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Calcium oxalate contribution to calcium cycling in forests of contrasting nutrient status

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ABSTRACT

Calcium oxalate (Ca oxalate) is an insoluble biomineral that forms in plants and fungi, and occurs in soils across many types of ecosystems. Assessing how Ca oxalate may shape ecosystem Ca cycling requires information on the distribution of Ca oxalate among plant biomass, detritus, and mineral soil, and how it varies with ecosystem Ca status. We compared two Douglas-fir forests of contrasting ecosystem Ca availability, and found that Ca oxalate was partitioned similarly among plant biomass, detritus and mineral soil major ecosystem compartments at both sites, and total pools of Ca oxalate were greater in the high-Ca forest. However, the proportional importance of Ca oxalate was greater in the low-Ca than high-Ca forest (18% versus 4% of actively cycling ecosystem Ca, respectively). And calcium oxalate in mineral soil, which is of particular interest as a potential long-term Ca reservoir, was a larger portion of total available Ca (exchangeable Ca plus Ca oxalate Ca) in the low-Ca site than the high-Ca site (9% versus 1% of available soil Ca, respectively). Calcium oxalate was the dominant form of Ca returned from plants to soil as leaf litterfall at the high-Ca site, yet calcium oxalate disappeared rapidly from decomposing litter ($0.28 \, yr^{-1}$ or faster) at both sites. We conclude that accumulation of Ca oxalate in forest ecosystems appears most closely related to overall Ca supply for live biomass pools, and that the accumulation of Ca oxalate in forest floor and mineral soil is limited by rapid microbial degradation of putatively unavailable Ca oxalate.

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1. Introduction

Calcium (Ca) is an essential macronutrient that is increasingly recognized as a biogeochemical factor that influences ecosystem structure and function (Schaberg et al., 2001; Lautner and Fromm, 2010). In some forests Ca from mineral weathering may be insufficient for plant growth particularly on soils that are highly weathered (Cuevas and Medina, 1988; Bockheim and Langley-Turnbaugh, 1997), subject to chronic acid rain or nitrogen deposition (Driscoll et al., 2001; Bigelow and Canham, 2007; Liu et al., 2011), or in stands undergoing sequential whole-tree harvests (Perakis et al., 2006; Siemion et al., 2011). Progress in understanding the sustainability of ecosystem Ca supply has been hampered by a lack of information on the various forms and pools of Ca in forest ecosystems. For example, while there has been

considerable interest and methodological development in understanding how various chemical forms of other macronutrients such as nitrogen and phosphorous are distributed in plants and soils, comparable approaches for investigating Ca forms and partitioning in plant–soil systems are poorly developed (Sparks, 1996). In fact, nearly all ecosystem and nutrition studies measure plant tissues as bulk-Ca and in forest floor and soils via exchangeable Ca (Mead, 1984; Sparks, 1996), which makes it difficult to identify specific biogeochemical and physiological processes that influence Ca dynamics.

Calcium-oxalate (Ca oxalate) is a biomineral form of Ca that may play an important ecosystem role, but its contribution to pools of Ca in forests is poorly understood. Calcium oxalate is created by nearly all plants (Franceschi and Nakata, 2005) and by both mycorrhizal and saprophitic fungi (Cromack et al., 1979; Arnott, 1995; Dutton and Evans, 1996). Both plants and fungi internally create Ca oxalate crystals to sequester Ca away from the cytoplasm, where ionic Ca is kept in micromolar concentrations (Arnott, 1995; Franceschi and Nakata, 2005). Plants and fungi also release oxalic acids into soil or detritus to regulate pH and

cation–anion balance of their external microenvironment (Jellison et al., 1997) or to enhance mineral weathering or decomposition for nutrient uptake, especially phosphorous (Ryan et al., 2001; Arvieu et al., 2003). Oxalic acid can subsequently bind to free soil Ca ions forming highly insoluble Ca oxalate crystals (K_{sp} 2.57×10^{-9}; Ringbom, 1963), whereas other metal-oxalate complexes are more soluble (Baran and Monje, 2008; Echigo and Kimata, 2011). Concentrations of crystalline Ca oxalate are rarely quantified in soil, yet Ca oxalate is often present where examined (Graustein et al., 1977; Cromack et al., 1979), particularly associated with decomposing leaf litter (Arnott, 1982; Horner et al., 1995), ectomycorrhizal fungal mats (Cromack et al., 1979; Entry et al., 1991) and lichen (Fujii et al., 2013). Despite progress in identifying mechanisms and controls on Ca oxalate formation in plants (Franceschi and Nakata, 2005), understanding the significance of Ca oxalate occurrence at the ecosystem-scale requires more detailed information on Ca oxalate contribution to vegetation, detritus and mineral soil Ca pools.

Ca oxalate creation in plant tissue and soil, and the subsequent longevity of insoluble Ca oxalate crystals, may influence both short- and long-term Ca supply and biotic recycling in ecosystems. In plants, once Ca^{2+} is deposited in Ca oxalate crystals it becomes unavailable for other physiological processes unless the Ca supply is completely truncated (Franceschi, 1989; Volk et al., 2002). Because general Ca mobility in plant tissue is low, the sequestration of available Ca^{2+} in Ca oxalate crystals may become a limiting process for other plant functions involving Ca such as cell wall formation or cell signaling. As leaves age, Ca oxalate accumulates (Borer et al., 2004; Littke and Zabowski, 2007; Smith et al., 2009). If highly insoluble Ca oxalate from leaf litter, roots and fungi accumulates in soil and then resists degradation, it could potentially reduce soil Ca availability overall for processes such as biotic uptake. The rate of Ca oxalate degradation in soil is unknown; some authors suggest that Ca oxalate is a "slow-release" form of Ca that is retained in soils and an important long-term source in regions where ecosystem Ca inputs are relatively low (Bailey et al., 2003; Smith et al., 2009), while others indicate that Ca oxalate may turnover quickly in leaf litter microcosms (O'Connell et al., 1983). Information on rates of Ca oxalate delivery from plants to soils via leaf litter, on the longevity of Ca oxalate in decaying detritus, and on how these fluxes may vary with site Ca status, are significant unknowns in resolving the importance of Ca oxalate to ecosystem Ca dynamics overall.

Douglas-fir forests of the Oregon Coast Range exhibit wide variation in plant and soil Ca availability due to high nitrogen from symbiotic N fixation and associated Ca loss (Perakis et al., 2006) with Ca limiting growth in some stands (Mainwaring et al., 2014) and provide an opportunity to examine how forest Ca status influences Ca oxalate partitioning and dynamics. The objectives of this study were: (1) to quantify the portion of Ca oxalate in major ecosystem compartments of living biomass, detritus and mineral soil at high and low Ca sites, (2) to examine which factors explain Ca oxalate concentrations across ecosystem pools, and (3) to determine the potential role of foliar Ca oxalate in shaping subsequent Ca dynamics during leaf litter decomposition.

2. Methods

2.1. Study sites

We estimated Ca oxalate in major ecosystem compartments of living biomass, detritus and mineral soil, and sub-compartments (smaller parts of the major compartment e.g., branches, foliage, bark, roots, bole-wood, forest floor, fine woody debris, coarse woody debris, soil at different depth increments) at two sites of contrasting Ca status in the north-central Oregon Coast Range. The two sites are part of a larger study of Ca depletion along a soil nitrogen gradient (Perakis et al., 2006, 2013; Perakis and Sinkhorn, 2011). The high-Ca site (site 5) is located at N44°38′W123°48′; the low-Ca site (site 16) is located at N45°10′W123°55′. Soils at both sites are classified as Andic Dystrudepts, and are derived from Tyee and Yamhill formations, respectively, which are chemically similar and differentiated by the thickness of interbedded sandstone layers. The geology of these sites is described in more detail in Perakis et al. (2013). Both sites experience maritime, temperate climates with cool, wet winters and warm, dry summers. Prior to the current stands, both sites were mixed conifer stands with red alder present. The vegetation at these two sites is dominated by Douglas-fir (*Pseudotsuga menziesii*) planted in 1977 at the high-Ca site and 1980 at the low-Ca site (Table 1). During planting, the sites were clear-cut, broadcast burned, and an initial herbicide treatment was applied to control vegetation competing with Douglas-fir seedlings. There has been no fertilization at the two sites. The two sites have been well documented as having significant differences in Ca cycling, loss, biotic retention and limitation concomitant with differences in soil N content, providing a context to compare site differences in Ca oxalate (Perakis et al., 2006, 2013; Mainwaring et al. 2014).

2.2. Tree tissue sampling and biomass estimation

We collected samples for total Ca and Ca oxalate in live Douglas-fir from previously established 0.5 ha plots at the high and low-Ca sites. Sun-exposed foliage was composited by site from three trees at the end of the growing season (September and October 2007), and separated into age cohorts (5 age cohorts at the high-Ca site and 4 age cohorts at the low-Ca site). Branch samples were collected in 2007 from three trees at each site. Tree cores were collected in May 2010 from three trees at each site using an increment borer, discarding any heart-wood, and separating the remainder into bark and bole-wood tissues. Four additional bark samples were taken with a 2 cm diameter corer. Samples were composited into one bark and one bole-wood sample per site. Root samples were collected in June 2010 by taking a composite of three 6.7 cm diameter soil cores to 10 cm depth, followed by sieving and water washing to isolate fine root samples of <2 mm

Table 1
Site characteristics.

Site	Soil exchangeable Ca 0–10 cm depth	Soil exchangeable Ca 0–100 cm depth	Soil pH (H$_2$O) 0–10 cm depth	Soil texture (%) 0–10 cm depth			Ca in aboveground plant biomass	Tree age	Total aboveground biomass	ANPP
	kg ha^{-1}	kg ha^{-1}		Sand	Silt	Clay	kg ha^{-1}	years	mg ha^{-1}	mg ha^{-1} yr^{-ha}
High-Ca (5)	853	5680	5.50	53	23	25	172	30	163	21.5
Low-Ca (16)	93	280	4.61	30	31	39	98	27	122	14.3

Characteristics of two sites in the Coast Range of Oregon including soil exchangeable Ca pools to 100 cm depth, soil pH and texture in surface soils, Ca in plant biomass, tree age in 2007 and total aboveground biomass. ANPP calculated as the net annual aboveground in stems, branches, foliage mass plus litterfall (Perakis and Sinkhorn, 2011).

and 2–10 mm. Leaves, bark and bole-wood samples were flash frozen in an −80 °C cooler and subsequently freeze-dried (Virtis 35L Genesis Super XL, SP Industries, Warminster, PA, USA). Branches and roots were dried at 65 °C for 48 h. All samples were ground to fine powder prior to chemical analysis. Biomass pool sizes were estimated using allometric equations determined from these sites (Maguire and Hann, 1990; Maguire et al., 2002; Weiskittel et al., 2010). The root biomass of <2 mm and 2–10 mm diameter fine roots was determined at both sites from 0 to 10 cm deep soil cores in June 2006. To calculate root density to 80 cm depth, we assumed root density measured in our 0–10 cm cores attenuated at the same rate as in Dauer et al. (2007) in 10–20 cm depths, and used estimates of <2 mm and 2–10 mm root biomass for the 20–80 cm depth determined from 16 cores at a nearby coastal Douglas-fir site (Lee et al., 2007).

2.3. Detrital sampling and biomass estimation

We measured Ca oxalate in detrital pools as fine and coarse woody debris and forest floor. Forest floor and fine (1–10 cm diameter) and coarse (>10 cm diameter) woody detritus were collected from five 16 m^2 subplots at each site. Each forest floor sample was collected using a 30 × 30 cm template. Fine downed wood was collected from a 1 m^2 subplot in each of the five main woody debris subplots and separated into decay classes for each site (Waddell, 2002). All coarse woody debris, both downed wood and stumps, in the subplot were measured for height and width (cm) and a representative tissue sample was collected. Ten litter traps (1.4 m^2 each) were placed in each plot in June 2010, during the summer dry season, and collected litter prior to the onset of fall rains in September 2010. Needle litter was separate from twigs and other debris for chemical measurement, and converted to fluxes based on 2004–2006 monthly measurements (Perakis and Sinkhorn, 2011). All detritus samples were dried at 65 °C for 48 h prior to weighing, grinding, and chemical analysis. Forest floor samples were also corrected for weight of mineral contamination using a measurement of dry ash content, and corrected for Ca concentration of contaminating mineral soil with a sample of 0–3 cm mineral soil collected directly beneath each forest floor sample. Coarse downed wood biomass was estimated by calculating each sample's volume with Huber's formula (Husch et al., 1972), corrected for density using the mean moisture content measured on the fine downed wood and published data on Douglas-fir specific gravity (Forest Products Laboratory, 2010), multiplied by a softwoods decay class reduction factor (Waddell, 2002).

2.4. Sequential extraction of Ca

For biomass and detritus samples we used a sequential extraction technique to separate Ca into soluble Ca^{2+} ions (Ca_{aq}), structurally bound Ca in Ca–pectin and Ca–lignin (Ca_{st}) and insoluble Ca originating in Ca oxalate (Ca_{ox}) as used in previous studies (Fink, 1991; Borer et al., 2004; Littke and Zabowski, 2007). Briefly, ground tissues (5 g subsamples) were sequentially extracted for Ca using three 10 mL solutions of nanopure water (Ca_{aq}), 2 N acetic acid (Ca_{st}), and 2 N hydrochloric acid (Ca_{ox}). Each extraction was centrifuged for 8 min and 11,000 rpm, the supernatant reserved for analysis, and the remaining solid rinsed with 40 mL nanopure water and dried at 60 °C prior to extraction of the next sequential fraction. This sequential extraction method, developed for biomass, was also applied to determine Ca fractions in forest floor and 0–3 cm depth mineral soil (see above). The % error across replicate samples was less than 10% for all sample materials that were extracted using this method. For total Ca concentrations (Ca_t), 2 mg of dried tissue or forest floor were weighed into a glass scintillation vial and burned for 12 h at 500 °C, digested with 12 N HCl,

diluted to 2 N HCl with nanopure water, and measured for calcium on an Perkin Elmer AAnalyst 200 atomic absorption spectrometer (Waltham, MA, USA). The sum of the three extractions (Ca_{sum}) was compared to Ca_t to determine the completeness of the Ca extraction.

2.5. Ca oxalate in mineral soil

We estimated Ca_{ox} concentrations (amount of Ca as Ca oxalate crystals) in 0–100 cm depth soil by measuring acid extractable oxalate anion concentrations and converting these to Ca_{ox} and to moles of Ca oxalate using stoichiometric relationships. Mineral soil samples were collected in summer 2005 in depth increments of 0–10 cm, 10–20 cm, 20–30 cm and 70–100 cm from three locations per site and sieved to 2 mm. Lacking a standard method for Ca oxalate determination in mineral soil, we selected a conservative oxalate extraction to reduce the possibility of release of mineral Ca that was based on relatively dilute HCl and short extraction times, and corrected for spike-recovery of Ca oxalate addition to each sample (see Dauer and Perakis, 2013; Dauer, 2012 for more discussion of measuring Ca oxalate in soils). This approach assumes that the oxalate measured by dilute acid extraction was bound primarily to Ca in Ca oxalate crystals, as previously reported in similar soils (Cromack et al., 1979). Soil samples of 5 g were extracted with 0.5 M HCl for 5 h (Lilieholm et al., 1992). Duplicate samples were spiked with 10 mg of monohydrate Ca oxalate and extracted as above. Spike recovery ranged from 33% to 61% on individual samples, and was used to correct for non-extracted Ca oxalate. Low recovery rates may be due to the deliberately low acidity and short time of the extraction. Standard error of duplicate samples for each depth (0–10, 10–20, 20–30 and 70–100 cm, respectively) in the high-Ca site was 3%, 3%, 8%, 2% and in the low-Ca site was 7%, 19%, 7% and 3%. Oxalate ions were measured by Dionex Ion Chromatography system with a carbonate–bicarbonate eluent with the ASRS-4 mm suppressor and AS4-A analytical column (4 × 250 mm) with AG4-A guard column (4 × 250 mm). The concentration of the Ca_{ox} soil pool was calculated using the stoichiometric relationship between oxalate and Ca in anhydrous Ca oxalate. We estimated concentrations for 30–50 and 50–70 cm depths by interpolation (Dauer, 2012), and used estimates of Ca_{exch} and soil bulk density determined in prior work at these sites (Perakis and Sinkhorn, 2011; Perakis et al., 2013).

2.6. Ca oxalate in decomposing leaf litter

To examine whether Ca oxalate in the forest floor is selectively concentrated or lost from bulk Ca during litter decomposition, we used a two-year leaf litter decomposition litter-bag study with Douglas-fir needles with varying Ca status, one high-Ca (14.2 mg Ca per g^{-1} dry weight of plant material) and one low-Ca (7.2 mg Ca per g^{-1} dry weight of plant material, Perakis et al., 2012). The two source litters were collected from sites (high Ca: 45.5261°N, 123.3841°W, low-Ca: southeast of Toledo, OR approximately 44.5977°N, 123.8873°W) that reflect the high and low range of landscape variation in nitrogen status, and hence Ca status, across the Oregon Coast Range (Perakis et al., 2006, 2013). The two source litters were decomposed at four Douglas-fir dominated study sites in the Oregon Coast Range for two years (as described by Perakis et al. (2012)); the four sites were used as experimental replication. Source needles and the two-year collection in November 2005 were used for this study, with ground samples analyzed for Ca_{ox} by sequential extraction, as above. Subsamples of dried whole needles were also measured for pH by combining needles with nanopure water (1_{needle}:10_{water}, by weight) for 10 m, followed by measurement with a pH probe (Fisher Scientific Accumet AR20 meter). Images of unground and

finely sliced open initial and decomposed needles were imaged on a Philips CM-12 scanning transmission electron microscope (STEM). To confirm the presence of Ca in crystals, chemical composition analysis was performed using X-ray Energy Dispersive Spectrophotometry.

2.7. Data analysis

Both Ca_{ox} and Ca_t (total Ca, kg Ca ha^{-1}) concentrations in tree biomass and detritus were converted to pools by multiplying against biomass estimates. Soil pools of Ca_{exch} (Perakis et al., 2013) and Ca_{ox} were estimated by multiplying concentrations with soil bulk density for each sampling depth for the two sites. We also report Ca_s as the sum of mineral soil Ca_{ox} and Ca_{exch} (Cromack et al., 1979). The proportional contribution of Ca_{ox} to actively cycling pools of Ca (i.e. percent of Ca_t existing as Ca_{ox}) was estimated for each compartment and sub-compartment, for biomass and detritus based on Ca_t, and for mineral soil based on Ca_s. The Ca_{ox}-proportion contained in total biomass, detritus, and mineral soil compartments of each site was calculated as the summed weighted average of Ca_{ox}-proportion contained in each individual sub-compartment. For example, for live biomass:

$100 * \Sigma_{\text{foliage, branches, bole-wood, bark, <2mm roots, 2-10mm roots}}$

Ca_{ox} (kg Ca ha^{-1})/$\Sigma_{\text{foliage, branches, bole-wood, bark, <2mm roots, 2-10mm roots}}$

Ca_t (kg Ca ha^{-1}).

Due to the time-intensive nature of sequential extractions for Ca, replicate samples from each site were not measured, so no statistical analysis was performed. We used a linear regression to examine the relationship between Ca_t concentrations (mg Ca g^{-1}) and Ca_{ox} concentrations (mg Ca g^{-1}) of ecosystem pools across the two sites within biomass and soil compartments, and between Ca_s concentration (µg g^{-1}) and Ca_{ox} concentration (µg g^{-1}) across the two sites' mineral soil depths. Additionally, the mean residence time (MRT, in years) of Ca_t and Ca_{ox} in leaf litter calculated as forest floor pool kg Ca ha^{-1}/leaf litter input kg Ca ha^{-1} yr^{-1}.

Leaf litter (1) mass loss percentage and (2) Ca loss percentage were estimated with the following equations:

(1) Mass loss (%) = $100 * (M_0 - M_2)/M_0$
(2) Ca loss (%) = $100 * (Ca_0 - (Ca_2 * M_2/M_0))/Ca_0$

where M = litterbag dry weight mass, Ca = Ca concentration, subscripts denote time of sampling in years. A nutrient release rate was determined for Ca_{ox} using a single exponential model:

$\ln(Ca_2/Ca_0) = k * t$

where Ca = Ca concentration, subscripts denote time of sampling in years, k = release rate per year, t = time in years (Olson, 1963; Cuevas and Medina, 1988) and averaged within litter source types. We chose a single-exponential model as it best fits decomposition of the Douglas-fir needles used in this study (Perakis et al., 2012). This approach assumes consistent exponential decomposition behavior over time, with mass loss approaching zero as time progresses, as has been observed for Ca (Rustad, 1994). The exponential model allowed us to calculate the time required to lose 95% of the Ca_{ox} ($3/k$) (Gosz et al., 1973) assuming proportional loss of Ca_{ox} to be constant through the decay process.

In the leaf litter decomposition experiment, effects of source litter, decomposition stage, and sequentially extracted Ca pool on Ca concentrations were analyzed using a three-way analysis of variance (ANOVA), with decomposition sites as replicates ($n = 4$). Differences in percent loss of either Ca or mass by source litter or sequential extraction were determined with a two-way ANOVA. Models were fit to the data using PROC GLM in SAS v9.2 (SAS

Institute Inc., Cary, NC), and data were unbalanced due to a missing data point for Ca_t, so LSMEANS was used to estimate means and the differences between means. Statistical significance for all models was at the level of $\alpha = 0.05$.

3. Results

Total ecosystem Ca was 12-times larger at the high-Ca site (6285 kg Ca ha^{-1}) than in the low-Ca site (520 kg Ca ha^{-1}), whereas total ecosystem Ca_{ox} was 3-times larger at the high-Ca site (260 kg Ca ha^{-1}) than the low-Ca site (93 kg Ca ha^{-1}) (Fig. 1A and B). All measured sub-compartment pools of Ca_{ox} were larger at the high-Ca site, yet the relative partitioning of Ca_{ox} among major compartment pools of live biomass, detritus, and mineral soil was fairly comparable between the sites. Live biomass was the largest pool of Ca_{ox}, comprising ~40% of total ecosystem Ca_{ox} at both sites, with branches particularly important at the low-Ca site. Combined detrital pools contributed roughly one-third of total ecosystem Ca_{ox}, mostly in the forest floor and coarse size classes of woody debris. Mineral soil averaged roughly one-fourth of whole-ecosystem Ca_{ox} at both sites, with contributions concentrated in surface soils (Table 2). Leaf litterfall rates of Ca_{ox} were 5.0 kg Ca ha^{-1} yr^{-1} at the high Ca site, and 0.7 kg Ca ha^{-1} yr^{-1} at the low-Ca site (not shown), reflecting larger litterfall Ca fluxes and larger proportional Ca_{ox} contribution at high-Ca site (Table 2).

In general, Ca_{ox} concentrations increased with total Ca concentrations, both within major ecosystem compartments, and across
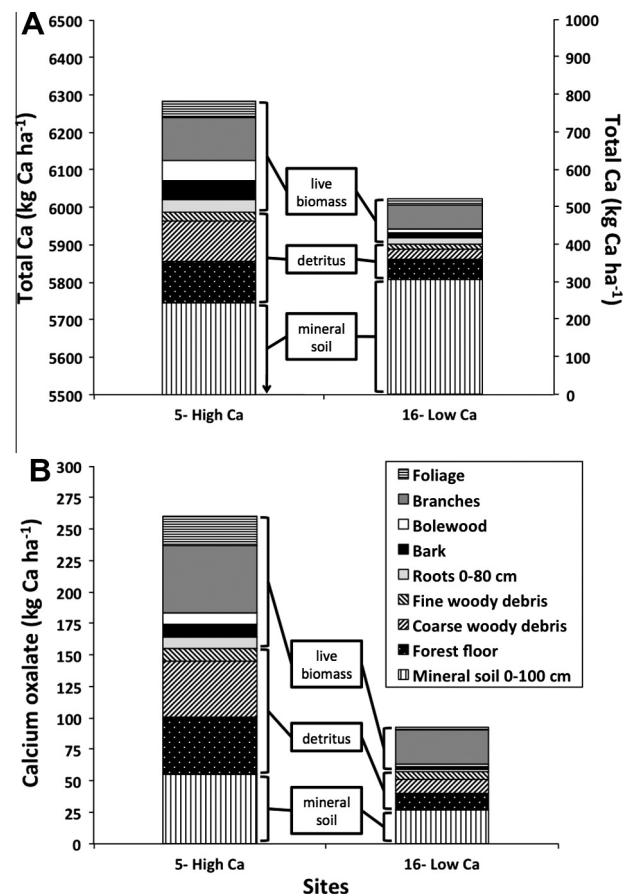
Fig. 1. (A) Ca content (kg Ca ha^{-1}) and (B) Ca_{ox} content (kg Ca ha^{-1}) in different ecosystem pools at a high Ca and low Ca site. Note the different starting-point of the scales for the high-Ca (left) and low-Ca (right) sites in (A). The total Ca in the mineral soil (0–100 cm) in the high Ca site makes up the vast majority (5745 kg Ca ha^{-1}) of ecosystem Ca, so the scale of (A) for the high-Ca site starts at 5500 in order to include more detail and easier comparison.

Table 2
Ecosystem Ca concentration and proportion Ca oxalate at two sites.

Ecosystem compartment	Ca concentration		Ca_{ox} concentration		Ca_{ox} proportion	
	High-Ca Site (5)	Low-Ca Site (16)	High-Ca Site (5)	Low-Ca Site (16)	High-Ca Site (5)	Low-Ca Site (16)
	Tissue Ca_t mg Ca g^{-1}		Tissue Ca_{ox} mg Ca g^{-1}		Tissues Ca_{ox} % of Ca_t	
Foliage 1 yr	3.2	1.5	1.4	0.1	43	10
Foliage 2 yr	7.1	2.3	3.0	0.4	42	17
Foliage 3 yr	8.5	2.9	4.1	0.9	48	30
Foliage 4 yr	11.5	2.7	6.4	0.8	56	30
Foliage 5 yr	12.9	n.a.	6.6	n.a.	52	n.a.
Branches	6.2	4.9	3.0	2.1	48	42
Bole-wood	0.3	0.2	0.0	0.0	16	15
Bark	1.5	1.6	0.3	0.3	20	16
Roots < 2 mm	2.2	1.4	0.5	0.2	20	16
Roots > 2 mm	4.3	1.6	1.5	0.3	35	16
Mean live biomass					34	29
Annual leaf litter	10.3	3.0	7.3	1.0	70	34
FWD decay 2	4.6	3.3	2.5	1.5	55	45
FWD decay 3	2.9	2.7	1.1	0.9	37	35
FWD decay 4	2.8	1.9	0.8	0.7	28	34
CWD decay 4	1.8	1.8	0.6	0.9	40	44
Forest floor	9.1	4.3	3.7	1.0	41	23
Mean detritus					41	25
	Soil Ca_{exch} µg Ca g^{-1}		Soil Ca_{ox} µg Ca g^{-1}		Soil Ca_{ox} % of Ca_s	
Soil 0–10 cm	2031	194	37.4	15.9	1.8	8.2
Soil 10–20 cm	1047	55	23.7	5.1	2.3	9.3
Soil 20–30 cm	767	29	9.0	1.8	1.2	6.0
Soil 30–50 cm	641	38	5.3	3.3	0.8	8.6
Soil 50–70 cm	570	38	3.5	2.6	0.6	6.9
Soil 70–100 cm	521	27	2.1	5.4	0.4	19.8
Mean mineral soil					1.0	8.9
Mean ecosystem total					4	18

sites. We found significant positive relationships between Ca_{ox} and Ca_t concentrations (mg Ca g^{-1}) of all live biomass sub-compartment pools (Ca_t mg Ca g^{-1} = 0.58 Ca_{ox} mg Ca g^{-1} – 0.62, R^2 = 0.98, $P < 0.001$) and of all detritus sub-compartment pools (Ca_t mg Ca g^{-1} = 0.53 Ca_{ox} mg Ca g^{-1} – 0.46, R^2 = 0.88, $P < 0.001$) across the two sites. When comparing sites, concentrations of total Ca and Ca_{ox} in sub-compartment pools were generally higher in the high-Ca site than the low-Ca site, with the exception of coarse woody debris and bark for total Ca, and coarse woody debris and 70–100 cm mineral soil for Ca_{ox} (Table 2). The highest concentration of Ca_{ox} in living biomass at the high-Ca site occurred in 4–5 year-old leaves, whereas branches contained the highest Ca_{ox} concentrations in living biomass at the low-Ca site. Bolewood displayed the lowest concentration of Ca_{ox} in living biomass at both sites. Leaf litter Ca_{ox} concentrations exceeded those of the oldest live leaf cohort at both sites. Concentrations of Ca_{ox} in fine woody debris generally decreased with increased stage of decay. There was also a significant positive relationship between Ca_{ox} and Ca_s concentrations (µg Ca g^{-1}) in all mineral soil depth increment pools excluding the interpolated data (Ca_s µg Ca g^{-1} = 0.16 Ca_{ox} µg Ca g^{-1} + 3.19, R^2 = 0.78, $P = 0.004$) across the two sites.

We estimated the potential importance of Ca_{ox} to ecosystem Ca pools as the proportion of Ca_{ox} in Ca_t in biomass and detritus, as the proportion of Ca_{ox} in Ca_s in mineral soil, and as the proportion of Ca_{ox} in the ecosystem overall (Table 2). At the ecosystem level, Ca_{ox} accounted for a higher proportion of total Ca in the low-Ca site (18%) than the high-Ca site (4%); this ecosystem-level pattern largely reflected the importance of Ca_{ox} to Ca_s in mineral soil at the low Ca site. In contrast, for combined detritus and forest floor pools, we found that Ca_{ox} contributed more to total Ca in the high-Ca site (41%) than the low-Ca site (25%). Within detritus pools the least decayed fine wood (<2 mm and 2–10 mm, decay class 2) contained the highest Ca_{ox} proportion at both sites. In addition, the proportion of leaf litter Ca flux as Ca_{ox} was twice as large at the

high-Ca site compared to the low-Ca site (Table 2). Living biomass had comparable proportional contributions of Ca_{ox} to total Ca in both sites. The MRT of Ca_{ox} was estimated as 9 and 17 years at the high- and low-Ca sites, respectively (for example: 45.6 kg Ca_{ox} ha^{-1}/5.1 kg Ca_{ox} ha^{-1} yr^{-1} = 9 yrs). In contrast, the MRT of Ca_t was estimated as 15 and 25 years at the high-and low-Ca sites, respectively.

STEM image analysis revealed the presence of Ca oxalate crystals in newly shed leaf litter of high-Ca concentration (Fig. 2A) but not in decomposed litter (Fig. 2B). Calcium oxalate crystals were not found in the low-Ca source un-decomposed litter, and no Ca oxalate crystal images were found in decomposed litter examined from any of the sites. X-ray Energy Dispersive Spectrophotometry was used to confirm the presence of Ca in the crystals in the image.

Sequential chemical fractionation of decomposing litter revealed significant differences in leaf chemical fractions (Ca_{aq}, Ca_{st}, Ca_{ox}) and total (Ca_{sum} and Ca_t) concentrations that varied with time between high and low-Ca source litter (source * time * pool interactive effects, F-value = 158.08, DF = 78, $P < 0.001$, three-way ANOVA, Table 3). In the high-Ca source litter, concentrations of Ca_{ox} decreased during decomposition ($P < 0.001$, Table 3), while Ca_{st} increased ($P < 0.001$), and Ca_t, Ca_{sum} and Ca_{aq} remained constant. In low-Ca source litter, Ca_{ox}, Ca_{aq}, and Ca_t concentrations remained constant during decomposition, but Ca_{sum} ($P = 0.011$) and Ca_{st} ($P = 0.026$) significantly increased (Table 3). The high-Ca litter had a mean initial pH of 5.32 (0.03 S.E.), which was not significantly different from decomposed litter pH of 5.24 (0.08 S.E.). The low-Ca litter had a mean initial pH of 5.48 (0.09 S.E.), which decreased in decomposed litter to a pH of 4.77 (0.10 S.E.; $P = 0.002$).

After two years of leaf litter decomposition, percent Ca loss differed significantly among Ca pools in a way that varied by Ca source litter ($F = 7.46$, DF = 5, pool * source interaction, two-way
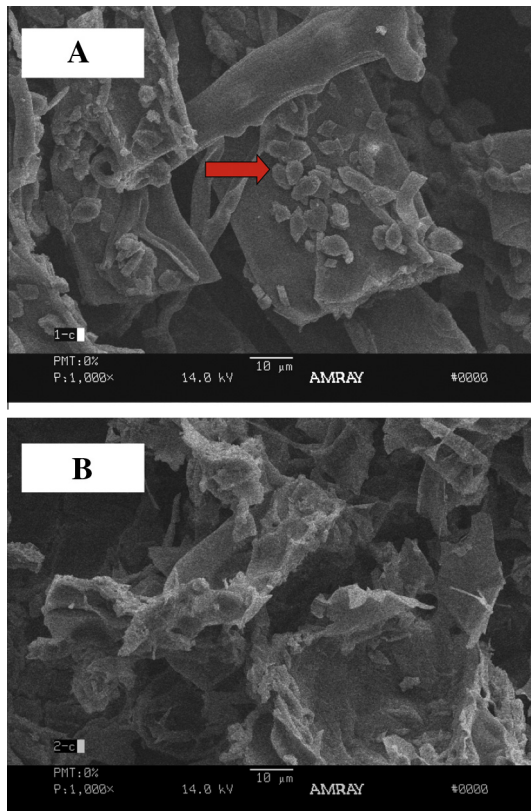
Fig. 2. High-Ca source litter (A) before decomposition and (B) after 2 years decomposition, imaged with STEM and magnified 1000 times. Calcium oxalate crystals are present in image (A) as indicated by arrow, and Ca content of the crystals in (A) were determined by X-ray Energy Dispersive Spectrophotometry.

ANOVA, $P < 0.001$, Table 3). Ca_{ox} had a higher percent loss than Ca_{st} in the low-Ca source litter and a higher percent loss than both Ca_{st} and Ca_{aq} in the high-Ca source litter (Table 3). Ca_{st} increased significantly (negative % loss) over two years in both source litters, but Ca_{st} percent loss did not differ from Ca_t or mass loss in low-Ca source litter only. The percent of Ca_t loss (43% in high-Ca source litter, 26% in low-Ca source litter) did not differ from mass loss (42% in high-Ca source litter, 38% in low-Ca source litter, Table 3). The rate of Ca_{ox} disappearance was 0.28 yr^{-1} and 0.04 yr^{-1} in high- and low-Ca source litter respectively; assuming the difference between initial and final source litters is representative of long-term behavior. From this Ca removal rate, we estimate a 95% disappearance of Ca_{ox} ($3/k$) after 15 years and 72 years for the high- and low-Ca source litters, respectively. The loss of Ca_{ox} in 2 years was equivalent to 2.0 (0.5 S.E.) mg Ca_{ox} g^{-1} yr^{-1} in the high-Ca source

litter, and 0.12 (0.11 S.E.) mg Ca_{ox} g^{-1} yr^{-1} in the low Ca source litter (for example: [10 mg Ca_{ox} g^{-1} initial minus 6 mg Ca_{ox} g^{-1} final]/ 2 years = 2 mg Ca_{ox} g^{-1} yr^{-1}). In contrast the loss of Ca_t in 2 years was equivalent to 0.2 (0.2 S.E.) mg Ca g^{-1} yr^{-1} in the high-Ca source litter, and due to Ca_t *accumulation* over time a *negative loss* of Ca_t in 2 years −0.7 (−12.3 S.E.) in the low-Ca source litter.

4. Discussion

4.1. Calcium oxalate ecosystem-level patterns

We know of no study that has evaluated ecosystem-level patterns of Ca oxalate accumulation in forests, although prior work has shown that Ca supply influences Ca oxalate accumulation in tree foliage (Borer et al., 2004; Littke and Zabowski, 2007). We found two lines of evidence to support the idea that high Ca levels promote Ca oxalate accumulation in ecosystems. First, we found that in the major ecosystem compartments living biomass, detritus, and mineral soil concentrations of Ca oxalate increased significantly with total Ca. Second, contrasts of low- versus high-Ca sites revealed that Ca oxalate pools were larger in the high-Ca site in both individual major compartments (e.g., living biomass, detritus, mineral soil) and their sum to whole-ecosystem pools (Fig. 1). However, the differences in ecosystem-level Ca oxalate pools we observed between sites was lower than expected given the large difference in total ecosystem Ca; the high-Ca site had 12-times more Ca, but only 3-times more Ca oxalate than the low-Ca site, suggesting that total soil Ca does not proportionally influence biologically-active pool sizes of Ca oxalate, and that biotic controls on Ca oxalate formation and decomposition dynamics may influence whole ecosystem Ca oxalate accumulation. These two sites are part of previous work that indicated significant differences in Ca cycling, loss, biotic retention and Ca limitation between these two sites (Perakis et al., 2006; Perakis and Sinkhorn, 2011; Mainwaring et al., 2014). The background context provided by these studies lends confidence in the trends we observed in this study without replication are most likely significant site differences in Ca status and Ca oxalate status.

4.2. Contribution of Ca oxalate to ecosystem Ca

The proportion of the total Ca that resides as Ca oxalate (Table 2) provides information on potential leverage that Ca oxalate exerts on ecosystem (or compartment and sub-compartment) Ca dynamics. At the ecosystem scale, Ca oxalate contributed 4–5 times as much to total Ca in the low- than high-Ca site (18% versus 4% Ca_{ox} proportion of Ca_t, Table 2). This difference was driven by the much smaller proportion of Ca oxalate in mineral soil Ca_s at the high-Ca site (1.0%) compared to the low-Ca site (8.9%), and reflects the

Table 3
Calcium dynamics in leaf litter decomposition Ca concentrations (mg g^{-1}) of Ca_t, Ca_{sum} and Ca fractions and mass (g) of initial and two-years decomposition of high-Ca and low-Ca Douglas-fir source litter at four sites in western Oregon. Values represent arithmetic means with standard errors in parentheses. Bold numbers represent significant differences between Ca (mg g^{-1}) initial and two-years decomposition (three-way ANOVA, $P < 0.001$). Percent loss of Ca and mass after two-years decomposition reported in italics. Net accumulation of Ca in Ca_{st} occurred, resulting in negative "loss" values. Letters in lower-case represent within-source litter differences in percent loss (two-way ANOVA, $P < 0.001$). Ca_{aq}, Ca_{st} and Ca_{ox} Ca concentrations were determined by sequential extraction on the same dried tissue, Ca_{sum} is the sum of those fractions, and Ca_t was determined independently by digestion of the whole tissue.

Ca fraction	High Ca source litter			Low Ca source litter		
	Initial mg Ca g^{-1} (g)	Two years mg Ca g^{-1} (g)	*Loss (%)*	Initial mg Ca g^{-1} (g)	Two years mg Ca g^{-1} (g)	*Loss (%)*
Ca_t	14.0 (0.1)	13.6 (0.4)	*43 (4) ac*	7.4 (0.2)	8.8 (0.5)	*26 (5) ab*
Ca_{sum}	12.5 (0.1)	13.0 (0.5)	*40 (4) ac*	**6.7 (0.1)**	**8.8 (0.2)**	*18 (5) ab*
Ca_{aq}	0.9 (0.0)	1.5 (0.1)	*4 (10) a*	0.7 (0.1)	1.1 (0.3)	*6 (20) ab*
Ca_{st}	**1.6 (0.0)**	**5.9 (1.1)**	*−102 (27) b*	**2.4 (0.0)**	**4.4 (0.4)**	*−13 (10) a*
Ca_{ox}	**10.0 (0.1)**	**6.0 (1.2)**	*66 (5) c*	3.6 (0.1)	3.3 (0.2)	*42 (4) b*
Mass	4.5 (0.0)	2.6 (0.1)	*42 (3) ac*	4.5 (0.0)	2.8 (0.1)	*38 (2) ab*

greater importance of mineral soil to total ecosystem Ca in the low-Ca site. The greater proportion of Ca_{ox} relative to Ca_s in mineral soils at lower Ca sites was also observed more broadly across a Ca gradient of nine sites that includes the two sites reported here (Dauer, 2012). Assuming similar Ca oxalate dynamics among ecosystems pools between high versus low-Ca sites, a greater proportion of total ecosystem Ca may be cycled through Ca oxalate pools in low-Ca sites, suggesting that Ca oxalate is more important in sustaining Ca supplies in forests where Ca is relatively scarce.

In contrast to mineral soil, we found a larger proportion of total ecosystem Ca oxalate in biomass and detritus at high-Ca sites than low-Ca sites, indicating that Ca oxalate exerts greater leverage in shaping ecosystem Ca dynamics via biomass and detritus pools in where Ca is abundant. However, low-Ca sites may be more reliant on Ca oxalate in biomass and detritus for Ca returns via mineralization pathways. Forests in this region are thought to conserve ecosystem Ca in low Ca-sites by retaining a larger proportion of total available ecosystem Ca in living biomass (Perakis et al., 2013), so low-Ca sites are more dependent on Ca returns from decomposing leaf litter and woody tissue. Therefore, Ca oxalate decomposition dynamics are important for either restricting or amplifying leaching loss and providing a pool of Ca for tree growth, depending on the turnover rates of Ca oxalate in relation to other forms of Ca. Consequently, we infer that sites with low-Ca may be more reliant on Ca oxalate as a potential to buffer short-term Ca loss from mineral soil and provide a long-term pool of stored Ca for tree growth. However, a low absolute quantity of Ca oxalate in low-Ca sites limits the ability of this pool to buffer ecosystem Ca depletion overall.

In living biomass the proportional contribution of Ca oxalate to total Ca was somewhat equitable between sites, with slightly more Ca oxalate contribution to total Ca in high-Ca sites. Ca_{ox} accounted for 34% mass-weighted proportion of Ca_t (up to 56% in sub-compartments) in the high-Ca sites, compared to 29% Ca_{ox} mass-weighted proportion of Ca_t in the low-Ca site (up to 42% in sub-compartments, Table 2), although these calculations are strongly influenced by wood which was the largest live biomass pool (69% of the live biomass at the high-Ca site and 50% of the live biomass at the low-Ca site).

Calcium oxalate was also a substantial portion of total Ca (Ca_t) in detritus. The proportion of Ca_{ox} in Ca_t within the forest floor was 41% at the high-Ca site and 23% at the low-Ca site. In woody debris across size classes, Ca oxalate made up from 23% to 55% of Ca_t (Table 2). Additionally, leaf litterfall can account for a sizeable input of Ca oxalate; while the overall leaf litterfall biomass is comparable between sites (0.69 mg ha^{-1} yr^{-1} high Ca site; 0.71 mg ha^{-1} yr^{-1}, low Ca site), the majority of the Ca in leaf litter flux is in the form of Ca oxalate at the high-Ca site (70% of Ca_t), compared to about a third of the total Ca in leaf litter flux at the low-Ca site (34% of Ca_t). Calcium recycled through litterfall and forest floor is thought to be a dominant source of Ca for plant uptake (Blum et al., 2008; Fahey and Blum, 2011) and our data further suggest that Ca oxalate may be a significant yet underappreciated component of this flux.

Despite the relatively small proportional contribution of Ca oxalate to ecosystem total Ca (4–18%), the amount of Ca oxalate we measured in mineral soil (27–55 kg Ca ha^{-1}) could be significant in terms of plant needs or export from many regions. For example, at the high and low-Ca sites in our study, the annual uptake of Ca was estimated to be 5.7 and 3.6 kg Ca ha^{-1} respectively (Perakis et al., 2013), stream export was estimated to be 1.5–2.6 kg Ca ha yr^{-1} in a northeastern hardwood forest (Bailey et al., 1996), and supply of Ca from mineral soil was estimated to be 33–47 kg Ca ha^{-1} yr^{-1} at Hubbard Brook Experimental Forest (Hamburg et al., 2003). The larger proportion of mineral soil Ca as Ca oxalate in the low-Ca site may also contribute to ecosystem

Ca conservation depending on the dynamics of Ca oxalate compared to other chemical forms of Ca. Overall, this evidence supports the idea that Ca oxalate comprises an ecologically significant pool of available Ca.

4.3. Ca oxalate concentrations across ecosystem pools

In mineral soil, Ca_{ox} increased with Ca_s ($R^2 = 0.78$, $P = 0.004$) although this relationship was less strong compared to biomass and detritus suggesting that Ca oxalate accumulation in mineral soil may be less directly influenced by total Ca supply compared to biomass and detritus. Variation in mineral soil Ca oxalate concentrations was predictable based on soil depth combined with either soil pH or soil exchangeable Ca, across nine sites including the high and low-Ca sites (Dauer, 2012). Because there is a strong interrelationship between soil-exchangeable Ca and soil pH, a controlled experiment is necessary to determine what specific chemical factor(s) most strongly control Ca oxalate presence in soils. Mineral soil Ca oxalate concentrations generally increased with increasing soil pH (from pH = 4.2 to 5.8) although, the relationship was strongest in 0–20 cm depths and broke down in lower soil depths. Ca oxalate concentrations were highest in 0–20 cm surface soils (Table 2), which supports the idea that Ca oxalate primarily accumulates biologically in upper horizons (Certini et al., 2000; Fujii et al., 2013) either via plant or fungal exudation of oxalate and concomitant Ca oxalate precipitation or via direct inputs of Ca oxalate created within plant and fungal tissue.

Across individual live biomass pools of both sites, concentration of Ca oxalate increased with increasing total Ca concentrations ($R^2 = 0.98$, $P < 0.001$) as observed in other studies (Borer et al., 2004; Littke and Zabowski, 2007). Additionally, in detritus pools, Ca_{ox} concentrations increased with higher Ca_t across both sites ($R^2 = 0.88$, $P < 0.001$). Similar to the distribution of whole-ecosystem Ca oxalate, these patterns suggest that Ca oxalate may be equally or slightly more important in regulating plant Ca dynamics at Ca-rich sites. However the influence of Ca oxalate concentration in plant tissues on aboveground productivity or resiliency is unknown. It is hypothesized that Ca oxalate crystal formation in plants regulates bulk Ca levels (Franceschi and Nakata, 2005), as a defense to herbivory (Hudgins et al., 2003; Korth et al., 2006) and detoxifies aluminum or other heavy metals (Ma et al., 2001; Mazen, 2004). Because the high-Ca site tree tissues accumulated a slightly higher proportion of Ca oxalate than the low-Ca site, with much higher absolute concentrations of Ca oxalate as well, the high-Ca site may have increased mechanisms for herbivory defense or detoxification of heavy metals, especially in young foliage where differences in Ca oxalate proportions are the most extreme between sites (Table 2). These findings also support the idea that Ca supply is an important driver for differential Ca in live biomass and ultimately detrital Ca oxalate production at these sites.

4.4. Ca oxalate turnover in decomposing leaf litter

Studies of Ca in decomposing materials have primarily investigated bulk Ca content, and often indicate that Ca_t is lost at the same rate as mass in decomposing leaf tissue (Attiwill, 1968; Gosz et al., 1973; Staaf and Berg, 1982). However, the rate of Ca release or immobilization in leaf litter can also vary by species (Bockheim et al., 1991; Rustad, 1994; Dijkstra, 2003), which may be related to the variation in species Ca concentration in leaf and root litter (Hobbie et al., 2006). In this study, Ca_t loss from litterbags was similar to mass loss after 2 years, with no significant differences between mass loss and Ca_t loss within either high or low-Ca source litters (Table 3). Therefore, the large difference between the two litter sources in Ca_t loss (43% in high-Ca source litter versus 26% in low-Ca source litter) was due entirely to differences in

initial chemical Ca pools and variations in the overall rate of litter degradation, and not to differences in bulk Ca loss relative to mass loss.

We found that the dynamics of different chemical forms of Ca within leaf litter differed greatly during decomposition. In particular, Ca_{ox} decomposed rapidly losing 66% in the high-Ca and 42% in the low-Ca source litters in two years, indicating the potential for quick Ca oxalate degradation (Table 3). In comparison, for both litter sources, Ca_t and Ca_{aq} concentrations remained constant between initial and decomposed litter. However, the Ca_{st} pool increased nearly 4-fold in the high-Ca and nearly 2-fold in the low-Ca litter over two years (Table 3). The Ca_{st} pool, extracted with 2 N acetic acid, targets structurally bound Ca in pectin and lignin of fresh tissue but also likely includes ionic Ca that is electrostatically bound to cation exchange sites. Exchange sites increase with time in decomposing conifer litter (Staaf and Berg, 1982) and may be the proximal fate for Ca that is released from decomposing litter.

The quick turnover of Ca oxalate in decomposing tissues is additionally supported by the rate of Ca_{ox} release, which we estimated to be 0.28 yr^{-1} for high-Ca litter, and 0.04 yr^{-1} for low-Ca litter. Our data of Ca oxalate percent loss (e.g. 66% in high-Ca source litter, Table 3) are comparable to Eucalyptus litter decomposition that lost 70% of Ca oxalate in the first wet season during which time NH_4Cl-extractable exchangeable Ca increased (O'Connell et al., 1983). Decaying Ca oxalate may not be released immediately from decomposing litter systems, but instead may bind to organic matter exchange sites, and be available for either plant uptake or leaching loss. This indicates one way that Ca oxalate can influence dynamics of Ca soil availability.

The rapid release of Ca_{ox} from decomposing litter was not expected, considering that Ca oxalate is a highly insoluble crystal (K_{sp} 2.57 × 10^{-9}; Ringbom, 1963). It has been speculated that Ca oxalate pools remain intact until soil pH decreases (Bailey et al., 2003), however we found that litter pH did not decrease significantly in litter that experienced appreciable Ca oxalate loss (high-Ca source litter initial pH = 5.32 versus final pH = 5.24). These data overall do not support the idea that pH was the main factor driving Ca oxalate degradation during leaf litter decomposition, raising the possibility that microbial degradation may have been more important. Microbial degradation of crystalline Ca oxalate has been observed in petri cultures (Jayasuriya, 1955; Morris and Allen, 1994; Braissant et al., 2004) and implied for *Streptomyces* in soil fungal mats (Knutson et al., 1980). Additionally, petri-culture evidence suggests that some species of white rot and ectomycorrhizal fungi are also capable of dissolving Ca oxalate crystals, especially when Ca supply is low (Tuason and Arocena, 2009; Guggiari et al., 2011).

In contrast to our direct evidence of Ca oxalate disappearance from decomposing needle litter, comparison of Ca oxalate concentrations in decomposing woody tissues to live tissues indicate that wood rot fungi contribute to overall accumulation of Ca oxalate. Live bole-wood had lowest, and branches had the highest Ca oxalate concentrations among live woody biomass at both sites (Table 2). Ca oxalate concentrations of coarse woody debris had higher concentrations of Ca oxalate than live bole-wood, which was approximately equal to fine woody debris despite difference in Ca oxalate concentrations of their original live material. Additionally, fine woody debris (1–10 cm diameter) had lower Ca oxalate concentrations than live branches, suggesting that decomposition processes in these pools may be more similar to litter than bole-wood, with no accumulation of additional Ca oxalate during decomposition. We speculate that different microbial communities may be associated with different types of decomposing tissues; microbes that use Ca oxalate in leaf litter versus wood-rot decay fungi that accumulate Ca oxalate in woody debris (O'Connell et al., 1983; Dutton and Evans, 1996).

Forest floor pools and leaf litter fall flux at the two study sites can be used to estimate residence time of total Ca and Ca oxalate in the forest floor, as a comparison to the leaf litter decomposition study. Mean residence time was shorter for Ca_{ox} than for Ca_t at both sites (high-Ca site: 9 years for Ca_{ox}, 15 years for Ca_t; low-Ca site: 17 years for Ca_{ox}, 25 years for Ca_t) indicating that Ca oxalate does not accumulate over time in the forest floor. Both the leaf litterfall data at the two sites and the decomposition study support the idea microorganisms do not decompose Ca oxalate more rapidly in lower Ca status sites, which would potentially, and thus do not appear to amplify the supply to vegetation via the Ca oxalate pathway. Overall, emerging lines of evidence supported by our observation of rapid Ca oxalate disappearance from litter merit further examination of Ca oxalate properties and dynamics *in situ*, to determine its importance as a Ca source in organic and mineral soil systems.

5. Conclusions

Ca oxalate is ubiquitous in temperate forest ecosystems, existing in live and decomposing biomass pools, as well as in organic and mineral soil horizons. It makes up a portion of ecosystem Ca (4–18% at our sites), enough that it may have a profound effect on the long-term availability of Ca. The pool size of total Ca of a site can influence both the absolute amount of Ca oxalate present in each ecosystem pool, as well as the proportion of Ca that occurs as Ca oxalate, although Ca oxalate accumulation in some woody debris, forest floor or soil pools can be influenced by other more complex factors like decomposer communities. Despite lower absolute amounts of Ca oxalate in the low-Ca site compared to the high-Ca site, the low-Ca site had a larger portion of total available ecosystem Ca in the form of Ca oxalate, suggesting that Ca oxalate is an important Ca pool in forests where Ca is relatively scarce. We found that despite high initial quantities of Ca oxalate in high-Ca leaf litter, and a large pool of Ca oxalate in the forest floor, Ca oxalate does not appear to accumulate over time as an insoluble crystal. Instead Ca oxalate loss paralleled or exceeded bulk Ca loss during decomposition, indicating that Ca oxalate release from decomposing leaf litter in the forest floor is an important pathway for Ca return to plants. This may be especially important at high-Ca sites, where Ca oxalate is more abundant in leaf litter, leading to faster turnover of Ca oxalate during leaf litter decomposition.

Processes involving Ca oxalate are poorly understood, but may be important in shaping long-term Ca availability of ecosystems under stress from acidic atmospheric deposition, repeated biomass harvest or, as in our study sites, naturally high N sites that result in soil acidification and Ca loss. The role of Ca oxalate and tree function and health has rarely been studied, especially in a forest ecosystem context. Ca oxalate may be involved in resistance to herbivory, permanent or impermanent sequestration of Ca in tissues, deposition of waste products from carbon metabolism or other physiological processes. With more mechanistic information in plants and soils about this form of crystalline Ca, we can more fully understand single physiological and biogeochemical processes involved in Ca cycling. A clearer understanding of these processes may be useful in understanding ecosystem response to perturbations and strategies for sustainable nutritive management of forested ecosystems.

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