Biogeochemistry (2008) 88:195–203
DOI 10.1007/s10533-008-9208-5

ORIGINAL PAPER

Test of soil extractants for their suitability in predicting Ca/Sr ratios in leaves and stems of sugar maple seedlings

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Received: 16 November 2007 / Accepted: 30 April 2008 / Published online: 16 May 2008
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Abstract Differential uptake and translocation of Ca and Sr in organisms have been reported, calling into question the use of Sr to track Ca cycling in the environment. We investigated the relationship between Ca/Sr ratios in soil extracts of various strengths (H_2O, NH_4Cl, and NH_4EDTA) and seedlings of sugar maple (*Acer saccharum* Marsh.) grown from natural regeneration on 37 sites. Our objectives were to determine if Ca/Sr ratios in soil extracts are correlated with those in sugar maple tissues, and what soil extractant best duplicate plant tissue Ca/Sr ratios. Leaves had higher Ca/Sr ratios than stems and the extractants did not produce equal Ca/Sr ratios: H_2O had the lowest Ca/Sr, and NH_4EDTA the highest. The relationships between soil extract Ca/Sr ratios and leaf and stem Ca/Sr ratios were significant and linear, but the slopes differed among extractants. The lowest slope (0.45) was observed for the water extract/leaves and the highest (2.15) for the NH_4EDTA extract/stem with discrimination factors ranging from 0.22 with NH_4EDTA to 1.59 for water. Leaf extracts were more strongly correlated with soil Ca/Sr than stem extracts (R^2 of 0.57–0.7 vs. R^2 of 0.45–0.6, respectively). These findings support the use of Ca/Sr ratios in plants to track their source of soil Ca, but they highlight the need to calibrate the relationships for the plant tissue and soil extractant used.

Keywords *Acer saccharum* · Calcium · Strontium · Absorption · Translocation · Tracer

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Introduction

Calcium availability is limited in acidic soils, especially those that are base poor (Bailey 2000). This has been particularly problematic for sugar maple (*Acer saccharum* Marsh.) trees growing in base poor soils that have also had heavy anthropogenic acid deposition (Bernier et al. 1989; Horsley et al. 2002; Bailey et al. 2004). An understanding of Ca dynamics in sugar maple is being sought by researchers in order to better manage and predict the occurrence of sugar maple declines (Dijkstra and Smits 2002; Bailey et al. 2004; Dasch et al. 2006).

Stable, naturally occurring isotopes are invaluable tools for tracking the flow of elements (e.g. N, S, Pb, O, H) from different pools within ecosystems (Griffiths 1998; Rubenstein and Hobson 2004; Fry 2006; Michener and Lajtha 2007). Stable isotopes of Ca do exist and have been used recently to track its flow in plants (Wiegand et al. 2005). However, their applicability is still problematic and researchers have mostly relied on the stable isotopes of Sr as a proxy to Ca. Calcium and Sr are sufficiently chemically similar to be incorporated into plants and other organisms in a like manner (Capo

et al. 1998). Strontium has the advantage of having two isotopes (^{86}Sr and ^{87}Sr) that are found in different concentrations in two pools contributing significantly to the soil solution: minerals and precipitation. The soil matrix is usually enriched in ^{87}Sr by decomposition of radiogenic ^{87}Rb in minerals containing Rb. Precipitation, on the other hand is usually enriched in ^{86}Sr because of the contribution from oceanic Sr which is relatively higher in ^{86}Sr than soil/mineral sources (Graustein and Armstrong 1983; Poszwa et al. 2000; Drouet et al. 2005). Both of these factors generally contribute to vertical gradients of $^{87}Sr/^{86}Sr$ ratios in soils (Dambrine et al. 1997; Poszwa et al. 2004). Once the isotopic composition of the different sources is known, their relative contribution to tree uptake can then be calculated with mixing equation (Graustein and Armstrong 1983; Capo et al. 1998).

More direct quantification of Ca cycling with Sr isotopes can be made by measuring Ca/Sr in the different pools to be used in mixing equations (Capo et al. 1998). Tracking of Ca sources with Ca/Sr ratios is particularly useful when end members (sources) do not differ in $^{86}Sr/^{87}Sr$ (Blum et al. 2002). The preferential uptake and translocation of Ca over Sr by plants on acid soils (Poszwa et al. 2000) is however problematic for the interpretation of results with Ca/Sr ratios. Typical increases in Ca/Sr by a factor of 1.0–1.6 in plant tissue compared to soil (Åberg et al. 1989; Blum et al. 2000) and higher Ca/Sr ratios in leaves than wood (Drouet and Herbauts 2008) are clear indications that both elements are used differently in living systems. In a recent paper by Blum et al. (2002), differences in Ca/Sr ratios among sugar maple, American beech (*Fagus grandifolia* Ehrh.) and red spruce (*Picea rubens* Sarg.) were attributed to interspecific differences in their capacity to tap Ca from the Ca-rich apatite deep in the soil. It was however acknowledged that part of the differences in tree Ca/Sr could be attributed to differences in preferential uptake of Ca over Sr among species and that more research on plant uptake of Ca and Sr was needed to support their conclusion.

Different soil fractions can have different Ca/Sr ratios (Poszwa et al. 2000; Blum et al. 2002). The heavier Sr, with its similar radius for the same charge, should be more tightly bound to exchange sites than Ca (Koranda and Robison 1978; Capo et al. 1998). However, the type of soil exchange sites, pH and concentration of other ions can change how strongly these elements are held with Ca being retained more strongly than Sr in many soils (Baes and Bloom 1988). Stronger extractants should therefore displace larger amounts of both elements but proportionately more Ca. Different soil extractants are therefore likely to yield different preferential uptake/discrimination factor estimates. Discrimination factors of Ca over Sr have been determined using different fractions of soil Ca and Sr such as zero-tension lysimeter solutions, salt extracts and digests (Runia 1987; Poszwa et al. 2000). The use of different fractions could make comparison of discrimination factors between species difficult.

The ideal soil extractant should duplicate as closely as possible the capacity of plants to extract both elements. Ammonium chloride (NH_4Cl) is commonly used to estimate the plant-available Ca and Sr in Sr isotope studies (Poszwa et al. 2004). Other salts such as $BaCl_2$ and $CaCl_2$, which are also used to estimate nutrient availability in forest soils (Hendershot and Duquette 1986), are not as suitable due to the likely presence of impurities of other alkaline earth elements in the case of $BaCl_2$, and of the obvious interference by $CaCl_2$. The ammonium salt of EDTA, used to estimate plant-available trace metals, is potentially useful as a strong extractant because it also extracts significant amounts of Ca (Manouchehri et al. 2006).

Clearly, a better assessment and understanding of the preferential uptake of Ca over Sr in different tree species and soils would improve the applicability of the Ca/Sr approach to track the sources of Ca in forest ecosystems. In this study, we investigated the relationship between soil Ca/Sr ratios and those of the leaf and stem tissue of sugar maple seedlings sampled from a wide range of sites with different soil conditions in order to maximize the variability in soil and tissue Ca/Sr ratios. Three extractants with a range of strength were tested: NH_4EDTA as an ammonium salt and strong extractant, NH_4Cl, of medium extractive strength, and water, a weak extractant. We hypothesized that: (1) leaf Ca/Sr would be higher than stem Ca/Sr and, therefore, have a higher discrimination factor, (2) the stronger soil extractant would have higher Ca/Sr and (3) plant tissue Ca/Sr would be a good predictor of soil Ca/Sr with discrimination factors decreasing with the strength of the extractant.

Methods

A field approach was chosen over a greenhouse trial to assess the relationship between seedling Ca/Sr and soil Ca/Sr to duplicate the conditions of temperature, moisture and nutrient regimes/cycles, and the microbial interactions that mature trees experience. A total of 37 sites with sugar maple regeneration present were chosen throughout south-western Quebec and south-eastern Ontario (approx. 45°27′ lat. and 74°17′ long.). Sites were chosen to provide a wide range of soil chemistry and fertility, and ultimately of Ca/Sr ratios in soils and plants. Sites had different soil types, bedrock/surficial geology, dominant tree species in the stand, and topography (Table 1). The surficial geology of the sites was either: marine sediments of clay and silt, alluvial sand, glacial lake deposited sand, glacial till, bedrock with thin veneers of unconsolidated rock, and organic matter (Richard et al. 1978; Richard 1982; Fulton 1995). The bedrock was either limestone, sandstone, anorthosite, gabbo-anorthosite, dolimite, shale, granite, granite-gneiss, or sedimentary gneiss (Geological Survey of Canada 1943). Soil texture, rockiness, nutrient availability (P, K, Ca, Mg and Al) based on Mehlich extractions, percent organic matter based on loss on ignition and pH in water for the 37 sites are listed in Table 1. Of particular interest is the wide range of Ca availability that varied from 130 mg kg^{-1}on a sandy soil to more than 4,400 mg kg^{-1} on a clay loam.

Sampling was carried out during the last week of July in 2005 and 2006. At each site, a group of seedlings (1–3 plants <30 cm tall) growing within a 2-m radius was dug up with their root ball. Seedlings were shaken gently first to remove the soil weakly attached to the roots. Roots were then shaken vigorously over a clean plastic container to collect the soil attached to the network of roots. The sampled soil was therefore representative of the soil used by the seedling through the rhizosphere or root zone soil, as well as the soil more loosely explored by the network of mycorrhizal hyphae that are known to be able to uptake nutrients directly from organic matter and minerals (Blum et al. 2002). Roots extended ca. 10 cm deep and approximately 100 cm^3 of soil was collected per group of seedlings. The small rooting depth meant that on some sites, most of the soil sampled was from the surficial organic horizon. In most cases, however, the soil sampled encompassed both the organic and top of the mineral soil horizons. Due to the small size of seedlings and small volume of soil sampled, one composite sample was made for each group of seedlings. The seedlings were naturally occurring regeneration. Seedling age was determined by counting the number of leaf scars. Seedlings ranged from 1 to 28 years old, a demonstration of the great capacity of sugar maple to survive in the shade for extended periods.

Soils were air dried, ground to break course aggregates, and passed through a 2 mm nylon mesh. Leaves and stems (wood plus bark) were dried at 65°C for 48 h and then ground. Soil samples were extracted using NH_4EDTA (0.05 M, pH 7.0) as described by Hendershot et al. (2007), or were extracted with water or NH_4Cl (1.0 M), following the same procedure as NH_4EDTA, except that water extractions were shaken for 2 h instead of one. Extractions were carried out in duplicate. Average coefficients of variation for Ca/Sr ratios ranged from 3% for NH_4Cl and NH_4EDTA, to 14% for water. Leaves and stems were digested in hot $HNO_3 + H_2O_2$. One tenth of a gram of tissue was incubated for 15 h in a test tube with 2 ml of 70% trace metal grade HNO_3. Tubes were covered with Parafilm^{TM} to minimize contamination. H_2O_2 was then added (0.1 ml) and allowed to react at room temperature for 1 h. The temperature was then increased gradually to 120°C and digested at this temperature for 5 h. Samples were diluted to a volume of 10 ml.

All lab ware was soaked in HCl (15%, 10 min) and HNO_3 (15%, 15 h) as part of the washing procedure, as well as given a final rinse with double deionised water. Ammonium EDTA and NH_4Cl extracts, and leaf and stem digests were analysed on an inductively coupled plasma atomic emission spectrometer (Perkin–Elmer OPTIMA 43000 V) for Ca and Sr. Soil water extracts were measured on an inductively coupled plasma mass spectrometer (Varian UltraMass 700) for Sr, and on an atomic absorption flame spectrometer (Perkin–Elmer 2380) for Ca. The use of multiple instruments to determine Ca and Sr concentrations was necessary because of the wide variation in Ca and Sr concentrations generated by the different strengths of extractant used. Multiple dilutions would have had to be done which would have canceled any gain in precision that the use of a single instrument would have provided. Quality control and standards were run in each batch. National Institute of Standards and Technology (reference number 1537) peach leaves were used as a quality control. Ca and Sr values were all within the certified ranges for this standard.

Table 1 Site and seedling characteristics

Dominant tree species[a]	Seedling age range[b]	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Al (mg kg^{-1})	pH	Organic matter (%)	Soil texture[c]	Rockiness	Latitude/longitude
SM BW WA	5	150	1916	315	838	5.7	7.0	Clay loam	High	45.26.510 N; 74.07.000 W
WC WP SM WB	3–9	57	779	119	1269	4.8	8.8	Sandy loam	Low	45.27.041 N; 74.07.443 W
SM BW WA	22	104	2451	285	670	5.7	11.0	Clay loam	Moderate	45.27.012 N; 74.07.012 W
WB SM YB	6–11	64	1505	113	1154	5.4	7.8	Sandy loam	Low	45.26.933 N; 45.07.388 W
SM WA WO	6–10	107	2539	324	850	4.9	12.8	Silt	Low	45.26.738 N; 74.07.160 W
SM	6–12	75	3172	440	439	7.0	7.2	Silt/gravel	Low	45.26.676 N; 74.07.271 W
SM WA WE	7–12	155	1130	149	1120	4.7	15.2	Loam	Moderate	45.23.723 N; 73.58.877 W
SM AB RO	5–9	202	1329	244	670	4.2	26.4	Sandy loam	Moderate	45.23.680 N; 73.58.709 W
YB SM BW	10–13	314	1496	185	259	4.5	14.4	Loam	Moderate	45.23.661 N; 73.58.633 W
SM	11–20	86	1514	243	1043	5.2	8.9	Loam	Low	45.26.097 N; 73.56.790 W
SM	17	95	3303	422	641	6.4	8.7	Loam	Moderate	45.26.303 N; 73.56.851 W
NS EL	4–23	152	4462	930	540	6.8	5.2	Clay loam	None	45.26.197 N; 73.56.989 W
SM	5–11	60	1569	221	749	5.6	5.2	Clay loam	Low	45.26.126 N; 73.57.122 W
SM RM AB RO	10–25	252	780	233	1110	5.1	5.7	Sandy clay	None	45.26.068 N; 73.57.247 W
NS WS	6	172	1881	427	900	5.4	6.2	Loamy clay	Low	45.25.895 N; 73.57.109 W
RP NS	7–8	125	879	157	1019	4.7	7.0	Sandy loam	None	45.25 N; 73.57 W
AB SM	4–6	80	620	138	880	4.4	12.2	Sand	None	45.25.891 N; 73.56.873 W
SM	6	105	827	160	797	5.2	5.2	Sand	None	45.25.905 N; 73.56.812 W
AB SM	11–20	527	1254	421	376	3.5	81.5	Sand	None	45.25 N; 73.57 W
AB SM	4–17	488	1777	365	626	3.6	71.9	Sand	None	45.25 N; 73.57 W
AB SM	4–23	190	851	87	1091	3.7	25.2	Sand	None	45.25 N; 73.57 W
RO WP RM	1	21	130	8	1289	5.1	3.6	Sand	None	45.24.148 N; 74.14.589 W
RO WP RM	6	57	828	65	838	5.0	7.8	Sand	None	45.24.135 N; 74.14.540 W
RO WP RM	6	35	662	38	1153	5.3	4.1	Sand	None	45.24.135 N; 74.14.540 W
RO WP RM	7–12	432	1434	228	923	4.2	44.5	Organic	High	45.26.401 N; 74.15.990 W
SM AB	11–18	133	280	34	2299	4.6	28.1	Loam	Moderate	45.26.505 N; 74.15.743 W
SM AB	6–11	201	2610	321	710	4.8	25.4	Loam	High	45.26.655 N; 74.15.666 W
AB SM	8–16	278	1925	176	742	4.0	57.1	Sandy loam	Moderate	45.25 N; 73.57 W
SM AB RM YB	6–13	110	1010	64	1250	4.8	51.1	Sandy loam	None	45.58.972 N; 74.01.069 W
SM AB RM YB	4–14	138	528	36	1654	4.5	21.6	Sandy loam	Moderate	45.58.990 N; 74.01.080 W
AB SM BW RE	22–28	86	3149	568	870	5.5	23.5	Clay	Low	45.21.856 N; 74.25.203 W
SM	6	111	2900	208	710	5.2	13.2	Clay loam	Low	45.18.987 N; 74.26.320 W
YB RM SM	7–12	119	1142	127	1092	4.8	21.2	Sandy loam	Low	46.05.254 N; 74.16.756 W
YB RM SM	7–8	36	392	42	1536	5.3	3.8	Sandy loam	Low	46.05.255 N; 74.16.737 W
YB RM SM BF	10–20	40	626	82	1311	5.5	4.1	Sandy loam	Low	46.05.278 N; 74.16.815 W
YB RM SM	7–8	NA	NA	NA	NA	NA	NA	Sandy loam	Low	46.05.254 N; 74.16.756 W
YB RM SM WA	6–17	65	1621	60	1251	4.7	11.9	Loam	Moderate	45.32.411 N; 74.39.469 W

[a] AB = American beech (*Fagus grandifolia* Ehrh.), BF = balsam fir (*Abies balsamea* L.), EL = European larch (*Larix decidua* Mill.), BW = basswood (*Tilia americana* L.), NS = Norway spruce (*Picea abies* (L.) Karst.), SM = sugar maple (*Acer saccharum* Marsh.), STM = striped maple (*A. pennsylvanica)*, RA = red ash (*Fraxinus pennsylvanica* Marsh.), RE = red elm (*Ulmus rubra* Muhl.), RM = red maple (*Acer rubrum* L.), RO = red oak (*Quercus rubra* L.), RP = red pine (*Pinus resinosa* Ait.), WA = white ash (*Fraxinus americana* L.), WB = white birch (*Betula papyrifera* Marsh.), WE = white elm (*Ulmus americana* L.), WO = white oak (*Quercus alba* L.), YB = yellow birch (*Betula alleghaniensis* Britt.)

[b] Age determined by number of bud scar

[c] Soil texture of the upper B horizon

To compare the ability of each extractant to duplicate leaf and stem Ca source, simple linear regressions between leaf and stem Ca/Sr ratios, and each soil extractant were computed independently. An analysis of residuals was made to rank the extractants for accuracy of prediction across the range of soil Ca/Sr measured. Discrimination factors were computed as the slope of the regression of soil Ca/Sr versus tissue Ca/Sr. Uncertainties in discrimination factors were reported as the standard error of the slope. Correlation coefficients between soil Ca, Sr, Ca/Sr and soil pH and organic matter were computed for the different soil extractants to assess the contribution of the mineral and organic pools to Ca and Sr availability and Ca/Sr ratios. The statistical program STATISTICA 6 (Statsoft 2004) was used for these calculations.

Results

Average soil Ca for the 37 sites increased with the strength of the extractant (0.89, 38.7 and 55.5 mg kg^{-1}) whereas average Sr was highest with NH_4Cl and lowest with water (2.9, 80.0 and 55.7 mg kg^{-1}). Soil Ca was positively correlated with soil pH with all three extractants ($r = 0.39$–0.56) but not with soil organic matter content. In contrast, soil Sr was primarily associated with soil organic matter with correlations of 0.53, 0.56 and −0.38 for water, NH_4Cl, and NH_4EDTA, respectively. Ca/Sr ratios were positively correlated with soil pH for water ($r = 0.60$) and NH_4Cl ($r = 0.58$) extracts, and with soil organic matter with NH_4EDTA ($r = 0.44$).

Leaf and stem Ca/Sr ratios increased with increases in soil extract Ca/Sr (Fig. 1). The range of soil Ca/Sr observed was lowest for water extracts and maximum for NH_4EDTA, and Ca/Sr was higher for leaves than for stems (Fig. 1). All regressions were highly significant with R^2 varying from 0.45 for stem Ca/Sr versus soil NH_4EDTA Ca/Sr to 0.70 for leaf Ca/Sr versus water or NH_4Cl Ca/Sr (Table 2). Standard errors of the estimates were lowest with water and highest with NH_4EDTA (Table 2). Residuals increased from negative values at low soil Ca/Sr with increasing soil Ca/Sr ratios for all soil extractants and the two plant tissues (Fig. 2). Lowest residuals were observed at mid-range

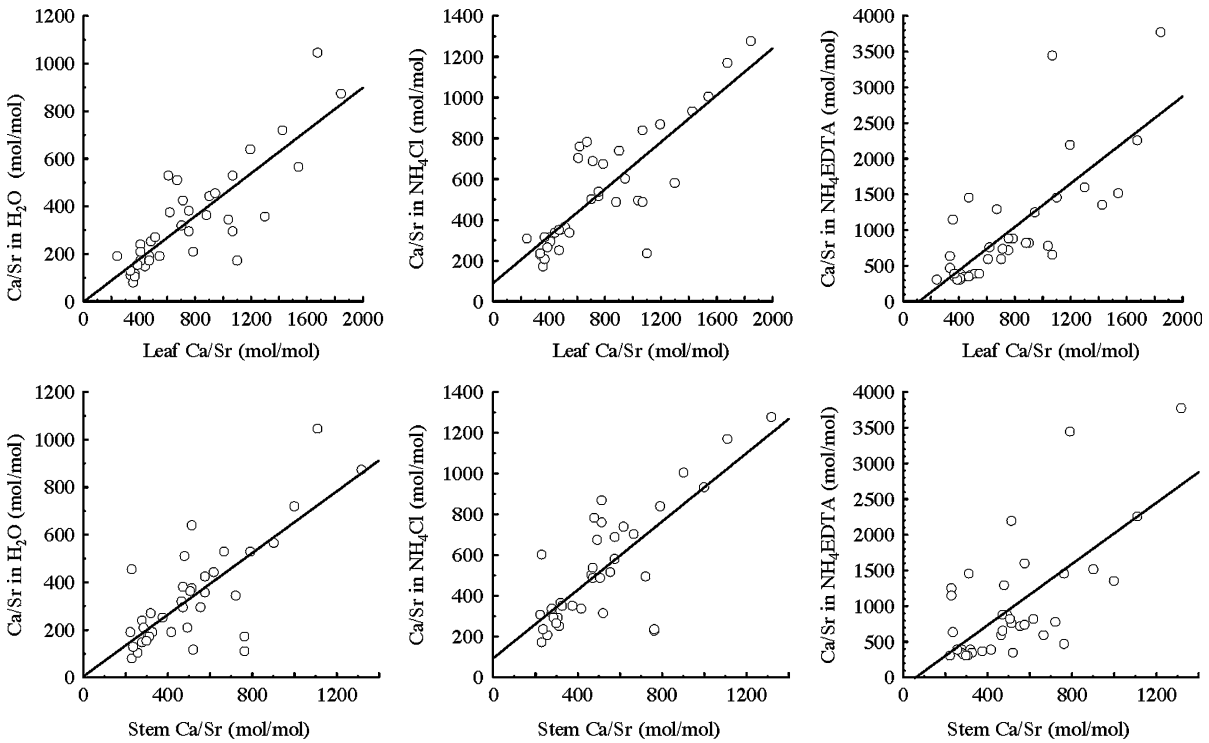

Fig. 1 Regressions of sugar maple leaf and stem Ca/Sr ratios versus ratios obtained with three types of soil extractants; for all $P < 0.001$

Table 2 Summary of regression equations of soil extract Ca/Sr versus plant tissue Ca/Sr

Tissue	Extraction	Equation[a]	Adjusted R^2	SEE[b]	Discrimination factor ± SE[c]
Leaf	H_2O	0.45X − 2.16	0.70	122	1.59 ± 0.17
	NH_4Cl	0.58X + 91	0.70	155	1.25 ± 0.14
	NH_4EDTA	1.52X − 174	0.57	538	0.39 ± 0.06
Stem	H_2O	0.65X + 5	0.58	144	0.91 ± 0.13
	NH_4Cl	0.84X + 94	0.60	180	0.72 ± 0.10
	NH_4EDTA	2.15X − 126	0.45	609	0.22 ± 0.04

[a] X = leaf Ca/Sr (mol/mol); $P < 0.0001$ for all equations

[b] Standard error of the estimate

[c] Slope of the regression of soil Ca/Sr versus tissue Ca/Sr, ± the standard error of the slope

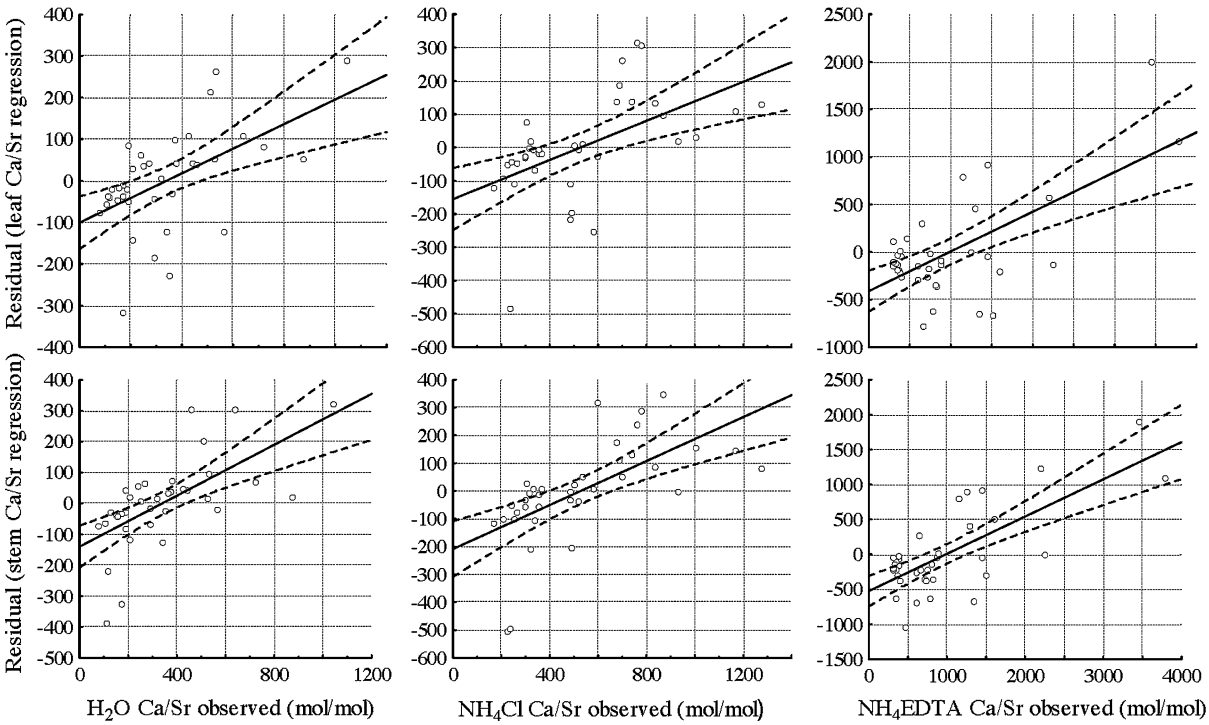

Fig. 2 Soil Ca/Sr residuals versus observed values for the three extractions and two tissues (mean ± 95% confidence interval)

that corresponded to Ca/Sr ratios of around 400, 500 and 1,000 for the water, NH_4Cl and NH_4EDTA extracts, respectively (Fig. 2). Largest residuals were observed for NH_4EDTA extracts.

The slopes of the relationships increased with increases in the strength of the extractant for both leaf and stem Ca/Sr (Table 2, Fig. 1). Slopes were below one for both water and NH_4Cl extracts and above one for the NH_4EDTA extracts (Table 1). Discrimination factors of Ca over Sr were on average above one for water, about one for NH_4Cl and below one for NH_4EDTA (Table 2).

Discussion

The different extractants produced different ranges of soil Ca/Sr ratios with stronger soil extractants producing higher Ca/Sr ratios. The difference in the range of Ca/Sr ratios observed was largest for

NH_4EDTA versus the other two extractants. Although differences in Ca/Sr between soil extractants were expected, the magnitude of the differences (>3×) was not, as both are often assumed to behave similarly in the soil–plant system. Our results suggest that Ca is more strongly held on soil exchangeable sites than Sr and that Ca availability is primarily mediated by soil pH and, therefore, the composition of the mineral soil. In contrast, the availability of Sr appears to be mediated more by the amount of soil organic matter, but no consistent pattern was observed between soil extractants. These results suggest that the different fractions of the soil organic matter have different Sr concentrations.

As hypothesized, all three soil extractants produced soil Ca/Sr ratios that were significantly correlated with plant Ca/Sr ratios. Because the stronger extractants yielded higher soil Ca/Sr ratios, the slope of the relationships between tissue Ca/Sr and soil Ca/Sr were higher for the stronger extractants, most markedly so for NH_4EDTA. In fact, the regressions with NH_4EDTA had slopes greater than one, and associated discrimination factors of less than one. Therefore Ca is impoverished over Sr along the soil–stem–leaf pathway based on NH_4EDTA extracts. This is contrary to what has been observed in many studies for the majority of plants, but there are exceptions (Runia 1987; Veresoglou et al. 1996; West et al. 2001). Both water and NH_4Cl regressions had slopes smaller than one and discrimination factors for leaves of more than one and, therefore, were more in line with the increasing gradient of Ca/Sr usually observed in the soil plant system (Poszwa et al. 2000; Dasch et al. 2006).

Overall, the NH_4Cl extractant, with an average discrimination factor of about one for the leaves and stems together, appears to best predict ratios of Ca/Sr taken up by sugar maple roots. Its discrimination factor of 1.25 ± 0.14 is also consistent with the discrimination factor measured by Dasch et al. (2006). In comparison, NH_4EDTA appears to generally extract an excess of Ca relative to Sr, and water an excess of Sr relative to Ca. In another study, however, NH_4Cl soil extracts Ca/Sr ratios were significantly lower than root and sapwood Ca/Sr ratios (Poszwa et al. 2004). Preferential uptake of Ca over Sr is dependent on soil acidity and/or mineral composition as well as tree species (Poszwa et al. 2000). Given the broad range of soil types/properties encompassed in this study and some of the large errors of Ca/Sr estimates measured, it is likely that sites with extreme/unusual soil conditions may require a soil extractant other than NH_4Cl to best predict plant Ca/Sr ratios.

Of the three extractants, water and NH_4Cl regressions had similar R^2 values and both had better-fit and smaller standard error of the estimates than NH_4EDTA. Although not commonly used to estimate soil nutrient availability, NH_4EDTA has been shown to be a good predictor of plant micro-element availability (Quevauviller et al. 1998). Moreover, the capacity of NH_4EDTA to extract elements in very low concentrations in the soil made it attractive for Sr, since solutions obtained have concentrations high enough to allow the use of less expensive instrumentation (ICP-AES in place of ICP-MS). Our results suggest that both water and NH_4Cl produce better predictive models than NH_4EDTA. Since NH_4Cl extracted enough Sr to be measured on a sensitive ICP-AES, the stronger extractive capacity of NH_4EDTA and the likely gain in analytical precision and/or operating cost associated with its use compared to NH_4Cl cannot overcome its shortcomings both in terms of quality of predictive models and capacity to match plant Ca/Sr ratios to justify its use with the plants and soils used in our study.

In terms of the most suitable plant tissue to use to assess the source of Ca, leaves had the best regressions (R^2 and standard error of the estimate) with the three extractants. This may be because stem samples were made of bark and wood: since the seedlings varied in thickness, the samples likely varied in the relative proportion of these two constituents. Since bark is more physiologically active than wood, it is likely to have higher Ca/Sr ratios than wood (Poszwa et al. 2000). Hence higher variability of Ca/Sr ratios for stems than for leaves was probably responsible for the poorer fit of the stem regressions. With better-fit regressions and easier access to sampling, leaves appear to have the edge over stems to predict Ca/Sr ratio of soils. Its discrimination factors above one is however less than ideal and since other species and larger plants may behave differently, no clear recommendation can be made as to which of the plant tissue, leaf or stem, should preferentially be used.

The differential translocation of Sr and Ca internally from the stem to the leaves observed in this study and in others (Poszwa et al. 2000; Dasch et al. 2006) raises questions about the applicability to mature trees of the relationships between soil and leaf Ca/Sr obtained with

seedlings. If the higher Ca/Sr ratios usually observed in leaves is linked to the chromatographic effect of the vascular column (xylem), then mature trees are not likely to follow the relationships determined for seedlings. The Ca/Sr ratio of mature sugar maple leaves from the canopy would be expected to be higher than those of seedlings with the same source Ca/Sr ratio. However, in red spruce, there was not a change of Ca/Sr with height in vascular tissue (Momoshima and Bondietti 1990). If the enrichment in sugar maple is primarily associated with the source/sink effect as suggested by the higher ratios in the physiologically active tissues, then the results of this seedling study may be applicable to mature trees.

The analysis of the regression residuals reveals that all regressions over-estimated soil Ca/Sr ratios at low leaf Ca/Sr and under-estimated soil Ca/Sr ratios at high leaf Ca/Sr ratios. Although not presented in this paper, exponential regressions were tested and had R^2 values similar to the linear regressions but better distribution of residuals especially with the NH_4EDTA extraction. Better results could therefore be obtained with logarithmic models if regressions have to be used for predicting soil Ca/Sr ratio at low or high leaf Ca/Sr ratios.

Conclusion

The relationship between plant tissue Ca/Sr ratios and soil Ca/Sr ratios is affected by both the soil extractants and the plant tissues used. Of the three extractants and two plant tissues tested, leaves in combination with water or NH_4Cl as soil extractants provided the best predictive models. NH_4Cl with its discrimination factor closer to one would however be the best predictor of plant-available soil Ca/Sr ratio with the soils and species tested.

The study highlights the need to calibrate the soil–plant predictive model for the plant tissue and soil extractant used before attempting to assess the source of soil Ca of a plant when using the Ca/Sr approach. Since different species often have different Ca/Sr ratios when growing in the same soil (Runia 1987; Veresoglou et al. 1996; West et al. 2001), the relationships between soil and plant tissue Ca/Sr are also likely to be species specific. Soil–plant predictive models of Ca/Sr ratios should therefore be determined for other combination of species and extractants in order to improve the applicability of the Ca/Sr ratio approach to assess/locate the source of soil Ca to plants.

Acknowledgements The research was supported in part by the Natural Sciences and Engineering Research Council of Canada (NSERC) and by the Fonds Québécois de la recherche sur la Nature et les Technologies (FQRNT). We would like to thank Hélène Lalande, Alain Brousseau, and Pierre-Olivier Quesnel for help in the field and in the laboratory.

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