Article

Calcium and potassium dynamics and biopurification in two populations of the subalpine evergreen shrub *Rhododendron ferrugineum*

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Abstract

Calcium (Ca) and potassium (K) are important acidity neutralizers in soils and essential elements for plants. These two elements are known to undergo a biopurification within the plant (i.e., discrimination against strontium (Sr) and barium (Ba) for Ca, and rubidium (Rb) for K). However variations in the magnitude of this process between plant populations have rarely been reported, especially in high altitude, nutrient-depleted habitats. Concentrations of Ca, Sr, Ba, K and Rb were measured in roots, stems and in the different leaf cohorts in two populations of the evergreen shrub *R. ferrugineum* located at a granitic high elevation site. Calcium and K concentrations in leaves were respectively ~5 and 3 times higher than in roots and stems. Ca concentration increased while K concentration decreased with leaf age. The ratios Ca/Sr, Ca/Ba and K/Rb increased from roots to leaves, revealing a significant biopurification especially between stems and leaves. This phenomenon was higher for Ca than for K, with Ca/Sr and Ca/Ba ratios more than twice and 4 times higher in leaves than in roots, respectively, while K/Rb ratio in leaves was only 50% higher than in roots. Ca/Sr ratio decreased whereas K/Rb increased with leaf age. While the first could result from a "chromatographic effect" of the vascular column, the latter suggests the existence of biopurification mechanisms during influx/efflux of K from the leaf. Surprisingly, the magnitude of Ca biopurification varied between populations on a small geographical scale suggesting that Ca/Sr ratio should be used cautiously for plant Ca source identification.

Keywords calcium, strontium; barium; potassium; rubidium; biopurification; ericaceous heathlands; *Rhododendron ferrugineum*

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1 Introduction

Base cations (Ca, Mg and K) play important roles in ecosystems (Prajapati, 2012), particularly by controlling the acid-base status of soils and the acid neutralizing capacity of surface water (Houle et al., 2006). Among base cations, K and Ca play an important role in ecosystems because they constitute the main part of the base cation reservoir and are required in large amounts for plant nutrition. In granitic sites, plagioclase feldspars weathering is thought to be the main source of Ca released in soil and stream water (Oliva et al., 2004) although the dissolution of carbonates, even at trace levels in the parental granitic rock, can constitute a large part of Ca exports due to its high solubility (Drever and Hurcomb, 1986; Jacobson et al., 2002; White et al., 1999). Most K in soils is found in primary minerals with low weathering rate (e.g., feldspars and micas), which releases K in the soil solution at a very slow rate, although biotite, a micaceous mineral, can release K in a relatively high rate. Only dissolved K in soil water is available to plants which represents a very small fraction (0.1-2%) of total soil K (Marschner, 1995).

In plants, K and Ca are major elements that can constitute up to 10% and 5% of shoot dry ash, respectively (Marschner, 1995; Véry and Sentenac, 2003). However, whereas Ca cytosolic concentration is maintained at submicromolar levels (White and Broadley, 2003), optimal cytosolic concentration of K^+ is in the range of 100 mM (Ashley et al. 2006) which makes K^+ the most abundant cation in the cytosol (Véry and Sentenac, 2003). Ca and K are taken up by roots from the soil solution as Ca^{2+} and K^+ ions and delivered to shoots via the xylem. The delivery of Ca to the xylem occurs via the apoplastic pathway in root tips and/or via a symplastic way when a Casparian band between endodermal cells is present (White and Broadley, 2003). Apoplastic pathway is not selective and is driven by transpiration rate or root pressure when transpiration is low, whereas symplastic pathway controls the rate and the selectivity of transport to the shoots (White, 2001). Ca is also adsorbed to fixed negative charges in the xylem walls and absorbed by adjacent cells. In this manner, the xylem cylinder operates as an exchange column so that Ca translocation cannot be explained simply in terms of mass flow, but rather by a process of exchange with negatively charged molecular groups. Indeed, in growing plant, Ca is translocated to the shoot apex, which is a low transpiring organ, presumably due to the formation of new cation exchange sites (Limami and Lamaze, 1991).

Several studies have shown that Sr dynamic is similar to that of Ca in the plant-soil system and hence Sr stable isotopes have been extensively used to study Ca dynamics in ecosystems (Åberg, 1995; Blum et al., 2002; Capo et al., 1998; Dambrine et al., 1997; Drouet and Herbauts, 2008; Drouet et al., 2005; Jacobson et al., 2002; Poszwa et al., 2000; Poszwa et al., 2004). This similarity results from their close ionic radii (1.00 and 1.18 Å for Ca and Sr, respectively), which can give them similar physical and chemical properties. Despite this similarity, several studies have shown that Ca/Sr ratio is often higher in plant tissues than in soil solution, revealing a process of discrimination against Sr during root uptake and subsequent ion translocation (Memon et al., 1983; Veresoglou et al., 1996). This discrimination has been qualified as a Ca “biopurification” process and has since been observed in several plant species and environments (Drouet and Herbauts, 2008; Poszwa et al., 2000, and references herein). Barium, another alkaline-earth with a close ionic radius (1.35 Å), is naturally present in soils at relatively high concentrations (Suwa et al. 2008) and is also discriminated by root uptake (Drouet and Herbauts, 2008).

Although Rb cannot replace K in its functions in plant metabolism, root uptake was thought to not distinguish between these two cations because of close ionic radii and similar valences (Marschner, 1995). As a consequence, ^{86}Rb has often been used as a radiotracer for K in plant root uptake studies. However, numerous studies have also shown that Rb was a poor tracer for K in higher plant systems because of a more or less strong discrimination against Rb (Britto and Kronzucker, 2008 and references herein). This

inconsistency suggests the similarity in K and Rb dynamics in the plant depend on plant species and experimental conditions.

The large majority of studies dealing with Ca and K dynamics have been conducted either in crops under controlled or semi-controlled conditions (Britto and Kronzucker, 2008; Broadley et al., 2003, and references herein) or in temperate forests (Beauregard and Côté, 2008; Blum et al., 2008; Dasch et al., 2006; Drouet and Herbauts, 2008; Poszwa et al., 2000). Overall, very few is known about Ca and K dynamics and the similarities of Ca vs. Sr and Ba, and K vs. Rb in high altitude ecosystems in which plant growth and root uptake are often limited by climatic conditions and nutrient availability. Moreover, these oligotrophic ecosystems are particularly at risk because atmospheric N deposition may change base cation availability such as Ca and K for which dynamics need to be investigated. In addition, the dynamics of these elements have been mostly investigated in short leaf lifespan deciduous species and rarely in broad and long leaf lifespan evergreen species.

Here, we investigated variations in Ca, Sr, Ba, K and Rb concentrations in roots, stems and different leaf cohorts of *Rhododendron ferrugineum*, an evergreen shrub characteristic of European high elevation granitic habitats. This species dominates most ericaceous heathlands of European mountains and contrary to most evergreen species of high altitude habitats *R. ferrugineum* is characterized by broad leaves.

Our first aim was to test covariations of Ca, Sr and Ba concentrations on one hand and of K and Rb concentrations on the other hand among the different plant organs to verify whether these elements have similar dynamics in the plant. Our second objective was to characterize the biopurification of Ca and K between the different plant organs, as well as during leaf aging. Calcium/Strontium ratio in plant tissues have often been used in association with $^{87}Sr/^{86}Sr$ ratio to identify plant Ca sources and determine their relative contributions (Blum et al., 2008; Blum et al., 2002; Drouet and Herbauts, 2008). Given the biopurification process that occurs at the step of root uptake and within the plant, species-specific correction factors have nevertheless to be applied (Beauregard and Côté, 2008; Drouet and Herbauts, 2008). However, how the magnitude of the biopurification process varies at the infra-specific level and through time has to our knowledge rarely been reported. Hence, the third objective was to investigate whether the concentrations, dynamics and biopurification of the studied base cations in the plant varied between populations on a small geographical scale.

2 Material and Methods

2.1 Study site

The study was conducted in the central French Pyrenees in the vale of Estaragne (42° 48’ N; 0° 9’ E), which is located in the Néouvielle massif. The Néouvielle pluton is an elliptical body with an area of 98 km^2 showing concentric petrogenic zonation (Alibert et al., 1988). The core of the massif is a biotite rich monzogranite (53% of the pluton). It is surrounded by a hornblende-rich graniodorite (47% of the pluton). The main components of the rock are quartz (30%), zoned plagioclases (35%) and K-feldspars (25%) (Oliva et al. 2004). Two types of soil develop over the granitic unit: ranker type soils under grassland and podzolic type soils mainly located on slopes and colonized by coniferous and ericaceous.

The valley is located at the border of the Néouvielle pluton oriented North-East / South-West (opening to the north) and stretches over 3 km between 1850 and 2500 m a.s.l. The vegetation is composed of a mosaic of meadows, shrubs and trees (*Pinus uncinata* Ram.) with long heathland/meadow ecotones. Heathlands are mainly composed of *Rhododendron ferrugineum* L. and *Vaccinium myrtillus* L. (Ericaceae). *Nardus stricta* L. and *Festuca eskia* Ram. are the main dominating species (Poaceae) of the meadows. The subalpine climate prevailing in the site is relatively mild due to Ibero-Mediterranean influences. Most atmospheric inputs

originate from the Atlantic Ocean but the Pyrenees are sometimes affected by dust-bearing precipitation events ("red rain" or "red snow") originating from Africa or Spain (Oliva et al., 2004). Snow cover usually persists from late October till early June. The average annual precipitation amounts to 1500 mm. Soils are acidic (pH = 4.7 ± 0.1, SD; total N: 0.5% ± 0.044, SD; bulk density: 0.65 ± 0.099, SD).

2.2 Studied species

The study was conducted into two *R. ferrugineum* populations (hereafter sites A and B) about 500 m apart on the North-western facing slope (slope of 34.0 ± 3.7 % and 29.8 ± 3.0 % for sites A and B respectively) of the mid-section of the valley (2100 and 2160 m a.s.l for sites A and B respectively). These two populations have been intensively studied and have shown differences in leaf turnover rate, internal N cycling and gas exchange rates (Marty et al., 2010; Marty et al., 2009; Pornon and Lamaze, 2007). For both sites, soil field capacities and organic matter contents are similar (0.82 ± 0.08 g g^{-1} DW and 11.75 ± 1.35 % for site A, and 0.81 ± 0.15 g g^{-1} DW and 12.10 ± 4.07 % for site B, respectively).

The species studied, *Rhododendron ferrugineum*, is an evergreen shrub, with well-branched trailing stems that reaches a height of 70-80 cm. It is widely distributed in the Alps and the Pyrenees between 1600 and 2200 m a.s.l. (Ozenda, 1985) where it can dominate plant communities especially in areas where grazing pressure has subsided.

2.3 Sampling design

At each site, plant analyses were conducted on five compartments: roots (R), stems (S) and current, 1 yr-old and 2 yr-old leaves (respectively L0, L1 and L2). Every compartment was collected three times in the vegetation period on mature shrubs: mid-June, mid-August and end-October. These periods match with the beginning of the vegetation period, the end of shoot growth and the end of the vegetation period respectively. At each vegetation period, five sub-areas (50 m^2 each) were delimited in each population. Inside each sub-area, plant compartment samples were collected on four shrubs and pooled together so that we obtained five replicates of each compartment from twenty individuals three times during the whole vegetation period. Samples were immediately refrigerated before they were meticulously rinsed with ultrapure water (Milli-Q integral system) in the laboratory. Then, they were oven-dried for 72 h at 60°C and ground in fine powder (diameter < 10 µm) in an agate mortar.

For each plant sample, a series of oxidizing acid attacks (bi-distilled HNO_3, HF and HCl) was conducted on 100 mg powder in Teflon reactors (Savillex®). The dry residual was then weighted and diluted in bi-distilled nitric acid (2%) for multi-elementary analyses. Details about the whole procedure are reported in (Viers et al., 2007).

2.4 Multi-elementary analysis

Concentrations of Ca and K in plant samples (mg g^{-1}) were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES; IRIS Intrepid II XDL). Concentrations of Sr, Rb and Ba were analyzed by inductively coupled plasma mass spectrometry (ICP-MS; 7500 CE, Agilent Technologies).

2.5 Biopurification of K and Ca in the plant

We assessed the discrimination of both Ba and Sr against Ca and Rb against K across the different compartments of the plant. In this respect, we calculated discrimination indexes between both roots and stems, and stems and leaves. For instance, Sr discrimination against Ca was calculated as follows:

$$DF_{S-R}^{Ca/Sr} = \frac{(Ca/Sr)_S}{(Ca/Sr)_R}$$

$$DF_{L-S}^{Ca/Sr} = \frac{(Ca/Sr)_L}{(Ca/Sr)_S}$$

where *S*, *R* and *L* are stem, root and leaf compartments respectively. For instance, $DF_{S\text{-}R}$ is the discrimination index from roots to stems. The higher $DF^{Ca/Sr}$ values the higher the Sr discrimination between two given compartments. This index was calculated for couples Ca/Sr, Ca/Ba and K/Rb.

2.6 Statistics

Normality of values and homoscedasticity were assessed with Shapiro-Wilk and Fligner-Killeen test respectively. Differences in both element concentrations and element ratios between plant compartments, and differences in discrimination factors (*DF*) were tested with one-way ANOVAs followed by Tukey post hoc analyses. The relationships between element concentrations in leaves, stems and roots were assessed with a Pearson's product moment correlation analysis. Linear and non-linear regressions were also performed to assess the variations in elemental concentrations and ratios with leaf age. An analysis of covariance (Ancova) was performed to estimate the significance of the "site" effect on the relationship between leaf age and Ca/Sr and K/Rb. All analyses were performed with R (R version 2.9.1; R Development Core Team, 2009).

3 Results

3.1 Concentrations in plant organs

Concentrations of Ca were similar in roots and stems and strongly lower (at least two times and up to 7 times) than in all leaf generations at both sites (Table 1). At both sites Ca concentrations in L1 and L2 were similar and significantly higher than in L0. In contrast, K concentrations in L0 were significantly higher than in L1 and L2 at both sites (Table 1).

Table 1 Concentrations (means (SD), n=62) in Ca and K in the different plant compartments of *R. ferrugineum* (mg g^{-1}).

	Ca			K		
	A	B	t-test (P)	A	B	t-test (P)
R	2.1 (0.8) *a*	2.7 (1.0) *a*	0.11	1.5 (0.2) *a*	1.6 (0.2) *a*	0.84
S	2.0 (0.3) *a*	2.0 (0.5) *a*	0.55	1.0 (0.2) *a*	1.0 (0.2) *a*	0.58
L0	5.7 (1.9) *b*	4.6 (1.8) *b*	< 0.001	5.8 (2.6) *b*	7.0 (3.4) *b*	< 0.005
L1	9.7 (1.7) *c*	7.7 (0.9) *c*	< 0.001	3.8 (0.7) *c*	4.3 (0.6) *c*	< 0.05
L2	11.0 (1.9) *c*	8.2 (0.6) *c*	< 0.001	3.2 (0.7) *c*	4.2 (0.4) *c*	< 0.001

Letters in italics: results of the comparison of compartments within each site (one-way Anova). *L0, L1, L2, R and T: current year, one yr-old, two yr-old leaves, roots and stems, respectively.*

Table 2 Correlations (r) between elemental concentrations in leaves, stems and roots of *R. ferrugineum*.

	[Ca] (mg g^{-1})			[Sr] (µg g^{-1})			[Ba] (µg g^{-1})			[K] (mg g^{-1})			[Rb] (µg g^{-1})		
	L	S	R	L	S	R	L	S	R	L	S	R	L	S	R
[Ca]	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-
[Sr]	0.9 ***	0.8 ***	0.9 ***	1	1	1	-	-	-	-	-	-	-	-	-
[Ba]	0.8 ***	0.2 ns	-0.5 *	0.9 ***	0.2 ns	-0.3 ns	1	1	1	-	-	-	-	-	-
[K]	-0.8 ***	0.5 **	0.2 ns	-0.6 ***	0.4 *	0.4 *	-0.6 ***	0.2 ns	-0.1 ns	1	1	1	-	-	-
[Rb]	-0.7 ***	-0.1 ns	-0.3 ns	-0.6 ***	0.1 ns	-0.1 ns	-0.5 ***	0.6 ***	0.5 ***	0.9 ***	-0.1 ns	0.3 ns	1	1	1

For leaves (L), stems (S) and roots (R), the numbers of samples (n) are respectively 85, 60 and 60. *** P < 0.005; **P < 0.01; * P < 0.05; ns P > 0.05.

Table 3 Mean Ca/Sr, Ca/Ba and K/Rb ratios in the different plant compartments at sites A and B.

	Ca/Sr			Ca/Ba			K/Rb		
	A	B	t-test (P)	A	B	t-test (P)	A	B	t-test (P)
R	239.5 (39.3) *a*	273.7 (40.7) *a*	ns	40.0 (15.3) *a*	58.3 (30.8) *a*	ns	176.9 (40.3) *a*	180.7 (41.6) *a*	ns
S	264.1 (22.6) *a*	284.7 (33.3) *a*	ns	41.1 (29.7) *a*	40.3 (12.9) *a*	ns	203.2 (76.6) *a*	213.5 (103.3) *a*	ns
L0	591.1 (53.9) *b*	774.5 (127.5) *b*	***	320.8 (335.31) *b*	110.9 (70.2) *b*	*	182.4 (44.3) *a*	203.5 (63.4) *a*	ns
L1	538.1 (67.5) *c*	682.5 (95.8) *c*	***	166.7 (100.46) *b*	177.9 (72.17) *c*	ns	268.9 (78.9) *b*	253.5 (77.4) *ab*	ns
L2	514.5 (57.8) *c*	654.6 (78.9) *c*	***	227.1 (206.3) *b*	136.3 (50.97) *bc*	ns	294.4 (94.4) *b*	298.1 (66.6) *b*	ns

For each site, values (mean (SD), n=15) that do not share the same letters are significantly different (P<0.05; one-way Anova followed by a Tukey HSD test). *L0, L1, L2, R and T: current year, one yr-old, two yr-old leaves, roots and stems, respectively. ns: not significant; * P< 0.05; ** P<0.01; *** P<0.001*

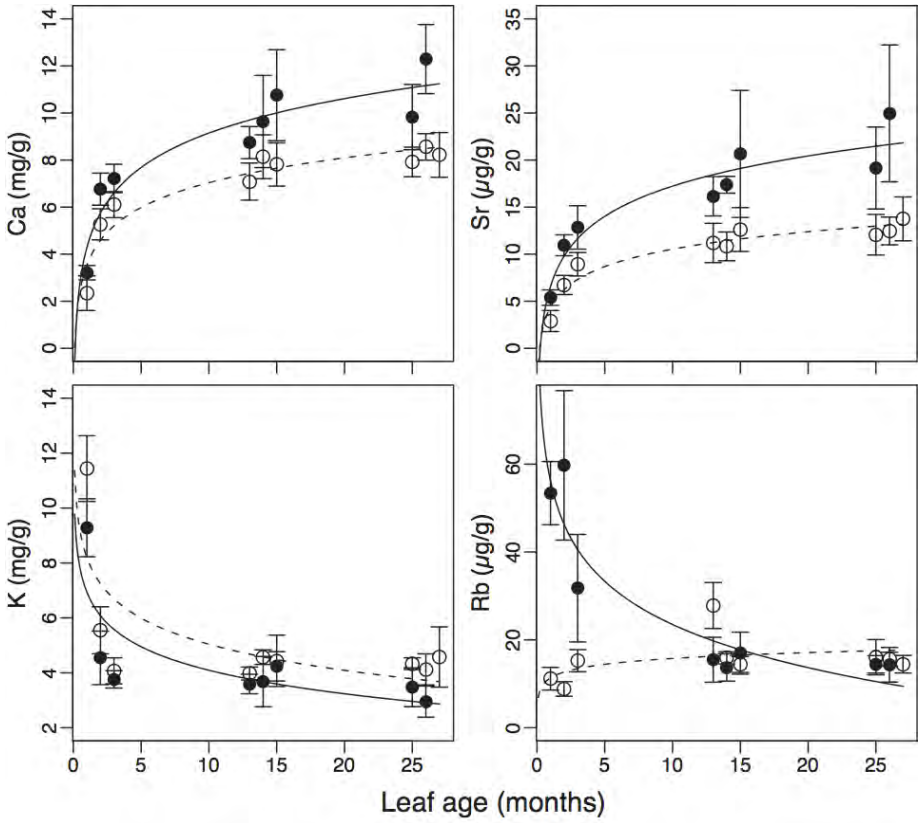

Fig. 1 Variations in Ca, K, Sr and Rb concentrations (mean ± SD, n = 5), expressed a on mass basis (mg g^{-1}) with leaf age. Closed and open circles are sites A and B, respectively. Equations are : *Top-left* – A : y = 2.1 log(x) + 4.3 (R^2=0.87***); B : y = 1.5 log(x) + 3.5 (R^2=0.89***); *Top-right* – A : y = 4.6 log(x) + 6.6 (R^2=0.88***); B : y = 2.7 log(x) + 4.4 (R^2=0.92***); A : *Bottom-left* – A : y = -1.2 log(x) + 6.9 (R^2=0.58***); B : y = -1.4 log(x) + 8.2 (R^2=0.51***); *Bottom-right* – A : y = -1.2 log(x) + 6.9 (R^2=0.86***); B : y = -1.4 log(x) + 8.2 (R^2=0.21 ns). ***: P<0.005.

Leaf Ca and Sr concentrations sharply increased during the first year, afterwards the increase declined and concentrations tended to stabilize around 11 mg g^{-1} and 20 µg g^{-1} in site A but only around 8 mg g^{-1} and 10 µg g^{-1} in site B (Fig. 2). In contrast, leaf K concentrations decreased drastically during leaf expansion and then tended to stabilize around 3.5 mg g^{-1} vs 4.2 mg g^{-1} at sites A and B, respectively. Consequently L1 and L2 were much more concentrated in Ca than in K. As for K, Rb concentrations at site A decreased strongly during the first year and then stabilized at ~15 µg g^{-1}. In contrast, Rb concentrations at site B were slightly lower in L1 (~12 µg g^{-1}) than in older leaves (15-19 µg g^{-1}).

In all leaf cohorts, Ca concentrations were significantly higher and K concentrations significantly lower at site A than at site B (Table 1), whereas both sites had similar K and Ca concentrations in stems and roots.

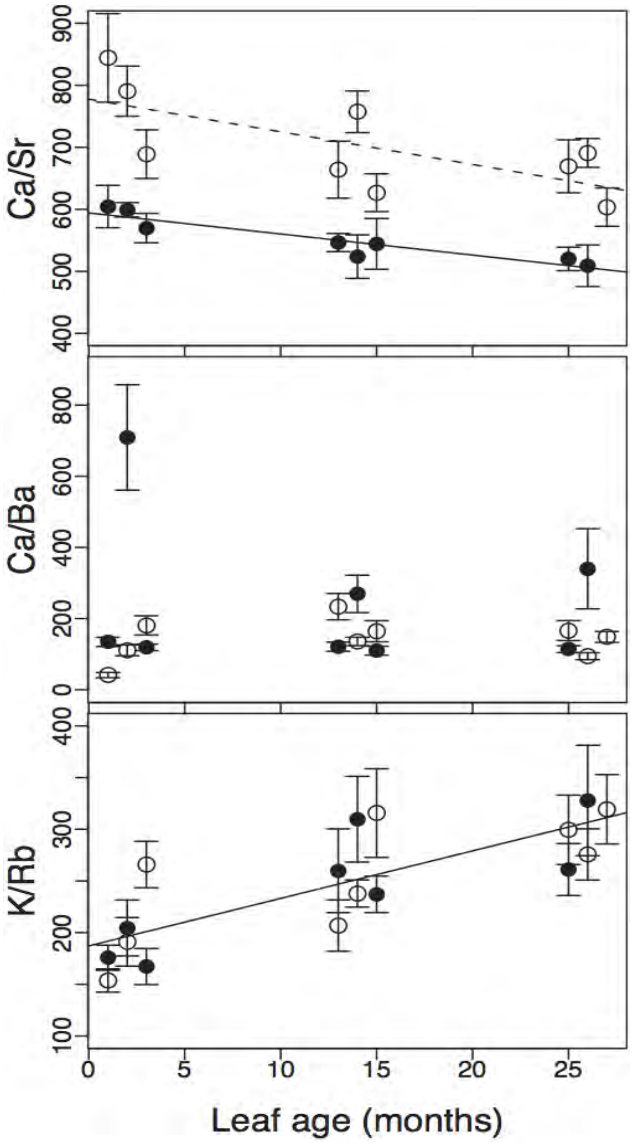

Fig. 2 Variations in leaf Ca/Sr, Ca/Ba and K/Rb ratios of *R. ferrugineum* with leaf age. Values are means ± SE (n = 5). Closed and open circles are site A and B respectively. Equations are: *Top*– A: y = -3.4x + 594.2 (R^2 = 0.87***); B: y = -5.3x + 777.5 (R^2 = 0.49*); *Bottom*– y = 4.6x + 187.1 (R^2 = 0.87***). ***: P<0.005.

3.2 Biopurification of Ca and K

There were strong positive correlations between Ca and Sr concentrations in leaves, stems and roots (Table 2). In contrast, the correlation between K and Rb concentrations was significant only in leaves. The correlations between Ca and Ba concentrations and Sr and Ba concentrations were also positive and strong in leaves but not significant in stems and tended to be negative in roots.

Ca/Sr and Ca/Ba ratios were markedly higher in leaves than in roots and stems (Table 3). In contrast, only leaves older than 1 year (both L1 and L2 at site A and only L2 at site B) had higher K/Rb ratios than roots and stems. In leaves, Ca/Sr ratio was significantly higher at site B than at site A. The difference was much less marked in roots and stems. In contrast, there was no difference in K/Rb and Ca/Ba among sites except in L0 where Ca/Ba was almost three times higher at site A than at site B.

Ca/Sr ratios declined whereas K/Rb ratios increased linearly with leaf age at both sites (Fig. 2). Ca/Sr ratios were higher and declined faster with leaf age at site B. In contrast, we did not find any "site" effect on the intercept and the slope of the K/Rb linear regression (Ancova, $P > 0.05$), so the data were pooled to fit the linear regression (Fig 2). In contrast, Ca/Ba was not correlated to leaf age ($R^2 = 0.04$, $P = 0.6$). At site A, the variation was very large within the same year with strikingly higher values in July (months 2, 14 and 26 on Fig 2).

Discrimination factors (*DF*) for Sr and Ba were low between roots and stems (only 1.09 for Sr; Table 4). There was even a positive discrimination of Ba since *DF* was < 1 (0.88), reflecting a decrease in the Ca/Ba ratio from roots to stems (Table 3). Discrimination factors between stems and leaves were at least twice (Sr) and four times (Ba) higher than between roots and stems (Table 4). Except between roots and stems, the discrimination against Ba ($DF^{Ca/Ba}$) was higher than that against Sr and Rb. The discrimination against Sr ($DF^{Ca/Sr}$) and Rb ($DF^{K/Rb}$) was similar between roots and stems and between stems and L2. In contrast, the discrimination against Sr was higher than that against Rb between stems and L0 and between stems and L1.

Table 4 Discrimination factors (*DF*) between the different plant compartments.

	$DF^{Ca/Sr}$	$DF^{Ca/Ba}$	$DF^{K/Rb}$
S-R	1.09 (0.17) *a*	0.88 (0.37) *b*	1.14 (0.30) *a*
L0-S	2.48 (0.38) *a*	4.88 (3.63) *b*	1.08 (0.49) *c*
L1-S	2.22 (0.31) *a*	4.68 (1.98) *b*	1.44 (0.61) *c*
L2-S	2.15 (0.25) *a*	3.80 (1.28) *b*	1.66 (0.56) *a*

For each compartment, *DF* values (mean (SD), n = 30) that do not share the same letter are significantly different ($P < 0.05$; one-way Anova followed by a Tukey HSD test).

4 Discussion

4.1 Elemental concentrations in plant tissues

There was no significant difference in Ca concentrations in both roots and stems between the two sites. In contrast, leaf Ca concentrations were significantly higher at site A. Due to the high toxicity of Ca in the cytosol and the phloem sap, this element is considered the most immobile cation in the plant and can only be weakly redistributed in the plant via the phloem (Marschner, 1995). As a consequence, developing tissues largely rely on Ca transport via the xylem, which is ultimately controlled by transpiration rate (White and Broadley, 2003). Consequently, water availability in the soil can impact Ca concentrations in leaves. It has actually been shown

that drought periods could decrease foliar Ca concentrations in several Mediterranean species (Sardans et al., 2008). Differences in leaf Ca concentrations between the two sites might therefore result from differences in transpiration rates, which could basically originate from differences in soil water reserves. This hypothesis is strengthened i) by the fact that soil is thicker and less rocky at site A, which might result in higher water availability at this site; ii) by leaf transpiration data obtained from a 2-year campaign of leaf gas-exchange measurement at both sites (Marty et al., 2010; Marty, 2009). Leaf transpiration (E; µmol H_2O m^{-2} s^{-1}) in L0 has been shown to be on average 25% higher and water use efficiency (WUE; µmol H_2O µmol^{-1} CO_2) 19-32% lower across all leaf generation at site A (Marty, 2009; data not shown).

Ca and Sr concentrations were strongly positively correlated in leaves and both elements exhibited the same change pattern with leaf age: concentrations increased strongly during the first year and then tended to plateau. The accumulation of Ca and Sr with leaf age results from their lack of mobility in the phloem sap, which prevents from remobilization to growing or storage organs (Marschner, 1995). Their decreasing rates of accumulation with leaf age in both populations might result from a reduction in transpiration rate with leaf aging as suggested by available leaf gas-exchange data, which have shown that transpiration rate was 12-39% lower in L2 than in L0. It has also been suggested that Ca decreasing rate of accumulation could result from an increasing exudation/leaching rate from leaves as leaf aged (Wyttenbach et al., 1995).

In contrast to Ca, leaf K and Rb concentrations at site A sharply decreased after bud break and reached their final value from the second month of their life. This decline in leaf concentration occurred during the short growth period of *R. ferrugineum's* leaves (Marty et al., 2009) and must correspond to the dilution of K in leaf biomass as they expanded. Actually, K leaf content (the product of K concentration by leaf mass) did not vary during this short period (data not shown) indicating that the amount of K in expanded leaves was already present in the buds before shoot growth.

Negative correlations between Ca (and also Sr and Ba) and K (and Rb too) concentrations (Table 2) in leaves might mainly result from their opposite dynamics in leaves (accumulation vs. resorption/dilution). However, significantly negative correlations were also found independently of their contrasting dynamics (at each measurement date), which might reflect an antagonism between the two elements in leaf cells. Calcium is mainly located in cell walls, whereas K is concentrated in cytoplasm. High Ca concentrations in older leaves could result from high cell wall content, which could reduce the proportion of cytoplasm in the total cell mass and consequently reduce K concentration in total leaf tissues, explaining the negative relationship between Ca and K concentrations in leaves.

4.2 Biopurification of Ca and K

Ca/Sr ratios in *R. ferrugineum*'s organs (Table 3) were in the range of those measured in beech (Drouet and Herbauts 2008; Drouet et al. 2005), maple and birch (Blum et al. 2002) but lower than in oak (Drouet and Herbauts 2008), fir and spruce (Blum et al., 2002). At both sites, this ratio was significantly lower in roots and stems than in leaves, indicating that from roots to leaves via the stems, Ca was "biopurified". Numerous studies have shown that biopurification occurs during root uptake and from roots to leaves (Drouet and Herbauts, 2008; Poszwa et al., 2000). Unfortunately we can't assess biopurification at the root uptake step with the present data. However, we show that within the plant most Ca and K biopurification occurred between stems and leaves, rather than between roots and stems. It has been known for long that, at least for forbs, stems contain an efficient trapping mechanism for Sr that removes the ion from the ascending flow (Emmert, 1965). The biopurification between stems and leaves could therefore result from a preferential capture of Sr and Ba (in the case of Ca biopurification) and Rb (in the case of K biopurification) by this mechanism, leading to higher Ca/Sr and Ca/Ba ratios or K/Rb in the ascending sap and consecutively in leaves. This "chromatographic effect" of the vascular column would lead to increasing Ca/Sr ratio with height in vascular

tissue (Beauregard and Côté, 2008). In *R. ferrugineum*, newly produced shoots (L0) are located at the top of the shoot produced the previous year (L1). Therefore, this mechanism could explain the decline in Ca/Sr with leaf age, but not the increase in K/Rb, which suggests the existence of specific mechanisms for K. As previously mentioned, K is highly mobile in the phloem and is as a consequence constantly exported from the leaf via the phloem. A discrimination effect against K at the step of phloem loading could explain the increase in K/Rb ratio with leaf leaf age, but this hypothesis should be tested.

4.3 Variations in Ca and K biopurification

K/Rb ratio in all plant compartments and Ca/Sr ratios in roots and stems were similar at both sites, but Ca/Sr ratio in leaves was significantly higher at site B (~30% higher). The reasons for such a discrepancy are unknown but it is unlikely that this difference resulted from different Ca sources because the two populations are only a hundreds of meters from each other and shrubs from both populations had similar Ca/Sr ratios in roots and stems. In addition, Sr stable isotopes ratios ($^{87}Sr/^{86}Sr$) measured in all plant compartments (see supplementary material) were found to be similar at both sites (Table S1), which strengthens the hypothesis of similar Ca sources at both sites. The difference between the two populations might rather result from a difference in Ca biopurification during ion translocation from roots to leaves. As previously suggested, this could result from a difference in the length of the vascular column (Beauregard and Côté, 2008), which may basically be the result of a difference in shrub age or growth between the two sites. This could also result from the lower rate of transpiration at site B as compared to site A leading to lower flow of water in the xylem of plants of site B therefore enhancing the "chromatographic effect" of the vascular column. Although these hypotheses remain to be tested, our results clearly indicate that the Ca/Sr ratio in leaves can significantly vary between populations with probably the same Ca sources on a very small geographical scale, and therefore that Ca/Sr ratio should be used with caution to assess differences in Ca sources among plant species.

In addition, our data show that biopurification between stems and leaves was higher for Ca than for K (Table 4), the magnitude of discrimination decreasing in the order Ba, Sr and Rb. The high discrimination against Ba might reflect its high toxicity in leaves as it is known to impair C assimilation (Suwa et al. 2008).

5 Conclusion

Our data show that in *R. ferrugineum*, Ca and Sr concentrations were very strongly positively correlated in all plant compartments, and K and Rb concentrations were strongly correlated in leaves, illustrating the similar dynamics of these two element couples in the plant. However, our data showed that after root uptake Ca and K were biopurified during their translocation from roots to leaves, resulting in higher Ca/Sr, Ca/Ba and K/Rb ratios in leaves. Most biopurification within the plant between stems and leaves rather than between roots and stems. Moreover, the discriminations against Sr and Rb were shown to respectively decrease and increase with leaf age. While the chromatographic effect of the vascular column could explain the increase in leaf Ca/Sr ratio with leaf age, this mechanism was unlikely to explain the increase in leaf K/Rb ratio with leaf age, suggesting the existence of mechanisms of biopurification at the step of leaf loading and/or the excretion of these elements from leaves with a discrimination effect. Surprisingly, we found that Ca and K concentrations and Ca/Sr ratio in leaves varied between two populations growing only a few hundred meters to each other. While variation in Ca concentrations might result from a difference in water availability, the origin of the difference in Ca/Sr ratio remains to be investigated. The proximity of the two populations as well as Sr stable isotope ratios strongly suggest that this difference does not originate from different Ca and Sr sources but rather from physiological processes and therefore that Ca/Sr ratio should be used cautiously for Ca sources identification in the field.

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