The relative uptake of Ca and Sr into tree foliage using a whole-watershed calcium addition

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Abstract. The use of strontium isotopes and ratios of alkaline earth elements (i.e., $^{87}Sr/^{86}Sr$ and Ca/Sr) to trace Ca sources to plants has become common in ecosystem studies. Here we examine the relative uptake of Ca and Sr in trees and subsequent accumulation in foliage. Using a whole-watershed Ca addition experiment at the Hubbard Brook Experimental Forest in N.H., we measured the uptake of Ca relative to Sr in foliage and roots of sugar maple (Acer saccharum), yellow birch (Betula alleghaniensis), American beech (Fagus grandifolia), and red spruce (Picea rubens). Vegetation was analyzed for Ca and Sr concentrations and the $^{87}Sr/^{86}Sr$ ratio. A comparison of the Ca/Sr ratio in the vegetation and the Ca/Sr ratio of the applied mineral allows for the calculation of a discrimination factor, which defines whether Ca and Sr are incorporated and allocated in the same ratio as that which is available. A discrimination factor greater than unity indicates preferential uptake of Ca over Sr; a factor less than unity reflects preferential uptake of Sr over Ca. We demonstrate that sugar maple (SM) and yellow birch (YB) have similar and small discrimination factors ($1.14 \pm 0.12, 1\sigma$ and $1.16 \pm 0.09, 1\sigma$) in foliage formation and discrimination factors of less than 1 in root formation (0.55–0.70). Uptake into beech suggests a larger discrimination factor (1.9 ± 1.2) in foliage but a similar root discrimination factor to SM and YB ($0.66 \pm 0.06, 1\sigma$). Incorporation into spruce foliage occurs at a much slower rate than in these other tree species and precludes evaluation of Ca and Sr discrimination in spruce foliage at this time. Understanding the degree to which Ca is fractionated from Sr in different species allows for refinement in the use of $^{87}Sr/^{86}Sr$ and Ca/Sr ratios to trace Ca sources to foliage. Methods from this study can be applied to natural environments in which various soil cation pools have different $^{87}Sr/^{86}Sr$ and Ca/Sr ratios. The results reported herein have implications for re-evaluating Ca sources and fluxes in forest ecosystems.

Introduction

In order to understand forest health and to evaluate the effects of acid rain on forest ecosystems, it is important to identify and differentiate between various plant nutrient sources. In forest ecosystems affected by acid deposition, macronutrients such as calcium, magnesium, and potassium, which are essential to all higher plants, are leached from the exchangeable pool (Federer et al. 1989; Likens et al. 1996; Likens et al. 1998; Huntington et al. 2000, Watmough and Dillon 2003b). It has been suggested that in acidified Ca-poor soils, depletion of Ca may directly or indirectly impact forest productivity. Sources of Ca to forest ecosystems include atmospheric deposition, as well as silicate and non-silicate mineral weathering. Differentiation and quantification of these sources is necessary in order to understand the biogeochemical cycling of Ca. In the Hubbard Brook Experimental Forest, NH, Ca/Sr and $^{87}Sr/^{86}Sr$ ratios have been defined for three ecosystem end-members (distinct sources of inputs to the system): silicate mineral weathering (Ca/Sr = 300, $^{87}Sr/^{86}Sr$ = 0.7370), apatite (Ca/Sr = 2,600, $^{87}Sr/^{86}Sr$ = 0.7153; Blum et al. 2002), and atmospheric deposition (Ca/Sr = 200, $^{87}Sr/^{86}Sr \sim 0.7100$; Bailey et al. 1996; Miller et al. 1993).

Strontium has been used as a proxy for Ca in studies of forest biogeochemistry, as it is believed to behave similarly during nutrient uptake due to its similar ionic radius (r_{Sr} = 0.113 nm vs. r_{Ca} = 0.099 nm) and charge (e.g., Graustein and Armstrong 1983; Miller et al. 1993; Vitousek et al. 1995). If Sr and Ca indeed do behave similarly, then ratios of Ca to Sr and other alkaline earth elements in plant parts can be compared

with ratios in ecosystem pools to determine sources. In addition, it has been shown that Sr isotopes are useful in determining sources of elements to surface and ground waters, and preliminary research suggests they are also useful nutrient tracers in vegetation (e.g., Miller et al. 1993; Capo et al. 1998; Vitousek et al. 1999; Blum et al. 2000). The ratio of ^{87}Sr to ^{86}Sr in rocks is dependent on the radioactive decay of ^{87}Rb to ^{87}Sr (half-life of 4.9×10^{10} years; Faure 1986) and the ratio of ^{86}Sr (a non-radiogenic isotope) to ^{87}Rb present in the original parent material. The $^{87}Sr/^{86}Sr$ of atmospheric deposition is controlled in coastal areas by the $^{87}Sr/^{86}Sr$ of the ocean water delivered via sea spray and by the regional influence of dust in areas farther inland (Graustein and Armstrong 1983; Miller et al. 1993). Cation pools in every ecosystem thus are represented by some combination of contributions from each of these sources. Therefore, at locations where the $^{87}Sr/^{86}Sr$ ratios of atmospheric deposition are distinct from that of mineral weathering, strontium (and by proxy calcium) sources in a watershed can be identified and fluxes can be quantified. Unlike many other isotopic systems, there is no significant chemical or biological isotopic fractionation of Sr (Aberg 1995), and any small degree of mass dependent isotopic fractionation is eliminated during analysis by normalizing to the $^{86}Sr/^{88}Sr$ ratio.

Although Sr has been used as a proxy tracer of Ca in many studies, other studies have suggested that the cycling of Ca in soils and plants may vary from that of Sr (Elias et al. 1982; Poszwa et al. 2000, 2004). A number of early studies measuring Ca and Sr uptake focused on herbaceous plants with mainly agricultural importance (Menzel and Heald 1955; Elias et al. 1982; Runia 1987; Veresoglou et al. 1996). These results may not be relevant to woody plants in which the ion transport pathway to foliage is significantly longer and the variance of Ca and Sr adsorption isotherms may have a larger impact. The pH of the soil solution may also affect preferential uptake of Ca or Sr in some species (Baes and Bloom 1988; Poszwa et al. 2000).

This study was conducted in the Hubbard Brook Experimental Forest (HBEF) in the White Mountains National Forest of central New Hampshire, USA. The area is underlain by a base-poor soil developed on granitiod and metamorphic glacial till, which makes it very susceptible to the leaching effects of acid deposition and provides an ideal location to study the effects of acid deposition on nutrient cycling. A large-scale manipulation experiment was conducted in the HBEF in which wollastonite ($CaSiO_3$) pellets with Ca/Sr ($2,870 \pm 36$, 1σ) and $^{87}Sr/^{86}Sr$ (0.70588 ± 0.00003, 2σ) ratios distinct from natural sources to the soil exchange complex were applied to an experimental watershed (Peters et al. 2004). In this study, we monitor the uptake of the wollastonite into trees by analyzing Ca/Sr and $^{87}Sr/^{86}Sr$ ratios in foliage and roots. Therefore, we can trace Ca through the forest ecosystem and quantitatively determine the extent of discrimination between Ca and Sr during uptake and foliage formation. Testing preferential uptake of Ca and Sr in a natural system allows us to determine some of the species-specific effects on Ca and Sr uptake and translocation, results that can then be applied to other environments. Thus, we can improve the utility of Sr isotopes for tracing Ca sources in foliage of various tree species.

Site description

This experiment was conducted in one of a series of experimental watersheds in the eastern half of the HBEF, which is underlain by the Silurian Upper Rangeley Formation (Barton et al. 1997). Soils are Spodosols with an average depth of 60 cm (Johnson et al. 1991, 2000) developed on 13,000 year-old glacial till derived from local igneous and high-grade metamorphic bedrock (Bailey and Hornbeck 1992).

The HBEF is dominated by second-growth northern hardwood forest comprised of sugar maple (*Acer saccharum* Marsh.), American beech (*Fagus grandifolia* Ehrh.), and yellow birch (*Betula alleghaniensis* Britt.). At the highest elevations and on ridgelines, red spruce (*Picea rubens* Sarg.), balsam fir (*Abies balsamea* (L.) Mill), and white birch [*Betula papyrifera* var. *cordifolia* (Marsh.) Regel] are dominant. Understory vegetation is dominated by wood fern (*Dryopteris spinulosa*) and *Viburnum* (*Viburnum alnifolium* Marsh.). Forest floor root abundance has been described previously; 44% of the forest's fine roots are in the forest floor horizons which average 7 cm in thickness (Fahey et al. 2005). Between 1907 and 1917, the area in and around the HBEF was heavily logged and then allowed to re-grow (Likens et al. 1994); a subsequent disturbance by a 1938 hurricane was followed by some salvage logging.
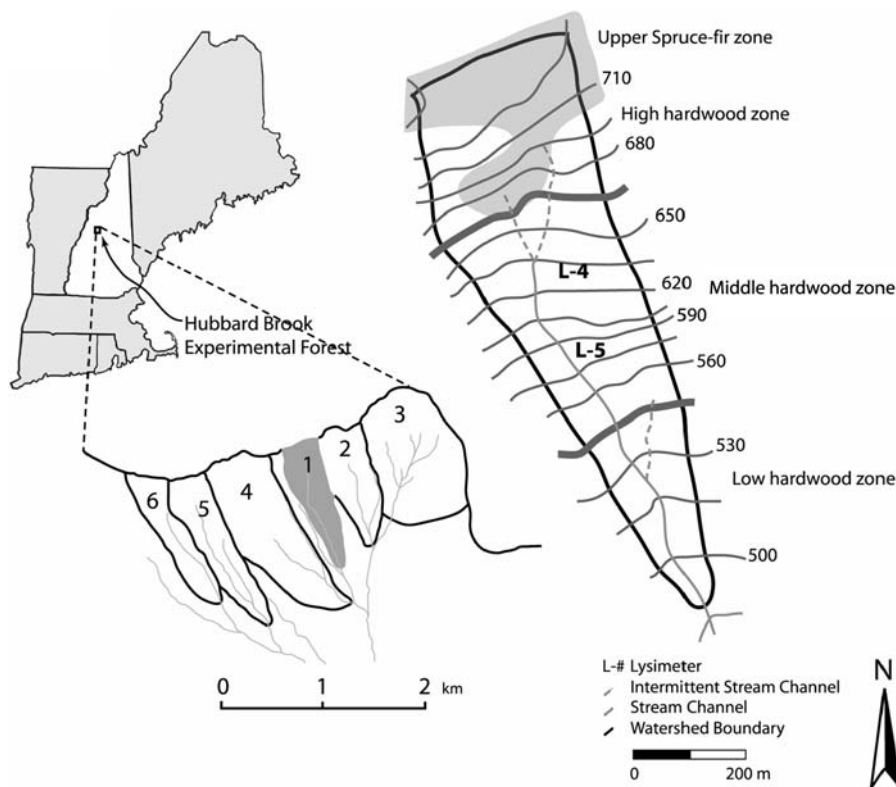
Figure 1. The Hubbard Brook Experimental Forest (HBEF), central NH, USA. Inset: Experimental Watershed 1 (W1) shaded in gray. W1 of HBEF; thick grey lines demarcate elevation divisions.

Samples and data collected are from Watershed 1 (W1), a south-facing 11.8 hectare, first order perennial catchment in the northeast corner of the HBEF (Figure 1) with a range in elevation from 450 to 750 m. In the HBEF, as in many other locations across the Northeast, a long-term depletion of Ca in the soil-exchange complex has been documented over the past 40 years (Likens et al. 1996, 1998). In October 1999, 55 tons (~ 420 g/m^2) of pelletized wollastonite ($CaSiO_3$) were added to W1 as part of a larger project to study the affects of calcium fertilization on a base-poor forest ecosystem (Peters et al. 2004). Over a period of 2 days, pellets were spread aerially by helicopter with a one-ton spreader. The products of wollastonite dissolution, with unique Ca/Sr and $^{87}Sr/^{86}Sr$ ratios, can be traced as they cycle through the ecosystem and can be used to determine fluxes, rates, sinks, and sources of Ca and Sr in the watershed.

Materials and methods

Tissue collection and analysis

Foliage
Composite samples of the understory species wood fern (WF) and *Viburnum* (VB) were collected in the summers of 1999 through 2004 along transects through W1 at four elevations designated low, mid, high, and upper (Figure 1). Within W1, overstory canopy foliage samples were collected by shotgun prior to senescence during August in 1999 through 2003 from the same five to ten individuals of sugar maple (SM), yellow birch (YB), and beech (BE) at low, mid, and high elevations. Due to limited distribution, red spruce (RS) needles were only collected from high elevations. Needles from spruce were separated by age class and current year needles were used for this study. Individuals were chosen on the basis of their proximity to

previously-installed lysimeters and throughfall collectors (Figure 1). Overstory samples from individual trees of each species were composited by mass according to species within each elevation. Collection and compositing methods were consistent over the course of the study. Composite samples of SM, YB, and BE from each elevation are unique due to spatial heterogeneity in soil, soil water chemistry, and species composition with elevation within the watershed.

Roots

Roots of BE, YB, and SM were collected in the summers of 1999 through 2003 in the forest floor of each of the low, mid, and high elevations in W1. Spruce roots were not consistently sampled over the course of the study and thus cannot be included in this analysis. Roots were shaken for 10 min in a 0.6% Tween 20 (polyoxethylene sorbitan monolaurate) solution and rinsed with deionized water to avoid contamination by soil particles or wollastonite. Roots were carefully examined and separated under a microscope to ensure accurate classification and then separated into size fractions of < 0.7 mm, 0.7–2.0 mm and > 2.0 mm. Only analyses of the < 0.7 mm fraction are reported in this paper due to the fast turnover rate of fine roots (Tierney and Fahey 2002) and the functional similarity to the rate of foliage turnover in deciduous species.

All foliage and roots were oven dried at 50 °C and then finely ground to increase sample homogeneity and facilitate digestion; 0.1–0.5 g of each foliar and root sample was then digested in ultra-pure HNO_3 and HCl using high-pressure microwave digestion in reinforced XP-1500 teflon vessels (MARS 5, CEM corporation, Matthews, NC), evaporated to dryness, and re-dissolved in ultra-pure 5% HNO_3 for analysis.

Stream and Lysimeter waters

Weekly streamwater data from October 1997 through October 2000 were used for this study. Streamwater samples were collected in acid washed LDPE bottles at weekly intervals from a small waterfall 10 m above the v-notch weir stream gauging station at the base of W1. Lysimeter waters from the Oa soil horizons were collected once a month by means of a peristaltic pump from two zero-tension lysimeters at the mid elevation in W1 (Figure 1). Additional details concerning the lysimeters can be found in Driscoll et al. (1988). Soil water was not present every month at each horizon. Lysimeter waters were filtered with polypropylene (0.45 μm) filters, acidified with ultra-pure hydrochloric acid, and an aliquot taken for elemental and isotopic analysis.

Wollastonite pellets

The wollastonite was mined from the No. 4 Quarry of the Valentine Mine, Adirondack Mountains, NY (Peters et al. 2004). Fifteen samples of the wollastonite pellets used to manipulate W1 were taken from a 1-ton storage bag prior to application. Approximately 0.5 g of pellets were pre-digested with H_2O_2 and then digested on a hotplate with ultrapure HF, HCl, and HNO_3 and analyzed for elemental ratios and $^{87}Sr/^{86}Sr$. Three additional pellet samples were collected post-application in both 2000 and 2001 from intact to partially dissolved pellets found on the forest floor of W1. Samples were pulverized in a tungsten carbide ring mill; 0.1 g of the powder was mixed in a graphite crucible with 1 g of technical grade $LiBO_2$ and heated for 20 min at 1100 °C to create a molten bead which was dissolved in 60 ml of trace metal grade 5% nitric acid. Solutions were analyzed as described below with the exception that $LiBO_2$ was added to the calibration to ensure the same matrix as that of the $LiBO_2$ digested pellet solutions.

Analyses

Analyses of elemental concentrations were conducted using inductively coupled plasma optical emission spectrometry (ICP-OES) (PE-3300DV, Perkin Elmer, Norwalk, CT). Five- to eight-point linear calibration

curves had r^2 values greater than 0.9999. For vegetation chemistry, one in-house standard and two High-Purity® solutions (Trace Metals in Drinking Water and CRM Soil Solution A) were analyzed for quality control before and after each 10-sample sequence. For water analyses, an in-house standard and one High-Purity® solution (Trace Metals in Drinking Water) were used. Analyses agreed with certified values to within ±5%.

Strontium isotope ratios were determined using a thermal ionization mass spectrometer (TIMS, MAT-262, Finnigan, Bremen, Germany) operated in static mode with the background measured at half mass. Digested samples in solution containing between 50 and 500 ng of Sr were evaporated, re-dissolved in 3 M HNO_3 and the Sr was then separated in a quartz cation exchange column using Sr-specific resin (Eichrom, Darien, IL, USA). Between 50 and 100 ng of Sr was evaporated to dryness, re-dissolved in 1 μl HCl and loaded onto tungsten single filaments with Ta_2O_5 powder and 1 μl 0.3 M H_3PO_4. $^{87}Sr/^{86}Sr$ ratios were normalized to $^{86}Sr/^{88}Sr = 0.1194$ to correct for instrumental mass fractionation. Between 100 and 200 isotope ratios were measured for each sample, yielding a mean $^{87}Sr/^{86}Sr$ ratio with an analytical uncertainty of less than ±0.000030 ($\pm 2\sigma$), unless reported otherwise. Repeated measurements of NBS-987 during the period of analysis resulted in a mean $^{87}Sr/^{86}Sr$ value of 0.710237 ± 0.000025 ($\pm 2\sigma$, $n = 138$).

Calculations

Contribution of wollastonite
A two-component isotope solute mixing model was used to determine the extent of the incorporation of Ca and Sr from wollastonite into foliage and roots (veg), with the 1999 pre-application vegetation values (pre-app) and the wollastonite (woll) value serving as the end member components. We first determined the fraction of Sr (X_{Sr}) in the vegetation from wollastonite using the following equation modified from Faure (1986):

$$X_{Sr} = \left[\frac{\left(\frac{^{87}Sr}{^{86}Sr}\right)_{veg} - \left(\frac{^{87}Sr}{^{86}Sr}\right)_{pre-app}}{\left(\frac{^{87}Sr}{^{86}Sr}\right)_{woll} - \left(\frac{^{87}Sr}{^{86}Sr}\right)_{pre-app}} \right] \tag{1}$$

To determine the fraction of Ca in the vegetation from wollastonite, we use Sr as a proxy for Ca and calculate the fraction of Ca (X_{Ca}) using the following equation modified from Capo et al. (1998):

$$X_{Ca} = \left[\frac{\left(\left(\frac{^{87}Sr}{^{86}Sr}\right)_{veg} - \left(\frac{^{87}Sr}{^{86}Sr}\right)_{pre-app} \right) \left(\frac{Sr}{Ca}\right)_{pre-app}}{\left[\left(\frac{^{87}Sr}{^{86}Sr}\right)_{veg} - \left(\frac{^{87}Sr}{^{86}Sr}\right)_{pre-app} \right] \left(\frac{Sr}{Ca}\right)_{pre-app} + \left[\left(\frac{^{87}Sr}{^{86}Sr}\right)_{woll} - \left(\frac{^{87}Sr}{^{86}Sr}\right)_{veg} \right] \left(\frac{Sr}{Ca}\right)_{woll}} \right] \tag{2}$$

Predicting Ca/Sr of uptake
Chemical analyses of vegetation plotted on a graph of $^{87}Sr/^{86}Sr$ vs. Ca/Sr should plot on a mixing line connecting pre-application values with the value of wollastonite. Because the Ca/Sr ratio in vegetation may be altered by uptake or allocation within the tree, a regression line projected to the $^{87}Sr/^{86}Sr$ value of wollastonite may or may not intercept with the Ca/Sr axis at the exact value of wollastonite. The intercept of this regression, however, does provide an estimate of the Ca/Sr ratio that is taken up or allocated within the vegetation and allows for the calculation of a discrimination factor.

Discrimination factor
We define a discrimination factor (DF) as the projected Ca/Sr ratio of vegetation at the $^{87}Sr/^{86}Sr$ value of wollastonite (Ca/Sr$_{proj}$) divided by the Ca/Sr ratio available for plant uptake (Ca/Sr$_{uptake}$) (Eq. 3a). We use wollasonite pellet values (Ca/Sr$_{woll}$) and streamwater chemistry (Ca/Sr$_{stream}$) as two alternate measures of

Ca/Sr$_{uptake}$ and consider the merits of each in the discussion section of this paper (Eq. 3b, c).

$$\text{(a) } DF = \frac{Ca/Sr_{proj}}{Ca/Sr_{uptake}} \qquad \text{(b) } DF_{woll} = \frac{Ca/Sr_{proj}}{Ca/Sr_{woll}}$$

$$\text{(c) } DF_{stream} = \frac{Ca/Sr_{proj}}{Ca/Sr_{steam}} \tag{3}$$

A discrimination factor greater than unity indicates preferential uptake of Ca over Sr; a factor less than unity reflects preferential uptake of Sr over Ca. Because initial vegetative $^{87}Sr/^{86}Sr$ values vary slightly with elevation, regressions result in different slopes; thus, regression through all data regardless of elevation may result in a miscalculation of the predicted Ca/Sr. To incorporate all data but maintain the predictive power of the smaller scale elevation data sets, we took an average of the intercepts predicted by the regression lines for each elevation to determine the most representative prediction for Ca/Sr$_{proj}$; error is reported as the standard deviation of the average.

Results

Wollastonite composition

$^{87}Sr/^{86}Sr$ ratios measured on the fifteen pellet samples varied little, with an average of 0.70588 ($\pm$ 0.00003, 2σ); the Ca/Sr ratio ranged from 2809 to 2953 with a mean of 2870 ($\pm$ 36,1σ). The small range in Ca/Sr ratios is most likely due to heterogeneity of the mined wollastonite and the incorporation of varying minor amounts of calcite (Ca(Mg)CO$_3$), prehnite (Ca$_2$Al$_2$Si$_3$), and diopside (Ca(Mg,Fe)Si$_2$O$_6$), but represents a difference of only three percent. The Ca/Sr ratios of the pellets collected in 2000 (Ca/Sr = 2697 $\pm$ 108, 1σ) and 2001 (Ca/Sr = 2855 $\pm$ 70,1σ) are not statistically different from each other or the initial 15 samples, suggesting that the 50 tons of wollastonite applied to W1 are well represented by the analyzed pellets and that differential dissolution of the pellets does not occur over time.

Streamwater chemistry

Weekly stream water samples for 1 year prior to wollastonite application establish a baseline from which to measure post-application changes in streamwater chemistry. Pre-application streamwater concentrations for all elements except Ca were statistically equivalent to long-term averages; calcium, however, has decreased significantly over the past 36 years (Likens et al. 1998; Peters et al. 2004). Over a 1 year-period immediately following the application of wollastonite, deviations in stream chemistry were due primarily to dissolution of wollastonite added directly to the stream (see Peters et al. (2004) for all streamwater data).

Streamwater values show a trend through time toward higher Ca/Sr ratios and lower $^{87}Sr/^{86}Sr$ following application of the wollastonite. Pre-application values of the streamwater Ca/Sr and $^{87}Sr/^{86}Sr$ were plotted with weekly post-application streamwater values and a regression line was drawn through these points (Figure 2). The intercept of the regression line with the Ca/Sr axis at the measured $^{87}Sr/^{86}Sr$ value for wollastonite (0.70588, $\pm$ 0.00003, 2σ) gives us an estimate of 3151 ($\pm$ 94,1σ) for the Ca/Sr$_{stream}$ ratio contributed to the streamwater by wollastonite dissolution (Figure 2).

Soil water chemistry

Analyses of lysimeter waters in the mid-elevation from 1999 through 2004 indicate that soil waters below the Oa horizon do not yet show a statistically significant effect from the wollastonite application. The Oa horizon soil water chemistry, however, is influenced by the wollastonite application (Figure 3). Linear regressions of Ca/Sr versus $^{87}Sr/^{86}Sr$ for two mid-elevation Oa horizon lysimeters are statistically significant
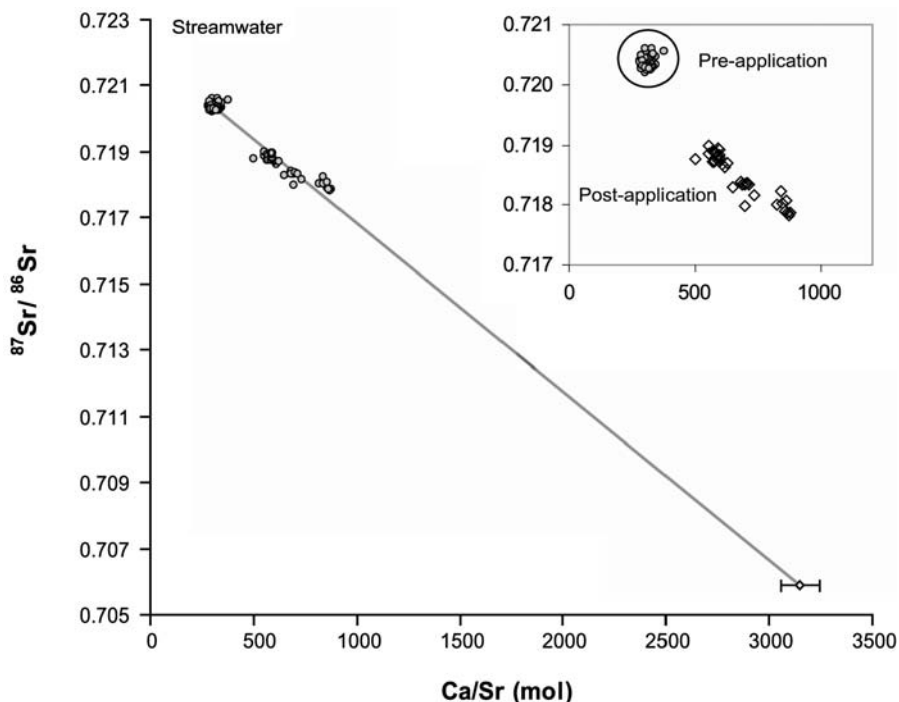
Figure 2. $^{87}Sr/^{86}Sr$ and Ca/Sr ratios of W1 streamwater. A linear regression is projected to intercept with the $^{87}Sr/^{86}Sr$ value of wollastonite, with a predicted Ca/Sr of 3,151 ± 94. Inset: 1 year of weekly pre-application data (circled) and 1 year of weekly post-application data (diamonds).

($r^2 = 0.903$; $r^2 = 0.962$; $p < 0.0001$), with projected Ca/Sr intercepts of 3212 (± 644,1σ) and 3436 (± 344,1σ) respectively (Figure 4a, b).

Foliar chemistry

$^{87}Sr/^{86}Sr$ and Ca/Sr in the pre-application foliage (Blum et al. 2002) contrast markedly with the ratios released by the dissolving wollastonite. Analyses of foliar tissues from 1999 through 2004 show a clear trend of decreasing $^{87}Sr/^{86}Sr$ and increasing Ca/Sr consistent with uptake and incorporation of wollastonite-derived Sr.

Understory

WF and VB $^{87}Sr/^{86}Sr$ ratios are correlated to time within each elevation ($p < 0.05$) (Table 1). The average of Ca concentrations from 2000 to 2004 is higher than 1999 Ca concentrations by 26, 41, and 50% in low, mid, and upper *Viburnum* and by 15, 47, 80, and 75% in low, mid, upper and high wood fern; there is no significant change in high elevation *Viburnum* Ca concentrations. In contrast, Sr concentrations show a decrease from 1999 to 2004 in wood fern at all elevations and in VB at the high elevation (Table 2). By 2004, the proportion of Ca from wollastonite calculated from the pellet value in the low, mid, upper, and high elevation WF reached 65.5, 68, 69, and 81% respectively; the proportion of Ca from wollastonite in the low, mid, upper, and high elevation VB reached 50, 48, 69, and 51% (Table 3). If the predicted ratio from the streamwater data is used instead of the pellet value, the percentages of Ca from wollastonite in foliage and roots change by less than 3% in all but two cases: VB upper 2004, which changes from 69 to 83.5% and YB high 2003, which changes from 40 to 57%. $^{87}Sr/^{86}Sr$ versus Ca/Sr linear regressions of WF data at each elevation individually are statistically indistinguishable from the average Ca/Sr ratio of 2870

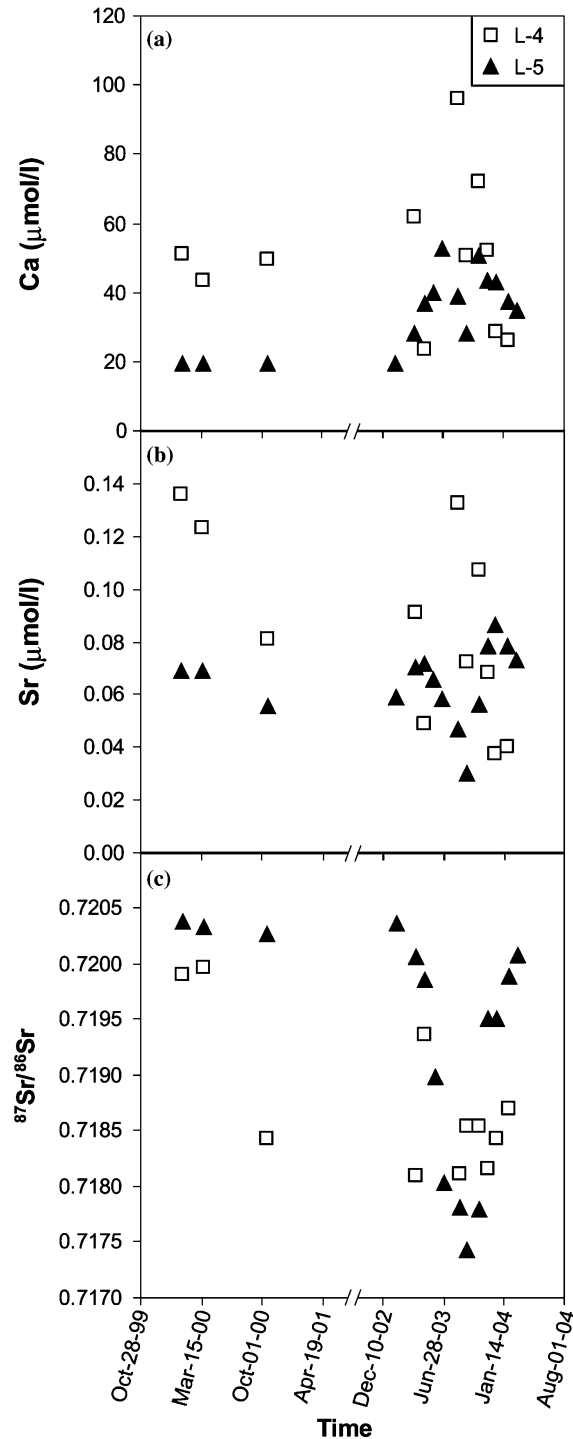*Figure 3.* Oa horizon soil water chemistry for Lysimeter 4 (open squares) and Lysimeter 5 (filled triangles). (a) Ca concentrations in mmol/l show increased variability and higher average concentrations after the wollastonite application. (b) Sr concentrations in mmol/l exhibit little change through time. (c) $^{87}Sr/^{86}Sr$ ratios decrease through time reflecting the influence of the applied wollastonite.

(a)

(b)
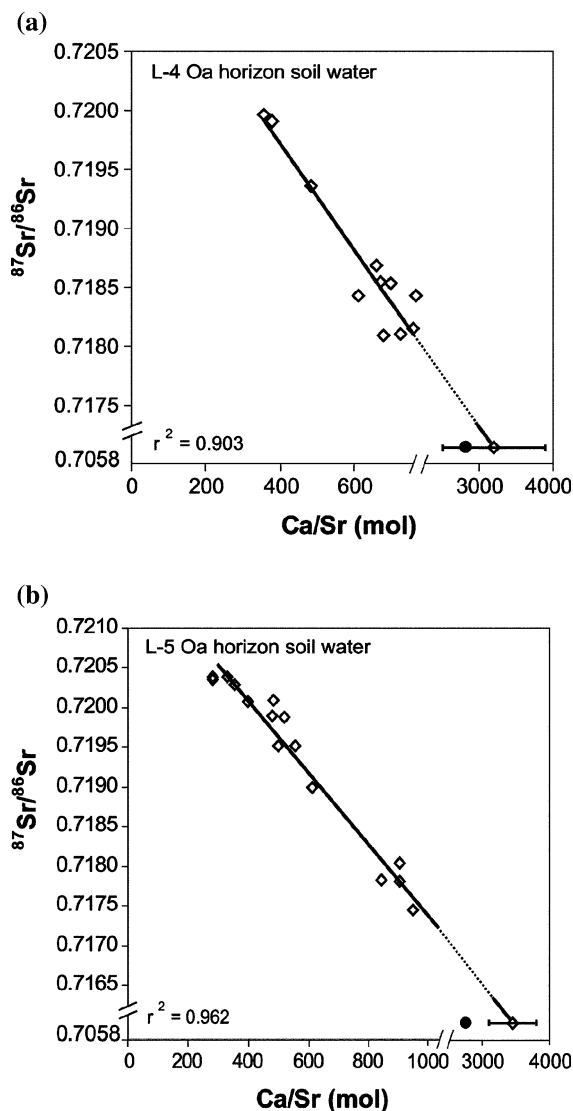
Figure 4. Oa horizon soil water chemistry with linear regression, projected intercept with error, and wollastonite pellet value (filled circle). Soil water projected Ca/Sr intercepts are statistically indistinguishable from streamwater Ca/Sr$_{proj}$. (a) Lysimeter 4 with linear regression (r^2 = 0.903) and projected Ca/Sr of 3212 ± 644. (b) Lysimeter 5 with linear regression (r^2 = 0.962) and projected Ca/Sr of 3436 ± 344.

($\pm$ 36) for the wollastonite pellets. The average of all projected intercepts from each elevation is 2582 ($\pm$ 244) for WF and 2388 ($\pm$ 464) for VB (Table 4).

Overstory
Incorporation of Ca and Sr from wollastonite into W1 trees is indicated by annual decreases in ^{87}Sr/^{86}Sr in foliage for which low-, mid- and high-elevation linear trends in YB and mid- and high-elevation linear trends in SM are highly significant (Table 1). Ca concentrations show a statistically significant ($p < 0.1$) increasing trend in foliage of YB from low (r^2 = 0.638) and mid (r^2 = 0.651) elevations and SM from low (r^2 = 0.738) and high (r^2 = 0.721) elevations; Sr shows no statistically significant increasing or decreasing trend with time (Table 5). The average of Ca concentrations from 2000 to 2003 is statistically higher than

Table 1. Linear regression of $^{87}Sr/^{86}Sr$ versus time for wood fern (WF), *Viburnum* (VB), yellow birch (YB), sugar maple (SM), American beech (BE), and red spruce (RS).

		r^2	p-value	n
WF	Low	0.940	0.0014	6
	Mid	0.836	0.0107	6
	Upper	0.682	0.0427	6
	High	0.889	0.0048	6
	All	0.512	0.0001	24
VB	Low	0.912	0.0030	6
	Mid	0.795	0.0160	6
	Upper	0.917	0.0027	6
	High	0.879	0.0057	6
YB	Low	0.991	0.0004	5
	Mid	0.937	0.0068	5
	High	0.931	0.0079	5
SM	Low	0.391	0.2600	5
	Mid	0.859	0.0234	5
	High	0.965	0.0029	5
BE	Mid	0.885	0.0171	5
	High	0.738	0.0622	5
	All	0.537	0.0160	10
RS	High	0.035	0.8134	4

Table 2. Ca and Sr concentrations and $^{87}Sr/^{86}Sr$ ratios for wood fern (WF) and *Viburnum* (VB).

Species & Elevation	Date	Ca (mg/g)	Sr (μg/g)	Ca/Sr (mol)	$^{87}Sr/^{86}Sr$
Viburnum low	1999	8.97	4.99	394	0.719818 (17)
	2000	10.59	5.34	434	0.719459 (24)
	2001	10.01	5.07	431	0.719535 (12)
	2002	13.14	5.82	494	0.719068 (14)
	2003	11.28	4.45	554	0.718812 (30)
	2004	11.64	4.40	578	0.718156 (22)
Viburnum mid	1999	9.00	4.32	456	0.719783 (16)
	2000	11.56	4.98	508	0.720634 (19)
	2001	12.12	4.81	551	0.719756 (12)
	2002	14.70	5.13	626	0.718871 (14)
	2003	12.32	4.33	623	0.718450 (10)
	2004	12.75	3.58	778	0.718003 (22)
Viburnum upper	1999	7.74	3.90	434	0.720001 (17)
	2000	11.69	4.46	574	0.720033 (21)
	2001	11.93	4.12	632	0.718877 (12)
	2002	15.38	4.43	759	0.718832 (15)
	2003	11.97	2.78	941	0.717055 (14)
	2004	7.00	1.42	1079	0.716420 (12)
Viburnum high	1999	16.56	7.27	498	0.720455 (18)
	2000	11.34	4.98	498	0.720512 (17)
	2001	13.54	4.73	626	0.719132 (13)
	2002	15.08	4.06	811	0.719441 (16)
	2003	12.47	3.23	844	0.718439 (14)
	2004	12.35	3.09	875	0.718244 (27)

Table 2. Continued.

Species & Elevation	Date	Ca (mg/g)	Sr (μg/g)	Ca/Sr (mol)	^{87}Sr/^{86}Sr
Wood fern low	1999	3.94	2.04	424	0.719911 (12)
	2000	5.17	1.90	597	0.718747 (19)
	2001	4.44	1.52	640	0.718356 (12)
	2002	4.52	1.29	766	0.717756 (18)
	2003	4.43	1.35	717	0.717697 (16)
	2004	4.08	1.06	847	0.716847 (15)
Wood fern mid	1999	3.24	1.67	425	0.719774 (15)
	2000	5.30	1.43	813	0.718925 (17)
	2001	5.34	1.22	961	0.717331 (14)
	2002	4.60	1.18	850	0.717507 (17)
	2003	4.37	1.14	840	0.717522 (34)
	2004	4.28	0.95	989	0.716463 (14)
Wood fern upper	1999	3.09	1.30	519	0.719634 (16)
	2000	6.61	1.41	1027	0.717778 (19)
	2001	5.82	1.18	1076	0.716168 (14)
	2002	6.11	1.19	1119	0.716212 (16)
	2003	4.45	0.94	1035	0.716774 (15)
	2004	4.87	0.83	1287	0.715769 (11)
Wood fern high	1999	2.64	0.87	664	0.720218 (17)
	2000	5.15	0.96	1173	0.717407 (24)
	2001	5.32	0.90	1295	0.715189 (05)
	2002	4.72	0.78	1323	0.715036 (14)
	2003	3.71	0.51	1580	0.712784 (18)
	2004	4.26	0.60	1548	0.713198 (15)

Numbers in parentheses represent 2 SE for the last two digits of the ^{87}Sr/^{86}Sr ratio.

Table 3. Percent Ca from wollastonite in foliage of wood fern (WF), *Viburnum* (VB), yellow birch (YB), sugar maple (SM), and American beech (BE) through time.

	1999	2000	2001	2002	2003	2004
WF low	0.0	38.0	46.0	55.0	56.0	65.5
WF mid	0.0	31.0	59.0	57.0	57.0	68.0
WF upper	0.0	46.5	65.0	65.0	59.0	69.0
WF high	0.0	51.0	70.0	71.0	82.5	81.0
VB low	0.0	16.0	13.0	29.5	36.0	50.0
VB mid	0.0	0.0	2.0	34.5	40.0	48.0
VB upper	0.0	0.0	36.5	37.5	63.5	69.0
VB high	0.0	0.0	37.0	30.0	48.0	51.0
YB low	0.0	10.0	22.0	31.5	36.0	n/a
YB mid	0.0	1.0	12.0	23.0	32.0	n/a
YB high	0.0	1.0	14.0	30.5	40.0	n/a
SM low	0.0	3.3	0.0	4.0	11.0	n/a
SM mid	0.0	3.0	5.0	28.0	34.0	n/a
SM high	0.0	7.0	19.0	25.0	37.5	n/a
BE low	0.0	0.0	0.0	0.0	0.0	n/a
BE mid	0.0	0.5	4.0	4.0	6.0	n/a
BE high	0.0	0.0	0.5	10.0	12.5	n/a

n/a = not available.

1999 foliar Ca concentrations by 41% in YB high and 38% in SM low. The average of 2000–2003 foliar Sr concentrations is statistically lower than 1999 values by 12, 4, and 25% for low and mid YB and mid BE; Sr concentrations are higher by 11, 28, 16, and 6% for YB high, SM low, SM high and BE high. Results from mixing calculations to determine the contribution of wollastonite to the total Ca in foliage show an increase

Table 4. Linear regression analysis of $^{87}Sr/^{86}Sr$ versus Ca/Sr for foliage of wood fern (WF), *Viburnum* (VB), yellow birch (YB), sugar maple (SM), and American beech (BE).

	n	r^2	p-value	Intercept	Error 1σ	DF_{woll}	Error 1σ	DF_{stream}	Error 1σ
Stream water	140	0.960	0.0000	3151	94	1.10	0.03	1.00	0.03
WF low	6	0.982	0.0001	2383	322	0.83	0.11	0.76	0.10
WF mid	6	0.797	0.0166	2623	1274	0.91	0.44	0.83	0.40
WF upper	6	0.897	0.0041	2910	900	1.01	0.31	0.92	0.29
WF high	6	0.967	0.0004	2409	305	0.84	0.11	0.76	0.10
WF all	24	0.879	0.0000	2716	296	0.95	0.10	0.86	0.09
WF average				2582	244	0.90	0.09	0.82	0.08
VB low	6	0.937	0.0015	2061	570	0.72	0.20	0.65	0.18
VB mid	6	0.748	0.0262	1927	1080	0.67	0.38	0.61	0.34
VB upper	6	0.935	0.0016	2694	720	0.94	0.25	0.85	0.23
VB high	6	0.811	0.0143	2870	1462	1.00	0.51	0.91	0.46
VB all	24	0.651	0.0000	2485	604	0.87	0.21	0.79	0.19
VB average				2388	464	0.83	0.16	0.76	0.15
YB low	5	0.984	0.0009	3064	544	1.07	0.19	0.97	0.17
YB mid	5	0.963	0.0030	3418	983	1.19	0.34	1.08	0.31
YB high	5	0.966	0.0027	3539	920	1.23	0.32	1.12	0.29
YB average				3340	247	1.16	0.09	1.06	0.08
SM low	5	0.839	0.0288	2893	1943	1.01	0.68	0.92	0.62
SM mid	5	0.839	0.0091	3365	1499	1.17	0.52	1.07	0.48
SM high	5	0.981	0.0011	3570	722	1.24	0.25	1.13	0.23
SM average				3276	347	1.14	0.63	1.04	0.57
BE mid	5	0.309	0.3305	4934	11313	1.72	3.94	1.57	3.59
BE high	5	0.670	0.0903	6396	7188	2.23	2.50	2.03	2.28
BE mid & high	5	0.540	0.0155	5464	3493	1.90	1.22	1.73	1.11

Discrimination factors (DF) calculated for a wollastonite source (DF_{woll}) and stream source (DF_{stream}). Standard errors (1σ) are given for all values with the exception of average values, for which standard deviation of the average is given.

Table 5. Ca and Sr concentrations and $^{87}Sr/^{87}Sr$ ratios for yellow birch (YB), sugar maple (SM), and American beech (BE) foliage.

Species & Elevation	Date	Ca (mg/g)	Sr ($\mu g/g$)	Ca/Sr (mol)	$^{87}Sr/^{86}Sr$
Yellow birch low	1999	9.14	3.20	624	0.719909 (23)
	2000	7.95	2.70	643	0.719593 (14)
	2001	8.21	2.37	758	0.719111 (16)
	2002	10.80	2.86	827	0.718638 (12)
	2003	13.54	3.32	892	0.718377 (13)
Yellow birch mid	1999	8.32	3.02	602	0.720114 (19)
	2000	7.74	2.98	568	0.720090 (17)
	2001	7.58	2.66	624	0.719720 (14)
	2002	9.93	2.96	733	0.719288 (14)
	2003	11.43	3.01	831	0.718806 (20)
Yellow birch high	1999	6.44	2.02	698	0.719559 (22)
	2000	8.80	2.73	705	0.719522 (13)
	2001	8.51	2.23	833	0.719043 (12)
	2002	10.40	2.20	1032	0.718237 (16)
	2003	8.62	1.77	1068	0.717639 (22)
Sugar maple low	1999	5.71	2.78	449	0.719712 (19)
	2000	6.13	2.80	479	0.719638 (13)
	2001	7.93	3.79	458	0.719820 (18)
	2002	9.34	4.20	486	0.719629 (14)
	2003	8.22	3.47	517	0.719445 (21)

Table 5. Continued.

Species & Elevation	Date	Ca (mg/g)	Sr (μg/g)	Ca/Sr (mol)	^{87}Sr/^{86}Sr
Sugar maple mid	1999	5.65	2.86	432	0.720400 (14)
	2000	5.37	2.39	490	0.720341 (17)
	2001	7.38	3.55	455	0.720282 (13)
	2002	14.02	5.34	575	0.719600 (14)
	2003	10.21	3.38	662	0.719377 (15)
Sugar maple high	1999	5.41	1.93	613	0.719754 (21)
	2000	4.61	1.51	668	0.719544 (13)
	2001	6.41	1.96	716	0.719101 (17)
	2002	11.46	3.11	805	0.718842 (13)
	2003	10.03	2.36	930	0.718180 (13)
Beech mid	1999	8.62	2.29	823	0.720320 (14)
	2000	6.41	1.80	781	0.720305 (19)
	2001	5.53	1.54	786	0.720153 (19)
	2002	6.66	1.87	777	0.720174 (13)
	2003	6.96	1.70	893	0.720060 (39)
Beech high	1999	4.90	1.47	731	0.720169 (12)
	2000	5.16	1.53	739	0.720364 (12)
	2001	5.35	1.52	770	0.720151 (14)
	2002	7.16	1.91	820	0.719771 (15)
	2003	6.18	1.28	1056	0.719676 (18)
Red spruce high	1999	1.57	0.09	4034	0.720071 (16)
	2000	4.48	0.38	2600	0.719457 (14)
	2001	1.58	0.09	3933	0.720037 (14)
	2002	2.55	0.16	3489	0.720019 (18)
	2003	2.78	0.23	2683	0.720145 (07)

Numbers in parentheses represent 2 SE for the last two digits of the ^{87}Sr/^{86}Sr ratio.

to 36, 32, and 40% in low-, mid-, and high-elevation YB and 11, 34, and 38% in low-, mid-, and high-elevation SM by 2003 (Table 3). Decreases in foliar ^{87}Sr/^{86}Sr for mid- and high-elevation BE are significant and nearly significant ($p = 0.06$) with 6 and 13% Ca from wollastonite in mid- and high-elevation foliage by 2003. Foliar ^{87}Sr/^{86}Sr in new needles of RS shows no change with time ($r^2 = 0.164$, $p > 0.1$) and less than 5% contribution of wollastonite to total Ca in foliage from any year, indicating slower rates of Ca uptake for RS from wollasonite-dominated soil solutions or within-tree Ca ion transport and allocation to foliage. Discrimination between Ca and Sr can only be addressed for those species for which statistically significant annual decreases in ^{87}Sr/^{86}Sr of foliage were recorded over the study period, therefore RS was excluded from further analysis; in the future a similar analysis should be possible for this species. Linear regressions of ^{87}Sr/^{86}Sr versus Ca/Sr for YB and SM at each of the low-, mid-, and high-elevations are statistically significant ($r^2 > 0.84$, $p < 0.03$; Figure 5a, b). Ca/Sr$_{proj}$ can be calculated for each species at each elevation (Table 4).

Root tissue chemistry

Decreases in the ^{87}Sr/^{86}Sr of roots of YB, SM, and BE after 1999 suggest that uptake of wollastonite into the forest floor roots occurred very quickly. Average Ca concentrations from 2000 to 2003 (Table 6), show statistically significant and large increases from the 1999 values for YB high (224%), SM low (117%), SM high (265%), BE low, mid and high (138, 251, and 298%). The proportion of Ca from wollastonite in the roots of all species from 2000 to 2003 averages 73% with no statistical increase or decrease in the percentage through time after the application (Table 7). Trends in ^{87}Sr/^{86}Sr with time are only significant in SM low- and mid-elevations ($r^2 = 0.97$, $p < 0.02$) and nearly significant for high elevation BE ($r^2 = 0.68$,
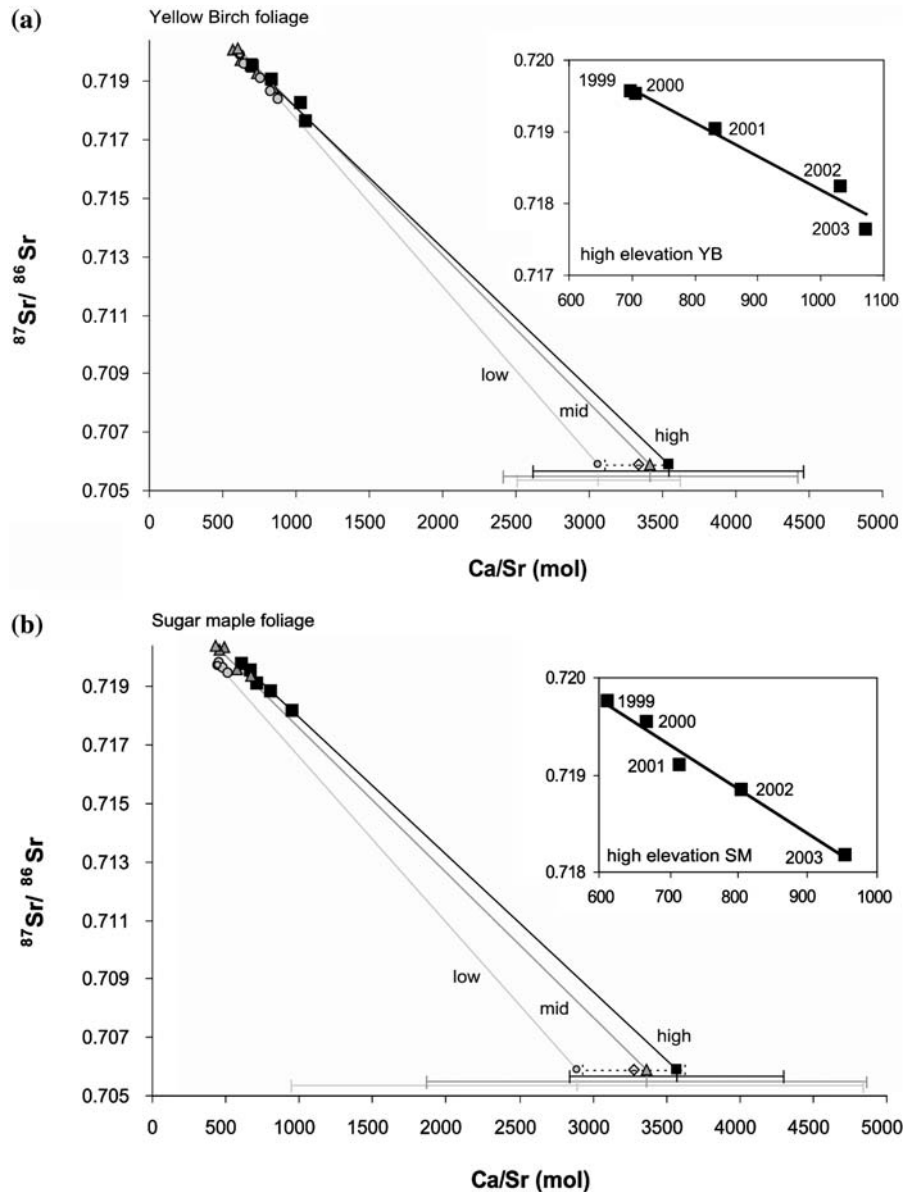
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Figure 5. Projected linear regressions of Ca/Sr versus $^{87}Sr/^{86}Sr$ for (a) Yellow birch foliage and (b) Sugar maple foliage by elevation showing individual intercept errors, the averaged intercept value, and the error on the average value. Low elevation in circles, mid in triangles, high in squares. Average intercept value shown in diamond with dashed error bars. Insets show a linear regression through high elevation foliage of YB and SM respectively.

$p = 0.09$). Linear regressions of $^{87}Sr/^{86}Sr$ versus Ca/Sr yield significant ($p \leq 0.1$, error is 1σ) trends for YB low (Ca/Sr$_{proj}$ = 2006 ± 1568), SM low (Ca/Sr$_{proj}$ = 1541 ± 1594), SM mid (Ca/Sr$_{proj}$ = 1731 ± 1110), and SM high (Ca/Sr$_{proj}$ = 1917 ± 2250), and BE mid (Ca/Sr$_{proj}$ = 2070 ± 1431) and BE high (Ca/Sr$_{proj}$ = 1805 ± 352). In all cases, Ca/Sr$_{proj}$ of roots is lower than Ca/Sr$_{proj}$ for the foliage of the same species.

Table 6. Ca and Sr concentrations and $^{87}Sr/^{87}Sr$ ratios for < 0.7 mm forest floor roots of yellow birch (YB), sugar maple (SM), and American beech (BE).

Species & Elevation	Date	Ca (mg/g)	Sr (μg/g)	Ca/Sr (mol)	$^{87}Sr/^{86}Sr$
Yellow birch low	1999	3.99	2.82	310	0.719024 (17)
	2000	13.07	3.25	876	0.715788 (15)
	2001	6.46	2.64	535	0.716248 (19)
	2002	4.04	2.55	347	0.718639 (11)
	2003	5.75	2.36	533	0.716453 (15)
Yellow birch mid	1999	3.12	2.26	302	0.719499 (25)
	2000	4.39	1.23	780	0.716050 (16)
	2001	4.92	2.05	525	0.716891 (14)
	2002	2.50	2.07	264	0.716458 (11)
	2003	8.89	3.04	641	0.716423 (21)
Yellow birch high	1999	2.24	1.30	378	0.719512 (18)
	2000	7.51	2.45	671	0.716386 (11)
	2001	9.50	1.80	1156	0.714628 (15)
	2002	4.75	1.21	861	0.719498 (17)
Sugar maple low	1999	2.52	2.01	275	0.718703 (19)
	2000	6.10	2.12	630	n/a
	2001	3.60	2.06	382	0.716971 (21)
	2002	6.31	2.47	560	0.716720 (14)
	2003	5.88	2.20	586	0.715399 (16)
Sugar maple mid	1999	2.15	1.70	277	0.719799 (15)
	2000	n/a	n/a	n/a	n/a
	2001	4.99	2.08	524	0.716329 (15)
	2002	8.48	1.94	955	0.714279 (14)
	2003	2.60	0.69	827	0.71383 (18)
Sugar maple high	1999	1.73	1.25	304	0.719130 (16)
	2000	n/a	n/a	n/a	n/a
	2001	6.02	1.37	959	0.713392 (17)
	2002	6.61	1.52	951	0.714345 (14)
Beech low	1999	2.04	1.81	248	0.719241 (17)
	2000	6.64	1.96	742	0.716609 (16)
	2001	4.34	2.00	475	0.716496 (16)
	2002	5.35	2.20	533	0.716749 (12)
	2003	3.16	1.52	455	0.716602 (27)
Beech mid	1999	1.56	1.69	203	0.719605 (19)
	2000	3.06	1.57	427	0.717278 (16)
	2001	4.74	1.93	537	0.716236 (16)
	2002	7.95	1.75	993	0.713953 (11)
	2003	6.26	1.72	797	0.717003 (28)
Beech high	1999	1.35	1.14	261	0.719695 (22)
	2000	3.79	1.30	640	0.715429 (15)
	2001	6.24	1.39	980	0.713559 (21)
	2002	5.86	1.37	933	0.713358 (11)
	2003	5.74	1.33	943	0.713814 (25)

Numbers in parentheses represent 2 SE for the last two digits of the $^{87}Sr/^{86}Sr$ ratio.
n/a = not available.

Discrimination factors (DF)

The determination of species-specific Ca/Sr_{proj} allows for the calculation of foliar discrimination factors (DF) for these species. There are two internally consistent estimates that can be used to calculate the plant-

Table 7. Percent Ca from wollastonite in < 0.7 mm forest floor roots of yellow birch (YB), sugar maple (SM), and American beech (BE).

	1999	2000	2001	2002	2003
YB low	0.0	76.0	71.5	22.0	69.5
YB mid	0.0	76.0	69.0	73.0	73.5
YB high	0.0	69.0	81.0	1.0	n/a
SM low	0.0	n/a	62.0	65.0	78.5
SM mid	0.0	n/a	77.5	87.5	89.0
SM high	0.0	n/a	86.0	84.0	n/a
BE low	0.0	74.0	75.0	72.5	74.0
BE mid	0.0	74.0	82.0	91.0	76.0
BE high	0.0	83.0	90.0	90.0	89.0

n/a = not available.

available Ca/Sr from wollastonite. The estimate we prefer is direct measurement of the wollastonite pellets (DF_{woll}), resulting in a plant-available Ca/Sr ratio of 2870 ($\pm$ 36). Alternately, the projected Ca/Sr of streamwater (DF_{stream} = 3151, $\pm$ 94, 1σ) can be used as an estimate of the average Ca/Sr of wollastonite applied to the watershed. Although calculation of species-specific discrimination factors from the Ca/Sr ratios of wollastonite pellets and streamwater averaging are independent means of assessing plant-available Ca/Sr, the discrimination factors are not significantly different when either estimate is employed (Table 4).

Foliar DF

Discrimination factors calculated for both SM and YB foliage are similar (DF_{woll} = 1.14 $\pm$ 0.12 and 1.16 $\pm$ 0.09 respectively; DF_{stream} = 1.04 $\pm$ 0.11 and 1.06 $\pm$ 0.08) (Figure 5a, b). Linear regressions of BE for mid- and high-elevations are not significant and nearly significant, respectively, and thus the calculated foliar discrimination factor has a large error associated with it. We chose to combine the mid- and high-elevation data for BE, sacrificing spatial variability, in order to gain some information about the general behavior of Ca and Sr uptake in BE. The linear regression for BE mid- plus high-elevation is statistically significant with a calculated foliar DF_{woll} of 1.90 ($\pm$ 1.22) and DF_{stream} is 1.73 ($\pm$ 1.11). The discrimination factor for BE has a much higher level of uncertainty than YB or SM such that clarification of the foliar discrimination factor for BE will need to await higher levels of wollastonite uptake.

Root DF

The uptake of wollastonite into the roots of YB, SM, and BE contrasts with that of the foliage. In the data sets for which linear regressions of $^{87}Sr/^{86}Sr$ versus Ca/Sr yield significant ($p \leq 0.1$) trends, preliminary discrimination factors can be calculated for each species by elevation (YB) and by the averaging method described above for foliage (SM and BE). In all cases, the discrimination factor is less than one and all values are statistically indistinguishable from one another. The DF_{woll} for YB (low) is 0.70 ($\pm$ 0.55, 1σ) and DF_{stream} is 0.64 ($\pm$ 0.50, 1σ). Sugar maple DF_{woll} calculated by averaging the projected Ca/Sr values is 0.60 ($\pm$ 0.07, 1σ) and DF_{stream} is 0.55 ($\pm$ 0.06, 1σ); beech root DF_{woll}, is 0.66 ($\pm$ 0.06, 1σ) and DF_{stream} = 0.60 ($\pm$ 0.05, 1σ). Although we have not yet quantitatively measured the DF for woody stem materials by species and elevation, preliminary data suggest that bole discrimination factors are similar to root tissues.

Discussion

Incorporation of wollastonite-derived cations

Foliage

Both SM and YB exhibit similar behavior in the incorporation of wollastonite-derived Ca into foliage over time, which suggests similar rates of Ca uptake and transport. In contrast, by 2003 only 6 and 13% of the

Ca in BE foliage was from wollastonite in mid- and high-elevations respectively, 25% less than the proportion of Ca from wollastonite in high elevation SM and YB foliage. These data can be explained by slower rates of Ca movement in BE relative to SM and YB and possibly by alternate methods of nutrient access employed by BE, which have ectomycorrhizal associations. Alternatively, lower concentrations of fine roots in the forest floor and a higher concentrations of roots in the deeper soil would result in the incorporation of less Ca from wollastonite in BE foliage.

Regardless of the relative rate of uptake, in each of the three major species discussed (YB, SM, and BE), an increasing proportion of Ca from wollastonite is incorporated with time and with increasing elevation (Table 3). Larger percentages at high elevations suggest that more wollastonite-derived Ca may be available for uptake, and thus that more dissolution has occurred at the higher elevations. This hypothesis is consistent with previous studies that have shown that mineral weathering rates are higher across the high elevation of W1 (Hogan and Blum 2003; Nezat et al. 2004). The shallower, organic-rich soils of the high elevation provide less acid neutralization (Johnson et al. 2000) and experience enhanced acid deposition relative to lower elevations due to cloud impaction and the year-round presence of foliage (Lovett et al. 1999). The net impact of these factors is that chemical weathering rates are significantly higher across the high elevation of W1, and this difference is most likely responsible for the greater amount of wollastonite-derived Ca in this region.

The relative uptake of Ca and Sr by spruce cannot be evaluated at this time as there has not been enough time since the application to show significant wollastonite-derived Ca and Sr transport to foliage. A lower concentration of roots in the forest floor and higher concentration of roots in deeper mineral soil could result in a slower rate of wollastonite-derived Ca and Sr incorporation into foliage. An additional explanation for the lack of wollastonite in the spruce needles is the ability of spruce to directly access mineral nutrients via ectomycorrhizal associations. Several studies have shown that ectomycorrhizal hyphae are able to enhance mineral uptake of Ca from minerals via exudates (Jongmans et al. 1997; Van Breemen et al. 2000; Smits et al. 2005), a phenomenon shown to be correlated to nutrient-poor sites (Hoffland et al. 2003). Blum et al. (2002) suggested that spruce might access apatite (calcium phosphate) directly, partially bypassing the soil exchange complex and possibly contributing to higher Ca/Sr ratios relative to the soil exchange complex.

Roots

In contrast to the stepwise increase in uptake of Ca and Sr from wollastonite through time displayed in the foliage, YB, SM, and BE roots incorporated ~70% of Ca from wollastonite after the first year and this amount remained relatively constant through time (Table 7). This pattern may reflect a steady state of contributions from the continuing dissolution of wollastonite and Ca input from atmospheric deposition, mineral weathering, and recycling of the forest floor. This contrasting observation suggests that some Ca and Sr is transported from root to leaf in a single year, but that significant amounts of Ca and Sr are also remobilized from within the tree during any given year. Complete turnover of a tree's Ca reservoir must take considerably longer than five years. Most of the delay in the movement of absorbed Ca through the tree is probably associated with ion exchange of Ca on fixed-charge exchange sites in the xylem cell walls (Ferguson and Bollard 1976).

Determination of Ca/Sr$_{uptake}$

To quantify the degree of discrimination between Ca and Sr by plants it is essential to have an accurate measure of the Ca/Sr ratio released by the wollastonite and available for plant uptake. Direct measurement of the wollastonite used in the application resulted in an average Ca/Sr$_{woll}$ value of 2870 ($\pm$ 36, 1σ). We assume congruent release of Ca and Sr from the wollastonite pellets but recognize that initial dissolution of minor impurities may give the appearance of incongruent release in the initial phase of the experiment. The minor impurities, however, are present in a small enough percentage as to not affect the overall influence of wollastonite on the watershed as a whole.

Non-woody understory species, such as wood fern (*Dryopteris spinulosa*), that regenerate their foliage each year, serve as indicators of rapid nutrient uptake from the forest floor; their foliar chemistry is not affected by a large ion exchange column such as that of woody species like YB and SM. Shallowly rooted ferns are thus a first-order monitor of nutrients available for uptake from the forest floor in any given year, and may not be as strongly influenced by soil water and the ion exchange pool as woody species. The Ca/Sr$_{proj}$ for WF (2582 ± 244, 1σ) is consistent with the Ca/Sr ratios measured on the wollastonite pellets values (2870 ± 36, 1σ). VB values are somewhat lower than ferns 2388 (± 464, 1σ) but within error, agree with WF data.

Changes in streamwater composition during the first year following application can be used as an alternative to using bulk chemical values for wollastonite to evaluate the Ca/Sr ratio of wollastonite dissolution in the watershed. The Ca/Sr$_{stream}$ from streamwater data is 3151 (±94) (Figure 2), which is significantly greater than measured bulk wollastonite values. Dissolution of minor accessory minerals such as calcite, which makes up less than 4% of the total pellet mass, would not provide an explanation for the observed Ca/Sr, as the calcite (Ca/Sr = 1320) would lower the overall Ca/Sr compared with bulk wollastonite. Analyses of Oa horizon soil waters also show slightly higher Ca/Sr ratios than bulk wollastonite (Figure 4a, b). Ca/Sr intercepts of 3212 (± 644, 1σ) and 3436 (± 344, 1σ) suggest that the soil water, like the stream water, is slightly elevated in Ca/Sr relative to bulk wollastonite.

One possible explanation for the difference in Ca/Sr$_{woll}$ and Ca/Sr$_{stream}$ might be a minor preference for Sr (over Ca) on exchange sites in the hyporheic zone (the saturated zone beneath the streambed), which could result in an elevated Ca/Sr$_{stream}$. Sr^{2+} is strongly adsorbed to organic matter, soils and phyllosilicates; a number of studies have shown a slight preference for Sr^{2+} over Ca^{2+} which could result in a small fractionation of Sr from Ca (e.g., Bruggenwert and Kamphorst 1982; Sposito 1989). If the adsorption isotherms for Sr and Ca are not the same (Isermann 1981), preferential loading of Sr over Ca on exchange sites could elevate the Ca/Sr ratio of streamwater above the Ca/Sr ratio of the applied wollastonite. Peters et al. (2004) suggested that the pH increase associated with the wollastonite application in W1 might have disturbed the streamed cation exchange complex equilibrium, resulting in a 20-day-period of Ca accumulation and re-equilibration. Additional studies have shown an increase in the loading of Ca and other base cations in streambed sediments associated with increases in pH (Hall et al. 2001; Likens et al. 2004).

Other studies have shown that cation exchange capacity, soil pH, and organic matter also affect the absolute and relative concentration of Ca and Sr on exchange sites and in soil solution (Baes and Bloom 1988; Veresoglou et al. 1996; Poszwa et al. 2000); Veresoglou et al. (1996) suggest that Sr is less available proportionally in soil solution than Ca, a difference that could explain elevated soil water Ca/Sr ratios in relation to that of bulk wollastonite. In W1 before the application, soil water had a Ca/Sr range of 300–500, and exchange sites had a range of 200–325, Blum et al. 2002) also suggesting that there is a preference for Sr over Ca on the exchange sites. After the application, Ca/Sr ratios in the Oa horizon soil waters more than double from 1999 pre-application values of 318 and 282 to 2004 values of 764 and 900.

Discrimination factors and implications for mineral Ca sources

Calculation of species-specific discrimination factors makes it possible to more accurately apply Ca/Sr and Sr isotope ratios in foliage to determine Ca sources in other ecosystems. Discrimination factors calculated for both SM and YB are similar (DF$_{woll}$ = 1.14 ± 0.12 and 1.16 ± 0.09, respectively; DF$_{stream}$ = 1.04 ± 0.11 and 1.06 ± 0.08) indicating that mechanisms of access and allocation of Ca and Sr are comparable in these two species (Figure 5a, b). Preliminary BE discrimination factors (DF$_{woll}$ = 1.90 ± 1.22 and DF$_{stream}$ = 1.73 ± 1.11) indicate that different mechanisms of allocation may be employed. Species-specific physiology needs to be studied in greater detail to understand the mechanisms behind partitioning of Ca and Sr in trees and to perhaps lend predictive power for application of discrimination factors to other species.

In recent studies, Blum et al. (2002) and Nezat et al. (2004) suggested that apatite weathering is a major contributor of Ca to HBEF. Blum et al. (2002) pointed out that published DFs for vegetation ranged from

1.0 to 1.4 but that there were no measurements for trees found in the northern forest. They also suggested that if there were no discrimination of Ca relative to Sr, that mixing calculations indicated that ~75%, ~60%, and ~80% of calcium respectively in YB, SM, and BE foliage could be derived from apatite. They further argued that these percentages were maximum values because of uncertainty in discrimination factors. They proposed that foliar discrimination factors would have to be as high as 2.9, 2.3,and 3.7 for YB, SM, and BE respectively to fully explain the foliar Ca/Sr ratios (Blum et al. 2002) if apatite was not a contributor of Ca. Based on our determination of discrimination factors, we can now more accurately recalculate the proportion of Ca from apatite in the foliage of YB, SM, and BE and suggest that values of 69%, 53%, and 52% from apatite is a more appropriate estimate. This study further clarifies the role of apatite as an ultimate source of calcium to the watershed, consistent with Nezat et al. (2004). Identifying apatite as a source of Ca to W1 does not prove that trees are directly accessing apatite via fungal hyphae as has been inferred (Wallander and Hagerberg 2004), but that over hundreds to thousands of years of nutrient cycling, annual incorporation by trees of small amounts of Ca from apatite has contributed significantly to the composition of the plant-available pool of nutrients.

The observation that discrimination factors are less than one for roots and greater than one for foliage has implications for understanding the role of vegetation in Ca cycling on a watershed scale. With a manipulation experiment such as the wollastonite application, the contrast in Ca/Sr ratios of foliage and roots has the potential result of temporarily elevating the Ca/Sr ratio of foliage, soil and streamwater above the value for wollastonite. One likely mechanism contributing to this discrimination would be preferential adsorption of Sr over Ca during ion exchange on xylem cell walls in a manner analogous to that noted for the soil exchange complex. However, Momoshima and Bondietti (1990) suggested that Sr moves at nearly the same rate as Ca during ascent of xylem sap in spruce. In any case, on longer time scales, the decomposition of woody tissue with a discrimination factor less than one and its return to the plant available pool would maintain the balance in the watershed-scale Ca cycle to reflect the original Ca/Sr of the sources.

Our findings may help to clarify the question of whether preferential uptake of Ca over Sr into foliage would result in a relative depletion of Ca relative to Sr in the soil exchange complex. Research has shown that Ca and other ions move by ion exchange processes within the xylem of woody plants (Ferguson and Bollard 1976). Our results suggest that significant preferential uptake of Ca over Sr does not occur at the root cell wall, but that instead, internal ion exchange favors Sr over Ca resulting in a relative enrichment of Sr in the wood and Ca in the foliage. These results are also in general agreement with previous studies that have noted that the Ca/Sr ratio may be changed by ion exchange, plant uptake or allocation (Runia 1987; Aberg et al. 1989). Also consistent with our results is a study, conducted over 60 years ago, of cation uptake and selective absorption in higher plants that shows data with a range of foliar discrimination factors between 1.02 and 2.01 for eleven different species (Collander 1941).

Although interpretation of the foliar and root chemistry in this study has elucidated the role of within-tree discrimination and allocation of Ca and Sr to the foliage and roots of several species of trees, interpretation of the chemistry from other organs is still quite uncertain. Large-scale variations in Ca/Sr ratios across plant parts within certain species have been shown in other studies (Poszwa et al. 2004; Watmough and Dillon 2003a), suggesting that experiments will need to be conducted for each tissue type for each tree species of interest. Analysis of tree ring chronologies for changes in alkaline element concentrations and $^{87}Sr/^{86}Sr$ ratios through time has been investigated as a possible means of assessing the impacts of acidification and rooting depth on nutrient acquisition (Aberg 1995; Bullen and Bailey 2005). Before these data can be used to understand changes in watershed Ca cycling, the mobility of Ca and Sr in woody tissues will need to be addressed in more detail.

Conclusions

Results from this study suggest that foliar chemistry is not necessarily a direct measure of the Ca/Sr ratio of plant-available sources. The majority of studies on Ca and Sr uptake in plants in relation to soil chemistry have been conducted on crop and pasture plants, the behavior of which may not parallel that of woody

plants. Understanding the species-specific behavior of Ca and Sr allocation within a tree is necessary in order to use alkaline earth element and Sr isotope ratios in vegetation to quantitatively track Ca sources. Discrimination factors of 1.14 ± 0.12, 1.16 ± 0.09, and 1.90 ± 1.22 are proposed for foliage of SM, YB, and BE respectively and data are currently insufficient to evaluate RS. Roots of the same species have discrimination factors less than one, suggesting that Sr is preferentially sequestered in roots. With the application of the discrimination factors outlined in this study, a more accurate assessment of the sources available to plants and the relative contribution of those sources can be determined.

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