotite from Bancroft (Canada) was selected as a second test mineral since this phyllosilicate is frequently present in acid soils and, in contrast to apatite, it contains only traces of Ca: $(Si_3Al)(Fe^{3+}_{0.12}Fe^{2+}_{0.61}Mg_{2.06}Mn_{0.02}Ti_{0.13})K_{0.88}Na_{0.06}O_{10}(OH_{0.98},F_{1.02})$.[36] Pure quartz crystals were used in order to strengthen the growing substrate. The minerals used in the study were pure and well crystallized. They were crushed, rinsed with Milli-Q water (18.2 MΩ;Millipore, Billerica, MA, USA), ultrasonicated, and sieved in order to keep only apatite, biotite and quartz grains with diameters of 0.51, 0.2–0.5, and 0.5–1/1–2 mm, respectively. They were finally sterilized by autoclaving (20 min at 120 °C) just before the experiments were started, following the protocol developed by Calvaruso *et al.*[36]

The bacterial strain *Burkolderia glathei* PML1(12) was chosen because of its known ability to alter minerals.[16,36] It was isolated from the ectomycorrhizosphere of oak *Scleroderma citrinum* in a temperate forest ecosystem (Breuil-Chenue; France).[41] *Pinus sylvestris* L., i.e. Scots pine (seeds from the Haguenau forest, France), was chosen for its rapid growth rate (germination procedure described in Koele *et al.*[42]).

Finally, sterile Milli-Q water as well as nutritive solutions and analytical grade acids were used during the course of the experiment. Two nutritive solutions representative of conditions found in soils of Breuil-Chenue (France) were prepared. One was Ca-free and consisted of 11.2 mg/L N, 4.2 mg/L K, 1 mg/L Mg, 1.3 mg/L S. The other was enriched in Ca and consisted of 1.5 mg/L Na, 2 mg/L P, 2.3 mg/L Ca, 1.9 mg/L S, 11.2 mg/L N, 1.5 mL Mo-Cu. Nitric and citric acids have, respectively, been used as analogues for inorganic acids and organic chelating agents, which are commonly produced by Scot pines[12] and the bacterial genus *Burkholderia*.[43] These acids were adjusted to pH values of both 2 and 3.5. A pH of 3.5 is representative of conditions found in temperate forest ecosystems (e.g. Strengbach watershed, France).[39] A pH of 2 is used to test the influence of highly acidic solutions on Ca isotopic fractionations.

The experimental design

In vitro column experiments were designed using chemically inert polypropylene tubes containing a 20 µm nylon filter and connected to a peristaltic pump providing either nutrient or acid solutions. The tubes were filled with quartz mixed with apatite or biotite in contact with the biological agents (pines and/or bacteria) (Fig. 1).

The experimental conditions are summarized in Fig. 1 and Table 1. Four abiotic experiments were performed. The abiotic experiments served as a reference to test the influence of the acidic nature under 'dissolution-forced' conditions. During these experiments apatite grains were in contact with nitric or citric acid at pH 2 and 3.5 injected by a peristaltic pump (0.5 mL/h) (Fig. 1(a)) for 5 weeks. Five biotic experiments were also performed over a period of 14 weeks. Three columns were filled with apatite grains and two with biotite grains. These columns received, respectively, Ca-free and Ca-rich solutions delivered by a peristaltic pump (0.8 mL/h) (Fig. 1(b)). Three experiments were performed with apatite: (1) apatite and pine $(P)_A$; (2) apatite with *B. glathei* PML1(12) inoculated pine $(P+B)_A$; and (3) apatite and *B. glathei* PML1 (12) bacterial strain alone $(B)_A$. Two column experiments were performed with biotite as the column packing: pines inoculated $(P+B)_B$ or not $(P)_B$ with *B. glathei* PML1(12). The columns were placed in a growth chamber with controlled day/night temperatures (25/18 °C), at a 17 h period of daylight and 60% humidity. Three experiments with apatite or biotite containing no biological material were also performed: the columns were eluted with a Ca-depleted and a Ca-enriched sterile nutritive solution (respectively, designed as $(N)_A$ and $(N)_B$) and with sterile Milli-Q water $(W)_A$.

The different drainage solutions were collected every week and some of them were selected for Ca isotope analyses. Initial and final pine seedlings originating from the apatite column experiments were separated into roots and shoot, dried at 65 °C for 1 week in an oven, minced, homogenized and analyzed for Ca isotopes.

Analytical methods

All isotopic analyses were performed under clean-laboratory conditions at the Laboratoire d'Hydrologie et de Géochimie de Strasbourg, Strasbourg, France (LHyGeS). About 100 mg of dried pine samples were digested in savillex™ vials (Savillex, Eden Prairie, MN, USA) using a hot oxidative acid method (double-distilled HNO_3/double-distilled HCl/suprapure H_2O_2). The collected liquid fractions were also treated with suprapure H_2O_2 in order to remove any organics before being processed for isotopic analysis.

The Ca isotopic compositions were determined following the procedure developed in the LHyGeS laboratory.[44] A typical mass of 1 µg of Ca from a $^{42}Ca/^{43}Ca$ double spike was added to 5 µg of Ca before the calcium purification was performed by high selectivity automated ion chromatography on a Dionex® ICS-3000 (Dionex, Voisins le Bretonneux, France) instrument with a high-capacity carboxylate-functionalized column (Dionex® CS16). After Ca separation, the samples were treated again with double-distilled HNO_3 and

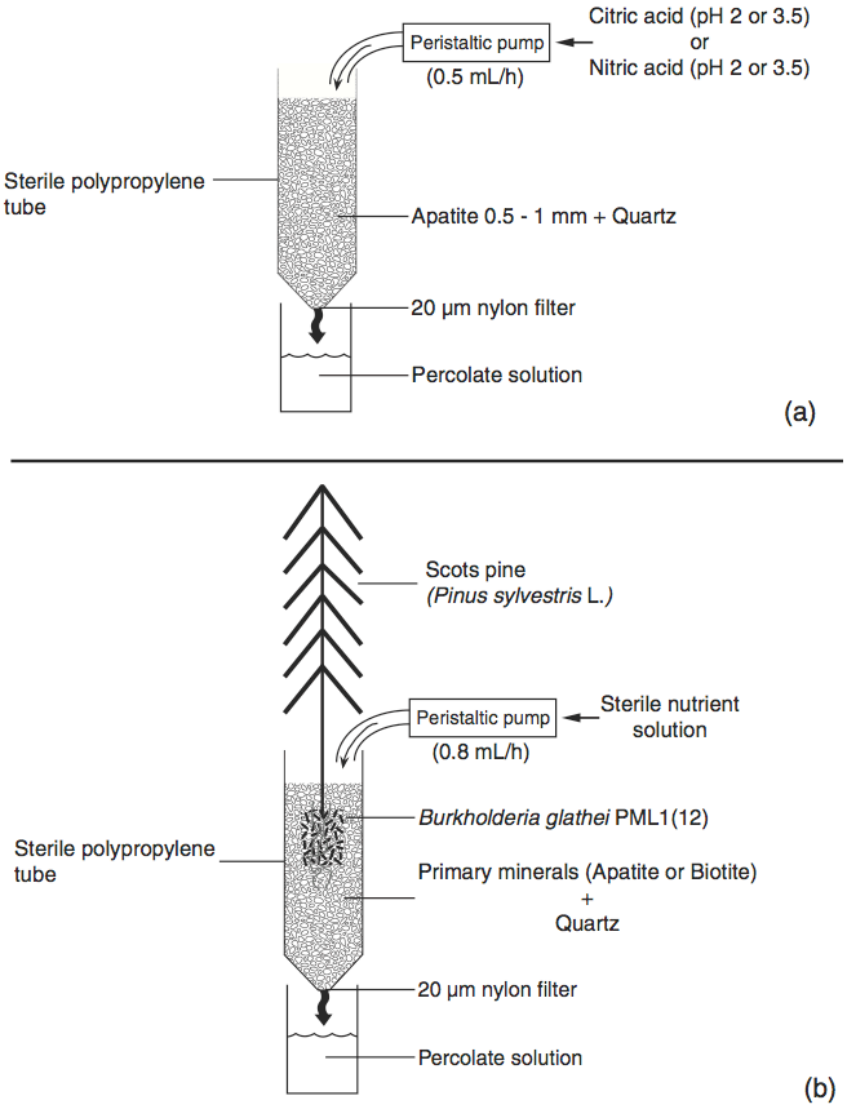

Figure 1. Schematic of experimental protocol used for the abiotic and biotic experiments: (a) abiotic columns filled with crushed apatite provided with nitric and citric acid at pH 2 and 3.5 and (b) biotic columns filled with apatite or biotite and biological material (Scots pine and/or *B. glathei* PML1(12)) provided with sterile nutritive solution.

Table 1. Nomenclature used for the different experimental settings

	Experimental agents		Apatite (Ca-enriched) columns	Biotite (Ca-free) columns
Abiotic	Nitric acid (pH 2–3.5)	5 weeks	nitric	
	Citric acid (pH 2–3.5)	"	citric	
Biotic	MilliQ water	14 weeks	$(W)_A$	
	Ca-free nutritive solution	"	$(N)_A$	
	Ca-enriched nutritive solution	"		$(N)_B$
	Scots pine	"	$(P)_A$	$(P)_B$
	B. glathei PML1(12)	"	$(B)_A$	
	Scots pine & *B. glathei* PML1(12)	"	$(P+B)_A$	$(P+B)_B$

suprapure H_2O_2 before being totally evaporated in order to eliminate any organic impurity from the cation-exchange resin breakdown and/or from the sample itself. The residue was dissolved in 1 µL 0.25 N HNO_3 and loaded without any further treatment on a single outgassed, and in partial vacuum oxidized, Ta filament (99.995% purity). The Ca isotopic compositions were measured by thermal ionization mass spectrometry (TIMS) on a Triton instrument (Thermo Fisher,

Table 2. $\delta^{44/40}$Ca values of drainage solutions, apatite, and Scots pine organs

Material	Treatment	Reference	Time (weeks)	$\delta^{44/40}$CaSRM$_{915a}$ (‰)	2SD[a]	N	Ca (ppm)	Ca (mg)	2SD	pH	Wet biomass (mg)	2SD
				INITIAL MATERIAL								
Apatite			initial	0.62	0.08	2	–	–	–	–	–	–
Sterile nutrient solution		$(N)_B$	initial	0.64	0.08	2	–	–	–	–	–	–
				ABIOTIC EXPERIMENTS								
Columns filled with apatite (pH 2)	Citric acid		1	0.66	0.08	2	–	–	–	–	–	–
	Citric acid		5	0.62	0.08	2	–	–	–	–	–	–
	Nitric acid		1	0.64	0.08	2	–	–	–	–	–	–
	Nitric acid		5	0.65	0.08	2	–	–	–	–	–	–
Columns filled with apatite (pH 3.5)	Citric acid		5	0.70	0.12	1	–	–	–	–	–	–
	Nitric acid		5	0.60	0.12	1	–	–	–	–	–	–
				BIOTIC EXPERIMENTS								
Columns filled with apatite	Sterile nutritive solution	$(N)_A$	8	0.71	0.08	2	–	–	–	–	–	–
	Sterile nutritive solution	"	14	0.71	0.08	2	–	–	–	–	–	–
	Sterile Milliq water	$(W)_A$	8	0.52	0.08	2	–	–	–	–	–	–
	Sterile Milliq water	"	14	0.64	0.08	2	–	–	–	–	–	–
	Scots pine	$(P)_A$	8	0.62	0.08	2	–	–	–	–	–	–
	Scots pine	"	12	0.70	0.08	2	–	–	–	–	–	–
	Scots pine	"	14	0.84	0.08	2	5.00	0.52	0.06	3.95	295	74
	Scots pine + *B. glathei* PML1(12)	$(P+B)_A$	8	0.64	0.08	2	–	–	–	–	–	–
	Scots pine + *B. glathei* PML1(12)	"	14	0.66	0.08	2	–	0.45	0.05	4.65	334	83
	B. glathei PML1(12)	$(B)_A$	8	0.59	0.08	2	–	–	–	–	–	–
	B. glathei PML1(12)	"	12	0.54	0.08	2	–	–	–	–	–	–
	B. glathei PML1(12)	"	14	0.51	0.08	2	–	–	–	–	–	–
Columns filled with biotite	Sterile nutritive solution	$(N)_B$	14	0.57	0.08	2	–	–	–	–	–	–
	Scots pine	$(P)_B$	14	0.60	0.19	1	–	0.50	25	3.5	–	–
	Scots pine + *B. glathei* PML1(12)	$(P+B)_B$	14	0.65	0.12	1	–	0.49	25	4.8	–	–
				SCOTS PINE ORGANS (FROM THE BIOTIC EXPERIMENT FILLED WITH APATITE)								
Needles + Stems	Scots pine		initial	0.37	0.15	1	–	1.70	0.10	–	–	–
Roots			initial	0.48	0.16	1	–	0.60	0.30	–	–	–
Needles + Stems	Scots pine		14	0.40	0.17	1	2230	7.90	1.80	–	–	–
Roots			14	0.51	0.16	1	1273	1.90	0.90	–	–	–
Needles + Stems	Scots pine + *B. glathei* PML1(12)		14	0.45	0.15	1	–	6.40	2.20	–	–	–
Roots			14	0.44	0.17	1	–	1.30	0.60	–	–	–

[a]2SD corresponds to $2\sigma_{mean}$ when N >1 and to $2\sigma_{error}$ when N = 1.

Bremen, Germany) operating in dynamic multicollection mode.[35,45] The filament was heated to ~1200 °C, and the beam current was adjusted to reach a typical value of 5.0 ± 10^{-1} A of ^{40}Ca, corresponding to a filament temperature ranging from 1380 to 1450 °C. Between 130 and 200 cycles were collected. Most of the samples were duplicated including the steps of digestion, Ca purification by ion chromatography and TIMS analysis. In order to check for possible isobaric interferences at masses 40 and 44, beams corresponding to $^{41}K^+$ and $^{88}Sr^+$ (proxy for $^{40}K^+$ and $^{88}Sr^{2+}$), were systematically checked. No interference was ever observed, so that no correction was necessary. The $\delta^{44/40}Ca$ values were calculated after the data had been reduced using the exponential fractionation law and the Newton-Raphson iteration technique.[46] This numerical treatment has the advantages of being quite robust and rapidly converging to precise analytical results. The data reduction was performed off-line using the optimization toolbox provided by the commercial Matlab® software (The MathWorks, Natick, MA, USA). The Ca isotope values are expressed as permil deviation relative to the NIST (Gaithersburg, MD, USA) SRM 915a standard solution:

$$\delta^{44/40}Ca = \left\{ \left(^{44}Ca/^{40}Ca\right)_{sample} / \left(^{44}Ca/^{40}Ca\right)_{SRM915a} - 1 \right\}.1000. \quad [29,33,47]$$

The Ca contamination for the total procedure was between 50 and 150 ng, which corresponds to a maximum of 3% of the amount of Ca from the samples. Ca contamination corrections were therefore negligible for all the samples. The global external repeatability is 0.12 ‰ (2SD, N = 165) and was determined from standard solutions (NIST SRM 915a, seawater, CaF_2) and repeated sample measurements. The accuracy of the measurements was tested by measuring two inter-laboratory shared solutions and the results (seawater: 1.91 ± 0.10 ‰ (2SD, N = 8), CaF_2: 1.45 ± 0.12 ‰ (2SD, N = 19)) were in good agreement with previously published values.[28,48–50]

RESULTS

The Ca isotopic compositions of the analyzed drainage solutions, minerals and tree organs are reported in Table 2.

Abiotic experiments

In order to determine the influence of apatite mineral dissolution on the Ca isotopic compositions of the drainage solutions, nitric and citric acid were used to adjust the pH of the entering solutions to either pH 2 or 3.5. The $\delta^{44/40}Ca$ value of the initial apatite was measured at the beginning of the experiments and the $\delta^{44/40}Ca$ of the percolate solution after 1 and 5 weeks (Table 2).

The $\delta^{44/40}Ca$ value of the initial apatite was 0.62 ± 0.08 ‰ and within error bars this is a similar value to that of the column output collected after 1 week at pH 2 with nitric (0.64 ± 0.08 ‰) and citric (0.66 ± 0.08 ‰) acid. After 5 weeks of experiment, no change in the $\delta^{44/40}Ca$ of the solution was observed with either nitric (0.65 ± 0.08 ‰) or citric (0.62 ±0.08 ‰) acid. Similarly, after 5 weeks of experiments, the $\delta^{44/40}Ca$ values measured at pH 3.5 in the outputs from both the nitric (0.60 ± 0.08 ‰) and the citric (0.70 ± 0.08 ‰) acid experiments remained undistinguishable from that of the initial apatite and of the experiments conducted at pH 2. Likewise, the $\delta^{44/40}Ca$ values of the abiotic control columns with apatite ($(W)_A$ and $(N)_A$), which were run in parallel to the biotic experiment, were within error bars similar to those of apatite (0.71 ± 0.08 ‰ and 0.62 ± 0.08 ‰, respectively).

Biotic experiments

A mass budget calculation based on the monitored Ca concentrations in the drainage solutions and the Ca concentrations in the mineralized biomass at the end of the experiments indicates that the pines accumulated about the same amount of Ca (7.5 mg) as is lost by drainage (9.4 mg) (Tables 2 and 3). Thus, the Scots pine have absorbed about 4 times the initial mass of Ca present in the pine, $(P)_A$ and $(P+B)_A$, during the 14 weeks of growth (Table 3).

The $\delta^{44/40}Ca$ values of the solutions collected in these two experiments after 8 weeks (0.54 ± 0.08 ‰ and 0.64 ±0.08 ‰, respectively), 12 weeks (0.54 ± 0.08 ‰, only $(B)_A$ treatment) and 14 weeks (0.51 ± 0.08 ‰ and 0.66 ± 0.08 ‰, respectively) remained similar to that of apatite (Figs 2(a) and 2(b)). This implies that the $(B)_A$ or $(P+B)_A$ conditions did not cause an observable Ca isotopic shift in the drainage solution along the course of the experiment (Table 2). In contrast, for experiment $(P)_A$, a significant ^{44}Ca enrichment was observed in the solution after 14 weeks (0.84 ± 0.08 ‰) compared with the $\delta^{44/40}Ca$ value of the initial apatite (Fig. 2(c)). However, in the case of the experiment including Scots pine and biotite $(P)_B$, the $\delta^{44/40}Ca$ of the drainage solution was 0.59 ± 0.08 ‰ and, therefore, the solution was not enriched in ^{44}Ca after 14 weeks of experiment (Table 2; Fig. 3). No variation in the $\delta^{44/40}Ca$ was observed for either the drainage solution after 14 weeks, or $(P+B)_B$ (0.65 ± 0.12 ‰; Table 2; Fig. 3). For $(P)_B$

Table 3. Amount of Ca in the percolate and Scots pine organs after 14 weeks of experiments for columns filled with apatite

Material	Treatment	Time (weeks)	Ca (mg)	2SD	Ca accumulated (mg)	2SD
Percolate	$(P)_A$				7.74	1.28
"	$(P+B)_A$				6.96	1.19
Needles + Stems	Scots pine	initial	1.7	0.1		
Roots	"	initial	0.6	0.3		
Needles + Stems	Scots pine	14			7.4	1.8
Roots	"	14			1.8	0.9
Needles + Stems	Scots pine + *B. glathei* PML1(12)	14			5.9	2.2
Roots	"	14			1.2	0.6

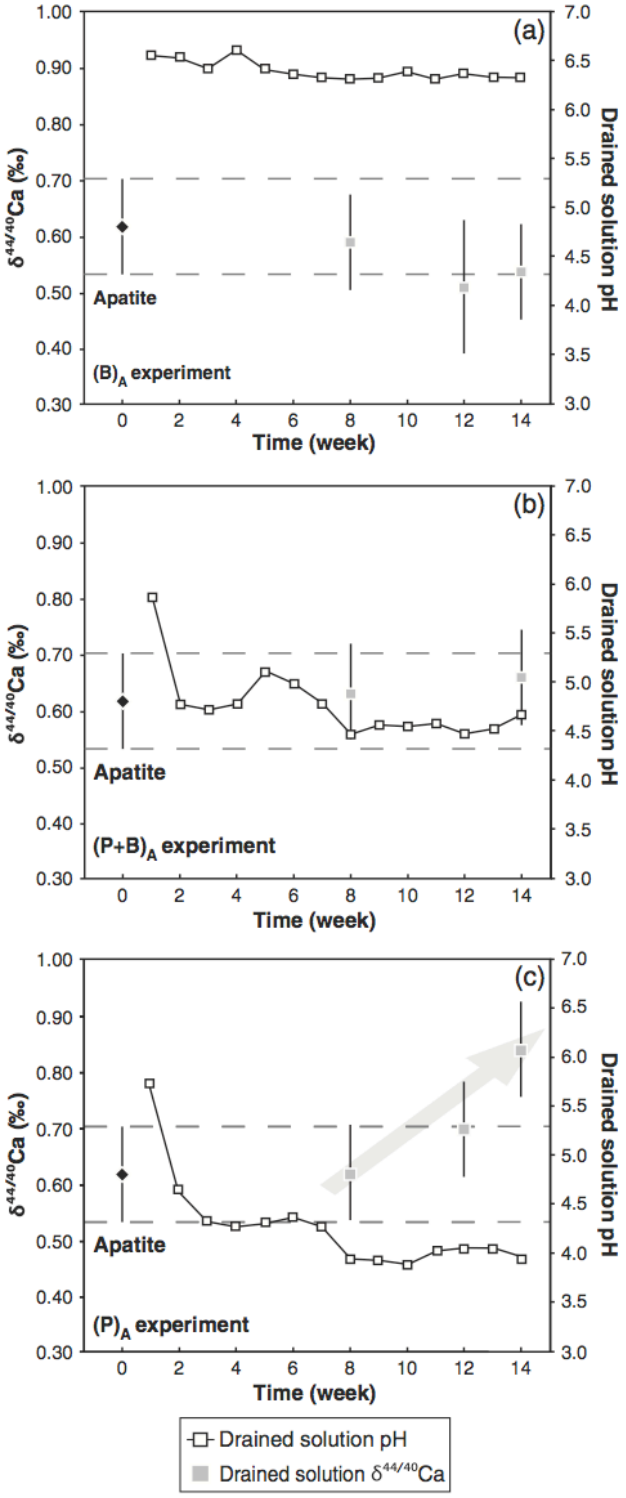

Figure 2. Evolution of Ca isotopic compositions and pH values of the collected percolates through time for columns filled with apatite and (a) *B. glathei* PML1(12) bacteria ($(B)_A$); (b) *B. glathei* PML1(12) inoculated Scots pine ($(P+B)_A$); and (c) not inoculated Scots pine ($(P)_A$).

or $(P+B)_B$, the $\delta^{44/40}Ca$ values of the solution were similar to that of the biotite control column $(N)_B$ after 14 weeks of experiments (0.57 ± 0.08 ‰; Table 2; Fig. 3).

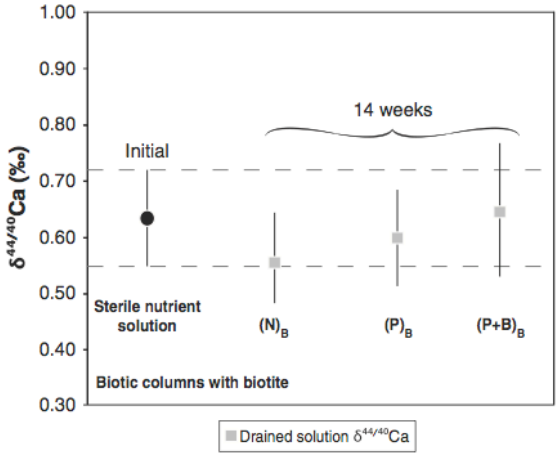

Figure 3. Ca isotopic compositions of the collected percolates after 14 weeks of experimentation for columns filled with biotite and not inoculated pine ($(P)_B$); *B. glathei* PML1(12) inoculated Scots pine ($(P+B)_B$); and Ca-enriched sterile nutritive solution ($(N)_B$).

The $\delta^{44/40}Ca$ values of separated organs from the initial Scots pine (roots and shoots) and those sampled after 14 weeks of growth on apatite ($(P+B)_A$ or $(P)_A$) covered a rather narrow range of values, scattering between 0.37 and 0.51 ‰ (Table 2).

DISCUSSION

Apatite and acids

The absence of any visible Ca isotopic shift in the drainage solution when apatite was exposed to nitric or citric acid confirms recent propositions that reactions of mineral dissolution do not cause any Ca isotopic fractionation.[51–53] Previous studies of closed-system fluorapatite dissolution by hydrochloric and citric acid at low pH (~2) point to local oversaturation with respect to apatite causing fluorapatite recrystallization.[54,55] Dissolution/recrystallization mechanisms occur after short times in both media and an apparent steady state is attained after 4 days in hydrochloric acid experiments and 34 days in citric acid experiments.[55] If such processes occurred in our open-system experiments, the absence of a visible $\delta^{44/40}Ca$ shift in solution would imply that the successive dissolution/recrystallization steps did not fractionate the Ca isotopes whatever the nature of the acid (mineral: nitric or organic: citric) or the pH value (2 and 3.5). Alternatively, this might also suggest that successive steps of apatite dissolution/recrystallization did not occur in our experiments. The main implication of our observations is that organic molecules such as those exuded by root and soil microbia did not cause any Ca isotopic fractionation during the weathering of phosphate minerals in soils.

Apatite and Scots pine

Plant organs are known to be enriched in the light ^{40}Ca isotope with respect to the soil solution from which they develop.[30,32–34,50] We might expect that the ^{40}Ca enrichments in plants would cause a complementary relative ^{44}Ca

enrichment in the residual solution. The comparison of the $\delta^{44/40}$Ca in apatite (0.62 ± 0.08 ‰) with that of the drainage solution in contact with apatite and Scots pines (0.84 ± 0.08 ‰) allowed us to observe a maximal Δ^{44}Ca enrichment of 0.22 ‰ in the last part of the experiment (after 14 weeks) ($(P)_A$; Table 2). Accordingly, the average $\delta^{44/40}$Ca values of Scots pine organs from $(P)_A$ and $(P+B)_A$ after 14 weeks may be slightly enriched in ^{40}Ca at the limit of statistical significance (roots: 0.48 ± 0.05, 2SD, N = 2, and shoots: 0.43 ± 0.07, 2SD, N = 2, Table 2) by comparison with apatite (0.62 ± 0.08, Table 2). The $\delta^{44/40}$Ca values measured in developed Scots pine organs (roots and shoots) are indistinguishable from each other and from those measured at the beginning of the experiment (0.37 ± 0.15 and 0.48 ± 0.16, Table 2). Therefore, the Ca isotopic compositions of the Scots pine shoots and roots remained identical despite the fourfold increase of Ca in the biomass and the $\delta^{44/40}$Ca gradient observed within the organs of forest trees.[33,34] The absence of visible fractionation within the Scots pine organs during the experiments may be explained by the fact that the $\delta^{44/40}$Ca value measured after 14 weeks within the Scots pine organs represents a cumulative plant value from week 1 to week 14. In contrast, the $\delta^{44/40}$Ca value measured in the percolate corresponds to an instantaneous value (1 week). Consequently, this absence of visible fractionation within the Scots pine organs during the experiments suggests that the distribution of Ca isotopes in field trees results from successive steps and long-term Ca transport which is not yet sufficiently developed in Scots pine after 14 weeks of growth.

Apatite, Scots pine and bacteria

In the presence of bacteria, no Ca isotopic fractionation was detected in the solutions along the course of the $(P+B)_A$ experiment. This is in strong contrast to the experiment involving Scots pines alone $(P)_A$. Bacteria possibly modify the process of Ca uptake in such a way that Ca isotopic fractionation is inhibited or counterbalanced by additional opposite fractionation. Two hypotheses may explain these observations: (1) bacteria influence the pH and, therefore, the number of active Ca-binding sites at the root surfaces, or (2) bacteria promote Ca bioavailability for pine and modify the plant nutrition strategy.

(1) During the course of the experiment, the pH of the drainage solution decreases and then stabilizes after 8 weeks at an average value of 3.98 for $(P)_A$ and 4.53 for $(P+B)_A$ (Figs. 2(b) and 2(c)). These pH values were more acid than those in the $(B)_A$ experiment (pH ≈ 6.33; Fig. 2(a)), perhaps because of the acidic root exudates. Probably, bacterial activity could produce compounds (organic acids, phenolic compounds, biofilms)[18,25,36,56–58,60] that increase the pH of the percolate solution or inhibit the production of acids by roots (Figs. 2(b) and 2(c)). In acidic environments, the number of available Ca-binding sites at the surface of the roots decreases by replacement of the $R\text{-}COO^-$ groups of polygalacturonic acid molecules (pectins) with R-COOH sites (pKa = 3.6).[21,56] Since Ca binding to $R\text{-}COO^-$ sites is expected to be the main $\delta^{44/40}$Ca fractionating process,[35] plant nutrition at lower pH would cause smaller $\delta^{44/40}$Ca isotopic fractionation. The Ca isotopes in solution would also be less fractionated than at higher pH, although this has not been observed in our experiments.

(2) The main difference between the $(P)_B$ and $(P)_A$ experiments is that in the first experiment Ca is immediately available for root uptake whereas in the second microcosm experiment the nutritive solution is Ca-free. Therefore, the plant roots have first to release Ca from apatite through mineral dissolution. As an alternative one might therefore suggest that bacteria weather the apatite, release Ca and transfer it directly to the plant roots through biofilms produced by bacteria. Indeed, the biofilms might minimize nutrient elements loss by drainage.[23] If this Ca supply by bacteria fully covers the plants' demand no fractionation occurs. Further investigations are necessary to verify these hypotheses.

CONCLUSIONS

The impact of bacteria and root exudation on Ca-rich and Ca-poor minerals and, therefore, on Ca availability in soils of forest ecosystems was investigated using *in vitro* abiotic and biotic column experiments. Abiotic apatite weathering indicates that no Ca isotopic fractionation occurs during apatite dissolution, whatever the acid and pH used. Similarly, organic molecules produced by root exudation and soil bacteria do not cause Ca isotopic fractionation.

Biotic microcosm experiments helped to test the influence of soil bacteria on the Ca isotopic signatures of Scots pine organs and of drainage solution. Our results show that the Ca isotopic composition of Scots pine organs remained constant through time. This may be related to the youth of the Scot pines, limiting the extent of the fractionating reactions. Similarly, the presence of bacteria did not cause any Ca isotopic fractionation of the collected solution after 14 weeks of experimentation. A ^{44}Ca enrichment was only recorded for a solution in contact with Scots pines grown on a bacteria-free apatite substrate. These preliminary results might suggest that bacteria influence the Ca isotopic signature by dissolving Ca from apatite more efficiently. Finally, this preliminary experimental study involving mineral, plant and bacteria suggests that Ca isotopes might be suitable for detecting bacteria-mediated processes in soils.

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Conclusion générale

I. Conclusion générale

L'objectif principal de cette thèse était d'identifier et de préciser les processus biotiques et abiotiques qui contrôlent le comportement du Ca et le fractionnement des isotopes du Ca à l'interface géosphère/biosphère/hydrosphère. La synthèse des résultats obtenus au cours de ce travail est présentée dans la Figure VI.1.

Suite à la mise en place d'un nouveau protocole automatisé innovant pour la séparation chimique du Ca préalable à toute analyse par TIMS, les variations des compositions isotopiques du Ca au sein de différents organes de végétaux cultivés en milieu nutritif liquide ont été étudiées. Les résultats de cette première investigation expérimentale mettent en avant des variations de composition isotopique du Ca au sein des différents organes de végétaux analysés. Les plantes sont toujours enrichies en isotopes légers ^{40}Ca par rapport à la composition isotopique de la source de Ca quelle que soit la condition expérimentale. L'étude approfondie de la répartition des compositions isotopiques du Ca au sein des végétaux a permi d'identifier trois niveaux de fractionnement des isotopes du Ca au sein de la plante : (1) Le premier a eu lieu au cours de l'adsorption préférentielle du ^{40}Ca sur les sites d'échanges cationiques de l'apoplasme des racines latérales; (2) Le second fractionnement s'est déroule pendant la translocation du Ca des racines primaires vers les feuilles. Durant ce trajet, le ^{40}Ca se fixe sur les acides polygalacturoniques (pectines) de la lamelle moyenne de la paroi cellulaire des vaisseaux xylémiques; (3) Le troisième et dernier fractionnement s'est produit au niveau des organes reproducteurs, il est aussi causé par des processus d'échange cationique avec la pectine des parois cellulaires de ces organes. Cependant, la structure des parois cellulaires des organes reproducteurs et/ou le nombre de sites d'échange cationique disponible dans les parois cellulaire paraissent différents de ceux de la paroi cellulaire des vaisseaux xylémiques. Ces trois mécanismes qui fractionnent les isotopes du Ca au sein de la plante semblent contrôlés par la physico-chimie du milieu nutritif (concentration en Ca, pH) et conduisent à un enrichissement en isotopes légers ^{40}Ca. L'amplitude du fractionnement des isotopes du Ca au sein des organes de végétaux est fortement dépendante du pH et de la composition de la solution nutritive, si cette dernière est considérée comme une source infinie de Ca.

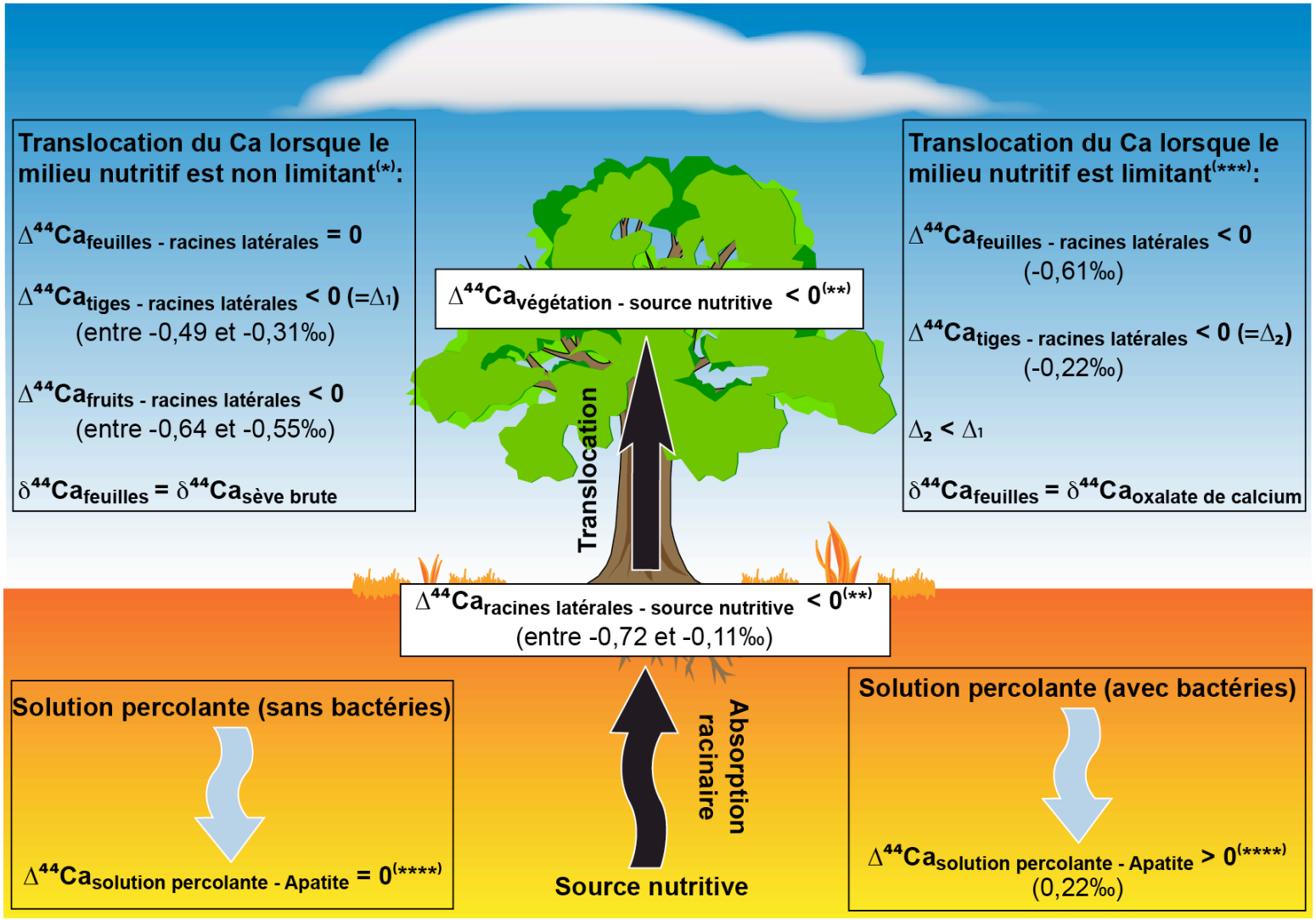

Figure VI.1 : *Récapitulatif et synthèse des principaux résultats de cette thèse. (*) Obtenu à partir des données du chapitre 3. (**) Obtenu à partir des données du chapitres 3, 4 et 5. (***) Obtenu à partir des données du Chapitre 4. (****) Obtenu à partir des données du chapitre 5.*

Ainsi, un faible pH (4) et une faible concentration (5ppm) n'entrainent pas d'effet sur la biomasse de plante produite. Cependant, cela induit une amplitude de fractionnement plus faible des isotopes du Ca au sein du plant de haricot comparée à celle observée pour les autres conditions expérimentales :

$$\Delta^{44}Ca_{\text{racines latérales-tiges}}\ (L4) < \Delta^{44}Ca_{\text{racines latérales-tiges}}\ (L6, H4 \text{ et } H6)$$

Une fois que les processus responsables des différents fractionnements des isotopes du Ca au sein des végétaux ont été identifiés, nous nous sommes intéressés à l'évolution de la signature isotopique en Ca de milieux nutritifs considérés comme non infinis en Ca (e.g. limitants ou non pour la croissance des plantes cultivées) et des différents organes de végétaux cultivés au cours du temps à partir de ces milieux nutritifs. Les résultats de cette deuxième étude expérimentale indiquent que lorsque les milieux nutritifs sont considérés comme non infinis en Ca (limitants ou non limitants pour la croissance des végétaux cultivés), la composition isotopique des solutions nutritives et des organes de plantes suit une loi de fractionnement des isotopes du Ca à l'équilibre (coefficient de fractionnement $\alpha_{\text{haricot-solution nutritive}}$=0,99858). De plus, l'enrichissement en ^{44}Ca observé au cours du temps pour les solutions nutritives non infinies en Ca (limitantes ou non limitantes pour la croissance des végétaux cultivés) engendre aussi un enrichissement en ^{44}Ca au sein des plantes cultivées. Nos résultats montrent également que, quelle que soit la quantité de Ca disponible dans le milieu nutritif (limitant ou non limitant pour la croissance des végétaux cultivés), les mécanismes responsables du fractionnement des isotopes du Ca lors de sa capture nutritive par les racines ou lors de sa translocation des racines vers les organes aériens sont identiques à ceux observés lors de la première étude expérimentale. Cependant, l'amplitude de fractionnement entre les différents organes change :

$$\Delta^{44}Ca_{\text{racines latérales-tiges}}\ (L6 \text{ limitant}) < \Delta^{44}Ca_{\text{racines latérales-tiges}}\ (L6 \text{ non limitant}) < \Delta^{44}Ca_{\text{racines latérales-tiges}}\ (L6 \text{ infini})$$

Enfin, lorsque la quantité de Ca devient limitante pour la croissance des plantes, nous proposons que ce soit uniquement les oxalates de Ca qui contrôlent la signature isotopique du Ca des feuilles.

La troisième et dernière étude expérimentale de culture de plantes, qui se déroule cette fois-ci sur un substratum solide (microcosme), nous a permis de tester l'influence des acides organiques et inorganiques des plantes et de l'activité bactérienne de la rhizosphère sur le rapport isotopique du Ca des solutions de sol et des organes de végétaux. Les résultats de

cette étude indiquent que le type d'acide (organique ou minéral) utilisé pour dissoudre le substratum solide composé d'apatite n'influence pas la signature isotopique en Ca de la solution percolante récupérée en sortie du dispositif expérimental. Au cours de cette expérience, seule l'activité combinée des bactéries de la rhizosphère et des racines de végétaux sur un substratum composé d'apatite influence la signature isotopique en Ca de la solution percolante, en enrichissant cette dernière de 0,22‰ en isotopes lourds ^{44}Ca.

Ce travail de doctorat a permis d'identifier et de préciser pour la première fois les processus biotiques et abiotiques qui influencent le fractionnement des isotopes du Ca dans le système sol/plante lors d'expériences en milieux contrôlés. Il a également permis de confirmer que la végétation engendre des fractionnements significatifs des isotopes du Ca et qu'elle semble pouvoir influencer le flux de Ca au cours de sa croissance. Cependant, les aspects présentés au cours de cette étude doivent être clarifiés ou confirmés lors de futures études sur le terrain. Ainsi, il est nécessaire de préciser ou de confirmer sur le terrain :

- le comportement des isotopes du Ca au sein des monocotylédones et des dicotylédones ;
- les différents niveaux de fractionnement des isotopes du Ca au sein des végétaux ;
- l'influence de l'activité combinée des bactéries de la rhizosphère et de l'activité racinaire sur la signature isotopique des solutions de sol ;
- l'impact de l'activité des bactéries de la rhizosphère sur la signature isotopique des végétaux qui leur sont associés ;
- le comportement des isotopes du Ca lors de la translocation du Ca des racines vers les organes aériens. En effet, au cours de nos différentes expérimentations, nous ne constatons aucun fractionnement vertical des isotopes du Ca au sein d'une même famille d'organes (p.ex : feuilles basales et feuilles apicales ou tiges basales et tiges apicales) lors de la translocation du Ca. Or, les études de terrain semblent indiquer le contraire ;
- le comportement des isotopes du Ca au niveau des organes reproducteurs, car peu d'éléments sont connus sur ce sujet ;
- le possible enregistrement des modifications physico-chimiques du milieu nutritif au cours de la vie des arbres dans leurs cernes ;

Pour conclure, les résultats présentés dans cette thèse confirment le fort potentiel des isotopes du Ca, à la fois en physiologie végétale et en biogéochimie. Ils montrent également

que les isotopes du Ca sont de très bons outils pour étudier les processus biotiques et abiotiques à l'interface sol- eau- plante.

II. Perspectives pour le cycle biogéochimique du Ca

Les expérimentations en milieu contrôlé (hydroponiques et microcosmes ; Chapitres 3, 4 et 5) réalisées au cours de cette thèse ont permis de mieux contraindre les mécanismes induisant des fractionnements isotopiques du Ca à l'interface solution nutritive/plante et au sein des plantes. Ainsi, nous pouvons maintenant essayer de transposer les interprétations résultantes de ces travaux à l'échelle du bassin versant et d'en déduire les applications potentielles des isotopes du Ca lors de futures études en milieu naturel.

II.1. Implications sur le cycle biogéochimique du Ca à l'échelle du bassin versant

La transposition de nos interprétations obtenues lors des expérimentations présentées chapitres 3, 4 et 5 à l'échelle du bassin versant, permettrait d'affirmer que la végétation va influencer le cycle biogéochimique du Ca à cette échelle. En effet, nos interprétations indiqueraient que la capture du Ca par les racines et le recyclage des feuilles et aiguilles de la litière forestière pourraient influencer le cycle du Ca à l'échelle du bassin versant. Ainsi, la capture nutritive du Ca par les racines enrichirait les solutions de sol en ^{44}Ca. Il semblerait également que l'amplitude de fractionnement $\Delta^{44}Ca_{racines\text{-}solution\ de\ sol}$ dans les écosystèmes forestiers soit contrôlée par la quantité de Ca disponible dans les solutions de sol. Cette amplitude de fractionnement des isotopes du Ca serait minimale entre les isotopes légers ^{40}Ca préférentiellement capturés par les végétaux, et les isotopes lourds ^{44}Ca qui s'accumulent dans le milieu nutritif lorsque la quantité de Ca présente dans les solutions de sol est considérée comme limitante pour la croissance des arbres. Le recyclage des feuilles de la litière forestière pourrait quant à lui apporter une grande quantité de ^{40}Ca aux solutions de sol à l'échelle d'un bassin versant, *via* la minéralisation de la litière forestière. La quantité de ^{40}Ca ainsi remise en circulation serait d'autant plus importante que les écosystèmes forestiers possèderaient des sols pauvres en Ca. Il semblerait aussi que la quantité de Ca présente dans les sols forestiers soit un des paramètres qui pourrait influencer la quantité de Ca provenant du recyclage de la litière forestière. Ainsi, le Ca des feuilles d'arbres ayant poussé sur des sols pauvres en Ca pourrait être majoritairement sous forme d'oxalate de Ca. Les cristaux d'oxalate de Ca sont

difficilement solubles dans le milieu naturel. Cela pourrait influencer le flux de Ca dans les sols à l'échelle d'un bassin versant.

D'après Cenki-Tok et al. (2009) et Holmden et Bélanger (2010), qui présentent les analyses les plus complètes sur l'étude de la variation isotopique du Ca au sein des végétaux, la variation de la composition isotopique du Ca au sein des feuilles et aiguilles d'arbres présentement mesurées est comprise entre 0,24 et 1,5‰ (Annexe 1). Or, il existe dans le milieu naturel des variations de composition isotopique du Ca au sein des feuilles d'un même arbre, entre les feuilles basales et les feuilles apicales ou entre de jeunes aiguilles et d'autres plus âgées (Cenki-Tok et al., 2009). Cependant, Cenki-Tok et al. (2009) montrent que la composition isotopique de la litière forestière est cohérente avec la composition isotopique moyenne du Ca au sein des feuilles d'un même arbre. C'est pourquoi il semble possible d'utiliser la composition isotopique du Ca de la litière forestière pour estimer l'impact de la végétation sur la signature isotopique actuelle du Ca des rivières d'un bassin versant. Nous savons que la signature isotopique actuelle moyenne du Ca des grands fleuves pondérée par les flux de Ca arrivant aux océans est égale à 0,76‰ (Tipper et al., 2010). Cependant cette valeur néglige l'influence potentielle de la végétation sur la signature isotopique en Ca des eaux des grands fleuves. De plus, nous savons également que la composition isotopique du Ca de la litière forestière varie entre 0,08 et 0,71‰ (Page et al., 2008 ; Cenki-Tok et al., 2009).

L'utilisation d'un modèle simple basée sur un calcul de mélange entre deux pôles est possible en première approche pour estimer l'influence de la litière sur la composition isotopique en Ca des grands fleuves à leur embouchure. Pour cela, nous utiliserons l'équation de mélange suivante :

$$\delta^{44/40}Ca_{\text{grands fleuves estimée}} = F* \delta^{44/40}Ca_{\text{litière}} + (1-F)* \delta^{44/40}Ca_{\text{moyenne grands fleuves}} \quad (VI.1)$$

où, F correspond à la fraction de Ca provenant du recyclage de la litière forestière.

D'après Zakharova et al. (2007) le recyclage de la litière forestière peut contribuer jusqu'à 35% du Ca présent dans les eaux de surface d'un petit bassin versant boréal. Si nous transposons cette valeur à l'échelle globale, nous nous apercevons que, pour une composition isotopique de la litière forestière ≤ 0,15‰, la signature isotopique des grands fleuves devient significativement différente des barres d'erreur et que la végétation va enrichir au maximum les grands fleuves en calcium léger d'environ 0,27‰ (Figure VI.2). Cela est plus de deux fois supérieur à la limite analytique actuelle de mesure des isotopes du Ca par TIMS au LHyGeS. Sachant que la valeur moyenne de litière forestière est d'environ 0,40‰ (Annexe 1), nos

estimations indiquent que la végétation n'impacterait que faiblement la signature isotopique des grands fleuves, ce qui est en accord avec les travaux de Tipper et al. (2010). En effet, ce dernier estime que la signature isotopique en Ca des grands fleuves est principalement contrôlée par la dissolution des carbonates de Ca sur les continents. Cependant, il n'existe pas de données suffisantes permettant de déterminer l'importance du recyclage de la litière sous d'autres conditions climatiques et d'autres environnements géologiques. Enfin, il faut noter que la variation maximale estimée du $\delta^{44/40}Ca$ des grands fleuves, induite par la végétation, est du même ordre de grandeur que la variation maximale estimée pour les isotopes du Mg et les isotopes du Si (Bolou-Bi et al., 2010 ; Hughes, 2011).

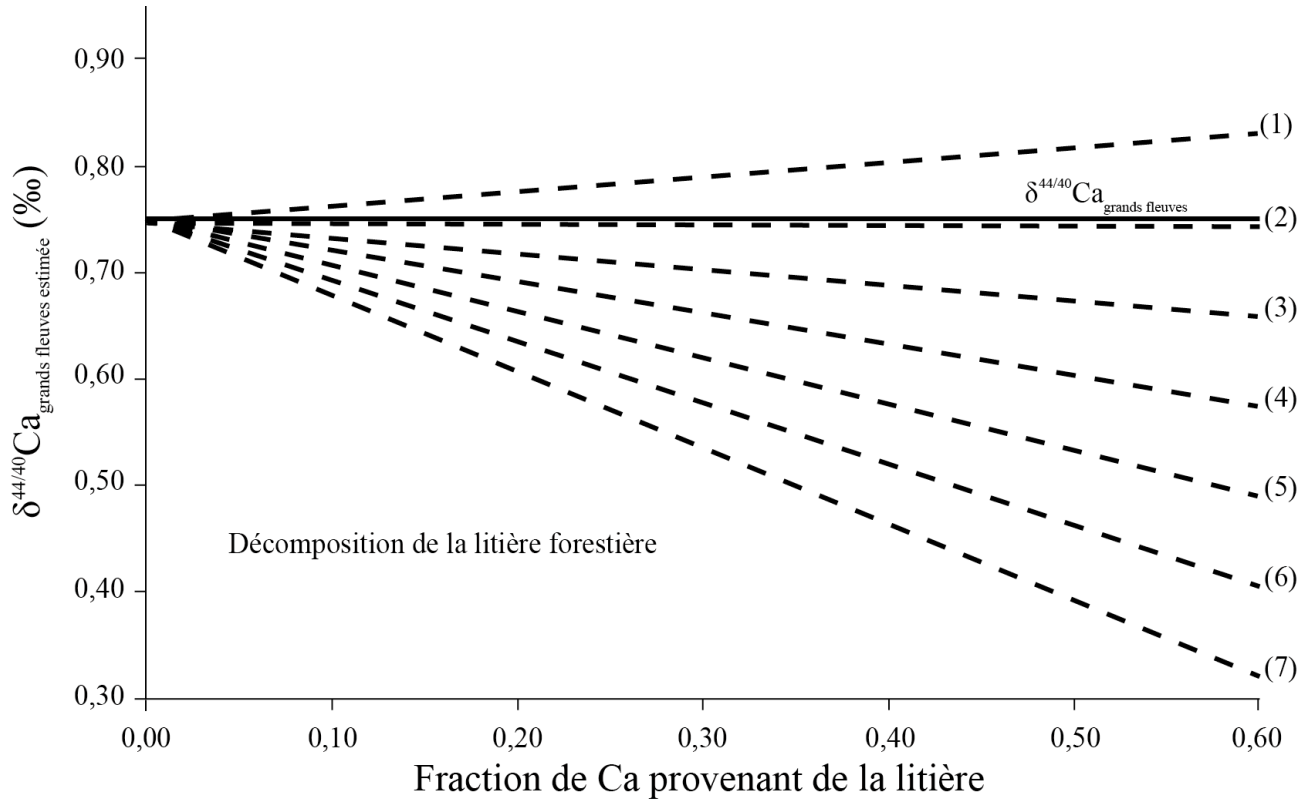

Figure VI.2 : *Évolution du $\delta^{44/40}Ca_{grands\ fleuves\ estimé}$ en fonction de la fraction de Ca issue de la litière forestière. La droite pleine correspond au $\delta^{44/40}Ca_{grands\ fleuves}$ (Tipper et al., 2010). Les droites en pointillés correspondent à différentes valeurs moyennes $\delta^{44/40}Ca_{végétation}$: 0,90‰ (1), 0,75‰ (2), 0,60‰ (3), 0,45‰ (4), 0,30‰ (5), 0,15‰ (6) et 0,00‰ (7).*

II.2. Potentiel des isotopes du Ca appliqués à des cernes d'arbres

Si l'on peut extrapoler les résultats de nos expérimentations présentés dans les chapitres 3 et 5 au milieu naturel, on pourrait suggérer que les cernes d'arbres enregistrent les modifications physico-chimiques des solutions nutritives au cours du temps. En effet, l'étude du fractionnement des isotopes du Ca au sein des plants de haricot semble indiquer que le pH et la quantité de Ca disponible dans la solution nutritive influencent les amplitudes de fractionnement des isotopes du Ca observées aux sein des plants de haricot. En effet, une solution nutritive avec un faible pH (4) et une faible concentration en Ca (5 ppm) entraine une faible amplitude du fractionnement des isotopes du Ca au sein des plants de haricot, comparée à celles observées lorsque le pH et la concentration en Ca sont élevés (Chapitre 3). De plus, la quantité de Ca disponible dans la solution nutritive pourrait également influencer l'amplitude de fractionnement des isotopes du Ca au sein des végétaux. Nos résultats indiquent que l'amplitude de fractionnement des isotopes du Ca au sein des plantes est la plus faible lorsque la quantité de Ca disponible dans le milieu nutritif est limitante pour la croissance des haricots, tandis que l'amplitude la plus forte s'observe lorsque la quantité de Ca dans le milieu nutritif est considérée comme infinie. Enfin, d'après nos interprétations, il semble que le $\delta^{44/40}$Ca moyen des plants de haricots est fonction de l'évolution du $\delta^{44/40}$Ca du milieu nutritif (Chapitre 4).

Ainsi, la transposition de ces interprétations au milieu naturel permet d'avancer l'hypothèse qu'il est possible d'utiliser les isotopes du Ca pour étudier les modifications physico-chimiques du réservoir de Ca au cours de la durée de vie des grands arbres. Cela est possible grâce à l'étude du fractionnement des isotopes du Ca dans les cernes d'arbres. En effet, les arbres enregistrent au cours de leur vie les évolutions physico-chimiques de leur environnement, au niveau de leurs cernes de croissance, ils sont donc considérés comme les « archives du passé » (p.ex. du climat, de l'influence anthropique ; Edwards et Fritz, 1986 ; De Niro et Cooper, 1990 ; Edwards, 1990 ; Hagemeyer et Schäfer, 1995 ; Nabais et al., 1999 ; Bindler et al., 2004 ; McCarroll et Loader, 2004 ; Battipaglia et al, 2008 ; Brüggemann et., 2011 ; Leavitt et al., 2008 ; Stille et al., 2012 ; Annexe 3).

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Annexe 1

Type	Numéro	Déscritption	Localisation	Échantillonage	$\delta^{40}Ca_{SRM915a}$	$2s_{mean}$	Ref
Précipitations							
Pluie	E 5448	–	Aubure, Fr	10/02-24/02/98	0.50	0.13	Schmitt et al. (2003)
Pluie	EPA 3	–	Aubure, Fr	11/05-08/06/99	1.01	0.15	Schmitt et al. (2003)
Pluie	EPS 5	–	Strasbourg, Fr	18/03-17/05/99	0.69	0.18	Schmitt et al. (2005)
Pluie	EPL 3	–	Luxembourg, Lux	27/06-06/10/99	0.27	0.15	Schmitt et al. (2005)
Pluie	E 6011	–	Berkeley, CA	25/03/01	0.38	0.13	Schmitt et al. (2005)
Pluie	E 5828	–	Normandie, Fr	17/07/01	0.73	0.27	Schmitt et al. (2005)
Pluie	E 6013	–	Zweisimmen, CH	04/07-16/07/2001	0.50	0.21	Schmitt et al. (2005)
Pluie	PA 6319	–	Aubure, Fr	22.May 2006	0.57	0.21	Cenki-Tok et al. (2009)
Pluie	PA 5560	–	Aubure, Fr	22.August 2005	0.93	0.17	Cenki-Tok et al. (2009)
Pluie	BP3	–	La Ronge, Ca	6 May 2005	0.57	0.04	Holmden et Bélanger (2010)
Pluie	BP3	–	La Ronge, Ca	30 May 2005	0.57	0.05	Holmden et Bélanger (2010)
Pluie	–	–	Damma glacier, CH	08072008	0.97	0.07	Hindshow et al. (2011)
Pluie	–	–	Damma glacier, CH	16092008	0.76	0.07	Hindshow et al. (2011)
Pluie	–	–	Damma glacier, CH	30092008	0.76	0.07	Hindshow et al. (2011)
Pluie	–	–	Wachusett Mt, USA	–	0.90	0.10	Farkas et al. (2011)
Neige	E 6012	–	Cadarache, Fr	02 March 2001	0.22	0.1	Schmitt et al. (2005)
Neige	E 6014	–	Japan	20 January 2001	0.68	0.17	Schmitt et al. (2005)
Neige	Nemeiben lake 1	–	La Ronge, Ca	–	0.65	0.05	Holmden et Bélanger (2010)
Neige	Nemeiben lake 2	–	La Ronge, Ca	–	0.66	0.05	Holmden et Bélanger (2010)
Neige	lac la Ronge 1	–	La Ronge, Ca	–	0.45	0.04	Holmden et Bélanger (2010)
Neige	lac la Ronge 2	–	La Ronge, Ca	–	0.61	0.04	Holmden et Bélanger (2010)
Neige	–	–	Damma glacier, CH	13052008	1.03	0.07	Hindshow et al. (2011)
Neige	–	–	Damma glacier, CH	28012008	1.22	0.07	Hindshow et al. (2011)
Neige	–	–	Wachusett Mt, USA	–	0.96	0.13	Farkas et al. (2011)
Pluviolessivat	PLH 5563	–	Aubure, Fr	22.August 2005	0.29	0.17	Cenki-Tok et al. (2009)
Pluviolessivat	PLH 6323	–	Aubure, Fr	22.May 2006	0.80	0.21	Cenki-Tok et al. (2009)
Pluviolessivat	–	–	Aubure, Fr	–	0.75	0.15	Schmitt et al. (2005)
Poussière	–	Poussière continentale	Hawai	–	-0.40	0.20	Wiegand et al. (2005)
Poussière	A2PM10	Aérosol <10 µm	Atacama	–	0.81	0.15	Ewing et al. (2008)
Poussière	A2>2.5	Aérosol 2.5<<12 µm	Atacama	–	0.81	0.15	Ewing et al. (2008)
Poussière	Y1	Dépôts total en 1 an	Atacama	–	0.70	0.15	Ewing et al. (2008)
Poussière	A1a	Dépôt	Atacama	–	0.95	0.15	Ewing et al. (2008)
Poussière	C2b	Dépôt	Atacama	–	0.70	0.15	Ewing et al. (2008)
Végétations							
Herbes	–	–	–	–	-0.22	–	Bullen et al. (2004)
Herbes	Site 2 A	–	–	–	-0.18	0.15	Chu et al. (2006)
Herbes	Site 2 B	–	–	–	-0.69	0.11	Chu et al. (2006)
Herbes	Site 4 A	–	–	–	-0.52	0.11	Chu et al. (2006)
Herbes	Site 4 B	–	–	–	-0.65	0.11	Chu et al. (2006)
Herbes	farm A	–	–	–	-0.94	0.11	Chu et al. (2006)
Herbes	farm B	–	–	–	-0.04	0.15	Chu et al. (2006)
Bruyère	A	–	–	–	-1.23	0.15	Chu et al. (2006)
Bruyère	B	–	–	–	-0.72	0.15	Chu et al. (2006)
Luzerne	–	alfalta SV	–	–	0.43	0.11	Skulan & DePaolo (1999)
Fétuque	–	Fescue alta	–	–	-0.19	0.20	Skulan & DePaolo (1999)
Hêtre	–	Feuilles	Aubure, Fr	2002	0.25	0.14	Schmitt et al. (2003)
Hêtre	LP40	Feuilles apicales	Aubure, Fr	13.Sept.2005	0.24	0.15	Cenki-Tok et al. (2009)
Hêtre	LP36	Feuille basales	Aubure, Fr	21.Sept.2005	0.64	0.21	Cenki-Tok et al. (2009)
Metrosideros	–	Feuilles	Thurston, USA	–	0.59	0.18	Wiegand et al. (2005)
Metrosideros	–	Feuilles	Kohala, USA	–	0.87	0.18	Wiegand et al. (2005)
Metrosideros	–	Feuilles	Molokai, USA	–	1.76	0.40	Wiegand et al. (2005)
Metrosideros	–	Feuilles	Kauai, USA	–	1.43	0.17	Wiegand et al. (2005)
Tremble d'aspen	Plot 1.1	Feuilles	La Ronge, Ca	–	0.58	0.05	Holmden et Bélanger (2010)
Tremble d'aspen	Plot 1.2	Feuilles	La Ronge, Ca	–	0.92	0.04	Holmden et Bélanger (2010)
Tremble d'aspen	Plot 1.3	Feuilles	La Ronge, Ca	–	0.99	0.04	Holmden et Bélanger (2010)
Peuplier baumier	Plot 1.3	Feuilles	La Ronge, Ca	–	0.98	0.04	Holmden et Bélanger (2010)
Laurier-rose des Alpes	RhL_Y_6_08	Feuilles	Damma glacier, CH	–	-0.48	0.07	Hindshow et al. (2011)
Laurier-rose des Alpes	RhL_Y_7_08	Feuilles	Damma glacier, CH	–	-0.40	0.07	Hindshow et al. (2011)
Laurier-rose des Alpes	RhL_Y_8_08	Feuilles	Damma glacier, CH	–	-0.46	0.07	Hindshow et al. (2011)
Épicéa	ELV5	Aiguilles jeunes	Aubure, Fr	15.May 2006	0.28	0.15	Cenki-Tok et al. (2009)
Épicéa	ELV3	Aiguilles vieilles	Aubure, Fr	15.May 2006	0.80	0.12	Cenki-Tok et al. (2009)
Pin gris	Plot 1.1	Aiguilles	La Ronge, Ca	–	1.24	0.06	Holmden et Bélanger (2010)
Pin gris	Plot 1.2	Aiguilles	La Ronge, Ca	–	1.23	0.07	Holmden et Bélanger (2010)
Pin gris	Plot 2.2	Aiguilles	La Ronge, Ca	–	1.50	0.06	Holmden et Bélanger (2010)
Épicéa noir (jeune)	Plot 1.1	Aiguilles	La Ronge, Ca	–	0.81	0.04	Holmden et Bélanger (2010)
Épicéa noir (jeune)	Plot 1.2	Aiguilles	La Ronge, Ca	–	0.57	0.06	Holmden et Bélanger (2010)
Épicéa noir (jeune)	Plot 1.3	Aiguilles	La Ronge, Ca	–	0.54	0.05	Holmden et Bélanger (2010)
Épicéa noir (vieux)	Plot 1.1	Aiguilles	La Ronge, Ca	–	0.68	0.04	Holmden et Bélanger (2010)
Épicéa noir (vieux)	Plot 1.2	Aiguilles	La Ronge, Ca	–	0.64	0.05	Holmden et Bélanger (2010)
Épicéa noir (vieux)	Plot 1.3	Aiguilles	La Ronge, Ca	–	0.57	0.05	Holmden et Bélanger (2010)
Épicéa noir	a Plot 2.1	Aiguilles	La Ronge, Ca	–	0.61	0.04	Holmden et Bélanger (2010)
Épicéa noir	a Plot 2.2	Aiguilles	La Ronge, Ca	–	0.45	0.06	Holmden et Bélanger (2010)
Épicéa noir	a Plot 2.3	Aiguilles	La Ronge, Ca	–	0.60	0.07	Holmden et Bélanger (2010)
Épicéa noir	b Plot 2.1	Aiguilles	La Ronge, Ca	–	0.84	0.04	Holmden et Bélanger (2010)
Épicéa noir	b Plot 2.2	Aiguilles	La Ronge, Ca	–	0.66	0.04	Holmden et Bélanger (2010)
Épicéa noir	b Plot 2.3	Aiguilles	La Ronge, Ca	–	0.45	0.04	Holmden et Bélanger (2010)
Épinette blanche	Plot 1.3	Aiguilles	La Ronge, Ca	–	0.56	0.05	Holmden et Bélanger (2010)
Hêtre	–	Branches	Aubure, Fr	2002	-0.57	0.12	Schmitt et al. (2003)
Hêtre	Catchment 14	Branches	Archer Creek, USA	–	-0.83	–	Page et al. (2008)
Hêtre	Catchment 15	Branches	Archer Creek, USA	–	-1.19	–	Page et al. (2008)
Hêtre	LP37	Branches apicales ø 1 cm	Aubure, Fr	21.Sept.2005	0.09	0.12	Cenki-Tok et al. (2009)
Érable à sucre	Catchment 14	Branches	Archer Creek, USA	–	-0.86	–	Page et al. (2008)
Érable à sucre	Catchment 15	Branches	Archer Creek, USA	–	-1.69	–	Page et al. (2008)
Tremble d'aspen	Plot 1.1	Branches	La Ronge, Ca	–	0.09	0.04	Holmden et Bélanger (2010)
Tremble d'aspen	Plot 1.2	Branches	La Ronge, Ca	–	0.44	0.06	Holmden et Bélanger (2010)
Tremble d'aspen	Plot 1.3	Branches	La Ronge, Ca	–	0.48	0.04	Holmden et Bélanger (2010)
Peuplier baumier	Plot 1.3	Branches	La Ronge, Ca	–	0.23	0.04	Holmden et Bélanger (2010)
Chêne rouge d'Amérique	wood 1	Cernes, 0 à 10 ans	Wachusett Mt, USA	–	-0.82	0.10	Farkas et al. (2011)
Chêne rouge d'Amérique	wood 2	Cernes, 10 à 20 ans	Wachusett Mt, USA	–	-0.73	0.12	Farkas et al. (2011)

Type	Numéro	Déscritption	Localisation	Échantillonage	$\delta^{40}Ca_{SRM915a}$	$2s_{mean}$	Ref
Chêne rouge d'Amérique	wood 3	Cernes, 20 à 30 ans	Wachusett Mt, USA	–	-0.81	0.14	Farkas et al. (2011)
Chêne rouge d'Amérique	wood 4	Cernes, 30 à 40 ans	Wachusett Mt, USA	–	-0.80	0.24	Farkas et al. (2011)
Chêne rouge d'Amérique	wood 5	Cernes, 40 à 50 ans	Wachusett Mt, USA	–	-0.58	0.27	Farkas et al. (2011)
Chêne rouge d'Amérique	wood 6	Cernes, 50 à 60 ans	Wachusett Mt, USA	–	-0.73	0.16	Farkas et al. (2011)
Chêne rouge d'Amérique	wood 7	Cernes, 60 à 70 ans	Wachusett Mt, USA	–	-0.81	0.08	Farkas et al. (2011)
Chêne rouge d'Amérique	wood 8	Cernes, 70 à 80 ans	Wachusett Mt, USA	–	-0.79	0.10	Farkas et al. (2011)
Chêne rouge d'Amérique	wood 9	Cernes, 80 à 90 ans	Wachusett Mt, USA	–	-0.92	0.11	Farkas et al. (2011)
Chêne rouge d'Amérique	wood 10	Cernes, 90 à 100 ans	Wachusett Mt, USA	–	-0.58	0.03	Farkas et al. (2011)
Chêne rouge d'Amérique	wood 11	Cernes, 100 à 110 ans	Wachusett Mt, USA	–	-0.90	0.00	Farkas et al. (2011)
Chêne rouge d'Amérique	wood 12	Cernes, 110 à 120 ans	Wachusett Mt, USA	–	-0.67	0.06	Farkas et al. (2011)
Chêne rouge d'Amérique	wood 13	Cernes, 120 à 130 ans	Wachusett Mt, USA	–	-0.57	0.12	Farkas et al. (2011)
Chêne rouge d'Amérique	wood 14	Cernes, 130 à 140 ans	Wachusett Mt, USA	–	-0.59	0.17	Farkas et al. (2011)
Chêne rouge d'Amérique	wood 15	Cernes, 140 à 150 ans	Wachusett Mt, USA	–	-0.44	0.06	Farkas et al. (2011)
Chêne rouge d'Amérique	wood 16	Cernes, 150 à 160 ans	Wachusett Mt, USA	–	-0.44	0.07	Farkas et al. (2011)
Chêne rouge d'Amérique	wood 17	Cernes, 160 à 170 ans	Wachusett Mt, USA	–	-0.43	0.04	Farkas et al. (2011)
Chêne rouge d'Amérique	wood 18	Cernes, 170 à 180 ans	Wachusett Mt, USA	–	-0.38	0.13	Farkas et al. (2011)
Chêne rouge d'Amérique	wood 19	Cernes, 180 à 190 ans	Wachusett Mt, USA	–	-0.41	0.03	Farkas et al. (2011)
Chêne rouge d'Amérique	wood 20	Cernes, 190 à 200 ans	Wachusett Mt, USA	–	-0.36	0.01	Farkas et al. (2011)
Chêne rouge d'Amérique	wood 21	Cernes, 200 à 210 ans	Wachusett Mt, USA	–	-0.38	0.12	Farkas et al. (2011)
Chêne rouge d'Amérique	wood 22	Cernes, 210 à 220 ans	Wachusett Mt, USA	–	-0.44	0.12	Farkas et al. (2011)
Chêne rouge d'Amérique	wood 23	Cernes, 220 à 230 ans	Wachusett Mt, USA	–	-0.58	0.07	Farkas et al. (2011)
Épicéa	ELV 6	Bois	Aubure, Fr	23.May 2006	0.42	0.15	Cenki-Tok et al. (2009)
Pin gris	Plot 1.1	Branches	La Ronge, Ca	–	0.74	0.06	Holmden et Bélanger (2010)
Pin gris	Plot 1.2	Branches	La Ronge, Ca	–	0.69	0.05	Holmden et Bélanger (2010)
Pin gris	Plot 2.2	Branches	La Ronge, Ca	–	0.99	0.04	Holmden et Bélanger (2010)
Épicéa noir (vieux)	Plot 1.1	Branches	La Ronge, Ca	–	0.30	0.05	Holmden et Bélanger (2010)
Épicéa noir (vieux)	Plot 1.2	Branches	La Ronge, Ca	–	0.20	0.05	Holmden et Bélanger (2010)
Épicéa noir (vieux)	Plot 1.3	Branches	La Ronge, Ca	–	0.28	0.06	Holmden et Bélanger (2010)
Épicéa noir	b Plot 2.1	Branches	La Ronge, Ca	–	0.62	0.04	Holmden et Bélanger (2010)
Épicéa noir	b Plot 2.2	Branches	La Ronge, Ca	–	0.07	0.04	Holmden et Bélanger (2010)
Épicéa noir	b Plot 2.3	Branches	La Ronge, Ca	–	0.23	0.04	Holmden et Bélanger (2010)
Épinette blanche	Plot 1.3	Branches	La Ronge, Ca	–	0.24	0.04	Holmden et Bélanger (2010)
Hêtre	LD	Sève brute	Aubure, Fr	–	0.16	0.15	Cenki-Tok et al. (2009)
Hêtre	Catchment 14	Racines	Archer Creek, USA	–	-2.11	–	Page et al. (2008)
Hêtre	Catchment 15	Racines	Archer Creek, USA	–	-1.11	–	Page et al. (2008)
Hêtre	LP39	Racines fines	Aubure, Fr	13.Sept.2005	0.17	0.21	Cenki-Tok et al. (2009)
Hêtre	LP34	Racines ⌀ 2-4 mm	Aubure, Fr	13.Sept.2005	-0.48	0.09	Cenki-Tok et al. (2009)
Hêtre	LP38	Racines ⌀ 1-1,5 cm	Aubure, Fr	13.Sept.2005	0.08	0.15	Cenki-Tok et al. (2009)
Érable à sucre	Catchment 14	Racines	Archer Creek, USA	–	-2.20	–	Page et al. (2008)
Érable à sucre	Catchment 15	Racines	Archer Creek, USA	–	-2.07	–	Page et al. (2008)
Tremble d'aspen	Plot 1.1	Racines ⌀ 2 mm	La Ronge, Ca	–	-0.30	0.10	Holmden et Bélanger (2010)
Tremble d'aspen	Plot 1.2	Racines ⌀ 2 mm	La Ronge, Ca	–	-0.15	0.03	Holmden et Bélanger (2010)
Tremble d'aspen	Plot 1.2	Racines ⌀ 0.5 mm	La Ronge, Ca	–	0.62	0.04	Holmden et Bélanger (2010)
Épicéa	EL V2	Racines ⌀ 2-4 mm	Aubure, Fr	23.May 2006	0.04	0.07	Cenki-Tok et al. (2009)
Pin gris	Plot 1.1	Racines ⌀ 2 mm	La Ronge, Ca	–	0.31	0.05	Holmden et Bélanger (2010)
Pin gris	Plot 2.2	Racines	La Ronge, Ca	–	0.34	0.04	Holmden et Bélanger (2010)
Épicéa noir	Plot 1.2	Racines ⌀ 2 mm	La Ronge, Ca	–	-0.23	0.04	Holmden et Bélanger (2010)
Épicéa noir	Plot 2.1	Racines ⌀ 2 mm	La Ronge, Ca	–	-0.23	0.04	Holmden et Bélanger (2010)
Épicéa noir	Plot 2.2	Racines ⌀ 2 mm	La Ronge, Ca	–	-0.32	0.03	Holmden et Bélanger (2010)
Hêtre	Catchment 14	Litière forestière	Archer Creek, USA	–	0.08	0.05	Page et al. (2008)
Hêtre	Catchment 15	Litière forestière	Archer Creek, USA	–	0.61	0.16	Page et al. (2008)
Hêtre	LHP (LP29)	Litière forestière	Aubure, Fr	–	0.50	0.10	Cenki-Tok et al. (2009)
Épicéa	LVP (LP24)	Litière forestière	Aubure, Fr	–	0.64	0.10	Cenki-Tok et al. (2009)
Érable à sucre	Catchment 14	Litière forestière	Archer Creek, USA	–	0.22	0.05	Page et al. (2008)
Érable à sucre	Catchment 15	Litière forestière	Archer Creek, USA	–	0.54	0.18	Page et al. (2008)
Frêne d'amérique	Catchment 14	Litière forestière	Archer Creek, USA	–	0.31	0.23	Page et al. (2008)
Frêne d'amérique	Catchment 15	Litière forestière	Archer Creek, USA	–	0.71	0.12	Page et al. (2008)
Tilleul	Catchment 14	Litière forestière	Archer Creek, USA	–	0.50	0.09	Page et al. (2008)
sols							
Solutions de sols	E4 6341	-10 cm	Aubure, Fr	22.May 2006	1.00	0.21	Cenki-Tok et al. (2009)
Solutions de sols	E5 6342	- 60 cm	Aubure, Fr	22.May 2006	1.47	0.21	Cenki-Tok et al. (2009)
Solutions de sols	E4 5356	-10 cm	Aubure, Fr	3.May 2005	0.97	0.17	Cenki-Tok et al. (2009)
Solutions de sols	E5 5357	- 60 cm	Aubure, Fr	3.May 2005	1.41	0.17	Cenki-Tok et al. (2009)
Solutions de sols	F-5 4443	- 5 cm	Aubure, Fr	28.Sept.2004	0.85	0.17	Cenki-Tok et al. (2009)
Solutions de sols	F-5 6194	- 5 cm	Aubure, Fr	7.April 2006	1.00	0.15	Cenki-Tok et al. (2009)
Solutions de sols	F-10 6195	- 10 cm	Aubure, Fr	7.April 2006	0.77	0.15	Cenki-Tok et al. (2009)
Solutions de sols	F-30 6196	- 30 cm	Aubure, Fr	7.April 2006	0.78	0.17	Cenki-Tok et al. (2009)
Solutions de sols	F-60 6197	- 60 cm	Aubure, Fr	7.April 2006	0.89	0.15	Cenki-Tok et al. (2009)
Solutions de sols	F-5 6337	- 5 cm	Aubure, Fr	22.May 2006	0.69	0.17	Cenki-Tok et al. (2009)
Solutions de sols	F-10 6338	- 10 cm	Aubure, Fr	22.May 2006	0.76	0.17	Cenki-Tok et al. (2009)
Solutions de sols	F-30 6339	- 30 cm	Aubure, Fr	22.May 2006	0.86	0.17	Cenki-Tok et al. (2009)
Solutions de sols	F-60 6340	- 60 cm	Aubure, Fr	22.May 2006	0.75	0.17	Cenki-Tok et al. (2009)
Solutions de sols	Plot 1.1	- 10 cm	La Ronge, Ca	–	0.86	0.04	Holmden et Bélanger (2010)
Solutions de sols	Plot 1.2	- 10 cm	La Ronge, Ca	–	0.83	0.05	Holmden et Bélanger (2010)
Solutions de sols	Plot 1.3	- 10 cm	La Ronge, Ca	–	1.03	0.04	Holmden et Bélanger (2010)
Solutions de sols	Plot 2.1	- 10 cm	La Ronge, Ca	–	0.85	0.04	Holmden et Bélanger (2010)
Solutions de sols	Plot 2.2	- 10 cm	La Ronge, Ca	–	1.05	0.04	Holmden et Bélanger (2010)
Solutions de sols	Plot 2.3	- 10 cm	La Ronge, Ca	–	0.97	0.03	Holmden et Bélanger (2010)
Solutions de sols	Plot 1.3	- 20 cm	La Ronge, Ca	–	1.33	0.06	Holmden et Bélanger (2010)
Solutions de sols	Plot 2.3	- 20 cm	La Ronge, Ca	–	0.98	0.03	Holmden et Bélanger (2010)
Solutions de sols	Plot 1.1	- 35 cm	La Ronge, Ca	–	1.09	0.05	Holmden et Bélanger (2010)
Solutions de sols	Plot 1.2	- 35 cm	La Ronge, Ca	–	1.19	0.06	Holmden et Bélanger (2010)
Solutions de sols	Plot 1.3	- 35 cm	La Ronge, Ca	–	1.43	0.03	Holmden et Bélanger (2010)
Solutions de sols	Plot 2.1	- 35 cm	La Ronge, Ca	–	1.00	0.04	Holmden et Bélanger (2010)
Solutions de sols	Plot 2.2	- 35 cm	La Ronge, Ca	–	1.10	0.07	Holmden et Bélanger (2010)
Solutions de sols	Plot 2.3	- 35 cm	La Ronge, Ca	–	1.07	0.06	Holmden et Bélanger (2010)
Sol total	–	–	Thurston, USA	–	0.86	0.67	Wiegand et al. (2005)
Sol total	–	–	Kohala, USA	–	0.72	0.25	Wiegand et al. (2005)
Sol total	–	–	Molokai, USA	–	1.55	0.40	Wiegand et al. (2005)
Sol total	–	–	Kauai, USA	–	0.73	0.25	Wiegand et al. (2005)
Sol total	MO33	0 à - 10 cm	Sud tibet, IND	–	1.07	0.05	Tipper et al. (2006)
Sol total	MO33	- 180 à - 190 cm	Sud tibet, IND	–	1.13	0.13	Tipper et al. (2006)
Sol total	–	0 à - 5 cm	Damma glacier, CH	–	0.86	0.07	Hindshow et al. (2011)
Sol total	–	- 5 à - 10 cm	Damma glacier, CH	–	0.99	0.07	Hindshow et al. (2011)
Sol total	–	–	Damma glacier, CH	–	0.92	0.07	Hindshow et al. (2011)

Type	Numéro	Déscritption	Localisation	Échantillonage	$\delta^{40}Ca_{SRM915a}$	2smean	Ref
Échangeable NH4Oac	–	–	Thurston, USA	–	0.81	0.12	Wiegand et al. (2005)
Échangeable NH4Oac	–	–	Kohala, USA	–	1.80	0.10	Wiegand et al. (2005)
Échangeable NH4Oac	–	–	Molokai, USA	–	2.77	0.52	Wiegand et al. (2005)
Échangeable NH4Oac	–	–	Kauai, USA	–	2.10	0.12	Wiegand et al. (2005)
Échangeable NH4Oac	–	- 5 cm	Oregon coast range, USA	–	0.06	0.20	Pérakis et al. (2006)
Échangeable NH4Oac	–	- 30 cm	Oregon coast range, USA	–	0.68	0.20	Pérakis et al. (2006)
Échangeable NH4Oac	–	- 60 cm	Oregon coast range, USA	–	1.08	0.20	Pérakis et al. (2006)
Échangeable NH4Oac	Catchment 14	Horizons Oe, Oa	Archer Creek, USA	–	-0.47	0.78	Page et al. (2008)
Échangeable NH4Oac	Catchment 14	- 15 cm	Archer Creek, USA	–	0.38	0.25	Page et al. (2008)
Échangeable NH4Oac	Catchment 14	- 50 cm	Archer Creek, USA	–	0.68	0.36	Page et al. (2008)
Échangeable NH4Oac	Catchment 14	- 70 à - 100 cm	Archer Creek, USA	–	0.50	0.56	Page et al. (2008)
Échangeable NH4Oac	Catchment 15	Horizons Oe, Oa	Archer Creek, USA	–	-0.04	0.88	Page et al. (2008)
Échangeable NH4Oac	Catchment 15	- 15 cm	Archer Creek, USA	–	-0.12	0.25	Page et al. (2008)
Échangeable NH4Oac	Catchment 15	- 50 cm	Archer Creek, USA	–	0.27	0.25	Page et al. (2008)
Échangeable NH4Oac	Catchment 15	- 70 à - 100 cm	Archer Creek, USA	–	-0.03	0.25	Page et al. (2008)
Échangeable BaCl2	Plot 1.1	- 50 à - 65 cm	La Ronge, Ca	–	1.08	0.03	Holmden et Bélanger (2010)
Échangeable BaCl2	Plot 1.2	- 50 à - 65 cm	La Ronge, Ca	–	1.32	0.04	Holmden et Bélanger (2010)
Échangeable BaCl2	Plot 1.3	- 50 à - 65 cm	La Ronge, Ca	–	1.20	0.03	Holmden et Bélanger (2010)
Échangeable BaCl2	Plot 2.1	- 50 à - 65 cm	La Ronge, Ca	–	1.04	0.03	Holmden et Bélanger (2010)
Échangeable BaCl2	Plot 2.2	- 50 à - 65 cm	La Ronge, Ca	–	0.94	0.03	Holmden et Bélanger (2010)
Échangeable BaCl2	Plot 2.3	- 50 à - 65 cm	La Ronge, Ca	–	0.97	0.03	Holmden et Bélanger (2010)
Échangeable BaCl2	AE	0 à - 7 cm	Wachusett Mt, USA	–	-0.08	0.03	Farkas et al. (2011)
Échangeable BaCl2	2E	- 7 à - 15 cm	Wachusett Mt, USA	–	-0.02	0.01	Farkas et al. (2011)
Échangeable BaCl2	3E	- 15 à - 22 cm	Wachusett Mt, USA	–	0.13	0.13	Farkas et al. (2011)
Échangeable BaCl2	4E	- 22 à - 29 cm	Wachusett Mt, USA	–	0.23	0.01	Farkas et al. (2011)
Échangeable BaCl2	5E	- 29 à - 36 cm	Wachusett Mt, USA	–	0.54	0.06	Farkas et al. (2011)
Échangeable BaCl2	6E	- 36 à - 45 cm	Wachusett Mt, USA	–	0.71	0.13	Farkas et al. (2011)
Échangeable NH4Cl	BL4a-1	–	Damma glacier, CH	–	0.61	0.07	Hindshow et al. (2011)
Échangeable NH4Cl	BL4a-2	–	Damma glacier, CH	–	0.84	0.07	Hindshow et al. (2011)
Échangeable NH4Cl	BL17a-1	–	Damma glacier, CH	–	0.59	0.07	Hindshow et al. (2011)
Échangeable NH4Cl	BL24a-1	–	Damma glacier, CH	–	0.88	0.07	Hindshow et al. (2011)
Minéraux							
Hornblende	76DSH-8	–	shasta dacite	–	0.87	0.21	Skulan et al (1997)
Apatite	–	–	Aubure, Fr	–	0.40	0.10	Schmitt et al. (2003)
Apatite	B1D1	–	Wachusett Mt, USA	–	0.64	0.14	Farkas et al. (2011)
Biotite	R07	–	Damma glacier, CH	–	1.05	0.07	Hindshow et al. (2011)
Biotite	B1D2	–	Wachusett Mt, USA	–	0.57	0.06	Farkas et al. (2011)
Plagioclase	76DSH-8	–	shasta dacite	–	0.71	0.06	Skulan et al (1997)
Plagioclase	R07	–	Damma glacier, CH	–	0.99	0.07	Hindshow et al. (2011)
Plagioclase	B1D3	–	Wachusett Mt, USA	–	0.58	0.08	Farkas et al. (2011)
Feldspathe-K	R07	–	Damma glacier, CH	–	0.76	0.07	Hindshow et al. (2011)
Roche							
Roche volcanique	D54G	–	basalt marianas dredge	–	0.65	0.07	DePaolo (2004)
Roche volcanique	KOO-10	–	tholeiitic basalt, koolau	–	0.81	0.22	DePaolo (2004)
Roche volcanique	KOO-21	–	tholeiitic basalt, koolau	–	0.88	0.19	DePaolo (2004)
Roche volcanique	KOO-55	–	tholeiitic basalt, koolau	–	0.89	0.14	DePaolo (2004)
Roche volcanique	GUG-6	–	basalt, marianas	–	1.19	0.22	DePaolo (2004)
Roche volcanique	ALV-1833	–	basalt, marianas	–	0.81	0.23	DePaolo (2004)
Roche volcanique	HK-02	–	alkali basalt haleakala	–	0.82	0.26	DePaolo (2004)
Roche volcanique	HU-24	–	alkali basalt hualalei	–	0.66	0.23	DePaolo (2004)
Roche volcanique	HK-11	–	alkali basalt haleakala	–	0.61	0.27	DePaolo (2004)
Roche volcanique	HU-05	–	alkali basalt hualalei	–	1.12	0.16	DePaolo (2004)
Roche volcanique	SUNY MORB	MORB	–	–	0.76	0.03	DePaolo (2004)
Roche volcanique	SUNY MORB	MORB	–	–	0.71	0.12	DePaolo (2004)
Roche volcanique	–	rhyolite	Lake Co Obsidian	–	0.75	0.05	DePaolo (2004)
Roche volcanique	KL2	–	Tholeiite Kilauea	–	0.76	0.14	Amini et al. (2009)
Roche volcanique	ML3B	–	Tholeiite Mauna Kea	–	0.62	0.14	Amini et al. (2009)
Roche volcanique	BHVO-2	tholeiitic basalt	–	–	0.75	0.08	Amini et al. (2009)
Roche volcanique	BIR-1	tholeiitic basalt	–	–	0.77	0.09	Amini et al. (2009)
Roche volcanique	BCR-2	tholeiitic basalt	–	–	0.81	0.14	Amini et al. (2009)
Roche volcanique	StHs6/80	Andesitic ash	–	–	0.77	0.14	Amini et al. (2009)
Roche volcanique	TML	Table mountain latite	–	–	0.73	0.14	Amini et al. (2009)
Roche volcanique	82LM66A (res)	magnesiocarbonatite	–	–	0.87	0.14	Amini et al. (2009)
Roche volcanique	82LM66A (leach)	carbonate phase	–	–	0.67	0.14	Amini et al. (2009)
Roche volcanique	83HV26 (res)	calciocarbonatite	–	–	0.69	0.14	Amini et al. (2009)
Roche volcanique	83HV26 (leach)	carbonate phase	–	–	0.74	0.14	Amini et al. (2009)
Roche cristaline	PP76-2	biotite granite pikes peak batholith	–	–	0.10	0.21	Marshall et DePaolo (1982)
Roche cristaline	RM 17 66	cretaceous 2 micas, Nevada	Nevada, USA	–	0.61	0.12	Marshall et DePaolo (1982)
Roche cristaline	RM 47 66	jurassic 2 micas, Nevada	Nevada, USA	–	0.44	0.12	Marshall et DePaolo (1982)
Roche cristaline	RM 47-66	PCIm	–	–	0.59	0.08	Marshall et DePaolo (1989)
Roche cristaline	79NV HP-2	MCIm	–	–	0.60	0.07	Marshall et DePaolo (1989)
Roche cristaline	79UT GH-2	MCIm	–	–	0.67	0.09	Marshall et DePaolo (1989)
Roche cristaline	W236	PCIm	–	–	0.48	0.10	Marshall et DePaolo (1989)
Roche cristaline	81AZ WG-1	PCIm	–	–	0.67	0.06	Marshall et DePaolo (1989)
Roche cristaline	81ID APGD-1	MCIm	–	–	0.46	0.12	Marshall et DePaolo (1989)
Roche cristaline	79UT LC-1	MCIm	–	–	0.56	0.15	Marshall et DePaolo (1989)
Roche cristaline	–	–	Sud tibet, IND	–	0.95	–	Tipper et al. (2006)
Roche cristaline	T1	quartz diorite	–	–	0.72	0.14	Amini et al. (2009)
Roche cristaline	BM90/21	Péridotite	–	–	1.01	0.08	Amini et al. (2009)
Roche cristaline	PCC-1	Péridotite	–	–	1.15	0.10	Amini et al. (2009)
Roche cristaline	DTS-1	dunite	–	–	1.44	0.09	Amini et al. (2009)
Roche cristaline	–	–	Damma glacier, CH	–	0.92	–	Hindshow et al. (2011)
Roche cristaline	–	–	Wachusett Mt, USA	–	0.58	0.08	Farkas et al. (2011)
Roche métamorphique	W-2	c.f. basalte ou gabbro	–	–	0.94	0.14	Amini et al. (2009)
Roche métamorphique	IAEA-CO1	Carrara marble	–	–	1.27	0.09	Amini et al. (2009)
Roche sédimentaire	2-8-E3	speleotherme (continental)	Israël	–	0.50	0.12	Halicz et al. (1999)
Roche sédimentaire	2-8-G	speleotherme (continental)	Israël	–	0.34	0.10	Halicz et al. (1999)
Roche sédimentaire	2-8-J	speleotherme (continental)	Israël	–	0.08	0.12	Halicz et al. (1999)
Roche sédimentaire	SA 310	Calcrete (continental)	Israël	–	0.88	0.10	Halicz et al. (1999)
Roche sédimentaire	SA 495	Calcrete (continental)	Israël	–	0.98	0.09	Halicz et al. (1999)
Roche sédimentaire	ett43 bulck	–	Sud tibet, IND	–	0.63	0.01	Tipper et al. (2006)
Roche sédimentaire	ace78	–	Sud tibet, IND	–	0.29	0.05	Tipper et al. (2006)
Roche sédimentaire	ace79	–	Sud tibet, IND	–	0.55	0.06	Tipper et al. (2006)
Roche sédimentaire	ace80	–	Sud tibet, IND	–	0.48	0.01	Tipper et al. (2006)

Type	Numéro	Déscritption	Localisation	Échantillonage	$\delta^{40}Ca_{SRM915a}$	2smean	Ref
Eaux de surface							
Fleuve Orénoque	OR451	–	–	–	0.71	0.14	Zhu et MacDougall (1998)
Fleuve Orénoque	OR750	–	–	–	0.74	0.14	Zhu et MacDougall (1998)
Fleuve Yangtze	GB9	–	–	–	1.25	0.10	Zhu et MacDougall (1998)
Fleuve Lena	UL102	–	–	–	0.69	0.14	Zhu et MacDougall (1998)
Fleuve Aldan	UL101	–	–	–	0.90	0.14	Zhu et MacDougall (1998)
Fleuve Yana	YN101	–	–	–	0.70	0.14	Zhu et MacDougall (1998)
Fleuve Kolyma	KY120	–	Embouchure	–	1.00	0.14	Zhu et MacDougall (1998)
Rivière Strengbach	1	–	Aubure, Fr	–	0.42	0.13	Schmitt et al. (2003)
Rivière Strengbach	1	–	Aubure, Fr	–	0.44	0.11	Schmitt et al. (2003)
Rivière Strengbach	1	–	Aubure, Fr	–	0.49	0.17	Schmitt et al. (2003)
Rivière Strengbach	1	–	Aubure, Fr	–	0.60	0.21	Schmitt et al. (2003)
Rivière Strengbach	1	–	Aubure, Fr	–	0.80	0.17	Schmitt et al. (2003)
Rivière Ohmbach	20	–	Ohmbach, Fr	–	0.42	0.18	Schmitt et al. (2003)
Fleuve Rhin	R	–	Rheinfelden, CH	–	1.09	0.16	Schmitt et al. (2003)
Fleuve Amazone	S302	–	–		0.55	0.20	Zhu et MacDougall (1998)
Fleuve Amazone	–	–	Embouchure	–	0.74	0.07	Schmitt et al. (2003)
Fleuve Ganges	GB9	–	Source	–	0.17	0.14	Zhu et MacDougall (1998)
Fleuve Ganges	GB13	–	Source	–	0.55	0.14	Zhu et MacDougall (1998)
Fleuve Ganges	GB18	–	Source	–	0.20	0.14	Zhu et MacDougall (1998)
Fleuve Ganges	GB20	–	Source	–	0.17	0.14	Zhu et MacDougall (1998)
Fleuve Ganges	BGP 65	–	Embouchure	–	1.16	0.17	Schmitt et al. (2003)
Fleuve Indus	AK 63	–	Embouchure	–	0.72	0.24	Schmitt et al. (2003)
Fleuve Huanghe	–	–	Embouchure	–	1.06	0.18	Schmitt et al. (2003)
Rivière Marsyandi	MT66	–	Sud tibet, IND	2002	1.13	0.06	Tipper et al. (2006)
Rivière Marsyandi	MT67	–	Sud tibet, IND	2002	1.16	0.04	Tipper et al. (2006)
Rivière Marsyandi	MT17	–	Sud tibet, IND	2002	0.97	0.04	Tipper et al. (2006)
Rivière Marsyandi	MT21	–	Sud tibet, IND	2002	0.80	0.02	Tipper et al. (2006)
Rivière Marsyandi	ett69	–	Sud tibet, IND	2002	0.88	0.04	Tipper et al. (2006)
Rivière Marsyandi	ett69rep	–	Sud tibet, IND	2002	0.86	0.04	Tipper et al. (2006)
Rivière Marsyandi	MT27	–	Sud tibet, IND	2002	0.82	0.03	Tipper et al. (2006)
Rivière Marsyandi	MT29	–	Sud tibet, IND	2002	0.97	0.01	Tipper et al. (2006)
Rivière Marsyandi	MT32	–	Sud tibet, IND	2002	0.88	0.06	Tipper et al. (2006)
Rivière Marsyandi	MT107	–	Sud tibet, IND	2002	0.92	0.08	Tipper et al. (2006)
Rivière Marsyandi	MT108	–	Sud tibet, IND	2003	0.99	0.06	Tipper et al. (2006)
Rivière Marsyandi	MT110	–	Sud tibet, IND	2003	0.78	0.05	Tipper et al. (2006)
Rivière Marsyandi	MT112	–	Sud tibet, IND	2003	0.90	0.05	Tipper et al. (2006)
Rivière Bhote Kosi	ace70	–	Sud tibet, IND	2003	0.67	0.01	Tipper et al. (2006)
Rivière Bhote Kosi	ace72	–	Sud tibet, IND	2003	1.34	0.01	Tipper et al. (2006)
Rivière Bhote Kosi	ace76	–	Sud tibet, IND	2003	1.18	0.04	Tipper et al. (2006)
Rivière Bhote Kosi	ace82	–	Sud tibet, IND	2003	0.95	0.04	Tipper et al. (2006)
Rivière	A	–	Damma glacier, CH	–	0.95	0.07	Hindshow et al. (2011)
Rivière	A	–	Damma glacier, CH	–	0.99	0.07	Hindshow et al. (2011)
Rivière	A	–	Damma glacier, CH	–	1.11	0.07	Hindshow et al. (2011)
Rivière	A	–	Damma glacier, CH	–	1.05	0.07	Hindshow et al. (2011)
Rivière	A	–	Damma glacier, CH	–	0.95	0.07	Hindshow et al. (2011)
Rivière	A	–	Damma glacier, CH	–	1.09	0.07	Hindshow et al. (2011)
Rivière	A	–	Damma glacier, CH	–	1.01	0.07	Hindshow et al. (2011)
Rivière	A	–	Damma glacier, CH	–	1.03	0.07	Hindshow et al. (2011)
Rivière	A	–	Damma glacier, CH	–	1.07	0.07	Hindshow et al. (2011)
Rivière	A	–	Damma glacier, CH	–	1.01	0.07	Hindshow et al. (2011)
Rivière	B	–	Damma glacier, CH	–	0.88	0.07	Hindshow et al. (2011)
Rivière	B	–	Damma glacier, CH	–	1.24	0.07	Hindshow et al. (2011)
Rivière	B	–	Damma glacier, CH	–	0.90	0.07	Hindshow et al. (2011)
Rivière	B	–	Damma glacier, CH	–	0.88	0.07	Hindshow et al. (2011)
Rivière	B	–	Damma glacier, CH	–	0.99	0.07	Hindshow et al. (2011)
Rivière	B	–	Damma glacier, CH	–	0.97	0.07	Hindshow et al. (2011)
Rivière	E	–	Damma glacier, CH	–	0.86	0.07	Hindshow et al. (2011)
Rivière	E	–	Damma glacier, CH	–	1.18	0.07	Hindshow et al. (2011)
Rivière	E	–	Damma glacier, CH	–	0.90	0.07	Hindshow et al. (2011)
Rivière	E	–	Damma glacier, CH	–	1.11	0.07	Hindshow et al. (2011)
Rivière	E	–	Damma glacier, CH	–	0.88	0.07	Hindshow et al. (2011)
Rivière	E	–	Damma glacier, CH	–	1.01	0.07	Hindshow et al. (2011)

Annexe 2

High performance automated ion chromatography separation for Ca isotope measurements in geological and biological samples

Anne-Désirée Schmitt,*[a] Sophie Gangloff,[b] Florian Cobert,[b] Damien Lemarchand,[b] Peter Stille[b] and François Chabaux[b]

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Natural mass-dependent fractionation of calcium isotopes is a promising tool for investigating Ca pathways and cycling in geological and biological materials. But since natural isotope fractionation of Ca appears to be extremely limited (~1.25‰/amu), excellent external precision and sensitivity are needed to make full use of its potential. Here, we describe a new Ca purification procedure that consists of a high selectivity automated ionic chromatography separation protocol, which is suitable for Ca isotope measurements by mass spectrometry and applicable to multiple natural matrixes (waters, mineral and organic samples). The analytical progress in this automated technique are multiple: (1) saving time with a minimum of handling, (2) unique operating protocol whatever the nature of the sample, (3) complete separation of Ca from K, Mg and Sr, avoiding isobaric interferences which are critical during TIMS analysis, and (4) Ca separation by peak recognition optimising the full recovery of Ca even if its retention time is shifted from one sample to another. The two latter advantages ensure a Ca recovery yield close to 100%, leading to the absence of any fractionation of Ca isotopes during the chemical clean-up. Thus, this chemical separation will be of special interest for applications not compatible with the use of the double spike technique such as MC-ICP-MS and ^{40}Ca excesses measurements. Additionally this procedure leads to a twofold improvement of the long-term repeatability of the Ca isotopes determination by TIMS (±0.11 $\delta^{44/40}$Ca, 2SD) as compared with a classical resin chemistry protocol and is similar to the best repeatability published so far (±0.10 $\delta^{44/40}$Ca, 2SD).

Introduction

During the past decade the calcium isotopic system became a promising tool for answering questions related to paleoclimate, global elemental cycling at the Earth's surface as well as environmental and paleontological geochemistry.[1–23] However, the extent of Ca isotopic variations in nature appears to be rather limited with respect to its large relative mass spread, with a maximum of ~1.25 ‰/amu. This feature increases the need for high analytical precision and accurate data in order to resolve small isotopic changes and implies special caution in avoiding any analytical bias. In particular, the presence of isobaric interferences at mass 40 and 44 caused by the ionisation of $^{40}K^+$ and $^{88}Sr^{2+}$, respectively, are critical issues. Moreover other aspects require special attention such as instrumental bias (mass discrimination, homogenisation of the sample during the filament loading and minimisation of ion optic effects through careful selection of cup configuration…). Much work has been carried out for improving the measurement protocol since the pioneering work of Russell *et al.*[24] Modern Thermo-Ionisation Mass Spectrometer (TIMS) instruments are indeed capable of measuring the Ca isotopic compositions at high precision as summarised in Fantle and Bullen,[25] but careful chemical separation of Ca still remains a primary critical condition for reliable measurements.

To date calcium separation from the sample matrix can be performed by several protocols all based on cation-exchange chromatography and developed by different laboratories using different cation exchange resins (*e.g.* AGMP-50; AG50W-X8; AG50W-X12; MCI gel).[15,18,19,23] Generally speaking, satisfactory separation is achieved for all cations, except for Sr and K, which are the closest elements to Ca in terms of chemical behaviour during the various separation steps. To minimise this problem, different strategies have been applied: (1) the samples have been processed twice through ion chromatography columns,[26,27] (2) the classical Ca cation-exchange chromatography is followed by an additional step using a Sr specific resin (Sr Spec) to separate Ca from Sr,[28,29] (3) the elution curve is truncated, leading to a recovery yield of about 70%[23] or greater than 80%.[30] In the latter case a double spike must be added prior to the chromatographic separation in order to avoid mass fractionation during ion exchange reactions as reported by Russell and Papanastassiou.[31] These techniques are however time-consuming and are based on the operator ability to rigorously reproduce the procedure from one sample to the other, including ionisation of Ca during the measurements. Moreover, none of those approaches is 100% satisfactory to avoid minor tailing of K,

[a]*Université de Franche-Comté – CNRS/UMR 6249, Chrono-environnement, 16, Route de Gray, F-25030 Besançon Cedex, France. E-mail: adschmit@univ-fcomte.fr; Fax: +33 (0)3 81 66 65 58; Tel: +33 (0)3 81 66 65 61*
[b]*Université de Strasbourg et CNRS, Laboratoire d'Hydrologie et de Géochimie de Strasbourg, Ecole et Observatoire des Sciences de la Terre, 1, rue Blessig, 67084 Strasbourg Cedex, France*

which can lead to isobaric interferences and Ca emission inhibition during the measurement protocol by TIMS.

For all the above reasons, we have developed a new Ca purification procedure, that consists of a high sensitivity automated ionic chromatography separation protocol suitable for Ca isotope measurements, which is applicable to multiple natural matrixes (waters, vegetation samples, soil carbonates, soils, rocks and minerals). This is a protocol pioneering for Ca isotope measurements and so far only one similar approach was published but is developed for the Sr separation from natural waters and carbonates for isotopic determinations.[32] In this work we also address the issues of measurement precision and sensitivity of this new method and the way of reporting replicate measurements.

Experimental

Sample description and preparation

To cover a very large range of natural matrixes, we analysed the following 21 natural samples (Table 1): liquid (2 rainwaters, 1 swamp, 3 nutritive solutions), mineral (4 soil carbonates, 1 apatite, 1 granite, 2 soils) and organic samples (1 leave, 1 cotyledon, 1 tegument, 1 root, 1 stem, 1 sap, 1 trunk; the samples come from different plants and trees).

Natural waters were filtrated using 0.22 μm Millipore® Teflon filters, acidified to pH = 1 with double distilled HNO_3 and evaporated to concentrate in calcium; the nutritive solutions were only evaporated. Soil carbonate samples were dissolved by leaching soil samples in 0.25 N HCl. The apatite sample was dissolved in 6 N HCl. Approximately 100 mg of crushed soil and granite powders were dissolved in savillex® vials following a procedure involving three acids (double distilled HNO_3-suprapure HF-suprapure $HClO_4$). Finally, 50 mg of dried vegetation samples were crushed in a clean agate mortar and digested in savillex® vials by a hot oxidative acid method (double distilled HNO_3 - double distilled HCl - suprapure H_2O_2).

An aliquot of each sample was analysed for Ca concentration by flame atomic absorption spectrometry with a Perkin Elmer 430 spectrometer to determine calcium concentration, so that the sample/double spike ratio could be optimised. Following Holmden,[23] a ^{42}Ca–^{43}Ca double spike was added to 5 μg of Ca prior to chromatographic clean-up for Ca to avoid hypothetical isotopic fractionation during ion exchange chromatography as earlier recognised by Russell and Papanastassiou.[31] Sample-spike homogenisation was attained before Ca purification from matrix elements. After evaporation the mixed solutions were dissolved in ultrapure (UHP) water.

No international standard with absolute Ca isotopic abundance is presently available for inter-laboratory comparison. However, several samples have been analysed by most of the laboratories interested in Ca isotopes, allowing inter-laboratory comparison among the different methods.[34] This is the reason why the NIST SRM 915a certified reference material, one seawater sample from North Atlantic,[35] and a solution obtained from a natural fluorite (CaF_2) used as a reference material in Bern[5] and Kiel[36] and provided by courtesy of Dr N. Gussone, have also been analysed in this study.

Chromatographic separation

When measuring Ca isotopes ranging from masses 40 to 44 by TIMS (see below), it appears that K (mass 40), MgF (masses 40, 43, 44) and double charged Sr (masses 42, 43, 44) could cause isobaric interferences that seriously hinder precise and accurate isotopic determination. Consequently we have developed an automated chemical separation because a careful Ca chemical purification is required before isotopic measurement. We have then compared the new Ca-matrix separation technique to a classical one used as a reference method by applying both methods to determine the Ca isotopic composition of aliquots of 12 selected samples.

Reference chemical separation. The chemical separation was carried out on a 0.6 cm internal diameter quartz column filled with 2 mL AG50W-X8, 200–400 mesh, Biorad® resin. 10 samples were usually run in parallel. 1.5 N HCl was used to separate successively magnesium (Mg), potassium (K), calcium (Ca), and strontium (Sr).[6,7,11,23,35] The Ca elution curve was truncated (~70% recovery yield) in order to minimise the contribution of K and Sr and optimise the Ca emission in the TIMS.[23] The whole elution duration including the Ca peak collection was about 10 hours, 24 more hours were necessary to clean and regenerate the resin. The resin was discarded after about 20 chemistries in order to avoid memory effects and ageing leading to shifts in the elution protocol. It should be noted that this protocol is not suitable for silicate matrixes.

Automated high pressure ionic chromatography (HPIC) separation. The analytical setup (ICS-3000, Dionex®) consists of an autosampler, an ion chromatograph (including the dual pump, the detector and the chromatography units) and a fraction collector (Fig. 1). The different components of this assemblage are connected with PEEK (PolyEther Ether Ketone) tubes. The whole system is controlled by the commercial Chromeleon® software, which is also intended for data acquisition.

The first part of the experimental setup consists of an autosampler (AS model). It allows a direct injection of 400 μL of the selected sample into the injection valve (V1). Its rack can handle up to 99 cleaned and disposable 1.5 mL polypropylene vials, closed by caps equipped with a Teflon septum, either filled with samples or UHP water. The vials and caps where cleaned in diluted double distilled HCl before being used.

The ion chromatograph is comprised of two units:

- The **DC unit** (Detector and Chromatography unit) consists of the chromatography and detector modules. It is comprised of two ovens, which regulate the column temperature in order to avoid temperature modifications that can cause variations in the peak positions during the elution.[37] The lower one contains the injection valve V1, the 400 μL injection loop and two 5 mm columns (a CG16 guard column to trap the impurities and a CS16 cation-exchange one), whose longevity is about 1500 samples. It is thermostated at 35 °C. The upper one is made up of the 4 mm CSRS-300 (Cation Self Regenerating Suppressor) and the conductivity detector. It is thermostated at 30 °C.

- The **DP unit** (Dual Pumps) consists of two pumps. They control the simultaneous circulations of the methanesulfonic acid (MSA) eluent (MSA, 27.4 mM, Flucka®) in isocratic mode at

Table 1 Comparison of $\delta^{44/40}Ca$ isotopic composition of multiple matrixes processed through the reference method and automated HPIC

		Automated HPIC			Reference method		
		$\delta^{44/40}Ca$	$2SE_{Int}$	N	$\delta^{44/40}Ca$	$2SE_{Int}$	N
Liquid samples							
CIE 002	rainwater	0.62	0.12	2	0.47	0.38	4
		0.55	0.11		0.59	0.28	
CIE 005	rainwater	0.57	0.12	2	0.71	0.47	
		0.60	0.09		0.90	0.29	
CIE 003	swamp	0.08	0.09	2			
		−0.04	0.08				
E 5109	Seawater	1.83	0.16	6	1.84	0.15	6
		1.90	0.18		1.97	0.18	
		1.91	0.09		1.76	0.39	
		1.79	0.15		1.93	0.20	
		1.69	0.07		2.01	0.21	
		1.67[a]	0.03[a]		1.69	0.49	
High Ca	nutritive solution	0.98	0.13	3	1.07	0.03	3
		0.91	0.13		1.08	0.11	
		0.91	0.11		1.10	0.09	
E4av3	nutritive solution	0.86	0.11	1	0.67	0.22	2
					0.94	0.10	
E4ap	nutritive solution	0.76	0.10	1	1.04	0.11	2
					0.76	0.15	
Mineral samples							
NIST SRM 915a	carbonate	−0.03	0.16	4			
		0.00	0.15				
		−0.01	0.09				
		0.04	0.12				
CIC 53less	carbonate	−0.63	0.12	2	−0.51	0.19	3
		−0.63	0.10		−0.63	0.29	
					−0.93	0.23	
CIC 62less	carbonate	−1.16	0.13	2	−1.09	0.14	2
		−1.23	0.08		−1.17	0.37	
Ir2 less	carbonate	−0.97	0.09	1	−1.03	0.17	2
					−1.01	0.21	
Ir1 less	carbonate	−0.92	0.09	1	−0.81	0.13	3
					−1.02	0.13	
					−1.18	0.34	
CaF_2	fluorure	1.36	0.10	6			
		1.37	0.14				
		1.40	0.10				
		1.36	0.10				
		1.39	0.10				
		1.48[a]	0.09[a]				
GranCI	granite	−1.36	0.13	2			
		−1.32	0.09				
ApatCI	apatite	0.77	0.08	2			
		0.90	0.08				
CIC 63res	soil	−1.12	0.09	2			
		−1.15	0.07				
CIC 59res	soil	−0.93	0.11	2			
		−1.08	0.11				
Organic samples							
feuilleg	leave	0.15	0.06	2			1
		0.34	0.10				
cotyng	cotyledon	−0.36	0.09	2	−0.46	0.05	3
		−0.37	0.07		−0.41	0.14	
					−0.34	0.07	
tégng	tegument	−0.90	0.11	2	−1.18	0.14	2
		−0.99	0.07		−1.00	0.23	
Aracines	root	0.01	0.09	2			
		0.07	0.18				
L4Tm	stem	0.02	0.16	2			
		−0.16	0.12				
sève CIless	sap	−0.07	0.09	2			
		−0.16	0.05				
Ir2 res	trunk	−1.92	0.18	2			
		−1.83	0.13				

[a] Double spiked after the chemical separation.

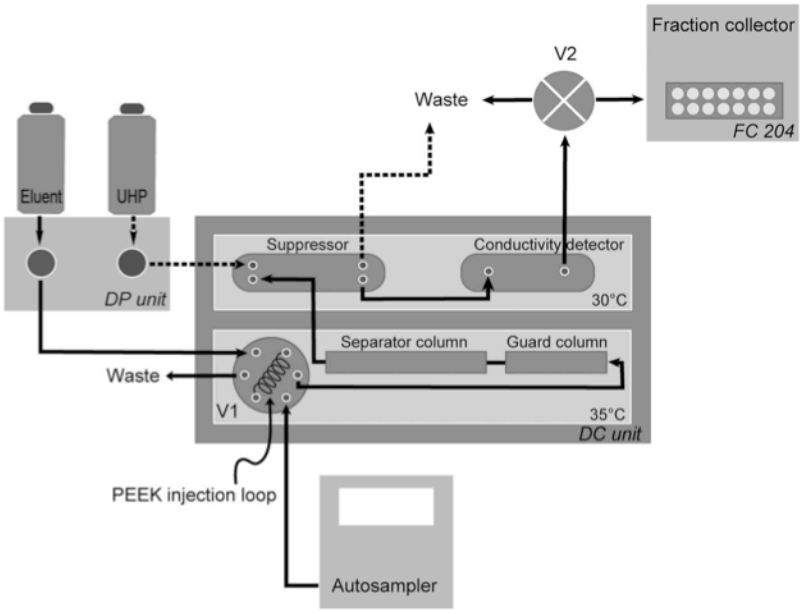

Fig. 1 Schematic description of the HPIC instrumental setup. It consists of three different units being connected with PEEK (PolyEther Ether Ketone) tubes: an autosampler, an ion chromatograph (including DP and DC units) and a fraction collector.

1 mL/min through the whole circuit and UHP water for continuously hydrating the CSRS. A 90 mA electrical potential is applied to the CSRS to produce OH- ions by electrolysis, which are intended to neutralize the H^+ ions supplied by the eluent. As a result, the suppressor delivers cations in a simplified water matrix. This step has two advantages: (1) it enhances the sensitivity of the conductivity signal, and (2) it rids the solution of the MSA eluent.

The oven temperatures of the DC unit, and the concentration and flow rate of the MSA eluent were chosen after trial and error experiments in order to optimise duration and selectivity of the Ca elution. In so doing, the chosen MSA concentration allowed for instance to permute the elution order of K and Mg as compared to the reference method, which helps minimise the K peak tailing contribution to the collected Ca fraction.

To collect the calcium fraction, the conductivity detector is connected to the collection valve V2, which has two positions: one is connected to the fraction collector (Gilson FC 204) and the other to the waste. The fraction collector is equipped with a 14 positions rack, and 15 mL cleaned Teflon beakers are used to collect the calcium fractions.

The overall elution takes 36 min. After each sample two UHP injections are performed in order to rinse the whole chromatographic system. The rinsing steps are sufficient to quantitatively remove traces of residual calcium released by the CS16 column (so-called "memory effect").

The software is written to allow the collection of individual elements according to peak characteristics (Ca retention time, slope, threshold…). We developed an automated Ca separation based on peak recognition rather than on time retention as previously proposed for the Sr purification.[32] The Ca elution peak comes out between $t_1 \approx 23$ and $t_2 \approx 32$ min. Thus, valve V2 is switched on to start collecting the Ca fraction when the slope is equal to 0.10 μS/s and the threshold 0.10 μS in this time interval. Valve V2 is switched off when the threshold reaches 0.50 μS. The collected volume is about 10 mL.

Mass spectrometry measurements

Ca-isotopic compositions were measured by thermal ionisation mass spectrometry (TIMS) using a Triton instrument (Thermo-Fisher) operating in dynamic multicollection mode. To resolve the natural differences in calcium isotope ratios, we used the double spike method initially proposed by Russell *et al.*[24] for Ca isotopes and later modified by Holmden[33] for multicollector TIMS. This allows correction for any procedural or instrumental mass discrimination that occurs after addition of the spiked solution. In doing so a new ^{42}Ca-^{43}Ca double spike solution[33] was prepared by mixing existing weighted double spikes, *i.e.*, the previously described ^{43}Ca–^{48}Ca and ^{42}Ca–^{48}Ca double spikes from Strasbourg[35] and Caltech,[10] respectively. The spiked solutions were mixed to obtain a $^{42}Ca/^{43}Ca$ ratio approximately equal to the normal $^{42}Ca/^{43}Ca$ value (~5). Optimization of the spike-sample ratio was attained by mixing a 5 μg Ca sample with 1 μg double spike Ca. Repeated measurements of raw calcium isotopic compositions of the NIST SRM 915a were carried out to achieve a precise isotopic composition for this standard ($^{40}Ca/^{42}Ca = 149.805$; $^{43}Ca/^{42}Ca = 0.20853$; $^{44}Ca/^{42}Ca = 3.22853$; not corrected for instrumental mass discrimination). This composition was then used as a reference to calibrate the double spike ($^{40}Ca/^{42}Ca = 0.16268$; $^{43}Ca/^{42}Ca = 0.47698$; $^{44}Ca/^{42}Ca = 0.039032$).

The spiked samples obtained after the chemical cleanup were then treated with a suprapure H_2O_2-double distilled HNO_3 mixture in order to eliminate any organic impurity from the cation exchange resin breakdown and/or from the sample itself, before being totally evaporated.[38] The residue was dissolved in 1 μL 0.25 N HNO_3. The latter solution was then loaded without

any further treatment on a single outgassed Ta filament (99.995% purity) and oxidized in partial vacuum. The filament was pre-heated to ~1200 °C, the temperature was adjusted to reach a typical beam of 5.5×10^{-11}A of $^{40}Ca^{+}$ corresponding to a filament temperature ranging from 1380 to 1450 °C. From 130 to 200 cycles were collected in a dynamic multicollection mode adapted from Holmden.[33] Most of the samples were run in replicates (N = 2 to 6) including chromatographic clean-up and mass spectrometric measurements. In order to check for possible isobaric interferences, beams at masses 41, 45 and 88, corresponding to $^{41}K^{+}$, $^{26}Mg^{19}F^{+}$ and $^{88}Sr^{+}$, respectively, were systematically checked. No interference was ever observed, so that no correction was necessary.

The data are reduced using the exponential fractionation law and the Newton-Raphson iteration technique to calculate $\delta^{44/40}Ca$ values.[39] The technique has the advantage in being quite robust and to rapidly converge to precise analytical results. The data reduction is performed off-line using the optimization toolbox provided by the commercial Matlab® software.

The Ca isotope values are expressed as permil deviation relative to the NIST SRM 915a standard solution:

$$\delta^{44/40}Ca = \{(^{44}Ca/^{40}Ca)_{sample}/(^{44}Ca/^{40}Ca)_{SRM915a} - 1\}.1000 \quad \text{(ref. 40).}$$

Results and discussions

This section aims at describing in detail the improvements, advantages and limits of this new automated chemical separation as well as providing evidence of its reliability and robustness.

Ca collection by peak recognition

The first advantage of the peak recognition technique compared to the reference method is saving time. Since analysis time for one sample is about 110 min, 12 samples can be analysed per day if one takes advantage of the fully automated system, which can operate overnight. This allows gaining time with a minimum of handling compared to classical chromatographic separations (usually one whole day of handling plus final cleaning of the resin) since one hour is enough to prepare and start the elution program. In addition one can much more efficiently separate Ca from alkaline and alkaline-earth elements such as K, Mg and Sr (Fig. 2a). In particular, this restricts the contribution of K and Mg by peak tailing whatever the initial concentrations of these elements in the samples. The K contribution to the collected Ca fraction is always the same (about 6 ng) and negligible when processing samples with contrasted initial K concentrations, *e.g.*, with a factor of 40 between the values (Table 2).

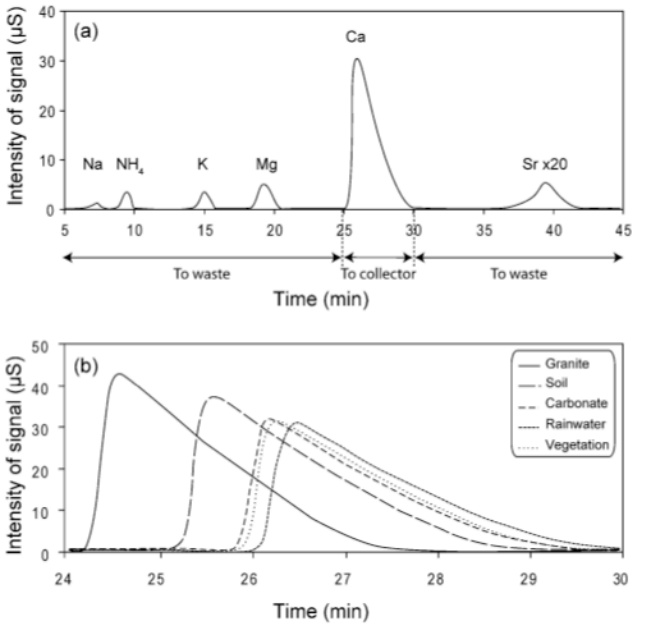

Fig. 2 (a) Elution chromatogram through time (example of a soil sample). The intensity of the signal is represented by the electrical conductance and is expressed in µS. (b) Eluted Ca fractions corresponding to several processed matrixes. By using a collection by peak, the beginning of the collection varies from about 2 minutes depending on the considered matrix.

Finally the Ca collection by peak recognition is monitored in real time and leads to a complete Ca recovery by adjusting the collecting interval. Therefore Ca collection no longer suffers from the shift of the retention time resulting from the sample matrix. Such variations were actually observed during our work when Ca elution peaks strictly coincide for samples with similar matrixes (not shown) whereas they may deviate for samples with different natures. For instance, the Ca retention time of silicate samples (granite, soils) is shortened by 1 or 2 minutes compared to that of other matrixes such as soil carbonates, rainwaters or vegetation samples (Fig. 2b). Repeated measurements of aliquots of a single sample run at different pH values indicate that the shift in retention time is caused by the sample matrix rather than its pH value (not shown).

Consequently, the combination of an efficient peak separation with a collection by peak recognition ensures a Ca recovery close to 100% as confirmed by isotopic dilution and avoids the interference of K and Sr peaks. This overrides the risk of any isotopic fractionation caused by Ca loss during the chemical clean-up. It was verified by comparing Ca isotopic compositions of two standard solutions (CaF_2 and seawater) in which the double spike had been added before and after the chromatographic

Table 2 K and Mg contributions in the Ca collected fraction after HPIC automated chemical clean-up of matrixes having contrasted K concentrations (average values and 2SD errors)

	Sample	Collected fraction volume (mL)	Initial K among (µg)	K contribution in the collected fraction (ng/mL)	Initial Mg among (µg)	Mg contribution in the collected fraction (ng/mL)
E 5109	Seawater	7.39	5.6	14.5 ± 0.3 (N = 2)	12	44 ± 6 (N = 2)
cotyng	cotyledon	7.09	220	13.1 ± 0.3 (N = 2)	15	37 ± 1 (N = 2)

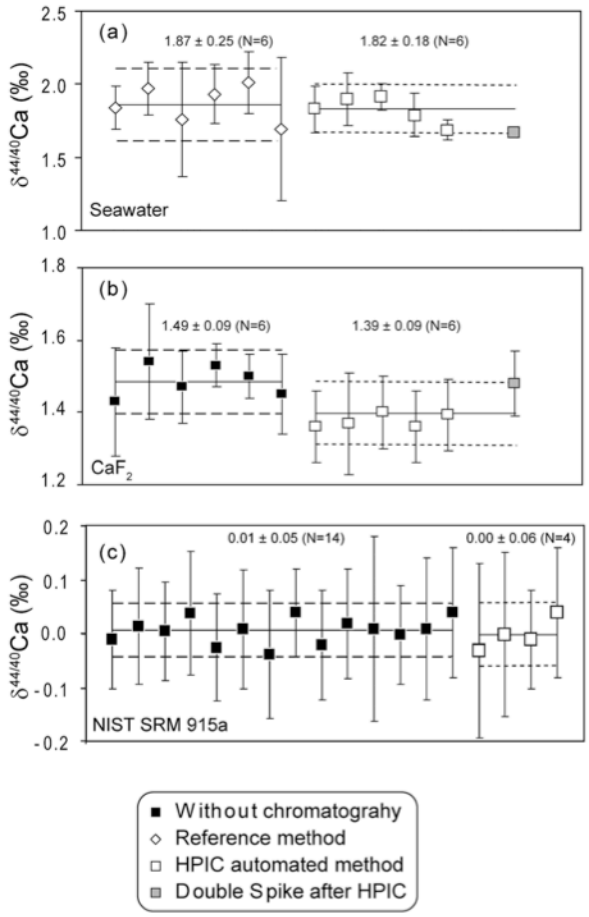

Fig. 3 Ca isotopic fractionation in several standards with and without chemistry, double spike added before and after the chemistry, reference method *versus* HPIC automated method for (a) Seawater, (b) CaF_2, (c) NIST SRM 915a.

clean-up (Fig. 3a, b). As a major advantage, this protocol can be useful for studies incompatible with the use of a double spike such as those measuring Ca isotopes by standard-sample bracketing on a MC-ICP-MS instrument or those interested in determining ^{40}Ca excesses.

Ca procedural contamination determination

In order to determine the overall procedural Ca contamination induced by the sample treatment with the automated HPIC, and also by the whole TIMS measurement, we have followed a classical isotopic dilution approach. Prior to the injection in the HPIC, we mixed ultrapure water with 10 µL of the Ca double spike solution into the Teflon savillex® beakers, cleaned by repeated bathes in dilute hot Normapur grade HCl (37%; 0.5 ppm Ca max) and HNO_3 (69%; 0.1 ppm Ca max) commercial acids. We determined that the overall Ca contribution of the estimated HPIC system was equal to ~30 ng, *i.e.*, ~ 0.5% of the processed Ca amount. We also performed the whole procedure before Ca isotope analyses by TIMS including the acids used for sample dissolution. The overall contamination is measured here at about 50 ng of Ca, *i.e.*, ~ 1% of the processed Ca among. It is slightly higher than the previous determination but includes the contamination brought by the procedure of mass spectrometry analysis, *e.g.*, acids used for samples dissolution, contact with vials, evaporation, HPIC, loading, filament. Consequently the maximal procedural contamination is estimated as ~50 ng or ~1% of the amount of processed Ca, and it can be neglected.

These values were confirmed by a HPIC concentration measurement protocol adapted for blank determinations since the previous technique is time-consuming and does not allow for an immediate evaluation of blanks. The improvement obtained by this method compared to classical HPIC concentration measurements was attained by injecting different standard volumes (from 5 to 200 µL) of a multi-elemental in-house standard (Mt Roucous, French mineral water), which allows the injection of weakly concentrated solutions. Corresponding calibration curve is presented in Fig. 4. It is validated by measuring the SLRS-4 certified solution (NRC-CNRC). We obtained a value of 325 ± 10 ng Ca, which is within error indistinguishable from the certified one, 310 ± 10 ng. Graphically we can infer from Fig. 4 that there exists a minimal amount (~30 ng) of Ca below which the calibration curve becomes horizontal. This value corresponds to the detection limit of the method. As a result we conclude that this instrumental setup is sensible enough to determine the procedural contamination induced by HPIC. It is less time consuming than classical isotope dilution and it allows for an immediate evaluation of blanks.

Precision, repeatability and accuracy of the measurements

Previous studies demonstrated the limited variability of Ca isotope ratios in natural materials, with a maximum of about 1.25‰/amu recorded for biological samples (between a puma bone and an egg white[1,3]). In general, the variability is even more limited and ranges between 0.25 to 0.5‰/amu for natural waters, soils, rocks and even vegetation samples.[6,7,11,12,15,17,19,23] For this

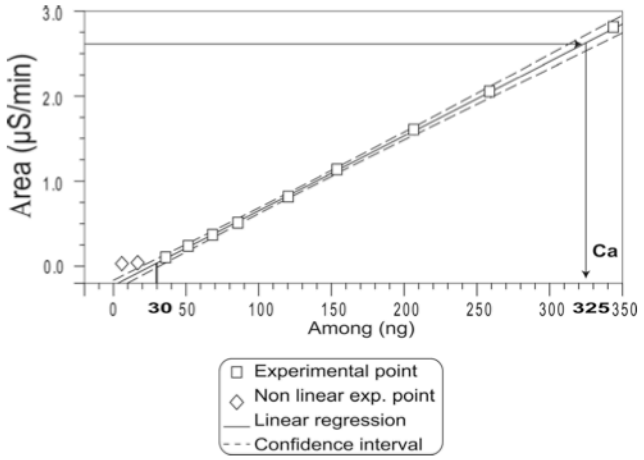

Fig. 4 HPIC calibration curve for Ca concentration measurements. The experimental points correspond to a multi-elemental internal standard (Mt Roucous, French mineral water) injected at different volumes (5, 10, 20, 30, 40, 50, 70, 90, 120, 150 and 200 µL). It should be noted that the two lower volumes do not define a linear trend in accord with that obtained for the other ones, which gives access to the detection limit of the method. The measured SLRS-4 value is also reported (lines with an arrow). The dashed lines correspond to the 2SD external error calculated from the linear calibration curve.

reason, it is absolutely paramount to acquire Ca isotope data with the utmost analytical precision.

In this study we expressed the $\delta^{44/40}$Ca value resulting from at least 2 independent measurements as the average Ca isotopic composition. Corresponding 2SE error was based on the external long term 2SD and the number of measurements performed for each sample. For samples only measured once, the worst value of the long-term 2SD or internal 2SE was reported.

Internal precision. The internal precision, *i.e.*, counting statistics of the measurements expressed as twice the standard error of the mean (2SE or $2\sigma_{mean}$) was comprised between 0.03 and 0.18‰ with an average of ±0.06‰ (2SD; N = 53; Table 1) for the newly developed automated procedure. This represents an improvement of a factor 4 compared to internal precision obtained for the reference resin separation where the variability was comprised between 0.03 and 0.49‰ with an average of ±0.24‰ (2SD; N = 32; Table 1).

As a result automated Ca separation is able to produce a much more stable beam during mass spectrometry analysis, which can be interpreted as a much more reproducible and/or complete chemical separation. Since the whole range of variability can be observed for repeated measurements of one single sample only processed through the automated HPIC, *e.g.*, seawater E 5109 or NIST SRM 915a (Fig. 3c), we can a priori suspect that it most likely originates from the mass spectrometry itself. This hypothesis is supported by the comparison with the internal repeatability obtained for standard solutions directly loaded onto the filament and not processed through the chromatographic separation. In that case the internal precision ranges from 0.06 to 0.17‰ with an average value of 0.11 ± 0.06‰ (2SD; N = 20; Table 3).

Table 3 Repeated measurements of NIST SRM 915a and CaF_2 not processed through the chemistry

		$\delta^{44/40}$Ca	$2SE^{int}$
NIST SRM 915a		−0.01	0.09
		0.01	0.11
		0.01	0.09
		0.04	0.11
		−0.02	0.10
		0.01	0.11
		−0.04	0.12
		0.04	0.08
		−0.02	0.10
		0.02	0.10
		0.01	0.17
		0.00	0.09
		0.01	0.13
		0.04	0.12
	Mean	*0.01*	
	2SD	*0.05*	
	N	*14*	
CaF_2		1.43	0.15
		1.54	0.16
		1.47	0.10
		1.53	0.06
		1.50	0.06
		1.45	0.11
	Mean	*1.49*	
	2SD	*0.09*	
	N	*6*	

External repeatability. The external repeatability was estimated from repeated measurements of standards and samples both processed or not through the automated chromatography (Tables 1 and 3; Fig. 3). $\delta^{44/40}$Ca external repeatability (average value, 2SD error) was equal to 0.01 ± 0.05‰ (N = 14) and 1.49 ± 0.09‰ (N = 6), for NIST SRM 915a and CaF_2, respectively, which yields a global 2SD external repeatability of ±0.06‰ (N = 20). When considering standards processed through the automated HPIC separation, we obtain 0.00 ± 0.06‰ (N = 4), 1.39 ± 0.09‰ (N = 6) and 1.80 ± 0.20‰ (N = 6) for NIST SRM 915a, CaF_2 and seawater, respectively. This yields a global 2SD external repeatability of ±0.13‰ (N = 16), which is twice worse than standards not chemically processed. Finally, the long-term 2SD external repeatability obtained for all the standard materials, processed or not through the automated chemistry is equal to ±0.11‰ (N = 35).

The external repeatability of 20 samples including the three studied standard materials processed through the automated HPIC chemistry is equal to ±0.11‰ (N = 51), and without the 3 standards it becomes ±0.10‰ (N = 35). Similarly the repeatability of 12 samples including the 6 seawater standards processed using the reference method is equal to ±0.22‰ (N = 32), and without the seawater standards, it remains unchanged.

As a major conclusion, the chemical protocol proposed in this study improves the external repeatability of the method by a factor of two. It should also be noted that the long-term 2SD external repeatability is similar for repeated measurements of standards processed through the chemistry and for samples (±0.11‰ $\delta^{44/40}$Ca). It is also similar to the presently best published external repeatability, that vary between 0.10 and 0.50‰ (see overview in Holmden[33]).

Accuracy. Since this automated technique was developed as a preparation in view of Ca isotopic measurements by TIMS, we analysed standard samples whose Ca isotopic ratios are certified or at least accepted by the international community (seawater and CaF_2 standard). We also analysed 12 samples that we

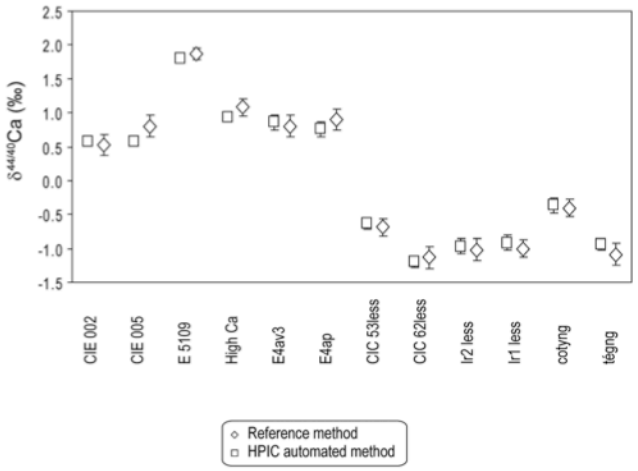

Fig. 5 Average and 2SE values based on replicates of 1 to 6 measurements for samples processed through automated HPIC and classical reference separations.

previously treated by both the automated HPIC and the reference techniques to verify the accuracy of the measured Ca isotopic compositions. In the latter case aliquots of each of the samples were separated and repeated up to 6 times by both the chromatographic separation methods to improve the statistical significance of the $\delta^{44/40}$Ca measurement. Results are quite conclusive and are presented in Table 1 and Fig. 3a and 5. No isotopic bias is observed whatever the analytical procedure is and, when expressed against the NIST SRM 915a, the values for the seawater and CaF_2 solutions (1.82 ± 0.18 ‰ (N = 6) and 1.44 ± 0.13 ‰ (N = 12), respectively) are identical to previously published values 1.88 ± 0.17 ‰ and 1.47 ± 0.19 ‰, respectively (*e.g.* Hippler *et al.*, 2003[34]).

The absence of bias between standards processed through the HPIC or not, measured in this study and by other laboratories, indirectly confirms the absence of any significant Ca contamination in the automated separation protocol. It also validates the absence of any significant isotope fractionation occurring during the column elution.

Conclusion

In this study we present a new Ca chemical separation protocol for isotope measurements using a fully automated chromatography technique. The technique's main advantages are (1) gaining time with a minimum of handling, (2) an unique operating protocol whatever the nature of the sample (water, rock, organic), (3) ~100% Ca recovery and complete chemical separation from K, Mg and Sr which are crucial issues when analysing Ca isotopes by TIMS, and (4) a twofold improvement of the external repeatability compared to the reference method used so far.

Measurements performed by using a ^{42}Ca–^{43}Ca double tracer on a TIMS instrument following the protocol described earlier by Holmden[33] allowed to diminish the $\delta^{44/40}$Ca external repeatability down to ±0.11‰ $\delta^{44/40}$Ca, similar to the smallest values presently published. This newly developed Ca separation technique will also prove useful for studies not compatible with the use of the double spike technique such as measuring Ca isotopes by MC-ICP-MS instruments or those determining ^{40}Ca excesses where an almost 100% recovery yield from the Ca column separation is requested.

Following the previous example of Sr separation, the efficiency of the present protocol in separating cations by peak detection and the relative low contamination it induces allows one to attempt its application to most of the cations analysed by mass spectrometry.

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Annexe 3

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The suitability of annual tree growth rings as environmental archives: Evidence from Sr, Nd, Pb and Ca isotopes in spruce growth rings from the Strengbach watershed

Le potentiel des anneaux de croissance des arbres en tant qu'archives environnementales : approche isotopique couplée Sr-Nd-Pb-Ca appliquée dans des cernes d'épicéas du bassin versant du Strengbach

Peter Stille [a,*], Anne-Désirée Schmitt [b], François Labolle [c], Marie-Claire Pierret [a], Sophie Gangloff [a], Florian Cobert [a], Eric Lucot [b], Florence Guéguen [a], Laure Brioschi [b], Marc Steinmann [b], François Chabaux [a]

[a] *LHyGeS-UMR7517, EOST, INSU/CNRS, université de Strasbourg, 1, rue Blessig, 67000 Strasbourg, France*
[b] *UMR 6249 chrono-environnement, université de Franche-Comté, 25030 Besançon, France*
[c] *Institut de zoologie et de biologie générale, université de Strasbourg, 12, rue de l'Université, 67000 Strasbourg, France*

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ABSTRACT

The combination of the Sr, Nd and Pb isotope systems, recognized as tracers of sources, with the Ca isotope system, known to reveal biology-related fractionations, allowed us to test the reliability of spruce (*Picea abies*) growth rings as environmental archives through time (from 1916 to 1983) in a forest ecosystem affected by acid atmospheric deposition. Sr and Pb isotopes have already been applied in former tree-ring studies, whereas the suitability of Nd and Ca isotope systems is checked in the present article. Our Sr and Nd isotope data indicate an evolution in the cation origin with a geogenic origin for the oldest rings and an atmospheric origin for the youngest rings. Ca isotopes show, for their part, an isotopic homogeneity which could be linked to the very low weathering flux of Ca. Since this flux is weak the spruces' root systems have pumped the Ca mainly from the organic matter-rich top-soil over the past century. In contrast, the annual growth rings studied are not reliable and suitable archives of past Pb pollution.

RÉSUMÉ

La combinaison des isotopes du Sr, du Nd et du Pb, reconnus comme étant des traceurs de sources avec le système isotopique du Ca, connu comme fractionnant lors de processus biologiques, nous a permis de tester le potentiel des cernes de croissance d'un épicéas (*Picea abies*) en tant qu'archives environnementales (entre 1916 et 1987) d'un écosystème affecté par des dépôts atmosphériques acides. Les isotopes du Sr et du Pb ont déjà été appliqués antérieurement dans des études de cernes d'arbres, alors que le potentiel des isotopes du Nd et du Ca appliqué à ces objets est étudié dans ce présent article. Nos

* Corresponding author.
E-mail addresses: pstille@unistra.fr (P. Stille), adschmitt@univ-fcomte.fr (A.-D. Schmitt).

résultats isotopiques en Sr et Nd indiquent une évolution dans l'origine des cations, les échantillons les plus anciens traduisant une source géogénique alors que les échantillons les plus récents ont une origine atmosphérique. Les isotopes du Ca montrent pour leur part une homogénéité isotopique au cours du temps qui pourrait être liée au très faible flux d'altération du Ca. Par conséquent le système racinaire des épicéas a certainement prélevé le Ca des horizons de sols superficiels riches en matière organique au cours du siècle passé. En revanche les cernes d'arbres étudiées ne sont pas des enregistreurs des pollutions passées en Pb.

1. Introduction

Acid atmospheric pollutants deposited on sensitive ecosystems like forested granitic catchments cause increasing acidification of surface waters and soils (Chapman et al., 2008; Nakano and Tanaka, 1997; Reuss and Johnson, 1986; Vogt et al., 2007). The sensitivity of silicate soils in the forested Strengbach catchment observatory (Vosges mountains; http://ohge.u-strasbg.fr) to acid deposition has been confirmed by several isotope and trace element studies (Aubert et al., 2001, 2002a,b; Dambrine et al., 1998; Probst et al., 2000; Viville et al., 1993).

The neutralization of acid atmospheric depositions in silicate-rich soils is, at a short timescale, mainly controlled by ion exchange processes because they are more rapidly compared to chemical weathering (Norton and Vesely, 2003). These processes release basic cations such as Mg^{2+}, Ca^{2+} and K^{+} into the draining surface waters and cause a depletion in these cations in the exchangeable pool of the upper (50 cm) soil compartments (Dambrine et al., 1998; Poszwa et al., 2003).

This might be harmful for such ecosystems, since the former cations are necessary for the growth of vegetation. Recent studies on soils from the Strengbach catchment have shown that with the exception of the leaf and needle litter layers, the upper 50 cm of the soils are strongly depleted in Ca, P and middle rare earth elements (REE) but strongly enriched in Al and Pb (Poszwa et al., 2003; Stille et al., 2009, 2011). The export of these elements from the watershed is partly interrupted due to their uptake by the plants' root system and storage in the vegetation cells. Indeed, isotope data of Ca, Sr and Nd (one of the REE metals) from the Strengbach catchment indicate that these elements are partly taken up by the vegetation from the soil solution and, therefore, become recycled after leaf excretion, leaf fall and leaf decomposition (Cenki Tok et al., 2009; Schmitt et al., 2003; Schmitt and Stille, 2005; Stille et al., 2006). According to the studies of Miller et al. (1993) and Holmden and Bélanger (2010) the proportion of recycled Ca from litterfall by vegetation might increase with decreasing weathering flux from 80% to 90% of the annual Ca input to the Ca cycle. Leaching experiments indicate that the uppermost soil samples contain an important quantity of adsorbed and leachable atmosphere and/or soil solution derived components with low $^{87}Sr/^{86}Sr$, $^{143}Nd/^{144}Nd$ and $^{206}Pb/^{204}Pb$ ratios, and that this mobile component, which is easily accessible for vegetation uptake, successively disappears with increasing depth, due to increasing pH of the soil solutions, the decrease of the soil cation exchange capacity and the increasing presence of primary, granite-derived minerals with depth; the mobile mainly atmosphere-derived component is replaced by another mobile reservoir with more geogenic, radiogenic Sr, Nd and Pb isotopic compositions (Stille et al., 2009, 2011). If a change of element sources occurred in the uppermost part of the soil system in the lifetime of a tree, then the change might have been recorded in its growth rings (Aberg et al., 1990; Drouet et al., 2005; Poszwa et al., 2003). Aberg (1995) and Aberg et al. (1990) observed that the Ca concentrations and $^{87}Sr/^{86}Sr$ ratios decrease from the center to the external part of the trunk and explained this phenomenon by the decrease of the contribution of Sr and Ca from the soil pool due to ongoing acidification and the constant contribution of rainwater Sr with a low $^{87}Sr/^{86}Sr$. The $^{87}Sr/^{86}Sr$ isotopic variation in tree-rings with time has been successfully modeled by Poszwa et al. (2003) using a mechanistic model based on long term variations of $^{87}Sr/^{86}Sr$ in trees and soils. The approach included soil acidification and root uptake. The results confirmed that the $^{87}Sr/^{86}Sr$ ratios of soils and trees decrease with soil acidification (Aberg, 1995) and suggested that spruce growth rings are archives of past atmospheric pollution and acidification. Presently, only two preliminary studies observe small Ca isotopic variations within tree-rings which could be the result of changing Ca sources and/or hydrological stress (Farkaš et al., 2011; Nielsen et al., 2011). However, Cobert et al. (2011) showed that Ca isotopes may also be fractionated by physiological processes. The laboratory experiments of Cobert et al. (2011) on bean plants indicate that low pH and low Ca concentrations in the nutritive solution cause smaller Ca isotopic fractionations between bean organs and solution than higher pH and Ca concentrations. However, at this point it is not clear how far an extrapolation of these experimental bean plant data to natural, soil-grown trees is appropriate.

Annual growth rings of *Picea abies* have also been used as archives of Pb pollution trends in Central Europe (Novak et al., 2010, and citations therein). However, how far dendrochemical patterns are indeed reliable archives of past atmospheric Pb pollution is still a matter of discussion and it appears that there are important disagreements between dendrochemical and other Pb archives (Bindler et al., 2004; Hagemeyer and Schäfer, 1995; Nabais et al., 1999).

The aim of the present study was to test and discuss the suitability of the annual growth rings as environmental archives of acid rain and past atmospheric pollution. To do

this, major, and trace element and Sr, Nd, Pb and Ca isotope determinations on growth rings of spruces from the forested Strengbach catchment have been performed. It is the first study on the Nd isotopic composition of growth rings. Earlier studies have shown that Nd isotopes are powerful tracers of atmospheric depositions on soils (Guéguen et al., 2012; Lahd Geagea et al., 2008a; Stille et al., 2006). It is also one of the first studies that reports and discusses the tree-ring Ca isotopic signatures. The sampled tree growth rings cover the period between 1916 and 1983 and, therefore, might have stored signs of the change in the relative contribution of Sr, Ca, Nd and Pb from the soil minerals and the atmosphere to the trees growth rings due to atmospheric pollution and increasing soil acidification.

2. Field settings

The forested Strengbach catchment is located in the Vosges mountains (north-east of France) (Fig. 1). It covers an 80 ha area at altitudes ranging from 880 m a.s.l. at the outlet to 1146 m a.s.l. at the top. The average annual pluviometry is around 1500 mm and the average temperature scatters around 6 °C; the climate is temperate mountainous and the west wind dominates. It has become a completely equipped environmental observatory with permanent sampling and measuring stations since 1986 (http://ohge.u-strasbg.fr).

The forest covers 90% of the area and corresponds to about 80% of spruces (mainly *Picea abies* L.) and 20% of beech (*Fagus sylvatica*). The bedrock is a hydrothermally altered Hercynian granite (Boutin et al., 1995). The soils are on average 80 cm deep, sandy and stony, and belong to a brown acidic to ochreous podzolic soil series (El Gh'Mari, 1995; Fichter et al., 1998a). Two neighbouring experimental soil profiles (PP and VP) at 1070 m a.s.l. altitude from the northern part of the watershed covered with spruces are regularly analysed (Fig. 1). Shallow soils (upper 50 cm) are acidic (pH = 3.7–5; Aubert et al., 2001; Poszwa et al., 2003; Solovitch-Vella et al., 2007). Their acidity is caused by bedrock lithology (Brezouard base-poor leucogranite), deposition of acid atmospheric pollutants (acid rain; Février et al., 1999; Party, 1999) and the formation of important quantities of organic acids derived from the

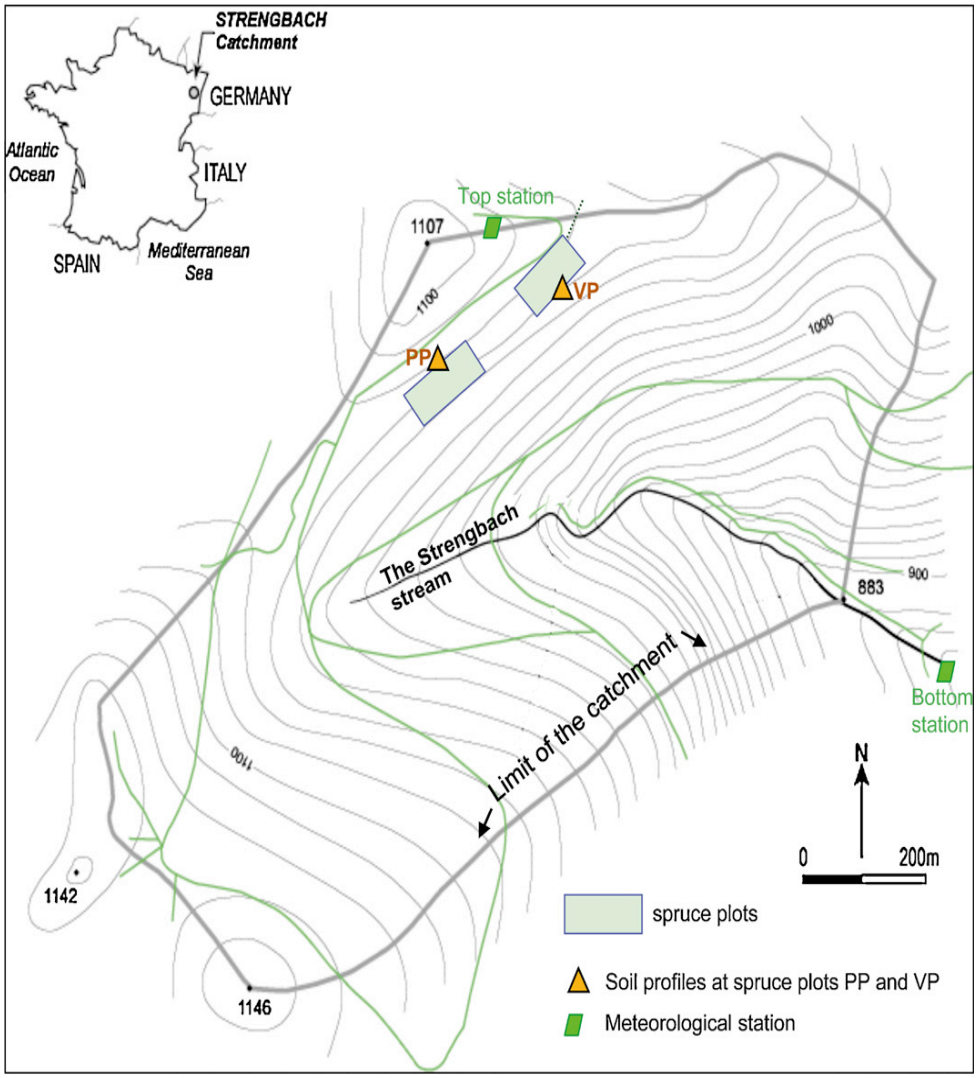

Fig. 1. Strengbach catchment with sampling sites at spruce plots PP and VP.

Fig. 1. Le bassin versant du Strengbach avec les sites d'échantillonnage PP et VP dans les parcelles recouvertes d'épicéas.

slow decomposition of organic matter (leaf and needle litter; Hansson et al., 2010; Lorenz et al., 2000). The studied spruces and soil solutions (Cenki Tok et al., 2009) are from the VP plot. Their ages range between 110 and 120 years and the tree density was 557 stems/ha (Biron, 1994). In 2008 we estimated a similar density of 575 stems/ha.

3. Sampling and analytical methods

Spruce trunk slices of 58–73 cm diameter were recovered in September 2005 from stumps (0 m height) of 3 spruce specimens freshly harvested for lumber from the VP plot (Fig. 1). Sampling at the standard height of 1.5 m was therefore not possible. The growth rings were counted at the Chrono-environment laboratory in Besançon. In order to recover at least 20 g of wood for the different chemical and isotope analyses (in particular for $^{143}Nd/^{144}Nd$), groups of growth rings rather than individual rings were sampled. Typically, groups covering 8 rings were extracted by splitting with an inox blade from the center (label 'C' in Table 1), the intermediate ('M'), and the external portion ('E') of each individual slice of trunk. The ages given in Table 1 are average values for each growth ring package. Growth rings younger than 1983 were not analyzed in order to avoid the active sapwood, which is an open isotopic system because of continuous re-equilibration by sap flow (Houle et al., 2008). For chemical and Sr, Nd and Pb isotope analyses, about 20 g of sample were split into match-scale splints and calcined using a step-wise heating procedure (350, 450, and 500 °C during 90, 90, and 120 min., respectively). The large sample size was necessary in order to have enough Nd for the determination of its isotopic composition. Nevertheless, two Nd isotope measurements failed due to the low Nd concentrations (< 5 ppb). Of course, this sampling strongly reduced the temporal resolution of the dendro-isotopical signal.

The ashed samples were digested under clean-lab conditions with 6 mL of 15 molar, distilled HNO_3 in closed teflon vessels. After 30 minutes at room temperature, the samples were progressively heated in a microwave stove (135, 160, and 100 °C during 10, 10, and 20 min., respectively), evaporated, taken up in 15 mL of 1 molar HNO_3 and divided into different aliquots for Nd, Sr, and Pb isotope analysis. The same digestion procedure was used for concentration analysis by ICP-MS, but with only 1 g of sample material.

For Ca isotope measurements about 100 mg of dried (not ashed) grinded trunk samples were digested in savillex^{TM} vials using a hot oxidative acid method (double distilled HNO_3, double distilled HCl and H_2O_2 suprapur).

The atmospheric particulate matter (PM) were collected with passive « Sigma-2 » samplers (Grobéty et al., 2010) close to the VP plot at the top station (Fig. 1; Guéguen et al., 2010, 2012). The sampling technique is based on the sedimentation principle (Stokes's law) and collects particles in the size range < 100 μm. Compared with the active samplers it allows a more precise trace element analysis since the PM can quantitatively be transferred, but it necessitates a much longer sampling time of two to four weeks.

The major and trace element concentrations were measured by ICP-AES and ICP-MS at the *Laboratoire d'Hydrologie et de Géochimie de Strasbourg* (LHyGeS, Strasbourg). The analytical error is < 5% and the detection limit is 0.01 μg/L (Chabaux et al., 2011; Steinmann and Stille, 1997; Stille et al., 2009). For all elements analyzed, our results were in good agreement with those of the certified reference materials (e.g. AGV-1, GSN, BEN and AIEA lichen standard).

The recovered solutions were evaporated and then prepared for Nd, Sr, Pb and Ca isotope analysis using standard techniques (Lahd Geagea et al., 2008a; Schmitt et al., 2009; Steinmann and Stille, 1997). Sr and Nd were separated from other elements using Eichrom's Sr Resin in series with Eichrom's TRU Resin and Eichroms Ln Resin according to Pin and Zalduegui, 1997. The Pb was separated from other elements using Bio-Rad AG1-X8 anion exchange resin and 0.6 N HBr and 6 N HCl as eluents. Ca was separated from matrix elements using a high selectivity automated ionic chromatography (HPIC, ICS-3000, DIONEX) (Schmitt et al., 2009). The procedural blanks were 0.35 ng, 0.13 ng and 0.005 ng for Pb, Sr and Nd, respectively. The procedural Ca blank was < 150 ng which corresponds to max. 2.5% of the total amount of Ca analyzed.

The Pb and Sr isotopic compositions were determined using a fully automatic VG Sector thermal ionization mass spectrometer at the LHyGeS with a 5-cup multicollector

Table 1
Ca, Sr, Pb and Nd concentrations in spruce tree-rings.

Tableau 1
Concentrations en Ca, Sr, Pb et Nd dans des cernes d'épicéas.

Sample	Age interval	Median age	Ca	Sr	Pb	Nd
	Years		ppm	ppm	ppm	ppb
VPE-1–E	1961–1969	1965	426.8	1.99	2.59	4.23
VPE-1-M	1934–1942	1938	429.0	1.79	1.88	4.65
VPE-1–C	1917–1925	1921	605.6	4.01	2.76	5.86
VPE-2–E	1968–1976	1972	329.0	1.08	3.91	5.32
VPE-2–M	1933–1941	1937	397.6	1.45	2.74	5.96
VPE-2–C	1918–1926	1922	709.8	3.24	3.68	9.51
VPE-5–E	1979–1987	1983	376.8	1.40	2.01	5.06
VPE-5–M	1951–1959	1955	382.1	1.26	6.18	4.93
VPE-5–C	1912–1920	1916	475.4	1.59	0.74	6.27

after enrichment and separation from the bulk sample. During the measurement period the NBS 987 standard yielded $^{87}Sr/^{86}Sr = 0.710259 \pm 0.000021$ (2σ, $n = 12$). The Pb isotopic data presented here were adjusted for mass fractionation by repeated analyses ($n = 30$) of the NBS 981 standard. The 2 σ-errors of the reported isotopic ratios are for $^{206}Pb/^{204}Pb \pm 0.021$, $^{207}Pb/^{204}Pb \pm 0.024$, $^{206}Pb/^{207}Pb \pm 0.00062$ and $^{208}Pb/^{206}Pb \pm 0.0021$. The Nd isotopic compositions were determined using a Nu instruments MC-ICP-MS at the branch of Isotope Geology at the University of Berne. The in-house standard yielded $^{143}Nd/^{144}Nd = 0.511061 \pm 0.000020$ (2σ, $n = 23$) corresponding to a value for the La Jolla standard of 0.511843. The Ca isotopic compositions were measured on a Triton thermal ionization mass spectrometer at the LHyGeS using a ^{42}Ca-^{43}Ca double spike and following a procedure adapted from Holmden, 2005 and presented in Schmitt et al., 2009. The results were expressed as $\delta^{44/40}Ca$ (‰) using the NIST SRM 915a standard (Eisenhauer et al., 2004). The 2SD long-term external reproducibility was found equal to ± 0.12‰ based on repeated standard and sample measurements.

4. Results

The Ca, Sr, Nd and Pb concentration and isotope data are given in Tables 1 and 2, respectively. The growth rings of the 3 spruce trunks show a similar record with decreasing Ca, Sr, and Nd concentrations from the core to the external portions of the trunk slides (Figs. 2a–c). The strongest relative concentration change is between the innermost growth rings, which formed in the same time period between 1916 and 1922, and the intermediate segments, which formed just before 1940. The Pb concentrations manifest no time-dependent progression (Fig. 2d).

The $^{87}Sr/^{86}Sr$ isotope ratios of the tree growth rings show a time-dependent evolution with high values in the innermost growth rings (1916–1922) that scatter comparatively weakly (0.7305–0.7315) and are significantly higher than those of the outermost segments (Figs. 3 and 4a). Growth rings of spruce from the VP site (Fig. 1) have previously been studied (Poszwa, 2000; Poszwa et al., 2003). Unfortunately, they only analyzed tree-rings from 1950 to 1990. Their $^{87}Sr/^{86}Sr$ ratios show, with the exception of one sample, a similar range of variation (Fig. 4a). In VPE-1 and, to a lesser extent, in VPE-5 samples, high $^{87}Sr/^{86}Sr$ ratios are associated with higher Ca and Sr concentrations (Fig. 3a,b).

The $^{143}Nd/^{144}Nd$ isotope ratios of the VPE-5 samples in the outermost (1983) and innermost (1916) segments are identical (Fig. 4b). However, innermost and outermost growth rings of VPE-1 and VPE-2 samples are significantly different with comparatively lower Nd concentrations and $^{143}Nd/^{144}Nd$ ratios in the outermost growth rings (Fig. 3c). Observable but less pronounced is the time dependent evolution of the $^{143}Nd/^{144}Nd$ ratios in the growth rings of trees VPE-1 and VPE-2 (Fig. 4b). Their outermost segments show lower Nd isotopic composition values than their innermost segments. The Sr and Nd isotopic compositions of atmospheric PM from urban environments and the catchment are significantly lower than those observed for tree-rings (Table 2).

In contrast to the Sr and Nd isotope ratios, the different segments do not allow us to recognize a time dependent evolution of the Pb isotope ratios (Fig. 4c). Similarly, Pb concentrations and Pb isotopic compositions show no covariation and high Pb concentrations and $^{208}Pb/^{204}Pb$ isotope ratios are observable for outermost and innermost segments (Fig. 3d). The $^{208}Pb/^{204}Pb$ and $^{206}Pb/^{204}Pb$ ratios of the growth rings are well correlated with each other and plot within the range of soil leachates from the uppermost 40 cm of the neighboured PP soil profile (Fig. 5) (Stille et al., 2011).

$\delta^{44/40}Ca$ values of the tree-rings are within error bars relatively homogenous between the three studied trees (Fig. 6). Their average value is equal to 0.42 ± 0.06 ($n = 9$, 2SE). Moreover, the 3 growth rings of each of the studied trees show rather small variations of their $\delta^{44/40}Ca$ values (0.50 ± 0.07, 0.37 ± 0.06, 0.38 ± 0.13, $N = 3$, 2SE, for VPE-1, VPE-2, VPE-5, respectively).

Table 2
Pb, Sr, Nd and Ca isotopic compositions in spruce tree-rings.

Tableau 2
Compositions isotopiques en Pb, Sr, Nd et Ca dans des cernes d'épicéas.

Sample	Median age (years)	$^{206}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{208}Pb/^{204}Pb$	$^{206}Pb/^{207}Pb$	$^{87}Sr/^{86}Sr$	$\pm 2s_{mean}$	$^{143}Nd/^{144}Nd$	$\pm 2s_{mean}$	$d^{44/40}Ca$	$\pm 2s_{mean}$[a]	n
VPE-1–E	1965	18.070	15.565	37.86	1.1610	0.72466	0.00001	0.51196	0.00002	0.49	0.08	2
VPE-1-M	1938	18.505	15.606	38.42	1.1858	0.72649	0.00001			0.56	0.08	2
VPE-1–C	1921	18.153	15.609	38.11	1.1629	0.73056	0.00001	0.51206	0.00003	0.44	0.08	2
VPE-2–E	1972	18.218	15.591	38.12	1.1684	0.72255	0.00001	0.51205	0.00002	0.31	0.08	2
VPE-2–M	1937	18.021	15.587	37.96	1.1562	0.72341	0.00001	0.51202	0.00002	0.40	0.08	2
VPE-2–C	1922	18.197	15.563	38.02	1.1692	0.73146	0.00001	0.51217	0.00007	0.41	0.08	2
VPE-5–E	1983	18.246	15.597	38.15	1.1698	0.72537	0.00001	0.51206	0.00004	0.42	0.08	2
VPE-5–M	1955	18.593	15.619	38.50	1.1904	0.72826	0.00001			0.46	0.08	2
VPE-5–C	1916	18.533	15.619	38.45	1.1865	0.73047	0.00001	0.51206	0.00003	0.25	0.08	2
PM catchment						0.71354	0.00003	0.51195	0.00010			
PM #1 urban						0.70919	0.00001	0.51198	0.00008			
PM #9 urban						0.70867	0.00001	0.51190	0.00001			

[a]Calculated from long term external 2 standard deviation reproducibility of 0.12.

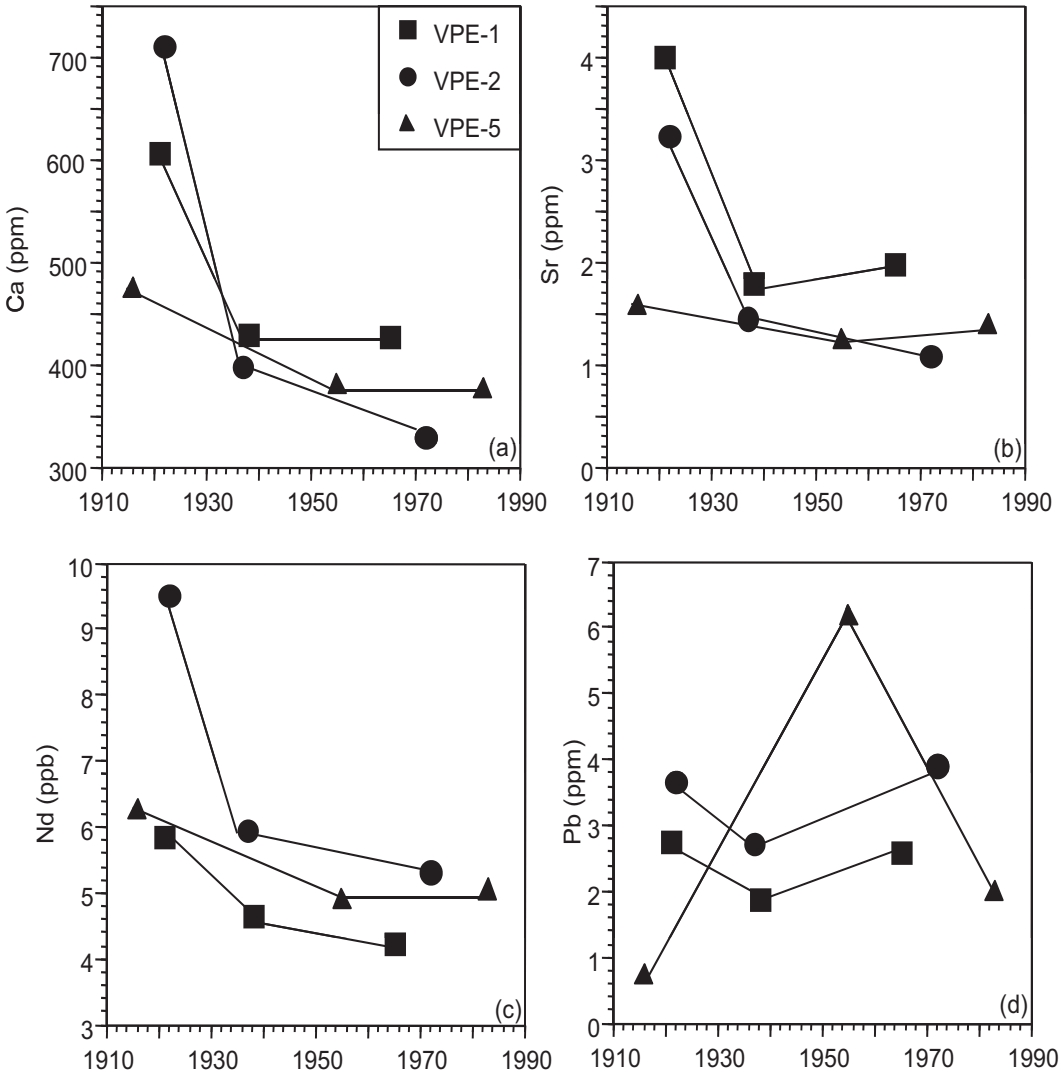

Fig. 2. Ca, Sr, Nd and Pb concentrations in tree growth rings through time (1916–1983).

Fig. 2. Concentrations en Ca, Sr, Nd et Pb dans les cernes d'épicéas au cours du temps (1916–1983).

5. Discussion

5.1. Biological effects on the tree-rings' chemical and isotopical composition

A key question is to what extent growth rings record changing environmental conditions. It is furthermore not clear whether growth rings are closed systems with no chemical exchange with the adjacent rings. A cross-section through a trunk might help to answer this question. The wood rays are indeed composed of rows of elongated living cells in the radial direction (Fig. 7). They provide a living link between the center and the periphery of the trunk. Parenchymal cells of wood rays are able to transfer substances, among others nutrients, from the center of the trunk to its living peripheral part or to store substances taken from the peripheral part in the older parts of the trunk. Some wood rays are continuous from the periphery of the wood to the center of the trunk, others are shorter and do not reach the center. Phloem rays have for their part the same structure as the rays of the wood. They connect the phloem to the outer part of the bark and thus allow the transfer of material from the cambium to the bark and vice versa. Radial-transport and re-equilibration of base-cations in growth rings have been discussed by several authors (Feretti et al., 2002; Drouet et al., 2005, and cit. therein). The study of Drouet et al. (2005) on beech from a limed and a not limed beech stand shows that the dendro-isotopical Sr pattern of the beech from the limed stand has been significantly influenced: the tree-rings foredate the liming application date by about 50 years. These experiments confirm on the one hand that growth rings are environmental archives, but on the other hand that radial transport might to some extent cause some isotopic re-equilibration between older and young growth rings. Thus, isotope ratios do not always allow a precise timing record of a polluting event. Similarly, the Ca, Sr and Nd concentration decrease from the inner to the outer part of the trees growth rings (Figs. 2a–c) might not only be or not at all linked with an

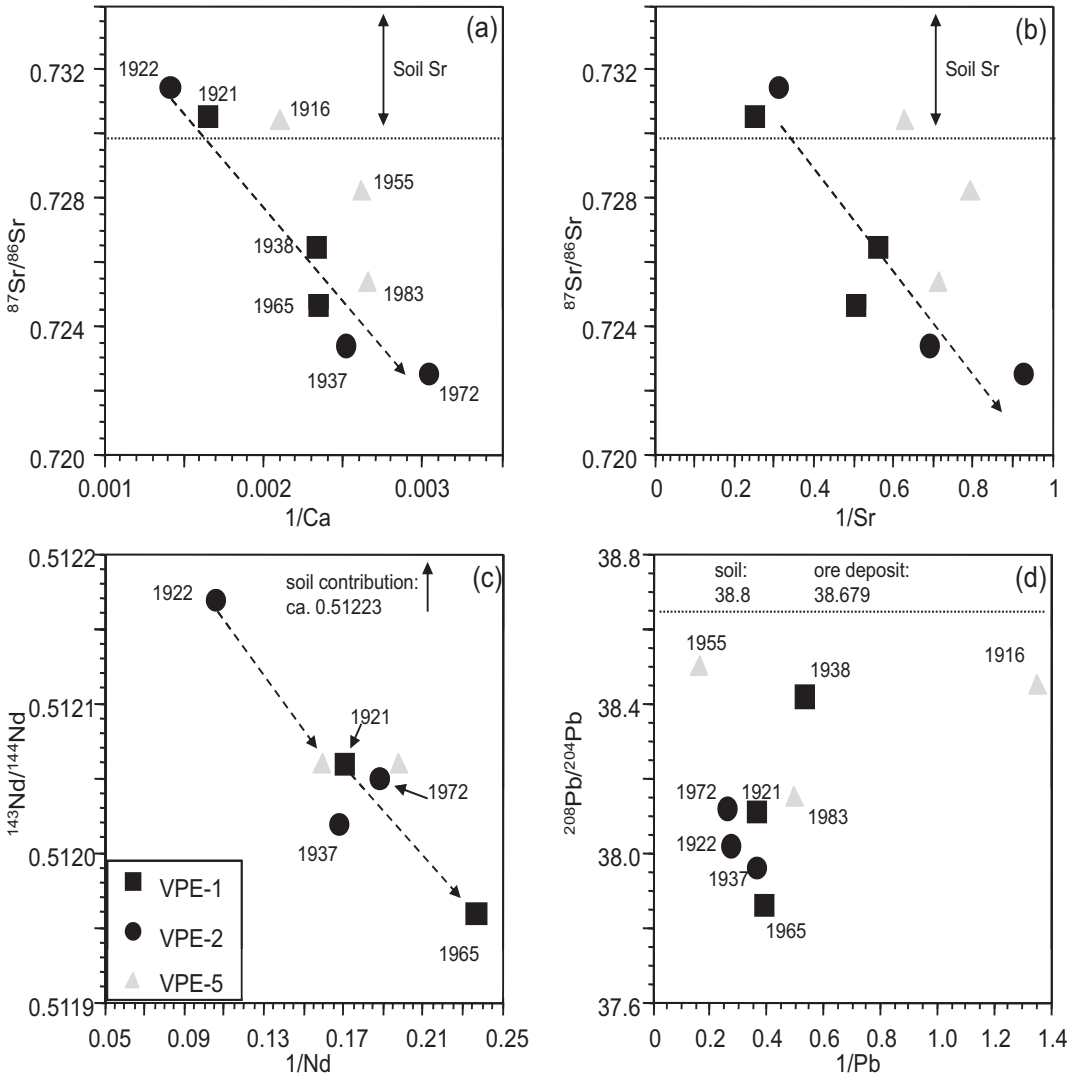

Fig. 3. Relationships between (a), (b) Ca-Sr concentrations and $^{87}Sr/^{86}Sr$ isotopic compositions, (c) Nd concentrations and $^{143}Nd/^{144}Nd$ ratios and (d) Pb concentrations and $^{208}Pb/^{204}Pb$ isotope ratios in spruce growth rings. Time dependent evolutions are observable for Ca, Sr and Nd but not for Pb.

Fig. 3. Relations entre (a), (b) les concentrations en Ca et en Sr et les compositions isotopiques du Sr, (c) les concentrations en Nd et les rapports isotopiques du Nd et (d) les concentrations en Pb et les rapports isotopiques du Pb dans les cernes d'épicéas. On observe une évolution au cours du temps pour les concentrations en Ca, Sr et Nd en fonction des rapports isotopiques du Sr et du Nd mais pas pour les concentrations en Pb en fonction des rapports isotopiques du Pb.

increasing cation depletion of the soils. Several studies have shown that outwardly decreasing Ca concentrations in oak and beech wood is not related to environmental changes, but to endogenous parameters such as wood binding capacity (cation exchange capacity) which decreases from pith to bark (Herbauts et al., 2002, and cit. therein). Other research on red spruce stemwood, however, showed that relative to the binding capacities increasing or decreasing Ca concentrations might occur in tree-rings, pointing to increasing cation mobilization, respectively decreasing availability of nutrient cations, in soils (Bondietti et al., 1990; Momoshima and Bondietti, 1990). Our study does not allow us to decide how far the Sr, Ca and Nd concentrations are really related to environmental changes and, therefore, shall not be included in the following discussions.

5.2. The Sr-Nd isotope record of the growth rings

The changing Sr and Nd isotopic compositions through time can be explained by an overall replacement of pedogenic elements by needle litter and atmosphere-derived Sr and Nd as a consequence of acidification. A similar model has been proposed by Drouet et al. (2005) who observed for forest sites of High Belgium with very acid soils and low concentrations of exchangeable Ca "a decrease of the $^{87}Sr/^{86}Sr$ ratio in growth rings from inner to outer wood, for beech and oak, suggesting that these forest ecosystems were abruptly affected by atmospheric inputs of strong acids around the 1920".

In both cases, it is the atmospheric contribution causing the shift toward less radiogenic isotopic composition values as emphasized in the Sr-Nd isotope diagram (Fig. 8), which allows one to distinguish between atmosphere-

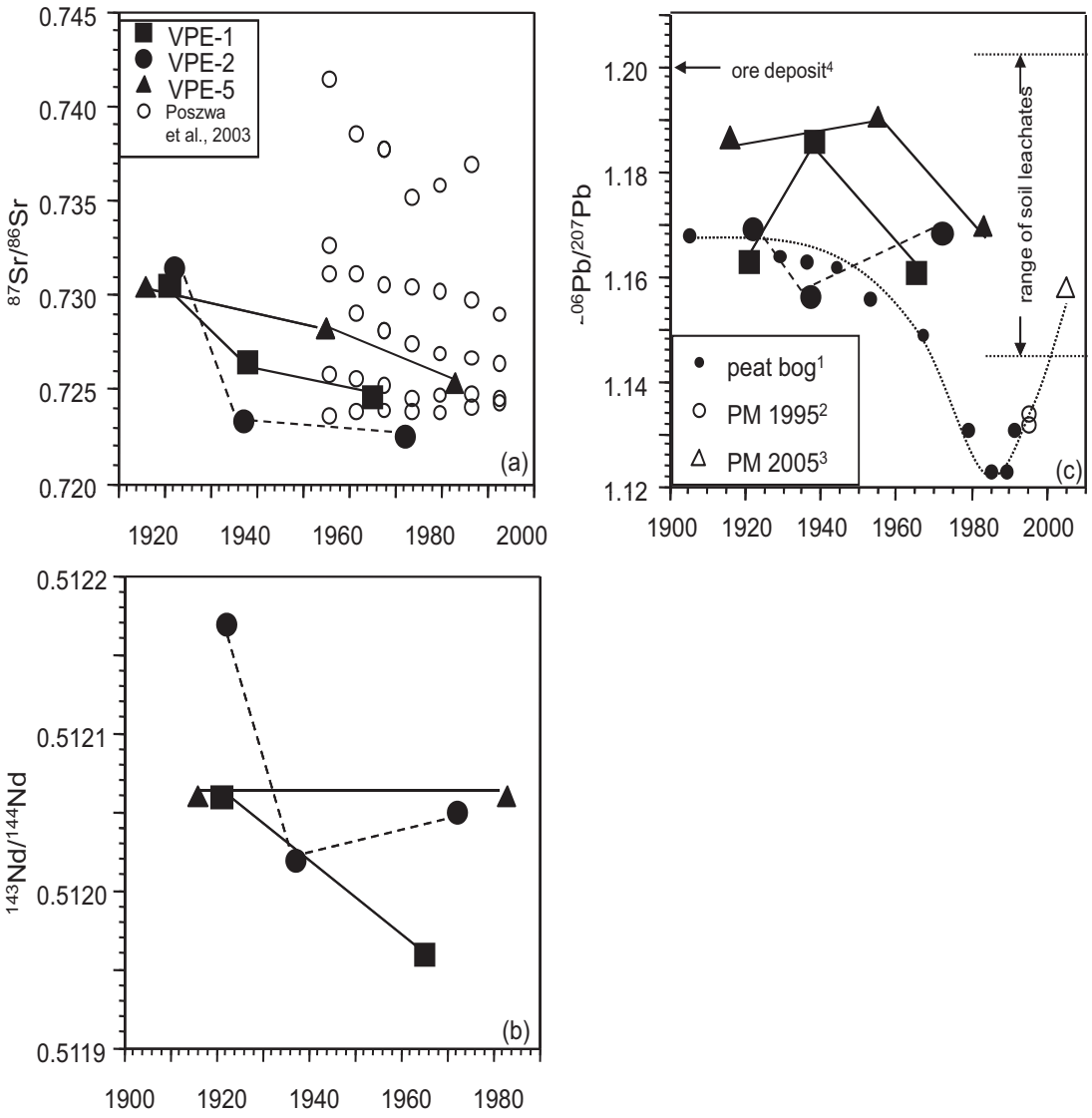

Fig. 4. (a), (b) $^{87}Sr/^{86}Sr$ and $^{143}Nd/^{144}Nd$ vs. time for spruce growth rings, (c) $^{206}Pb/^{207}Pb$ vs. time for spruce growth rings; comparison with the evolution of the atmospheric Pb isotopic composition through the 20th century derived from peat bog and atmospheric particulate matter (PM). The uppermost 40 cm of the soils are strongly enriched in Pb compared to the bedrock. At close to the surface the corresponding soil leachates carry a present-day anthropogenic Pb isotopic signature ($^{206}Pb/^{207}Pb$: 1.148 and 1.167) and at 30 to 40 cm depth an old anthropogenic Pb isotopic signature derived from ore mining and smelting 200 to 300 years ago ($^{206}Pb/^{207}Pb$: 1.197 and 1.225; Stille et al., 2011) (1: Shotyk et al., 2001; 2: Monna et al., 1997; 3: Lahd Geagea et al., 2008a; 4: Marcoux, 1987).

Fig. 4. (a), (b) Évolution au cours du temps de $^{87}Sr/^{86}Sr$ et $^{143}Nd/^{144}Nd$ dans les cernes de croissance d'épicéas, (c) $^{206}Pb/^{207}Pb$ en fonction du temps pour les cernes d'épicéas ; comparaison avec l'évolution de la composition atmosphérique en Pb au cours du XX^{e} siècle déterminée à partir de la composition isotopique en Pb de tourbières et de particules atmosphériques (PM). Les 40 cm supérieurs des sols sont fortement enrichis en Pb par rapport à la roche mère. Près de la surface les lessivats des sols montrent une signature isotopique en Pb similaire à la signature anthropique actuelle ($^{206}Pb/^{207}Pb$: 1,148 et 1,167). Vers 30–40 cm de profondeur les signatures correspondent à d'anciennes signatures anthropiques, similaires aux mines et industries d'il y a 200 à 300 ans ($^{206}Pb/^{207}Pb$: 1.197 et 1.225; Stille et al., 2011). (1 : Shotyk et al., 2001 ; 2 : Monna et al., 1997 ; 3 : Lahd Geagea et al., 2008a ; 4 : Marcoux, 1987).

and bedrock-derived contributions (Aubert et al., 2001; Stille et al., 2009). Pure soil-derived materials plot upon or to the upper right of this mixing curve. In contrast, Sr-Nd isotopic compositions to the lower left of the curve are indicative for the presence of only atmospheric contributions (Stille et al., 2009). The Sr-Nd isotopic compositions of our spruce trunk samples, that plot to the lower left of the curve of bedrock alteration products (apatite-plagioclase mixing curve; Aubert et al., 2001), thus demonstrate that they do not only contain bedrock-derived Sr and Nd, but important quantities of atmospheric compounds. They are isotopically similar to those of lichen, tree bark, root and leaf samples from beech, leaf litter and throughfall. This interpretation is in accord with observations that show that soil acidification induces a rise of the fine root system of spruce towards the topsoil (Poszwa et al., 2003, 2004). Poszwa et al. (2004) have especially shown that the average depth of Sr and probably Ca absorption for spruces in boreal forest ecosystems is about 20 cm. Similar depths have been found by Ladouche (1997) for spruce from the Strengbach catchment using oxygen isotopes. The rise of the root system allows spruces in Ca-depleted soils to mobilize Ca accumulated in the organic matter-rich topsoil (Berger et al., 2006; Berner et al.,

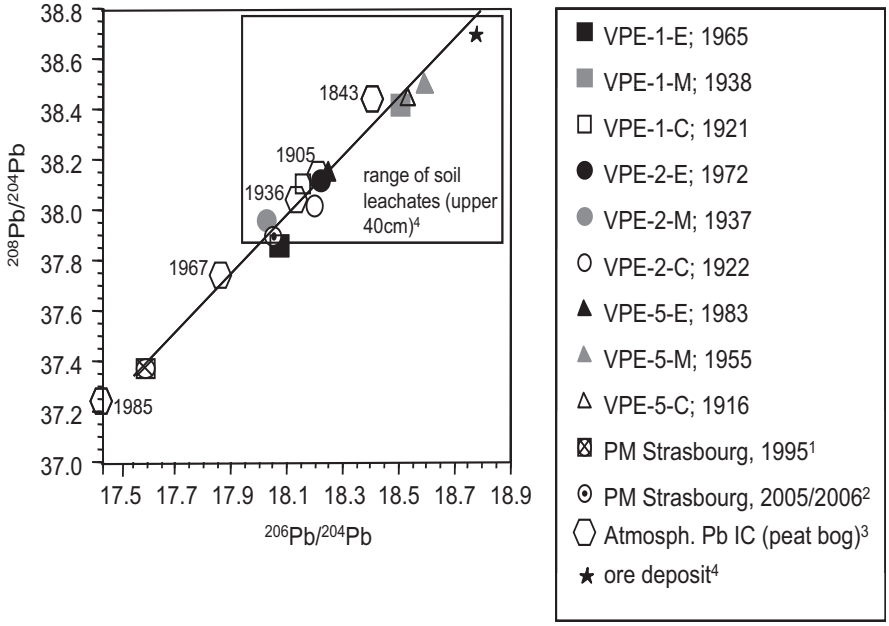

Fig. 5. $^{208}Pb/^{204}Pb$ vs. $^{206}Pb/^{204}Pb$ for spruce growth rings which show the same range of Pb isotopic compositions as the leachates from the uppermost soil compartments (Stille et al., 2011). Also shown are atmosperic Pb isotopic compositions derived from particulate matter from an urban environment (cities of Strasbourg and Kehl situated 80 km north of the catchment) (1: Monna et al., 1997; 2: Lahd Geagea et al., 2008a) and peat bog (3: Shotyk et al., 2001). Pb isotopic signature of ore deposit from 4: Marcoux (1987).

Fig. 5. $^{208}Pb/^{204}Pb$ vs. $^{206}Pb/^{204}Pb$ pour des cernes d'épicéas qui présentent une amplitude de variation similaire à celle des compositions isotopiques en Pb des lessivats des compartiments superficiels des sols (Stille et al., 2011). Les compositions isotopiques en Pb dérivées de matières particulaires provenant d'un environnement urbain (villes de Strasbourg et de Kehl situées à 80 km au nord du bassin versant) (1 : Monna et al., 1997 ; 2 : Lahd Geagea et al., 2008a), ainsi que de tourbières (3 : Shotyk et al., 2001) sont également représentées. La signature isotopique en Pb des mines provient de 4 : Marcoux (1987).

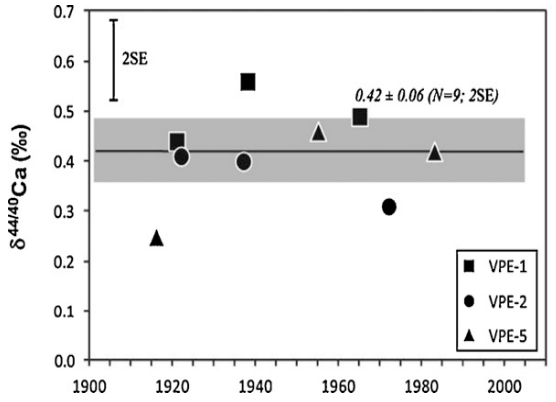

Fig. 6. $\delta^{44/40}Ca$ values determined on spruce growth rings. The 2SE amplitude of Ca isotopic replicate measurements is indicated in the top left corner. The black line and the shaded area correspond to the average value of the nine measured tree-rings and its corresponding 2SE.

Fig. 6. Compositions isotopiques en Ca mesurées dans les cernes d'épicéas. La reproductibilité externe des mesures (2SE) est représentée dans le coin en haut à gauche. Le trait noir et le rectangle grisé correspondent respectivement à la valeur moyenne et au 2SE des neuf valeurs de cernes.

2007; Poszwa et al., 2000). This is certainly also the case for other trace elements such as Nd and Pb.

The $^{87}Sr/^{86}Sr$ and most of the $^{143}Nd/^{144}Nd$ ratios of the outermost growth rings of VPE-1 and VPE-2 samples are significantly lower than those of the innermost ones. However, most significant is the change in Sr and Nd isotopic compositions between the innermost and intermediate segments. Thus, similar to the study of Drouet et al., 2005, one might suggest that the greatest rate of acidification occurred in the period between 1922 and 1938. This has to be tested in another, more detailed study. The low Nd-Sr isotopic compositions of the youngest growth rings of spruce thus reflect a dominating atmospheric contribution. They are comparable with isotopic compositions of urban PM and PM collected in the catchment, with those of industrial emissions from the Rhine valley (not shown; Lahd Geagea et al., 2007, 2008a,b) and with those of North African dusts (Fig. 8) (Grousset and Biscaye, 2005). In this scenario, atmospheric acid deposition would have led to a general cation depletion of the soil due to desorption and to dissolution of soil minerals, resulting in nutritional deficiencies for vegetation (Probst et al., 1990). This causes the progressive substitution of pedogenic cations by atmospheric inputs.

Thus, our study confirms for the first time that not only the Sr but also the Nd isotopic compositions of tree-rings record the chemical and isotopical changes in the soil system and, therefore, allow one to recognize the principal sources of Sr and Nd in the wood.

5.3. The origin and the behaviour of Ca isotopes in the trees growth rings

In contrast to Sr and Nd isotopes, the Ca isotopic homogeneity of the three growth segments of each of the trees ranging from 1916 to 1983 ($\delta^{44/40}Ca$: 0.42 ± 0.06, $n = 9$, 2SE) might suggest that the Ca sources remained the same or isotopically similar during this timespan (Fig. 6). The important nutrient environment for trees are soil solutions

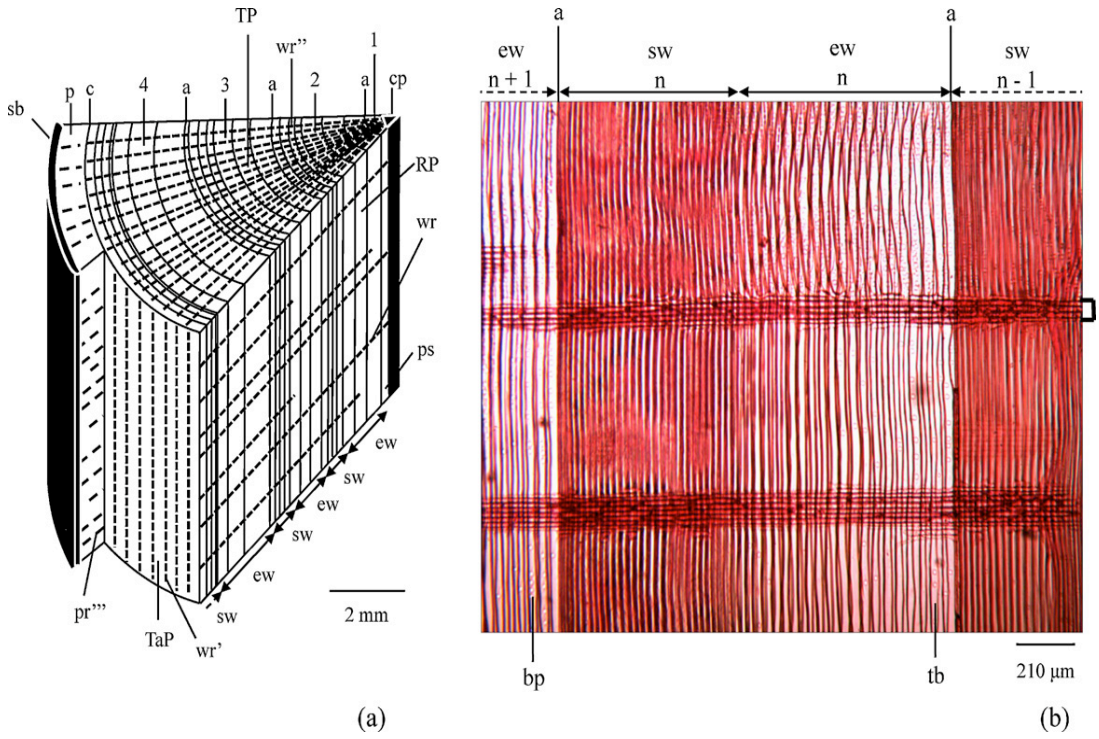

Fig. 7. (a) Simplified block diagram of a portion of the trunk of a conifer in the late summer of the fourth year, of growth, and (b) radial cut in the trunk of a conifer (adapted from Bresinsky et al., 2008). Microscopic preparation: Turtox No BW 1.1, Gen. Biol. Sup. House Chicago, Illinois, USA, safranin coloration; Microscope: Olympus CH-BI45-T-2; Shooting: caméra DCM 130E, 1.3 Mpixels. Note that the wood rays are continuous from year to year in both parts of the Figure. TP: transverse plane; RP: radial plane; TaP: tangential plane; 1, 2, 3, 4: successive years; *n*–1, *n*, *n* + 1: successive years; ew: earlywood; sw: summer wood; cp: pith; ps: primary and secondary tissues of the first year; a: delineation of a growth ring; wr: wood ray (parenchyma cells) seen in the radial plane; wr': wood ray (parenchyma cells) seen in the tangential plane; wr'': wood ray (parenchyma cells) seen in the transverse plane; c: cambium; p: phloem; pr''': phloem ray; sb: suberized bark; tb: tracheid with bordered pits; bp: bordered pits.

Fig. 7. (a) Bloc-diagramme simplifié d'une partie de tronc de conifère en fin d'été de quatrième année ; et (b) coupe radiale dans un tronc de conifère (adapté de Bresinsky et al., 2008). Préparation microscopique : Turtox No BW 1.1, Gen. Biol. Sup. House Chicago, Illinois, USA, coloration à la safranine ; Microscope : Olympus CH-BI45-T-2 ; Shooting : caméra DCM 130E, 1,3 Mpixels. Noter que les rayons ligneux sont continus d'une année à l'autre dans les deux parties de la figure. TP : plan transversal ; RP : plan radial ; TaP : plan tangentiel ; 1, 2, 3, 4 : années successives ; *n* –1, *n*, *n* + 1 : années successives ; ew : bois de printemps ; sw : bois d'été ; cp : moelle centrale ; ps : tissus primaires et secondaires de la première année ; a : delimitation d'un cerne annuel ; wr : rayon ligneux (cellule parenchyme) vu dans le plan radial ; wr' : rayon ligneux (cellule parenchyme) vu dans le plan tangentiel ; wr'' : rayon ligneux (cellule parenchyme) vu dans le plan transverse ; c : cambium ; p : liber ; pr''' : rayon phloémien ; sb : écorce subérifiée ; tb : trachéide à ponctuation aréolée ; bp : ponctuation aérolée.

which carry pedogenic and needle litter derived cations. The needle litter itself contains important quantities of cations derived from nutrient cycling through vegetation and atmospheric deposition. Current atmospheric inputs such as rainwater and snow, analysed at the Strengbach catchment, have $\delta^{44/40}$Ca values scattering largely between 0.57‰ and 1.29‰ (Cenki Tok et al., 2009; Schmitt and Stille, 2005). This local scale scattering is consistent with atmospheric rain and snow deposits sampled all over the world (Ewing et al., 2008; Hindshaw et al., 2011; Holmden and Bélanger, 2010; Schmitt and Stille, 2005). We also note that the Ca taken up by roots from soil solutions at the spruce plot has a $\delta^{44/40}$Ca value similar to that of atmospheric deposits (0.82 ± 0.19; 2SE, $n = 9$) (Table 3) (Cenki Tok et al., 2009). Thus, the tree-rings are enriched in the light isotope compared to current soil solutions. This is consistent with other studies which have observed that the Ca uptake by vegetation causes the enrichment in the heavy ^{44}Ca isotope in soil solutions and in the light ^{40}Ca isotope in the vegetation (Cenki Tok et al., 2009; Cobert et al., 2011; Hindshaw et al., 2011; Holmden and Bélanger, 2010; Page et al., 2008; Platzner and Degani, 1990; Schmitt and Stille, 2005; Schmitt et al., 2003; Wiegand et al., 2005). The geogenic Ca source is for its part mainly controlled by apatite dissolution ($\delta^{44/40}$Ca = 0.4‰, Schmitt et al., 2003). Consequently, the rise of the fine root system of spruce towards the topsoil due to soil acidification (Poszwa et al., 2003, 2004) and the herewith induced change of Ca source from primary minerals (e.g. apatite; $\delta^{44/40}$Ca~0.4‰) to top soil-derived Ca ($\delta^{44/40}$Ca~0.8‰) has apparently no significant influence on the $\delta^{44/40}$Ca values of the trees growth rings.

This is confirmed by two mass balance equations. In the first case, it is assumed that all Ca loss between soil solutions *F*-5 cm (F_1) and *F*-30 cm (F_2) results from plant immobilization (F_{tree}) and that the weathering flux brought from primary soil minerals to soil solution (F_{alt}) is still operating; in this case the soil solutions at 5 cm and 30 cm depths obey the following mass balance equation:

$$F_1 + F_{alt=}F_2 + F_{tree} \quad (1)$$

Using the Ca fluxes (Table 3) yields a weathering flux (F_{alt}) of 1.78 kg ha^{-1} yr^{-1} which is high compared to the model estimations of Fichter et al. (1998b) and Dambrine

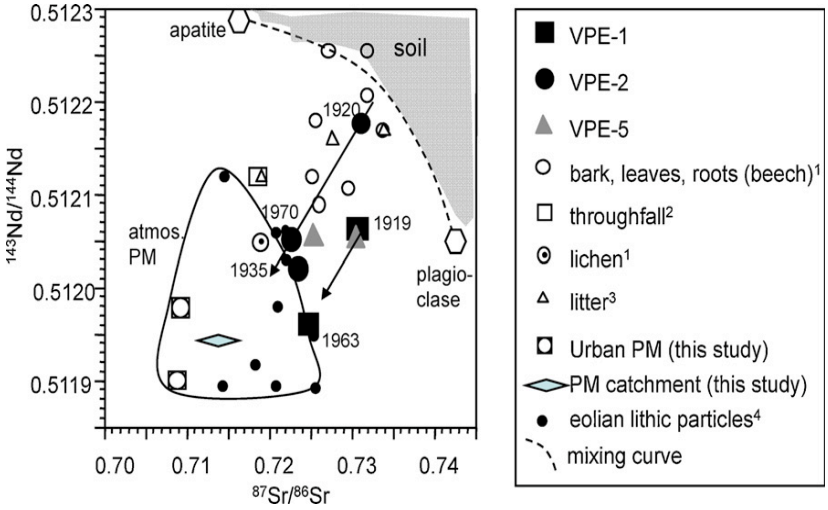

Fig. 8. Comparison of Sr and Nd isotope data of spruce growth rings with soil, North African dust and particulate matter from an urban environment (cities of Strasbourg and Kehl) and from the Strengbach catchment (Table 2). Also given is the mixing curve of alteration products defined by apatite and plagioclase separating possible granite derived soil mineral contributions (to the right of the mixing curve) (Aubert et al., 2001) from atmospheric contributions (field below curve). (1: Stille et al., 2006; 2: Aubert et al., 2002a; 3: Stille et al., 2009; 4: Grousset and Biscaye, 2005). PM refers to Particulate Matter.

Fig. 8. Comparaison des signatures isotopiques en Sr et en Nd des cernes d'épicéas avec des valeurs de sols, des poussières provenant d'Afrique du Nord et des matières particulaires provenant d'un environnement urbain (villes de Strasbourg et Kehl) et du bassin versant du Strengbach (Tableau 2). La droite de mélange correspondant aux produits d'altération définis par l'apatite et le plagioclase et séparant la contribution des minéraux issus de l'altération du granite (à la droite de la courbe de mélange) (Aubert et al., 2001) des contributions atmosphériques (domaine en-dessous de la courbe) est également représentée. (1 : Stille et al., 2006 ; 2 : Aubert et al., 2002a ; 3 : Stille et al., 2009 ; 4 : Grousset and Biscaye, 2005). PM est l'abréviation de matière particulaire.

Table 3
Ca and Sr annual fluxes and isotopic compositions at VP sampling site (spruces).

Tableau 3
Flux annuels de Ca et de Sr et compositions isotopiques associées pour le site d'échantillonnage VP (épicéas).

Sample	Ca	$\delta^{44/40}$Ca	Sr	$^{87}Sr/^{86}Sr$
	kg/ha/yr		g/ha/yr	
Soil sol. 5 cm (F1)	7.95[a]	0.85[a]	36[d]	0.7227[d]
Soil sol. 10 cm	5.59[a]	0.77[a]	35[d]	0.7286[d]
Soil sol. 30 cm (F2)	4.73[a]	0.82[a]	30[d]	0.7237[d]
Soil sol. 60 cm	3.34[a]	0.82[a]	33[d]	0.7241[d]
Modelled flux of weathering (MF_{alt})	0.2[b]	0.4[e]		
Tree uptake	12[c]		18[c]	
Litter recycling	7[c]		7[c]	
Immobilization (F_{tree})	5[c]	0.42[a]/0.41	11[c]	0.7252/0.7415
Calculated weathering flux (F_{alt})	1.78		5	0.77[b]
Calculated supplementary flux (F_{ex})	1.58	−0.54		

Immobilisation corresponds to the net elemental annual flux stock in vegetation = tree uptake flux − litter recycling flux. F2 = F1 + F_{alt} − F_{tree}. The global weathering flux between F1 and F2 = F2-F1 + F_{tree} = F_{alt}. The suplementary flux F_{ex} = calculated flux − modelled flux = F_{alt}-MF_{alt}.
[a] Cenki Tok et al. (2009).
[b] Fichter et al. (1998b) and Dambrine et al. (1998).
[c] Poszwa (2000).
[d] Aubert (2001) and Aubert et al. (2002b), values with no sign refer to calculated value.
[e] Schmitt et al. (2003).

et al. (1998). Using their weathering flux (MF_{alt}) of 0.2 kg ha^{-1} yr^{-1} (Table 3) one observes that an additional Ca flux (F_{ex}) of 1.58 kg ha^{-1} yr^{-1} is necessary to equilibrate the Ca budget of the soil solutions. Including the Ca isotopic compositions in the mass balance equations yields:

$$F_1 \times \delta Ca_{5cm} + MF_{alt} \times \delta Ca_{alt} + F_{ex} \times \delta Ca_{ex} = F_2 \times \delta Ca_{30cm} + F_{tree} \times \delta Ca_{tree} \quad (2)$$

Using the flux and the corresponding $\delta^{44/40}$Ca values (Table 3) yields a $\delta^{44/40}$Ca value of −0.54 for the supplementary flux (F_{ex}). This flux is strongly enriched in the light ^{40}Ca and might be linked to a Ca desorption flux e.g. from colloidal phases. Preliminary data of a study actually running in our laboratory on soils and soil solutions from the same VP soil profile indicate that the colloïdal phases appear to be strongly enriched in the light ^{40}Ca. In the second simplified case we assume that the weathering flux stopped and all Ca originates from the top

soil (needle litter, atmosphere) and from the supplementary flux (F_{ex}):

$$F_1 \times \delta Ca_{5cm} + F_{ex} \times \delta Ca_{ex} = F_2 \times \delta Ca_{30cm} + F_{tree} \times \delta Ca_{tree} \quad (3)$$

This relationship allows us to derive a similar δCa_{tree} of 0.41‰. Thus, if the desorption flux remained stable then, due to the very low weathering rate for Ca (Dambrine et al., 1998), the δCa_{tree} remains for the 2 cases also unchanged. Consequently, the rise of the fine root system toward the topsoil and the herewith induced small change of Ca source did not cause a change in the Ca isotopic composition of the spruce growth rings. Since the isotopic composition of the trees growth rings remained unchanged it also appears that, compared to Cobert et al. (2011), no physiology related Ca isotopic fractionations occurred. This might simply be due to the fact that still enough Ca was bioavailable. The isotopic homogeneity further suggests that the $\delta^{44/40}Ca$ of the soil solution probably remained rather unchanged during lifetime of the trees, and that given the very small Ca weathering rates, most of the Ca was taken up in the organic matter-rich top-soil during past century.

The Sr isotope system, however, behaves differently. Using the Sr fluxes of soil solutions and tree uptake (Table 3), one derives a weathering flux of 5 g ha^{-1} yr^{-1} for Sr from Eq. (1). These fluxes and the corresponding Sr isotopic compositions allow one to calculate a tree $^{87}Sr/^{86}Sr$ ratio of 0.7415 from Eq. (4) and values from Table 3.

$$F_1 \times \delta Sr_{5cm} + F_{alt} \times \delta Sr_{alt} + F_{ex} \times \delta Sr_{ex} = F_2 \times \delta Sr_{30cm} + F_{tree} \times \delta Sr_{tree} \quad (4)$$

Decreasing weathering flux of Sr and decreasing Sr uptake by spruce decreases the tree-rings Sr isotopic composition. For example, if the weathering flux decreases to 1 g ha^{-1} yr^{-1} and the tree uptake to 7 g ha^{-1} yr^{-1}, then the $^{87}Sr/^{86}Sr$ ratio of the corresponding tree-ring reaches a value of 0.7252.The Pb isotope record of the growth rings

The Pb isotopic composition of the atmosphere has dramatically changed during the past 100 years as deduced among others from Pb isotope determinations on ombrotrophic peat bogs from the Jura mountains, about 80 km south of the catchment (Rosman et al., 2000; Shotyk et al., 2001; Vallelonga et al., 2002). The atmospheric $^{206}Pb/^{207}Pb$ composition was about 1.17 in 1900 and decreased to 1.12 in 1985 (Fig. 4c). After 1995 the values increased again, mainly due to the diminution of industrial emissions and to the introduction of unleaded gasoline. Today's urban particulate matter (PM) from the cities of Strasbourg and Kehl (80 km north of the Strengbach catchment) yield an average $^{206}Pb/^{207}Pb$ ratio of 1.16 (Lahd Geagea et al., 2008a), which is clearly above PM values from 1985 (1.13; Monna et al., 1997). The Pb isotope data of the growth rings from the Strengbach catchment plot with a few exceptions above the peat bog-derived evolution line of the atmospheric Pb isotopic composition.

This discrepancy between Pb isotope data from growth rings and atmospheric composition is, for different reasons, conceivable:

- Above ground tree organs such as foliage or bark are indeed important sinks for airborne particles and have successfully been used as biomonitors (Lahd Geagea et al., 2007, 2008a). However, Pb is accumulated for more than 10 years in the bark (Guéguen et al., 2012) and the Pb, if some of it is transferred from the bark to the outermost growth ring, will therefore have a mixed isotopic signature. This is illustrated by tree barks collected recently in an urban environment (Strasbourg, France; Kehl, Germany), which furnished intermediate Pb isotopic compositions between those of actual airborne PM and those from 1995 (Grobéty et al., 2010);
- The Pb isotope data of the growth rings plot with a few exceptions above the peat bog-derived evolution line of the atmospheric Pb isotopic composition (Shotyk et al., 2001). However, their isotope ratios are in the range of soil leachates from the uppermost 40 cm of the corresponding acid soils (Figs. 4c and 5; Stille et al., 2011), demonstrating that the Pb isotopic composition of the growth rings is significantly influenced by root absorption. This is supported by the fact that the uppermost 40 cm of the soils in the Strengbach catchment are strongly enriched in Pb compared to the bedrock. Pb isotope ratios of these leachates indicate that the Pb from close to the surface corresponds to a very recent isotopic signature of the atmosphere and that the Pb from 30 to 40 cm depth corresponds to Pb airborne PM derived from historical mining activities 100 to 150 years ago (Stille et al., 2011). The soils are very acid with pH values of 3.5, 3.9 and 4.2 at 0–10 cm depth, 10–30 cm depth, and 30–50 cm depths, respectively (http://ohge.u-strasbg.fr). Likewise it has previously been shown that Pb in oak tree-rings seem to be more related to soil pH than bedrock type and that soils with low pH have higher tree-ring Pb concentrations than soils with higher pH (Bukata and Kyser, 2008). Therefore, some of the Pb is easily mobilized in these soils and bioavailable (Stille et al., 2011). Accordingly, it is still likely, as suggested by Watmough and Hutchinson, 2002, that tree-rings of *Picea abies* L. growing on a soil with higher pH than that observed for the VP soils in the catchment and with much less bioavailable Pb monitor historical changes in atmospheric Pb deposition.

Consequently, our current observations do not confirm that the Pb of growth rings originates entirely from absorption of airborne particles at aboveground parts of the tree as suggested by Novak et al. (2010). On the contrary, we suggest, like Bindler et al. (2004), that growth rings integrate mixed isotopic signals resulting from absorption of atmospheric Pb through aboveground tree organs and/or from root uptake of atmosphere-derived Pb accumulated over years in the topsoil. Similarly Bellis et al. (2004) found that beech trees accumulate Pb from soils via roots in the annual growth rings and Watmough and Hutchinson (2002) suggest, based on their Pb isotope study on tree-rings from *Acer pseudoplatanus*, "that soil Pb accumulates within rings of diffuse porous wood over a number of years" and, therefore, that the dendroisotopical Pb record is not a reliable archive to reconstruct past atmospheric Pb pollution. Likewise, in their study on *Acer*

pseudoplatanus, Patrick and Farmer (2006) conclude that "in areas with no local point source of lead, accurate records of changes in atmospheric lead concentration and isotopic composition are not preserved in the annual growth rings of sycamore trees".

6. Conclusion

Growth rings of spruce can be to a certain extent archives for the reconstruction of the chemical evolution of the uppermost soil compartments which are accessible for root uptake. The innermost rings show Nd and Sr isotopic compositions closer to those of soils and soil minerals whereas outermost growth rings have significantly lower Sr and Nd isotopic compositions and approach values similar to those of industrial or pre-industrial "natural" aerosols like Saharan dust. Thus, both the Sr and Nd isotopic compositions of spruce growth rings record the chemical and isotopical changes in the soil and, therefore, allow one to identify the principal sources of Sr and Nd in the wood. However, due to re-equilibration processes, isotope ratios do not always allow a precise timing record of a polluting event. Mass balance calculations clearly indicate that decreasing alteration flux causes a decrease of the $^{87}Sr/^{86}Sr$ ratio in the trees growth rings.

In contrast, mass balance calculations for Ca indicate that the alteration flux alone does not allow one to equilibrate the Ca budget of the soil solutions. An additional ^{40}Ca enriched flux of 1.6 kg ha^{-1} yr^{-1} ($\delta^{44/40}Ca$:–0.54) is necessary; it might be linked to a Ca desorption flux from an instable pool (e.g. colloidal phases) in the soil. However, the rise of the fine root system of spruce toward the topsoil due to soil acidification had no impact on the Ca isotopic composition of the growth rings, suggesting that still enough Ca was bio-available, and that during past century Ca was taken up from the upper soils rich in organic matter.

Compared with Sr, Nd and Ca, the Pb isotope ratios and concentrations behave differently. The Pb isotope data of the trees growth rings show that Pb carries a mixed isotopic signal which resulted from: (1) absorption of atmospheric Pb through direct aerial atmospheric interception and/or through root uptake from a large pool of atmosphere-derived; and (2) isotopically variable Pb accumulated over years in the uppermost part of the soil profile. Therefore, we suggest that the dendroisotopical Pb record is not suitable for the study of past atmospheric Pb pollution in the Strengbach catchment.

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UNIVERSITÉ DE STRASBOURG

FLORIAN COBERT

Ecole et Observatoire des Sciences de la Terre

PROCESSUS ET MÉCANISMES PHYSICO-CHIMIQUES ET BIOLOGIQUES RESPONSABLES DU FRACTIONNEMENT DES ISOTOPES DU CALCIUM

Résumé

Cette thèse a pour but d'identifier et de préciser les processus biotiques et abiotiques qui contrôlent le comportement du Ca et plus spécifiquement le fractionnement des isotopes du Ca à l'interface géosphère-biosphère-hydrosphère en combinant différentes études expérimentales (hydroponiques et microcosmes).

Les résultats obtenus lors des expérimentations hydroponiques ont permis d'identifier 3 niveaux de fractionnement des isotopes du Ca au sein des végétaux, ces 3 niveaux de fractionnement sont induits par des mécanismes physico-chimiques qui enrichissent les organes de végétaux en ^{40}Ca. Les résultats des expérimentations hydroponiques montrent également que l'évolution de la composition isotopique du Ca de la solution nutritive et des plantes suit une loi de fractionnement des isotopes du Ca à l'équilibre ($\alpha_{plantes/solution\ nutritive}$ = 0,99858).

Les expérimentations abiotiques en microcosmes, quant à elles, indiquent que la dissolution de l'apatite par des acides organiques ou inorganiques n'influence pas la signature isotopique en Ca de la phase dissoute résultante. À l'inverse, lors des expérimentations biotiques en microcosmes, seule l'action combinée des racines de pins et des bactéries sur un substratum d'apatite enrichit la solution qui percole en ^{44}Ca de 0,22‰.

Mots clés : Isotopes du calcium, fractionnement isotopique, facteur de fractionnement, expérimentations hydroponiques, expérimentations en microcosmes, plantes, bactéries, sols

Abstract

The aim of this thesis is to identify and to specify biotic and abiotic processes affecting the Ca behavior and specifically the Ca isotope fractionation at the geosphere/biosphere/hydrosphere interface by combining different experimental studies (hydroponic and microcosm).

Results of hydroponic experiments allow to identify three Ca isotopic fractionation levels, which are driven by physico-chemical mechanisms and enrich the plant organs in the light ^{40}Ca isotope. Moreover, the evolution of Ca isotopic composition of nutrient solution and plants follow an equilibrium law ($\alpha_{plants/nutrient\ solution}$ = 0.99858).

Abiotic column experiments show no Ca isotopic fractonation during apatite disolution, whatever the nature of the acid (mineral or organic). At the opposite, during biotic microcosm experiments, only the combined action of roots of scots pines and bacteria on apatite substratum enrich the percolate solution in heavy ^{44}Ca of 0.22‰.

Key words : Calcium isotopes, isotopic fractionation, fractionation factor, hydroponic experiments, column filled experiments, plants, soils, bacteria