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Calcium isotope fractionation in alpine plants

R. S. Hindshaw · B. C. Reynolds · J. G. Wiederhold · M. Kiczka · R. Kretzschmar · B. Bourdon

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Abstract In order to develop Ca isotopes as a tracer for biogeochemical Ca cycling in terrestrial environments and for Ca utilisation in plants, stable calcium isotope ratios were measured in various species of alpine plants, including woody species, grasses and herbs. Analysis of plant parts (root, stem, leaf and flower samples) provided information on Ca isotope fractionation within plants and seasonal sampling of leaves revealed temporal variation in leaf Ca isotopic composition. There was significant Ca isotope fractionation between soil and root tissue $\Delta^{44/42}Ca_{root-soil} \approx -0.40\,‰$ in all investigated species, whereas Ca isotope fractionation between roots and leaves was species dependent. Samples of leaf tissue collected throughout the growing season also highlighted species differences: Ca isotope ratios increased with leaf age in woody species but remained constant in herbs and grasses. The Ca isotope fractionation between roots and soils can be explained by a preferential binding of light Ca isotopes to root adsorption sites. The observed differences in whole plant Ca isotopic compositions both within and between species may be attributed to several potential factors including root cation exchange capacity, the presence of a woody stem, the presence of Ca oxalate, and the levels of mycorrhizal infection. Thus, the impact of plants on the Ca biogeochemical cycle in soils, and ultimately the Ca isotope signature of the weathering flux from terrestrial environments, will depend on the species present and the stage of vegetation succession.

Keywords Calcium · Stable isotope fractionation · Glacier forefield · Alpine plants

R. S. Hindshaw (✉) · B. C. Reynolds · J. G. Wiederhold · M. Kiczka · B. Bourdon
Institute of Geochemistry and Petrology, ETH Zurich, Clausiusstrasse 25, 8092 Zurich, Switzerland
e-mail: ruth.hindshaw@ngu.no; hindshaw@erdw.ethz.ch

R. S. Hindshaw · J. G. Wiederhold · M. Kiczka · R. Kretzschmar
Institute of Biogeochemistry and Pollutant Dynamics, ETH Zurich, CHN, Universitätstrasse 16, 8092 Zurich, Switzerland

Introduction

The presence of vegetation has a major impact on the biogeochemical cycles of many elements, through uptake, re-cycling and the acceleration of weathering rates (Drever 1994; Bormann et al. 1998). As one of the essential plant macronutrients, calcium has been particularly intensively studied (Likens et al. 1998; McLaughlin and Wimmer 1999; White and Broadley 2003).

Calcium plays an essential role in plant growth and development. Calcium is used for signalling (in response to stimuli, changes in intracellular Ca concentrations induce physiological changes),

maintaining membrane integrity, stomatal regulation, enzyme activation and for the structural integrity of the plant, where it is a major component of cell walls (Marschner 1995; McLaughlin and Wimmer 1999).

Uptake of Ca occurs by mass flow of Ca, driven by transpiration, into the vicinity of cells walls, where it is adsorbed, typically by the carboxyl groups of cell wall pectins (Haynes 1980). Within the roots, a small fraction of Ca flow is symplastic (through cells) as it is required within cells for signalling, but the majority of flow is non-metabolic (Drew and Biddulph 1971) and apoplastic (between cells) (White and Broadley 2003). The vast majority of Ca transport in the plant is through the xylem and the accumulation of Ca is determined by the transpiration rate and the root CEC, both of which are species dependent (White and Broadley 2003). Plant leaves are a sink for Ca and unlike most other plant nutrients, Ca is not retranslocated later in the growing season. Calcium is the least mobile of the plant macronutrients in phloem tissue and plants lose Ca through leaf fall and leaching (Marschner 1995; McLaughlin and Wimmer 1999).

Calcium uptake by vegetation has previously been investigated using a combination of elemental ratios (typically Ca/Sr and Ca/Ba) and radiogenic Sr (Bailey et al. 1996; Poszwa et al. 2000; Bullen and Bailey 2005; Dasch et al. 2006; Blum et al. 2008; Drouet and Herbauts 2008; Pett-Ridge et al. 2009). The results from these studies show that, in addition to mineral weathering, atmospheric deposition is an important source of Ca for plants (Bullen and Bailey 2005; Blum et al. 2008; Pett-Ridge et al. 2009). In densely vegetated environments or in highly weathered soils, the majority of Ca released when a plant dies is taken up by other plants (biologically recycled) and only a small fraction is exported to streams (Bullen and Bailey 2005; Blum et al. 2008). Although radiogenic Sr can in theory act as an analogue for Ca (Capo et al. 1998), the sources of Sr and Ca are not exactly the same and the use of elemental ratios is complicated by varying discrimination factors within the plant (Poszwa et al. 2000; Drouet and Herbauts 2008).

Radioactive ^{45}Ca has a long history of use in plant nutrition studies (Drew and Biddulph 1971; Ferguson and Bollard 1976). However, it is only recently that stable Ca isotopes have been utilised in vegetation studies (e.g. Holmden and Bélanger 2010). Stable isotopes have the potential to refine our understanding of the biological cycling of elements and act as tracers for the impact of vegetation in biogeochemical cycles. Within the biogeochemical Ca cycle, uptake by vegetation is one of the few processes known to induce large stable Ca isotope fractionation in nature (Wiegand et al. 2005; Page et al. 2008; Cenki-Tok et al. 2009; Holmden and Bélanger 2010; Hindshaw et al. 2011). Calcium isotopes thus have the potential to trace Ca cycling within ecosystems, but first, the exact processes inducing Ca isotope fractionation between plant tissues and during uptake need to be investigated.

Previous studies of Ca isotope fractionation in vegetation have concentrated on tree species within forested ecosystems. These studies have clearly shown that within-plant Ca isotope fractionation occurs, with root samples exhibiting the lightest Ca isotopic compositions in the plant (lighter than source Ca) and leaves the heaviest (Wiegand et al. 2005; Page et al. 2008; Holmden and Bélanger 2010; Cenki-Tok et al. 2009). It is not clear at present what causes the large Ca isotope fractionation between soil and roots. Identifying the source of Ca is complicated in forested ecosystems due to the presence of chemically (and isotopically) distinct soil horizons and different rooting depths (Holmden and Bélanger 2010). As different plant species will have different demands for calcium (Marschner 1995) this will likely lead to species specific fractionation patterns. In order to investigate Ca isotope fractionation in a wider range of plant families, we analysed several species of small alpine plants. The small size of alpine plants allowed us to collect complete plant samples and obtain whole plant Ca isotopic compositions, in addition to those of individual tissues. In order to directly relate these results to previous work on Ca isotope fractionation in an alpine environment (Hindshaw et al. 2011), plant samples were taken from the same fieldsite (Damma glacier). This allowed us to also investigate the effect of soil heterogeneity and mycorrhiza on plant Ca isotopic compositions. The aims of the study were to investigate species differences, within plant fractionation and seasonal differences in plant Ca isotopic compositions.

Fieldsite and methods

All plant samples were collected from the Damma glacier forefield, which is the focus of a large

interdisciplinary study on initial weathering, soil formation and ecosystem development (BigLink Project, Bernasconi et al. 2011). The Damma glacier fieldsite is a small (10.7 km^2), glaciated, granitic alpine catchment which is snow covered for approximately six months of the year. Due to the retreat of the glacier, a ~1.5 km long soil chronosequence has formed in the glacier forefield which spans approximately 150 years of soil development. The soils in the forefield were classified as Hyperskeletic Leptosols following the World Reference Base for Soil Resources (WRB 2006). In the glacier forefield area, three distinct zones of vegetation development were observed. The youngest zone (ice-free since 1992) had very little vegetation cover apart from a few pioneer plant species, e.g. *Leucanthemopsis alpina*. In the middle zone (exposed between 1927 and 1950) the vegetation was typified by herbs and grasses, e.g. *Agrostis gigantea* and *Rumex scutatus*. The oldest zone (exposed before the early 1900s) was dominated by woody vegetation such as *Rhododendron ferrugineum*. A range of different species from different plant orders were analysed in this study (Table 1).

Three sets of samples were collected from the forefield in 2008. The first set of samples consisted of tissues (root, stem, leaf and flower samples) from individual specimens of *Rhododendron* and *Rumex* to investigate within-plant Ca isotope fractionation. Two different specimens of *Rhododendron* were analysed (RhA and RhB), in addition to the roots of a third (RhC). The complete root systems of the *Rhododendron* specimens were unable to be collected due their great areal extent. RhA and RhB were from soil which was 75 years old, and RhC was from soil which was 110 years old (at the time of sampling in 2008). Three types of soil material surrounding RhB were analysed in order to investigate whether the immediate soil environment was influenced by the plant. The three types of soil samples were: organic soil (uppermost layer below the litter horizon), bulk soil (homogenised soil sample from 0 to 5 cm below the organic soil layer) and rhizosphere soil (soil which had to be shaken off plant roots). The *Rumex* specimen grew on soil which was approximately 10 years old and here the complete root system was obtained. The root samples of *Rumex* and RhC were peeled to give a stele sample (innermost part of the root) and a cortex sample (outer part of the root) (see Fig. 1a). This sample set has previously been described and analysed for iron isotopes by Kiczka et al. (2010).

The second set of samples was used to investigate seasonal variations in plant Ca isotopic composition. These samples were collected from a site where the soil was approximately 75 years old and consisted of leaves from *Rhododendron* and *Oxyria*, and leaves and flowers from *Agrostis*. Leaf samples were collected at one month intervals over the growing season. Each sample was a composite sample of leaves from several individual plants. Due to the difficulty of separating *Agrostis* leaves from the stem, *Agrostis* 'leaf' samples contain both stem and leaf tissue.

The third set of samples served to investigate if the age of the soil affected the above-ground biomass Ca isotopic composition. The above-ground plant parts of *L. alpina, Salix h.* and *Salix r.* were collected from soils of different ages along the chronosequence. The infection of plant roots by mycorrhizal fungi may alter the plant Ca isotopic composition. In order to investigate this, the percentage of roots infected by mycorrhizal fungi was estimated microscopically

Table 1 List of plant species analysed in this study

Full species name	Abbreviated name used in text	Family	Type	Samples analysed		
				PT	L	CR
Rhododendron ferrugineum	*Rhododendron*	Ericaceae	Woody	×	×	
Salix helvetica	*Salix h.*	Salicaceae	Woody			×
Salix retusa	*Salix r.*	Salicaceae	Woody			×
Oxyria digyna	*Oxyria*	Polygonaceae	Herb		×	
Rumex scutatus	*Rumex*	Polygonaceae	Herb	×		
Leucanthemopsis alpina	*L. alpina*	Asteraceae	Herb			×
Agrostis sp.	*Agrostis*	Poaceae	Grass		×	

PT different plant tissues, *L* seasonal leaf samples, *CR* above-ground plant samples from along the chronosequence

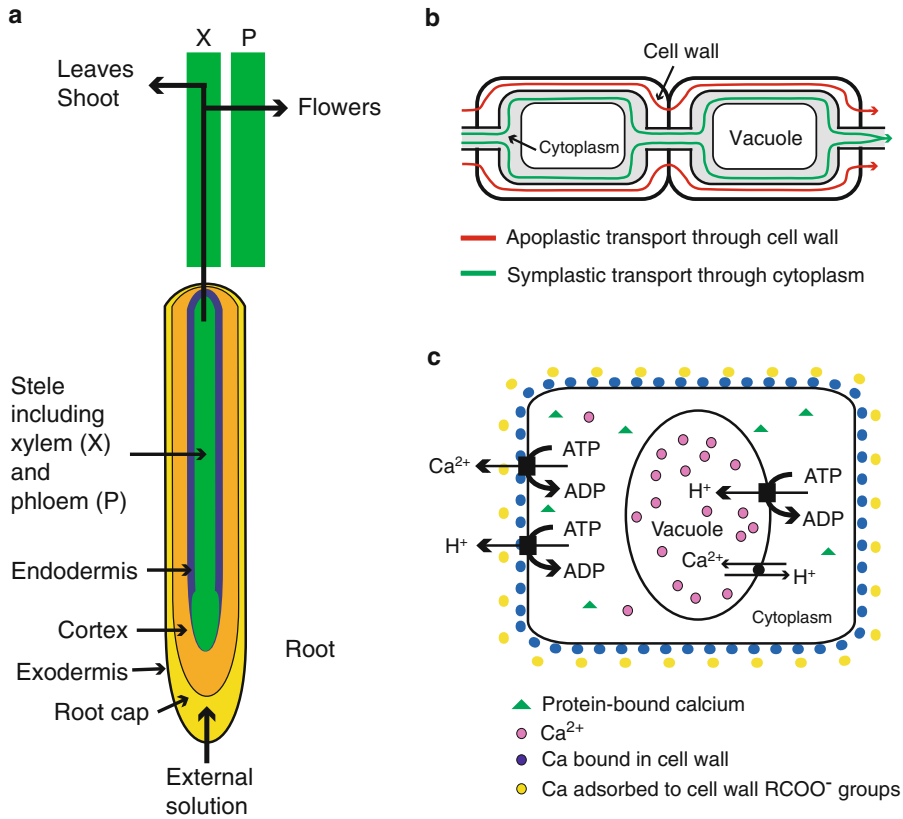

Fig. 1 **a** Plant uptake and movement through plant. Calcium enters the plant at the root tip and moves by apoplastic flow (see **b**) to the xylem which transports Ca to the rest of the plant. The majority of Ca uptake occurs at the root tips where the endodermis (which prevents apoplastic flow) is not yet developed. **b** Transport pathways. Apoplastic flow occurs within the cell walls and symplastic flow occurs within the cytoplasm. For Ca to enter phloem tissue it must use the symplastic pathway. Due to the toxicity of high cytoplasmic Ca concentrations the vast majority of Ca transport in the plant is through the apoplasm. **c** Distribution of Ca in a cell and the active transport processes which may induce Ca isotope fractionation. The majority of Ca is located inside the vacuole and in the cell wall. Additional Ca is adsorbed onto carboxyl groups located on cell walls. In order to maintain low cytoplasmic Ca concentrations Ca ATPase and Ca^{2+}/H^{+} antiporters move Ca out of the cytoplasm and into the vacuole or the apoplasm. Figures based on Marschner (1995) and Karley and White (2009)

using the gridline intersection method (Giovannetti and Mosse 1980). In addition, sporocap (fruiting body) samples from two different species of mycorrhiza were sampled.

All plant samples were washed with deionized water, dried at 35 °C and ground using either a tungsten carbide rotary disc mill or a zirconium oxide mixer mill depending on the sample size. The first and second set of samples were digested using distilled 15 M HNO_3 and H_2O_2 (30 %) in a microwave oven (Kiczka et al. 2010) and the third set of samples were ashed in silver crucibles at 550 °C and then dissolved in distilled 15 M HNO_3 and H_2O_2 (30 %).

Potential sources of Ca for plants have previously been described for this field site by Hindshaw et al. (2011). The Ca isotopic composition of porewaters, bulk soils and soil sequential extracts were measured. A five-step sequential extraction procedure was used which targeted the following pools: exchangeable, organically bound, phyllosilicates and two residual soil pools.

Elemental concentrations were analysed by inductively-coupled plasma optical emission spectrometry (ICP-OES) (Vista-MPX, Varian Inc., USA). Measured major cation concentrations of the standard NCS DC 73349 were within 20 % of certified values and

average precision for Ca^{2+} was 5 %, Sr^{2+} 2 % and Ba^{2+} 10 % (Kiczka et al. 2010, electronic annex). Aliquots of each sample, containing approximately 3 μg of Ca, were processed through a four-stage ion exchange column chemistry procedure in order to remove interfering elements prior to analysis by thermal ionisation mass spectrometry (Triton, Thermo Fischer Scientific) using a ^{43}Ca–^{46}Ca double-spike technique to correct for instrumental mass bias. Each sample was measured at least twice. The Ca separation and mass spectrometry procedures were identical to those described by Hindshaw et al. (2011). Calcium isotope ratios are reported in delta notation:

$$\delta^{44/42}\mathrm{Ca}(‰) = 1000\left\{\frac{\left(\frac{^{44}\mathrm{Ca}}{^{42}\mathrm{Ca}}\right)_{\mathrm{sample}}}{\left(\frac{^{44}\mathrm{Ca}}{^{42}\mathrm{Ca}}\right)_{\mathrm{SRM915a}}} - 1\right\} \quad (1)$$

The external $2\sigma_{SD}$ reproducibility ($n = 79$) of NIST SRM 915b was 0.07 ‰ and, as this was greater than internal errors, this was used as the error for all samples.

Whole plant Ca isotopic compositions were calculated using the following mass balance equation:

$$\delta^{44/42}\mathrm{Ca}_{\mathrm{plant}} = \frac{\sum_i m_i[\mathrm{Ca}]_i\delta^{44/42}\mathrm{Ca}_i}{\sum m_i[\mathrm{Ca}]_i} \quad (2)$$

where i is a plant part (root, stem, leaf, flower), m is the dry mass and [Ca] is the Ca concentration. The root mass of *Rhododendron* was estimated to be 25 % of the whole plant mass based on a study of *Rhododendron arboreum* (Rana et al. 1989). The Ca isotopic composition of the root cortex was not analysed but was calculated by mass balance using the following equation:

$$\delta^{44/42}\mathrm{Ca}_{\mathrm{cortex}} = \frac{\delta^{44/42}\mathrm{Ca}_r[\mathrm{Ca}]_r - \delta^{44/42}\mathrm{Ca}_s[\mathrm{Ca}]_s f_s}{[\mathrm{Ca}]_r - [\mathrm{Ca}]_s f_s} \quad (3)$$

where r is the complete root, s is the stele (peeled root) and f_s is the fraction of root mass contributed by the stele.

Results

Soil pools

In this fieldsite, soil Ca concentrations were typically around 10 g kg^{-1} and the average Ca isotopic composition was 0.44 ± 0.07 ‰ ($n = 49$) (Hindshaw et al. 2011). The range of soil Ca isotopic compositions was 0.28–0.57 ‰ and this is the range indicated in the figures. All of the soil sequential extraction steps yielded $\delta^{44/42}$Ca values which were within error of bulk soil values (Hindshaw et al. 2011) and the measured Ca isotopic compositions of porewater samples ($\delta^{44/42}\mathrm{Ca} = 0.31-0.42$ ‰) were also within range of soil values. The lightest Ca isotope value measured in the soils (0.28 ‰) was heavier than all of the whole plant Ca isotopic compositions measured (Table 3). The $\delta^{44/42}$Ca value of the rhizosphere soil was not significantly different from the bulk or organic soil samples of the same soil age (Table 2) and this value (0.40 ‰) is used as the soil $\delta^{44/42}$Ca value in the calculation of plant–soil Ca isotopic differences.

Whole plant analyses

The Ca concentrations of *Rumex* tissues increased from root (9.34 g kg^{-1}) to stem (10.8 g kg^{-1}) to leaf (31.4 g kg^{-1}). A similar increase in concentration was observed for the two *Rhododendron* specimens analysed, but the absolute concentrations were lower, for example the highest leaf Ca concentration was only 9.19 g kg^{-1} (Table 2). The Ca concentration of the leaves of both species was more than three times that found in the whole root. Flower tissue had higher Ca concentrations than stem tissue in *Rhododendron*, but in *Rumex*, flower tissue had lower Ca concentrations than both the stem and the root. Within the root, the cortex (outer root) had higher Ca concentrations than the stele (inner root) for both species. In *Rumex*, the cortex was seven times more concentrated in Ca than the stele, whereas in *Rhododendron* it was only twice as concentrated. The alkali earth element ratios Sr/Ca and Ba/Ca decreased along the transpiration stream (stele to leaf) in *Rumex* and the same general trend was observed in *Rhododendron* (stem to leaf) (Fig. 2a; Table 2).

In both species there was significant Ca isotope fractionation (> 0.36 ‰) between the soil pool ($\delta^{44/42}\mathrm{Ca} = 0.40$ ‰) and the roots (Fig. 3). Within the *Rhododendron* specimen RhB there was little isotope fractionation from root ($\delta^{44/42}\mathrm{Ca} = 0.04$ ‰) to stem (0.09 ‰) to leaf (0.00 ‰), the same was observed for RhA but the tissues were isotopically lighter than those of RhB (Fig. 3). The flowers of both

Table 2 Ca concentrations, $\delta^{44/42}$Ca and element ratios for different plant tissues and soils

Sample	Total weight (g)	[Ca] (g/kg)	Sr/Ca (mmol/mol)	Ba/Ca (mmol/mol)	$\delta^{44/42}$Ca $2\sigma_{SD} = 0.07$ ‰	$\Delta^{44/42}Ca^{a}_{plant-soil}$ $2\sigma_{SD} = 0.10$ ‰
Rhododendron A						
Root	9.53[b]	1.59	1.39	6.09	−0.09	−0.49
Stem	20.7	2.02	1.09	7.35	−0.16	−0.56
Leaf	5.51	9.29	0.49	2.20	−0.11	−0.51
Flower	2.39	3.63	0.76	4.61	−0.31	−0.71
Rhododendron B						
Bulk soil	–	10.6	7.23	17.4	0.38	
Organic soil	–	12.2	6.44	13.1	0.38	
Rhizosphere soil	–	8.03	7.52	28.2	0.40	
Root	2.79[b]	1.16	1.66	12.2	0.04	−0.36
Stem	5.36	2.52	1.41	13.2	0.09	−0.31
Leaf	2.59	7.14	0.76	4.43	0.00	−0.40
Flower	0.42	3.03	1.04	9.40	−0.20	−0.60
Rhododendron C						
Whole root	–	1.05	1.65	15.7	0.01	−0.39
Cortex[c]	–	*1.45*			*0.03*	−0.37
Stele	–	0.65	1.81	16.6	−0.02	−0.42
Rumex						
Whole root	2.53	9.34	2.16	1.34	−0.11	−0.51
Cortex	*0.80*	*22.4*	*2.05*	*0.48*	*−0.17*	−0.57
Stele	1.74	3.33	2.51	1.80	0.08	−0.32
Stem	1.99	10.8	1.76	1.69	−0.05	−0.45
Leaf	1.35	31.4	0.83	0.83	0.41	0.01
Flower	0.82	6.43	0.69	0.76	0.40	0.00

Values in italics are calculated from mass balance equations

[a] Relative to rhizosphere soil $\delta^{44/42}$Ca = 0.40 ‰

[b] Assumes roots contribute 25 % to total plant mass

[c] Assumes the cortex and stele contribute 50 % each to the root mass

Table 3 Whole plant $\delta^{44/42}$Ca compositions (‰) relative to soil ($\Delta^{44/42}Ca_{plant-soil}$) based on mass balance

Fraction	RhA	RhB	*Rumex*
Complete plant	−0.54	−0.37	−0.23
Ste + St + L + F	–	–	−0.14
St + L + F	−0.55	−0.37	−0.13
L + F	−0.54	−0.42	0.01

Ste stele, *St* stem, *L* leaf, *F* flower

specimens were 0.2 ‰ lighter than the leaves. For *Rumex*, the $\delta^{44/42}$Ca value of root and stem tissue was nearly identical to that of *Rhododendron* but the leaves and flowers had higher $\delta^{44/42}$Ca values ($\delta^{44/42}$Ca = 0.40 ‰), in the range of soil $\delta^{44/42}$Ca values (Fig. 3). The cortex of *Rumex* roots was 0.25 ‰ lighter than the stele. As the complete root system of a *Rhododendron* specimen could not be obtained, the fraction of root mass in the stele is not known. However, given the measured isotopic compositions and concentrations of the stele and the whole root, it was calculated using Eq. 3, that the mass fraction of stele could change from 10 to 90 % and the resulting isotopic composition of the cortex ($\delta^{44/42}$Ca = 0.01−0.05 ‰) would not differ, within error, from that of the stele ($\delta^{44/42}$Ca = −0.02 ‰).

Whole plant Ca isotopic compositions, calculated using Eq. 2, were 0.23–0.54 ‰ lighter than soil

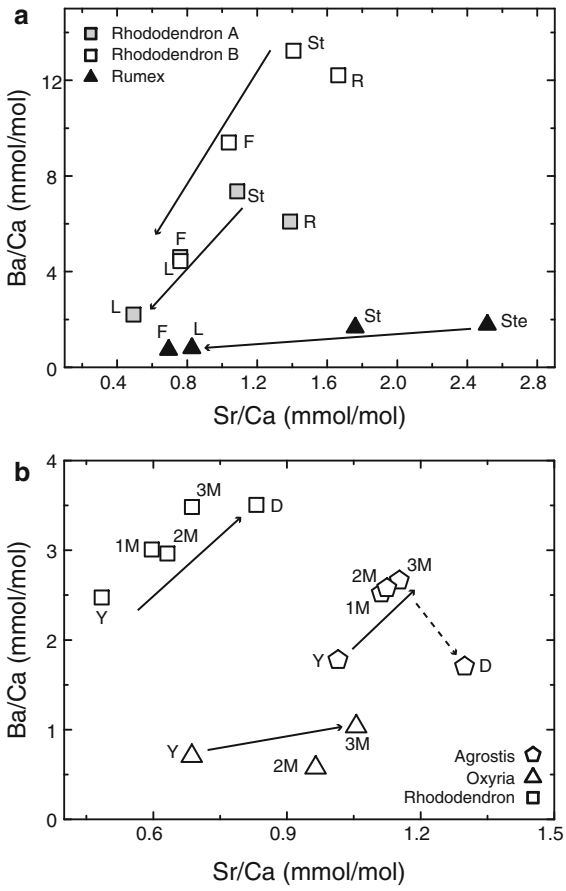

Fig. 2 Change in Ba/Ca and Sr/Ca ratios **a** along the transpiration stream and **b** over the growing season in the leaves. *Arrows* highlight the direction of change. In (**b**) the *dotted arrow* points to a straw (dead leaf) sample which may be influenced by leaching processes and decomposition on the ground. *R* root; *St* stem; *F* flower; *L* leaf; *Ste* stele; *Y* young leaves; *1M*, *2M* and *3M* 1, 2 and 3 month old leaves respectively; *D* dead leaves

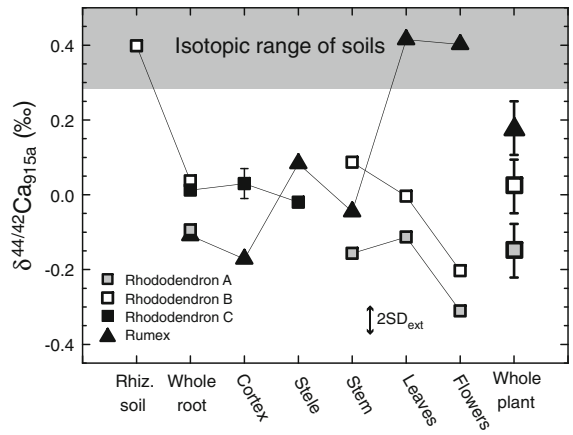

Fig. 3 Variation of $\delta^{44/42}$Ca between different plant tissues for two specimens of *Rhododendron* and one specimen of *Rumex*. *Rhiz. soil* stands for rhizosphere soil—the soil in direct contact with the plant roots. The Ca isotopic composition of the cortex is calculated using Eq. 3. The range in *Rhododendron* cortex $\delta^{44/42}$Ca is the range obtained when the mass fraction of the cortex is varied from 10 to 90 %. The error in whole plant isotopic compositions is 0.14 ‰. For *Rhododendron*, the whole plant isotopic compositions are calculated assuming the roots contribute 25 % to the total mass of the plant

(Fig. 3), unambiguously demonstrating that the uptake of Ca from soils by plants is accompanied by an enrichment of light Ca isotopes. In addition to calculating the whole plant Ca isotopic composition, Eq. 2 was used to calculate the isotopic composition of the whole plant at each step of the transpiration stream. The results (Table 3) confirm the negligible within-plant fractionation in the *Rhododendron* specimens, but highlight the increase in Ca isotope ratios along the transpiration stream in *Rumex*.

Seasonal variation

The Ca concentration of leaves increased with age, with 3 month old leaves up to six times as concentrated in Ca as young leaves (Fig. 4; Table 4). The increase in leaf Ca concentration with age has previously been observed in deciduous trees (Guha and Mitchell 1966) and is due to the lack of retranslocation of Ca, resulting in steady accumulation with time, together with the retranslocation of other nutrients and water loss from older leaves. The observed increase in concentration was most pronounced in *Rhododendron* leaves and least in *Agrostis* leaves (Fig. 4). There was an increase in the Ca concentration of *Agrostis* flowers with age (1.3–2.3 g kg^{-1}).

Overall the Sr/Ca and the Ba/Ca ratios in the leaves (and flowers) increased with age for all species analysed (Fig. 2b). The only exception to this general trend was the Ba/Ca ratio of the dead *Agrostis* leaves which was lower than the 3 month old leaf sample and may have been influenced by leaching and decomposition processes on the ground.

Leaf samples from *Rhododendron* had a constant Ca isotopic composition for the first two months ($\delta^{44/42}$Ca $= -0.20$ ‰), then became progressively enriched in the heavier isotopes as the leaves aged (Fig. 4; Table 4). In contrast, there was no clear change in the leaf Ca isotopic compositions of *Agrostis* and *Oxyria* as the leaves aged. Both

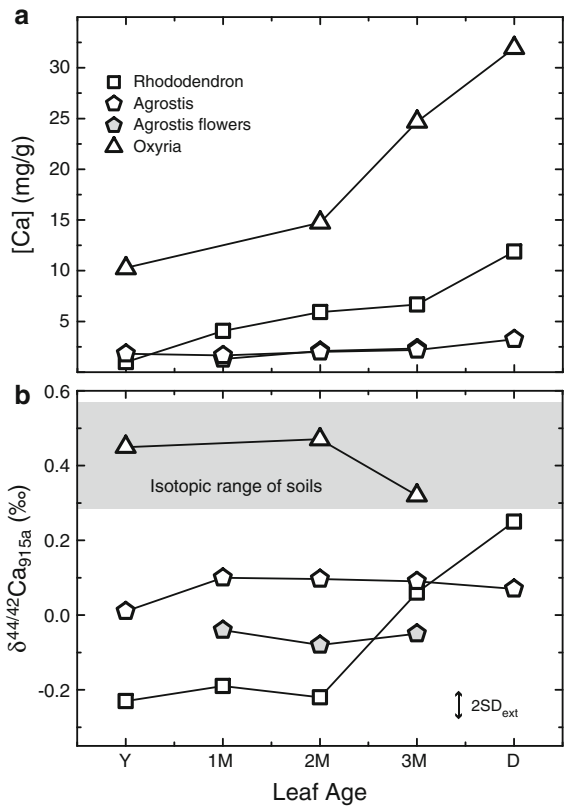

Fig. 4 Variation of calcium concentration (**a**) and $\delta^{44/42}$Ca (**b**) in leaves and flowers (*Agrostis* only) versus tissue age. The range of $\delta^{44/42}$Ca values in the soils is indicated by the *grey area*. For leaf age, *Y* denotes young leaves sampled in June; *1M*, *2M* and *3M* denote leaves sampled 1 2 and 3 months after young leaves were sampled; and *D* denotes dead leaves which for *Rhododendron* were still attached to the plant and for *Agrostis* were collected as straw

Rhododendron and *Agrostis* leaves were always isotopically lighter than average soil (0.44 ± 0.07 ‰) whereas *Oxyria* leaves had an indistinguishable Ca isotopic composition to soil. No increase in Ca isotopic composition with age was observed in the flowers of *Agrostis* and there was a constant Ca isotopic difference between the leaves and flowers of ~ 0.15 ‰.

Variation along the chronosequence

Three species were sampled at various points along the chronosequence: *L. alpina, Salix h.* and *Salix r.* (Table 5). Calcium concentrations of *L. alpina* tended to decrease with soil age, whereas those of the two *Salix* species tended to increase with soil age (Fig. 5a). For all species there was no correlation between the Ca isotopic composition of the above-ground plant parts and soil age (Fig. 5b). The degree of mycorrhizal infection may be expected to influence the Ca isotopic composition of the plant. There was a general increase of root colonization with soil age across both species (Table 5), but within each species there was a wide range of Ca isotopic compositions which were not correlated with the percentage of roots infected with mycorrhizal fungi. However, one sample which was not infected had a significantly higher $\delta^{44/42}$Ca value than all of the infected samples (Fig. 6). Across all species with above-ground biomass data, Ca concentration was correlated with Ca isotopic composition ($r^2 = 0.52$, $p < 0.01$ across all species, Fig. 7) with a stronger enrichment in light Ca isotopes relative to soil at lower Ca concentrations. Sr/Ca and Ba/Ca ratios were neither correlated with soil age, Ca isotopic composition nor Ca concentrations in the above-ground biomass of these plants.

Discussion

It is clear from the variation in above-ground Ca isotopic compositions that Ca isotope fractionation is species dependent and that there is also significant intra-species variability in above-ground Ca isotopic compositions (Fig. 7). We will first discuss Ca uptake processes and then discuss possible causes of within-plant Ca isotope fractionation.

Calcium uptake processes

Significant Ca isotope fractionation occurs between the soil and the roots of *Rhododendron* (Fig. 3), as was also observed for several tree species (Wiegand et al. 2005; Page et al. 2008; Cenki-Tok et al. 2009; Holmden and Bélanger 2010). Fractionation is either occurring at the soil–plant interface, or the plant accesses a source of isotopically light Ca.

A specific soil pool, for example exchangeable Ca, could be a potential source of light Ca. However, all the root samples were more than 0.24 ‰ lighter in $\delta^{44/42}$Ca than the lightest $\delta^{44/42}$Ca value reported from the porewater and soil sequential extraction measurements (Hindshaw et al. 2011) and therefore additional fractionation must occur between these pools and

Table 4 Ca concentrations, $\delta^{44/42}$Ca and element ratios for samples collected at various times throughout the season

Sample	[Ca] (g/kg)	Sr/Ca (mmol/mol)	Ba/Ca (mmol/mol)	$\delta^{44/42}$Ca $2\sigma_{SD} = 0.07‰$
Rhododendron ferrugineum leaves				
Young	0.99	0.48	2.47	−0.23
1 month	4.07	0.60	3.01	−0.19
2 months	5.93	0.69	3.48	−0.22
3 months	6.67	0.63	2.96	0.06
Dead	11.9	0.83	3.51	0.25
Oxyria digyna leaves				
Young	10.2	0.96	0.58	0.45
2 months	14.7	0.69	0.71	0.47
3 months	24.7	1.06	1.04	0.32
Agrostis leaves				
Young	1.81	1.01	1.78	0.01
1 month	1.65	1.15	2.67	0.10
2 months	1.99	1.11	2.52	0.10
3 months	2.19	1.12	2.58	0.09
Dead	3.24	1.30	1.71	0.07
Agrostis flowers				
1 month	1.30	0.90	2.29	−0.04
2 months	2.09	1.25	2.85	−0.08
3 months	2.32	1.16	3.03	−0.05

Table 5 Ca concentrations, $\delta^{44/42}$Ca and element ratios for plants (above-ground biomass only) along the chronosequence

Sample	Soil age (years)	[Ca] (g/kg)	Sr/Ca (mmol/mol)	Ba/Ca (mmol/mol)	$\delta^{44/42}$Ca $2\sigma_{SD} = 0.07‰$	% of mycorrhizal root colonization
L. alpina	7	11.8	1.42	1.27	0.34	0
	8	12.1	1.80	1.18	0.19	34
	14	9.82	1.45	0.97	0.21	38
	59	7.58	2.45	3.03	0.13	55
	65	7.44	2.63	4.16	0.24	66
	68	6.46	1.57	1.58	0.23	47
	73	8.32	1.65	2.46	0.15	72
	76	6.77	1.55	2.25	0.16	30
Salix h.	7	2.89	0.99	0.42	0.11	28
	67	5.29	2.08	4.12	0.05	22
	73	7.31	1.72	1.38	0.14	47
	78	3.52	1.69	3.13	0.06	23
	79	4.87	1.94	1.73	-0.02	35
Salix r.	59	1.43	3.19	6.28	-0.04	39
	129	4.91	1.71	0.34	0.10	70
Ectomycorrhizal sporocaps						
Laccaria montana		0.61	2.59	7.95	0.21	
Russula exalbicans		0.32	3.44	9.07	0.23	

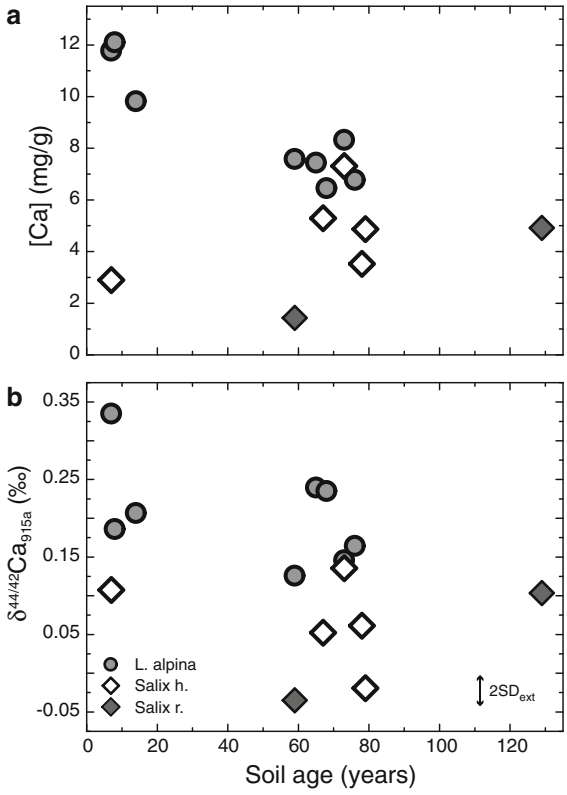

Fig. 5 Ca concentration (**a**) and Ca isotopic composition (**b**) of the above-ground biomass of three plant species versus soil age. Across all three species soil age had no effect on the Ca concentration or the Ca isotopic composition of the plant

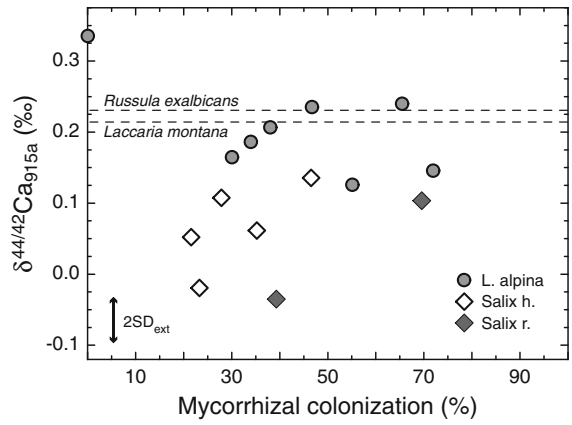

Fig. 6 $\delta^{44/42}$Ca in above-ground biomass plotted against the percentage of roots infected with mycorrhizal fungi for three different plant species. The measured Ca isotopic composition for the sporocaps of two species of ectomycorrhizal fungi collected from the forefield are indicated by the *dotted lines*. There is no clear relationship between the percentage of roots infected and above-ground $\delta^{44/42}$Ca values, but a single specimen of *L. alpina* which has no mycorrhiza is notably heavier than the rest of the specimens

plant roots. Mycorrhiza act as an additional interface between the soil and the plant, thus the extent of Ca fractionation in a plant might be expected to depend on the percentage of root area infected with mycorrhiza. However, no clear relationship between the extent of root infection and above-ground Ca isotopic composition was found (Fig. 6), suggesting that mycorrhiza are not the main control of plant Ca isotopic compositions.

We consider it likely that the isotopic difference between plant roots and soil arises due to a fractionation process occurring during the uptake of Ca into plant roots. Ca uptake is by mass flow, i.e. passive uptake driven by transpiration (Marschner 1995) and this process is not expected to cause Ca isotope fractionation. Within the root, Ca transport is predominantly apoplastic (between cells, Fig. 1b), partly due to the toxicity of high levels of cytoplasmic Ca (e.g. McLaughlin and Wimmer 1999). The presence of the endodermis, a hydrophobic layer of cells in fully developed root cells, prevents apoplastic Ca transport from the cortex (outer root) to the stele (inner root) (Fig. 1a). Transport across the endodermis is symplastic (within cell) requiring transport proteins (White and Broadley 2003). This would be expected to induce measurable isotope fractionation between the cortex and the stele as was recently observed for iron (Kiczka et al. 2010). The fact that the cortex and the stele of *Rhododendron* had the same isotopic composition strongly supports the view that Ca uptake is primarily apoplastic (between cells) and the majority of uptake must therefore occur at the root apices where the endodermis has not yet developed (e.g. Karley and White 2009). Calcium isotope fractionation must therefore occur in the cortex. Studies utilising isotopically labelled solutions have shown biphasic uptake behaviour for divalent cations (Epstein and Leggett 1954; Drew and Biddulph 1971) with an adsorbed and an absorbed component. As defined by Epstein and Leggett (1954), adsorption is a passive process which results in a readily exchangeable component and absorption is an active process resulting in an essentially non-exchangeable component. Both adsorption and absorption processes could fractionation Ca isotopes, resulting in the difference in isotopic composition observed between roots and soil.

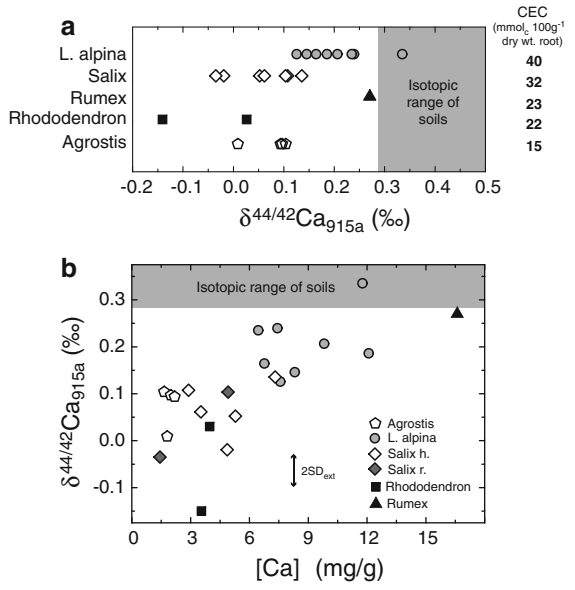

Fig. 7 **a** Summary of above-ground Ca isotopic compositions of species analysed. The root CEC for the different plant species at the order level (White and Broadley 2003) is shown for comparison. Within the dicotyledonous species (all except *Agrostis*) the whole plant Ca isotopic composition appears to increase with increasing root CEC. *Rumex* does not follow the trend of increasing $\delta^{44/42}$Ca values with increasing root CEC observed in the other dicotyledonous species. This may be due to the precipitation of Ca oxalate in the root cortex. **b** Across all species, there is a correlation ($r^2 = 0.52$, $p < 0.01$) between the concentration of Ca (dry weight) and the above-ground Ca isotopic composition. This suggests that root level Ca uptake processes, i.e. the amount of Ca adsorption, determined by root CEC, could control the overall plant Ca isotopic composition

The absorbed phase is essentially irreversibly bound Ca which has undergone active transport into cells. Due to low cytoplasmic Ca concentrations, an electrochemical gradient exists from the apoplasm (mmol L^{-1} Ca) into the cell (nmol L^{-1} Ca). Calcium in the cytoplasm is actively removed by pumping it into the vacuole or back out of the cell across the cell membrane. This is achieved by calcium ATPase and Ca^{2+}/H^{+} antiporters (Evans et al. 1991; Fig. 1c). We hypothesise that these active processes will favour the transport of light Ca as a result of incomplete kinetic reactions, resulting in an accumulation of light Ca inside the cell vacuole.

The adsorbed phase is readily exchangeable with the soil solution and it has been shown that the adsorption of magnesium by root cells induces isotope fractionation with the adsorbed phase enriched in heavy Mg isotopes (Bolou-Bi et al. 2010). Therefore, it is reasonable to assume that Ca isotopes will also fractionate during adsorption. Adsorption is likely to be an equilibrium fractionation process whereby the stronger bonding environment is enriched in the heavier isotopes. The most common adsorption sites for Ca, and other metals, in the apoplasm are carboxyl groups located in the cell walls (Marschner 1995). Recent root desorption experiments indicate that the adsorbed phase is enriched in light Ca isotopes (Cobert et al. 2011) and this is supported by estimates of bond lengths, which are a proxy for the strength of the bond. During equilibrium fractionation, the heavy isotopes are enriched in the stiffer (shorter) bonds (Bigeleisen 1965). Spectroscopic methods and density functional theory calculations indicate that the Ca–O bond length of hydrated Ca (2.4 Å) (Carugo et al. 1993; Kaufman Katz et al. 1996; Pavlov et al. 1998; Jalilehvand et al. 2001) is shorter than that of the Ca–O bond in a bidentate Ca carboxylate crystal structure (2.5 Å) (Einspahr and Bugg 1981; Carugo et al. 1993; Wong et al. 2006). Based on these results, the adsorbed phase with the weaker, longer bond should be enriched in light Ca. The extent of this fractionation would be dependent on the number of available exchange sites, the functional groups present and the extent of equilibration with the soil solution. Thus, both adsorption and absorption are capable of causing isotope fractionation in the same direction and could be responsible for the light Ca isotopic composition of root tissue compared to soil.

Within-plant fractionation processes

The processes involved in fractionating Ca isotopes once Ca is within the plant are not well understood and we have no definitive explanations for the fractionation patterns detected. In the interest of guiding future research, we propose some potential processes in the next section which could be responsible for the within-plant fractionation patterns we observed.

Root to leaf and reproductive organs

In the two *Rhododendron* specimens analysed, excluding the flowers, negligible within-plant Ca isotope fractionation along the transpiration stream was observed (Table 3), whereas internal fractionation was observed in *Rumex*.

Rumex and *Oxyria* are closely related species and both had heavier Ca isotopic compositions ($\delta^{44/42}$Ca = ~0.40 ‰) in their leaves and flowers compared to root tissue. With respect to the whole plant Ca isotopic composition, the roots were 0.28 ‰ lighter and the leaves 0.24 ‰ heavier. These two plants are known to contain high levels of oxalate in order to deter grazers (Landolt and Urbanska 2003) and Ca oxalate crystals can occur in all parts of the plant (Doaigey 1991). Calcium oxalate crystals were observed under a microscope in root samples from *Rumex*. Ca oxalate contains a Ca–carboxylate bond and is predicted to be enriched in light Ca isotopes, based on bond-length arguments outlined above (see "Calcium uptake processes" section). The concentration of Ca in the cortex was extremely high (22 g/kg, cf. 1 g/kg for *Rhododendron*) and this, together with the 0.25 ‰ increase in $\delta^{44/42}$Ca between the cortex and the stele, strongly suggests that calcium oxalate is precipitating within the cortex. We suggest that the $\delta^{44/42}$Ca of xylem fluid is further increased during passage through the stem due to further oxalate precipitation, resulting in the observed heavy isotopic composition of the leaves and flowers.

Previous studies (Wiegand et al. 2005; Page et al. 2008; Holmden and Bélanger 2010), observed an increase in $\delta^{44/42}$Ca from root to leaf, consistent with laboratory ion exchange experiments by Russell and Papanastassiou (1978) which showed that light Ca isotopes were preferentially retained. The decrease of Ba/Ca and Sr/Ca ratios from stele to stem to leaf in *Rhododendron* (Fig. 2a) is consistent with the occurrence of ion exchange during transport through the xylem (Ferguson and Bollard 1976; Van de Geijn and Petit 1979) and has been observed in plants from a wide range of ecosystems (Bailey et al. 1996; Poszwa et al. 2000; Drouet and Herbauts 2008; Pett-Ridge et al. 2009). However, we observed no clear increase in $\delta^{44/42}$Ca values from root to leaf in *Rhododendron*. Russell and Papanastassiou (1978) predicted that longer ion exchange columns would induce more Ca isotope fractionation than shorter columns, as this amplifies the difference in transit time between light and heavy isotopes. By analogy, since the stem of *Rhododendron* is much shorter than in trees, we suggest that the length of the xylem 'ion-exchange column' was not long enough to produce significant Ca isotope fractionation. This is analogous to the explanation proposed by Viers et al. (2007) for the different Zn isotopic compositions of herbs and trees.

The flowers of *Rhododendron* were 0.20 ‰ lighter in $\delta^{44/42}$Ca in both specimens analysed compared to the bulk plant isotopic composition. The flowers of *Agrostis* were also lighter by 0.15 ‰ than the leaf+stem $\delta^{44/42}$Ca value. The flowers contain the reproductive organs of the plant and these tissues acquire Ca from the phloem (Marschner 1995). Ca reaches the phloem by symplastic transport (Marschner 1995; Fig. 1a) and is thus expected to be isotopically light due to kinetically controlled isotope fractionation during transport of Ca across cell membranes.

Seasonal variation in leaf Ca isotopic composition

Significant increases over the growing season were observed in the Ca isotopic compositions and Ca concentrations of *Rhododendron* leaves, but no increase in $\delta^{44/42}$Ca was observed in *Oxyria* or *Agrostis* leaves.

Unlike most other plant nutrients, the retranslocation of Ca from leaves is negligible (Marschner 1995), thus changes in leaf $\delta^{44/42}$Ca with age must either arise from a change in the Ca isotopic composition of the xylem fluid, which is the source of Ca to the leaf, or as a result of a change in whole leaf Ca isotopic composition during the loss of Ca through leaching by rainwater.

As leaves age, they take up less Ca from the xylem (Clarkson 1984) and the leaching rate increases due to an increase in the permeability of cell membranes (Marschner 1995). A reduction in the uptake of Ca could explain the increase in Ba/Ca and Sr/Ca ratios observed in all three species (Fig. 2b). The Ca inside cells is expected to be isotopically light due to absorption. If this isotopically light Ca was leached it could explain the increase in the Ca isotopic composition of *Rhododendron* leaves. The lack of change in leaf $\delta^{44/42}$Ca of *Oxyria* and *Agrostis* could be due to differences in leaf morphology and physiology.

Alternatively, a change in the Ca isotopic composition of the xylem fluid could change *Rhododendron* leaf $\delta^{44/42}$Ca over the season. Ferguson and Bollard (1976) demonstrated for trees, using a ^{45}Ca tracer, that

in autumn, compared to spring, xylem fluid transport was retarded and that this was due to lignin deposition increasing the CEC of the xylem. An increased CEC of the stem would be expected to result in decreasing Ba/Ca and Sr/Ca ratios with leaf age, as the heavier cations are more strongly retained on the exchange sites. Such a seasonal decrease was observed by Guha and Mitchell (1966) in sycamore leaves (*Acer pseudoplatanus*). In autumn, secondary xylem growth results in smaller cells compared to growth in spring with a corresponding increase in cell wall growth, for which Ca is a major component (McLaughlin and Wimmer 1999). The relative increased demand for Ca from the stem tissue in autumn (involving active uptake) could lead to an increased $\delta^{44/42}$Ca value of the xylem fluid and the increase in leaf Ba/Ca and Sr/Ca ratios observed (Fig. 2b). If correct, this seasonal change in leaf $\delta^{44/42}$Ca values would only be observed in plants with woody stems.

Intercomparison of alpine plant species

Root CEC is thought to be important in controlling Ca uptake (Haynes 1980) and because above-ground $\delta^{44/42}$Ca values and Ca concentrations were correlated (Fig. 7b), root CEC may also control above-ground Ca isotopic compositions. Within the dicotyledonous species there is a trend of increasing above-ground Ca isotopic composition and Ca concentration with increased root CEC (Fig. 7). The greater the number of adsorption sites (higher CEC) the closer the Ca isotopic composition of the plant is to soil (source Ca). *Rumex* does not lie on this trend, which may be due to the additional effect of oxalate precipitation. The heterogeneity in soil $\delta^{44/42}$Ca values and variations in hydrology could contribute to the observed variability in above-ground Ca isotopic compositions within a single species. *Agrostis* was the only monocotyledonous species analysed and its relatively high above-ground Ca isotope ratio could be due to differences in both the composition of the root cell walls (Hose et al. 2001) and demand for Ca between monocotyledonous and dicotyledonous species. Thus, whole plant $\delta^{44/42}$Ca values could be largely determined by the root CEC which is species specific, but this hypothesis requires further experimental verification.

Comparison with Sr

Strontium and calcium are expected to behave similarly during plant uptake and translocation, due to their similar chemical properties. It is also expected that the stable isotopes of these two elements should have similar within-plant fractionation patterns. However, a previous study of stable Sr isotope fractionation within *Rhododendron* plants at the Damma glacier fieldsite (de Souza et al. 2010) concluded that Sr fractionated in the opposite sense to Ca during within-plant fractionation based on a comparison with previously published Ca isotope data on trees (Wiegand et al. 2005; Page et al. 2008). This study measured *Rhododendron* from the same fieldsite (different specimens) allowing the stable isotope fractionation of Sr and Ca to be directly compared in the same species. Our results indicate that the discrepancy is probably due to comparison between different species, and directly comparing stable Ca and Sr isotope ratios relative to soil from the same species (*Rhododendron*) shows that the direction of stable isotope fractionation between soil and different tissues is the same (Table 6). In *Rhododendron*, both within leaves of different age and between different tissues, there is a strong correlation of Ca and Sr concentrations ($r^2 = 0.97$ and 0.80 respectively), suggesting that these two elements are behaving similarly within the plant. The similarity between Sr and Ca stable isotope fractionation in plants indicates that either the active transport mechanisms cannot distinguish between Ca and Sr

Table 6 Comparison of stable Ca and Sr isotope ratios relative to soil in *Rhododendron* (different specimens)

‘	Sr (‰)		Ca (‰)	
	$\Delta^{88/86}$Sr[a]	95 % CL[b]	$\Delta^{44/42}$Ca	$2\sigma_{SD}$
Root–soil	−0.29	0.14	−0.34	0.10
	−0.18	0.10	−0.47	0.10
			−0.37	0.10
Flower–soil	−0.54	0.14	−0.58	0.10
			−0.69	0.10
Stem–soil	−0.24	0.14	−0.29	0.10
			−0.54	0.10

[a] All Sr data from de Souza et al. (2010)

[b] CL stands for confidence limit

and/or that adsorption fractionates both cations similarly as a result of both cations competing for identical adsorption sites (Epstein and Leggett 1954).

Effect of plants in the biogeochemical Ca cycle

Plants strongly fractionate Ca isotopes compared to soil pools, and would thus be expected to impact soil pool Ca isotope values. No influence of plants on soil, water or porewater $\delta^{44/42}$Ca was detected in this catchment, due to the very low vegetation density (Hindshaw et al. 2011). However, the effect of plants on Ca cycling is likely to change as vegetation succession proceeds. Calcium from litterfall can be mobilised and re-used by plants, but its composition is species dependent: old *Rhododendron* leaves had a Ca isotopic composition identical to soil whereas those of *Agrostis* had a lighter isotopic composition. In addition, as vegetation succession proceeds an increasing proportion of Ca is incorporated into biomass, especially into woody tissues. In the late stages of vegetation succession the size of the Ca pool in plants can exceed that of the exchangeable pool (Johnson 1992). Thus, it is conceivable that during succession, the Ca isotopic composition of plant–available soil pools will change as a result of vegetation development and this could potentially affect porewater and thus stream water isotopic compositions. However, it is unclear how important vegetation succession versus mineral weathering is in controlling the dissolved fluxes of streams (Gorham et al. 1979; Taylor and Velbel 1991). Ultimately the Ca isotopic composition of stream water will depend on the relative size of the respective fluxes. For example, Holmden and Bélanger (2010) found that 80 % of the Ca in stream water in a forested watershed in Saskatchewan was biologically derived despite 90 % of Ca being internally recycled. Calcium isotope variability in the Strengbach catchment (France) has been attributed to seasonal vegetation control (Schmitt et al. 2003; Cenki-Tok et al. 2009). On the other hand, Tipper et al. (2006, 2008) concluded that vegetation did not affect the Ca isotopic compositions of Himalayan rivers. These results are not contradictory, Saskatchewan is located on the Precambrian Shield and the mineral weathering rates of shield terrains are generally low. The Himalaya however, are typified by high chemical (and physical) weathering rates. Thus, where mineral weathering rates are low, fractionated reservoirs of Ca due to biological activity may be observed. In such locations, large-scale ecosystem disturbances such as forest fires or clear cutting, which are known to cause high transient Ca fluxes as Ca is released from decaying humic matter (Likens et al. 1970; Balogh-Brunstad et al. 2008), could cause significant changes in soil pool and runoff $\delta^{44/42}$Ca values.

Conclusions

Several species of plants collected from a recently glaciated, granitic catchment were shown to have a bulk Ca isotopic composition which was isotopically lighter than soil, but the magnitude of $\Delta^{44/42}Ca_{plant-soil}$ was strongly species dependent and was not dependent on soil age. The Ca isotope fractionation patterns observed in *Rhododendron* can be explained by two processes: equilibrium fractionation during adsorption of Ca onto carboxyl groups located on cell walls, resulting in an isotopically light adsorbed phase and kinetic fractionation during active uptake by cells resulting in the accumulation of light Ca inside cells. Comparing all species, the higher the Ca concentration of the plant, the smaller the Ca isotope fractionation relative to soil, with high Ca concentrations linked to high root CEC. This observation suggests that the amount of adsorption of Ca by roots may be an important factor in determining the Ca isotopic composition of the plant.

Calcium is an essential plant nutrient and stable Ca isotopes have the potential to refine our understanding of plant uptake and utilisation processes. This study only analysed bulk plant tissues. The next step would be to analyse plant tissues in finer detail e.g. tree rings and xylem fluid and exploit the combination of stable isotopes and radioactive tracers. In addition, Ca isotope fractionation during adsorption remains to be experimentally verified and the form of binding during adsorption investigated. The results from this study indicate that stable Ca isotopes are a promising tool for understanding the biogeochemical Ca cycle in terrestrial environments and elucidating species differences in Ca utilisation by plants.

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