Contribution of Calcium Oxalate to Soil-Exchangeable Calcium

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Abstract: Acid deposition and repeated biomass harvest have decreased soil calcium (Ca) availability in many temperate forests worldwide, yet existing methods for assessing available soil Ca do not fully characterize soil Ca forms. To account for discrepancies in ecosystem Ca budgets, it has been hypothesized that the highly insoluble biomineral Ca oxalate might represent an additional soil Ca pool that is not detected in standard measures of soil-exchangeable Ca. We asked whether several standard method extractants for soil-exchangeable Ca could also access Ca held in Ca oxalate crystals using spike recovery tests in both pure solutions and soil extractions. In solutions of the extractants ammonium chloride, ammonium acetate, and barium chloride, we observed 2% to 104% dissolution of Ca oxalate crystals, with dissolution increasing with both solution molarity and ionic potential of cation extractant. In spike recovery tests using a low-Ca soil, we estimate that 1 M ammonium acetate extraction dissolved sufficient Ca oxalate to contribute an additional 52% to standard measurements of soil-exchangeable Ca. However, in a high-Ca soil, the amount of Ca oxalate spike that would dissolve in 1 M ammonium acetate extraction was difficult to detect against the large pool of exchangeable Ca. We conclude that Ca oxalate can contribute substantially to standard estimates of soil-exchangeable Ca in acid forest soils with low soil-exchangeable Ca. Consequently, measures of exchangeable Ca are unlikely to fully resolve discrepancies in ecosystem Ca mass balance unless the contribution of Ca oxalate to exchangeable Ca is also assessed.

Key words: Calcium oxalate, exchangeable Ca, soil analysis, soil calcium extraction

Calcium (Ca) is a macronutrient essential to ecosystem structure and function that undergoes intense recycling between plants and soils in terrestrial ecosystems (McLaughlin and Wimmer, 1999). Ca availability can, however, decrease because of acidic deposition (Bailey et al., 2005; Johnson et al., 2008; Bedison et al., 2010), nitrogen fertilization (Lucas et al., 2011), and repeated biomass harvest (Hornbeck and Kropelin, 1982; Perakis et al., 2006; Siemion et al., 2011). In the soil, Ca may exist in multiple chemical forms, including soluble Ca, exchangeable Ca, Ca bound in organic matter, mineral Ca, or biomineral Ca (Sparks, 2003). However, detailed approaches to investigating the various chemical forms, dynamics, and plant availability of Ca in soil are lacking relative to other macronutrients such as nitrogen and phosphorous. Soil Ca dynamics are characterized most frequently for exchangeable Ca, which can be defined mechanistically or

operationally. Mechanistically, exchangeable Ca is defined as hydrated Ca^{2+} ions that are held by negatively charged soil surfaces through relatively weak electrostatic and nonspecific interactions (Essington, 2004). Operationally, exchangeable Ca can be defined as it is measured; an ion that is removed from the soil by a solution containing a neutral salt, as opposed to soluble Ca, for example, which can be removed by water. Mechanistic and operational definitions of exchangeable Ca are only equivalent if exchangeable Ca procedures target only cations that are nonspecifically adsorbed to negative soil surfaces, with no additional contribution from other potential soil Ca sources (Essington, 2004).

Ca oxalate is by far the most abundant ionic biomineral in natural environments and commonly occurs in living plants, animals, and fungi, as well as nonliving carbonate concretions, marine and lake sediments, hydrothermal veins, and lignite coal (Baran and Monje, 2008; Echigo and Kimata, 2011). In terrestrial ecosystems, the presence of Ca oxalate is attributed to formation within tissues of plants and fungi (Arnott, 1995; Franceschi et al., 2005) and in soil via Ca^{2+} precipitation with oxalate anion exudates from plants and fungi (Dutton and Evans, 1996; Jones, 1998; Ryan et al., 2001). Ca oxalate has been shown to accumulate in several soil types (Graustein et al., 1977; Verrecchia and Dumont, 1996; Certini et al., 2000) and is particularly observed in association with ectomycorrhizal fungal mats (Cromack et al., 1979) and in detritus with wood-rot fungi (Dutton and Evans, 1996). Quantification of Ca oxalate in soils has been attempted in only a few studies (Fox and Comerford, 1990; Lilieholm et al., 1992; Certini et al., 2000), and the size of the Ca oxalate pool has been compared with exchangeable Ca even less frequently (Cromack et al., 1979; Dauer, 2012). Yet, it has been suggested that Ca_{ox} may contribute substantially to ecosystem Ca budgets (Bailey et al., 2003) and play a significant role in the long-term availability and retention of Ca (Perakis et al., 2006; Smith et al., 2009).

The crystalline structure and attendant chemical properties of Ca oxalate would suggest that it is unlikely to contribute exchangeable Ca in soils. Ca oxalate crystals have no charge, thus preventing electrostatic binding to negatively charged soil surfaces. Hence, Ca oxalate does not fit the mechanistic definition of exchangeable Ca. Also, Ca oxalate crystals are sparingly soluble, especially in the typical pH range of soils (Cromack et al., 1979; Gadd, 1999) and therefore should not release free Ca during typical extractions for soil-exchangeable Ca (Sparks, 1996). Therefore, Ca oxalate is assumed to be unrecorded in standard measures of soil-exchangeable Ca, making it a "missing" form of Ca (Cromack et al., 1979; Bailey et al., 2003; Perakis et al., 2006). Because of this assumption, researchers have invoked Ca oxalate to account for mass balance discrepancies between soil Ca availability and leaching from watersheds (Bailey et al., 2003). However, crystal solubility may increase with increasing ionic strength of the solution because of higher entropy, known as the neutral salt effect. Accordingly, the solubility of Ca oxalate has been observed to increase when subjected to high ionic strength tissue cultures (Hoover and Wijesinha, 1945; Belliveau and Griffin, 2001), suggesting that some portions of Ca oxalate may dissolve during salt extraction and be measured as exchangeable

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Ca, although this has not been tested explicitly. The potential inclusion of soluble Ca originating as calcium oxalate (Ca_{ox}) in measurements of exchangeable Ca may introduce bias in studies that assume Ca oxalate can resolve discrepancies in Ca mass balance. It may also introduce difficulties in interpreting mechanisms of Ca cycling because the dynamics of crystalline Ca oxalate are likely to differ from the ionic form of exchangeable Ca. For example, the soil pool of exchangeable Ca is commonly implied to be available for plant uptake by delivering Ca ions to the soil solution (White, 2001; Sparks, 2003), whereas crystalline Ca oxalate is unlikely to be absorbed by plant roots because of its large size and is unlikely to readily diffuse in the soil solution.

The objectives of this study were twofold: (i) determine the solubility of Ca oxalate in solutions commonly used to extract exchangeable Ca and in response to variation in solution ionic strength and (ii) determine the contribution of Ca oxalate to measurements of exchangeable Ca in low-Ca and high-Ca soils using both standard and modified soil extractions. We conducted two laboratory experiments to address these objectives; one experiment evaluated the solubility of Ca oxalate crystals in pure extraction solutions without soil, and the second experiment evaluated extraction recovery of Ca oxalate spikes added to soils. Based on the potential for a neutral salt effect, we expected Ca oxalate to increasingly dissolve in stronger ionic strength solutions of soil extractants. We also expected that high-Ca soils with higher soil pH would have larger Ca oxalate contributions to exchangeable Ca.

MATERIALS AND METHODS

Experiment 1: Ca Oxalate Solubility in Salt Solutions

To investigate how much Ca oxalate dissolves in salt solutions typically used to measure soil-exchangeable Ca, we determined the equilibrium constants of Ca oxalate in unbuffered ammonium acetate (NH_4OAc), ammonium chloride (NH_4Cl), and barium chloride ($BaCl_2$) solutions without any soil added. We used a range of molarity (0.1, 1, and 2 M) of a common extractant, NH_4OAc, to test how extractant concentration influences Ca oxalate dissolution. We also determined Ca oxalate solubility in solutions of hydrochloric acid (HCl) and nitric acid (HNO_3) as controls intended to dissolve/dissociate the majority of Ca oxalate crystals (Lilieholm et al., 1992; Bullen and Bailey, 2005) and in solutions of acetic acid (HOAc) intended to dissolve only Ca contained in pectin and lignin, adapted from an extraction scheme developed for Ca in plant tissues (Fink, 1991). Ca oxalate crystals (monohydrate crystals, 99.9% pure; Sigma-Aldrich) were prepared by drying for 12 h at 45°C. We then added Ca oxalate crystals in amounts equivalent to 0, 25, 50, 500, and 2,500 mg Ca oxalate per liter of extracting solution of 0.1 M NH_4OAc, 1 M NH_4OAc, 2 M NH_4OAc, 1 M NH_4Cl, 0.1 M $BaCl_2$, 0.5 N HCl, 1 N HNO_3, 2 N HOAc, and nanopure water (H_2O), using replicate ($n = 3$) 20-mL batches in 50-mL centrifuge tubes. If Ca oxalate was completely dissolved, solutions would contain 0, 0.20, 0.39, 3.9, or 20 mmol Ca_{ox} L^{-1}, which encompasses the range of Ca_{ox} in forest soils estimated in the literature (Dauer, 2012). Samples were shaken at approximately 23°C for 20 h, then filtered through disposable 25-mm Acrodisc 1-μm glass fiber filters attached to 60-mL syringes. Ca concentrations were measured using atomic absorption spectrophotometry on an AAnalyst 200 Spectrometer (Perkin Elmer, Waltham, MA). The solubility product of Ca oxalate in water (K_{sp}) was calculated as $\gamma^2[Ca^{2+}][C_2O_4^{2-}]$, where γ = activity coefficients of ions (for water, $\gamma = 1$). For all other solutions, equilibrium concentration products were calculated as $[Ca^{2+}][C_2O_4^{2-}]$.

The molarity of oxalate anions was assumed via stoichiometry where within Ca oxalate molecules, 1 mol of Ca^{2+} binds to 1 mol of oxalate. After the dissolution, solution pH was measured with a pH probe (Fisher Scientific Accumet AR20 meter, with an AccuFast pH combination electrode), except for solutions of 0.1 M NH_4OAc and 0.1 M $BaCl_2$, which were estimated based on the original solution. The ionic strength of the extractant solution (I) was estimated with the equation:

$$I = \frac{1}{2} \sum_{i=1}^{n} c_i z_i^2$$

where c_i is the molar concentration of ion i, z_i is the charge number of ion i, and the sum is taken over all the ions (n) in the solution.

Experiment 2: Ca Oxalate Solubility During Soil Extraction

We tested the dissolution of Ca oxalate crystals during routine extractions of exchangeable Ca from soils. To understand how soil Ca concentration may influence the dissolution of Ca oxalate crystals, we used two forest soils representing high and low levels of exchangeable Ca. Both sites were Andic Dystrudepts with sandstone parent material located in the north-central Oregon Coast Range. The high-Ca site (Site 5) contained concentrations of 1 M NH_4OAc mineral soil-exchangeable Ca (0- to 10-cm depth) of 2,031 μg Ca g^{-1}. The low-Ca site (Site 16) contained concentrations of 1 M NH_4OAc soil-exchangeable Ca (0- to 10-cm depth) of 194 μg Ca g^{-1}. These 10-fold differences in surface 0 to 10 cm exchangeable Ca concentrations lead to 9-fold differences in 0- to 10-cm exchangeable Ca pools between sites and contribute to 20-fold site differences in exchangeable Ca pools measured to 100-cm soil depth (Perakis et al., 2013). Plant tissue Ca concentrations and pool sizes, as well as annual plant Ca uptake, are also roughly twice as high in the high-Ca site than those in the low-Ca site. Soils at the high-Ca site had 53% sand, 23% silt, 25% clay, and a $pH_{(H2O)}$ of 5.50 in 0- to 10-cm mineral soils, and soils at the low-Ca site had 30% sand, 31% silt, and 39% clay, and a $pH_{(H2O)}$ of 4.61 in 0- to 10-cm mineral soils (Perakis and Sinkhorn, 2011). In June 2010, we collected three samples of 0- to 10-cm soil from each site using a 5.1-cm-diameter soil corer. Soils were sieved and homogenized (2 mm), and the three samples were composited by site and stored at 4°C for less than 1 week.

We examined the dissolution of Ca oxalate in soil extractions in response to several extraction concentrations and temperatures (summarized in Table 1). We used unbuffered 1 M NH_4OAc for these tests, which is among the most common extractants used for exchangeable Ca in forest soils (Sparks, 1996). To investigate the amount of Ca oxalate that could be dissolved during soil extraction, we used a Ca_{ox} spike recovery technique calculated as the difference in Ca concentration between a soil spiked with Ca oxalate and a control unspiked sample. The percent recovery indicates the amount of the spike dissolved during the extraction. We used 10-fold higher Ca oxalate spikes in the high-Ca soil than the low-Ca soil commensurate with approximate differences in exchangeable Ca between the two sites. To determine if temperature may play a role in dissolving Ca oxalate during extraction either by changes in entropy or changes in enzymatic activity of microbes, we compared a cold treatment (4°C) to extraction at room temperature (23°C). We also determined the specificity of our analysis to different concentrations of Ca oxalate in the soil with a treatment of extremely high Ca oxalate spikes. We also used $CaCl_2$ as a highly soluble spike to examine the inherent reproducibility and efficacy of spike recovery tests in these soils

TABLE 1. Extraction Treatments and Associated Ca Oxalate Spikes to Low-Ca and High-Ca Soils (Experiment 2)

Treatment	Extractant	Ca Oxalate Spike, mg Ca_{ox}		Ca Oxalate Spike, mg Ca_{ox}	
		Low-Ca Soil	High-Ca Soil	Low-Ca Soil	High-Ca Soil
1) Control 1M	1 M NH$_4$OAc	0	0	0	0
2) Ca oxalate spike 1M	1 M NH$_4$OAc	10	100	3	31
3) Cold (4 °C) Ca$_{ox}$ spike	1 M NH$_4$OAc	10	100	3	31
4) 5× Ca oxalate spike	1 M NH$_4$OAc	50	500	16	158
5) 10× Ca oxalate spike	1 M NH$_4$OAc	100	1,000	32	322
6) CaCl$_2$ spike	1 M NH$_4$OAc	10	100	3	28
7) Control 0.1M	0.1 M NH$_4$OAc	0	0	0	0
8) Ca oxalate spike 0.1M	0.1 M NH$_4$OAc	10	100	3	31

Extraction treatments and associated Ca oxalate spikes (reported both as Ca oxalate and Ca) for low-Ca and high-Ca soils. Ca oxalate spikes were approximately 10 times greater in the high-Ca soil than those in the low-Ca soil to match background differences in standard 1-M NH$_4$OAc–exchangeable Ca. Spikes were added to field moist soil equivalent of approximately 10 mg of dry-weight soil, with each treatment occurring in two sequential 25-mL extractions. All samples were extracted at 23°C, except the cold Ca oxalate spike, which was extracted at 4°C.

and also to determine if Ca precipitation or adsorption occurs subsequent to extraction, which would interfere with the ability to relate Ca oxalate dissolution in solution experiments to those with soil. We tested the potential for a lower–ionic strength extraction solution to remove exchangeable Ca without dissolving a large amount of Ca oxalate and therefore offer a more accurate estimate of exchangeable Ca by examining 0.1 M NH$_4$OAc as an extractant. Each treatment was replicated three times (2 soils × 8 treatments × 3 reps = 48 samples total). For the above treatments, we adapted standard methods of soil Ca extraction (Sparks, 1996) by weighing 10 g (dry weight) of moist soil into Falcon filters fitted with 0.7-μm particle retention Whatman GF/F filters, adding 25 mL of extracting solution, gently stirring with a glass rod, then suction filtering after 10 min. Subsequently, 25 mL of extractant was added and left at 23°C for 12 h (except cold treatment samples that were left at 4°C), then suction filtered and added to the first 25-mL extraction (following Sparks, 1996). Calcium concentrations were measured on all extractants by atomic adsorption spectrophotometry as above, although we note that neither atomic adsorption nor emission spectrophotometry, the two most common methods for measuring extractable Ca, will discern free Ca^{2+} ions from dissolved complexed Ca in measurements of exchangeable Ca. After the extraction, extractant solution pH was measured with a pH probe.

Data Analysis

In Experiment 1 tests of Ca oxalate dissolution in salt solutions without soil, we used two-way analysis of variance (ANOVA) to determine the effect of extractant and Ca oxalate addition level on the percent dissolution of Ca oxalate additions and on equilibrium concentration products. Comparisons were made between solutions across Ca oxalate addition levels between Ca oxalate addition levels within a solution and between solutions within a Ca oxalate addition level using LSMEANS in SAS version 9.2 (SAS Institute Inc., Cary, NC). In Experiment 2 evaluating spike recovery of Ca oxalate in soils, separate one-way ANOVA were used for the low-Ca and high-Ca soils to determine differences in soluble Ca among treatments. Comparisons between each treatment and the control were performed using LSMEANS to determine whether the treatment would dissolve Ca oxalate. The low-Ca and high-Ca soils were known to differ in exchangeable Ca concentrations (Perakis et al., 2013) and so were not directly compared with one another. All models were fit to the data using PROC MIXED in SAS version 9.2 (SAS Institute Inc.).

RESULTS

Experiment 1: Ca Oxalate Dissolution in Salt Solutions

Ca oxalate partially dissolved in several common unbuffered pH-neutral salt solutions that are used in the determination of soil-exchangeable Ca. There were significant interactive and main effects of solution type and amount of Ca oxalate added to solution on percent dissolution (two-way ANOVA, $P < 0.001$; Table 2). Ca oxalate dissolved to a greater extent in all salt (NH$_4$OAc, NH$_4$Cl, BaCl$_2$) and acidic (HCl, HNO$_3$, HOAc) solutions than in water (two-way ANOVA, $P < 0.001$; Table 2). The maximum observed dissolved Ca$_{ox}$ from salt solutions was 36.1 mg Ca$_{ox}$ L^{-1} in 0.1 M BaCl$_2$. Generally, Ca oxalate dissolution increased as the molarity of a given salt solution increased. Ca oxalate maximum dissolution increased two-fold between 0.1 M and 1 M NH$_4$OAc and 1.4-fold between 1 M and 2 M NH$_4$OAc, although there was a significant increase in percent dissolution across a range of Ca additions only between 0.1 and 1 M NH$_4$OAc and not between 1 and 2 M NH$_4$OAc (two-way ANOVA, $P < 0.01$; Table 2).

The fraction of added Ca oxalate that dissolved in salt solutions declined as the amount of Ca oxalate addition increased. At the lowest rate of Ca oxalate addition, we found that 76% to 104% of Ca oxalate dissolved into salt solutions, but only 1% to 5% of Ca oxalate dissolved at the highest rate of addition (Table 2). The weak acid 2 N HOAc dissolved a similar amount of Ca oxalate as 1 and 2 M NH4OAc extraction solutions. In contrast, Ca oxalate dissolution was nearly complete at all addition rates in the strong acids 0.5 M HCl and 1 N HNO$_3$. The absolute amount of Ca that dissolved from Ca oxalate crystals after the 20-h extraction in each solution increased as the Ca oxalate addition increased, so that the maximum observed concentration of Ca dissolved from Ca oxalate (Ca$_{ox}$ mg L^{-1}) was at the highest level of Ca$_{ox}$ crystals added to solution (2,500 mg L^{-1}). For each of the nine solutions, an observation of 782 mg Ca$_{ox}$ L^{-1} would be approximately 100% dissolved (reported in the bottom row of Table 2).

Calculations of equilibrium concentration products revealed significant interactive and main effects of solution type and amount of Ca oxalate added to solution (two-way ANOVA, $P < 0.001$; Table 3). Only the strong acid solutions (0.5 N HCl and 1 N HNO$_3$) had significant differences between equilibrium concentration products among Ca oxalate addition amounts ($P < 0.001$; Table 3). The mean pH of the extractions ranged from 7.42 to 2.29 in the salt solutions and 0.98 to 0.11 in the acid solutions (Table 3). The ionic

TABLE 2. Percent Dissolution of Ca Oxalate Crystals in Water, Extraction Solutions, and Acid Solutions Across a Range of Ca Oxalate Additions and the Maximum Resulting Concentration of Dissolved Ca (Experiment 1)

Ca Oxalate Added, mg L^{-1}	H$_2$O	0.1 M NH$_4$OAc	1 M NH$_4$OAc	2 M NH$_4$OAc	1 M NH$_4$Cl	2 N HOAc	0.1 M BaCl$_2$	0.5 N HCl	1 N HNO$_3$
	A	B	C	C	D	C	E	F	G
25	28 (2.5) a	76 (1.2) a	98 (1.0) a	99 (0.5) a	100 (1.3) a	97 (2.4) a	104 (1.2) a	99 (5.3)	120 (5.8) a
50	14 (0.7) b	46 (1.3) b	98 (1.9) a	98 (0.8) a	81 (1.0) b	101 (0.9) a	98 (1.7) b	96 (0.4)	108 (0.9) b
500	2 (0.1) c	5 (0.2) c	9 (0.5) b	13 (0.1) b	10 (0.3) c	13 (0.6) b	27 (2.8) c	94 (0.1)	98 (0.3) c
2,500	0 (0.0) c	1 (0.0) c	2 (0.1) c	3 (0.0) c	2 (0.0) d	3 (0.1) c	5 (0.7) d	94 (1.3)	97 (0.4) c
Max. Ca dissolved, mg Ca$_{ox}$ L^{-1}	3.2	7.2	14.6	20.6	14.6	18.8	36.1	633.2	706.1

Percent dissolution of Ca oxalate across a range of additions and the maximum resulting concentration of dissolved Ca (mg Ca L^{-1}) derived from Ca oxalate in nine solutions after 20 h at 23°C. Percent values reported as means, with SE in parentheses ($n = 3$). There were significant interactive and main effects of solution type and amount of Ca oxalate added to solution on percent dissolution (two-way ANOVA, $P < 0.001$). Different lowercase letters indicate significant effects of Ca$_{ox}$ addition level on percent dissolution within a solution ($P < 0.05$), and values without letters are nonsignificantly different. Different uppercase letters in the second row indicate significant differences in percent dissolution among solutions across Ca oxalate addition levels ($P < 0.001$). The bottom row provides the amount of Ca dissolved from Ca oxalate for the highest level of Ca addition (2,500 mg L^{-1} Ca oxalate) or the maximum observed amount of Ca dissolved for each solution (an observation of 782 mg Ca$_{ox}$ L^{-1} would be ~100% dissolved).

strength (*I*), calculated based on oxidation states and concentration of solution, ranged from 0 in water to 2 in 2 M NH$_4$OAc and 2 N HOAc (Table 3).

Experiment 2: Ca Oxalate Solubility During Soil Extraction

Spike additions of Ca oxalate crystals to the low-Ca soil significantly increased the amount of Ca extracted by 1 M NH$_4$OAc when compared against unspiked controls (one-way ANOVA, $P > 0.001$; Fig. 1A). For the low-Ca soil, 12% to 31% of added Ca oxalate was extractable as dissolved Ca with 1 M NH$_4$OAc across the range of Ca oxalate additions. The cold spiked treatment extracted at 4°C from the low-Ca soil yielded less Ca from Ca oxalate than the same spike amount and extraction conducted at 23°C (one-way ANOVA, $P = 0.03$; Fig. 1A). The exchangeable Ca concentration extracted using the more dilute 0.1 M NH$_4$OAc (0.1 M control treatment) was 61% less than that extracted by 1 M NH$_4$OAc (1 M control treatment, one-way ANOVA; $P < 0.001$) in the low-Ca soil. More dilute 0.1 M NH$_4$OAc also extracted a significant portion of the Ca oxalate spike (15%) (compared within

0.1 M NH$_4$OAc controls, one-way ANOVA, $P < 0.001$; Fig. 1A). Before extraction, the pH$_{H_2O}$ was 4.6 in the low-Ca soil, and after extraction, the solution pH was 6.2 in 1 M NH$_4$OAc and 5.7 in 0.1 M NH$_4$OAc.

In the high-Ca soil extractions, we could not detect additional Ca from the dissolution of 100 mg Ca oxalate spikes because of the high background exchangeable Ca concentration of 2 mg g^{-1} soil (i.e., 10-fold higher than that of the low-Ca soil; Fig. 1B). To illustrate, the predicted 0.74 mg of dissolved Ca$_{ox}$ in two 25-mL extractions with 1 M NH$_4$OAc (based on the maximum Ca$_{ox}$ dissolution for 1 M NH$_4$OAc in Table 2) was only 3.5% of the amount of exchangeable Ca present in the high-Ca control soil. This amount falls below the average range of experimental variability in replicated extraction measurements at the high-Ca soil (±10%). Only the CaCl$_2$ in 1 M NH$_4$OAc and the high Ca oxalate spikes (i.e., 500 mg and 1,000 mg Ca oxalate in 1 M NH$_4$OAc) were higher than the 1 M NH$_4$OAc control (one-way ANOVA, $P < 0.001$; Fig. 1B), yielding mean spike recovery rates of 92% (100 mg Ca oxalate), 2.5% (500 mg Ca oxalate), and 2.6% (1,000 mg Ca oxalate) for each treatment, respectively.

TABLE 3. Mean Equilibrium Concentration Products, pH, and Ionic Strength of Each Solution

Ca Oxalate Added, mg L^{-1}	H$_2$O	0.1 M NH$_4$OAc	1 M NH$_4$OAc	2 M NH$_4$OAc	1 M NH$_4$Cl	2 N HOAc	0.1 M BaCl$_2$	0.5 N HCl	1 N HNO$_3$
	A	B	C	D	E	F	G	H	I
25	2.6×10^{-9}	2.3×10^{-8}	4.2×10^{-8}	4.3×10^{-8}	4.7×10^{-8}	5.0×10^{-8}	5.2×10^{-8}	3.7×10^{-8} a	5.1×10^{-8} a
50	2.6×10^{-9}	2.7×10^{-8}	1.3×10^{-7}	1.5×10^{-7}	1.1×10^{-7}	1.3×10^{-7}	1.5×10^{-7}	1.2×10^{-7} a	1.6×10^{-7} a
500	3.4×10^{-9}	2.7×10^{-8}	1.3×10^{-7}	2.4×10^{-7}	1.2×10^{-7}	2.1×10^{-7}	9.8×10^{-7}	1.4×10^{-5} b	1.3×10^{-5} b
2,500	6.7×10^{-9}	3.2×10^{-8}	1.3×10^{-7}	2.7×10^{-7}	1.3×10^{-7}	2.2×10^{-7}	8.4×10^{-7}	2.5×10^{-4} c	3.1×10^{-4} c
pH of extraction	6.00	7.42	7.32	7.23	5.89	2.29	5.53	0.98	0.11
I	0	0.1	1	2	1	2	0.4	0.5	1

The mean equilibrium concentration products [Ca^{2+}] [C$_2$O$_4^{2-}$] of each solution calculated at each amount of Ca oxalate (mg L^{-1}) added. For water, where ionic strength = 0 and activity coefficients = 1, K_{sp} = (activity coefficient) 2[Ca^{2+}][C$_2$O$_4^{2-}$] was estimated. There were significant interactive and main effects of solution type and amount of Ca oxalate added to solution (two-way ANOVA, $P < 0.001$). Different lowercase letters indicate significant differences between equilibrium concentration products within a solution ($P < 0.001$), and values without letters are not significantly different. Different uppercase letters in the second row indicate significant effects of Ca oxalate addition level on equilibrium concentration products among solutions across Ca oxalate addition levels ($P < 0.001$). The mean solution pH after extraction is also given, except for 0.1 M NH$_4$OAc and 0.1 M BaCl$_2$, which were estimated from preextraction values. Ionic strength (*I*) was calculated based on oxidation states and concentration of solution.
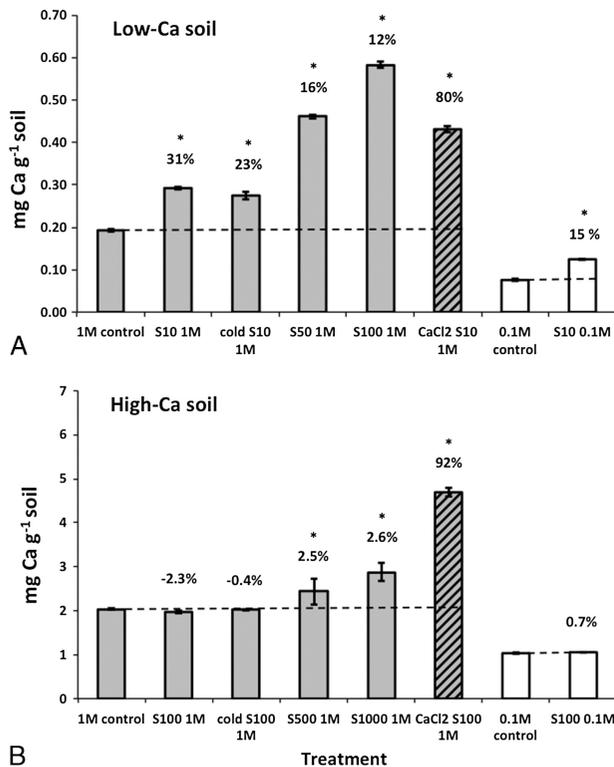
FIG. 1. Extractable Ca concentrations (mg Ca g^{-1} soil) in control soil and in soil spiked with Ca oxalate or Ca chloride, as determined for (A) low-Ca soil and (B) high-Ca soil. Calcium spikes occurred as Ca oxalate (S10 = 3 mg Ca_{ox}; S50 = 16 mg Ca_{ox}; S100 = 32 mg Ca_{ox}; S500 = 158 mg Ca_{ox}; S1000 = 322 mg Ca_{ox}) or $CaCl_2$ ($CaCl_2$ S10 = 3 mg Ca from $CaCl_2$; S100 = 28 mg Ca from $CaCl_2$, striped bars), and spiked soils were extracted with either 1 M NH_4OAc (1M, gray bars) or 0.1 M NH_4OAc (0.1M, white bars). The percent recovery of the spike is indicated above each bar and was calculated by comparing the concentration of the spiked sample to the concentration of the control. Bars with (*) indicate significantly different from their respective 1 or 0.1 M NH_4OAc control (one-way ANOVA, $P < 0.05$).

Similar to the low-Ca soil, the exchangeable Ca concentration using the concentrated extractant solution, 0.1 M NH_4OAc (0.1 M control treatment) was 49% less than that extracted by 1 M NH_4OAc (1 M control treatment, one-way ANOVA; $P < 0.001$) in the high-Ca soil. Before extraction, the pH_{H2O} was 5.5 in the high-Ca soil, and after extraction, the solution pH was 7.1 in 1 M NH_4OAc and 5.7 in 0.1 M NH_4OAc.

DISCUSSION

Solubility Experiments

Ca oxalate has been considered inert in soil chemical analyses that occur at neutral pH (Cromack et al., 1979; Bailey et al., 2003). However, we found that Ca oxalate partially dissolved in several common unbuffered pH-neutral salt solutions that are used in the determination of soil-exchangeable Ca, and that dissolution was greater in salt solutions than that in nanopure water. Crystals dissolve until a chemical equilibrium is reached with the salt solution, which may result in complete dissolution even in low ionic strength solutions if the amount of Ca oxalate is small. For example, addition of 5 mg L^{-1} of Ca_{ox} was 98% dissolved in 0.1 M $BaCl_2$ (Table 2). Ionic concentrations of exchangeable cation

extractants (I = 0.1–2; Table 3) are several orders of magnitude higher than native soil solution (e.g., 0.006 M) (Gillman and Bell, 1978). This disparity in ionic strength of soil versus extraction solutions and the resultant effects on Ca oxalate solubility highlights the dramatic chemical shift that the addition of the highly concentrated extractants may have on Ca oxalate dissolution in soil. Our results therefore show that common salt extractants to measure soil-exchangeable Ca, which is typically considered available for plant uptake and/or leaching loss, may release some Ca_{ox} into solution as free Ca in excess of mechanistically defined exchangeable Ca.

In our solubility experiment with water and strong acids, Ca oxalate crystal dissolution behaved as expected, with low dissolution in water and near-complete dissolution in strong acids. The solubility products in H_2O that was estimated from lower levels of Ca oxalate addition (2.6×10^{-9}; Table 3) closely matched the K_{sp} reported in literature (2.57×10^{-9}) at an ionic strength (I) of zero and at room temperature (Ringbom, 1963). In contrast, Ca oxalate dissolved completely in strong acid solutions (i.e., HCl, HNO_3) probably via both dissolution and dissociation from acidic reactions (97% and 94% dissolution, respectively; Table 2).

A neutral salt effect is defined as a change in solubility of a solute in a salt solution that has no ion in common with the solute and results from an increase in solution entropy (Mendham et al., 2000). The neutral salt effect likely explains our observations of Ca oxalate dissolution in salt solution where the ionic strength of the salt influenced the amount of Ca oxalate dissolution. As expected, when the concentration of the salt solution increased in molarity, the dissolution of Ca oxalate increased. Extraction using NH_4OAc at low concentration (0.1 M; Table 2) was the most similar to Ca oxalate dissolution in H_2O, which raises the possibility that 0.1 M NH_4OAc could be an alternative exchangeable Ca extractant that does not dissolve a substantial amount of Ca oxalate. Ca oxalate dissolution increased with higher concentrations of NH_4OAc (Table 2), although not as much as proportional differences in solution molarity might suggest; the 10-fold change in concentration between 0.1 and 1 M NH_4OAc solution dissolved only two-fold more Ca_{ox} in the three highest levels of Ca oxalate addition (5, 50, and 250 mg L^{-1}). The two-fold increase between 1 and 2 M NH_4OAc in extraction solution concentration dissolved only a 1.4-fold increase in Ca_{ox} in the highest Ca oxalate level. This non-1:1 relationship is likely caused by thermodynamics of dissociation that have equilibrium constants that are much lower than chemical reactions that cause complete dissolution, such as strong acids like HCl and HNO_3.

The molarity of extractant solutions affected dissolution similarly regardless of the type of counter anion involved; for example, the dissolution of Ca oxalate crystals was similar in 1 M NH_4OAc and 1 M NH_4Cl at the two highest levels of Ca oxalate addition (Table 2). In contrast, the type of cation in extractant solutions markedly influenced Ca oxalate dissolution. In our experiment, 0.1 M $BaCl_2$ dissolved nearly four times more Ca oxalate on average than 0.1 M NH_4OAc (0.1 M). The divalent charge and lower ionic radius (1.35 Å) of barium (Ba^{2+}) as compared with those of monovalent ammonium (NH_4^+) with a higher ionic radius (1.43 Å) yield a four-fold higher ionic potential (2.96 in Ba^{2+} vs. 0.70 in NH_4^+), which allows Ba^{2+} to more strongly attract oxalate anions. In addition, the ionic strength of the $BaCl_2$ solution was four-fold higher than the NH_4OAc solution (0.4 in $BaCl_2$ vs. 0.1 in NH_4OAc) because of its divalent charge (Table 3).

Although we tested only monohydrate Ca oxalate in this experiment, crystal solubility is also influenced by crystal phase properties. It is therefore possible that dihydrate Ca oxalate would have a different dissolution behavior from the monohydrate form we evaluated. However, monohydrate Ca oxalate (or whewellite)

is more common and more stable than dihydrate Ca oxalate (weddellite) and is much more prevalent in natural systems (Dana, 1997; Baran and Monje, 2008). Therefore, dissolution information provided in this study may be a conservative assessment of Ca oxalate behavior overall.

Ca Oxalate Contribution to Exchangeable Ca During Soil Extraction

We have thus far discussed the dissolution of Ca oxalate in pure solutions of water, acids, and extraction salts used in standard soil analyses. To better evaluate the amount of Ca oxalate that could dissolve in actual soil extractions for exchangeable cations, we compared Ca_{ox} concentrations in soil extractions with 1 M NH_4OAc to estimates of equilibrium Ca_{ox} dissolution determined from pure solution experiments. We used the Ca_{ox} concentration at the highest level of Ca oxalate addition in the solution experiment as an approximation of the amount of dissolved Ca_{ox} at equilibrium. So, we estimate that 1 M NH_4OAc has the potential to dissolve up to 14.6 mg Ca_{ox} L^{-1} in aqueous solutions (Table 2). We compared this Ca_{ox} concentration to soil extractions, which used two sequential 25-mL solutions to extract 10 g of soil (Sparks, 1996). If Ca oxalate dissolution was the same in both the solubility experiment and the extraction of field soils, we would predict a maximum dissolution potential of 0.37 mg Ca_{ox} for each of two extractions of field soil, totaling 0.74 mg Ca_{ox} for 10 g of soil. In the 1 M NH_4OAc extraction of the low-Ca soil spiked with 3 mg Ca_{ox}, we extracted 0.98 mg Ca_{ox} (31% dissolution; Fig. 1A), which is more Ca than the predicted equilibrium dissolution of 0.74 mg Ca_{ox} determined in aqueous 1 M NH_4OAc. Therefore, the Ca_{ox} dissolution in soil extractions was higher than the estimates of Ca_{ox} dissolution for 1 M NH_4OAc. The higher dissolution of Ca oxalate in 1 M NH_4OAc soil extraction than in pure solution could be caused by the lower pH and/or the higher ionic strength of the soil extraction.

Portions of the Ca oxalate spikes to the low-Ca soil were dissolved with 1 M NH_4OAc in all levels of Ca oxalate addition in the low-Ca soil (Fig. 1A). The dissolution of Ca oxalate in soil extractions with NH_4OAc indicates that if Ca oxalate were present in the low-Ca soil, a substantial portion of it would be dissolved and reported as "exchangeable Ca" using standard exchangeable Ca methods. The maximum equilibrium amount of Ca oxalate that dissolved in 1 M NH_4OAc (0.10 mg Ca g^{-1} soil) would increase measured exchangeable Ca in these low-Ca soils (0.19 mg Ca g^{-1} soil) by 52%. A portion of the Ca oxalate spike (15%) was even detected in a 10 times less-concentrated extractant (0.1 M NH_4OAc), indicating the susceptibility of crystals to dissolution in this type of procedure (Fig. 1A). This suggests that molarity adjustments to conventional soil extraction solutes are unlikely to entirely eliminate dissolution of Ca oxalate in the determination of soil-exchangeable Ca. In addition, the exchangeable Ca concentration using the more dilute 0.1 M NH_4OAc extractant was less than 1 M NH_4OAc by 49% in the high-Ca soil and 61% in the low-Ca soil, suggesting that more dilute extractants will yield substantially less exchangeable Ca, yet without eliminating the dissolution of Ca oxalate.

Forest soils are unlikely to have Ca oxalate concentrations as high as the spikes used in this experiment. The highest reported Ca oxalate literature value was 3.32 mg Ca_{ox} g^{-1} soil in ectomycorrhizal mats (Cromack et al., 1979), whereas our high spikes in the high-Ca soil added 16 and 32 mg Ca_{ox} g^{-1} soil (spike treatment 4 and 5; Table 1). In comparison, the lower-level spikes that we added to the low-Ca soil (0.3 mg Ca_{ox} g^{-1} soil, spike treatment 3; Table 1), which more closely resemble native soil Ca oxalate concentrations, underwent 31% dissolution and increased total soil-exchangeable Ca levels by 52%. Therefore, unless Ca oxalate soil concentrations are extremely high, our results suggest that

high-Ca soils may be less susceptible than low-Ca soils to bias by Ca oxalate dissolution in measurements of exchangeable Ca. The wide variance in dissolution properties of Ca oxalate in standard exchangeable cation methods may make cross-site comparisons difficult when background Ca levels also vary and add complexity to calculating soil Ca pools for mass balance budgets.

Our results also indicate that dissolution of Ca oxalate in soil extraction is temperature dependent, as the cold spiked treatment extracted at 4°C from the low-Ca soil yielded less Ca from Ca oxalate than the same spike and extraction conducted at 23°C (Fig. 1A). Several factors may explain this difference. Temperature affects solubility by changes in entropy, and as an endothermic reaction, solubility is predicted to increase with increasing temperature (Le Chatelier Principle). In addition, the lower-temperature extraction may have decreased oxalotrophic microbial activity, which is primarily enzymatic (Khammar et al., 2009; Svedruzic et al., 2005) and is therefore likely suppressed at lower temperatures. Soil microbes that use oxalate for energy, electron transfer, and carbon are widespread (Verrecchia et al., 2006; Sahin, 2003), and although mechanisms by which carbon in crystalline Ca oxalate is accessed by microbes are unstudied, oxalate originating as Ca oxalate has been observed to be degraded by microbes at relatively rapid rates in Petri cultures (Jayasuriya, 1955; Braissant et al., 2004). Additional work is needed to determine the actual mechanism(s) of temperature effects on Ca oxalate dissolution, as such effects may also be relevant in field degradation of Ca oxalate in soils.

Ca oxalate solubility is highly pH dependent (Gadd 1999), and differences in soil pH may also influence Ca oxalate dissolution during soil extraction. The initial pH_{H2O} of the two soils was more acidic (4.6 in the low-Ca soil, 5.5 in the high-Ca soil) than the extraction pH values (6.2 in 1 M NH_4OAc and 5.7 in 0.1 M NH_4OAc in the low-Ca soil and 7.1 in 1 M NH_4OAc and 5.7 in 0.1 M NH_4OAc in the high Ca soil) because of the more than 7 pH of NH_4OAc extraction solutions. The higher pH measured in 1 M than 0.1 M NH_4OAc extractions may be caused by greater buffering by the more concentrated extractant after the addition of soil. Despite the consistently lower soil pH that resulted from extraction with the more dilute 0.1 M NH_4OAc, this extraction nevertheless dissolved a smaller percentage of the Ca oxalate addition than 1 M NH_4OAc, suggesting that differences of extraction pH in the range of 5 to 7 had less of an effect than the 10-fold difference in ionic concentrations of extractant on the amount of Ca oxalate dissolved during extraction. Previous work has emphasized that soil pH may regulate Ca oxalate solubility in soil (Cromack et al., 1979; Bailey et al., 2003), and our work raises the possibility that soil solution ionic strength may also be an important factor shaping Ca oxalate solubility in the field.

Addition of $CaCl_2$ spikes to the soil was intended to provide an easily dissociated form of Ca that could be used to estimate soil effects on extractable Ca recovery independent of effects of Ca oxalate addition. Ca chloride is expected to dissolve 745 mg $CaCl_2$ L^{-1} at 20°C and 1 atm, so we expected that 0.2 mg $CaCl_2$ L^{-1} (low-Ca soil) and 2.0 mg $CaCl_2$ L^{-1} (high-Ca soil) would be 100% dissolved in two sequential soil extractions with 25 mL of 1 M NH_4OAc. We measured 80% and 92% Ca recovery from $CaCl_2$ spikes in the low-Ca soil and high-Ca soil (respectively), suggesting that there may be a small amount of interference in soil that complexes or adsorbs some free Ca^{2+} ions (8%–20% of Ca in a spike). This may have happened to a greater extent in the low-Ca soil because of a higher clay content (39% vs. 25% at the high-Ca site).

Determining an alternative solution for exchangeable cation extraction that excludes Ca oxalate proves to be a difficult problem. Extracting at a higher pH (7) may have dissolved less Ca oxalate in

our low-Ca soil and may provide a way to exclude Ca oxalate from exchangeable Ca extractions. However, extractions that occur at a high pH in buffered NH_4OAc are known to overestimate CEC and underestimate exchangeable Ca as compared with ammonium salts (Borge, 1997; Skinner et al., 2001) because increasing the negative charges on the surface of soil results in stronger adsorption of Ca^{2+} ions. Where Ca oxalate is expected to contribute substantially to measurements of exchangeable Ca, it may be possible to independently measure Ca oxalate by measuring both oxalate and Ca ions and use the stoichiometry of Ca oxalate to account for Ca_{ox} contributions to extracted Ca. And further research to resolve dynamics of different Ca forms could occur using Ca isotope–enriched Ca oxalate or free Ca added as spikes to soil, then evaluated with soil extractions or plant uptake measurements.

How much does Ca oxalate potentially contribute to measures of exchangeable Ca across forest soils? Assuming a bulk density of 0.48 g cm^{-3} in 0–10 cm in low-Ca soil where this sample was taken (Perakis and Sinkhorn 2011) and using data from our high-Ca spike (100 mg, treatment 5), the amount of Ca_{ox} dissolved during exchangeable Ca measurements could be as high as 188 kg Ca_{ox} ha^{-1} in the top 10 cm of soil. This amount of Ca_{ox} exceeds annual vegetation Ca uptake by aggrading Douglas-fir on the forest soils we sampled ($10-30 \text{ kg Ca ha}^{-1} \text{ year}^{-1}$) (Perakis et al., 2013) and is thus a nontrivial factor in estimating plant-available Ca pools. Overall, concentrations of Ca oxalate in soils are relatively unstudied (Lilieholm et al., 1992; Certini et al., 2000; Dauer, 2012), and estimates from forests near our study site range from 95 to 1,895 kg Ca_{ox} ha^{-1}, with higher values from mycorrhizal mats in relatively Ca-rich sites (Cromack et al., 1979). Ca oxalate can vary even more when other soil types are considered, yet, generally, mechanisms of Ca oxalate accretion and dissolution remain poorly characterized, so the applicability of our results to other soil types is unknown. However, given that values of exchangeable Ca reported in some temperate forest surface soils range from 160 and 3,600 kg ha^{-1} (Cole and Rapp, 1981), even limited quantities of Ca oxalate have the potential to be important for understanding Ca dynamics, especially in low-Ca soils.

CONCLUSIONS

This work challenges the notion that Ca oxalate does not contribute to measurements of soil-exchangeable Ca, indicates how Ca oxalate could contribute to soluble Ca to measures of exchangeable Ca, and highlights the importance of independently measuring this pool of Ca to determine its role in ecosystem Ca dynamics and availability. Measurements of exchangeable Ca are often interpreted mechanistically or to indicate soil function, such as quantifying the pool of cations available for plant uptake, leaching loss or other processes in the soil. Yet our findings show that standard methods for the measurement of exchangeable soil cations likely include some portion of the Ca_{ox} pool, contrary to the assumptions of previous ecosystem Ca research. The contribution of Ca oxalate to exchangeable pools may be especially large (as a fraction of total) in low-Ca soils, which are typically the soils of greatest risk of Ca depletion from acidic deposition and biomass removal. Additional work is needed to determine how cycling dynamics differ between crystalline Ca oxalate compared with soluble Ca or exchangeable Ca. Such information will help resolve discrepancies in mass balance budgets on an ecosystem scale and their relative importance to ecosystem biogeochemical processes.

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