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The ratio of germanium to silicon in plant phytoliths: quantification of biological discrimination under controlled experimental conditions

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Abstract Slight differences in the chemical behavior of germanium (Ge) and silicon (Si) during soil weathering enable Ge/Si ratios to be used as a tracer of Si pathways. Mineral weathering and biogenic silicon cycling are the primary modifiers of Ge/Si ratios, but knowledge of the biogenic cycling component is based on relatively few studies. We conducted two sets of greenhouse experiments in order to better quantify the range and variability in Ge discrimination by plants. Graminoid species commonly found in North American grassland systems, *Agropyron smithii*, *Schizachyrium scoparium*, and *Andropogon gerardii* were grown under controlled hydroponic environmental conditions. Silicon leaf contents were positively correlated with solution Si and ambient temperature but not with nutrient solution pH, electrical conductivity, or species. The Ge/Si ratio incorporated into phytoliths shows a distribution coefficient $[(Ge/Si)_{phytolith}/(Ge/Si)_{solution}]$ of about 0.2 and is remarkably invariant between species, photosynthetic pathway, and solution temperature. Ge seems to be discriminated against during the uptake and translocation of Si to the opal deposition sites by about a factor of five. In the second experiment, a wider range of graminoid species (*Agropyron smithii*, *Bouteloua gracilis*, *Buchloe dactyloides*, *Oryzopsis hymenoides*, *Schizachyrium scoparium* and *Andropogon gerardii*) were grown in two different soil mediums. Plant phytoliths showed a distribution factor of about 0.4 for field grown grasses, and 0.6 for potting soil grown grasses with no clear trends among the species. Evidence of the direction and degree of biological Ge discrimination during plant uptake provides a geochemical finger print for plants and improves the utility of Ge/Si ratios in studies of terrestrial weathering and links between Si cycles in terrestrial and marine systems.

Keywords Germanium · Ge/Si · Graminoid · Phytolith · Silicon

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Introduction

Germanium exists at trace levels in the earth's crust and exhibits similar biogeochemical behavior to silicon. Germanium/silicon (Ge/Si) ratios have been used

to trace silicon sources in marine sediment cores, where the lack of biologic discrimination of Ge/Si during assimilation by diatoms (Froelich et al. 1992; Bareille et al. 1998) allows interpretation of the marine opal record in terms of Si inputs and cycling (Froelich et al. 1992). Ge/Si ratios have also been utilized to examine weathering processes in tropical and temperate ecosystems (Murnane and Stallard 1990; Filippelli et al. 2000; Kurtz et al. 2002), where Ge is often enriched in secondary minerals and depleted in biogenic minerals. Given the differences in Ge/Si between plants and soils, plant cycled Si leaking into stream systems can be identified using Ge/Si (Derry et al. 2005). The low Ge/Si ratios of ferns (*Cybotium* sp.) reported by Derry et al. (2005), suggest discrimination against Ge during plant uptake. However more data are needed to verify the magnitude and direction of this discrimination with higher plants across a variety of ecosystems, in order to increase the utility of biologically mediated Ge/Si in studies of terrestrial Si cycling.

The importance of silicon in plants is well documented (e.g., Raven 1983; Epstein 1999) and often manifest under environmental stress (Ma et al. 2001). Silicon benefits plants by increasing mechanical strength, yield, enzyme activity, and resistance to disease and pests (Epstein 2001). Silicon also enhances salt tolerance, cold hardiness, resistance to metal toxicity, and promotes nodule formation in legumes (Sangster and Hodson 1992; Epstein 2001).

Plants extract silicon from soil as undissociated monosilicic acid (H_4SiO_4), and transport it via the transpiration stream into stems and leaves where it precipitates as amorphous opal (Jones and Handreck 1967; Epstein 1999). Both passive and active transport mechanisms govern silicic acid movement into plants, and varying among plant families (e.g., Prychid et al. 2003; Hodson et al. 2005). For many plants, Si uptake involves passive diffusion across the lipid component of the root cell membrane (Raven and Edwards 2001). However Si concentrations greater than those accounted for by diffusion and transpiration alone, imply active transport. The physiology of active Si uptake in vascular plants (often against concentration gradients) has yet to be elucidated (Raven 2001), though association with proteinaceous material has been demonstrated (Perry and Keeling-Tucker 2000; Ma et al. 2006). In plants, silicic acid in excess of physiological needs polymerizes into amorphous silica bodies that are stored primarily in cell walls, cell lumina, and intercellular spaces near evaporating surfaces (Raven 1983; Sangster and Hodson 1992; Prychid et al. 2003). Commonly known as plant opal or phytoliths, amorphous silica bodies are present in most plants, ranging in content (on a dry weight basis) from 0.5% or less in most dicotyledons, 1–3% in many dryland grasses, and up to 10–15% in some wetland plant species. Phytolith-Si is returned to soil upon plant death and decomposition, where its concentration is regulated by the balance between plant production and the rate of chemical weathering (Alexandre et al. 1997).

There are still relatively few data regarding discrimination of Ge from Si during plant uptake. Field based studies by Derry et al. (2005) showed lower Ge/Si plant phytolith ratios compared to concomitant soil solutions for ferns (*Cybotium* sp.) in Hawaii, and a similar trend has been reported by Blecker (2005) for grasses of the Great Plains. Under controlled growing conditions, differences in Ge and Si uptake have been shown in wheat and rice, but at Ge levels much higher than those typically found in natural systems (Takahashi et al. 1976; Rains et al. 2006). Though these studies have inferred biological discrimination against Ge, the magnitude and direction have yet to be quantified under controlled growing conditions, with Ge levels representative of natural systems. The objective of this study was to examine the magnitude and direction of Ge discrimination in ecologically diverse grassland species under controlled conditions in order to further quantify the role of vegetation in the terrestrial biogeochemical Si cycle. We designed a series of experiments utilizing hydroponic solutions and soils. We selected native plants that are either dominant or co-dominant in the grassland regions of North America representing both C_3 and C_4 photosynthetic systems.

Methods

Experiment 1—hydroponic study

Seeds from western wheatgrass (*Agropyron smithii*) a plant possessing the C_3 photosynthetic carbon fixation pathway, little bluestem (*Schizachyrium scoparium*) and big bluestem (*Andropogon gerardii*) both of which possess the C_4 photosynthetic pathway were germinated in a dilute nutrient solution (Table 1) on

polypropylene mesh in the dark. Seedlings were transplanted 14 d after germination to 20-l PVC growth containers aerated with compressed air passed through plastic tubing. The seedlings (approximately 8–12 per tank) were supported by polystyrene disks that floated on the solution surface. Two adjoining greenhouses were used for the study to simulate a range in growing temperatures, a 'cooler' greenhouse (optimum for C_3 grasses) with an ambient air temperature range of approximately 18–20°C and a 'warmer' greenhouse (optimum for C_4 grasses) with an ambient air temperature range of approximately 24.5–28°C. For *Agropyron smithii*, three tanks each with concentrations of 10 mg Si l^{-1} (0.36 mM Si) or 50 mg Si l^{-1} (1.76 mM Si) were set up in both greenhouses. For *Schizachyrium scoparium* and *Andropogon gerardii*, three tanks each with Si levels of 1.76 mM Si were set up in the 'warmer' greenhouse. The Si levels were chosen to represent monosilicic acid levels near the lower and upper concentrations in soil solution (Lindsay 1979; Marschner 1995; Epstein 1994). Relative humidity levels in both greenhouses ranged between 20% and 75%. Conductivity and pH levels were monitored throughout the study using an Orion 105A conductivity meter and an Orion 720A pH meter. Nutrient solutions were monitored continuously and changed every 5–7 d in order to maintain adequate nutrient levels and relatively constant Si concentrations. Subsamples of nutrient solution were taken throughout the study to measure Ge/Si levels. A slight yellowing of the leaves on most plants, likely indicative of minor nutrient deficiencies, was observed during the study. *Agropyron smithii* plants were harvested at 84 d, just as a few of the plants started to set seed. *Schizachyrium scoparium* and *Andropogon gerardii* plants were harvested at 65 d, prior to the plants setting seed. Roots (combined into one sample by species where collected), leaves and stems were separated and oven dried at 60 °C immediately after harvest.

Experiment 2—soil study

An experiment using two diverse soil media was initiated to further examine the magnitude, direction and variability of biologic Ge discrimination. Seeds from a wide range of grassland plants, namely, *Agropyron smithii*, *Bouteloua gracilis*, *Buchloe dactyloides*, *Oryzopsis hymenoides*, *Schizachyrium scoparium* and *Andropogon gerardii* were planted in two different soils: surface horizon soil from a common soil within the shortgrass steppe in eastern Colorado (Blecker 2005) and potting mix consisting of vermiculite, Canadian sphagnum peat moss, perlite, and dolomitic limestone (Sun Gro Metro-Mix 200, Sun Gro Horticulture, Bellevue, WA). Three replicates of each species were planted in each soil. All plants were grown in the 'warmer' greenhouse. Soil moisture was maintained near field capacity with a diluted nutrient solution that contained no added silica (Table 1). Plants were harvested at 60 d, prior to the plants setting seed. Leaves and stems were separated from roots and oven dried at 60°C immediately after harvest. Soil water for chemical analysis was extracted by saturated paste equilibration (Lajtha et al. 1999). Subsamples of soil were brought to near saturation with deionized water and allowed to equilibrate at room temperature for 48 h. Soil water was extracted via Buchner vacuum funnel using Whatman no. 42 filter paper and further filtration through a 0.45 μm polycarbonate membrane.

Plant phytolith extraction

Plant samples from both studies were cleaned to remove soil contamination, dry ashed, then treated to remove non-siliceous material in a method adapted from Piperno (1988), Kelly (1990), and Parr et al. (2001). Oven-dried plant material was cut into 2–3 cm lengths, and washed in a mixture of 5% sodium hexametaphosphate, 1M hydrochloric acid and deionized water. After thorough rinsing with deionized water, the sample was washed in 70% ethanol, and again rinsed with deionized water. An oven-dried subsample was weighed into a Ni crucible, ashed at 500°C for 1 h, allowed to cool in a desiccator and weighed. The ash was washed in warm, 1M hydrochloric acid, rinsed with deionized water, washed in hot 30% hydrogen peroxide, filtered through a 0.20 μm filter, and rinsed thoroughly with deionized water. After drying at 55–60°C, the sample was allowed to cool in a desiccator, weighed, and stored in a plastic vial. Phytolith concentration (on a % dry weight plant basis) was determined gravimetrically. Other plant constituents are also reported on a % dry weight plant basis.

Table 1 Nutrient solution composition

Macronutrients and silicon		Micronutrients[a]	
Compound	Concentration in solution (mM)	Compound	Concentration in solution (μM)
$Ca(NO_3)_2$	4	H_3BO_3	20
KNO_3	6	$MnSO_4 \cdot H_2O$	1.2
$NH_4H_2PO_4$	0.9	$ZnSO_4 \cdot 7H_2O$	1.7
$MgSO_4$	1	$CuSO_4 \cdot H_20$	0.5
Na_2SiO_3[b]	0.36 or 1.78	H_2MoO_4	0.5
		$Ni(NH_4)_2(SO_4)_2 \cdot 6H_2O$	0.2
Ge/Si $\times 10^{-6}$	0.48–0.53[b]	Fe-EDDHA	200

[a] Micronutrients were mixed in a concentrated form in solution then added to the water, producing the final concentration listed

[b] Na_2SiO_3 was added to the water, followed by 1M HCl to adjust pH to approximately 5.5–6.0, then the nutrients. Deionized water was used to prepare the nutrient solutions. The Ge was derived from the Na_2SiO_3. Ge/Si ratios are mol/mol

Si and Ge analyses

Plant phytolith samples were dissolved in 2 M NaOH and analyzed for Si concentration by spectrophotometry at 812 nm using a blue silicomolybdous acid method (Mortlock and Froelich 1989). Measured Si content was compared to expected Si content (based on a 10% water content: $SiO_2 \cdot 0.4H_2O$) to ensure complete dissolution. Aliquots of the dissolved phytolith were neutralized with dilute nitric acid prior to Ge analysis. Ge analysis was performed by isotope dilution hydride generation mass spectrometry (Jin et al. 1991; Mortlock and Froelich 1996). Samples were spiked with an enriched ^{70}Ge tracer solution. In some cases two splits from a sample were spiked separately to obtain different final $^{70}Ge/^{74}Ge$ ratios, with target values for $^{70}Ge/^{74}Ge$ of ≈ 3 and ≈ 10. After equilibration for at least 24 h but typically longer approximately 1 ml of sample was introduced into an on-line continuous flow hydride generator, modified from (Klaue and Blum 1999), along with 2% $NaBH_4$ solution and a Tris–HCl buffer at pH 6. Evolved $GeH_4(g)$ was separated using a Teflon membrane and swept into the plasma source of a Finnigan Element 2 sector ICP-MS in low resolution (ca. 400) setting. Signal at masses 70, 72, and 74 was acquired on an electron multiplier, and standards and blanks were run between every four samples. The data for each peak was corrected for background using the blank data. The blank does not have the isotopic composition of natural Ge, principally because of the presence of ArNO interference at mass 70, which is not resolved at the low resolution setting of the Element 2. The background correction thus includes both the effects of reagent blanks and molecular interferences in the ICP-MS. The background correction was normally 2–3% on mass 74, and less on mass 70 because of the high ^{70}Ge content of spiked samples. After background subtraction mass bias was corrected by interpolation of the $^{70}Ge/^{74}Ge$ and $^{72}Ge/^{74}Ge$ ratios measured on a 100 ppt Ge standard in HNO_3. Mass bias is not constant but fell in the range 1.2–1.4% per amu. The Ge content of the sample was calculated by normal isotope dilution arithmetic and corrected for sample dilution. The agreement between differently spiked sample splits was always within 5%; normally it was better than 3%. We did not analyze for methyl-Ge species, and assume that they are either present in very low abundance and/or unreactive in this system (Lewis et al. 1989). Si concentration in the nutrient solutions and saturated soil paste extracts were analyzed using the same silicomolybdous blue method. Germanium concentrations in the nutrient solution and saturated paste extracts were also analyzed by isotope-dilution hydride-generation ICP-MS.

Statistical analyses

All statistical tests were performed using the Proc GLM model in SAS software version 9.1 (SAS institute 2002). Differences were examined using

one-way analysis of variance (ANOVA) at a significance level of 0.05, and all means were expressed as one standard error of the mean.

Results

Hydroponic study

Agropyron smithii

For the 'cool' greenhouse, average minimum (16.7°C), maximum (20.9°C), and daily average temperatures (18.7 ± 0.15°C) were significantly lower than those of the 'warm' greenhouse (min. 19.2°C, max. 25.1°C, daily avg. 22.1 ± 0.14°C; $P < 0.0001$). Average relative humidity throughout the experiment was slightly higher in the warm greenhouse (42.6%) compared to the cool greenhouse (38.1%), fluctuating between 25% and 70% in both greenhouses. Average nutrient solution pH values were statistically similar among the treatments ($P = 0.868$) ranging from 5.7 to 6.0. Average electrical conductivity values were slightly lower among the 10 mg Si l^{-1} containers (2.37 ± 0.02 dS m^{-1}) compared to the 50 mg Si l^{-1} containers (2.66 ± 0.03 dS m^{-1}; $P < 0.0001$), the latter containing a greater amount of sodium metasilicate. Throughout the course of the study, the actual concentration of the 10 mg Si l^{-1} containers averaged 10.82 ± 0.16 mg Si l^{-1}, and the actual concentration of the 50 mg Si l^{-1} containers averaged 53.33 ± 0.37 mg Si l^{-1}, when measured immediately after nutrient solution preparation. In both the 10 mg Si l^{-1} and 50 mg Si l^{-1} containers, Si concentration decreased slightly over time (Fig. 1a and b), likely due to increasing Si uptake with increasing plant growth. Over the course of the experiment, average Ge/Si ratios for the 10 mg Si l^{-1} nutrient were slightly higher than average Ge/Si ratios for the 50 mg Si l^{-1} nutrient solution (0.53 ± 0.01 and 0.48 ± 0.015, respectively; $P = 0.026$).

Table 2 presents a summary of *Agropyron smithii* leaf, stem, and root data. On average, the plant leaves grown in 50 mg Si l^{-1} contained twice as much plant phytolith (6.1 ± 0.42%) as those grown in 10 mg Si l^{-1} (3.1% ± 0.19; $P = 0.0005$). This result has been seen in numerous studies of plant Si uptake (Van der Vorm 1980; Jarvis 1987; Rafi and Epstein 1999, Ma et al. 2001) and suggests an active Si uptake mechanism. Stem phytolith concentrations were roughly 2.5 times less than those of the plant leaves within the same solution. Root phytolith concentration was 1.0%, less than either leaf or stem concentrations. Molar Si and Ge concentrations and Ge/Si ratios for plant material are presented in Table 2. The average Ge/Si ratios for 50 mg Si l^{-1} leaves (0.081 ± 0.007, were statistically similar to the 10 mg Si l^{-1} leaves (0.065 ± 0.006; $P = 0.1485$). Average stem Ge/Si ratios were also statistically similar between the 10 mg Si l^{-1} and 50 mg Si l^{-1} systems (0.105 ± 0.005 vs. 0.131 ± 0.014, respectively; $P = 0.191$), but collectively were about two times greater as compared to leaf Ge/Si ratios (0.120 ± 0.010 vs. 0.074 ± 0.005, respectively;

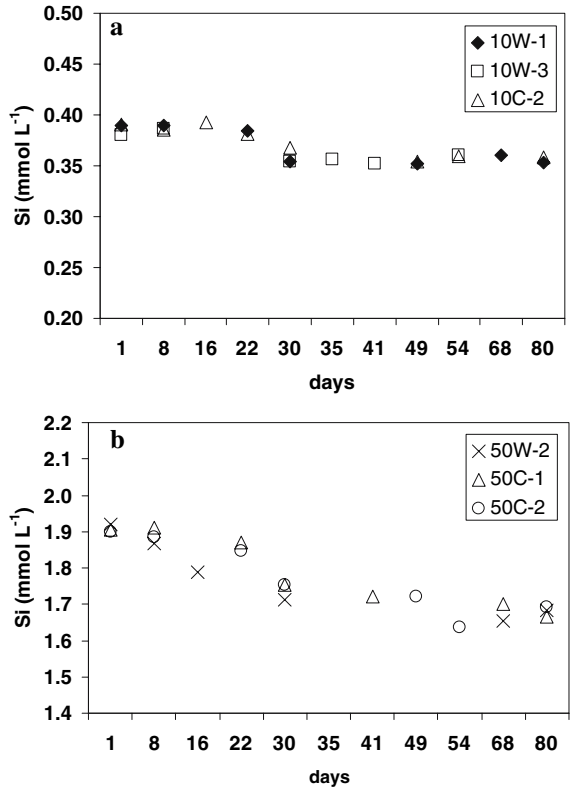

Fig. 1 (**a**) Nutrient solution low Si concentrations for *Agropyron smithii* over the course of the experiment. '10' represents low-Si containers; 'C' represents containers located in the 'cool' greenhouse; 'W' represents containers located in the 'warm' greenhouse. 1, 2, or 3 represent replicates. (**b**) Nutrient solution high Si concentrations for *Agropyron smithii* over the course of the experiment. '50' represents high-Si containers; 'C' represents containers located in the 'cool' greenhouse; 'W' represents containers located in the 'warm' greenhouse. 1,2,or 3 represent replicates

$P = 0.0005$). The root Ge/Si ratio (0.12) was similar to that of the stems. Leaf Ge/Si ratios were slightly lower for the warm vs. the cool treatments (0.063 ± 0.006 vs. 0.084 ± 0.007; $P = 0.038$).

Schizachyrium scoparium and Andropogon gerardii

Average electrical conductivity was slightly higher in the 50 mg Si l^{-1} *Agropyron smithii* experiment (3.09 ± 0.035 vs. 2.66 ± 0.035 dS m^{-1} respectively; $P < 0.0001$). Average pH values were statistically similar between the *Agropyron smithii* experiment (5.8 ± 0.06) and this experiment (5.5 ± 0.24; $P = 0.2000$). Nutrient solution Si concentrations throughout the course of the experiment followed a similar pattern to those of *Agropyron smithii* experiment, with average initial Si levels of 52.7 ± 0.20 mg Si l^{-1} decreasing to an average of 49.4 ± 0.11 mg l^{-1} as plant growth increased. Table 3 presents a summary of plant data. Average plant phytolith contents between *Schizachyrium scoparium* and *Andropogon gerardii* were similar (4.5% ± 0.59 vs. 4.2% ± 0.09, respectively; $P = 0.643$). Ge/Si ratios of the combined leaf and stem tissues of these species were also similar to the average combined leaf and stem Ge/Si ratios for *Agropyron smithii* plants grown in 50 mg Si l^{-1} (0.08 ± 0.01 and 0.091 ± 0.007, respectively).

Soil media study

Table 4 lists plant data for the soil media grown graminoid Ge/Si uptake experiment. Plant phytolith values were roughly 2.5 times greater for grasses grown in field soil compared to potting soil (4.8% ± 0.67 vs. 1.9% ± 0.28, respectively; $P = 0.002$). This trend can only be partially explained by the higher concentration of soluble Si associated with the field soil compared to the potting soil (23.8 ± 2.04 vs. 17.7 ± 0.61 mg Si l^{-1}, respectively; $P = 0.045$). The Ge/Si ratios of the soluble Si in the field soil were approximately double those for the potting soil (0.33 ± 0.02 vs. 0.16 ± 0.02, respectively; $P < 0.0001$). The Ge/Si ratios of the plants grown in field soil was higher than that of their counterparts grown in potting soil, but by a significantly smaller margin (0.12 ± 0.004 vs. 0.09 ± 0.007, respectively; $P = 0.002$).

Table 2 Hydroponic study—*Agropyron smithii*, selected plant data

Sample	Plant phytolith[a] (%)		Si[b] (μmol/g)		Ge (pmol/g)		Ge/Si[c] × 10^{-6}	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
10C-1	3.0	1.1	8.0	6.4	0.58	0.64	0.07	0.10
10C-2	2.7		4.7		0.39		0.08	
10W-1	2.7		8.0		0.51		0.06	
10W-2	3.5	1.5	10.3	7.3	0.46	0.73	0.04	0.10
10W-3	3.6	1.7	7.5	6.3	0.46	0.73	0.06	0.12
50C-1	5.5	2.7	10.9	9.2	0.77	1.13	0.07	0.12
50C-2	5.6	2.6	7.9	7.9	0.68	0.95	0.09	0.12
50C-3	5.6		6.7		0.77		0.11	
50W-1	5.4	2.1	8.1	5.0	0.62	0.87	0.08	0.17
50W-2	7.9		10.7		0.72		0.07	
50W-3	7.0	2.6	10.9	8.3	0.76	0.90	0.07	0.11
Roots[d]	1.0		4.2		0.53		0.12	

[a] Based on dry weight of plant

[b] Determined spectrophotometrically after dissolution in 2 M NaOH

[c] Ge/Si values are a molar ratio

[d] Roots represent a composite subsample of all the plant samples; empty cells—not determined

'C' represents containers located in the 'cool' greenhouse; 'W' represents containers located in the 'warm' greenhouse. '10' represents the low-Si treatments of 10 mg Si l^{-1}; '50' represents the high-Si treatments of 50 mg Si l^{-1}; '1', '2', or '3' represent replicates

Table 3 Hydroponic study—and *Andropogon gerardii* (Ag) and *Schizachyrium scoparium* (Ss), selected plant data

Sample	Plant phytolith[a] (%)	Si[b] (μmol/g)	Ge (pmol/g)	Ge/Si × 10^{-6}
Ag-1	4.2	8.9	0.62	0.07
Ag-2	4.4			
Ag-3	4.1	7.2	0.48	0.07
Ss-1	4.8	6.0	0.60	0.10
Ss-2	5.4			
Ss-3	3.4	6.3	0.46	0.07

[a] Based on dry weight of plant

[b] Determined spectrophotometrically after dissolution in 2 M NaOH

Table 4 Selected plant data for the soil grown graminoids

Sample	Plant phytolith[a] (%)		Si[b] (μmol/g)		Ge (pmol/g)		Ge/Si[c] × 10^{-6}	
BOGR	7.3	2.4	10.4	6.1	1.29	0.49	0.12	0.08
BUDA	5.9	2.0	10.8	8.1	1.48	0.93	0.14	0.11
AGSM	2.5	0.9	8.0	6.5	.94	0.56	0.12	0.09
ORHY	4.8	1.3	9.0	7.0	1.1	0.51	0.11	0.07
ANGE	4.1	2.1	9.8	5.8	1.25	0.47	0.13	0.08
SCSC	4.5	2.7	9.9	9.6	1.16	1.06	0.12	0.11

Photosynthetic pathway follows species abbreviation: BOGR (C_4) = *Bouteloua gracilis*, BUDA (C_4) = *Buchloe dactyloides*, AGSM (C_3) = *Agropyron smithii*, ORHY (C_3) = *Oryzopsis hymenoides*, ANGE (C_4) = *Andropogon gerardii*, and SCSC (C_4) = *Schizachyrium scoparium*. For each parameter, the left-hand column represents plants grown in field soil, the right-hand column represents plants grown in potting soil; Si concentrations for the field grown soil solution and potting soil solution were 0.85 mmol Si l^{-1} and 0.63 mmol Si l^{-1} respectively; Ge/Si values for the field soil and potting soil were 0.33 and 0.16 respectively

[a] based on dry weight of plant

[b] Betermined spectrophotometrically after dissolution in 2 M NaOH

[c] Ge/Si values are a molar ratio

Discussion

A comparison of the greenhouse experimental data, to data from recent field studies (Blecker 2005; Derry et al. 2005) provides another avenue of examining biologic Ge discrimination (Fig. 2). Though field soil was used as one of the growing mediums in the greenhouse study, it was taken from a single horizon, whereas in the field, soluble Si sources available to the plant contain soluble Si from multiple horizons with potentially different Ge/Si signatures. From Blecker (2005), a bioclimosequence spanning grassland sites across the Great Plains, soil water Ge/Si ratios for the surface horizons averaged 0.69 ± 0.10, and plant phytolith Ge/Si ratios averaged 0.31 ± 0.02, a fractionation factor (distribution coefficient) (K_D) of 0.45, given by the equation

$$K_D = \left(\frac{(Ge/Si)_{phyto}}{(Ge/Si)_{aq}} \right) \quad (1)$$

where $(Ge/Si)_{phyto}$ is the measured ratio in plant opal and $(Ge/Si)_{aq}$ is the ratio in the source solution. Dominant grasses in that study included *Bouteloua gracilis* and *Buchloe dactyloides* at shortgrass sites to *Andropogon gerardii* at tallgrass sites. Differences in Ge/Si among the species in that study indicate potential species-specific differences in Si uptake and/or utilization of different Si sources. Similar graminoid species grown in the greenhouse soil media study exhibited similar discrimination against Ge; field soil soluble-Si Ge/Si values averaged 0.33 ± 0.06 and plant phytolith Ge/Si values averaged 0.12 ± 0.02, for a distribution coefficient of 0.38; potting soil soluble-Si averaged 0.16 ± 0.004

and plant phytolith Ge/Si averaged 0.09 ± 0.007, for a distribution coefficient of 0.56. Though plant silicon concentration of ferns (Polypodiaceae) varies widely (Piperno 1988; Ma et al. 2001), the *Cybotium*, *Dicranopteris* and *Diplazium* genera reported in Derry et al. (2005) have relatively low plant-Si concentrations (avg. = 1.0% Si, $n = 11$) and low Ge/Si ratios (Fig. 2). In these initial studies of grasses and ferns, it appears that extent of Si accumulation does not greatly affect the mechanisms responsible for plant Ge discrimination. Despite the completely different climate and soils associated with Hawaiian ferns compared to Great Plains grasses, both plant groups appear to show similar levels of discrimination against Ge (Fig. 2), resulting in fairly low Ge/Si values in biogenic opal. In addition a leaf sample from hydroponically grown *Zea mays* exhibited a similarly low Ge/Si ratio of 0.07 (Kurtz 2000).

Despite differences in plant phytolith concentration, conductivity, nutrient solution Si concentration, Ge/Si plant phytolith ratios for the *Agropyron smithii* leaves were statistically similar, averaging 0.06 for the low-Si leaves and 0.08 for the high-Si leaves ($P = 0.145$). Warm greenhouse *Agropyron* leaves appear to have slightly lower Ge/Si ratios than the corresponding cool greenhouse samples, but the difference is not statistically significant. Temperature-driven discrimination from kinetic or physiological (e.g., transpiration) differences may underlie these differences, but greater numbers of

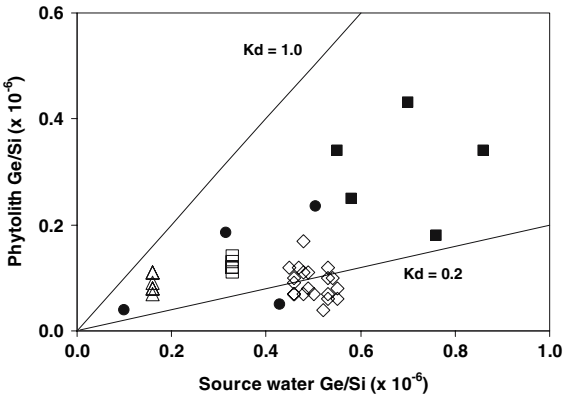

Fig. 2 Ge/Si units for both phytolith and source water values are a molar ratio. Open symbols represent samples from the greenhouse study (diamonds = nutrient solution, squares = field soil, triangles = potting soil), closed symbols represent samples from field studies (squares = grasses from Blecker 2005, circles = ferns from Derry et al. 2005)

replicates and species are necessary to clarify this potential trend. The slightly higher average nutrient solution Ge/Si ratio for the low-Si systems (0.53) compared to the high-Si systems (0.48) could be a reflection of the greater Ge discrimination by the plants grown in the low-Si solution, creating a nutrient solution slightly more enriched in Ge compared to the high-Si systems. Despite the slight drop in nutrient solution Si concentration over time for both low- and high-Si systems the Ge/Si ratios did not drift appreciably over time, implying that weekly replacement of the nutrient solution was adequate to minimize any impact of a Rayleigh distillation type process. *Agropyron smithii* stems had lower plant phytolith concentrations and higher Ge/Si ratios than the leaves, with a 0.20 distribution coefficient for the low-Si systems and 0.28 for the high-Si systems (Table 2 and Fig. 3). Though only one composited root sample was measured, the low Si concentration and Ge/Si ratio (Table 2) suggests that Ge is excluded at the solution/root interface and not accumulated in the root. A lack of root Si accumulation has been reported in other greenhouse studies (Jarvis 1987; Ma et al. 1989) as the root tends to reach equilibrium Si levels fairly early in growth, while stem and leaf Si concentrations tend to increase during the growing cycle. In a study of plant Si uptake, Takahashi et al. (1976) noted that Ge tends to be concentrated in the stem, which we also observed in the greater Ge/Si stem ratios compared to the leaves of *Agropyron smithii* (Table 2).

Plant phytolith concentrations were similar among *Schizachyrium scoparium* and *Andropogon gerardii* and slightly lower than *Agropyron smithii*, which could be a function of species and experiment duration (85 vs. 60 d for the former). Similarities in phytolith Ge/Si ratios between *Schizachyrium scoparium* and *Andropogon gerardii* (Table 3), and the similarity of the Ge/Si ratios among the three hydroponically grown graminoid species, suggest a similar magnitude and direction of biologic Ge discrimination. Takahashi et al. (1976) also showed biologic discrimination against Ge in greenhouse experiment examining the Si uptake by various plants. Starting with a heavily Ge-spiked nutrient solution (Ge/Si = approximately 39 mmol mol^{-1}), phytoliths extracted from rice and wheat exhibited distribution coefficients of 0.47 and 0.45, respectively. In the current study, taken as a group,

Agropyron smithii, *Schizachyrium scoparium* and *Andropogon gerardii* showed an average distribution coefficient of 0.18. Differences in species and Ge concentrations compared to the experiments of Takahashi et al. (1976) could account for the differences in discrimination. More recently, in a hydroponic study of *Triticum aestivum* L 'Yecoro Rojo', Rains et al. (2006) reported diminished Ge uptake when Ge and Si were both present at concentrations <0.05 mM and similar uptake between the two elements when they were present at concentrations >0.05 mM.

The limited variability among phytolith Ge/Si values for the grass species *within* each soil medium, suggests a lack of species-driven discrimination against Ge, at least among grasses. Nor did any significant difference in Ge discrimination exist when the grasses were compared by photosynthetic pathway (i.e., C_3 vs. C_4), which could affect transpiration and therefore Si uptake. Differences between the magnitude of Ge discrimination or grasses grown in potting soil (distribution factor = 0.56) and grasses grown in field soil (distribution factor = 0.37), suggest that different growing conditions and/or Si sources may affect Ge speciation or reactivity and consequently Ge discrimination. The three species common to both the nutrient solution and soil studies also showed different degrees of Ge/Si discrimination compared to the source Ge/Si values (Tables 2 and 3; Fig. 2). *Agropyron smithii*, *Schizachyrium scoparium* and *Andropogon gerardii* grown in nutrient solution showed greater discrimination against Ge than their soil-grown counterparts. Differences in Si levels between nutrient solution (approximately 0.36 mM Si and 1.78 mM Si), field soil (approximately 0.82 mM Si) and potting soil (approximately 0.64 mM Si) along with other differences in growing conditions (e.g., Si source, transpiration rate, nutrient status) may have contributed to differences in the magnitude of Ge discrimination.

Differences in Ge and Si physiology, speciation and reactivity may play a role in Ge discrimination mechanisms. Though Ge is generally toxic to plants at high levels (Sankhla and Sankhla 1967; Halperin 1995), such levels were not measured in this study and therefore not a likely mechanism of Ge discrimination. Germanium physiology warrants consideration, as it has been shown to serve physiological functions (Loomis and Durst 1992; Cakmak et al. 1995) that

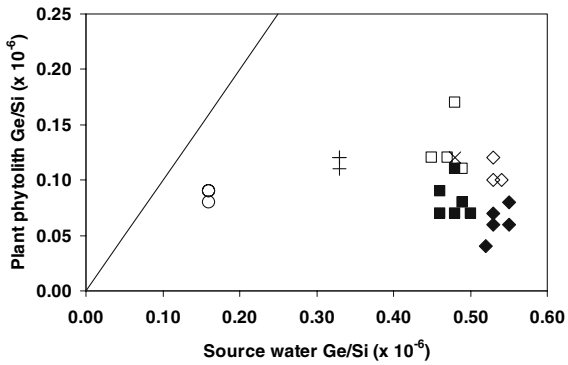

Fig. 3 A comparison of Ge/Si data for hydroponic and soil grown *Agropyron smithii*. Soil grown plants are represented by 'circle' = potting soil and '+' = field soil; Hydroponic grown plants are represented by 'x' = root; 'solid square' = 50 mg Si l^{-1} leaves; 'open square' = 50 mg Si l^{-1} stems; 'solid diamond' = 10 mg l^{-1} leaves 'open diamond' = 10 mg Si l^{-1} stems. The solid line represents a 1:1 relationship between source water and phytolith Ge/Si (i.e., no discrimination). Ge/Si values are a molar ratio

differ from those of Si (Epstein 2001). Competitive interactions between Ge and Si during uptake and internal silicification in diatoms (Azam and Volcani 1981), points to the potential similarity in Ge behavior in these and other metabolic pathways in Si accumulating plants, such as grasses. Another consideration relates to minor differences in dissociation chemistry, as aqueous Si is largely present as monosilicic acid (pK1 = 9.7 and pK2 = 11.9), and Ge as monogermanic acid (pK1 = 8.5 to 8.8 and pK2 = 12.7: Ingli 1963; Glockling 1969) under a wide range of environmentally common pH values. Ge is stable in both tetrahedral and octahedral coordination, and can form complexes with common organic ligands (Pokrovski and Schott 1998; Pokrovski et al. 2000), whereas Si exhibits a much weaker affinity for aqueous organic ligands. However, Kubicki and Heaney (2003) proposed a silicon transport and precipitation mechanism via hypercoordinate Si-organic complexes, which may be more prevalent in nature than previously thought (Pokrovski and Schott 1998; Poulson et al. 1997). Thus further research into Si and Ge physiology and chemical activity, more intensive measures of plant pools (e.g., stem water), and manipulation and measurement of environmental factors such as transpiration may elucidate the mechanisms behind Ge discrimination and improve the utility of Ge/Si ratios in studies of terrestrial Si cycling.

Conclusions

Biologic Ge discrimination was observed in studies of grasses grown in both nutrient solution and soil, with the former exhibiting a greater magnitude of Ge discrimination. Discrimination of Ge during uptake likely occurs at the root/solution interface as evidenced by both the lower root and stem Si concentrations and slightly higher root and stem Ge concentrations, compared to leaves of the same plant, an observation that requires further study to elucidate discrimination mechanisms. Among the graminoids studied, discrimination appears to be independent of species, photosynthetic pathway, and environmental factors of pH and EC. Temperature appears to have a limited affect on Ge discrimination, based on the greater differences in Si concentrations compared to Ge among the cool and warm treatments. Si concentration appears to have a slight though statistically significant impact on Ge discrimination. Regardless of the discrimination mechanism (e.g., physiological, kinetic), inductively and deductively observed biologic Ge discrimination increases the understanding of terrestrial Ge/Si relationships and the use of Ge/Si as a tracer in terrestrial weathering studies.

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