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Effects of Calcium on the Rate and Extent of Litter Decomposition in a Northern Hardwood Forest

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Abstract

Cross-site syntheses of litter decomposition studies have shown that litter calcium (Ca) concentration may have a role in controlling the extent of decomposition of tree foliage. We used an ongoing watershed $CaSiO_3$ addition experiment at the Hubbard Brook Experimental Forest in New Hampshire, USA, to test the hypotheses that increased Ca in litter would have no effect on the initial rates of litter decay but would increase the extent or completeness (limit value) of foliar litter decomposition. We tested these hypotheses with a 6-year litter decomposition experiment using foliar litter of four tree species that are prominent at this site and in the Northern Hardwood forest type of North America: sugar maple (*Acer saccharum* Marsh), American beech (*Fagus grandifolia* Ehrh.), yellow birch (*Betula alleghaniensis* Britt.), and white ash (*Fraxinus americana* L.). The experiment used a reciprocal transplant design with the Ca-treated watershed and a control site providing two sources of litter and two placement sites. The litter from the Ca-treated site was 10–92% higher in Ca concentration, depending on species, than the litter from the control site. After about 3 years of decomposition, the Ca concentrations in the litter reflected the placement of the litter (that is, the site in which it was incubated) rather than the source of the litter. The source of the litter had no significant effect on measures of initial decomposition rate, cumulative mass loss (6 years), or limit value. However, the placement of the litter had a highly significant effect on extent of decomposition. Some litter types responded more than others; in particular, beech litter placed in the Ca-treated site had a significantly higher limit value, indicating more complete decomposition, and maple litter in the Ca-treated site had a marginally higher limit value. These results indicate that Ca may influence the extent of litter decomposition, but it is the Ca at the incubation site rather than the initial litter Ca that matters most. The results also suggest that loss of Ca from the soil due to decades of acid deposition at this site may have impeded late-stage litter decomposition, possibly leading to greater soil C storage, especially in forest stands with a substantial component of beech. Likewise, de-acidification may lead to a reduction in soil C.

Key words: decomposition; litter; calcium; sugar maple; American beech; white ash; yellow birch.

Introduction

In terrestrial ecosystems, decomposition of plant litter is a fundamental process that serves to recycle nutrients held in dead plant biomass and add carbon to soils. Studies of the regulation of litter

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decomposition rates have identified climatic controls (for example, Berg and others 1993) as well as litter chemistry controls (for example, Fogel and Cromack 1977). Of the latter, lignin and nitrogen are recognized as primary constituents controlling short-term rates of litter decomposition (Melillo and others 1982) although tannins (Kraus and others 2003) and other litter constituents (Berg 2000) have been identified as being important in some cases. The activity of soil fauna (Petersen and Luxton 1982) and the composition of the community of microbial decomposers (Austin and others 2014) can also be important factors controlling litter decomposition. Early research modeled the decomposition of a cohort of litter as a simple exponential decay governed by the litter decay constant k (for example, Olson 1963). More recent modeling conceptualizes the process as either a multi-stage exponential decay, with separate decay constants for different litter fractions, or as exponential decay to an asymptote (Adair and others 2008; Harmon and others 2009). When plotted as cumulative percent mass lost versus time, the asymptote (that is, the maximum mass loss) represents the labile fraction of the litter cohort, and has been termed the ''limit value'' (Berg and others 1996). The complement of the limit value is the recalcitrant fraction, which includes lignocelluloses and other complex compounds that can be present in the plant litter and can also be formed in situ during the process of decomposition (Berg and McClaugherty 2014). This recalcitrant fraction may be a prime contributor to the buildup of humus and sequestration of carbon in soils (Berg and Dise 2004). Different factors may influence the limit value as opposed to the initial decay rate (k). For instance, unlike k, the limit value shows little sensitivity to climate and is not correlated to initial lignin concentration in the litter (Berg and McClaugherty 2014). Litter nitrogen (N) concentration is inversely correlated to the limit value, such that higher N is associated with less complete decomposition (Berg and others 2000). In a synthesis of decomposition rates of forest foliar litter, initial concentrations of manganese (Mn) and calcium (Ca) were shown to be positively correlated with limit values (Berg and others 2000).

The reasons for lignin and N control on initial rates of litter decomposition seem relatively straightforward. Lignin is a class of compounds containing multiple phenolic structures that are difficult for microbes to degrade, whereas N is an important microbial nutrient that is in short supply in fresh litter having high C:N ratios. Thus, more lignin or less N should impede the decomposition process, and the lignin:N ratio is inversely correlated to k (Melillo and others 1982). The reasons for chemical control of the limit value are less clear, but it has been hypothesized that N is involved with the chemical stabilization of late-stage decomposition products (Berg and McClaugherty 2014), and N has also been shown to suppress the production of lignin-degrading enzymes such as phenol oxidase (Sinsabaugh and others 2005; Weand and others 2010). Manganese is a component of an important lignolytic enzyme, manganese peroxidase, produced by fungi (Berg and others 2010). Calcium, which is shown in a data synthesis to be positively related to the limit value, especially in deciduous litter, is an important constituent of fungal cell walls and has been shown to increase growth of white rot fungi, which can degrade lignin (Berg and others 1996; Berg and McClaugherty 2014). Although litter Ca has been shown to be correlated to late-stage decomposition in cross-site comparisons (Berg and others 2000; De Santo and others 2009), there has never been a direct test of its influence on limit values in a manipulative study, to the best of our knowledge.

Calcium is a plant nutrient of particular interest in forests exposed to acid deposition, because acidic anions such as sulfate and nitrate, if leached from a forest, can remove Ca^{2+} from soil exchange sites (Likens and others 1998). To test the influence of Ca on the functioning of a Northern Hardwood forest, wollastonite ($CaSiO_3$) was added to a forested watershed at the Hubbard Brook Experimental Forest in central New Hampshire, USA. This long-term experiment was intended to replenish the amount of Ca that was estimated to have been leached from the soil exchange complex over six decades of acid precipitation at the site (Cho and others 2010). Results from the study have shown that forest growth, leaf area index, and evapotranspiration increased in the Ca-treated watershed relative to a reference watershed (Green and others 2013). Sugar maple (*Acer saccharum* Marsh), a dominant tree at this site, has shown increased vigor, growth, flowering, seed production, and decreased metabolic indicators of stress in response to Ca addition (Juice and others 2006; Minocha and others 2010; Halman and others 2013). Measurements of forest floor chemistry in 2006 and 2010 (during the course of the litter decomposition experiment reported here) showed that the Oi and Oe horizons had increased pH, base saturation, exchangeable Ca^{2+} and effective cation exchange capacity and decreased exchangeable H^+, relative to pre-treatment (1998) in the watershed (Johnson and others 2014). In addition, the Ca treatment

appeared to increase the ability of the plants to compete for available N in the soil, relative to the microbes (Groffman and Fisk 2011). This ''tightening'' of the N cycle resulted in declines in the N content of the microbial biomass, potential net and gross N mineralization rates, and soil inorganic N pools in the Oi + Oe horizons of the treated watershed (Groffman and others 2006).

We took advantage of this Ca manipulation to test whether additional Ca influenced the decomposition of deciduous leaf litter, either the initial decomposition rates or the limit values. We performed a 6-year litter bag decomposition experiment, using litter from the Ca-treated watershed and a control area that was not treated with Ca, in a reciprocal transplant design. Leaf litter decomposition experiments of this length are rare, but they are necessary for understanding late-stage decomposition dynamics. We hypothesized that elevated Ca levels in litter from the Ca-treated site would not affect initial decomposition rates of the litter, but would increase the limit values, leading to a greater extent (completeness) of decomposition.

Methods

Research Site

The Hubbard Brook Experimental Forest (HBEF, 43°56′N, 71°45′W) comprises most of the Hubbard Brook Valley in the White Mountain National Forest of central New Hampshire, USA. The eastern portion of the HBEF, including the sites discussed here, is underlain by a complex assemblage of metasedimentary and igneous rocks. The major map unit is the Silurian Rangeley Formation, consisting of quartz mica schist and quartzite interbedded with sulfidic schist and calc-silicate granulite. The retreat of the continental glacier approximately 14,000 years ago left a mantle of till 0–3 m thick. Soils are primarily acidic (pH 3.9), well-drained Haplorthods formed from this till. Earthworms are largely absent from these sites, and no earthworms were detected during our sampling.

The HBEF is vegetated primarily by forests of the Northern Hardwood association dominated by sugar maple (*Acer saccharum* Marsh), American beech (*Fagus grandifolia* Ehrh.), yellow birch (*Betula alleghaniensis* Britt.), and white ash (*Fraxinus americana* L.). Spruce-fir-birch forest, including red spruce (*Picea rubens* Sarg.), balsam fir (*Abies balsamea* (L.) Mill), and paper birch (*Betula cordifolia* Regel Fern.), dominates above 730 m elevation.

Mean annual precipitation at the HBEF is 1423 mm (SD = 187, range 1107–1824 mm in the period 1964–2000), and mean annual temperature (1955–2000) at 450 m elevation is 5.6°C (−8.3°C in January and 18.7°C in July) (Bailey and others 2003).

Experimental Design and Field and Laboratory Methods

We used a reciprocal transplant design employing two sites, the calcium-treated watershed (Watershed 1) and an untreated, control site of similar elevation, age, and species composition about 500 m away. Foliar litter of four species was collected at the two sites, and incubated in mesh decomposition bags at both sites for 6 years. The four focal species were sugar maple, American beech, yellow birch, and white ash, henceforth referred to as maple, beech, birch, and ash. A subset of bags was collected annually, weighed, and chemically analyzed.

The wollastonite addition to the Ca-treated watershed occurred in October 1999. Fifty-six metric tons of VANSIL-10, a commercially available form of wollastonite, was crushed, pelletized with a lignin sulfonate binder (approximately 2% wet weight), and applied by helicopter to the 11.8-ha watershed. Collectors placed throughout the watershed confirmed even application of the mineral. The Ca application rate from the wollastonite was 1028 kg Ca/ha (Battles and others 2013). The treatment increased the Ca^{2+} export in streamwater immediately due to wollastonite dissolution in the stream channel, and over the course of the subsequent 9 years, the loss of wollastonite-derived Ca^{2+} declined and stabilized at about 11 kg of Ca per year, representing about 30% of the Ca^{2+} in streamwater (Nezat and others 2010).

Foliar litter was collected using nets suspended under the canopy at both sites (Ca-treated and untreated) during the autumn of 2005, 6 years after the Ca addition. Litter was collected weekly and composited by site, then sorted to isolate the four focal species and air-dried. Litter bags were constructed of 1.6-mm mesh fiberglass window screening heat-sealed to create 20 × 20 cm pockets. Each litter bag received 10 g of litter of a single species x treatment combination, plus an identifying tag, and then was heat-sealed shut.

At each of the two sites, five 3 × 3 m plots were selected for incubation of the litter. These replicate plots were chosen based on having low to moderate slopes and few surface rocks. Replicate bags of each species and treatment were included in each plot. Bags were deployed in a randomized order within the plot and staked to the ground with stainless

steel stakes. Bags were initially staked at the surface of the forest floor, but by the end of the 6-year incubation several cm of forest floor had accumulated on top of most of the remaining bags. All handling of litter and bags (sorting, deployment, and collection) was done wearing latex or nitrile gloves.

Henceforth, we use the term ''source'' to refer to the site from which the litter was collected and ''placement'' to refer to the site in which the bags were incubated. For maple and birch, 12 bags (2 sources × 6 collection times) were set out in each plot. For beech and ash, only 10 bags (2 sources × 5 collection times) were set out in each plot due to insufficient litter of those species. For the entire experiment, there were 440 bags (2 sources × 2 placements × 4 species × 5 plots × 5 or 6 collection times). In addition, 5 bags of each combination of species × source × placement were brought to the field and then immediately returned to the lab as time 0 samples. A larger sample of each species × source combination was dried and ground for later analysis of initial concentrations of lignin and fiber.

Bags were set out on November 9–10, 2005, and samples were collected every autumn for the next 6 years. At each collection time, one bag of each species x source combination was collected from each plot; thus for each collection time, there were 5 replicates of each species x source x placement. Ash and birch bags were collected only in years 1, 2, 3, 4, and 6. Actual collection times in days since November 10, 2005 were 321, 706, 1055, 1447, 1791, and 2170 days for years 1–6, respectively.

Bags were collected in the field by brushing away covering litter and severing any seedlings or roots that had grown through the bag. The bags were returned to the laboratory and stored in a refrigerator at about 4°C for up to 4 weeks until initial analysis. To analyze the contents, the bags were cut open and the contents were carefully picked through to remove any roots growing inside the bag. The remaining contents were oven-dried at 60°C for 48 h and weighed to calculate mass remaining. The sample was then ground and analyzed for C and N by an Elementar Vario MAX CNS analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). For analysis of Ca, K, Mg, and P, as well as Mn for initial time 0 samples, samples were combusted at 500°C for 12 h, and then digested in 6 N HNO_3. Solutions were analyzed for Ca, K, Mg, and Mn by GBC Avanta atomic absorption spectrophotometer (GBC Scientific Equipment LLC, Hampshire, IL). Solutions were analyzed for PO_4^{3-} on a Technicon AAII auto-analyzer, and Al was analyzed by ICP (Varian Vista-Pro, Agilent Technologies, Santa Clara, CA). The sample that was reserved for fiber analysis was analyzed for acid detergent fiber (ADF, including lignin, cellulose, and lignin-bound protein), neutral detergent fiber (NDF, including lignin, cellulose, and hemi-cellulose), and lignin at the Dairy One Laboratory in Ithaca, NY, using an ANKOM Fiber Analyzer (ANKOM, Inc., Macedon, NY). This instrument uses a modified and partially automated version of the Van Soest technique (Van Soest and others 1991).

Statistical Analysis

To test whether the source or the placement of the litter influenced the initial rate or the ultimate extent of decomposition, we calculated four response variables. Two of these were directly measured: the mass loss at the year 1 collection (ML1, in percent) is an estimate of the initial decomposition rate, and the cumulative mass loss to year 6 (ML6, in percent) is an estimate of the extent of decomposition. The other two response variables were derived by fitting the mass loss sequence for every litter species, source, placement, and plot to an asymptotic decomposition equation:

$$\%\,\mathrm{ML}_t = LV\left(1 - e^{-k_A t}\right), \qquad (1)$$

where $\%\mathrm{ML}_t$ is cumulative % mass loss to time t (in years), LV is the limit value in percent (the labile fraction of the litter), and k_A is an asymptotic decomposition constant (y^{-1}). Equation (1) simply states that there is an exponential decomposition of the labile fraction (limit value) of the litter. The remainder of the litter (1-LV) is assumed to be stable. Note that k_A in equation (1) is not the same as the decomposition constant k from a simple exponential decay model (for example, Olson 1963).

The mass loss sequences were fit to equation (1) using Proc NLIN in the SAS Statistical program (SAS Version 9.2, SAS Institute, Inc.) and estimates of k_A and limit value were calculated for each combination of species, source, placement, and plot, using the Marquardt method to fit the nonlinear regression model (SAS Institute Inc. 2004). Because the limit value cannot conceptually exceed 100%, we bounded the limit value parameter estimate to remain below or equal to 100% in the regressions, which affected eight of the 80 decomposition sequences. Thus, the mean limit value estimates are biologically reasonable, but the standard errors determined from the bounded estimates may slightly underestimate uncertainty for the limit value parameter. Statistical analyses using

bounded versus unbounded estimates of the limit value and associated k_A yielded similar conclusions regarding the effects of species, litter source, and litter placement on decomposition rate and extent.

These calculations produced four principal response variables for mass loss: two measures of the initial decomposition rate (ML1 and k_A) and two measures of the extent of litter decay (ML6 and limit value). Species and treatment effects on these four variables and chemical concentrations in litter were determined using analysis of variance. Initially mixed-model ANOVAs (Proc MIXED) were performed on the data using species, source, and placement as main effects and plot as a random blocking variable, nested within the placement effect. In no case was plot a significant effect, nor were there any significant interactions involving plot, so plot was dropped from further analyses. The final analyses used Proc GLM to analyze the main effects species, source, and placement and their interactions, with contrasts to determine the effect of source or placement for each species. For the Time 0 litter, only species and source were used as main effects. For the mass loss and chemical variables, individual collection years were analyzed separately.

Results

Initial Litter Chemistry

Initial (Time 0) litter chemistry differed significantly among species and between the two different sources of litter (control vs. +Ca site) (Table 1). As expected, Ca was significantly elevated in the +Ca litter, but level of the enhancement differed among tree species—maple showed the greatest litter Ca response to the treatment and beech the least. All species except beech also showed highly significant increases in N concentration, and maple and birch also showed significant increases in K concentration. The +Ca treatment also caused significant decreases in some constituents: Mg concentration declined in all species; Mn concentration declined in beech, ash, and birch; and *P* declined in beech and birch. There were no significant effects of the treatment on Al or C concentrations. Species varied considerably in fiber and lignin content (Table 1), with lignin concentration decreasing in the order beech > birch > ash > maple. Differences in lignin concentration between control and +Ca litter were less than 1%.

Mass Dynamics

The cumulative mass loss generally followed an asymptotic curve, with the shapes of the curves and the asymptote varying among species (Figure 1).

For the two measures of initial decomposition rate, ML1 and k_A, there were highly significant species effects ($P < 0.0001$) but no significant effects of litter source or placement, nor were there any significant interaction terms. Mean percent mass loss at the year 1 collection (321 days) was 18.0, 28.9, 38.4, and 39.9% for beech, maple, birch, and ash, respectively. Mean k_A values were 0.32, 0.50, 0.58, and 0.69 y^{-1} for the same species.

For the two measures of the extent of litter decay, ML6 and limit value, there were significant species and placement effects, but no litter source effect. Cumulative mass loss at year 6 (ML6, measured at 2170 days) showed highly significant effects of species and placement but no significant effects of litter source nor any interaction terms (Figure 2a). For all species considered together, mean ML6 was greater in the +Ca site than in the control site. Considering species individually, this effect was highly significant for beech ($P = 0.0011$), marginally significant for maple ($P = 0.08$), and not significant for birch and ash. Litter limit value also showed significant species and placement effects and no significant interaction terms (Figure 2b). For individual species, the placement effect on limit value was significant for beech ($P = 0.0017$) and marginally significant for maple ($P = 0.08$), but not for the other species. Considering all species together, the limit value was greater in the +Ca placement site than in the control site.

Chemical Dynamics

The significance of the treatment effects on litter chemistry varied through time and different chemical constituents behaved differently (Table 2). For C, a strong species effect was evident throughout the incubation. In year 1, the +Ca litter had a higher %C than the control litter, but by the end of the incubation this pattern had reversed. Effects of placement were weak or nonexistent. For N, strong species and source effects persisted through year 3, after which the species effect remained strong and the source effect weakened. The moderate placement effect observed in year 1, with litter in the +Ca site having greater %N than the control litter, was highly significant by year 6. For Ca, significant species, source, and placement effects persisted through year 3, after which the source effect on the litter Ca concentration was lost but the placement effect remained, and was highly significant in year 6. For Mg, K, and P, species, source, and placement effects were inconsistent in magnitude and direction over time (Supplementary material Table S1).

Table 1. Initial Litter Chemistry Expressed as Mean (SD)

Species	Source	C	N	Ca	Mg	K	P	Al	Mn	ADF[1]	NDF[1]	Lignin[1]
American Beech	Control	44.5 (2.0)	0.83 (0.02)	8.06 (0.25)	1.62 (0.08)	5.37 (0.28)	0.38 (0.02)	34.8 (1.1)	753 (12)	43.7	62.2	20.0
	+Ca	44.8 (1.9)	0.87 (0.03)	8.90 (0.18)*	1.24 (0.04)****	5.57 (0.12)	0.24 (0.01)	37.0 (2.9)	597 (12)****	42.9	62.4	19.5
Sugar Maple	Control	42.3 (2.6)	0.58 (0.02)	6.33 (0.24)	0.82 (0.05)	4.74 (0.14)	0.35 (0.02)	20.1 (0.4)	653 (14)	26.6	34.0	11.6
	+Ca	43.9 (1.9)	0.87 (0.04)****	12.14 (0.15)****	0.46 (0.04)****	5.62 (0.11)***	0.36 (0.02)	23.9 (1.5)	656 (08)	26.9	38.1	10.9
White Ash	Control	47.9 (1.3)	0.79 (0.02)	6.96 (0.20)	0.96 (0.05)	8.41 (0.09)	0.36 (0.14)	26.0 (0.6)	340 (12)	37.6	51.6	13.4
	+Ca	49.1 (0.5)	1.14 (0.02)****	10.85 (0.16)****	0.14 (0.01)****	8.18 (0.15)	0.20 (0.02)	25.9 (1.6)	299 (11)*	36.3	53.8	12.6
Yellow Birch	Control	43.3 (1.7)	1.04 (0.04)	12.17 (0.29)	2.02 (0.06)	6.27 (0.22)	0.93 (0.06)	40.3 (13.4)	1028 (10)	33.3	48.7	16.1
	+Ca	44.7 (1.6)	1.37 (0.05)****	18.35 (0.37)****	1.01 (0.06)****	8.61 (0.16)****	0.64 (0.04)***	38.1 (1.9)	797 (22)****	32.4	48.1	16.0
Species effect		*	****	****	****	****	****	**	****			
Source effect			****	****	****	****	***		****			
Species*source interaction			****	****	****	****			****			

C, N, acid detergent fiber (ADF), neutral detergent fiber (NDF), and lignin are in units of % dry mass; Ca, Mg, K, and P are in mg/g, and Al and Mn are in μg/g. Three rows at bottom give the significance of ANOVA tests of the main effects of species and litter source (+Ca vs. control site) and their interaction.

[1]*ADF, NDF, and lignin were measured on a single composite sample of each species x treatment combination, so no standard errors or ANOVA tests are available*

*Significance of the treatment effect is given adjacent to the +Ca entry for each species and constituent, as follows: blank = not significant, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.*

Table 2. Results of ANOVA on C, N and, Ca in Litter

Year		C	N	Ca
1	Species	****	****	****
	Source	**** (T > C)	**** (T > C)	**** (T > C)
	Placement		** (T > C)	**** (T > C)
	Interactions	sp*so		sp*so
2	Species	****	****	****
	Source		**** (T > C)	*(T > C)
	Placement	* (C > T)	** (T > C)	** (T > C)
	Interactions	sp*so, sp*p	sp*so, sp*p	sp*so, sp*p
3	Species	****	****	****
	Source		**** (T > C)	**** (T > C)
	Placement		* (T > C)	**** (T > C)
	Interactions		sp*so	sp*p
4	Species	****	****	****
	Source	**** (C > T)		
	Placement			**** (T > C)
	Interactions	sp*so*p	sp*so	
6	Species	****	****	**
	Source	* (C > T)	** (T > C)	
	Placement		**** (T > C)	**** (T > C)
	Interactions	sp*so, sp*p		

*Significance of main effects (species, source, placement) are given as blank = not significant, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. Significant ($P < 0.05$) interaction terms are listed with the abbreviations sp = species, so = source, and p = placement. In cases where source or placement main effects are significant, the direction of the effect is noted with T = +Ca treatment and C = Control. Year 5 is not included because not all species were collected in that year.*

As an example, patterns of chemical change are illustrated for Ca and N in maple and beech litter in Figure 3. (These two elements were chosen because Ca was the focus of the experiment and N is also known to influence litter decomposition dynamics.) In general, as decomposition proceeded, Ca concentrations increased for 2–3 years and then declined, whereas N concentrations increased and leveled off. For maple, Ca concentrations were initially much higher in litter from the +Ca source site, but by year 2, those differences disappeared and by year 6 litters incubated in the +Ca site were much higher in Ca than those incubated in the control site, regardless of source (Figure 3a). For beech, the Ca pattern was similar except the initial differences in concentration were much smaller (Figure 3c) and the litter placed in the +Ca site did not decline in concentration nearly as much as litter in the control site in the later years of the study. For N, initially the maple litter from the +Ca site had higher concentrations than litter from the control site, but by year 3, the differences had become non-significant or were inconsistent in direction from year to year (Figure 3b). The placement effect was generally non-significant except for a weakly significant effect in year 6. For N in the beech litter, a significant placement effect was evident in years 2, 4, and 6, with litter incubated in the +Ca site being higher in N (Figure 3d). There was also a significant source effect in years 4 and 6, but the direction is opposite in the 2 years, making it difficult to interpret.

Discussion

We hypothesized that the +Ca treatment would have little effect on the initial decomposition of the litter, and that hypothesis was supported by the data. Neither mass loss in the first year (ML1) nor the derived k_A value showed a significant effect of the treatment as reflected in either the source of the litter or the placement of the decomposition bags.

We also hypothesized that the high-Ca litter from the +Ca treatment would have a greater limit value, making the litter decomposition more complete after 6 years. Our data did not support this hypothesis, in that we found no significant effects of litter source on the limit value or mass loss after 6 y (ML6). However, we did discover that litter placed in the +Ca site, regardless of source, had higher limit value and ML6 than litter placed in the control site. Considering species individually, the effect was highly significant for beech litter and

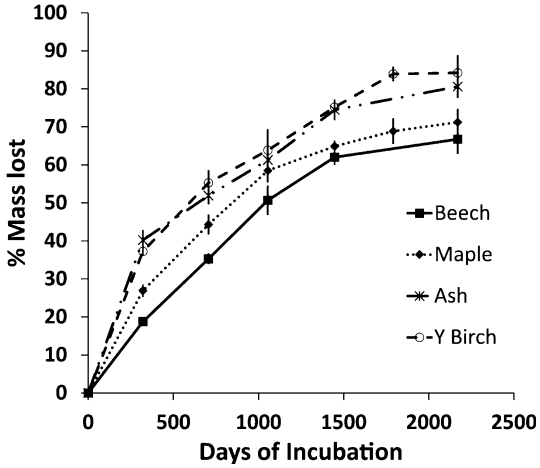

Figure 1. Cumulative mass loss for control site litter of the four species, placed at the control incubation site. *Vertical bars* represent plus or minus 1 standard error of the mean. There were highly significant ($P < 0.0001$) species effects on cumulative mass loss at all collection times except time 0.

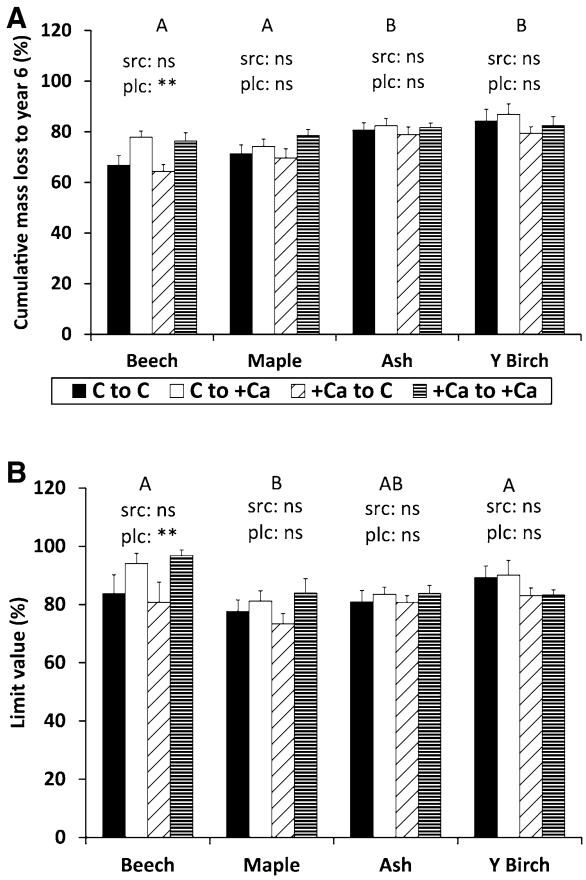

Figure 2. Mean cumulative mass loss in year 6 (**A**) and limit value (**B**) calculated for each species and treatment group. *Error bars* represent 1 standard error. *Upper-case letters* denote significant differences among species; species sharing the *same letter* are not significantly different. Legend format: ''litter source'' to ''placement,'' that is, ''C to +Ca'' indicates litter from the control site placed in the +Ca site. *Notations* above each cluster of *bars* are the significance of the main effects source (*src*) and placement (*plc*) in the ANOVA for that species: ns $P > 0.05$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

marginally significant for ML6 in maple litter. This indicates that $CaSiO_3$ addition can indeed affect the late-stage decomposition of foliar litter, and that this effect is species specific.

The reason that there is a placement effect but no litter source effect on litter decomposition is obvious from the chemical dynamics of the litter. Although there was initially a strong source effect on litter Ca concentration, over time that effect faded and the litter Ca took on the characteristics of the site in which it was placed (Figure 3). Thus, site chemistry matters more than initial litter chemistry in determining the late-stage litter decomposition rates.

The mechanism by which the $CaSiO_3$ addition affected the late-stage decomposition is not clear. Several chemical characteristics of the forest floor changed in concert after the addition of the wollastonite, including a greater than 3-fold increase in exchangeable Ca^{2+} and a 30% decline in exchangeable acidity ($H^+ + Al^{3+}$) (Johnson and others 2014). This experiment cannot distinguish among the effects of Ca, acidity, or some other chemical change in the soil. However, a direct effect of Ca on the extent of litter decomposition has been postulated by Berg and McClaugherty (2014), who note that Ca is an important constituent of fungal cell walls and that lack of Ca may limit fungal biomass formation, particularly for the white rot fungi that are capable of degrading lignin. There were no earthworms at these sites, so the effects of Ca on litter decomposition were not mediated by earthworm abundance as has been shown at other sites (Hobbie and others 2006; Melvin and Goodale 2013). Further, the greater extent of decomposition occurred despite a reduction in microbial biomass N and microbial functions such as N mineralization and nitrification in the forest floor of the treated watershed (Groffman and others 2006).

We found that addition of Ca changed not only Ca concentration of litter, but other constituents of the litter as well. In particular, N was increased in the litter from the +Ca treatment, consistent with the reported increase in N concentration in green foliage from this site (Green and others 2013). Although N can also influence the limit value of decomposition (Berg and McClaugherty 2014), we do not believe that N caused the patterns we attribute to Ca, for two reasons. First, the limit value and ML6 showed a placement effect rather than a

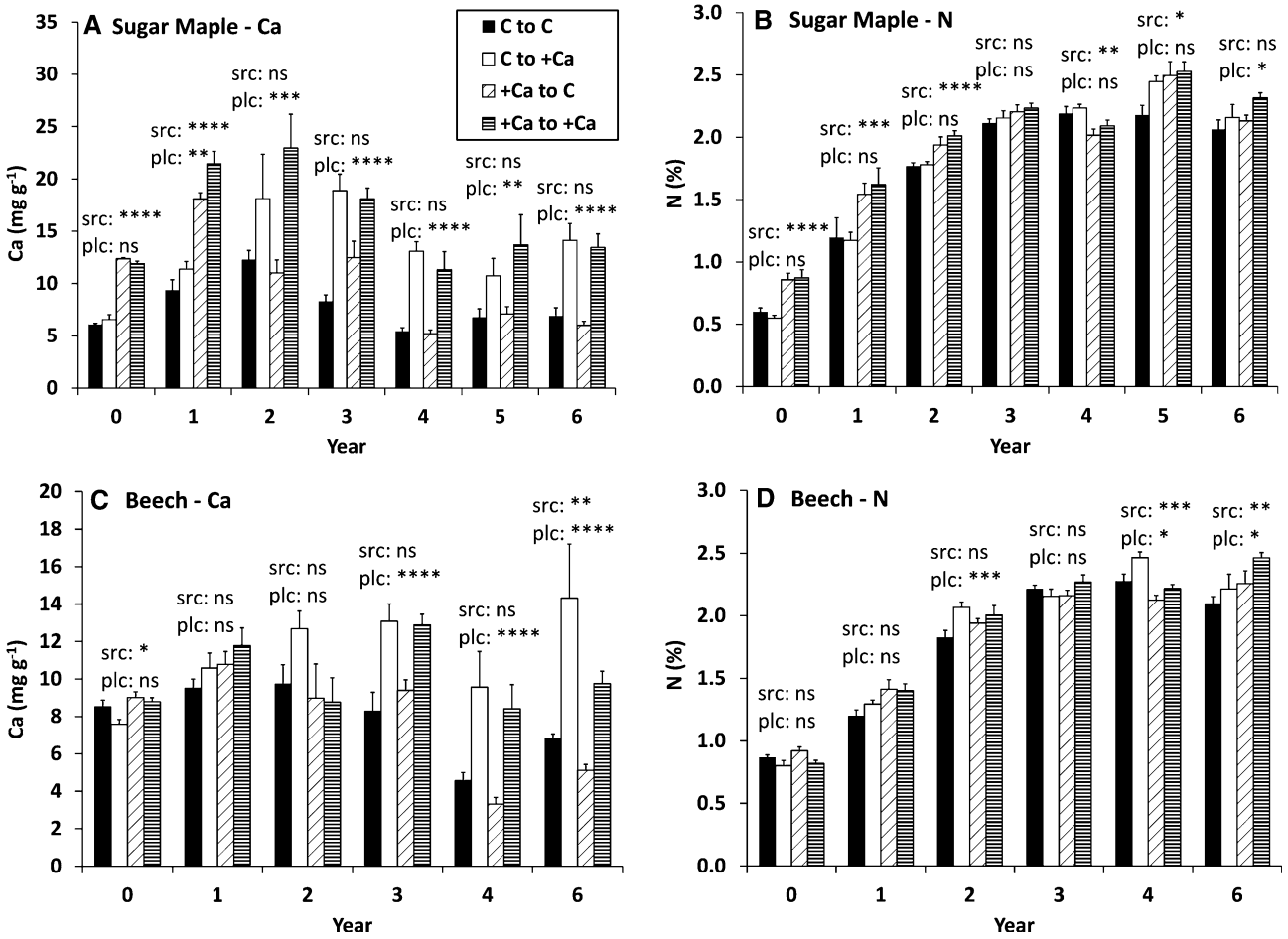

Figure 3. Trends in concentrations of maple litter Ca (**A**), maple litter N (**B**), beech litter Ca (**C**), and beech litter N (**D**). *Error bars* represent 1 standard error. Legend format as in Figure 2. *Notations* above each cluster of *bars* are the significance of the main effects source (*src*) and placement (*plc*) in the ANOVA for that time period: ns $P > 0.05$, *P < 0.05, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

litter source effect, and placement effects on N concentration in late-stage beech litter were very weak compared to placement effects on Ca (Figure 3). Second, +Ca site placement increased beech litter N concentration, which should have decreased the limit value, but instead we found the opposite response, that is, beech limit value increased in the +Ca site. Thus, the effects of site Ca on late-stage decomposition that we observed for beech are unlikely to have been caused by N, but the higher initial N in litter from the +Ca site may have reduced late-stage decomposition and obscured some effects of initial litter Ca on the extent of decomposition. It is also possible that some of the other species may have shown increases in limit value in the +Ca site were it not for the confounding effect of higher N concentration of the litter in that site.

Beech litter had the lowest overall mass loss in the control treatments, and it had the greatest increase in limit value and ML6 in the +Ca site relative to the control. Maple had the second-lowest overall mass loss, and maple limit value and ML6 were marginally sensitive to placement in the +Ca site. Birch and ash had greater overall mass loss and the effects of Ca on the limit value and ML6 for those species were not statistically significant. This suggests the hypothesis that site Ca effects on extent of litter decomposition may be most strongly manifested in litters that are slow to decay or have low intrinsic limit values. In contrast, the opposite hypothesis is suggested by the results of De Santo and others (2009), who found that Ca concentration was positively correlated with late-stage litter decomposition in broadleaf litters but not coniferous needles, which were higher in lignin and slower to decompose. More experimental data on a greater range of species would be necessary to test these hypotheses.

If addition of Ca increases the extent of decomposition, as observed in this study, the likely result

would be a reduced forest floor mass. Indeed, forest floor mass at this site declined by 18% and forest floor C pool by 28% in the first 11 years after the wollastonite addition, compared to the pre-treatment levels (Johnson and others 2014). This suggests that acid deposition, which can strip Ca from forest soils (for example, Likens and others 1998), can reduce the extent of litter decomposition and increase forest floor mass, at least for some litter types. The relationship between acidic deposition and humus accumulation was postulated over 30 years ago by Bernhard Ulrich and colleagues in Germany (Ulrich 1983). This mechanism provides a link between air pollution and soil C storage, and suggests that reduced Ca availability, which is a long-term legacy of acid deposition in many forests, may be affecting the sequestration of C in soil. This is consistent with the results of Oulehle and others (2011), who found that de-acidification of a forest in the Czech Republic due to reductions in airborne sulfur pollution was coincident with a decline in forest floor mass. On the other hand, Melvin and others (2013) observed the opposite effect in the Adirondack Mountains of New York, where liming of a forest greatly increased forest floor mass by slowing the decomposition of the humus layer. It is difficult to reconcile these studies; perhaps the very high dose of Ca provided by liming induces other effects, such as Ca-binding of organic matter, that overwhelm the effects on litter limit values. The mechanisms of these calcium responses, including both microbially mediated and purely physicochemical effects, clearly need further research.

The results of this study indicate that the Ca status of a site can affect the extent of decomposition of foliar litter, with higher Ca resulting in more complete decomposition, and that this effect may be most evident in litters with low limit values. This suggests that in acid-impacted forests in the Northeastern U.S., some decomposers may be limited by lack of Ca, and this limitation may result in a buildup of forest floor mass and carbon. Further, our results suggest that the ecosystem-level response of a forest to declines in emissions and deposition of acidifying substances will be influenced by the tree species composition of the forest.

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