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ORIGINAL PAPER

# Controls over leaf and litter calcium concentrations among temperate trees

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**Abstract** Four-fold variation in leaf-litter Ca concentration among 14 tree species growing in a common garden in central Poland was linked to variation in soil pH, exchangeable Ca, soil base saturation, forest floor turnover rates, and earthworm abundance. Given the potential importance of tissue Ca to biogeochemical processes, in this study we investigated potential controls on leaf Ca concentrations using studies of both laboratory seedlings and 30-year-old trees in the field. We first assessed whether species differences in Ca concentration of green leaves and leaf litter were due to differences in Ca uptake, plant growth, or Ca translocation to different organs, by measuring seedlings of 6 of the 14 species grown under controlled conditions of varying Ca supply. We also investigated whether trees species with high Ca concentrations in green leaves and leaf litter access soil Ca to a greater extent than low-Ca species by growing more fine roots in high-Ca soil horizons. Root distribution in the field was determined in all 14 tree species by profile wall mapping and soil sampling of excavated pits. There was no correlation between horizon root count density (number of roots $m^{-2}$) and exchangeable soil Ca, nor was there a correlation of stand-level leaf litter Ca with density of roots 45–100 cm deep in the soil, suggesting that a deeper root distribution does not result in greater Ca acquisition among these species. Variation among species in leaf Ca concentration of greenhouse seedlings was positively correlated with leaf Ca concentrations of mature trees, indicating that the same ranking in leaf Ca among species existed under controlled Ca supply. Species also differed in seedling growth response to Ca supply. *Tilia*, the species with the highest leaf Ca in the field, generated only 10% as much biomass and height at low relative to high Ca supply, whereas the other species exhibited no significant differences. Species exhibited differences in (i)

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partitioning of whole plant Ca and biomass to leaf, stem and root organs and (ii) the pattern of such partitioning between high and low Ca treatments. Our data support the hypothesis that although soil Ca supply can contribute to variation among trees in leaf and litter Ca concentration, innate physiological differences among species also can be a major cause for species variation.

**Keywords** Calcium · Partitioning · Physiology · Root distribution · Seedling · Tree species

## Introduction

Organisms modify nutrient movement within ecosystems in species-specific ways (Chapin et al. 1997; Jones et al. 1997; Eviner et al. 2006). The mechanisms by which plants influence nutrient movement, however, are poorly understood (Binkley and Giardina 1998; Hobbie 1992). One way in which tree species influence forest ecosystem structure and function is by differential cycling of calcium (Finzi et al. 1998; McLaughlin and Wimmer 1999; Dijkstra and Smits 2002; Reich et al. 2005). Calcium (Ca) cycling is of particular interest as current studies show that Ca may be lost from forest soils in the northeastern United States and in Central Europe due to acidic atmospheric deposition and whole tree harvesting (Hedin et al. 1994; Likens et al. 1998; Jandl 2004). Additionally, through its effect on soil properties, Ca exerts a significant control on forest ecosystem properties (McLaughlin and Wimmer 1999). High concentrations of Ca in leaf litter can increase base saturation and soil pH in the forest floor and surface mineral soil horizons (Reich et al. 2005). Higher pH is often associated with greater microbial biomass and higher rates of litter decomposition, soil respiration, and net nitrogen mineralization (Persson et al. 1989; Simmons et al. 1996; Andersson et al. 2000). High-Ca inputs to the soil can influence soil aggregate formation (Muneer and Oades 1989; Chan and Heenan 1999), stabilization of soil organic matter (Oades 1988; Oyonarte et al. 1994; Oste et al. 2002), and earthworm abundance (Muys and Lust 1992; Pop et al. 1992; Reich et al. 2005). All of these factors can influence carbon and nutrient cycling in forest ecosystems.

Tree species may be associated with specific spatial patterns of soil acidity and cation cycling within forests (Boettcher and Kalisz 1990; Eriksson and Rosen 1994; Finzi et al. 1998; Washburn and Arthur 2003). Several studies have suggested that differential Ca uptake and allocation to tree biomass pools are the main cause for changes in Ca content in the surface soil (Alban 1982; Johnson and Todd 1990; Bockheim 1997; Dijkstra and Smits 2002). For example, in our prior study (Reich et al. 2005) we found tree species growing in replicated monoculture plots caused large changes in soil acidity and fertility in just 30 years because of differences in leaf-litter Ca concentration. Because Ca mobility is limited in the phloem, little Ca is resorbed during leaf senescence (Killingbeck 1986; Marschner 1995), and accordingly, we found that differences in litter Ca concentration were closely associated with differences in green leaf Ca concentration. Regardless of total annual litter fall, tree species that had high leaf-litter Ca concentrations were associated with relatively high soil pH, exchangeable Ca, percent base saturation, forest floor turnover rates and earthworm abundance (Reich et al. 2005). While the idea of plant influence on Ca distribution in the soil has become widely accepted, the mechanisms underlying species variation in leaf and root Ca are not well understood (McLaughlin and Wimmer 1999). The present work comprises a field and greenhouse study in which we investigated the contrasting, but not exclusive, possibilities that variation in interspecific leaf Ca concentration is due to (i) differential access to Ca supply in the soil by tree roots or (ii) differential Ca accumulation at a specific Ca soil solution concentration and (iii) differential partitioning of total plant Ca to leaves versus roots or stems.

Species may exhibit differences in their ability to distribute roots and acquire Ca in Ca-rich soil horizons. Small increases in root numbers in a given soil horizon high in Ca may increase the potential for Ca uptake because Ca is mobile in the soil and primarily arrives to the root surface by mass flow (Troeh and Thompson 2005). Several studies have suggested that species with deeper roots have access to more Ca and nutrients due to their greater vertical extent in the soil (Dijkstra and Smits 2002; Lehmann 2003; Jobbágy and Jackson 2004), which may influence leaf Ca accumulation. We examined root distribution of trees in the 36-year-old common

garden described previously by Reich et al. (2005) in order to determine the extent that species variation in leaf Ca concentration could be explained by either variation in root abundance in Ca-rich soil horizons or root distribution in the soil profile. We predicted that species with high-leaf-Ca concentrations distribute higher numbers of roots in high-Ca soil horizons and higher numbers of roots deeper in the soil profile than species with low-leaf-Ca concentrations.

Species may also vary widely in their inherent growth requirement for Ca and the degree to which Ca is partitioned in leaves versus stems and roots. Compared to other leaf macronutrient concentrations in angiosperms, Ca shows the highest degree of interspecific variability (Thompson et al. 1997) and consistent differences among plant functional types (Broadley et al. 2003). However, it is unknown to what degree these differences are due to differences in Ca requirement for growth, root foraging capacity in Ca-rich soil layers, or are simply a byproduct of other physiological traits, such as xylem cation exchange capacity or plant water use. Therefore, in the greenhouse study, we compared growth, Ca uptake and Ca partitioning among leaves, stems and roots, under a range of Ca supply that covered the range from the mean to low end of Ca measured in the soil solution of the common garden field site. We predicted that the variation among species in green leaf and leaf litter Ca concentration in the field experiment would be positively correlated with that observed in leaves of seedlings grown under greenhouse conditions where the supply of Ca to the root system was controlled. Additionally, we predicted that species showing high-leaf-Ca concentrations in the field would exhibit a greater increase in growth rate in high-Ca relative to low-Ca treatment when grown in the greenhouse, indicating a greater capacity to use Ca for growth, as compared to low-leaf-Ca species. Lastly, we predicted that compared to low-Ca species, high-Ca species would partition a greater proportion of total Ca to leaves.

## Materials and methods

### Field experiment

The study area is located in a common garden of 14 tree species in the Siemianice Experimental Forest in central Poland (51°14.87′ N, 18°06.35′ E, altitude: 150 m), a nutrient-poor site. Soils were formed from sandy glacial outwash overlying finer textured glaciofluvial sediments and are generally loamy sands (83 ± 8% (SD) sand) with a gradient of soil texture occurring across the site. Prior to planting the different tree species in 1970 and 1971, a *Pinus sylvestris* stand on the site was harvested and plowed up to a depth of 60 cm. Fourteen species, including six gymnosperms (*Abies alba*, *Picea abies*, *Pinus sylvestris*, *P. nigra*, *Larix decidua*, *Pseudotsuga menziesii*) and eight angiosperms (*Acer platanoides*, *A. pseudoplatanus*, *Betula pendula*, *Carpinus betulus*, *Fagus sylvatica*, *Quercus robur*, *Q. rubra*, *Tilia cordata*) were planted in 1970 and 1971 in 1 × 1 m spacing in two adjacent plantings. Each planting had nine of the species, replicated three times, in a total of 27, 20 × 20 m monospecific plots (see Szymanski 1982; Withington et al. 2003, 2006; Reich et al. 2005; Hobbie et al. 2006 for details).

During 1 week in the summer of 2002, pits 1 m wide, 1.8 m long and 2 m deep, were excavated in each plot (from 3 to 6 plots of each of 14 species, 53 pits total). Soils were sampled to 1 m depth by genetic horizon and described using standard techniques (described below). There was a gradient in soil texture across the two sites with high clay soils occurring to a greater extent in one planting. A subsurface clay-rich Bt horizon was found in about one-third (17) of the 53 plots within 1 m of the soil surface and these existed only in the planting with greater clay.

### Image collection and analysis for root distribution

Root distribution was mapped by the classic approach of trenching and profile wall mapping (Böhm 1979; Caldwell and Virginia 1989). Because of the time constraints of working at a distant field site and the requirement to work ahead of soil scientists who were sampling soil profiles in each pit, it was not possible to map roots with the conventional plastic over-lay method of profile wall mapping (Böhm 1979; Caldwell and Virginia 1989), which may take half a day or more for a pit this size (Eissenstat, personal observation). While some have used photographic approaches to assist profile wall mapping such as using a digital camera, this has potential problems

associated with camera angle affecting scale and appropriate light source (Caldwell and Virginia 1989). Dong et al. (2003) described a method of monitoring root growth of apple trees growing in greenhouses by pressing a scanner to a transparent acrylic sheet on one side of a root box. We modified this scanner-based approach of image acquisition to document large areas of roots growing in field conditions. We used a scanner to capture images of roots on one wall of our soil pits in Poland, which allowed us to obtain images of root distribution on a pit face of approximately 1 $m^2$ in about 1 h. In each monoculture plot at our field site, one single face of each pit was prepared by smoothing the soil with a flat shovel and cement knife. Soil was brushed away to expose the roots. All roots were clipped to <3 cm in length. The face of the exposed wall was sprayed with water to increase the color contrast between roots and soil, wash soil from the roots, and stabilize the sandy soil on the side of the pit. The wall was grid-marked with pins in squares 30.5 × 22.8 cm (fit to scanner window) to cover the area of the pit face (1 × 1 m). Images of each rectangle were taken at 200 dpi using an Epson Perfection 1250 (Seiko Epson Corporation) desktop scanner. The lid of the scanner was removed and the glass surface was covered with acetate and taped to protect it from being scratched. The scanner had a depth of field of approximately 3.5 cm. At 200 dpi, roots were magnified 2.2 times. After the images were obtained, soil horizons in each pit were characterized and sampled for chemical and physical analysis.

## Image analysis

Scanner images of the soil profile were used to determine the number of roots intersecting a given area of soil in each soil horizon excluding the O horizon. In Adobe Photoshop 7.0 (Adobe Systems Incorporated, San Jose, CA, USA), a new image indicating roots and horizons was created using a digital transparent layer added to each scanned image. One dot was marked to indicate a root each time it transected an imaginary vertical plane. Horizons were outlined and labeled on each image. Image J 1.28 (Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA) software was used to analyze the number of root intersections (dots) in the image for a given area (or number of roots in a given horizon). This approach allowed us to compare root count densities with changes in chemical and physical soil properties as indicated by soil horizon and by depth.

## Soil and leaf Ca analysis

Soils were sampled from zero (surface) to 1 m depth by genetic horizon in the excavated soil pits described above. A total of 262 mineral horizons were sampled (ranging from 4 to 6 horizons per pit). Samples collected across the horizontal extent of each mineral genetic horizon in the pit were composited, and then transported to the University of Arizona, where they were air-dried, and the fine earth (<2 mm) fraction isolated by sieving prior to further analysis. Water soluble ions ($Al^{3+}$, $Ca^{2+}$, $H^+$, $Mg^{2+}$, $Na^+$, $Cl^-$, $SO^{4-}$, $PO^{4-}$, $NO^{3-}$, $NO^{2-}$) were measured in saturated soil paste extracts using ultrapure (18 mΩ) water (Rhoades 1996) and exchangeable ions ($Mg^{2+}$, $Al^{3+}$, $K^+$, $Ca^{2+}$, $Fe^{3+}$, $Mn^{2+}$, $Sr^{2+}$) were measured following extraction in unbuffered $BaCl_2$ solution (Amacher et al. 1990). Cation concentrations in both extracts were measured using inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer DRC II) and anion concentration in the water extracts were measured using ion-chromatography (Dionex DX-500). Soil pH was measured by combination electrode. Field litter samples were collected in 0.38 $m^2$ litter traps from May 1, 1996 through April 30, 1997 using eight litter traps placed in each plot. Litter traps were emptied at monthly intervals or more frequently as needed. Samples were sorted, composited, and analyzed by year. Litter collected in other years (1996–2004) was similar in production and chemistry, but we used the 1996–1997 dataset as it was the most complete in terms of all litter measures. Green leaves were collected in August of 2001 from 3 to 5 individual trees in each of 30 (out of 53) plots. From each tree, current year foliage was sampled in high and low light positions and combined. Litter and leaf tissues were dried (65°C for 48 h) and ground in a Kikro-Feinmühle Culatti mill (IKA Labortechnik Staufen, Germany). Foliar concentrations of P, K, Ca, Mg, and Al were analyzed simultaneously with an ICP—dry ash method (ICP-AES, model ARL 3560, Fisons,

Sunland, CA) at the University of Minnesota Research Analytical Laboratory, St. Paul MN, USA.

## Greenhouse experiment

Six tree species, *Larix decidua*, *Pseudotsuga menziesii, Picea abies*, *Pinus sylvestris*, *Tilia cordata*, and *Betula pendula*, were chosen from the 14 common garden species for a greenhouse study. Species were selected to maximize phylogenetic distance and the range of leaf Ca concentrations observed at the Siemianice field site (see Table 1 in Reich et al. 2005). Seeds were collected in Poland and stratified (if required) for germination. All six species were germinated at University Park, Pennsylvania, USA, in cone-tainers™ (164 ml, Stuewe and Sons, Corvallis, OR) filled with vermiculite. Thirty individuals of each tree species were selected for uniformity and transplanted to individual Deepots™ (656 ml,

**Table 1** Percentage of whole plant biomass and Ca in leaves, roots and stems of six species (Tico, *Tilia cordata*; Bepe, *Betula pendula*; Piab, *Picea abies*; Psme, *Pseudotsuga menziesii*; Pisy, *Pinus sylvestris*; Lade, *Larix decidua*) grown with high (313 μmol $l^{-1}$), medium (31.3 μmol $l^{-1}$) and low (3.13 μmol $l^{-1}$) Ca nutrient solution and harvested after 15 weeks

| Species | Ca treatment | Percentage of whole plant biomass | | | Percentage of whole plant Ca | | |
|---|---|---|---|---|---|---|---|
| | | Leaves | Stems | Roots | Leaves | Stems | Roots |
| Tico | high | 29 (2) | 29 (1) | 42 (2) b | 51 (4) a | 29 (2) | 21 (3) b |
| | med | 25 (1) | 23 (2) | 52 (3) a | 33 (2) b | 29 (3) | 38 (2) ab |
| | low | 26 (3) | 25 (2) | 49 (3) ab | 46 (4) a | 26 (1) | 27 (4) a |
| | *mean* | 27 (1) D | 26 (1) A | 48 (2) A | 43 (3) B | 28 (1) A | 29 (3) A |
| Bepe | high | 42 (5) | 25 (4) | 32 (3) | 55 (9) | 18 (4) | 27 (6) |
| | med | 38 (2) | 20 (2) | 41 (2) | 72 (2) | 12 (2) | 16 (3) |
| | low | 44 (2) | 21 (2) | 35 (2) | 75 (2) | 12 (2) | 13 (2) |
| | *mean* | 42 (2) C | 22 (2) AB | 36 (2) B | 68 (4) A | 14 (2) B | 19 (3) B |
| Piab | high | 51 (7) | 19 (1) | 30 (7) | 74 (2) a | 8 (1) | 18 (2) b |
| | med | 56 (1) | 18 (2) | 26 (1) | 67 (2) ab | 9 (1) | 25 (2) ab |
| | low | 45 (5) | 20 (1) | 35 (5) | 58 (4) b | 11 (1) | 32 (4) a |
| | *mean* | 51 (3) B | 19 (1) B | 30 (3) B | 68 (2) A | 9 (1) B | 24 (2) AB |
| Psme | high | 60 (1) | 20 (1) | 20 (1) | 76 (4) p | 13 (2) | 11 (2) q |
| | med | 60 (2) | 19 (1) | 22 (2) | 67 (3) pq | 15 (2) | 18 (2) p |
| | low | 58 (2) | 19 (2) | 23 (1) | 59 (2) q | 17 (1) | 25 (0) p |
| | *mean* | 59 (1) AB | 19 (0.5) B | 22 (1) C | 68 (3) A | 15 (1) B | 17 (2) B |
| Pisy | high | 62 (1) | 19 (2) | 19 (1) | 68 (2) | 14 (1) | 18 (1) |
| | med | 58 (4) | 18 (2) | 23 (2) | 70 (5) | 13 (1) | 17 (5) |
| | low | 51 (3) | 24 (2) | 25 (2) | 63 (6) | 19 (6) | 18 (6) |
| | *mean* | 57 (2) A | 20 (1) B | 22 (1) C | 67 (2) A | 15 (2) B | 18 (2) B |
| Lade | high | 59 (2) | 20 (2) | 21 (1) | 52 (3) q | 12 (1) | 36 (3) p |
| | med | 58 (2) | 18 (2) | 24 (2) | 77 (3) p | 12 (1) | 11 (3) q |
| | low | 60 (1) | 16 (1) | 24 (1) | 73 (4) p | 17 (2) | 12 (2) q |
| | *mean* | 59 (1) A | 18 (1) B | 23 (1) C | 66 (4) A | 13 (1) B | 20 (4) B |
| Ca treatment | | 0.2361 | 0.0417 | 0.0091 | 0.6883 | 0.7445 | 0.7014 |
| Species | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Ca treatment × species | | 0.2685 | 0.3331 | 0.2314 | <0.0001 | 0.4941 | <0.0001 |

Standard error in parentheses. Bottom portion of table shows *P*-values of Ca treatment, tree species and the species × treatment interaction (two-way ANOVA). Differences in upper-case letters represent significant differences among species means (one-way ANOVA, Tukey HSD, $P < 0.05$); within any species, differences in lower-case letters denote significant differences among Ca treatments (one-way ANOVA, Tukey HSD, $P < 0.05$)

Stuewe and Sons, Corvallis, OR) with 4:1 sand:perlite mixture at the age of 2 months.

Ten individuals of each species were irrigated with nutrient solutions at three different Ca concentrations: 313, 31.3 and 3.13 μmol $l^{-1}$. The high-Ca treatment was comparable to the mean Ca in the soil solution at the Poland field site, which averaged 337 μmol $l^{-1}$ in mineral soil to a depth of 1 m. All nutrient solutions were adjusted to pH 5.0 and contained the same background nutrient concentrations (in mmol $l^{-1}$): N, 3.21; P, 0.807; K, 0.639; Mg, 0.206; S, 0.022, and micronutrients (in μmol $l^{-1}$): Fe, 1.12; B, 0.787; Cu, 0.018; Mn, 0.069; Mo, 0.0104; and Zn, 0.046. Calcium was adjusted in each treatment with $CaNO_3$ and the difference in N was adjusted with $NH_4NO_3$. Total N for all three treatments was constant but with a ratio of nitrate/ammonium/urea of 5.6/1.0/2.5, 1.5/1.1/1.0, and 1.4/1.2/1.0 for high-, medium- and low-Ca treatments respectively, so that no form of N should have been growth limiting. Trees were watered with nutrient solution twice daily with enough nutrient solution so that the electrical conductivity of the pot leachate matched that of the stock nutrient solution, ensuring complete replenishment, constant pH, and no accumulation of excess salts.

Half of the trees (five reps) were harvested 8 weeks after treatment initiation and the remainder 15 weeks after initiation of Ca treatments. At harvest, each seedling was measured for height, stem diameter at root collar, and number of leaves. Seedlings were separated into roots, stems, and leaves after washing roots of media. A sub-sample of leaves was measured for leaf area by taking an image at 200 dpi with a desktop scanner (HP ScanJet 5400c, Hewlett-Packard), and estimating the total area with Image J 1.28 (Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA). All plant samples were oven-dried for 3 days at 65°C and dry masses determined. The sequential harvest permitted determination of relative growth rate (RGR).

$$\mathrm{RGR} = (\ln W_2 - \ln W_1) \times (t_2 - t_1)^{-1}$$

where W = total dry mass (g) at the first harvest ($t_1$) or the second harvest ($t_2$). Tree leaves, roots, and stems were dried at 65°C and small samples were ground in a mixer/mill (Spex Industries, Metuchen, NJ, USA), whereas larger samples were ground with a Cyclotec 1093 Sample Mill (Foss Tecator, Eden Prairie MN). Calcium concentration was analyzed by atomic absorption spectrophotometry (AAnalyst 100 Spectrometer, Perkin-Elmer Instruments, Wellesley MA, USA) after dry-ashing at 550°C.

### Statistical analyses

All regression and ANOVA statistics were conducted using the GLM procedure in SAS statistical software (SAS Institute Inc., Cary, NC). We used linear regression to examine the relationship of exchangeable Ca with various soil factors including percent clay, water soluble Ca, pH, exchangeable Al, and organic C. Regressions were also performed to determine the relationship between leaf litter Ca and green leaf Ca in the field. Rank correlation was calculated between field leaf litter Ca as well as greenhouse seedling leaf-Ca. We used a separate slopes analysis of covariance to investigate clay (covariate) and species interactions on leaf litter Ca in the field, and Ca treatment and species interactions on leaf, stem, and root Ca concentrations, total Ca, height and mass of seedlings in the greenhouse. A one-way ANOVA with Tukey HSD post-hoc tests within a species was used to determine significant differences among Ca treatments on leaf, stem, and root Ca concentrations, total Ca accumulated, height and dry mass of seedlings in the greenhouse.

## Results

### Field study

#### *Leaf Ca concentration in relation to Ca availability*

In all soil horizons within and among 53 pits at variable depths, but all within the 0–100 cm deep zone ($n = 262$), there was a strong positive linear relationship between exchangeable Ca and percent clay (Fig. 1; $r^2 = 0.85$, $P < 0.001$). Weaker trends existed for exchangeable Ca with other soil characteristics including negative relationships with $H^+$ ions ($r^2 = 0.19$, $P < 0.001$), exchangeable Al ($r^2 = 0.16$, $P < 0.001$) and organic C ($r^2 = 0.17$, $P < 0.001$), and a positive relationship with water soluble Ca ($r^2 = 0.17$, $P < 0.001$) (data not shown). Mineral soil horizons with the highest exchangeable Ca (on a mass

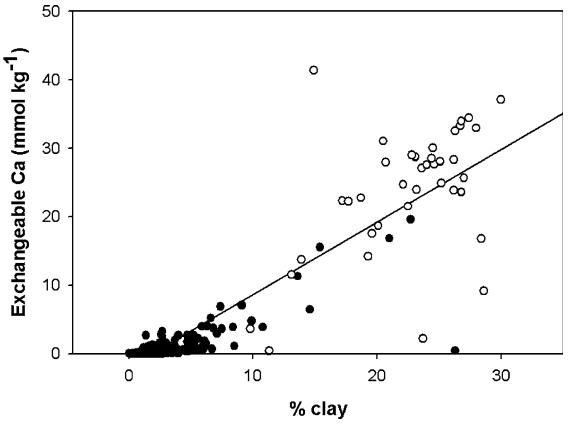


**Fig. 1** Relationship between exchangeable calcium (mmol $kg^{-1}$) and percent clay for soils beneath 14 conifer and hardwood species ($r^2 = 0.82$, $P < 0.001$, $y = 1.06x - 1.93$). Each point represents one soil horizon sampled within 1 m-deep soil pits across two plantings in Siemianice, Poland. All horizons present to 1 m depth are included and high-clay (Bt) soil horizons are indicated with *open circles*; all other horizons (e.g., A, E) are *closed circles*

basis) were Bt horizons (mean 23.8 mmol $kg^{-1}$, ±1.6SE) that occurred from 40 to 80 cm deep and only in the high-clay planting site, and soil A horizons that occurred in all plots within the top 1–30 cm depths (mean 1.03 mmol $kg^{-1}$, ±0.20SE). Subsurface Bt horizons comprised the majority of high-Ca zones in the soil and contained as much as 40 times greater Ca than non-Bt horizons (Fig. 1).

Because our sampling of green leaf data was modest and did not extend to all plots per species, we used leaf litter Ca collected in all plots as a surrogate for green leaf Ca in our analyses, based on a predictive positive relationship between green leaf Ca and litter Ca ($r^2 = 0.97$, $P < 0.001$, data not shown). Variation in soil texture across our two plantings allowed us to assess whether differences in leaf litter Ca concentrations were associated with increased supply of soil Ca due to higher CEC, using the four species each grown in a total of six plots among both plantings. Leaf litter Ca of the four species grown in both plantings (*Quercus robur*, *Picea abies*, *Pseudotsuga menziesii* and *Larix decidua*) was plotted with depth-weighted mean percent clay of each plot (data not shown). Species, but not clay were significant in leaf litter Ca (separate slopes analysis of co-variance, $P < 0.001$ for species, $P = 0.184$ for clay, respectively) although all the species indicated increasing leaf litter Ca with increasing clay. There was no significant difference in slope between species, indicating that species responded similarly to differences in soil clay content (species × clay interaction, separate slopes analysis of covariance, $P = 0.38$). These data were consistent with the hypothesis that in the common garden, leaf litter Ca was more strongly influenced by species than by Ca availability.

### *Root distribution in relation to Ca availability*

Across and within species there were no significant correlations between root count density (number of roots $m^{-2}$ of profile wall) in a soil horizon and exchangeable Ca (mmol $kg^{-1}$) of that horizon (data not shown). We examined if high-leaf-Ca species exhibited preferential root growth near the Ca-rich Bt horizon to a greater extent than low-leaf-Ca species. Four species growing on plots with Bt horizons were chosen to represent a range of field leaf litter Ca concentrations (*Tilia cordata*: 2.24; *Picea abies*: 1.1; *Pseudotsuga menziesii*: 0.97 and *Larix decidua*: 0.7 [in percent dry mass]; Reich et al. 2005). A baseline response of root count attenuation with soil depth was created by plotting a regression line of the relationship of root count density (logarithm of number of roots $m^{-2}$) with soil depth of a given horizon (excluding Bt horizons) (Fig. 2). We then compared root count density in Bt horizons with that of the baseline response (i.e., asking whether densities were higher than predicted by depth alone, which would indicate preferential root growth at a given soil depth). Only in *Larix* (Fig. 2d), a low-Ca-accumulating species, was root count density in all Bt horizons higher than the 95% confidence interval. There was no evidence that high-Ca-accumulating species (*Tilia* and *Picea*) had significantly greater root count density than low-Ca-accumulating species (*Pseudotsuga* and *Larix*) for a given soil exchangeable-Ca concentration. Likewise, among the 14 species in the field, no relationship existed between mean Ca concentrations of leaf litter with depth-weighted mean root density in horizons 45–100 cm deep ($r^2 = 0.04$, $P = 0.52$, data not shown), nor the slope of the regression line of root attenuation with depth ($r^2 = 0.00$, $P = 0.99$), indicating high-Ca species do not preferentially access resources at lower soil depths than low-Ca species.

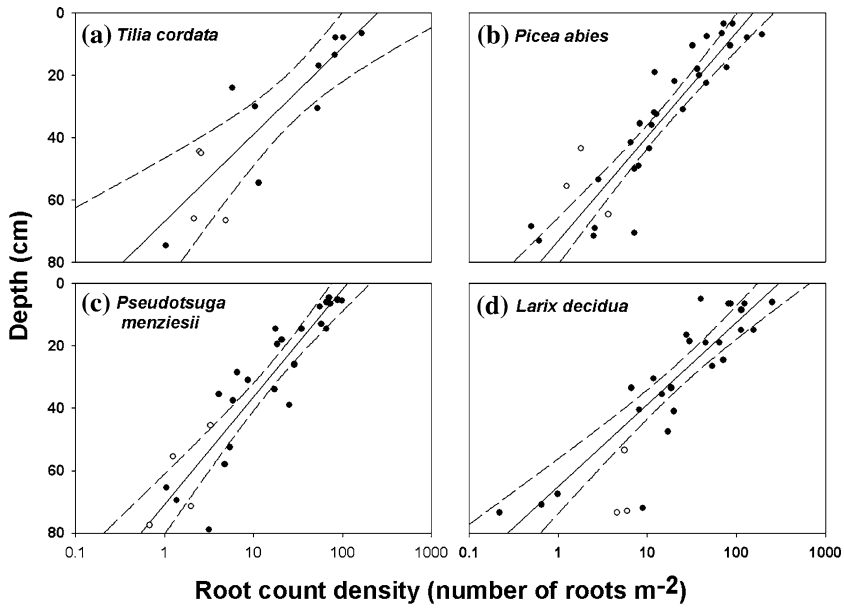


**Fig. 2** The relationship of root count density (number of roots $m^{-2}$) on a log scale to mean depth of a soil horizon for four species: (**a**) *Tilia cordata*, (**b**) *Picea abies*, (**c**) *Pseudotsuga menziesii* and (**d**) *Larix decidua*, growing in the high-clay planting. Each point represents one sampled soil horizon in any of the 3–6 soil pits for each species. Regression line (±95% confidence interval) calculated using only non-Bt horizons (*closed circles*). Root count density in the high-clay (Bt) horizon (*open circles*) is also indicated

Greenhouse study

*Plant growth and Ca uptake at constant Ca supply*

Leaf Ca concentrations of greenhouse seedlings in the high-Ca treatment were significantly rank correlated with leaf litter Ca concentrations of mature trees in the field ($P = 0.02$) with greenhouse seedlings exhibiting approximately half the leaf Ca concentrations in young green leaves as that of leaf litter of field trees (Fig. 3). Leaf Ca concentration in seedlings ranged from 1.19% dry mass in *Tilia cordata* to 0.31% in *Larix decidua* and *Pinus sylvestris* (Fig. 4a). Supply differentially affected leaf, stem, and root Ca concentration among species (species × Ca treatment interaction, $P$ 0.001, two-way ANOVA; Fig. 4a–c). For all species, leaf, stem, and root Ca concentrations tended to decline with a decrease in Ca supply (Fig. 4a–c), although only *Larix* leaves and *Picea* stems had significant differences among every level (one-way ANOVA, Tukey HSD, $P < 0.05$). *Tilia cordata* had significantly higher stem Ca and leaf Ca concentrations in the high-Ca treatment than did all or most of the other species, respectively ($P < 0.05$, Fig. 4a, b). *Larix*, the species with the lowest leaf Ca concentrations, had the highest root Ca concentration in the high-Ca treatment ($P$ 0.05, Fig. 4c).

In general, whole-plant RGR did not differ greatly among Ca treatments for most species between week 8 and week 15 (data not shown). An exception was *Tilia cordata*, which exhibited a RGR of 29.3 mg $g^{-1}$ $day^{-1}$ in the high-Ca treatment and 3.9 mg $g^{-1}$ $day^{-1}$ in the low-Ca treatment. Increases in Ca supply differentially increased total biomass ($P < 0.001$) and height ($P < 0.001$) among species by the end of the experiment (species × treatment interaction, two-way ANOVA; Fig. 5a, b). Total biomass and height at week 15 were significantly smaller with each decreasing Ca treatment for *Tilia* (one-way ANOVA, Tukey HSD, $P < 0.05$; Fig. 5a, b). None of the other species exhibited significant differences in total biomass or height at the end of the experiment among the Ca treatments (Fig. 5a, b).

At the end of the greenhouse experiment with 15-week-old seedlings of six species, Ca treatment differentially affected whole-plant (leaves, stem and roots combined) Ca accumulation depending on species (species × treatment interaction, two-way ANOVA, $P < 0.0001$). All species except *Picea* significantly differed in whole-plant Ca accumulated among treatments (one-way ANOVA, Tukey HSD, $P < 0.05$; Fig. 5c).

*Partitioning of Ca to leaves, stems and roots*

To investigate species differences in Ca partitioning, the percent of whole-plant Ca and whole-plant

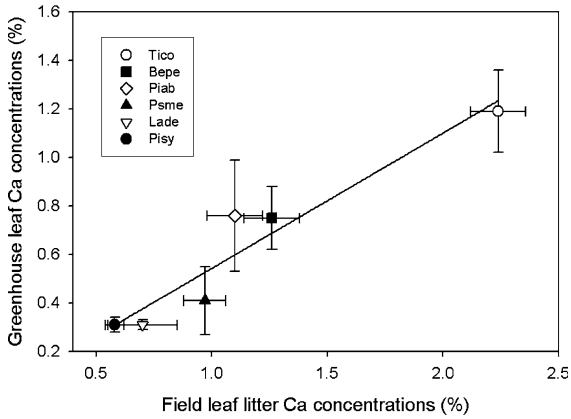


**Fig. 3** Relationship of leaf litter Ca concentrations from field observations in a common garden in Siemianice, Poland (from Reich et al. 2005) with green leaf Ca concentrations of seedlings of the same species in a greenhouse experiment at University Park, Pennsylvania ($n = 5$; ±SE). The Ca supply of greenhouse-grown 15-week-old seedlings was 313 μmol $l^{-1}$ Ca nutrient solution (i.e., high Ca supply) ($r^2 = 0.92$ $P < 0.01$, $y = 0.558x - 0.015$). (Tico, *Tilia cordata*; Bepe, *Betula pendula*; Piab, *Picea abies*; Psme, *Pseudotsuga menziesii*; Pisy, *Pinus sylvestris*; Lade, *Larix decidua*)

biomass were estimated for leaves, stems, and roots in the greenhouse seedlings at the end of the experiment (15 weeks). Species differed in both whole-plant biomass and Ca partitioning among leaves, stem and roots (two-way ANOVA, $P$ 0.0001; Table 1), whereas Ca treatment effects were only significant in whole-plant biomass partitioning to stems ($P = 0.04$) and to roots ($P = 0.009$). The effect of species on the proportion of whole-plant Ca partitioned to leaves and to roots depended on treatment (Ca treatment × species interaction, two-way ANOVA, $P > 0.001$), whereas there were only species effects in Ca partitioning to stems (two-way ANOVA, $P < 0.001$; Table 1). Species, while in some cases affected by Ca supply, differed in mean organ partitioning by as much as 25% for Ca (leaves) and 32% for biomass (leaves) (Table 1).

All species had significantly higher concentrations of Ca in the high compared to low Ca treatment in all organs (Fig. 4); yet, species showed differences in partitioning of Ca to various organs in the high- compared to the low-Ca treatment. For example, when Ca supply was high, *Tilia*, *Picea*, *Pseudotsuga* and *Pinus* distributed a greater percent of Ca in leaves than roots, whereas *Betula* and *Larix* distributed a greater amount of Ca in roots than leaves (Table 1).

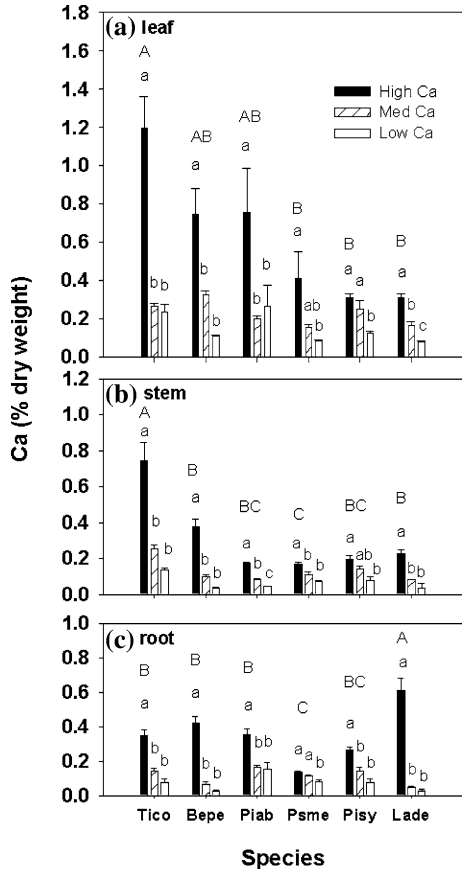


**Fig. 4** (**a**) Leaf, (**b**) stem and (**c**) root Ca concentrations at week 15 of six tree species grown under high (313 μmol $l^{-1}$), medium (31.3 μmol $l^{-1}$) and low (3.13 μmol $l^{-1}$) Ca nutrient solution (Tico, *Tilia cordata*; Bepe, *Betula pendula*; Piab, *Picea abies*; Psme, *Pseudotsuga menziesii*; Pisy, *Pinus sylvestris*; Lade, *Larix decidua*). Within a species, differences in lower-case letters signify significance; differences in upper-case letters signify significant differences among species in the high-Ca treatment (one-way ANOVA, Tukey HSD, $P < 0.05$)

A greater partitioning of Ca to a particular organ may be an indirect result of a shift in biomass partitioning. We illustrate both these factors in Fig. 6 in order to assess their relative importance in a given plant organ. We plotted the effect size of a change from high- to low-Ca treatments, on the shift in Ca or in biomass partitioning ([percent of whole plant biomass in the high Ca treatment − percent whole plant biomass in the low Ca treatment] compared with [percent of whole plant Ca in the high Ca − percent whole plant Ca in the low Ca treatment]) of leaves, roots and stems for all six species (Fig. 6). A comparison between high-medium and high-low Ca treatments showed similar trends, so only high-low

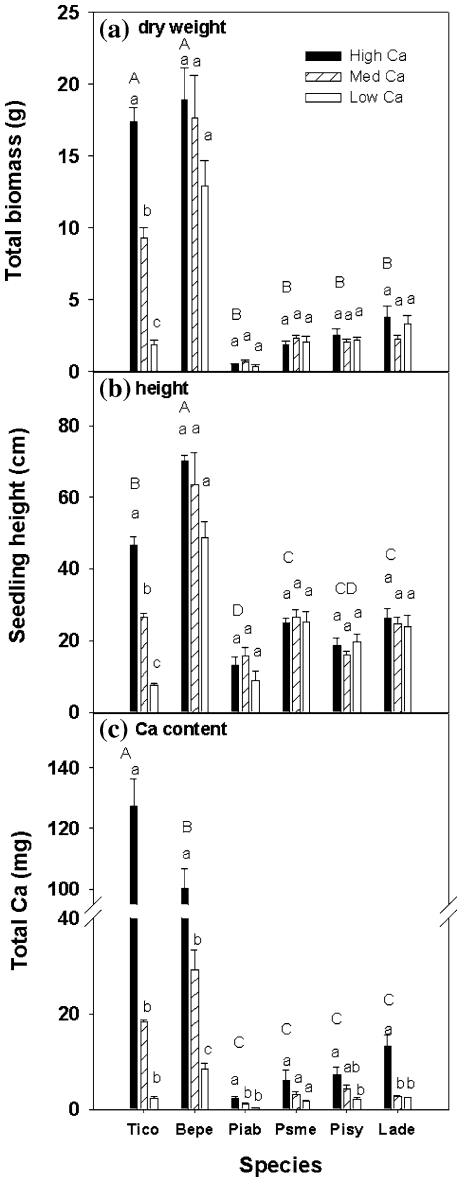


**Fig. 5** (**a**) Total biomass, (**b**) Total seedling height and (**c**) total Ca content at week 15 of six tree species (see Fig. 4 for species name abbreviations) grown under high (313 μmol $l^{-1}$), medium (31.3 μmol $l^{-1}$) and low (3.13 μmol $l^{-1}$) Ca nutrient solution. Statistical significance as described in Fig. 4

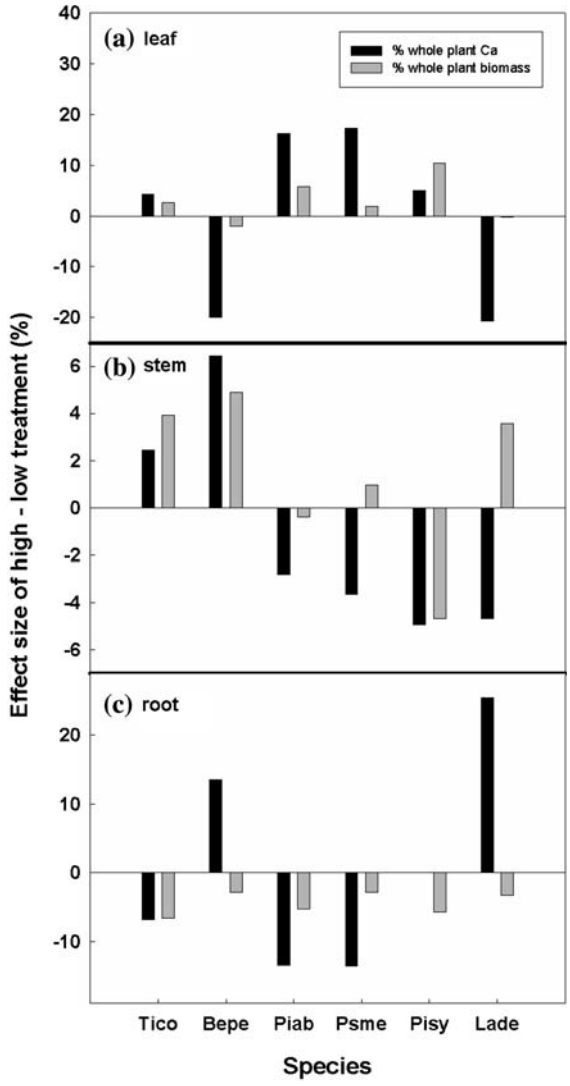


**Fig. 6** Effect size of high-Ca minus low-Ca treatment in the percent of whole plant biomass and percent of whole plant Ca of leaves (**a**), stems (**b**) and roots (**c**) of six species (see Fig. 4 for species name abbreviations). (e.g., for Tico leaves, 51% whole plant Ca in high treatments − 46% whole plant Ca in low treatment = 5%). Seedlings were grown in a greenhouse at high (313 μmol $l^{-1}$) and low (3.13 μmol $l^{-1}$) Ca nutrient solution and harvested after 15 weeks.

Ca treatment comparisons are shown for simplicity. Small shifts in biomass partitioning with a change in Ca supply accompanied by large shifts in Ca partitioning would be indicative of Ca accumulation in particular organs with an increase in supply. *Betula* and *Larix* show high positive effect sizes of Ca partitioning and negative effect sizes of biomass partitioning in roots, suggesting that high Ca concentration of roots in individuals in the high-Ca treatment is related to both a higher Ca partitioning and a lower biomass partitioning than roots of individuals in the low-Ca treatment. In contrast, *Larix* exhibited a relative increase in stem biomass partitioning with an increase in Ca supply without a similar increase in stem Ca partitioning. Especially in

leaves and roots, Ca partitioning was more affected than biomass partitioning with an increase in Ca supply (Fig. 6).

## Discussion

Greenhouse-grown seedling leaf-Ca concentrations were correlated with leaf litter Ca of mature trees in the field (Fig. 3), indicating that a similar rank order variation in leaf Ca concentrations among species exists even when supply is held constant. The seedlings grown in the greenhouse had approximately 50% lower Ca concentration in their leaves than did senesced leaves of trees in the field (Fig. 3). Lower Ca concentration in greenhouse leaves was likely due to seedling immaturity, or to comparing younger (15 weeks) leaves of seedlings versus end of season leaf-litter (~24 weeks for deciduous species, 3–8 years for evergreen species) of mature trees, as Ca is known to accumulate throughout the lifespan of the leaf (Oleksyn et al. 2000, 2002). Despite a 50-fold range in exchangeable Ca in the soil of the common garden and a 100-fold range in Ca solution concentration in the greenhouse, both mature field trees and greenhouse seedlings exhibited only a 4-fold difference in leaf Ca concentrations among species. Species, as well as supply, exert a significant influence on leaf Ca concentrations and on leaf, stem and root partitioning of Ca, leading us to conclude that physiological differences in uptake and translocation among species are a major influence on leaf Ca concentrations in the field.

All six species grown as seedlings in the greenhouse showed declining Ca concentrations in leaves, stems and roots with decreasing Ca supply, even when growth did not respond to Ca supply. Only *Tilia cordata* showed evidence of seedling growth limitation by Ca within the concentration range used in this study. Compared to the high-Ca treatment, growth rate, total biomass and height of *Tilia* were 84–89% lower in the low-Ca treatment. Although the growth of *Betula* did not decline significantly, two of the five individuals in the low-Ca treatment exhibited cupped leaves, a sign of Ca deficiency (Taiz and Zeiger 2002) and may have exhibited a decrease in vigor if the experiment continued. In contrast, gymnosperm species showed virtually no change in growth rates, height or total accumulated biomass at the end of the 15-week treatment. Differences in growth rate and total biomass in just 15 weeks in *Tilia* is an indication that some tree species are sensitive to Ca supply as very young seedlings. Likewise, Bigelow and Canham (2007) found variation in growth response to Ca among seedling tree species in northern hardwood forests, with a response rate to Ca equal to that of nitrogen for some species.

Species exhibited differences in partitioning of whole plant Ca to leaf, stem and root organs by Ca supply (Table 1; Fig. 6). *Larix* and *Betula* demonstrated markedly different trends than the other species, with low leaf and high root Ca partitioning in the high Ca treatment which could not be accounted for by similar shifts in biomass partitioning between high- and low-Ca treatments (Fig. 6). This pattern in *Larix* and *Betula* suggested a greater tendency to accumulate Ca in roots than leaves with an increase in Ca supply. In contrast, in stems *Betula* partitioned a greater portion, and *Larix* a smaller portion, of total Ca under increasing Ca supply (Fig. 6). These patterns of differential Ca partitioning to specific organs further indicate species-specific physiological control of leaf Ca.

We examined root distribution in relation to soil chemistry for 14 tree species using several different approaches and found little indication that active root proliferation contributed to greater access to soil Ca. A review of literature indicates few studies of the direct effects of localized Ca enrichment on root proliferation; the few studies where root proliferation has been shown could be due to indirect effects of pH or increased availability of other nutrients (Cuevas and Media 1988; Raich et al. 1994). Areas of high exchangeable Ca were influenced by soil texture and its effects on cation exchange capacity (Fig. 1), but there was no indication of higher density of roots in these soil horizons. Contrary to our hypothesis, only in *Larix decidua*, a low-leaf-Ca-accumulating species, did root density in the Bt horizons exceed the expected root density at a particular depth (Fig. 2d). In addition, we found no evidence that having deeper roots conferred an advantage in acquiring Ca at this site as there was no correlation of Ca concentration in the leaf litter in the field with root density in deep soils (horizons from 45 to 100 cm deep). In contrast to our findings, Dijkstra and Smits (2002) used a budget model to determine that *Acer saccharum*, a

species with high leaf Ca and more fine roots than *Tsuga canadensis*, had more potential to acquire Ca from deep soil. It is possible root distribution may play a role as root distribution and root activity for Ca uptake may not be equal across species or with depth. The number of roots that would constitute a significant difference in ability to acquire Ca from a given soil zone is unknown. Calcium uptake by the roots in addition to root length, may depend on other root traits including root age, mycorrhizal associations, and specific rates of water influx.

Data from the two plantings at our field site indicate that a gradient in soil Ca availability in the mineral soil corresponded with the gradient in soil clay content. However, for the four species that occur in both plantings there was no evidence of increasing leaf-litter Ca concentration with increasing soil clay content ($P = 0.18$) nor was there any evidence of a significant interaction between clay and species ($P = 0.38$), suggesting that these species did not show strong differences in degree of responsiveness to clay content in the soil. Moreover, species was overall a better predictor of leaf litter Ca concentrations than soil percent clay, further indicating the importance of intrinsic differences among species accounting for differences in leaf Ca levels.

The mechanisms underlying differences in tree species regulation of Ca uptake and translocation are still not completely understood (McLaughlin and Wimmer 1999; Reich et al. 2005). In particular, it is not known why some species have a greater growth response to Ca supply, whereas other species apparently restrict Ca movement to the leaves. However, the process of Ca uptake and partitioning is a physiological characteristic that may have large variation among species (Killingbeck 1986; Marschner 1995; Meerts 2002). While soil Ca availability and plant access to soil Ca likely play a role in leaf-Ca accumulation, the wide range of Ca concentrations in leaves due to inherent species-specific physiological differences needs to be recognized, both in the context of nutrient status and in investigations examining tree species effects on forest soil nutrition and chemistry. Studies of ecosystem nutrient dynamics, particularly those pertaining to Ca, are incomplete without an understanding of the potential unique contributions of particular plant species (Hobbie 1992). Pairing our field study with a controlled greenhouse experiment allowed us to not only describe patterns, but importantly, to address mechanisms for species differences in leaf-Ca accumulation. This study demonstrates the importance of plant differences in Ca uptake and Ca partitioning among roots, stems and leaves as major contributors to leaf-Ca variation and cycling among species.

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## References

Alban DH (1982) Effects of nutrient accumulation by aspen, spruce and pine on soil properties. Soil Sci Soc Am J 46:853–860

Amacher MC, Henderson RE, Breithaupt MD et al (1990) Unbuffered and buffered salt methods for exchangeable cations and effective cation exchange capacity. Soil Sci Soc Am J 54:1036–1042

Andersson S, Nilsson SI, Saetre P (2000) Leaching of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in mor humus as affected by temperature and pH. Soil Biol Biochem 32:1–10

Bigelow SW, Canham CD (2007) Nutrient limitation of juvenile trees in a northern hardwood forest: calcium and nitrate are preeminent. For Ecol Manage 243:310–319

Binkley D, Giardina C (1998) Why do tree species affect soils? The warp and woof of tree-soil interactions. Biogeochemistry 42:89–106

Bockheim JG (1997) Soils in a hemlock-hardwood ecosystem mosaic in the Southern Lake Superior Uplands. Can J For Res 27:1147–1153

Boettcher SE, Kalisz PJ (1990) Single-tree influence on soil properties in the mountains of Eastern Kentucky. Ecology 1990:1365–1372

Böhm W (1979) Methods of studying root systems. Springer-Verlag, New York, p 188

Broadley MR, Bowen HC, Cotterill HL et al (2003) Variation in the shoot calcium content of angiosperms. J Exp Bot 54:1431–1446

Caldwell MM, Virginia RA (1989) Roots systems. In: Pearcy RW et al (ed) Plant physiological ecology. Chapman and Hall, London

Chan KY, Heenan DP (1999) Lime-induced loss of soil organic carbon and effect on aggregate stability. Soil Sci Soc Am J 63:1841–1844

Chapin FS, Walker BH, Hobbs RJ et al (1997) Biotic control over the functioning of ecosystems. Science 277:500–503

Cuevas E, Medina E (1988) Nutrient dynamics within amazonian forests: II. Fine root growth, nutrient availability and leaf litter decomposition. Oecologia 76:222–235

Dijkstra FA, Smits MM (2002) Tree species effects on calcium cycling: the role of calcium uptake in deep soils. Ecosystems 5:385–398

Dong SF, Neilsen D, Neilsen GH et al (2003) A scanner-based root image acquisition technique for measuring roots on a rhizotron window. Hortscience 38:1385–1388

Eriksson HM, Rosen K (1994) Nutrient distribution in a Swedish tree species experiment. Plant Soil 164:51–59

Eviner VT, Chapin FS, Vaughn CE (2006) Seasonal variations in plant species effects on soil N and P dynamics. Ecology 87:974–986

Finzi AC, Canham CD, Breeman N (1998) Canopy tree-soil interactions within temperate forests: species effects on pH and cations. Ecol Appl 8:447–454

Hedin LO, Granat L, Liken GE et al (1994) Steep declines in atmospheric base cations in regions of Europe and North America. Nature 367:351–354

Hobbie SE (1992) Effects of plant species on nutrient cycling. Trends Ecol Evol 7:336–339

Hobbie SE, Reich PB, Oleksyn J, Ogdahl M, Zytkowiak R, Hale C, Karolewski P (2006) Tree species effects on decomposition and forest floor dynamics in a common garden. Ecology 87:2288–2297

Jandl R, Alewell C, Prietzel J (2004) Calcium loss in Central European forest soils. Soil Sci Soc Am J 68:588–595

Jobbágy E, Jackson RB (2004) The uplift of soil nutrients by plants: biogeochemical consequences across scales. Ecology 85:2380–2389

Johnson DW, Todd DE (1990) Nutrient cycling in forests of Walker Branch Watershed, Tennessee: roles of uptake and leaching in causing soil changes. J Environ Qual 19:97–104

Jones CG, Lawton JH, Shachak M (1997) Positive and negative effects of organisms as physical ecosystem engineers. Ecology 78:1946–1957

Killingbeck KT (1986) Litterfall dynamics and element use efficiency in a Kansas gallery forests. Am Midl Nat 116:180–189

Lamont BB, Groom PK (2002) Green cotyledons of two *Hakea* species control seedling mass and morphology by supplying mineral nutrients rather than organic compounds. New Phytol 152:101–110

Lehmann J (2003) Subsoil root activity in tree-based cropping systems. Plant Soil 255:319–331

Likens GE, Driscoll CT, Buso DC, Siccama TG, Johnson CE, Lovett GM, Fahey TJ, Reiners WA, Ryan DF, Martin CW, Bailey SW (1998) The biogeochemistry of calcium at Hubbard Brook. Biogeochemistry 41:89–173

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic Press, London

McLaughlin SB, Wimmer R (1999) Tansley review no. 104: calcium physiology and terrestrial ecosystem processes. New Phytol 142:373–417

Meerts P (2002) Mineral nutrient concentrations in sapwood and heartwood: a literature review. Ann For Sci 59:713–722

Muneer M, Oades JM (1989) The role of Ca-organic interactions in soil aggregate stability. I. Laboratory studies with $^{14}C$-glucose, $CaCO_3$ and $CaSO_4$-$2H_2O$. Aust J Soil Res 27:389–399

Muys B, Lust N (1992) Inventory of the earthworm communities and the state of litter decomposition in the forests of Flanders, Belgium, and its implications for forest management. Soil Biol Biochem 24:1677–1681

Oades JM (1988) The retention of organic matter in soils. Biogeochemistry 5:37–70

Oleksyn J, Zytkowiak R, Reich PB, Tjoelker MG, Karolewski P (2000) Ontogenetic patterns of leaf $CO_2$ exchange, morphology and chemistry in *Betula pendula* trees. Trees 14:271–281

Oleksyn J, Reich PB, Zytkowiak R, Karolewski P, Tjoelker MG (2002) Needle nutrients in geographically diverse *Pinus sylvestris* L. populations. Ann For Sci 59:1–18

Oste LA, Temminghoff EJM, Van Riemsdijk WH (2002) Solid-solution partitioning of organic matter in soils as influenced by an increase in pH or Ca concentration. Environ Sci Technol 38:208–214

Oyonarte C, Perez-Pujalte A, Delgado G, Delgado R, Almendros G (1994) Factors affecting soil organic matter turnover in a Mediterranean ecosystem from Sierra de Gador (Spain): an analytical approach. Commun Soil Sci Anal 25:1929–1945

Persson T, Lundkvist H, Wirén A, Hyvönen R, Wessén B (1989) Effects of acidification and liming on carbon and nitrogen mineralization and soil organisms in mor humus. Water Air Soil Pollut 45:77–96

Pop VV, Postolache T, Vasu A, Craciun C (1992) Calcophilous earthworm activity in soil; an experimental approach. Soil Biol Biochem 24:1483–1490

Raich JW, Riley RH, Vitousek PM (1994) Use of root-ingrowth cores to assess nutrient limitations in forest ecosystems. Can J For Res 24:2135–2138

Reich PB, Oleksyn J, Modrzynski J, Mrozinski P, Hobbie SE, Eissenstat DM, Chorover J, Chadwick OA, Hale CM, Tjoelker MG (2005) Linking litter calcium, earthworms, and soil properties: a common garden test with 14 temperate tree species. Ecol Lett 8:811–818

Rhoades JD (1996) Salinity: electrical conductivity and total dissolved salts. In: Sparks DL et al (eds) Methods of soil analysis. Part 3. SSSA Book series No 5. ASA and SSA, Madison, pp 417–436

Simmons JA, Yavitt JB, Fahey TJ (1996) Watershed liming effects on the forest floor N cycle. Biogeochemistry 32:221–224

Szymanski S (1982) Growth of some forest tree species in the first 10 years on fairly poor mixed conifer sites. Sylwan 126:11–29 (in Polish)

Taiz L, Zeiger E (2002) Plant physiology, 3rd edn. Sinauer, Sunderland

Thompson K, Parkinson JA, Band SR, Spencer RE (1997) A comparative study of leaf nutrient concentrations in a regional herbaceous flora. New Phytol 136:679–689

Troeh FR, Thompson LM (2005) Soils and soil fertility, 6th edn. Blackwell

Washburn CSM, Arthur MA (2003) Spatial variability in soil nutrient availability in an oak-pine forest: potential effects of tree species. Can J For Res 33:2321–2330

Withington JM, Elkin AD, Bulaj B, Olesinski J, Tracy KN, Bouma TJ, Oleksyn J, Anderson LJ, Modrzynski J, Reich PB, Eissenstat DM (2003) The impact of material used for minirhizotron tubes for root research. New Phytol 160:533–544

Withington JM, Reich PB, Oleksyn J, Eissenstat DM (2006) Comparisons of structure and life span in roots and leaves among temperate trees. Ecol Monogr 76:381–397