

# Evaluation of the mobility and discrimination of Ca, Sr and Ba in forest ecosystems: consequence on the use of alkaline-earth element ratios as tracers of Ca

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**Abstract** A comprehensive understanding of Ca cycling in an ecosystem is desirable because of the role of this element in tree mineral nutrition and its status as a major base cation on the soil exchange complex. The determination of the origin of Ca in forests is particularly indicated in regard of important changes linked to acid inputs and intensive logging. Natural strontium isotopes are increasingly used as tracers of Ca in forest ecosystems for qualitative and quantitative assessments. Nevertheless this method is limited to relatively simple systems with two sources of nutrients. Some recent studies coupled Sr/Ca or Sr/Ba ratios to Sr isotopic measurements in order to solve more complex systems. Such method has however associated with it some uncertainties: this approach assumed that Ca, Sr and Ba behave similarly throughout the ecosystem and does not take into account the Ca biopurification processes occurring in some tree's organs which can alter element ratio. The present work focuses on two deciduous species covering large areas in Europe: European beech (*Fagus sylvatica* L.) and

pedunculate oak (*Quercus robur* L.). In order to test the similarity of behaviour between Ca, Sr and Ba, their concentrations were measured extensively in the major compartments of two forest ecosystems. In parallel, the discrimination process inside tree organs was studied in 23 stands for beech and 10 stands for oak. We found that Sr and Ca behave similarly in all soil and tree compartments. By contrast, Ba and Ca appear to have contrasting behaviours, especially in streams, soil solution and soil exchange complex (no correlations between element concentrations). Sr/Ba and Ba/Ca ratios must therefore be used with care as tracer of Ca. The Ca biopurification is absent in roots and slight in bole wood but is large in bark, twigs and leaves. The discrimination factors (DF) between wood and leaves are characteristic of the two species studied and do not change significantly as a function of the soil Ca status (acidic or calcareous soils). Therefore, strontium–calcium DF can be used as a correction factor of the Sr/Ca ratio of leaves when this ratio is used in connection with Sr isotopic ratios. This correction allows to solve systems of tree nutrition with more than two sources of Ca.

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**Keywords** Barium · Calcium · Discrimination ratio · *Fagus sylvatica* L. · Forest ecosystems · Strontium · *Quercus robur* L. · Tree nutrition

## Abbreviations

DF Discrimination factor

## Introduction

Calcium is an essential element in forest ecosystems, as a nutrient for vegetation and as a buffer against acidic inputs in soil. In most ecosystems, Ca nutrition of the vegetation is supplied via two sources: mineral weathering and atmospheric precipitation. Natural strontium isotopes have been used to determine the relative contributions of these two sources for Ca nutrition of forest stands (e.g., Åberg et al. 1990; Miller et al. 1993; Bailey et al. 1996; Blum et al. 2002; Drouet et al. 2005a). This method is based on the fact that Sr and Ca have close geochemical behaviour and secondly, that any Sr isotopes fractionation which does occur in the soil–vegetation–atmosphere interface can be corrected by the mass spectrometry data processing procedure. The standard mixing model commonly used (Capo et al. 1998; Phillips and Gregg 2003) is mathematically limited to systems with two sources of Ca. This model includes a mass equilibrium equation as well as a second equation maintaining the integrity of proportions. However, in some ecosystems, an additional source of Ca is involved in tree nutrition. This is the case, for example when soil contains Ca-minerals with distinct  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in different horizons. Moreover, in many situations, tree roots have access to several soil horizons with contrasting Sr isotopic signature. These variations in the isotopic composition may be linked to mineralogical differences and/or to changes in weathering rates among horizons (Drouet et al. 2007). The presence of a third Ca source leads therefore to a system with two equations and with more than two unknown, which is mathematically undetermined.

Nevertheless, some studies have shown the possibility, in some cases, to overcome this problem. In order to determine the Ca sources of a forest stand (Hubbard Brook Experimental Forest), Blum et al. (2002) have used Ca/Sr ratios as tracer of Ca coupled with measurement of Sr isotopes. The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios measured in the sources and the vegetation allowed the authors to discriminate between Ca inputs from silicate weathering and atmospherically-derived Ca. In complement, the proportion of Ca coming from apatitic inclusions in some minerals was quantified by measurement of leaf Ca/Sr ratio.

However, a physiological process of segregation of Sr relative to Ca occurs in plants. This process discriminates Sr (Poszwa et al. 2000; Blum et al.

2000; Dasch et al. 2006) but also barium as well as lead (Elias et al. 1982). This discrimination among alkaline-earth elements with their incorporation into different types of tissues strongly modifies the value of the Sr/Ca ratio, which provides a characteristic signature of the relative proportions of the supplying sources. This modification could therefore compromise the use of such element ratios as tracer of Ca (Watmough and Dillon 2003). In order to mitigate the effect of Ca discrimination against Sr, Bullen and Bailey (2005) have used the Sr/Ba ratio, considered by these authors as insensitive to physiological modification, to support the hypothesis that, consecutively to a soil acidification episode, depth of root prospecting of a spruce stand has diminished, and concentrates today in the top soil horizons.

The objective of this study is to evaluate the possibility of using the Sr/Ca, Ba/Ca and Sr/Ba element ratios, measured in leaves of European beech (*Fagus sylvatica* L.) and pedunculate oak (*Quercus robur* L.), as tracers of Ca sources for the nutrition of these species.

The use of these element ratios to trace Ca is based on several hypotheses: (1) movements of Ca, Sr, and Ba in the soil compartment of the ecosystem are similar; (2) the element ratios measured in the tree organs must reflect the relative proportions of the supplying sources; (3) Sources must have different element ratios to quantify their respective contributions. In a first step, we will focus on the variation of sufficiently contrasting concentrations and element ratios in the main organs of beech and oak in a large number (24) of stands growing on soils with a wide range of Ca content. The aim is to determine which organ of beech and oak (roots, bole wood, bark, twigs, and leaves) are influenced by Ca discrimination and to quantify this process. We hypothesise that discrimination intensity between two organs is constant for a given tree species. Therefore, the determination of a unique discrimination factor, characteristic to each species allow to correct the Sr/Ca, Ba/Ca and Sr/Ba ratios measured in tree leaves and to reconstruct the element proportions at the time of root uptake from the soil solution (mixing of sources). Indeed, tree leaves are relatively easy to collect, present little risks of contamination by soil particles (contrary to roots) and their sampling is not damaging for trees (contrary to wood coring or bole sampling). In a second step we will study the behaviour of Ca, Sr and Ba cations in the entire

forest ecosystem to confirm the similarity of behaviour among these elements in two forest stands with contrasting Ca status. We will verify that the measured element ratios result from the mixing of the different feeding sources and that the influence of other processes (preferential sorption on soil components, Ca discrimination in the vegetation...) is minor. The final objective is to validate the use of these element ratios as tracers of Ca, in complement to the Sr isotopic method.

## Materials and methods

### Study sites

Twenty-four forest stands were selected in central and high Belgium (Table 1). Stands cover a large range of parent material and of soil Ca reserves. Two sites, in central Belgium (MES) and in high Belgium (POUR), have already been studied for Ca nutrition by the Sr isotopic method (Drouet et al. 2005a). The exploration of the relationship between tree leaves (beech and oak) and soil chemistry was extended to 22 other forest stands. The main characteristics of the sites studied are summarized in Table 1. Eighteen sites (mainly beech stands) are situated in central Belgium (Soignes Regional Forest) on loessic substrate or on outcroppings of Tertiary sand. Two stands were selected in high Belgium on Lower Devonian shales (SMD, POUR). Four other forest stands are situated in high Belgium, three on Jurassic limestone or clay from decarbonation (COT I, COT II, MUS), and one on sand from the weathering of Lower Liassic sandstone (MEIX). Complete description of these sites can be found in Drouet (2005), Herbauts (1981, 1982), and Herbauts and Tanghe (1987).

### Sampling

For each stand, five or ten beech trees (and an equivalent number of oaks when presents), were selected for leaf sampling. During September 2001–2004, three twigs (diameter <0.5 cm) of ca. 25 leaves were sampled on each tree in the crown periphery. Bole wood and bark were sampled during previous dendroecological and dendrochemical studies (Penninckx et al. 1999, 2001; Drouet et al. 2005b). Additional wood samples were collected by tree coring. In the MES stand, beech bole of a recent windfall was sampled each 2 m on a length

of 16 m with a tree corer. Five beech boles in the DIEP stand were also sampled with a corer at two heights (1 and 8 m). At MES, HUB I, HUB II, DIEP, LND, BEQ, FNT, POUR and SMD sites, beech and/or oak roots of different soil layers (MES:  $A_h$ , E and  $B_{2t}$  horizons, POUR:  $A_h$  and  $B_{2w}$  horizons, only  $A_h$  horizons for the other sites) and leaf litter were also sampled. In each site, samples of the main soil horizons ( $A_h$ , E/ $A_h$ B,  $B_t$ / $B_w$ / $B_s$ ) were collected with an auger or from pits. In addition, five samples of two holorganic horizons (fragmentation  $O_f$  and humified  $O_h$  horizons) were collected in the MES site. Tension water samplers (Teflon/quartz porous probes, Prenart equipment Aps., Fredriksberg, Denmark) were installed in situ to collect soil solution in the MES and POUR site. Soil solution was extracted by connecting the probes to a collection bottle with –50 kPa vacuum and was sampled monthly. Sources and spring waters were collected monthly from September 2003 to June 2005 in the POUR site. Bulk precipitation samples in central and high Belgium were obtained from another study (Drouet et al. 2005a). Throughfalls were collected under beech of the MES site after ten rain events by means of ten polyethylene funnels connected to polyethylene bottles.

### Sample preparation and analytical methods

Root samples were rinsed with copious amounts of water, washed with successive agitation in demineralised water and methanol treatments. Separation between leaf veins and limbs of beech and oak leaves from the MES site as well as separation of bark and wood of twigs were made under binocular using a stainless-steel scalpel. All vegetation material was dried at 65°C and ground in a Retsch ZM100 mill using a 750- $\mu$ m screen. Mineralization of about 1–2 g of plant sample was done by dry ashing in covered zirconium crucibles (16 h at 450°C in a muffle furnace). Ashes were dissolved in suprapur HCl and heated on a hot plate for 10 min.

Air-dried soil samples were sieved to a particle size of <2 mm and homogenized. Exchangeable cations were extracted with 1 M  $\text{CH}_3\text{COONH}_4$  pH 7 solutions. Element concentrations Ca, Mg, K, Sr and Ba were measured with an inductively coupled plasma optical emission spectrometer (ICP-OES). The precision and accuracy of analysis for element concentrations were monitored using BCR-100 as well as in house standards. Analyses agreed with certified values to within  $\pm 5\%$ .

**Table 1** Main characteristics of the stands studied

Site	Coordinates		Elevation (m a.s.l.)	Mean annual precipitation (mm)	Mean annual temperature (°C)	Tree species	Stand age (years)	Soil type <sup>a</sup>	Parent material
	Latitude	Longitude							
Central Belgium									
'Mésanges' (MES)	50°47'13"N	4°27'01"E	114	780	9.8	Fagus, Quercus	140–145	Dystric podzolvisol	Pleistocene loess
'Tumult' (TUM)	50°46'40"N	4°24'55"E	125	780	9.8	Fagus	193	Dystric podzolvisol	Pleistocene loess
'Relais des Dames II' (REL II)	50°47'30"N	4°26'33"E	110	780	9.8	Fagus	192–202	Dystric podzolvisol	Pleistocene loess
'Tir aux Pigeons' (PIG)	50°49'41"N	4°28'11"E	108	780	9.8	Fagus, Betulus, Acer	38	Dystric podzolvisol	Pleistocene loess
'Tambour' (TAM)	50°47'37"N	4°26'41"E	105	780	9.8	Fagus	25	Dystric podzolvisol	Pleistocene loess
'Relais des Dames III' (REL III)	50°47'29"N	4°26'10"E	110	780	9.8	Fagus	33–58	Dystric podzolvisol	Pleistocene loess
'Comité de Flandre I' (CTE I)	50°48'06"N	4°26'34"E	92.5	780	9.8	Fagus	40 (180)	Dystric podzolvisol	Pleistocene loess
'Comité de Flandre II' (CTE II)	50°47'20"N	4°25'45"E	120	780	9.8	Fagus	155–175	Dystric podzolvisol	Pleistocene loess
'St Hubert I' (HUB I)	50°46'35"N	4°24'11"E	128	780	9.8	Fagus	140	Dystric podzolvisol	Pleistocene loess
'St Hubert II' (HUB II)	50°46'29"N	4°24'35"E	110	780	9.8	Fagus	125–140	Dystric podzolvisol	Pleistocene loess
'Moulin' (MLN)	50°47'09"N	4°26'27"E	120	780	9.8	Fagus	170–175	Dystric podzolvisol	Pleistocene loess
'Terrest' (TER)	50°47'59"N	4°27'46"E	85	780	9.8	Fagus	130	Dystric podzolvisol	Pleistocene loess
'Rouge-Cloître' (CLO)	50°48'56"N	4°27'12"E	90	780	9.8	Fagus, Quercus	20	Dystric podzolvisol	Pleistocene loess
'Vallon des Chênes' (CHE)	50°47'16"N	4°26'45"E	100	780	9.8	Fagus, Quercus	120	Dystric podzolvisol	Pleistocene loess
'Belle Etoile' (BEQ)	50°44'56"N	4°24'52"E	132	780	9.8	Quercus	~100	Dystric podzolvisol	Pleistocene loess
'Trois Fontaines' (FNT)	50°48'17"N	4°26'27"E	80	780	9.8	Fagus	~30	Cambic calcisol	Tertiary calcareous sandstone
'Diependelle' (DIEP)	50°47'56"N	4°26'04"E	100	780	9.8	Fagus	185–195	Stagnic luvisol	Tertiary clayey sand
'Léonard' (LND)	50°48'22"N	4°27'58"E	95	780	9.8	Fagus	215	Orthic podzol	Tertiary sand
High Belgium									
'Poursumont' (POUR)	49°47'57"N	5°16'26"E	410	1,200	7.8	Fagus, Quercus	~160	Dystric cambisol	Lower Devonian shales
'Smuid' (SMD)	50°02'07"N	5°15'52"E	358	1,100	8.2	Fagus	~160	Dystric cambisol	Lower Devonian shales
'Meix-devant-Virton' (MEIX)	49°38'03"N	5°28'20"E	335	900	9.1	Fagus, Quercus	>100	Cambic umbrisol	Sand from the decarbonation of Lower Lias calcareous sandstone
'Bois de la Côte I' (COT I)	49°32'14"N	5°32'11"E	325	870	9.1	Fagus	>100	Mollic leptosol	Jurassic limestone
'Bois de la Côte II' (COT II)	49°32'10"N	5°33'14"E	390	870	9.1	Fagus, Quercus, Acer	>100	Dystric podzolvisol	Clay from the decarbonation of Jurassic limestone
'Musson' (MUS)	49°37'14"N	5°42'49"E	380	870	9.1	Fagus	>100	Dystric podzolvisol	Clay from the decarbonation of Jurassic limestone

<sup>a</sup> FAO classification

## Calculations

The Ca discrimination factors (DF) against Sr were calculated between organs of the tree. They are expressed by the following equations:

$$DF_{W-L}^{Sr/Ca} = \frac{(Sr/Ca)_W}{(Sr/Ca)_L};$$

$$DF_{W-T}^{Sr/Ca} = \frac{(Sr/Ca)_W}{(Sr/Ca)_T};$$

$$DF_{W-B}^{Sr/Ca} = \frac{(Sr/Ca)_W}{(Sr/Ca)_B};$$

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

where discrimination factors apply to the element ratio indicated in superscript. Letters in subscript designate: *W*, bole wood, *L*, leaves, *T*, twigs and *B*, bark. For example, *W-L* subscript indicates the discrimination from bole wood to leaves. The higher is the DF value the higher is the biopurification of Ca. Discrimination factors related to the Ba/Ca and Sr/Ba ratios are calculated following the same formulation. To quantify the element segregation (Ca, Sr and Ba) from an organ to another inside the tree, we determined the DF for each species studied (beech and oak). In order to use the DF values as correcting factors, we calculated the characteristic DF factor for each species as the arithmetic mean DF determined in several stands. We do not observe any marked radial trend of alkaline earth ratios along tree-ring chronologies (~100 years, unpublished data) for beech (CV<12, 25 and 26% for Sr/Ca, Ba/Ca and Sr/Ba respectively, 5 sites) and oak (CV<23, 28 and 31% for Sr/Ca, Ba/Ca and Sr/Ba respectively, 2 sites). However, for homogeneity with the tree core sampling, we only used analyses of the 15 last tree-rings for wood alkaline-earth ratios in DF calculations.

## Statistical analyses

One-way ANOVAs (leaf parts, twigs or soil compartment) were performed on element concentrations (Ca,

Sr, Ba) and element ratios (Sr/Ca, Ba/Ca, Sr/Ba). Multiple mean comparisons were subsequently made by the Tukey's HSD or unequal *n* HSD. To test differences of element ratios among trees organs, the homogeneity of variance (Levene test) could not be obtained after transformations (inverse, square root, logarithmic). Therefore, an equivalent, non-parametric test was used (Kruskal–Wallis), followed by mean rank comparisons for each pair (multiple comparison of mean ranks). In the main compartments of two forest ecosystems (MES and POUR sites), relationships between Ca, Sr and Ba concentrations were evaluated by the calculation of the Spearman rank coefficient of correlation ( $r_s$ ). The comparison of several parameters between species was performed with Student paired or unpaired *t*-tests following the case (see results). Relationships between discrimination factors and Ca amounts of the substrate were tested by means of linear regressions.

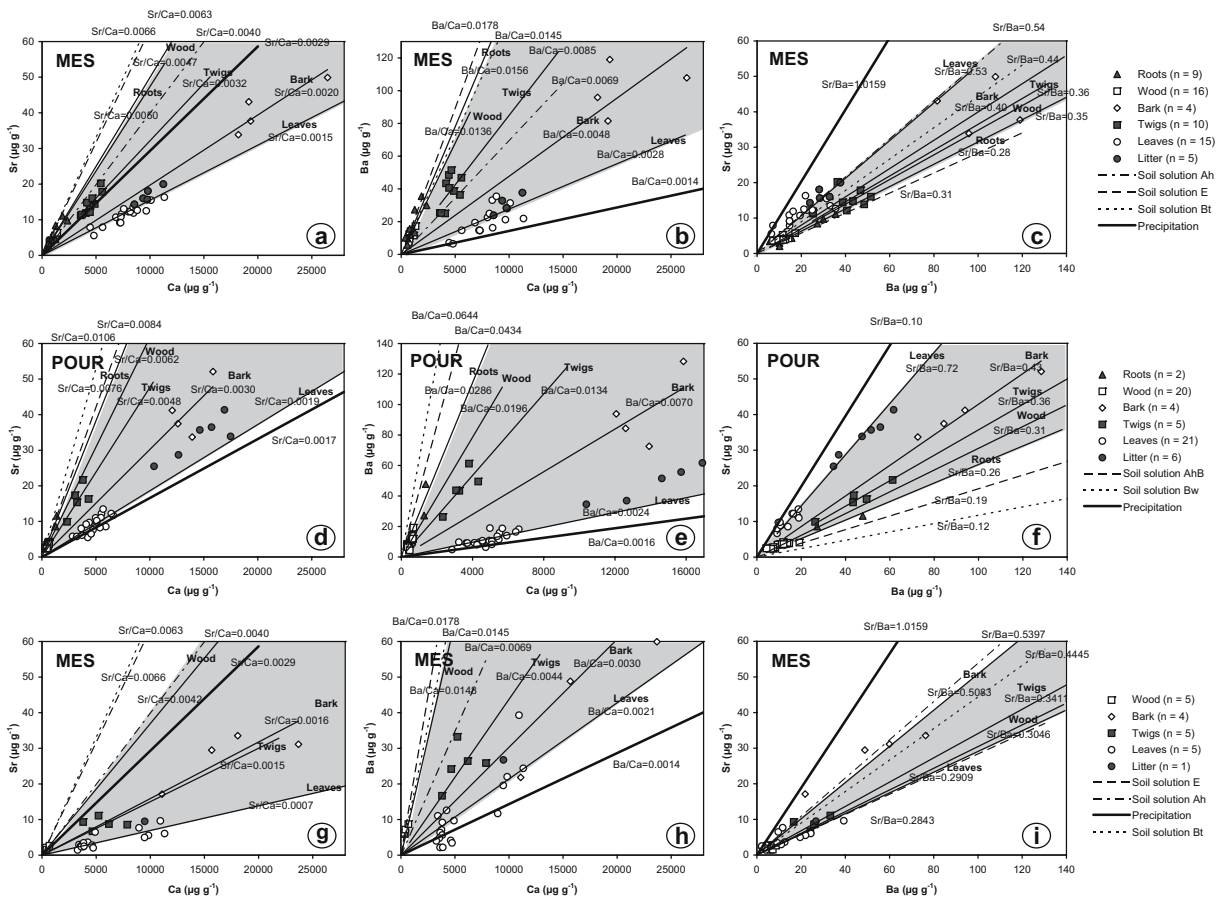
## Results and discussion

### Ca discrimination process

Figure 1a–f shows the results related to the discrimination processes in the main organs of beech (roots, bole wood, bark, twigs, leaves) in two stands, one in central Belgium (MES), the other in high Belgium (POUR). In oak, discrimination for Ca in the MES stand is shown in Fig. 1g–i. Measurements extended to a larger number of stands, on a limited number of organs (leaves, twigs and bole wood) are listed in Table 2, discriminations factors are calculated in Tables 3 and 4.

### Sr–Ca discrimination

We can observe that the Sr/Ca element ratios between soil solution and roots are confounded, for the two species studied (Fig. 1). Within a same horizon, Sr/Ca ratios of roots and soil solution are not significantly different (*t*-test,  $P>0.10$ , MES site). This is in line with the data from Tyler (2004) that point out a similar root selectivity for Sr and Ca in beech on podzol. Figure 1 shows a slight Sr discrimination from roots to bole wood. This difference of element ratios is however not significant for the MES and POUR sites (Table 5). Next, we observe a progressive Ca discrimination from



**Fig. 1** Effect of the alkaline-earth discrimination on (a, d, g) the Sr/Ca ratio, (b, e, h) the Ba/Ca ratio and (c, f, i) the Sr/Ba ratio during their route through the organs of *F. sylvatica* (a–f) or *Q. robur* (g–i). Straight lines show the element ratio

corresponding to each organ. Shaded area underlined the magnitude of the discrimination process from roots/bole wood to leaves

bole wood to leaves, with differences of intensity between beech (Fig. 1a, d) and oak (Fig. 1g). This difference between the two tree species is the more marked between bole wood and twigs, as indicated by the discrimination factor calculated from several stands ( $DF_{W-T}^{Sr/Ca} = 1.36 \pm 0.23$  and  $2.53 \pm 0.67$ ; median  $\pm$  SD for beech and oak, respectively, Tables 3 and 4). This difference between species is highly significant ( $t$ -test,  $P < 0.001$ ). To explain the mechanism of Ca discrimination between bole and twigs, a segregation of Sr and Ba in the trunk, as a function of the tree height (from the soil) could be put forward. However the sampling and chemical analyses of a beech bole each 2 m on a length of 16 m performed in a site of central Belgium (MES) does not show a substantial change of element ratios (Ca, Sr and Ba, data not shown). This observation is comforted by measurements of Sr/Ca ratios in beech

boles in a neighbouring site (DIEP) that are not significantly different between 2 and 8 m height (paired  $t$ -test,  $P > 0.10$ ;  $n = 5$ ; data not shown). Momoshima and Bondietti (1990) have also observed constant Sr/Ca ratio at different trunk heights of *Picea rubens* Sarg. The pathway from bole wood to bark has a similar effect ( $t$ -test,  $P > 0.10$ ) on the Sr/Ca ratio for the two species studied ( $DF_{W-B}^{Sr/Ca} = 1.90 \pm 0.46$  and  $2.61 \pm 0.28$ , mean  $\pm$  SD for beech,  $n = 9$ , and oak,  $n = 5$ , respectively; Tables 3 and 4). The Sr/Ca ratio change similarly from twigs to leaves in the two species ( $DF_{T-L}^{Sr/Ca} = 2.18 \pm 0.35$  and  $2.66 \pm 0.45$ , mean  $\pm$  SD for beech,  $n = 18$ , and oak,  $n = 9$ , respectively). But within a same site, the discrimination factor is significantly lower for beech (paired  $t$ -test,  $P = 0.019$ ,  $n = 8$ ). Therefore, oak discriminates Sr slightly more than beech in the pathway of alkaline earth from twigs to leaves.

**Table 2** Ca, Sr and Ba concentrations and Sr/Ca, Ba/Ca and Sr/Ba ratios (mean±SD) in leaves, twigs and bole wood of *Fagus sylvatica* and *Quercus robur*

Tree species and sites	Leaves						Twigs						Bole wood							
	n	Ca	Sr	Ba	Sr/Ca	Ba/Ca	n	Ca	Sr	Ba	Sr/Ba	Ba/Ca	n	Ca	Sr	Ba	Sr/Ca	Ba/Ca	Sr/Ba	
		µg g <sup>-1</sup>		µg g <sup>-1</sup>	g g <sup>-1</sup> ±SD	10 <sup>-4</sup>		µg g <sup>-1</sup>		µg g <sup>-1</sup>	g g <sup>-1</sup> ±SD	10 <sup>-4</sup>		µg g <sup>-1</sup>		µg g <sup>-1</sup>	g g <sup>-1</sup> ±SD	10 <sup>-4</sup>	g g <sup>-1</sup> ±SD	
<i>F. sylvatica</i>																				
MES	10	8,500±1,600	12.1±2.7	22.8±8.1	0.0014±1	0.0027±8	10	4,500±700	14.4±3.0	38.1±10.0	0.56±0.13	0.0027±3	0.0085±19	5	790±50	4.0±0.2	11.4±2.0	0.0050±1	0.0144±21	0.36±0.06
TUM	5	7,900±700	13.9±2.5	22.9±2.4	0.0018±2	0.0029±2	3	3,900±500	13.3±2.6	33.6±12.1	0.61±0.12	0.0034±3	0.0087±29	3	850±0.11	4.0±0.7	12.4±1.8	0.0049±7	0.0151±26	0.33±0.02
REL II	5	6,300±1,300	9.7±1.8	18.6±5.7	0.0016±1	0.0029±3	3	5,800±700	20.9±4.9	48.8±17.9	0.54±0.09	0.0036±4	0.0083±22	3	250±0.12	2.6±0.26	8.7±0.3	0.0049±0.03	0.0167±0.3	0.3
PIG	5	9,200±2,700	16.3±5.4	33.0±10.2	0.0018±2	0.0036±6	3	5,000±400	18.6±1.8	72.5±11.8	0.51±0.12	0.0037±5	0.0145±24	1	521±0.09	2.6±0.8	8.7±0.3	0.0049±0.03	0.0167±0.3	0.3
TAM	5	6,900±1,200	9.5±2.2	20.3±3.4	0.0014±1	0.0030±8	1	2,783±9.3	9.3	29.9	0.48±0.14	0.0033	0.0108	3.1	0.0037±0.12	0.145±0.12	0.48±0.14	0.0033	0.0108	3.1
REL III	5	7,800±1,200	10.5±2.5	18.2±5.4	0.0013±2	0.0023±5	4	3,100±200	11.2±1.5	34.7±7.5	0.61±0.19	0.0037±7	0.0113±24	3	640±0.55	3.5±0.4	7.5±0.8	0.0055±3	0.0118±15	0.47±0.05
CTE I	5	7,200±1,800	14.2±4.6	22.2±8.2	0.0020±4	0.0031±8	5	4,900±1,200	19.1±5.6	35.0±10.6	0.66±0.13	0.0038±4	0.0071±13	3	100±0.09	0.4±0.3	0.8±0.3	0.0055±3	0.0118±15	0.47±0.05
CTE II	5	6,300±2,000	12.8±3.1	22.7±10.1	0.0021±3	0.0035±5	1	0.61±0.16			0.61±0.16			1	814±0.65	3.1±0.32	10.7±0.29	0.0039±0.032	0.0132±0.29	0.29
HUB I	5	6,800±900	11.1±2.5	18.7±5.4	0.0016±3	0.0027±5	5	5,400±800	16.0±2.9	28.4±12.1	0.70±0.40	0.0028±9	0.0088±19	1	814±0.65	3.1±0.32	10.7±0.29	0.0039±0.032	0.0132±0.29	0.29
HUB II	5	8,000±300	13.3±1.7	22.3±7.5	0.0017±2	0.0028±9	5	5,400±800	16.0±2.9	28.4±12.1	0.70±0.40	0.0028±9	0.0088±19	1	814±0.65	3.1±0.32	10.7±0.29	0.0039±0.032	0.0132±0.29	0.29
MLN	5	6,400±1,700	9.8±3.9	15.2±8.2	0.0015±3	0.0023±8	3	4,100±800	17.2±5.5	60.6±14.2	0.69±0.14	0.0032±10	0.0088±19	1	814±0.65	3.1±0.32	10.7±0.29	0.0039±0.032	0.0132±0.29	0.29
TER	5	7,700±1,300	12.4±3.2	24.8±10.0	0.0016±3	0.0032±10	5	4,900±1,100	16.2±4.2	43.5±13.6	0.54±0.14	0.0032±10	0.0088±19	1	814±0.65	3.1±0.32	10.7±0.29	0.0039±0.032	0.0132±0.29	0.29
CLO	2	6,400±200	13.4±1.3	21.9±3.3	0.0021±1	0.0034±4	2	5,300±400	19.6±0.2	66.3±13.5	0.61±0.03	0.0034±4	0.0126±35	3	660±0.53	3.5±0.6	9.7±1.0	0.0055±0.04	0.0147±0.40	0.41
CHE	5	5,800±800	8.4±1.1	16.9±2.7	0.0015±2	0.0030±8	5	3,500±800	12.2±3.3	41.1±15.7	0.51±0.11	0.0034±5	0.0119±38	1	756±0.31	4.3±0.10	10.4±0.6	0.0057±0.0137	0.0137±0.41	0.41
FNT	5	15,200±7,200	83.6±37.9	14.4±7.3	0.0055±2	0.0010±5	5	7,800±1,300	72.9±12.9	11.1±3.6	6.50±2.36	0.0015±8	0.0094±5	1	1,072±7.33	11.4±3.05	1.7±0.6	0.0106±0.0016	0.0016±0.6	6.56
DIEP	5	7,100±1,500	9.5±2.1	24.1±2.5	0.0013±2	0.0035±6	3	4,900±1,100	14.5±2.4	42.1±12.6	0.39±0.07	0.0035±6	0.0089±31	5	540±0.36	2.5±0.5	6.3±1.9	0.0048±0.0055	0.0118±0.22	0.08
LND	5	5,400±1,300	9.0±1.4	13.2±4.2	0.0017±4	0.0024±3	5	3,400±1,000	13.4±3.6	25.5±5.8	0.73±0.23	0.0041±11	0.0077±12	3	660±0.53	3.5±0.6	9.7±1.0	0.0055±0.0047	0.0147±0.39	0.39
POUR	16	6,000±1,700	11.3±3.5	16.1±8.3	0.0020±7	0.0027±8	5	3,400±800	16.1±4.2	44.8±12.6	0.77±0.21	0.0027±8	0.0048±8	4	500±0.36	3.4±0.3	9.7±1.0	0.0068±0.0068	0.0203±0.36	0.36
SMD	10	5,500±2,000	14.6±4.9	26.2±15.8	0.0027±3	0.0046±9	5	2,500±400	16.8±1.9	35.4±7.4	0.61±0.12	0.0046±9	0.0145±45	4	430±0.50	3.0±0.4	10.8±2.4	0.0069±0.0069	0.0251±0.28	0.28
MEIX	10	8,300±2,500	8.0±2.0	23.4±6.4	0.0010±2	0.0029±6	4	0.35±0.05			0.35±0.05			1	0.17±0.17	0.4±0.4	2.4±0.2	0.0069±0.0069	0.0251±0.28	0.28

Table 2 (continued)

Tree species and sites	Leaves						Twigs						Bole wood							
	n	Ca	Sr	Ba	Sr/Ca	Ba/Ca	n	Ca	Sr	Ba	Sr/Ca	Ba/Ca	n	Ca	Sr	Ba	Sr/Ca	Ba/Ca	Sr/Ba	
	µg g <sup>-1</sup>	µg g <sup>-1</sup>	µg g <sup>-1</sup>	µg g <sup>-1</sup>	g g <sup>-1</sup> ±SD	g g <sup>-1</sup> ±SD×10 <sup>-4</sup>	µg g <sup>-1</sup>	µg g <sup>-1</sup>	µg g <sup>-1</sup>	µg g <sup>-1</sup>	g g <sup>-1</sup> ±SD	g g <sup>-1</sup> ±SD×10 <sup>-4</sup>	µg g <sup>-1</sup>	µg g <sup>-1</sup>	µg g <sup>-1</sup>	µg g <sup>-1</sup>	g g <sup>-1</sup> ±SD	g g <sup>-1</sup> ±SD×10 <sup>-4</sup>	g g <sup>-1</sup> ±SD	
COT I	5	16,900±4,700	15.0±4.1	28.2±9.0	0.00089±0	0.0009±1	5	12,600±2,000	18.2±2.6	13.7±3.2	0.0015±0.4	0.0011±2	5	710±40	2.1±0.1	3.2±0.6	0.0030±1	0.0044±6	0.69±0.10	
COT II	5	10,300±1,500	10.5±1.5	21.3±2.0	0.0010±1	0.0021±4	5	5,500±1,300	10.4±2.1	23.9±4.2	0.0019±2	0.0045±12	5	560±280	2.1±0.6	6.8±1.2	0.0041±11	0.0141±52	0.32±0.11	
MUS																				
<i>Q. robur</i>																				
MES	5	10,100±1,000	6.8±1.9	23.4±10.1	0.0007±2	0.0023±8	5	5,500±1,600	8.9±1.6	25.3±5.9	0.0017±6	0.0047±11	5	560±280	2.1±0.6	6.8±1.2	0.0041±11	0.0141±52	0.32±0.11	
PIG	3	6,800±2,200	5.8±1.3	21.9±9.5	0.0009±1	0.0033±11	3	4,900±700	10.3±0.4	32.2±7.8	0.0021±3	0.0066±12	3	294±70	1.2±0.6	8.7±0.6	0.004±0.06	0.0227±0.18	0.18±0.06	
HUB II	3	5,300±400	3.9±1.1	9.1±4.2	0.0007±3	0.0017±7	2	3,900±900	10.0±2.6	23.8±15.8	0.0027±13	0.0058±27	2	356±13	2.1±0.49	6.9±0.49	0.0059±0.13	0.0190±0.30	0.30±0.13	
CLO	5	6,800±1,000	8.3±1.4	18.5±5.6	0.0012±1	0.0027±8	5	4,000±500	12.2±0.7	48.2±12.9	0.0031±4	0.0123±40	5	278±12.9	1.5±0.06	9.5±0.06	0.0054±0.0343	0.0343±0.16	0.16±0.06	
CHE	5	7,500±600	4.4±1.0	15.4±3.1	0.0006±1	0.0020±3	5	4,300±1,000	6.9±1.5	23.6±6.0	0.0016±3	0.0056±10	5	170±13	1.4±0.13	6.9±0.13	0.0059±0.04	0.0190±0.04	0.04±0.13	
BEQ	10	7,600±1,400	6.5±2.0	22.2±8.1	0.0009±2	0.0028±7	5	4,100±1,100	8.2±3.3	37.5±11.7	0.0019±3	0.0091±19	5	356±19	2.1±0.04	6.9±0.04	0.0059±0.04	0.0190±0.04	0.04±0.13	
POUR	5	4,100±500	4.0±1.1	12.2±5.6	0.0010±3	0.0029±13	5	3,800±700	11.2±3.9	40.4±14.8	0.0029±7	0.0105±34	5	170±50	1.4±0.4	4.9±1.4	0.0081±7	0.0301±109	0.31±0.13	
SMD	6	4,200±800	5.8±1.6	14.1±4.9	0.0014±3	0.0033±7	5	3,100±1,200	11.8±5.0	41.5±17.5	0.0037±8	0.0131±33	5	420±80	3.0±0.5	9.7±2.1	0.0071±8	0.0234±55	0.31±0.05	
MEIX	10	4,700±1,500	3.2±1.0	17.8±5.9	0.0007±2	0.0039±11														
COT II	5	9,400±2,700	5.4±1.9	47.5±12.94	0.0006±1	0.0052±10	5	4,700±700	5.9±1.0	43.7±15.3	0.0013±2	0.0095±35	5	153±700	0.15±0.07	5.9±0.07	0.0095±0.07	0.015±0.07	0.07±0.07	

n number of samples



**Table 3** Discrimination factors (DF) between the organs of *Fagus sylvatica*

Sites	DF Sr/Ca				DF Ba/Ca				DF Sr/Ba			
	Bole wood → Leaves	Twigs → Leaves	Bole wood → Twigs	Bole wood → Bark	Bole wood → Leaves	Twigs → Leaves	Bole wood → Twigs	Bole wood → Bark	Bole wood → Leaves	Twigs → Leaves	Bole wood → Twigs	Bole wood → Bark
MES	3.52	2.25	1.57	2.52	5.37	3.17	1.70	2.92	0.68	0.71	0.95	0.87
TUM	2.78	1.95	1.39	1.64	5.19	3.00	1.73	0.67	0.54	0.65	0.82	2.00
REL II		2.33				2.86				0.82		
PIG	2.76	2.09	1.34	1.63	4.64	4.02	1.15	1.35	0.61	0.52	1.17	1.25
TAM		2.44				3.60				0.66		
REL III		2.73				4.87				0.56		
CTE I	2.77	1.96	1.42		3.80	2.30	1.65		0.73	0.85	0.86	
CTE II												
HUB II	2.30	1.78	1.29		1.78	1.89	0.93		0.49	0.94	0.52	
MLN		2.73				6.42				0.44		
CLO		1.77				3.69				0.48		
CHE	3.87	2.35	1.68		4.57	3.97	1.15		0.82	0.59	1.39	
FNT	1.93	1.71	0.88	1.63	1.60	1.50	1.07	1.14	1.13	1.13	1.00	1.36
DIEP	3.48	2.21	1.57	1.88	3.37	2.55	1.32	2.51	1.04	0.87	1.19	0.68
LND	3.21	2.39	1.34	1.25	6.06	3.18	1.91	0.64	0.57	0.77	0.74	2.05
POUR	3.60	2.54	1.42	2.26	7.54	4.94	1.53	2.93	0.52	0.51	1.01	0.83
SMD	2.68	2.52	1.06	1.68	5.19	3.16	1.64	1.42	0.56	0.85	0.66	1.18
COT I		1.63				1.21				2.50		
COT II		1.87				2.18				0.89		
MUS				2.62				3.17				0.82
Mediane	2.78	2.23	1.39	1.68	4.64	3.16	1.53	1.42	0.61	0.74	0.95	1.18
Mean	2.99	2.18	1.36	1.90	4.47	3.25	1.44	1.86	0.70	0.82	0.94	1.23
SD	0.60	0.35	0.23	0.46	1.76	1.30	0.32	1.02	0.22	0.46	0.25	0.51
CV (%)	19.9	16.1	16.8	24.2	39.4	40.1	22.5	54.7	30.9	55.9	27.1	41.2

The effect of the route from the conducting vessels (leaf veins) to parenchyma tissue on alkaline-earth proportions appears clearly in Table 8. We observe a highly significant difference in the Sr/Ca ratios between leaf veins and limb in beech ( $Sr/Ca = 0.0031 \pm 0.0004$  and  $0.0014 \pm 0.0002$ ) and in oak trees ( $Sr/Ca = 0.0015 \pm 0.0004$  and  $0.0006 \pm 0.0002$ , mean  $\pm$  SD). On the other hand, values of Sr/Ca ratios are very close between twigs and leaf conducting vessels both for beech ( $Sr/Ca = 0.0032 \pm 0.0003$  and  $0.0031 \pm 0.0004$ , respectively, mean  $\pm$  SD) and oak ( $Sr/Ca = 0.0017 \pm 0.0006$  and  $0.0015 \pm 0.0004$ , respectively). This shows clearly that Sr discrimination against Ca between twigs and leaves operates in the passage of the elements through the endoderm which separates conducting vessels (perivascular bundle) from the leaf mesophyll. Gierth et al. (1998) reported similar Sr accumulation over Ca in leaf endoderm of *Larix decidua* Mill. As a complementary mechanism,

Poszwa et al. (2000) hypothesized preferential leaching of Sr from leaves relative to Ca, which could also diminish the proportion of Sr in the mesophyll.

Sr is discriminated against Ca from bole wood to bark ( $DF_{W-B}^{Sr/Ca} = 1.90 \pm 0.46$  and  $2.24 \pm 0.28$  for beech and oak, respectively). The intensity of this discrimination is not significantly different between species. We also observe large difference of concentration levels and element ratios between twigs wood and twigs bark (Table 7). Partition between Sr and Ca occurs through the entry of these elements in the bark of the twigs. The consequence is a relative enrichment in Ca in the bark of this organ at the expense of Sr and, conversely, an accumulation of Sr in the wood of twigs. Consequently, we observe a diminution of the Sr/Ca ratio between bole wood and twigs (including wood and bark) from 0.0050 to 0.0032 for beech of the MES site (Table 2). However, if we only consider the wood of twigs without bark, Sr/Ca ratio change

**Table 4** Discrimination factors (DF) between the organs of *Quercus robur*

Sites	DF Sr/Ca				DF Ba/Ca				DF Sr/Ba			
	Bole wood → Leaves	Twigs → Leaves	Bole wood → Twigs	Bole wood → Bark	Bole wood → Leaves	Twigs → Leaves	Bole wood → Twigs	Bole wood → Bark	Bole wood → Leaves	Twigs → Leaves	Bole wood → Twigs	Bole wood → Bark
MES	6.09	2.53	2.41	2.49	6.09	2.03	3.00	4.76	0.97	1.21	0.86	0.55
PIG	4.54	2.43	1.87	1.82	6.88	2.00	3.43	1.41	0.62	1.21	0.53	1.29
HUB II		3.65				3.36				0.97		
CLO		2.56				4.55				0.56		
CHE	9.18	2.74	3.35	2.44	16.76	2.74	6.12	2.23	0.52	1.02	0.50	1.09
BEQ	6.56	2.11	3.11		6.79	3.25	2.09		0.97	0.75	1.36	
POUR	8.31	2.99	2.77	2.09	10.18	3.56	2.86	4.11	0.94	0.84	1.12	0.59
SMD	4.59	2.71	1.69	2.37	6.13	3.94	1.55	7.07	0.73	0.69	1.07	0.34
COT II		2.26				1.83				1.19		
Mediane	6.32	2.56	2.59	2.37	6.83	3.25	2.93	4.11	0.84	0.97	0.97	0.59
Mean	6.54	2.66	2.53	2.24	8.80	3.03	3.18	3.92	0.79	0.94	0.91	0.77
SD	1.90	0.45	0.67	0.28	4.18	0.95	1.59	2.23	0.19	0.24	0.34	0.40
CV (%)	29.1	17.0	26.3	12.5	47.5	31.2	50.2	56.9	24.5	25.6	37.5	52.4

**Table 5** Evolution of the Sr/Ca, Ba/Ca and Sr/Ba ratios (Mean±S.D.) in the main compartments of two forest ecosystems in central (MES) and high Belgium (POUR)

	MES site				POUR site			
	<i>n</i>	Sr/Ca g g <sup>-1</sup>	Ba/Ca	Sr/Ba	<i>n</i>	Sr/Ca g g <sup>-1</sup>	Ba/Ca	Sr/Ba
Bulk precipitation	17	0.0032±0.0008	0.0030±0.0023	1.71±1.33	14	0.0024±0.0010	0.0026±0.0018	1.11±0.34
Throughfall	9	0.0028±0.0002	0.0020±0.0004	1.42±0.21	–	–	–	–
Soil solution								
A <sub>h</sub> horizon	10	0.0039±0.0003	0.0065±0.0022	0.65±0.16	–	–	–	–
E or A <sub>h</sub> B horizon	9	0.0057±0.0009	0.0239±0.0100	0.32±0.21	5	0.0075±0.0019	0.0375±0.0105	0.20±0.03
B <sub>t</sub> or B <sub>w</sub> horizon	9	0.0066±0.0006	0.0148±0.0031	0.46±0.08	7	0.0102±0.0015	0.0956±0.0444	0.14±0.10
Exchangeable fraction								
A <sub>h</sub> horizon	6	0.0054±0.0012	0.0152±0.0058	0.38±0.09	–	–	–	–
E or A <sub>h</sub> B horizon	4	0.0030±0.0008	0.0575±0.0299	0.06±0.02	5	0.0078±0.0028	0.1132±0.0266	0.05±0.02
B <sub>t</sub> or B <sub>w</sub> horizon	5	0.0063±0.0008	0.1656±0.0538	0.04±0.02	5	0.0094±0.0018	0.5673±0.2301	0.02±0.01
Spring water	–	–	–	–	17	0.0097±0.0005	0.0007±0.0004	17.06±8.13
Stream water	–	–	–	–	18	0.0060±0.0002	0.0007±0.0005	10.77±4.76
<i>Fagus sylvatica</i>								
Roots	9	0.0053±0.0010 <sup>a</sup>	0.0170±0.0056 <sup>a</sup>	0.34±0.11 <sup>ab</sup>	2	0.0076±0.0012	0.0281±0.0096	0.28±0.05
Bole wood	16	0.0049±0.0003 <sup>a</sup>	0.0134±0.0064 <sup>a</sup>	0.34±0.08 <sup>a</sup>	21	0.0063±0.0005 <sup>a</sup>	0.0193±0.0046 <sup>a</sup>	0.35±0.09 <sup>a</sup>
Bole bark	4	0.0020±0.0002 <sup>b</sup>	0.0049±0.0010 <sup>ab</sup>	0.42±0.10 <sup>abc</sup>	4	0.0030±0.0004 <sup>b</sup>	0.0069±0.0013 <sup>bc</sup>	0.44±0.02 <sup>a</sup>
Twigs	10	0.0032±0.0003 <sup>c</sup>	0.0085±0.0019 <sup>b</sup>	0.39±0.09 <sup>c</sup>	5	0.0048±0.0008 <sup>c</sup>	0.0133±0.0020 <sup>b</sup>	0.36±0.03 <sup>a</sup>
Leaves	15	0.0014±0.0001 <sup>b</sup>	0.0027±0.0007 <sup>b</sup>	0.57±0.12 <sup>bc</sup>	15	0.0018±0.0004 <sup>d</sup>	0.0026±0.0008 <sup>c</sup>	0.75±0.19 <sup>b</sup>
Plantlets	10	0.0018±0.0001	0.0036±0.0006	0.51±0.07	5	0.0015±0.0002	0.0024±0.0004	0.63±0.04
Leave litter	5	0.0017±0.0001	0.0031±0.0003	0.56±0.06	6	0.0023±0.0002	0.0033±0.0004	0.71±0.05

Distinct superscripted letters in a same row indicate significant difference between mean values of tree's organs ( $P < 0.05$ , Multiple comparison of mean ranks)

**Table 6** Spearman rank correlation coefficients ( $r_s$ ) between Ca, Sr and Ba concentrations in the main compartment of two forest ecosystems of central (MES) and high Belgium (POUR)

	MES site				POUR site			
	<i>n</i>	Sr vs. Ca	Ba vs. Ca	Sr vs. Ba	<i>n</i>	Sr vs. Ca	Ba vs. Ca	Sr vs. Ba
Bulk precipitation	17	0.892***	0.495*	0.652**	14	0.807***	0.480	0.757**
Throughfall	9	1.000***	0.905**	0.905**	–	–	–	–
Soil solution								
A <sub>h</sub> horizon	10	1.000***	0.927***	0.927***	–	–	–	–
E or A <sub>h</sub> B horizon	9	0.983***	0.133	0.167	5	0.900*	0.900*	1.000***
B <sub>t</sub> or B <sub>w</sub> horizon	9	0.900***	0.200	0.417	7	1.000***	0.821*	0.821*
Exchangeable fraction								
A <sub>h</sub> horizon	6	0.714	0.257	0.200	–	–	–	–
E or A <sub>h</sub> B horizon	4	0.600	–0.800	–0.800	5	0.900*	–0.300	–0.100
B <sub>t</sub> or B <sub>w</sub> horizon	5	1.000***	0.900*	0.900*	5	0.700	–0.100	–0.500
Spring water	–	–	–	–	17	0.926***	0.240	0.196
Stream water	–	–	–	–	18	0.963***	0.120	0.200
<i>Fagus sylvatica</i>								
Roots	9	0.933***	0.833**	0.867**	2	NA	NA	NA
Bole wood	16	0.956***	0.747***	0.741***	21	0.891***	0.826***	0.916***
Bole bark	4	0.800	0.600	0.000	4	0.200	0.200	1.000***
Twigs	10	0.879***	0.515	0.576	5	0.500	0.800	0.900*
Leaves	15	0.879***	0.610*	0.797***	15	0.543*	0.561*	0.900***
Plantlets	10	0.806**	0.107	0.903***	5	0.700	0.600	0.900*
Leave litter	5	0.900*	0.600	0.700	6	0.700	0.700	1.000***

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; NA not applicable because of reduced effect.

from 0.0050 in the bole wood to a value of 0.0088 in the twigs wood (Table 7). This trend is even more marked for oak. Such high value is not present in other organs of the tree.

Overall, from bole wood (or roots) to leaves, the Ca discrimination process against Sr is twofold larger for oak ( $DF_{W-L}^{Sr/Ca} = 6.54 \pm 1.90$ ) than for beech ( $DF_{W-L}^{Sr/Ca} = 2.99 \pm 0.60$ ). In beech tree,  $38 \pm 17\%$  ( $n=11$ ) of this discrimination occurs between bole wood and twigs and  $62 \pm 17\%$  ( $n=18$ ) between twigs and leaves. Inversely, in oak tree, this discrimination mainly occurs from the bole wood to twigs ( $69 \pm 11\%$ , mean  $\pm$  SD,  $n=6$ ). This last proportion is significantly different from that of beech (unpaired  $t$ -test,  $P=0.001$ ).

#### Ba–Ca discrimination

For each species, the main Ba–Ca discrimination occurs in the same organs than that of Sr and following similar proportions from bole wood to twigs ( $35 \pm 19\%$  for beech and  $71 \pm 17\%$  for oak, mean  $\pm$  SD) and from twigs to leaves ( $65 \pm 19\%$  for beech,  $29 \pm 17\%$  for oak; Fig. 1b, e, h). These contrasting proportions between species are signifi-

cantly different ( $t$ -test,  $P=0.002$ ). The difference of total Ba segregation from bole wood to leaves is distinct between beech and oak ( $DF_{W-L}^{Ba/Ca} = 4.47 \pm 1.76$ ;  $n=11$ ; and  $8.80 \pm 4.18$ ;  $n=6$ , respectively). The pathway from bole wood to bark seems to have a contrasting effect between beech and oak on the Ba/Ca ratio. We observe statistically different DF between the two species ( $DF_{W-B}^{Ba/Ca} = 1.86 \pm 1.02$  for beech and  $3.92 \pm 2.23$  for oak, mean  $\pm$  SD, unpaired  $t$ -test,  $P=0.033$ ).

#### Sr–Ba discrimination

For the whole stands studied, beech trees are globally less discriminating for Sr than for Ba ( $DF_{W-L}^{Sr/Ba} = 0.70 \pm 0.22$ ; mean  $\pm$  SD;  $n=11$ ; Fig. 1c, f, i). But carbonated substrates seem to favour Ba at the expense of Sr, particularly from twigs to leaves ( $DF_{T-L}^{Sr/Ba} = 1.13$  and  $2.50$  in FNT and COT I sites, respectively, for a mean of all stands equal to  $0.82 \pm 0.46$ ;  $n=18$ ). Oak discriminates Ba over Sr from bole to twigs ( $DF_{W-T}^{Sr/Ba} = 0.88 \pm 0.25$ ) and is equally discriminating from twigs to leaves ( $DF_{T-L}^{Sr/Ba} = 0.94 \pm 0.24$ ; mean  $\pm$  SD). However, for the two species, segregation is larger for Ba than for Sr in the endoderm: from the

**Table 7** Ca, Sr and Ba concentrations ( $\mu\text{g g}^{-1}$ ) and ponderal element ratios Sr/Ca, Ba/Ca and Sr/Ba (mean $\pm$ SD) in leaves, mesophyll and veins and in twigs, stripped twigs and bark of twigs of *Fagus sylvatica* and *Quercus robur* in a site of central Belgium (MES)

	<i>n</i>	Ca $\mu\text{g g}^{-1}$	Sr	Ba	Sr/Ca $\text{g g}^{-1}$	Ba/Ca	Sr/Ba
<i>F. sylvatica</i>							
Leaves	7	9,800 $\pm$ 2,300 <sup>a</sup>	16.9 $\pm$ 5.1 <sup>a</sup>	30.0 $\pm$ 9.6 <sup>a</sup>	0.0017 $\pm$ 0.0002 <sup>a</sup>	0.0031 $\pm$ 0.0004 <sup>a</sup>	0.57 $\pm$ 0.07 <sup>ab</sup>
Limb	7	9,700 $\pm$ 2,000 <sup>a</sup>	13.7 $\pm$ 4.6 <sup>a</sup>	18.1 $\pm$ 9.0 <sup>a</sup>	0.0014 $\pm$ 0.0002 <sup>a</sup>	0.0018 $\pm$ 0.0007 <sup>a</sup>	0.85 $\pm$ 0.31 <sup>a</sup>
Leaf veins	7	11,300 $\pm$ 1,500 <sup>a</sup>	35.4 $\pm$ 6.0 <sup>b</sup>	71.2 $\pm$ 25.1 <sup>b</sup>	0.0031 $\pm$ 0.0004 <sup>b</sup>	0.0063 $\pm$ 0.0021 <sup>b</sup>	0.54 $\pm$ 0.16 <sup>b</sup>
<i>Q. robur</i>							
Leaves	7	3,900 $\pm$ 500 <sup>a</sup>	3.4 $\pm$ 1.4 <sup>ab</sup>	8.2 $\pm$ 3.0 <sup>a</sup>	0.0008 $\pm$ 0.0002 <sup>a</sup>	0.0021 $\pm$ 0.0007 <sup>a</sup>	0.43 $\pm$ 0.14 <sup>ab</sup>
Limb	6	4,000 $\pm$ 600 <sup>a</sup>	2.4 $\pm$ 0.7 <sup>a</sup>	4.2 $\pm$ 2.6 <sup>b</sup>	0.0006 $\pm$ 0.0002 <sup>a</sup>	0.0011 $\pm$ 0.0007 <sup>a</sup>	0.71 $\pm$ 0.34 <sup>a</sup>
Leaf veins	4	3,800 $\pm$ 800 <sup>a</sup>	5.8 $\pm$ 2.3 <sup>b</sup>	26.8 $\pm$ 15.3 <sup>c</sup>	0.0015 $\pm$ 0.0004 <sup>b</sup>	0.0068 $\pm$ 0.0028 <sup>b</sup>	0.25 $\pm$ 0.12 <sup>b</sup>
<i>F. sylvatica</i>							
Twigs	10	4,500 $\pm$ 700 <sup>a</sup>	14.4 $\pm$ 3.0 <sup>a</sup>	38.1 $\pm$ 10.0 <sup>a</sup>	0.0032 $\pm$ 0.0003 <sup>a</sup>	0.0085 $\pm$ 0.0019 <sup>a</sup>	0.39 $\pm$ 0.09 <sup>a</sup>
Stripped twigs	2	1,300 $\pm$ 400 <sup>b</sup>	11.5 $\pm$ 2.7 <sup>a</sup>	6.5 $\pm$ 2.1 <sup>b</sup>	0.0088 $\pm$ 0.0009 <sup>b</sup>	0.0049 $\pm$ 0.0000 <sup>a</sup>	1.81 $\pm$ 0.18 <sup>b</sup>
Twigs bark	3	18,900 $\pm$ 14,500 <sup>c</sup>	43.8 $\pm$ 11.9 <sup>b</sup>	65.0 $\pm$ 8.0 <sup>c</sup>	0.0031 $\pm$ 0.0018 <sup>a</sup>	0.0048 $\pm$ 0.0026 <sup>a</sup>	0.67 $\pm$ 0.11 <sup>c</sup>
<i>Q. robur</i>							
Twigs	5	5,550 $\pm$ 1,560 <sup>a</sup>	8.9 $\pm$ 1.6 <sup>a</sup>	25.3 $\pm$ 5.9 <sup>a</sup>	0.0017 $\pm$ 0.0006 <sup>a</sup>	0.0047 $\pm$ 0.0011 <sup>ab</sup>	0.37 $\pm$ 0.11 <sup>a</sup>
Stripped twigs	6	1,230 $\pm$ 650 <sup>b</sup>	18.9 $\pm$ 12.2 <sup>a</sup>	11.0 $\pm$ 6.4 <sup>b</sup>	0.0176 $\pm$ 0.0143 <sup>b</sup>	0.0088 $\pm$ 0.0020 <sup>a</sup>	1.90 $\pm$ 1.22 <sup>b</sup>
Twigs bark	5	17,950 $\pm$ 3,780 <sup>c</sup>	34.9 $\pm$ 7.9 <sup>b</sup>	59.2 $\pm$ 20.6 <sup>c</sup>	0.0019 $\pm$ 0.0002 <sup>a</sup>	0.0033 $\pm$ 0.0008 <sup>b</sup>	0.63 $\pm$ 0.20 <sup>c</sup>

*n* number of samples

Different superscripted letters following numbers denote significant differences between the means ( $P < 0.05$ , Tukey HSD or unequal *n* HSD)

leaf veins to the limb, Sr/Ba ratio change from 0.54 $\pm$ 0.16 to 0.85 $\pm$ 0.31 for beech and from 0.25 $\pm$ 0.12 to 0.71 $\pm$ 0.34 for oak (Table 7). More marked difference of discrimination between the two species occurs in the pathway from stemwood to bark ( $DF_{W-B}^{Sr/Ba} = 1.23 \pm 0.51$  and 0.77 $\pm$ 0.40 for beech,  $n = 9$  and oak,  $n = 5$ , respectively; mean $\pm$ SD, Tables 3

and 4). Table 8 also shows Sr–Ba segregation between wood and bark of the twigs.

In conclusion, both for beech and oak, Sr and Ba accumulate in the wood of twigs whereas Ca accumulates in leaf mesophyll. The consequence of such process on the cycle of alkaline-earth elements will be treated in the following paragraph.

**Table 8** Effect of the leaf litter fragmentation and mineralisation on the Ca, Sr and Ba concentrations and the element ratios (Mean $\pm$ SD) within a beechwood of central Belgium (MES)

	<i>n</i>	Ca $\mu\text{g g}^{-1}$	Sr	Ba	Sr/Ca $\text{g g}^{-1}$	Ba/Ca	Sr/Ba
Beech leaves	10	8,500 $\pm$ 1,600 <sup>a</sup>	12.1 $\pm$ 2.7 <sup>a</sup>	22.8 $\pm$ 8.1 <sup>a</sup>	0.0014 $\pm$ 0.0001 <sup>a</sup>	0.0027 $\pm$ 0.0008 <sup>a</sup>	0.56 $\pm$ 0.13 <sup>ab</sup>
O <sub>l</sub> horizon	5	9,700 $\pm$ 1,000 <sup>ab</sup>	16.8 $\pm$ 2.2 <sup>a</sup>	30.2 $\pm$ 5.3 <sup>a</sup>	0.0017 $\pm$ 0.0001 <sup>b</sup>	0.0031 $\pm$ 0.0003 <sup>a</sup>	0.56 $\pm$ 0.06 <sup>ab</sup>
O <sub>f</sub> horizon	5	12,100 $\pm$ 1,600 <sup>b</sup>	32.8 $\pm$ 3.9 <sup>b</sup>	71.4 $\pm$ 9.0 <sup>b</sup>	0.0027 $\pm$ 0.0002 <sup>c</sup>	0.0059 $\pm$ 0.0004 <sup>b</sup>	0.46 $\pm$ 0.02 <sup>abc</sup>
O <sub>h</sub> horizon	5	7,200 $\pm$ 3,000 <sup>a</sup>	28.1 $\pm$ 5.7 <sup>b</sup>	73.5 $\pm$ 17.2 <sup>b</sup>	0.0043 $\pm$ 0.0013 <sup>d</sup>	0.0113 $\pm$ 0.0039 <sup>cd</sup>	0.39 $\pm$ 0.05 <sup>ac</sup>
Soil solution A <sub>h</sub> horizon <sup>c</sup>	13	4,100 $\pm$ 900 <sup>f</sup>	15.8 $\pm$ 2.7 <sup>f</sup>	22.5 $\pm$ 7.7 <sup>f</sup>	0.0040 $\pm$ 0.0003 <sup>d</sup>	0.0062 $\pm$ 0.0021 <sup>bc</sup>	0.70 $\pm$ 0.19 <sup>b</sup>
Exchangeable fraction A <sub>h</sub> horizon	6	220 $\pm$ 60 <sup>f</sup>	1.2 $\pm$ 0.3 <sup>f</sup>	3.1 $\pm$ 0.6 <sup>f</sup>	0.0054 $\pm$ 0.0012 <sup>d</sup>	0.0152 $\pm$ 0.0058 <sup>d</sup>	0.38 $\pm$ 0.09 <sup>c</sup>
Calcination A <sub>h</sub> horizon <sup>e</sup>	6	230 $\pm$ 70 <sup>f</sup>	1.5 $\pm$ 0.5 <sup>f</sup>	ND	0.0067 $\pm$ 0.0021 <sup>d</sup>	ND	ND
Significance		0.0027	<0.001	<0.001	<0.001	<0.001	<0.001

<sup>a,b,c,d</sup> Non-overlapping letters designate statistically significant differences between means within columns ( $P < 0.05$ ). The significance of the vegetation–soil compartment factor was determined by Kruskal–Wallis tests.

<sup>c</sup> Concentrations in  $\mu\text{g L}^{-1}$

<sup>f</sup> Not integrated into the statistical analyses.

<sup>e</sup> Calcination at 450°C followed by ashes dissolution in 0.1 M HCl (data from Drouet et al. 2007).

ND not determined.

The use of Sr/Ca, Ba/Ca and Sr/Ba element ratios as tracers of Ca sources in forest stands

It must be reminded that several conditions have to be met to allow the use of element ratios as tracers of Ca:

- (1) Elements (Ca, Sr and Ba) must have the same behaviour throughout the ecosystem. This implies that the composition of the soil exchange complex must reflect the relative proportions of the sources that supplied it and that these proportions are not or poorly influenced by preferential sorption phenomena, returns of the vegetation which have experienced Ca discrimination, or other processes.
- (2) Values of the element ratios must be sufficiently distinct between the sources so that the ratio measured in the vegetation reflects the relative proportions of each source.
- (3) To quantify the respective inputs of the sources, the measured element ratio in a given organ of the tree must be proportional to that of the soil during the trees uptake. Thus, the discrimination phenomenon must be a major obstacle to the use of element ratios as tracers of the Ca sources in forest. However, we have previously shown that the discrimination between Ca, Sr and Ba can be quantified by means of discrimination factors (DF). In a next step, we will attempt to determine to which extent these discrimination factors can be used to correct the Sr/Ca, Ba/Ca and Sr/Ba values measured in the leaves of the two species studied.

#### Ca, Sr and Ba behaviour in forest ecosystems

Three phenomena are susceptible to control the proportions between alkaline earth in the cycle of elements in a forest ecosystem, (a) the preferential adsorption to the surface of minerals and soil organic matter, (b) the discrimination into the vegetation which increases the relative proportion of Ca in the biomass restitutions relative to Sr and Ba (c) the mineralization effect on the proportion of elements in the humic horizon.

#### *Preferential adsorption on the exchange complex*

The comparison between element ratios in the soil solution and in the exchangeable fraction can be used to determine sorption selectivities on the exchange

complex (Veresoglou et al. 1995). For example, a higher Sr/Ca ratio in the exchangeable fraction compared to that of the soil solution indicates a selectivity of the exchanger for Sr relative to Ca. We therefore compared Sr/Ca Ba/Ca and Sr/Ba ratios of the exchangeable pool to that of the soil solution (Table 5) in two stands (MES and POUR sites). It should be noted that other processes as preferential flowpaths along roots or soil aggregates could also reach to a disequilibrium between the soil water and the exchanger. However, tension probes used in the field reduce macropore water sampling and then minimize the effect of this last process. In the Dystric cambisol (POUR), the exchange complex of the  $A_hB$  horizon, rich in organic matter (~14%) and that of the deeper mineral horizon ( $B_{1w}$ ) display the same selectivity for the three elements. Sr/Ca ratios are not significantly different between the soil solution and the exchangeable fraction (*t*-tests,  $P > 0.10$  for the two horizons). In contrast, Ba/Ca ratio is significantly higher in the exchangeable fraction (*t*-tests,  $A_hB$ :  $P = 0.001$ ;  $B_{1w}$ :  $P < 0.001$ ) and the Sr/Ba ratio significantly higher in the soil solution (*t*-tests,  $A_hB$ :  $P < 0.001$ ;  $B_{1w}$ :  $P = 0.021$ ). We can deduce from these observations that the selectivity of the exchanger follows the sequence  $Ba \gg Ca = Sr$  both for organic and mineral horizons. Soil selectivity for Ca, Sr and Ba cations is quite different in the acid leached soil of central Belgium (MES). In the hemiorganic  $A_h$  horizon, Sr/Ca and Ba/Ca ratios are significantly greater in the exchangeable fraction (unpaired *t*-tests,  $P = 0.002$  and  $0.007$ , respectively), Sr/Ba ratio is greater in the soil solution ( $P = 0.002$ ). So, we can conclude to a preference for ions of great radius:  $Ba \gg Sr \geq Ca$  in the  $A_h$  horizon. In the E and  $B_{2t}$  mineral horizons, Sr/Ca ( $P < 0.001$  and  $0.04$ ) and Sr/Ba ratios ( $P = 0.04$  and  $< 0.001$ ) are greater in the soil solution; in contrast, Ba/Ca ratios are greater in the exchangeable pool ( $P = 0.009$  and  $0.002$  for E and  $B_{2t}$  horizons, respectively). The differences between these two soil compartments point out the preferential sequence  $Ba \gg Ca > Sr$  for the mineral horizons.

So, the exchanger selectivity for Ba with respect to Ca and Sr is constant for all the horizons of the two soils studied. This can be explained by the large ionic radius of Ba (1.35 Å) relative to that of Ca (0.99 Å) and of Sr (1.13 Å); the solvation mantle of  $Ba^{2+}$  ion is less developed and permit a greater accessibility of this ion to soil exchange sites. This explanation

cannot be put forward for Sr as, excepted for the organic horizon of the MES site, exchange complex is either less selective for Sr compared to Ca (mineral horizons of the MES, Dystric podzoluvisol) or shows no preference for these elements (POUR, Dystric cambisol).

In the humic horizons, we found in one case no selectivity for Ca and Sr, and in another case, preferential sorption of Sr (and Ba) compared to Ca. This last observation is in line with that of Elias et al. (1982) showing that these elements tends also to be accumulated in humic horizons, arguing that organic matter forms more stable complexes with Sr and Ba. By contrast, higher selectivity of the soil organic matter for Ca was invoked in another study (Poszwa et al. 2000). Our results differs also from the selectivity of peat material observed by Baes and Bloom (1988) displaying a selectivity pattern as Ba > Ca > Sr, but their theoretical work reveals that the selectivity of the exchanger may be very different depending on the nature of the organic matter.

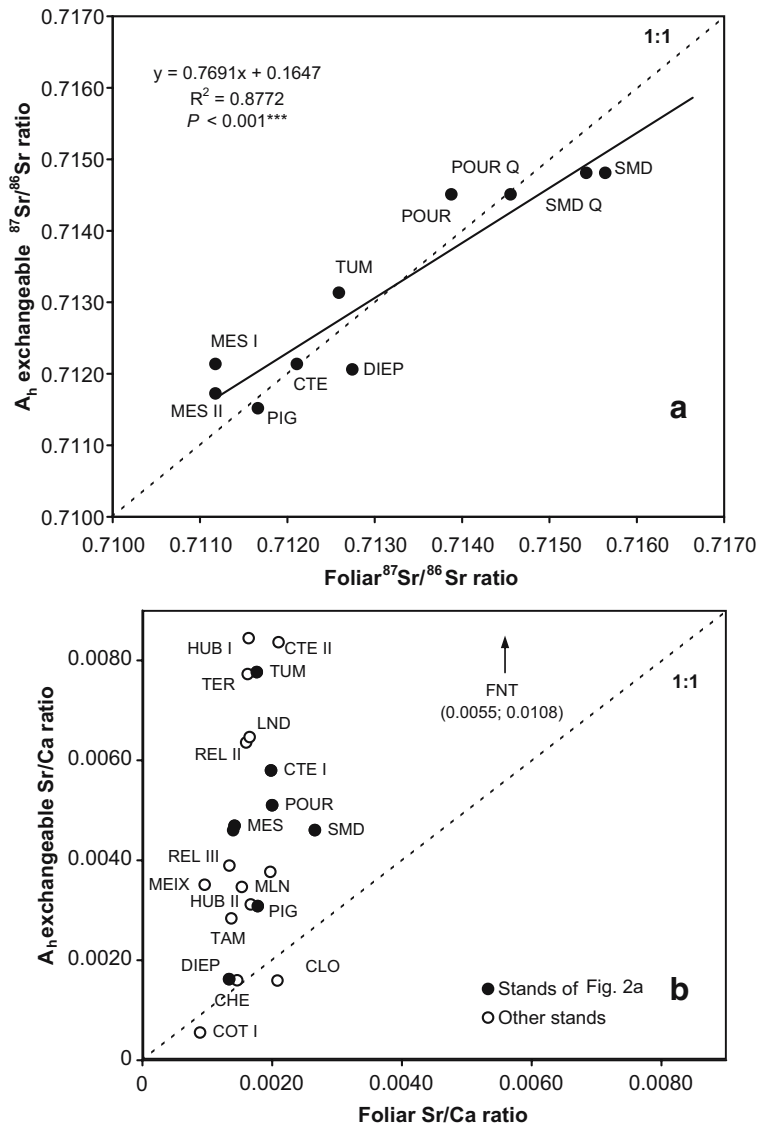
We can conclude that the selectivity of the exchange complex for these alkaline-earth elements may differ from a soil type to another and from horizon to another (humic or mineral). The selectivity of the exchanger is homologue in the Dystric cambisol and, in the Dystric podzoluvisol, Sr/Ca ratios between the soil solution and the exchangeable fraction are always of the same range, which indicates a common behaviour of Sr and Ca. In addition, we observe a high correlation between these elements in all the compartments of the two ecosystems (Table 6). By contrast, exchange selectivity for Ba is always clearly higher compared to that of Ca. This highlights the large difference of mobility between Ba and Ca and is in accordance with the weak correlations observed between the concentrations of these two elements in most of the compartments of the ecosystems studied, in particular precipitations, soil solution and soil exchangeable fraction (Table 6). Therefore, the use of Ba/Ca and Sr/Ba ratios as tracers of Ca must be made with care.

*Ca, Sr and Ba dynamics in holorganic and hemiorganic soil horizons: mineralization and OM selectivity effects*

The modifications of Sr/Ca, Ba/Ca and Sr/Ba ratios among tree leaves, leaf litter ( $O_1$ ), holorganic horizons

( $O_f$ ,  $O_h$ ) and the exchangeable fraction of the humic horizon ( $A_h$ ) are presented in Table 8. High level of Ca, Sr and Ba concentrations in leaves and holorganic horizons ( $O_f$ ,  $O_h$ ) suggests that the exchangeable fraction of the  $A_h$  horizon is mainly supplied by litterfall. Strontium isotopic measurements confirmed this fact for the MES beech stand, showing similar  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in leaves, exchangeable fraction and soil solution of this horizon (Drouet et al. 2007). The similar Sr isotopic composition between beech leaves and the exchangeable fraction of the  $A_h$  horizon also indicates that the progressive change of element ratios during the litter mineralization is not caused by an increasing influence of weathering-derived Sr with higher  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio. The effect of Ca biopurification by vegetation could favour Ca against Sr and Ba on the exchange complex, via the leaf returns. However, the opposite is observed (Table 8) and this explanation has to be excluded. The hypothesis of a barrier against Sr entry during the absorption by a mechanism of root selectivity can also be discarded. This could increase the proportions of Sr and Ba relative to Ca in the horizons concerned by plant uptake. However the Sr/Ca and Ba/Ca ratios of roots are very close to that measured in the exchangeable fraction of the humic horizons (Table 5), which does not support the hypothesis of preferential root absorption of Ca. Moreover, data from Tyler (2004) on beech trees shows close root selectivity between Sr and Ca (but a selectivity for Ba).

Strontium isotopes are not fractionated by exchange processes and constitute therefore a valuable criterion to determine the origin of Sr. Figure 2a shows a significant linear relationship between the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of the exchangeable fraction in the  $A_h$  horizon and that measured in beech and oak leaves in seven stands of central and high Belgium. The very close isotopic signatures of these two compartments of the ecosystem confirm that the litter restitutions are controlling the Sr (and Ca) supply of the hemiorganic horizons ( $A_h$ ). The lack of correlation between the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio in the exchangeable fraction of the mineral horizons (>10 cm depth) and that in tree leaves (data not shown) indicates that the influence of the biomass returns vanishes with depth. Contrary to the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios, we do not observe any correlation between the Sr/Ca ratio in the exchangeable fraction of the  $A_h$  horizon and that of leaves (Fig. 2b). The inter-stand variation of the leaf Sr/Ca ratio is



**Fig. 2 a** Relation between the Sr isotopic composition of the  $A_h$  soil exchangeable fraction and that of the leaves of *Fagus sylvatica* and *Quercus robur* (Q) from central and high

Belgium. **b** Sr/Ca ratio of the  $A_h$  soil exchangeable fraction as a function of Sr/Ca ratio of *Fagus sylvatica* and *Quercus robur* (Q) leaves from central and high Belgium

relatively low (from 0.0010 to 0.0025) whereas this ratio is generally higher in the exchangeable fraction of the  $A_h$  horizon and varies in a broad range from stand to stand (from 0.0005 to 0.0080). The apparent contradiction between the close  $^{87}\text{Sr}/^{86}\text{Sr}$  values of the leaves and the  $A_h$  exchangeable pool, and the divergence of the element ratios (Sr/Ca but also Ba/Ca and Sr/Ba) could be explained by a higher selectivity of the organic matter for Sr, associated to a discriminating effect of leaf mineralization on the alkaline-earth concentrations. Table 8 shows the

evolution of Sr/Ca, Ba/Ca and Sr/Ba ratios in the different steps of the litter fragmentation and mineralization in the MES site (central Belgium).

The study of alkaline-earth discrimination in plants has highlighted a segregation of Sr and Ba relative to Ca in the pathway from leaf veins to mesophyll of beech and oak (Table 7). The consequence of this discrimination is an accumulation of Sr and Ba in leaf veins, probably in the endoderm cells at the entry of the perivascular bundle. Inversely, we observe an increase of the relative proportions of Ca in the

mesophyll relative to Sr and Ba. Therefore, we can assume differences of mobility among these elements during the leaf litter decomposition. Indeed, perivascular bundles, which contain higher amounts of Sr and Ba, are protected by sclerenchyma and mucilage cells as well as tannins, which slow down their mineralization relative to that of the mesophyll. This mechanism could explain for a great part the change of the Sr/Ca ratio from  $0.0017 \pm 0.0001$  in the beech litter ( $O_l$ ) to a value of  $0.0043 \pm 0.0013$  in the holorganic horizon ( $O_h$ ) (MES site, Table 8). In addition, a greater selectivity of the exchange complex for Sr relative to Ca could explain the greater relative proportions of Sr in the exchangeable fraction. A substantial turnover of roots, in which element discrimination is absent, could take part in the conservation of a high Sr/Ca ratio in the  $A_h$  horizon (0.0050). The proportionally greater restitutions of Ca could also be compensated in the long-term over a complete revolution or when the Sr-richer twigs are returned.

Relationships among element ratios measured in tree leaves and those of weathering and atmospheric sources

The use of the Sr/Ca ratio as tracer of Ca requires that the proportions between elements, which are characteristic of the mixing of atmospheric and weathering sources (reflected by the composition of the soil solution) must be preserved in the initial step of root absorption (no root selectivity). Poszwa et al. (2000) have shown that the Sr/Ca ratio of the roots provided a good estimation of the Sr and Ca proportions in the bioavailable fraction of the soil. In most of the stands studied, only tree leaves (and twigs) were sampled and analysed. However, leaf Sr/Ca ratio is not representative to that of the soil solution uptake by roots because calcium present in these organs has been biopurified against Sr. The next step is to reconstitute the value of the Sr/Ca ratio during the roots uptake on the basis of the Sr/Ca ratio measured in the tree leaves. For this purpose, the discrimination factor  $DF_{R-L}$  that expresses the change of the element ratio between roots and leaves could be used as correcting factor. The limited number of  $DF_{R-L}$  calculated in the present study does not permit to show the constancy of this ratio for the species studied. In addition, we observed large variation of the root Sr/Ca

ratio through the soil profile. Therefore, the roots sampled in the surface horizons do not necessarily integrate the whole horizons of trees uptake (see further discussion).

Table 5 shows that there is no significant modification of the element ratios between roots and trunk in a stand of central Belgium (MES). Therefore, the discrimination factor  $DF_{W-L}$  that relies leaf and bole wood Sr/Ca ratios, can be calculated for a large number of stands and could be used as a correcting factor. This method assumed that discrimination factor  $DF_{W-L}$  is constant for a given species, and it has partly been demonstrated in regard of the low coefficients of variation (Tables 3 and 4). However, data from Watmough and Dillon (2003) reporting Ca and Sr concentrations in the main organs of nine North American tree species indicates the presence of high discrimination of Ca on behalf of Sr from the entry of roots to the stemwood ( $DF_{R-W}$  from 0.11 to 0.50). Works undertaken in the Cone Pond catchment, New Hampshire, confirmed this discrepancy for *Picea rubens* Sarg. ( $DF_{R-W}=0.16$ ; Bailey et al. 1996 or between 0.50 to 0.67; Bullen and Bailey 2005). By contrast, our measurements show that, for beech, Ca and Sr proportions are less affected through the pathway from the roots to wood tissues ( $DF_{R-W}=1.08$  and 1.20 in the MES and POUR sites, respectively). This is confirmed for beech, spruce and maple by the study of Poszwa et al. (2000) in stands with contrasting Ca soil reserves in sites from Jura and Vosges regions (mean  $DF_{R-W}=1.14$ , 1.01 and 1.21 respectively for *Fagus sylvatica*, 2 sites; *Picea abies*, 2 sites; and *Acer sp.*, 1 site). Likewise, data from Navrátil (2003), in a forest stand of Czech Republic, does not show large difference of Sr/Ca ratios between roots and stemwood ( $DF_{R-W}=1.17$  for beech and 1.10 for spruce). The contradiction between the literature data concerning woody species could be explained by the diameter of the sampled roots used for analyses (fine roots vs. roots of diameter > 5 mm), diameter which is not specified in the North American studies. However, Poszwa et al. (2000) did not observe differences of Sr/Ca with different root diameter for several European tree species. More likely, the depth of the root sampling seems to have a large influence on the Sr/Ca ratio measured in this tree compartment. Poszwa et al. (2004) reported root Sr/Ca ratios for *Picea abies* of  $\sim 0.0020$  at 0–10 cm depth and  $\sim 0.0060$  at 20–70 cm



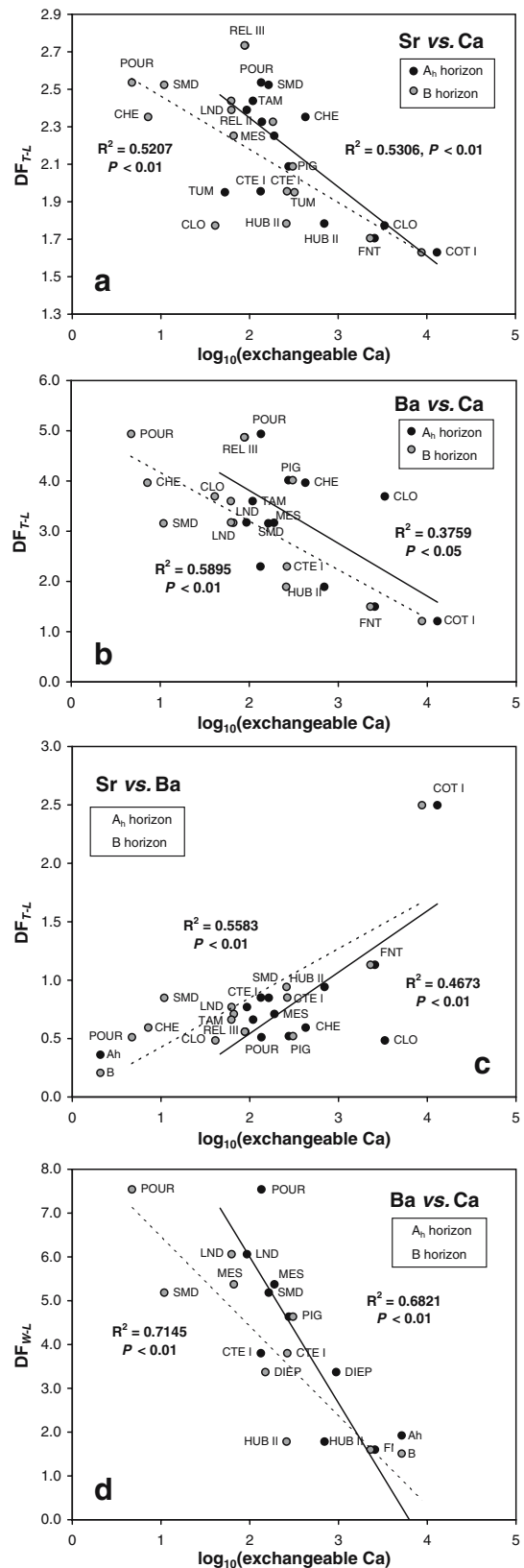
**Fig. 3** Relation between the discrimination factor from twigs to beech leaves ( $DF_{T-L}$ ) and the logarithm of the exchangeable Ca concentration in the  $A_h$  horizon (straight line) and B horizon (dotted line). **a**  $DF_{T-L}$  Sr/Ca; **b**  $DF_{T-L}$  Ba/Ca; **c**  $DF_{T-L}$  Sr/Ba. B horizon corresponds to the  $B_t$  horizon in Podzoluvisols, to the  $B_w$  horizon in the Cambisols and to the  $B_s$  horizon in the Podzol. **d** No significant relationship was found among the discrimination factors between bole wood and leaves ( $DF_{W-L}$ ) and the exchangeable Ca concentration except for Ba vs Ca

depth. This corresponds to  $DF_{R-W}$  of 1.75 and 0.58, respectively. Therefore, the sampling depth probably explains the highly contrasting value of  $DF_{R-W}$  calculated from the literature data (within a same red spruce stand: calculated  $DF_{R-W}=0.16$  after data from Bailey et al. (1996) and 0.50 to 0.67 after that of Bullen and Bailey (2005)). The case of a stand on a very homogeneous soil profile (peat) is in line with this interpretation: root data in Poszwa et al. (2004) gives  $DF_{R-W}=1.05$  for *Picea abies*, i.e. no discrimination between roots and bole. In addition, Poszwa et al. (2000) have shown that the Sr/Ca ratio of the bole wood (beech and spruce) was close to the tree weighted mean Sr/Ca ratio (taking into account the relative weight of each organ), and can be considered as a good estimator of the proportions of the two elements during uptake. The high variability of root chemistry comforts the use of  $DF_{W-L}$  rather than  $DF_{R-L}$  to quantify the modification of the element ratios from the absorption to tree leaves. Additional isotopic arguments will be discussed further.

Relationship between discrimination factors and the Ca richness of the substrate

The discrimination factors between twigs and leaves ( $DF_{T-L}^{Sr/Ca}$ ,  $DF_{T-L}^{Ba/Ca}$ , and  $DF_{T-L}^{Sr/Ba}$ ) varies significantly with the log of concentrations of exchangeable Ca in the  $A_h$  and the B horizon (Fig. 3a–c). By contrast, discrimination factors from wood to leaves are not significantly correlated with the amount of exchangeable Ca, except  $DF_{W-L}^{Ba/Ca}$  (Fig. 3d). Constant  $DF_{W-L}$  could be explained by the fact that high  $DF_{W-T}$  are compensated by proportionally lower  $DF_{T-L}$ .

So, the discrimination factors ( $DF_{W-L}$ ) within a same tree species are relatively constant, for a large range of soil Ca-status. In conclusion, the element ratios measured in tree leaves can be corrected with a constant factor ( $DF_{W-L}$ ) inherent to each species (2.99 for beech, 6.54 for oak in the case of the Sr/Ca ratio, Tables 3 and 4). This corrected value is characteristic

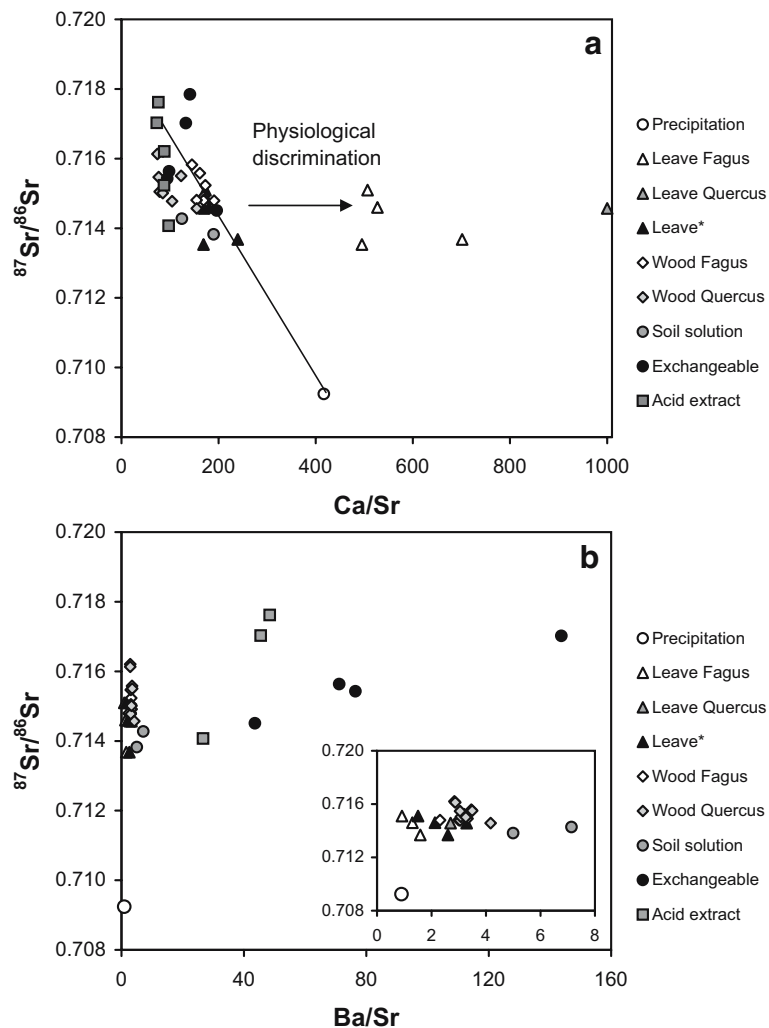


of the proportions of these elements during the absorption from the soil solution.

### $^{87}\text{Sr}/^{86}\text{Sr}$ vs. Ca/Sr and Ba/Sr diagrams

$^{87}\text{Sr}/^{86}\text{Sr}$  vs. 1/Sr diagrams are commonly used in geochemistry. In such diagram, the different mixing components of two sources of Sr plot along a mixing line connecting the end-members (Langmuir et al. 1978). However, in surficial systems, at the soil–vegetation–atmosphere interface, accumulation in the biomass and evapotranspiration can strongly increase the element concentrations. The increase of strontium

concentration in vegetation, in a tenfold proportion of that present in the feeding sources, may remove the linear relationship between the sources and their mixing. By contrast, as Sr and Ca behave similarly, they are equally affected by physical concentrations and the Ca/Sr ratio only depends on the mixing of the sources. Therefore,  $^{87}\text{Sr}/^{86}\text{Sr}$  vs. Ca/Sr diagram which is not affected by any concentration process has been used. Figure 4a displays such diagram with precipitation, soil acid extracts simulating natural weathering, soil exchangeable fraction, soil solution, wood, and corrected leaf values plotting along a mixing line connecting the two sources of Ca and Sr. The site



**Fig. 4**  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio vs. **a** Ca/Sr and **b** Ba/Sr ratios in atmospheric precipitation, plant, and soil compartments of a high Belgium forest ecosystem (POUR site). (\*) symbol indicates that leaf element ratios were corrected from the tree

physiological discrimination (after DF calculated in Tables 3 and 4, see text for explanation). Sr isotopic data are from Drouet et al. (2005a and b). Window of Fig. 4b focuses on low Ba/Sr values

chosen presents a shallow soil substrate which constrains the forest to a two feeding source system (atmosphere and mineral weathering). Foliar Ca/Sr were corrected from the biopurification process with the discrimination factors previously determined for the two species (*Fagus*:  $DF_{W-L}=2.99$ , *Quercus*:  $DF_{W-L}=6.54$ ). By contrast, strontium isotopes are not fractionated by chemical and biological processes and are therefore not sensitive to the discrimination phenomenon. Such diagram can be plot for other stands studied (Drouet et al., submitted).  $^{87}\text{Sr}/^{86}\text{Sr}$  vs. Ca/Sr diagrams may also be used to discriminate between three sources of Ca in forest ecosystems. In this case, Ca/Sr ratios are decoupled from the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio and can fully be used as complementary tracer (Drouet et al. submitted). Figure 4a confirms again: (a) the similar behaviour of Ca and Sr in soil compartment (exchangeable fraction, soil solution) (b) that the discrimination of Ca does not occur significantly between roots and bole wood, and that the Sr/Ca ratios measured in these organs are representative of the proportions of the two elements during their absorption in the soil solution (both for beech and oak) and (c) in a practical point of view, mean discrimination factors  $DF_{W-L}^{\text{Sr/Ca}}$  for beech and oak (Tables 3 and 4) permit to quantify the modification of the Sr/Ca ratio between bole wood and leaves in stands developed on large soil Ca status. By contrast,  $^{87}\text{Sr}/^{86}\text{Sr}$  vs. Ba/Sr ratio diagram (Fig. 4b) shows a very dispersed pattern and an absence of straight-line alignment which confirms that Ba behaves very differently compared to Sr (and to Ca, by inference).

## Conclusion

The similar behaviour of Sr and Ca in forest ecosystems has been confirmed. We extensively studied the process of Ca discrimination in two forest species: European beech and pedunculate oak. The segregation of Sr and Ba compared to Ca is generally more intense in oak trees. In beech trees, the greatest part (62%) of the total Ca discrimination (from bole wood to leaves) occurs between twigs and leaves, whereas for oak, discrimination occurs mainly from boles to twigs (69%). For these two species, the segregation against Ba occurs in the same organs and in the same proportions than that of Sr, but with a greater intensity.

The measurement of Ca/Sr ratios in conjunction with Sr isotopic ratio ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) which is not fractionated by biological processes, confirms the absence of segregation of Ca and Sr cations between the soil solution, the roots and the bole wood of European beech and pedunculate oak. The discrimination factors ( $DF_{W-L}$ ) are constant for a single species and are not dependant on the Ca richness of the substrate. After correction with this discrimination factors, Sr/Ca ratios of leaves may therefore be used as tracer of Ca in complement of  $^{87}\text{Sr}/^{86}\text{Sr}$  measurements to solve complex systems with more than two sources.

On the contrary, we show weak or no correlations between Ca and Ba concentrations in most of the soil compartments. Ba is more tightly bound on the exchange sites than Sr and Ca and is therefore much less accessible to roots. Moreover, simultaneous measurements of Ba/Sr ratio and of Sr isotopic ratio confirmed that Ba has globally a different behaviour than Sr and Ca in the forest ecosystem components. The use of Sr/Ba and Ba/Ca ratios as tracers of Ca must therefore be used with care.

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## References

- Åberg, G, Wickman T, Hamilton PJ (1990) Strontium isotopes in trees as an indicator for calcium availability. *Catena* 17:1–11
- Baes AU, Bloom PR (1988) Exchange of alkaline earth cations in soil organic matter. *Soil Sci* 146:6–14
- Bailey SW, Hornbeck JW, Driscoll CT, Gaudette HE (1996) Calcium inputs and transport in a base-poor forest ecosystem as interpreted by Sr isotopes. *Water Resour Res* 32:707–719
- Blum JD, Taliaferro EH, Weisse MT, Holmes RT (2000) Changes in Sr/Ca, Ba/Ca and  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios between trophic levels in two forest ecosystems in the northeastern U.S.A. *Biogeochemistry* 49:87–101
- Blum JD, Klaue A, Nezat CA, Driscoll CT, Johnson CE, Siccama TG, Eagar C, Fahey TJ, Likens GE (2002) Mycorrhizal weathering of apatite as an important calcium source in base-poor forest ecosystems. *Nature* 417: 729–731

- Bullen TD, Bailey SW (2005) Identifying calcium sources at an acid deposition-impacted spruce forest: a strontium isotope, alkaline-earth element multi-tracer approach. *Biogeochemistry* 74:63–99
- Capo RC, Stewart BW, Chadwick OA (1998) Strontium isotopes as tracers of ecosystem processes: theory and methods. *Geoderma* 82:197–225
- Dasch A, Blum JD, Eagar C, Fahey TJ, Driscoll CT, Siccama TG (2006) The relative uptake Ca and Sr into tree foliage using a whole-watershed calcium addition. *Biogeochemistry* 80:21–41
- Drouet Th (2005) Etude de l'origine du calcium dans les écosystèmes forestiers par les méthodes de géochimie et de dendrochimie isotopiques du strontium. PhD thesis, Université Libre de Bruxelles, pp 151
- Drouet Th, Herbauts J, Gruber W, Demaiffe D (2005a) Strontium isotope composition as a tracer of calcium sources in two forest ecosystems in Belgium. *Geoderma* 126:203–223
- Drouet Th, Herbauts J, Demaiffe D (2005b) Long-term records of strontium isotopic composition in tree-rings suggest changes in forest calcium sources in the early 20th century. *Global Change Biol* 11:1926–1940
- Drouet Th, Herbauts J, Gruber W, Demaiffe D (2007) Natural strontium isotope composition as a tracer of weathering patterns of exchangeable calcium sources in acid leached soils developed on loess of central Belgium. *Eur J Soil Sci* 58:302–319
- Elias RW, Hirao Y, Patterson CC (1982) The circumvention of the natural biopurification of calcium along nutrient pathways by atmospheric inputs of industrial lead. *Geochim Cosmochim Acta* 46:2561–2580
- Gierth M, Stelzer R, Lehmann H (1998) Endodermal Ca and Sr partitioning in needles of the European larch (*Larix decidua* (L.) Mill.). *J Plant Physiol* 152:25–30
- Herbauts J (1981) Evolution des sols sur les sables d'altération des calcaires gréseux du Lias inférieur dans le sud-est de la Belgique. *Science du Sol* 3:187–212
- Herbauts J (1982) Chemical and mineralogical properties of sandy and loamy-sandy ochreous brown earths in relation to incipient podzolization in a brown earth-podzol evolutive sequence. *J Soil Sci* 33:743–762
- Herbauts J, Tanghe M (1987) Relations entre formations superficielles, sols et associations forestières sur la cuesta bajocienne de Lorraine belge. L'exemple du bois de la Côte à Virton-Saint-Mard. *Bull Soc Roy Bot Belg* 120:93–105
- Langmuir CH, Vocke RD, Hanson GN, Hart SR (1978) A general mixing equation with applications to icelandic basalts. *Earth Planet Sci Lett* 37:380–392
- Miller EK, Blum JD, Friedland AJ (1993) Determination of soil exchangeable-cation loss and weathering rates using Sr isotopes. *Nature* 362:438–441
- Momoshima N, Bondietti EA (1990) Cation binding in wood: applications to understanding historical changes in divalent cation availability to red spruce. *Can J Forest Res* 20:1840–1849
- Navrátil T (2003) Biogeochemistry of the II.A group elements in a forested catchment. Dissertation, Faculty of Science, Charles University, Prague, pp 108
- Penninckx V, Meerts P, Herbauts J, Gruber W (1999) Ring width and element concentration in beech (*Fagus sylvatica* L.) from a periurban forest in central Belgium. *Forest Ecol Manag* 113:23–33
- Penninckx V, Glineur S, Gruber W, Herbauts J, Meerts P (2001) Radial variation in wood mineral element concentrations: a comparison of beech and pedunculate oak from the Belgian Ardennes. *Ann For Sci* 58:253–260
- Phillips DL, Gregg JW (2003) Source partitioning using stable isotopes: coping with too many sources. *Oecologia* 136:261–269
- Poszwa A, Dambrine E, Pollier B, Atteia O (2000) A comparison between Ca and Sr cycling in forest ecosystems. *Plant Soil* 225:299–310
- Poszwa A, Ferry B, Dambrine E, Pollier B, Wickman T, Loubet M, Bishop K (2004) Variations of bioavailable Sr concentration and the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio in boreal forest ecosystems. Role of biocycling, mineral weathering and depth of root uptake. *Biogeochemistry* 67:1–20
- Tyler G (2004) Ionic charge, radius, and potential control root/soil concentration ratios of fifty cationic elements in the organic horizon of a beech (*Fagus sylvatica*) forest podzol. *Sci Total Environ* 329:231–239
- Veresoglou DS, Barbayiannis N, Zalidis GC, Kalpakis S, Batianis E (1995) Transfer factors for Sr as influenced by species Ca uptake and soil Ca availability. *Plant Soil* 175:225–232
- Watmough SA, Dillon PJ (2003) Mycorrhizal weathering in base-poor forests. *Nature* 423:823–824