Chapter 9

Silicon and germanium uptake by plants: impact on Ge/Si fractionation *

Abstract

Plants strongly impact continental silicon cycle by taking up Si, form phytoliths and restitute biogenic opal to the soil. The similar behaviour of Ge with Si provides a useful tracer of Si pathways. However, Ge uptake and transport through plants and impact on Ge/Si ratio of phytoliths is still poorly understood. Here, we studied Ge uptake and accumulation and Ge/Si fractionation in all plants parts and solutions from (i) hydroponic banana and (ii) *in situ* sampled banana and (iii) horsetails. We further combine these data with δ^{29}Si measurements in banana plants. Ge/Si ratios were calculated after determination of Ge content by HR-ICP-MS and Si content by ICP-AES. Isotopic compositions were measured by MC-ICP-MS Nu Plasma in dry plasma mode with external Mg doping: δ^{29}Si vs. NBS28 $\pm$ 0.08‰ ($\pm$ $2\sigma_{SD}$). Banana and horsetails were shown to accumulate Ge in roots. No discrimination of Ge occurred at the root-solution interface. Low Ge/Si ratios in root phytoliths compared to high Ge/Si ratios in bulk roots suggest that Ge is organically trapped in roots. Shoots display lower Ge/Si ratios, without fractionation between shoot parts. This would indicate that Ge follows transpiration stream as silicon, and is not discriminated between shoot parts. This contrasts with discrimination of heavy Si isotopes at the root-solution interface, for xylem loading,

*Article in preparation: Delvigne et al.

and within the shoots, inducing heavier roots than shoots and heavier upper shoots compared to lower shoots. Combining Si stable isotopes and Ge/Si ratios in plants is thus very complementary to understand Si uptake and mobility through plant. Ge/Si fractionation induced by the plants would significantly impact Ge/Si ratio of phytoliths restituted to the soil, and hence could affect Ge/Si ratio of river waters.

9.1 Introduction

The silicon (Si) cycle is of major concern in global change studies due to its link with C cycle and the regulation of atmospheric CO_2 concentration through diatoms and silicate weathering (Raven and Edwards, 2001; Smetacek, 1999). As rivers represent the major source of dissolved Si to the oceans (Tréguer et al., 1995), the study of the continental Si cycle is of primary importance. In this context, plants contribute to weathering and strongly impact the Si cycle (Alexandre et al., 1997; Conley, 2002; Lucas, 2001). Silicon is taken up by plant roots as monosilicic acid H_4SiO_4 and reaches shoots following water flow (Raven, 1983). Due to plant transpiration, oversaturation induces Si polymerization and precipitation of silica as hydrated amorphous silica $SiO_2.nH_2O$, i.e. opaline phytoliths (Yoshida et al., 1962). Those phytoliths are restituted to soil by litter fall and plant death.

Germanium (Ge) is a trace element considered as a chemical analog to Si. In this respect, germanium/silicon ratio seems a promising tracer of weathering processes and plant impact (Blecker et al., 2007; Derry et al., 2005; Kurtz et al., 2002; Scribner et al., 2006). Ge/Si ratio of the continental crust (1.3μmol/mol; Mortlock and Froelich (1987)) is higher than the ratio of river waters (0.6μmol/mol; Froelich et al. (1992)). This is mainly attributed to Ge retention in secondary weathering products (Kurtz et al., 2002; Murnane and Stallard, 1990) including Ge adsorption onto secondary iron oxides (Scribner et al., 2006). No discrimination of Ge has been observed during Si assimilation by diatoms (same Ge/Si ratio in diatoms than seawater) (Bareille et al., 1998; Froelich et al., 1992). Similarly, Ge is incorporated in sponge spicules with Si (Ellwood et al., 2006; Simpson, 1981) and their Ge/Si ratio was shown to be related to seawater Ge/Si ratio, and hence can be used to reconstruct paleo-Si and Ge concentrations in ocean (Ellwood et al., 2006). In contrast, phytoliths display very low Ge/Si ratio which was interpreted as a discrimination process against Ge by higher plants (Blecker et al., 2007; Derry et al., 2005). In large amounts, Ge is considered as a toxic element for plants and affects plant growth (Lewin and Reimann,

1969; Puerner et al., 1990; Sankhla and Sankhla, 1967). Though, small amounts of Ge have no effect or may stimulate the plant during the first stages of growth (Geilmann and Brunger, 1935; Halperin et al., 1995). This toxicity could partly be explained by Ge specific competitive inhibition of Si metabolism (Sankhla and Sankhla, 1967; Takahashi et al., 1976), which contrasts with the beneficial effects of Si for crop plants, e.g. enhancing pest and pathogen resistance, increasing photosynthetic capacity, minimizing water loses, and improving heavy-metal tolerance (Ma et al., 2001).

Stable Si isotopes are very useful in soil-plant studies (Opfergelt et al., 2006ab; Ziegler et al., 2005ab) (Chapter 7; Appendix C), as plants were shown to take up preferentially light isotopes (Ding et al., 2005b 2008; Opfergelt et al., 2006ab; Sun et al., 2008), as diatoms (De La Rocha et al., 1997) and sponges (De La Rocha, 2003). Recent studies evaluated a fractionation factor ($^{29}\varepsilon$) for banana between plant and source of -0.40 ± 0.11‰ (Opfergelt et al., 2006b) and highlighted an intra-plant fractionation with discrimination against heavy isotopes inducing relatively heavier isotopic compositions in upper shoots (Ding et al., 2005b; Opfergelt et al., 2006ab) (Chapter 7; Appendix C). Based on the similarities between Ge and Si, comparing both tracers Ge/Si ratio and stable Si isotopes in a soil-plant system and within the plant would be very helpful to understand the role of plants in the Si-Ge cycles.

Here we study the Ge uptake and transport by plants and its impact on Ge/Si fractionation. Hydroponic experiments were combined with sampling in natural conditions to provide new insights to answer four open questions (Blecker et al., 2007; Derry et al., 2005): (1) Is Ge rejected by plant roots? (2) Is there a Ge/Si fractionation within the plant? (3) How does this affect Ge/Si ratio of phytoliths? (4) How does this impact the Ge-Si cycles? This study focuses on two Si-accumulating plants (banana and horsetails) characterized by (i) contrasted Si content (banana = 0.7 to 3.8%, Jauhari et al. (1974); horsetails = 4.7-7.0%; Marschner (1995)), and (ii) contrasted phylogenetic relation to the plant kingdom, as horsetails are primitive plants (typical of Carboniferous), while banana are monocotyledons. Within plant species, most Si-accumulating plant are monocotyledons (especially *Gramineae* and *Cyperaceae*), but a few dicotyledones, Pteridophyta and Bryophyta are also concerned (Ma et al., 2001). Our study involves Si and Ge quantification in all plant parts and comparison with δ^{30}Si measurements in banana.

9.2 Materials and methods

9.2.1 Hydroponic experiment on banana in a closed system

Banana plantlets of *Musa acuminata* Colla, cv Grande Naine were grown in hydroponics under controlled conditions of light (448μE $m^{-2}s^{-1}$ photon density flux 12h per day), humidity (80% relative humidity), temperature (28/25°C day/night) and nutrients during six weeks, in a closed system with a finite pool of Si and Ge. Nutrients requirements were adapted to plant need and continuously supplied as dilute nutrient solutions with peristaltic pumps at a rate of 104 ml $h^{-1}pot^{-1}$ (Rufyikiri et al., 2001). Initial nutrient solutions were doped both in Si (0.42mM or 13.5mg.l^{-1}) and Ge (0.004mM or 0.32mg.l^{-1}). These Ge concentrations are much higher than in natural waters (rivers and ocean: $0.8.10^{-3}\mu$M; Burton et al. (1959)), but this should not disturb the Ge-Si uptake by plant as the ratio between Ge and Si was fixed at 1%Ge. At Ge/Si<0.01, Ge was shown to do not inhibit diatoms growth (Azam and Volcani, 1981; Froelich et al., 1992). At higher level, Ge could be toxic for plants when confused with Si (Ma et al., 2002).

Si was provided as H_4SiO_4 obtained from sodium metasilicate after Na^+-H^+ exchange by using H^+-resin (Amberlite IR120), and Ge as H_4GeO_4 from $GeCl_4$ reaction with NaOH (Azam, 1974). Si concentration was below solubility limit at given temperatures ($\leq$ 28°C), and pH ranged between 5 and 6.5. In such conditions, Si and Ge were stable as silicic and germanic acid respectively (Bernstein, 1985; Stumm and Morgan, 1996). The solution was continuously recovered in order to keep a closed Si and Ge reservoir. All other nutrients were adjusted on a weekly basis following plant need. An aliquot of the nutrient solution was sampled every week to follow the evolution of the Si and Ge concentrations.

The number of leaf emerged per week was monitored (usually one or two). After a six-week growth period, roots (R) and pseudostems (PS) were sampled in bulk per plant (two banana replicates with Ge and Si in the finite pool: GS1 and GS2). Midribs-petioles (MP) and lamina (L) were separated to form one group per week (e.g. L1 correspond to the lamina of all the leaves formed during week 1, MP1 to the midribs and petioles of these leaves). Plant materials were dried at 60°C and grinded. Control banana plantlets were grown in parallel (with only Ge, with only Si, and without Si and Ge) and will not be presented in the present study. Plant growth was limited for some plantlets due to variable adaptation to nutrients readjustment in the finite Si and Ge

pool; e.g. the leaf area increased from 40 to 782 cm^2 in 43 days from leaf 0 to leaf 8 for GS1, against a mean of 476 cm^2 for the final leaf area for other plants. For this reason, more Si was taken up by GS1 inducing a larger Si depletion in the finite pool (43%) compared to other plants (10%). GS1 and GS2 were both analysed for Ge/Si ratio, and isotopic measurements were performed on GS1.

9.2.2 Plant collecting *in situ*

Two Si-accumulating plants were sampled: banana (*Musa* spp., Cameroon) and horsetail (*Equisetum* sp., Belgium).

Banana plant parts (*Musa acuminata* Colla, cv Grande Naine) were collected in November 2005 in the Mungo plain (Njombé, Cameroon) where climatic conditions are the following: rainy season from March to November, 2700 mm total average annual rainfall, mean annual temperature between 22.4°C (870m) and 27.5°C (40m) (Martin and Sieffermann, 1966). The soils devoted to intensive banana cropping derive from basaltic pyroclastic deposits and were previously described in details (Delvaux et al., 1989). Two stations were selected from a poorly developped (PD) to a strongly weathered (SW) volcanic soils (vs), Dia-Dia (DD) and Djungo (DJ), respectively (Chapter 8). In each station, five plants were selected at flowering stage according to the international foliar sampling procedure (Martin-Prével, 1984) involving the sampling of petiole (P), midrib (M), internal and external lamina (IL, EL) of leaf III. Among the five, one plant was sampled from root to fruit: root (R), internal pseudostem (PSi), external pseudostem (PSe), P, M, IL, EL, peduncle (Pe) and fruit (F). The other four plants were used to build up four composite foliar samples (bulk P, bulk M, bulk IL, bulk EL). Corresponding soils were also sampled (bulk soil 0-3cm). Clay fractions were recovered after sonication and dispersion with Na^+-saturated resins (Rouiller et al., 1972) from surface and deep horizons and will be considered as a mean sample in the present study.

Two horsetail species (*Equisetum telmateia* (T) and *Equisetum kamchaticum* (K)) were collected in September 2006 in the Jean Massart botanical garden (Bruxelles, Belgium) on sandy-clay soils. Climatic conditions are the following: 780 mm total average annual rainfall, mean annual temperature about 9.7°C with mean maximum temperature of 13.5°C and minimum of 6.3°C. Three composite samples of *Equisetum telmateia* specie were collected in a wetland part of the botanical garden, whereas five composite samples of *Equisetum kamchaticum* were sampled along the path of the orchard. The following different plant parts were considered: rootstock (Rs), roots (R), stem (S), leaf

(L), and ramifications (Ram) (Ram only for *Equisetum telmateia*). Corresponding soils (surface horizon) were sampled (three soils for T and one soil for K).

Both for banana and horsetail, plant materials were cleaned to remove soil contamination. However, a complete root cleaning is very difficult to achieve without root destruction. Samples were dried at 60°C and grinded.

9.2.3 Germanium and silicon content analyses

The Ge and Si contents were measured in solutions, various plant parts, and soils. Plant parts were calcinated at 450°C during 16 hours, and soils (<2mm fractions) were dried at 105°C. Samples were then dissolved by borate fusion (sample:$LiBO_2$ in 1:1 ratio) at 1000°C during 1 hour in platinum crucible. The dissolution of fusion beads was carried out in 5% HNO_3 solution. The obtained solution was used for further analysis. To minimize Si-Ge contaminations, only plastic material (PP) was used. Ge was measured by HR-ICP-MS in medium resolution with indium (In) as internal standard to correct for the instrumental drift, with a reproductibility of ± 4% ($2\sigma_{SEM}$) (n=6). Accuracy was checked with rock standards. Detection limit in solution (10.1ng.l^{-1}) was determined by measuring 9 procedure blanks (borate fusion). Si content was analyzed by ICP-AES with gold (Au) and yttrium (Y) as internal standards and a reproductibility of 2.7% (n=5). Detection limit in solution (0.35 mg.l^{-1}) was determined by measuring 6 procedure blanks.

Due to very low Ge content of natural plant parts, a pre-concentration step was necessary. An aliquot of the HNO_3 solution (10ml) was evaporated and the dry residue was dissolved to reach a Ge content of approximately 1ng.l^{-1} in 2% HNO_3 solution. Due to high Ge and Si content in clay minerals, underground plant parts may have been contaminated by clay minerals. As shown for banana (Chapter 8), Al content in roots is much higher than in shoots. The contribution of Al^{3+} adsorbed onto root surfaces is likely negligible at soil pH above 7, as in these soils, despite the fact that proton extrusion by banana roots may decrease pH in the surrounding environment and mobilize Al from mineral dissolution (Rufyikiri et al., 2004). Here, we consider that root-Al is in fact a signature of clay minerals adhering to root surfaces. Therefore, a correction factor has to be applied to remove clay contribution using following equations:

$$[\mathrm{Ge}]_{\mathrm{corrected}} = [\mathrm{Ge}]_{\mathrm{measured}} - (\mathrm{Ge/Al} \times [\mathrm{Al}]_{\mathrm{measured}})$$

$$[\mathrm{Si}]_{\mathrm{corrected}} = [\mathrm{Si}]_{\mathrm{measured}} - (\mathrm{Si/Al} \times [\mathrm{Al}]_{\mathrm{measured}})$$

where Ge/Al and Si/Al are calculated with Ge, Si and Al content measured in SWvs and PDvs clay fractions for DJ and DD plant parts respectively, and for horsetails with kaolinite clay ratio (Ge/Si= 5.9μmol/mol, Kurtz et al. (2002); Si/Al= 1.05mol/mol, Newman (1987)). This correction is about 30% and 13% of measured concentrations for Ge and Si, respectively. Being aware of the large uncertainty on this correction (few samples were rejected resulting in 1 banana and 6 horsetails available), the difference in Ge concentration between roots and shoot discussed in the present study is much larger than the correction applied, and would not be affected by this uncertainty.

9.2.4 Silicon isotopes analyses

Si isotopic analyses were performed on GS1 plant (all plant parts) and nutrient solutions (sampled every week) from the hydroponic experiment. Banana plants collected *in situ* considered in the present study were previously isotopically characterized (Chapter 8). From plant aliquot, silicon was recovered after: (1) mineralization at 450°C during 24 hours in Pt crucibles, (2) opal dissolution by 0.2 M NaOH leaching at 100°C during 2 hours (adapted from Ragueneau et al. (2005)). Dissolved Si from plant and water samples was purified by triethylamine molybdate (TEA-moly) co-precipitation and combustion in covered Pt crucibles at 1000°C (De La Rocha et al., 1996), and dissolved in dilute suprapur HF-HCl mixture (Cardinal et al., 2003).

The Si-isotopic compositions were measured using a Nu Plasma multicollector plasma source mass spectrometer (MC-ICP-MS) operating in dry plasma mode, with an external Mg doping to correct mass bias (Cardinal et al., 2003). To avoid matrix effects, purity of the samples (absence of major elements and Mo) was controlled by ICP-AES before MC-ICP-MS analyses. Data were obtained by the sample-standard bracketing technique relative to the NBS28 silica sand standard (National Institute of Standard and Technology RM #8546) for silicon isotopes. The analytical method was supported by an inter-laboratory comparison exercise and proved to be accurate on secondary reference materials (Reynolds et al. (2007); Chapter 6). In this study, our results

were expressed as δ^{29}Si vs. NBS28 (‰) with an average precision and accuracy below 0.08‰ ($\pm$ $2\sigma_{SEM}$) following:

$$\delta^{29}\mathrm{S}i = \left[\frac{\left(\frac{^{29}\mathrm{S}i}{^{28}\mathrm{S}i}\right)_{\mathrm{sample}}}{\left(\frac{^{29}\mathrm{S}i}{^{28}\mathrm{S}i}\right)_{\mathrm{NBS28}}} - 1 \right] \times 1000$$

9.3 Results

9.3.1 Germanium and silicon contents

In vitro banana

Banana plants grown in a closed system induced a Si depletion of the reservoir of 43% and 8% and a Ge depletion of 52% and 15%, for GS1 and GS2, respectively. Along the experiment, Si and Ge concentrations in nutritive solutions generally decrease, with a parallel decreasing of Ge and Si in solution through time (Figure 9.1). Following these results, Ge/Si ratios of nutrient solutions do not vary significantly through time, except for GS1 at week 3 where Si and Ge concentrations slightly increased, and for GS2 with Si concentration increasing at week 6 (Table 9.1). This parallel depletion of Ge and Si can be observed for each respective plantlet, despite the difference of depletion between GS1 and GS2 (no enrichment of one element in the remaining solution compared to the other).

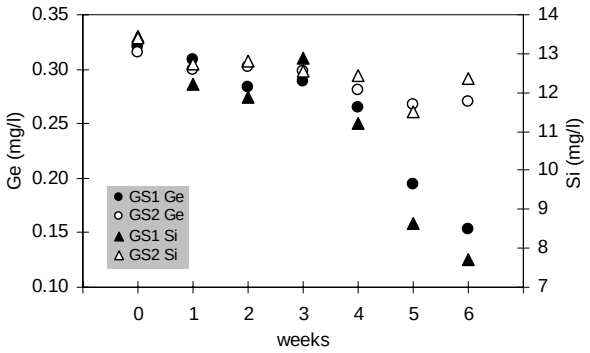

Figure 9.1: Evolution of the Ge and Si concentration in the nutrient solution (finite pool) during the hydroponic experiment with banana plantlets established from a weekly monitoring. The error bar is included in symbols.

Ge concentrations in banana plantlets ranged from 56 to 434 mg.kg^{-1} and Si concentrations from 1.0 to 18.5 g.kg^{-1} (Table 9.1). Along Si pathway through plant, Si concentrations progressively increased from root to shoots (Figure 9.2a) to reach a maximum value in leaves (GS1 mean L = 12.1 g.kg^{-1}; GS2 mean L = 7.3 g.kg^{-1}), which confirms the Si gradient observed in banana plantlets (Henriet et al., 2006). On the opposite, Ge concentration was high in roots, then lower in pseudostem and increased up to leaves (Figure 9.2b). As a result, roots displayed very high Ge/Si ratio in contrast with shoots where Ge/Si ratio gradually decreased up to leaves (Figure 9.2c). Moreover, roots exhibited much larger Ge/Si ratio than nutrient solutions (Table 9.1).

Table 9.1: Ge and Si concentrations and Ge/Si ratios of (i) various plant parts of hydroponic banana plantlets (GS1 and GS2) and (ii) nutrient solutions sampled every week. R = roots; PS = pseudostem; MP = midrib and petiole; L = lamina; NS = nutrient solution; 0 to 6 = week number.

	GS1			GS2		
Sple ID	Ge mg/kg	Si g/kg	Ge/Si mmol/mol	Ge mg/kg	Si g/kg	Ge/Si mmol/mol
R	123	1.6	29.6	158	1.0	61.9
PS	86	3.2	10.3	86	2.6	12.8
0MP	271	10.9	9.6	134	5.3	9.9
1MP	184	7.1	10.0	108	3.9	10.7
2MP	164	7.6	8.3	82	3.2	10.1
3MP	131	5.6	9.1	103	4.1	9.6
4MP	118	5.1	9.0	87	3.5	9.6
5MP	84	3.9	8.4	78	3.2	9.3
6MP	56	2.4	9.1	61	2.2	11.0
0L	434	18.5	9.0	229	11.6	7.6
1L	352	16.9	8.1	188	9.1	7.9
2L	333	13.7	9.4	133	6.1	8.4
3L	316	12.9	9.4	142	6.5	8.4
4L	260	10.6	9.5	174	7.5	9.0
5L	203	8.4	9.3	188	7.5	9.7
6L	86	3.6	9.2	97	3.5	10.8
	Ge mg/l	Si mg/l	Ge/Si mmol/mol	Ge mg/l	Si mg/l	Ge/Si mmol/mol
NS week0	0.32	13.5	9.2	0.32	13.4	9.1
NS week1	0.31	12.2	9.0	0.30	12.7	9.2
NS week2	0.28	11.9	8.6	0.30	12.8	8.5
NS week3	0.29	12.9	4.6	0.30	12.5	8.3
NS week4	0.26	11.2	9.1	0.28	12.4	8.7
NS week5	0.20	8.6	9.0	0.27	11.5	9.0
NS week6	0.15	7.7	7.7	0.27	12.4	8.4

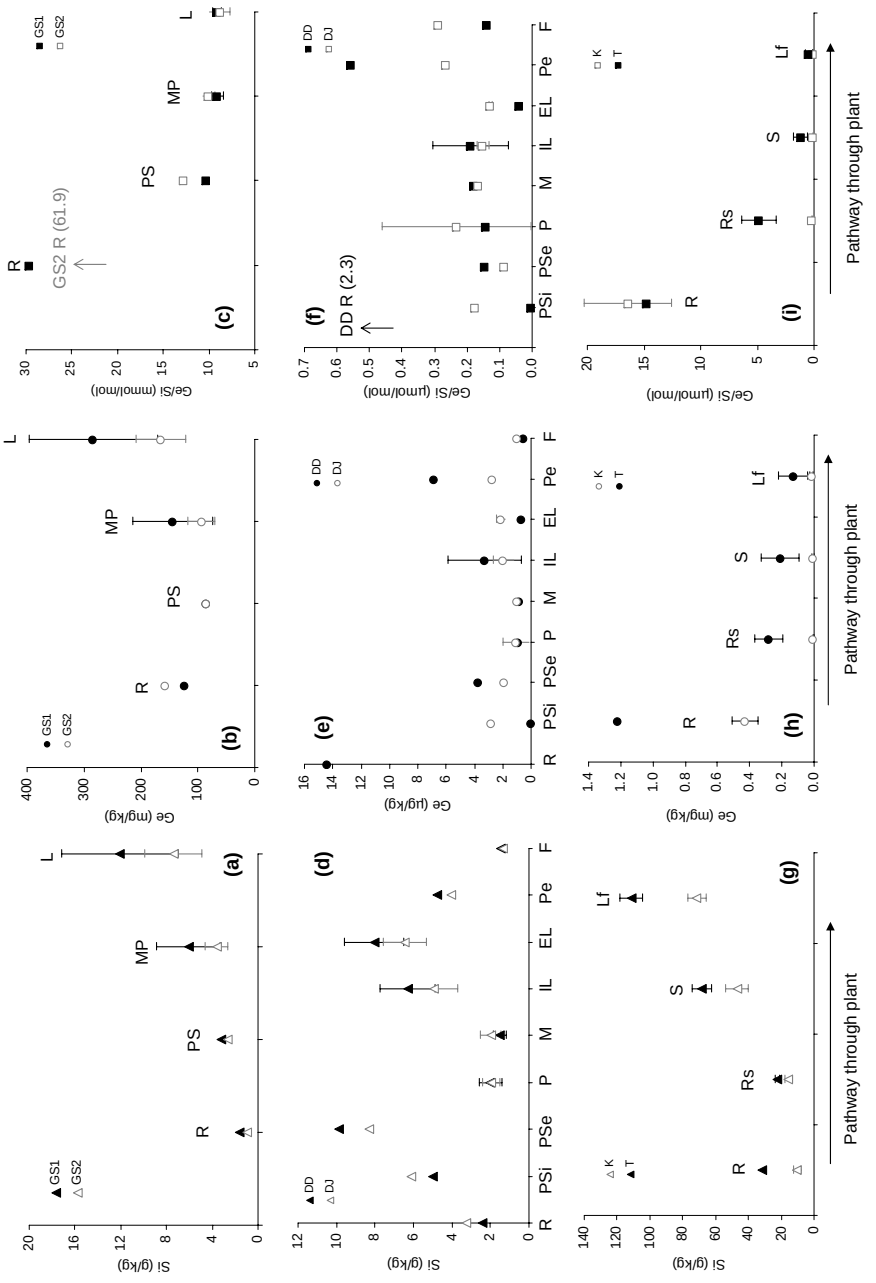

Figure 9.2: Evolution of Ge and Si concentrations and Ge/Si ratio (with standard deviation from different samples) on pathway through plant: (a-b-c) Hydroponic banana plantlets (GS1 and GS2). R = roots; PS = pseudostem; MP = midrib and petiole; L = lamina. (d-e-f) Mature banana plants from Cameroon (DD and DJ). R = roots; PSi, PSe = internal and external pseudostem; P = petiole; M = midrib; IL, EL = internal and external lamina; Pe = peduncle; F = fruit. (g-h-i) Horsetails (T and K) R = roots; Rs = rootstock; S = stem; Lf = leaf.

In situ banana

Si concentrations in Cameroon banana plants ranged between 1.3 and 9.9 g.kg^{-1} and Ge content varied from 0.03 to 14.44μg.kg^{-1} (Table 9.2), which is smaller than concentrations in hydroponic banana plantlets. As observed in hydroponics, roots were the most Ge enriched plant part (DD R = 14.44 μg.kg^{-1}; Table 9.2) and displayed low Si concentrations (DD R = 2.4 g.kg^{-1}; DJ R = 3.3 g.kg^{-1}; Table 9.2). This gives the highest Ge/Si ratio in roots (DD R = 2.30 μmol/mol; Table 9.2) (Figure 9.2f). In shoots (aerial parts), Ge and Si concentrations exhibited a similar profile for DD and DJ, with high Si content in external pseudostem and lamina (Figure 9.2d) and no increasing trend for Ge content (Figure 9.2e).

The corresponding surface soils of DD and DJ (bulk 0-3 cm) differed in their Ge content (1.16 and 1.82mg.kg^{-1}, respectively; Table 9.2) whereas Si content was roughly equivalent (Table 9.2). The corresponding Ge/Si ratio is higher in SWvs (DJ = 3.80 μmol/mol) compared to PDvs (DD = 2.47 μmol/mol). Mean clay fractions displayed lower Ge/Si ratio at DD (4.69 μmol/mol; Table 9.2) than

Table 9.2: Ge, Si, Al concentrations and Ge/Si ratios in plant parts of mature banana (Cameroon). R = roots; PSi, PSe = internal and external pseudostem; P = petiole; M = midrib; IL, EL = internal and external lamina (bulk = composite samples of respectively P, M, IL and EL from four banana leaves); Pe = peduncle; F = fruit; bulk soil = 0-3cm; DL = analytical detection limit.

	Dia-Dia				Djungo			
Sple ID	Ge μg/kg	Si g/kg	Al mg/kg	Ge/Si μmol/mol	Ge μg/kg	Si g/kg	Al mg/kg	Ge/Si μmol/mol
R	14.44*	2.4*	816	2.30	-	3.3*	527	-
PSi	0.03	5.0	28	0.00	2.79	6.1	37	0.18
PSe	3.72	9.9	50	0.15	1.87	8.3	74	0.09
P	0.88	2.4	19	0.14	0.42	2.3	23	0.07
M	0.81	1.7	12	0.18	1.01	2.4	13	0.16
IL	5.13	7.3	58	0.27	2.45	5.8	43	0.16
EL	-	9.1	76	-	2.32	7.2	12	0.12
bulk P	<DL	1.6	22	-	1.69	1.7	23	0.39
bulk M	<DL	1.3	24	-	<DL	1.6	27	-
bulk IL	1.48	5.3	50	0.11	1.46	4.0	40	0.14
bulk EL	0.72	7.0	72	0.04	1.93	5.7	78	0.13
Pe	6.87	4.7	31	0.56	2.78	4.1	79	0.26
F	0.53	1.5	20	0.14	1.03	1.4	104	0.29
bulk soil	1.16**	181	77	2.47	1.82**	184	109	3.80
mean clay	0.81**	67	63	4.69	2.05**	134	122	5.88

* corrected by Al factor (see section 9.2.3)
** Ge concentration in mg/kg

at DJ (5.88 μmol/mol; Table 9.2).

Horsetails

Si content in horsetails ranged between 8.5 and 115.7g.kg^{-1} (Table 9.3) which is much higher than in banana. Horsetails were also Ge enriched compared to banana with a range from 0.01 to 1.22 mg.kg^{-1} (Table 9.3). In horsetails, low Si content was related to high Ge/Si ratio (Figure 9.3). As in banana, roots and shoots can be distinguished based on their Ge and Si content: roots are Si-poor and Ge-rich compared to shoots. Again roots displayed larger Ge/Si ratio and lower Si content than shoots (Figure 9.2i and 9.3). On the opposite, leaves displayed higher Si content and very low Ge/Si ratio. Across their pathway through plant, Ge and Si showed distinct profiles: as in banana, Si content progressively increased from roots to shoots to reach a maximum in leaves (Figure 9.2g); in contrast, Ge concentrations decreased from roots to leaves (Figure 9.2h).

Bulk soils displayed similar Ge/Si ratio of 1.73 and 1.92 μmol/mol for T and K respectively (Table 9.3).

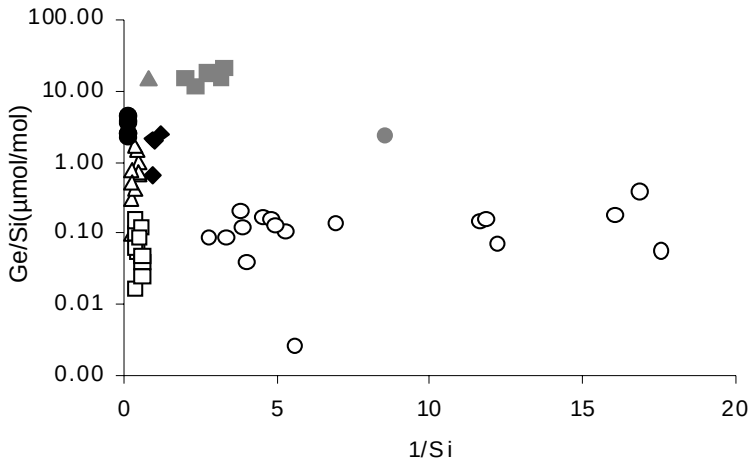

Figure 9.3: Relation between Ge/Si ratios and Si accumulation (1/Si) for mature banana (circle), *Equisetum telmateia* (triangle) and *Equisetum kamchaticum* (square). Differenciation between soils (black), roots (grey) and shoots (open symbol). Horsetails soils are grouped (black diamond).

Table 9.3: Ge, Si, Al concentrations and Ge/Si ratio in horsetails plant parts for *Equisetum telmateia* (T, n=3) and *Equisetum kamchaticum* (K, n=5). R = roots (except T1 and T3); Rs = rootstock; Ram = ramifications (only for *Equisetum telmateia*); S = stem; Lf = leaf; bulk soil = surface horizon; DL = analytical detection limit.

Sample ID	Ge mg/kg	Si g/kg	Al g/kg	Ge/Si μmol/mol
Equisetum telmateia				
T1 Rs	0.38*	22.2*	0.5	6.65
T1 Ram	0.08	99.9	0.1	0.30
T1 S	0.08	71.0	0.1	0.45
T1 Lf	0.03	115.7	0.3	0.10
T2 R	1.22*	31.8*	8.9	14.76
T2 Rs	0.22*	21.0*	1.3	4.05
T2 Ram	0.10	54.0	0.1	0.71
T2 S	0.23	61.6	0.1	1.44
T2 Lf	0.21	103.2	0.2	0.77
T3 Rs	0.24*	23.4*	4.0	3.91
T3 Ram	0.13	52.0	0.8	0.96
T3 S	0.31	72.4	0.5	1.68
T3 Lf	0.15	114.7	1.1	0.52
T bulk soil**	1.18	273.0	35.7	1.73
Equisetum kamchaticum				
K1 R	0.33*	8.6*	3.0	15.06
K1 Rs	-	15.5*	0.4	-
K1 S	0.02	47.6	0.1	0.12
K1 Lf	0.02	73.2	0.6	0.10
K2 R	0.47*	9.9*	3.0	18.26
K2 Rs	-	13.1*	0.4	-
K2 S	0.00	42.9	0.1	0.04
K2 Lf	0.03	70.2	0.7	0.15
K3 R	0.52*	13.4*	3.1	15.00
K3 Rs	0.01*	18.8*	0.3	0.21
K3 S	<DL	42.9	0.1	-
K3 Lf	<DL	71.1	0.3	-
K4 R	0.35*	11.7*	3.2	11.74
K4 Rs	0.01*	15.9*	0.4	0.18
K4 S	0.01	43.0	0.1	0.05
K4 Lf	0.01	63.3	0.4	0.06
K5 R	0.47*	8.2*	2.7	21.95
K5 Rs	0.00*	14.5*	0.5	0.10
K5 S	0.01	58.4	0.1	0.09
K5 Lf	0.01	79.0	0.5	0.07
K bulk soil	1.37	275.9	27.3	1.92

* corrected by Al factor (see section 9.2.3)
** mean values of three soil samples

9.3.2 Silicon isotopic variations

Systematic isotopic compositions on each plant parts of GS1 indicated that within the plant, roots were heavier than the shoots, and pseudostem lighter than lamina (Figure 9.4), which is similar to the intra-plant fractionation previously reported in continuous flow device (Opfergelt et al. (2006b); Chapter 7) and in mature banana plant (Chapter 8). The δ^{29}Si isotopic composition of the finite Si pool evolved from +0.05‰ at the beginning of the experiment to +0.28‰ after 6 weeks showing depletion in light isotopes (from week 0 to week 6 (‰): W0=+0.05, W1=-0.09, W2=-0.04, W3=+0.08, W4=+0.00, W5=+0.26, W6=+0.28). Based on the isotopic compositions of each plant parts of GS1 plant, on their SiO_2 content and on their biomasses, a bulk plant isotopic composition can be calculated (-0.30‰). The fractionation factor between the Si source and the plant can be approached by a difference between the Si-isotopic composition of the source ($\delta^{29}Si_{source}$ = +0.05‰) and the bulk Si-isotopic composition of the plant ($\delta^{29}Si_{plant}$ = -0.30‰). The calculated plant-source fractionation factor $^{29}\varepsilon$ (-0.35‰) confirms the fractionation factor previously calculated on juvenile banana plantlets in continuous flow device (-0.40 ± 0.11‰; Opfergelt et al. (2006b); Chapter 7). This provides a good confidence to interpret Ge/Si fractionation presently studied in a closed system in term of plant plant uptake, excluding any impact from the closed system design.

9.4 Discussion

9.4.1 Germanium-silicon fractionation by plants

The weekly monitoring of nutritive solutions highlighted a parallel decreasing of Ge and Si in the nutrient solution (Figure 9.1). This presumably implies that no fractionation process occurs during banana plant uptake, which is in good agreement with the similar Ge and Si uptake control reported for rice roots (Ma et al., 2001; Takahashi et al., 1976), and with the competitive effect of Ge on Si observed for wheat (Rains et al., 2006). Roots exhibited a much higher Ge/Si ratio compared to nutritive solutions and other plant parts (Table 9.1, Figure9.2c) pointing to a Ge accumulation in roots and a significant Ge discrimination between roots and shoots compare to Si.

From the low Ge/Si ratio measured in grass phytoliths (from shoots, one measurement in roots) compared to solution, Blecker et al. (2007) deduced that a Ge discrimination should occur at the root-solution interface, and that Ge should be excluded from the plant. However, the

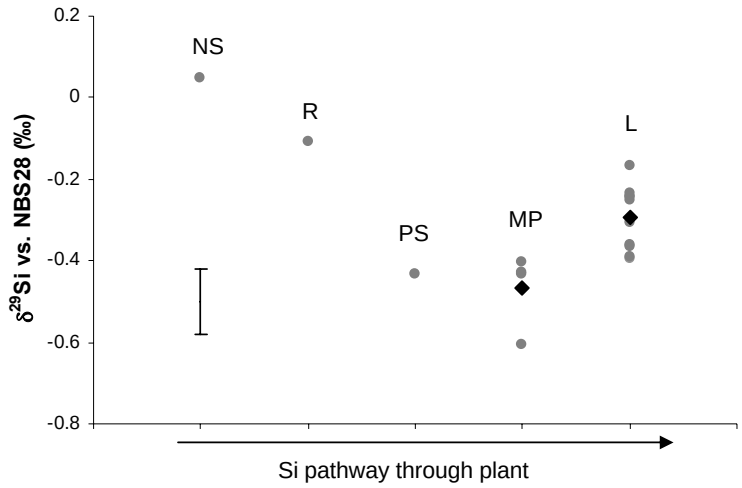

Figure 9.4: Isotopic compositions of various plant parts of a hydroponic banana plantlet (GS1) grown with a finite Si pool: single δ^{29}Si measurements (grey circle) and their average (black diamond). Bracket shows the average standard deviation of full replicates ($\pm$ $2\sigma_{SD}$). NS = nutrient solution; R = roots; PS = pseudostem; MP = midrib and petiole; L = lamina.

hydroponic experiment has shown that no Ge discrimination occured at the root-solution interface (Figure 9.1). We suggest that Ge is likely organically entrapped in roots (section 9.4.2). This would reconcile the low Ge/Si ratio observed in pure phytoliths (mineral Si-rich phases; Blecker et al. (2007)) and the high Ge concentration and Ge/Si ratio in bulk root (present study). We further suggest that the Ge accumulation in roots constitutes a Ge pool underestimated until now. Depending on the turnover of root decomposition in soils, this could potentially retard Ge export to rivers. Dissolution of phytoliths in soil surface would affect Ge/Si ratio exported if Ge pool from roots is stable.

As highlighted by the present study, no discrimination occurs at the root-solution interface but a discrimination should occur between root and shoot. Following transpiration stream, Ge and Si are both passively taken up with no obvious fractionation, with Si precipitation in major transpiration sites (Sangster and Parry, 1971). The low Ge/Si ratios measured in shoots reflect the early separation of Ge and Si in plant roots. High Ge concentrations and high Ge/Si ratios measured in roots of mature banana and horsetail support a Ge accumulation plant roots (Figure 9.2e-f and h-i). This suggests a mechanism of Ge-Si fractionation common for several higher plants, especially when

considering that horsetails are very primitive plants.

9.4.2 Proposed mechanism for Ge accumulation in roots

Specific Si-transporters were identified in rice plant, at the root-solution interface and for xylem loading allowing an influx and efflux of Si through cell, and hence transfer from roots to shoots (Ma et al., 2006 2007). Those transporters were suggested to be responsible for discrimination against heavy Si isotopes at the root-solution interface and for xylem loading (Opfergelt et al. (2006b); Chapter 7), as a discrimination against heavy isotopes was occurring at the root interface and between root and shoots. As no Ge-Si fractionation is induced by plant uptake (Figure 9.1), these Si-transporters should not be responsible for any discrimination against Ge. This suggests that within the plant, Ge/Si ratio and Si isotopes are not affected by the same processes, which is supported by the absence of trend between δ^{29}Si and Ge/Si within the plant, both in hydroponics plantlets (Figure 9.5a) and in mature plants (Figure 9.5b).

However, high Ge concentrations in roots strongly suggest that Ge should be trapped in roots while Si is not retained, leaving a residual solution Ge-depleted transferred to shoot through xylem loading without Ge-Si fractionation. Ge-organic complexes readily form in aqueous solutions, while Si-organic complexation is significantly weaker or does not occur (Pokrovski and Schott, 1998). Moreover, evidence of Ge organic complexes in plants were found in peat (Manskaya et al., 1961) and Ge was shown to form germanate diol diesters in pumpkin plants (Ishii et al., 2002). On the opposite, no organo-silicon compounds have been identified so far under natural physiological conditions (Casey et al., 2003; Knight and Kinrade, 2001) and in natural waters (Kubicki and Heaney, 2003). The formation of Ge-organic complexes is the most likely mechanism to account for Ge trapping in roots. Residual uncomplexed Ge would then be transferred to the shoot with Si, inducing very low Ge/Si ratio in shoots. This hypothesis is in good agreement with the organically trapped Ge proposed by Blecker et al. (2007), different from the minerally trapped Ge in phytoliths.

9.4.3 Intra-plant germanium-silicon fractionation

In shoots, Si and Ge were shown to be accumulated in leaves, where phytoliths formed in the transpiration sites (Sangster and Parry, 1971). Ge and Si are both passively transferred without obvious fractionation through aerial pathway. In contrast with hydroponics

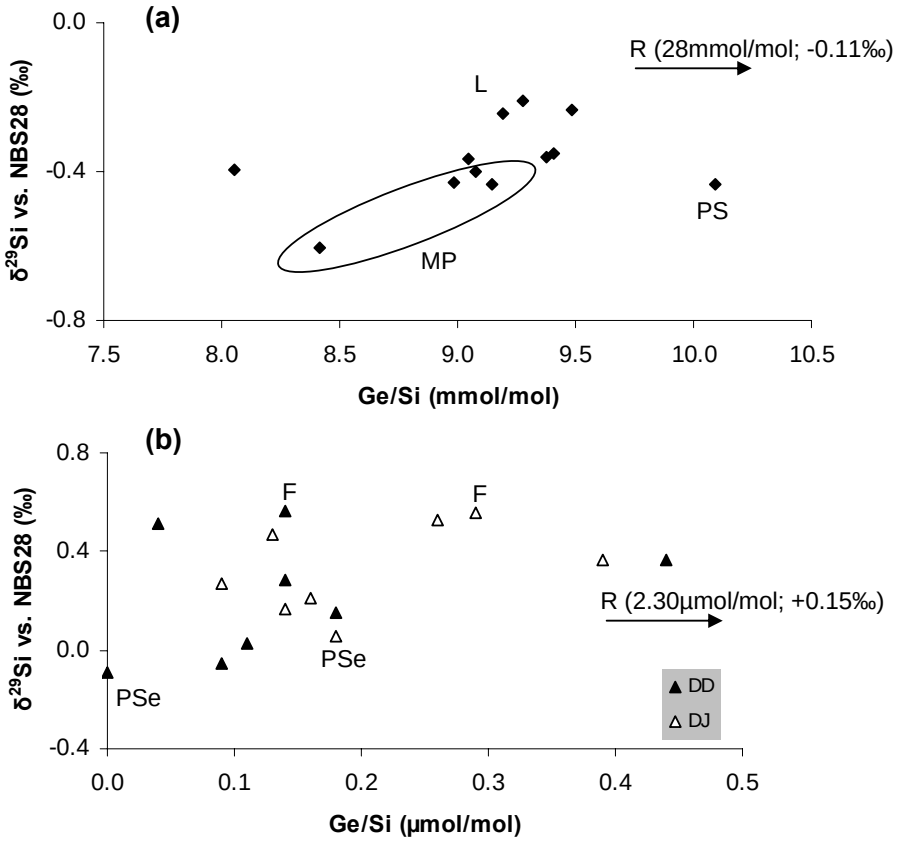

Figure 9.5: Relation between Ge/Si and δ^{29}Si in (a) hydroponic banana (GS1). R = roots; PS = pseudostem; MP = midrib and petiole; L = lamina - (b) mature banana plants (DD, DJ: δ^{29}Si from Chapter 8). R= roots; PSe = external pseudostem; F = fruit. Both roots values are outside the graph (values are indicated).

banana plantlets, Ge and Si accumulation were found in pseudostem of mature banana, constituted of old leaf sheaths where Si accumulation is related to ageing of tissues (Motomura et al., 2004). In banana and horsetails, Ge was found to be accumulated in roots compared to shoots. Though discrimination mechanism is common to both plant species, the fractionation magnitude could be affected by many factors, notably the extent of Si accumulation. Indeed a higher Si accumulation was found in horsetails compared to banana (Figure 9.3). A relation with Si accumulation could be explained by the density of Si transporters

varying between plant species (Ma et al., 2006). High Si-accumulating plants are characterized by an active Si uptake mechanism and by a high density of Si transporters: Si is taken up in larger quantity than that predicted by mass flow (Takahashi et al., 1990) with no Ge-Si discrimination. We suggest that similarly to a toxic element, Ge taken up in considerable amounts by confusion with Si has to be stocked with a higher efficiency in high Si-accumulating plants. Consequently, these plants would be characterized by a larger intra-plant fractionation.

9.4.4 Impact of the soil

Banana plants from Cameroon were sampled on two contrasted soils, a PDvs (DD) and a SWvs (DJ). To identify whether the soil would impact on the Ge/Si ratio of the shoot (restituted to the soil), a bulk shoot Ge/Si value can be computed, based on Ge/Si values of each shoot parts. This value is calculated by balancing the Ge/Si value of each shoot part by the Si proportion of each shoot part in the total shoot Si content (as for bulk plant Si isotopic composition; Chapter 8). Si proportion of each shoot part is calculated using the Si content measured in this study and the biomasses measured by Martin-Prével and Montagut (1966) in each respective plant part of banana. Roots were excluded from this calculation as no biomass data were acquired. For this calculation, the potential high Ge pool from roots is considered as more stable compared to shoots which are restituted to the soil at harvesting time. Obtained bulk shoot Ge/Si values are not significantly different for DD (0.130 ± 0.008 μmol/mol) than DJ (0.126 ± 0.008 μmol/mol).

Yet, DD and DJ bulk soils (0-3cm) displayed contrasted Ge/Si ratio, with a higher Ge/Si ratio in Djungo (3.80 μmol/mol; Table 9.2) compared to Dia-Dia (2.47 μmol/mol; Table 9.2). These soils Ge/Si ratios reflect a higher clay content in DJ compared to DD (72 against 25%; Chapter 8), as secondary weathering products are known to exhibit high Ge/Si ratio (e.g. kaolinite = 5.9 μmol/mol; Kurtz et al. (2002)). This is supported by Ge/Si ratio in clay fractions, higher in DJ (5.88 μmol/mol; Table 9.2) than in DD (4.69 μmol/mol; Table 9.2). Similar bulk plant Ge/Si ratios in DD and DJ indicates that contrasted soils would not impact the bulk plant Ge/Si ratio. In other words, the bulk plant Ge/Si ratio would not reflect the soil weathering stage, in opposition to bulk plant Si isotopic signature (Chapter 8).

In contrast, Ge/Si ratio in phytoliths were shown to depend on the Ge/Si ratio of the source (Blecker et al., 2007). Phytoliths from ferns enriched in silica ($5\%SiO_2wt$) grown in Si-rich soils ($Ge/Si_{soil} = 2.9\mu mol/mol$) displayed low Ge/Si ratios ($Ge/Si_{phyto} = 0.05\mu mol/mol$)

compared to phytoliths from ferns grown in Si-depleted soils (Ge/Si_{soil} = 10 to 21μmol/mol) with lower silica content (0.5-1.5%SiO_2 wt) (Ge/Si_{phyto} = 0.1-0.4μmol/mol) (Kurtz and Derry, 2004). This strongly suggests that in contrast to bulk shoot Ge/Si ratio, phytolith Ge/Si ratio (<0.5μmol/mol, Derry et al. (2007)) might reflect the soil weathering stage. As phytoliths are restituted to the soil (considering a stable Ge pool in roots), the Si recycling through plants would progressively produce a Si source very depleted in Ge in soil surface (Derry et al., 2005). This confirms that Ge/Si ratio is a very useful tracer of the impact of plants on the Si cycle. Through this Si bio-recycling, plants play a major role on weathering (Alexandre et al., 1997; Lucas, 2001).

9.4.5 Implications

Our data provide unequivocal answers to the questions of crucial natural significance raised in the Introduction. (1) Ge is taken by plant similarly to Si without any rejection, despite the low Ge/Si ratios reported for phytoliths. (2) There is a large Ge/Si fractionation between roots and shoots as Ge is trapped in roots but no Ge/Si fractionation occurs within shoots. (3) Due to Ge trapping in roots, the xylem sap entering the shoots would be depleted in Ge, which explains the low Ge/Si ratios reported in phytoliths precipitated in shoots (Blecker et al., 2007; Derry et al., 2005). (4) As phytoliths with low Ge/Si ratios are restituted to the soil by decomposition of vegetal debris, dissolution of phytoliths would provide a Ge depleted source of silica. This was shown to be very helpful to trace biogenic impact on soil solutions (Derry et al., 2005). Our data thus indicate that Ge uptake by plant can be useful to understand Si mobility through plant, as Si isotopes (Opfergelt et al. (2006b); Chapter 7), and that Ge/Si fractionation by plants would impact Ge-Si fluxes to ocean (Ellwood and Maher, 2003).

Tracing silicon into plant: Ge/Si vs. Si isotopes

Ge/Si ratio and Si isotopes were used to trace Si mobility within the plant. Both tracers are not correlated (Figure 9.5) as they are impacted by very different processes. At the root-solution interface, Ge is not discriminated contrary to heavy Si isotopes. Light Si isotopes would be preferentially taken up by Si transporter at the root interface (Opfergelt et al. (2006b); Chapter 7). Roots were shown to accumulate Ge and to be enriched in heavy Si isotopes. Ge would be trapped as Ge organic complex, as inferred from the present study, while heavy Si isotopes would be discriminated by Si transporters responsible for

xylem loading (Opfergelt et al., 2006b). Shoots display similar Ge/Si ratios but significantly heavier Si isotopic compositions in lamina than in pseudostem. This would indicate that Ge will follow transpiration stream as silicon, and not been discriminated between pseudostem and lamina in contrast with Si isotopes (Opfergelt et al. (2006ab); Chapter 7; Appendix C; Chapter 8).

In summary, Ge and heavy Si isotopes are both discriminated at root level but in shoots, only heavy Si isotopes are significantly fractionated. As previously highlighted (see section 9.4.4), the bulk shoot Ge/Si ratios in plants would not reflect the soil weathering stage contrary to Si isotopes (Chapter 8), but the phytolith Ge/Si ratio might be related with the soil weathering stage (Kurtz and Derry, 2004). Combining bulk shoot Ge/Si ratio and phytolith Ge/Si ratio provided the major information that Ge was not discriminated at the enter of the plant to induce low Ge/Si ratio in phytoliths but that plant roots were extremely enriched in Ge, and should organically trap Ge explaining the low Ge/Si ratio of phytoliths. Consequently, combining Si stable isotopes and Ge/Si ratios in plants is very useful to understand Si uptake by plant and Si mobility within the plant, as these two tracers do not reflect similar mechanism but can be very complementary.

Plant impact on Ge-Si ratio exported

The strong Ge/Si differenciation within plants has potentially important geochemical implications due to different residence times of leaves and roots. Leaves are rather quickly recycled into the litter at a seasonal time scale. As the litter-stocked phytoliths are rapidly dissolved at intermediate-alkaline surface water pH, their signature could be rapidly transferred to rivers. A rapid mass balance calculation demonstrated that river Ge/Si ratio (0.6μmol/mol, Froelich et al. (1992)) could be explained by a dissolution mixture of 89% of DD leaves (Ge/Si = 0.07μmol/mol) and 11% of dissolved DD clays (Ge/Si = 4.69μmol/mol; Table 9.2), or by 92% of DJ leaves (Ge/Si = 0.13μmol/mol) and 8% of dissolved DJ clays (Ge/Si = 5.88μmol/mol; Table 9.2). However, this does not take into account the Ge depletion induced by clay neoformation, and the contribution of groundwaters. A similar mass balance calculation with δ^{30}Si values indicates that dissolution of phytoliths and clay minerals would not explain the riverine δ^{30}Si signature. Clay neoformation and other abiotic processes should be taken into account. Nevertheless, this suggests that phytoliths dissolution can be an important contributor to the Ge/Si riverine ratio.

9.5 Conclusion

Hydroponics banana plantlets, mature banana and horsetails displayed Ge enrichment in roots compared to shoots, likely due to a organically trapped Ge accumulation in roots. This would explain the low Ge/Si ratio reported on phytoliths. In shoots Ge follow transpiration stream and no discrimination within shoots was observed. Further studies on the form of Ge in roots would be very helpful to better understand this mechanism. Bulk plant Ge/Si ratio seems not related to the soil weathering stage while the phytolith Ge/Si ratio varied with soil weathering stage. Combined with stable Si isotopes, the Ge/Si fractionation induced by plants would provide new insights on Si and Ge-uptake and mobility through plant, and contribute to identify the plant contribution relative to Si from mineral dissolution to Si exported.