Figure 1 Major-element ratios of sequential digests of six composite C-horizon soil samples and soil minerals. See Methods for details.

Mycorrhizal weathering of apatite as an important calcium source in base-poor forest ecosystems

Joel D. Blum*, Andrea Klaue*, Carmen A. Nezat*, Charles T. Driscoll†, Chris E. Johnson†, Thomas G. Siccama‡, Christopher Eagar§, Timothy J. Fahey|| & Gene E. Likens¶

* *Department of Geological Sciences, University of Michigan, 425 East University Avenue, Ann Arbor, Michigan 48109, USA*
† *Department of Civil and Environmental Engineering, Syracuse University, New York 13244, USA*
‡ *Yale School of Forestry and Environmental Studies, New Haven, Connecticut 06511, USA*
§ *Northeastern Research Station, USDA Forest Service, Durham, New Hampshire 03824, USA*
|| *Department of Natural Resources, Cornell University, Ithaca, New York 14853, USA*
¶ *Institute of Ecosystem Studies, Millbrook, New York 12545, USA*

The depletion of calcium in forest ecosystems of the northeastern USA[1–3] is thought to be a consequence of acidic deposition and to be at present restricting the recovery of forest and aquatic systems[4–7] now that acidic deposition itself is declining. This depletion of calcium has been inferred from studies[1–3] showing that sources of calcium in forest ecosystems—namely, atmospheric deposition and mineral weathering of silicate rocks such as plagioclase, a calcium-sodium silicate—do not match calcium outputs observed in forest streams. It is therefore thought that calcium is being lost from exchangeable and organically bound calcium in forest soils. Here we investigate the sources of calcium in the Hubbard Brook experimental forest, through analysis of calcium and strontium abundances and strontium isotope ratios within various soil, vegetation and hydrological pools. We show that the dissolution of apatite (calcium phosphate) represents a source of calcium that is comparable in size to known inputs from atmospheric sources and silicate weathering. Moreover, apatite-derived calcium was utilized largely by ectomycorrhizal tree species, suggesting that mycorrhizae may weather apatite and absorb the released ions directly, without the ions entering the exchangeable soil pool. Therefore, it seems that apatite weathering can compensate for some of the calcium lost from base-poor ecosystems, and should be considered when estimating soil acidification impacts and calcium cycling.

We studied the biogeochemistry of calcium in a mixed conifer/ hardwood forest within a 12-ha watershed (W-1) at the Hubbard Brook experimental forest (HBEF), New Hampshire, USA[2,7,8]. The main calcium-bearing minerals present in soil parent material are plagioclase, K-feldspar, hornblende, pyroxene and apatite. Sequen-
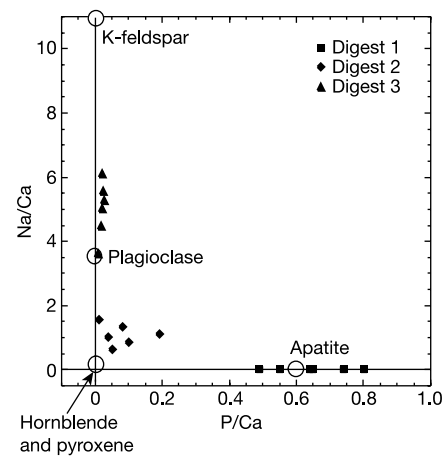
tial digestions of soil samples (see Methods) were used to give information on the weathering sources of Ca (Table 1). We first examined the molar ratios of P/Ca versus Na/Ca in C-horizon digestions in comparison with the average composition of the main Ca-bearing minerals (Fig. 1). 'Digest 1' consisted mainly of dissolved apatite, consistent with the much faster ($>10^3$ times) dissolution rate of apatite compared to hornblende, plagioclase and K-feldspar (at pH 2–5)[9–11]. 'Digest 2' consisted mainly of dissolved hornblende and pyroxene (Fig. 1), and 'digest 3' was mainly dissolved plagioclase and K-feldspar. About 12% of the Ca in the soil parent material occurs in apatite, another 12% is in hornblende and pyroxene, and 76% is in feldspars. The Ca/Sr ratio of digest 1 was very different to that of digests 2 and 3 (Table 1), owing to differences in Sr substitution for Ca in silicate versus phosphate minerals—providing a tracer of apatite-derived Ca. The Ca/Sr ratio of digest 1 for each soil horizon suggests that apatite was depleted from the Oa, E, Bh and Bs1 horizons, but is present in the Bs2 and C horizons (Fig. 2). If we assume that all of the Ca in the C-horizon digest 1 is derived from apatite (Fig. 1), then a Ca/Sr mixing calculation[12,13] shows that 86% of the Ca released from the Bs2-horizon soils by digest 1 is derived from apatite.

We used Ca/Sr and $^{87}Sr/^{86}Sr$ ratios to ascertain the original sources of Ca in ecosystem pools[13–17]. The $^{87}Sr/^{86}Sr$ ratio is not changed by ion exchange, plant uptake or transport[13–19], and the Ca/Sr ratio has generally been observed to be only slightly changed by these processes (a factor of 1.0–1.6)[14,18,19]. The C-horizon digest-1 fractions had average Ca/Sr and $^{87}Sr/^{86}Sr$ ratios (respectively 2,580 and 0.72164) that were distinct from the values for the digest-2 (279 and 0.73669) and digest-3 fractions (170 and 0.73352; Fig. 3). This pattern is consistent with our interpretation that digest 1 is dominated by dissolution of apatite, which has characteristically

Table 1 Average exchangeable and sequential digest concentrations

	Ca	K	Mg	Na	Si	P	Ca/Sr*
			μmol per g soil				
Exchangeable	0.01	0.50	<0.04	<0.04	0.09	0.02	281
Digest 1	24.0	1.45	6.21	0.27	36.9	16.0	2580
Digest 2	24.0	58.1	65.6	25.5	305	1.63	279
Digest 3	151	580	34.7	733	13200	3.04	170
Total soil (sum)	199	640	107	759	13500	20.7	202
			Percentage of total soil				
Exchangeable	0.05	0.08	<0.04	<0.01	<0.01	0.11	
Digest 1	12.0	0.23	5.83	0.04	0.27	77.3	
Digest 2	12.0	9.07	61.6	3.36	2.25	7.90	
Digest 3	75.9	90.6	32.5	96.6	97.5	14.7	

Data are shown for six composite C-horizon samples.
*Mole ratio.

high Ca/Sr and low $^{87}Sr/^{86}Sr$ (refs 20, 21). In comparison, atmospheric deposition near Hubbard Brook has an average Ca/Sr of 193 and $^{87}Sr/^{86}Sr$ of 0.71060 (refs 15, 16). The 'exchangeable' fractions (see Methods) of soil Oa and Bs1 horizons had Ca/Sr ratios similar to atmospheric and soil-digest values, but had $^{87}Sr/^{86}Sr$ close to the digest-1 values and intermediate between atmospheric and digest-2 and digest-3 values. Thus the soil cation exchange pool is dominated by a mixture of Ca and Sr from silicate weathering and atmospheric deposition.

In Fig. 3, the foliage samples plot along a line connecting the soil cation exchange pool and apatite, with different species occupying distinct fields. The Ca/Sr ratios were relatively low for sugar maple, intermediate for yellow birch and American beech, and high for balsam fir and red spruce. In contrast, foliar $^{87}Sr/^{86}Sr$ was nearly identical for all tree species, and was consistent with both the soil cation exchange pool and apatite weathering values (Fig. 3). Wood ferns, which are shallowly rooted and reflect forest floor values, had Ca/Sr ratios in the same range as maple and birch foliage (Fig. 3). Throughfall from the dominantly spruce/fir areas of the mixed conifer–hardwood canopy is a mixture of atmospheric deposition and spruce/fir foliage, whereas throughfall from the dominantly hardwood areas shows a stronger hardwood foliage influence. The range of soil water Ca/Sr and $^{87}Sr/^{86}Sr$ ratios for nine lysimeters sampled during each season of 1998, and the average streamwater Ca/Sr and $^{87}Sr/^{86}Sr$ for weekly samples collected in 1998, are also shown in Fig. 3.

To identify the sources of Ca in various ecosystem pools, we defined the Ca/Sr and $^{87}Sr/^{86}Sr$ ratios of three ecosystem endmembers. For the silicate weathering endmember we used the average value of the soil C-horizon digest 2, because it is the most readily weathered soil silicate fraction. For the apatite weathering endmember, we used the average of the C-horizon digest-1 fractions. The average atmospheric deposition endmember has been measured directly[15,16]. Apatite weathering does not contribute appreciably to exchangeable Ca in the Bs1 horizon, probably because it has been depleted in the upper horizons of the soil profile (Fig. 2). A mixing calculation for Ca/Sr and $^{87}Sr/^{86}Sr$ of two endmembers[12,13] shows that the Bs1 exchange pool contained 55% Ca from silicate weathering and 45% Ca from atmospheric sources (Fig. 3), similar to findings of previous studies[15,16].

The composition of the Oa exchange pool, stream water, and vegetation can be explained by a mixture of the Bs1 exchange pool

and apatite (Fig. 3). Mixing calculations provide an estimate of the relative quantity of Ca derived from the Bs1 exchange pool compared to the apatite weathering pool, and show that on average ~95% of the foliar Ca in spruce/fir foliage is derived from apatite, whereas ~80% of beech, ~75% of birch and ~60% of maple and fern Ca is derived from apatite (Figs 2, 3). Also, ~35% of the stream Ca loss from the watershed is estimated to be derived from apatite weathering and ~20% of the exchangeable Ca in the Oa horizon is derived from apatite. Trees at HBEF appear to be accessing Ca and Sr from apatite in the Bs2 horizon, and partially bypassing the soil cation exchange complex. Studies in base-poor northern European coniferous forests have suggested that ectomycorrhizal mycelia are able to penetrate micropores in silicate minerals and take up Ca and P directly from apatite inclusions, while remaining isolated from the soil solution[22]. This process has only been observed in conifer forests[22,23] and pot experiments[23,24], but may explain the high abundance of apatite-derived Ca in the foliage of the mixed conifer/hardwood forest at HBEF. The shallower soils on which conifers tend to grow may facilitate the higher proportion of apatite-Ca. Moreover, ectomycorrhizal fungi associated with the roots of conifers may be more effective at weathering apatite. In contrast, maple—the only major endomycorrhizal tree in the forest[25]—is least able to access the Ca-apatite and is also the most sensitive species to Ca depletion in forest soils of the northeastern USA[6,26].

An alternative explanation for the higher Ca/Sr ratios in foliage compared to those in the soil exchange complex would require that trees preferentially take up Ca over Sr by a factor of 2.3 for maple, 2.9 for birch, 3.7 for beech and 8.5 for spruce/fir. We consider this unlikely for several reasons, but acknowledge that additional research on plant uptake of Ca and Sr is needed. First, to maintain mass balance, preferential uptake of Ca over Sr would leave the soil exchange complex depleted in Ca relative to Sr compared to silicate minerals, and this is not observed (Fig. 3). Second, plants grown in nutrient solutions display only minimal preferential uptake of Ca over Sr (by a factor of 1 to 1.4)[18]. A study of trees growing on
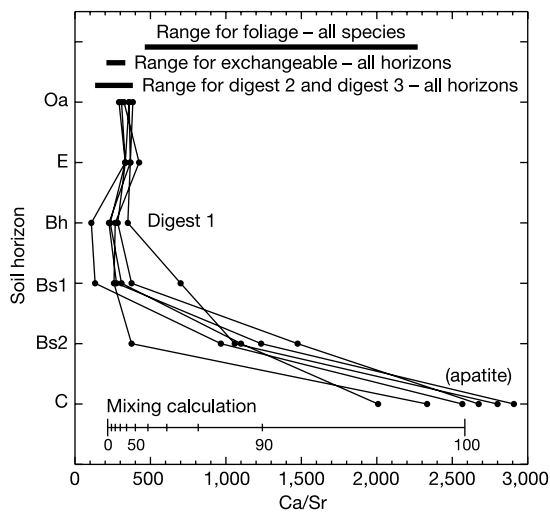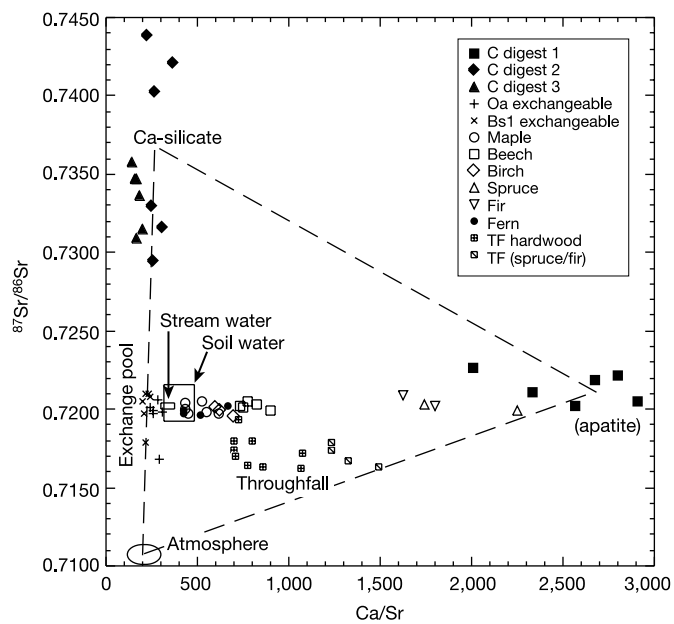

Figure 2 Ca/Sr ratios of the digest 1 for each soil horizon. The bars at the top show the total range of Ca/Sr ratios for all foliage, and for 'exchangeable', 'digest 1', 'digest 2' and 'digest 3' for all horizons (see Methods). The line at the bottom shows the results of a mixing calculation between the Bs1 horizon exchangeable and the average C-horizon digest 1 (that is, apatite) with tick marks for each 10% addition of Ca from apatite.

Figure 3 The Ca/Sr and $^{87}Sr/^{86}Sr$ ratios of all samples analysed. The small rectangular box represents the total variation in weekly streamwater samples collected during 1998, and the larger rectangular box represents the total variation in 28 soil water samples collected during each season in 1998. Dashed lines enclose the range of possible compositions for mixtures of Ca-silicate, apatite and atmospheric deposition. TF, throughfall.

carbonate bedrock suggested that spruce may assimilate Sr preferentially to Ca, whereas beech and maple do not display preferential uptake[27]. Other studies of the uptake of Ca and Sr by trees growing on soils derived from silicate minerals show higher Ca/Sr of foliage compared to the exchange complex by a factor of 4.7 for beech[27] and 7.9–8.4 for spruce[16,27]. We suggest that these values may reflect the presence or absence of apatite in the mineral soils, combined with rooting depth and mycorrhizal associations, rather than species-specific differences in plant uptake. Nevertheless, we consider the proportions of apatite-Ca in foliage given above to be maximum values, as some portion of the enhanced Ca/Sr may be due to preferential uptake of Ca over Sr.

Despite the importance of apatite-derived Ca to foliage, the Oa exchange complex contains only ~20% Ca from apatite. To shift the Ca/Sr ratio of the Bs1 exchange complex to the value of the Oa exchange complex, as much as 40% of the Ca would need to be supplied from maple litter or as little as 22% from spruce/fir litter. Most of the Ca in the Oa exchange complex (60–78%) is derived from a combination of silicate weathering and atmospheric deposition, in a 55:45 proportion. Ferns have Ca/Sr ratios consistent with maple foliage, suggesting that maple trees may access Ca from the forest floor without it entering an exchangeable form in the Oa horizon, as has been documented for P (ref. 28). In contrast, spruce and fir trees appear to extract Ca from apatite deep in the soil in addition to utilizing the forest floor pool.

We estimate that Ca in stream water is derived as follows: ~30% from the atmosphere, ~35% from silicate minerals, and ~35% from apatite. Previous research at HBEF[1–3,7,29] suggested, on the basis of Ca/Na ratios, that silicate weathering is insufficient to account for Ca losses in stream water, and that Ca is being depleted from exchangeable and organically bound pools in forest soils of the northeastern USA. Our data suggest that apatite weathering, possibly by the direct action of root mycorrhizal systems, may account for a significant portion of this Ca, which has no accompanying Na release. However, this apatite pool itself has largely been depleted from upper soil horizons. Based on the average mass of the Bs2 horizon per hectare (ref. 30), and the amount of Ca released by the digest 1 of each soil horizon, we estimate the apatite Bs2 Ca reservoir to be $14\,kmol\,ha^{-1}$. This pool is larger than the entire exchangeable Ca pool of the soil ($5\,kmol\,ha^{-1}$; ref. 2), and the forest floor Ca pool ($8\,kmol\,ha^{-1}$; ref. 2), but is less than the pool of Ca in above-ground biomass ($15\,kmol\,ha^{-1}$; ref. 2). Therefore, depletion of Ca from base-poor soils remains a considerable concern, but assessments and models of Ca depletion need to account for this important Ca source. □

Methods

Soil pits were excavated in 1997 at 47 sites in W-1 and sampled by horizon (Oa, E, Bh, Bs1, Bs2, C). Composite samples of the <2-mm size fraction were made for each horizon for six zones within W-1. Optical microscopy and electron microprobe analysis of soil parent material was used to determine the main Ca-bearing minerals and their compositions. Soils were subjected to a four-step sequential digestion on 0.5-g samples. Solutions were removed by centrifugation after each treatment, and are referred to in the main text as follows: 'exchangeable', 5 ml of 1 M NH_4Cl (pH = 7) for 20h; 'digest 1', 5 ml of 1 M HNO_3 at room temperature for 20 h; 'digest 2', 5 ml of 1 M HNO_3 at 200 °C for 30 min (by microwave at ~20 MPa); 'digest 3', 10 ml of concentrated HF and HNO_3 at 210 °C for 30 min (by microwave at ~20 MPa). Composite samples of understory foliage (UF) and overstory foliage (OF) were collected in 1999 from trees encountered along transects through each of four elevation zones. Foliage sampling included red spruce (*Picea rubens*, 2 UF), balsam fir (*Abies balsamea*, 2 UF), American beech (*Fagus grandifolia*, 3 UF and 2 OF), yellow birch (*Betula alleghaniensis*, 3 OF), sugar maple (*Acer saccharum*, 3 UF and 3 OF) and wood fern (*Dryopteris spinulosa*, 4 UF). Throughfall was collected during a single storm event on 29 July 1999 at 13 locations in the watershed; soil water was collected using zero tension lysimeters during April, June, October and December of 1998 from the Oa, Bh and Bs1 horizons at three locations in the watershed; and weekly stream water samples from the base of W-1 were analysed for the 1998 calendar year. Foliage samples (0.5 g) were ground, homogenized and digested in concentrated HNO_3 at 200 °C for 30 min (by microwave at ~25 MPa). Leaches, digests and waters were analysed for Ca, K, Mg, Na, Si, P and Sr (±2%) and $^{87}Sr/^{86}Sr$ (±0.002%) following established methods[12,14].

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Competing interests statement

The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to J.D.B. (e-mail: jdblum@umich.edu).