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Effects of Calcium Silicate Treatment on the Composition of Forest Floor Organic Matter in a Northern Hardwood Forest Stand

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Abstract

Calcium amendment can help improve forest sustainability in stands that have been impacted by chronic acid deposition. An important component of this improvement is the stimulation of the microbial activity that supports ecosystem nutrient cycling processes. To test the hypothesis that Ca treatment alters the structure and solubility of organic matter substrates, an important driver of microbial activity, we investigated the effect of wollastonite ($CaSiO_3$) treatment on soil organic matter (SOM) and hot-water-extractable organic matter (HWEOM). We found a decrease in the HWEOM content of forest floor soils within two years of treatment with a high dosage of wollastonite (4250 kg Ca/ha), but not at a low dosage (850 kg Ca/ha). High-dosage treatment did not reduce the biodegradability of HWEOM. Hence, a high dose of $CaSiO_3$ appears to reduce the solubility of organic matter in the forest floor but not the bioavailability of the extracted SOM. Nuclear magnetic resonance spectroscopy revealed no significant changes in the O-alkyl C content of SOM in response to wollastonite addition, but a reduction in the O-alkyl C content of HWEOM suggests that the extractability of carbohydrate structures was reduced by added $CaSiO_3$. Phosphorous treatment, when performed in combination with Ca, also decreased the O-alkyl C content of HWEOM, but had no effect when performed without Ca. The reduced solubility of SOM after Ca treatment may have been the result of bridging between Ca^{2+} and negatively charged sites on SOM, as suggested in other studies. Also, high concentrations of Si in soil solution, due to dissolution of the wollastonite, likely resulted in oversaturated conditions with respect to SiO_2 or kaolinite, perhaps leading to co-precipitation of soluble organic matter. Overall, our results suggest that added Ca and/or Si may react with SOM to reduce the accessibility of labile C forms to soil microbes.

Introduction

Calcium and magnesium amendments have been widely used as a remedial measure for acidic soils. These treatments have been found to increase base saturation, pH, cation exchange capacity, and available phosphorus and to enhance soil microbial activity (Ivarson 1977; Adams et al. 1978; Zelles et al. 1978; Lohm et al. 1984; Yavitt and Newton 1990; Illmer and Schinner 1991; Frostegard et al. 1993; Neale et al. 1997). Interest in Ca amendments is particularly strong in areas affected by acidic deposition, such as the northeastern U.S. (Driscoll et al. 2001).

Decades of acidic deposition at the Hubbard Brook Experimental Forest (HBEF) in the White Mountain National Forest in central New Hampshire have resulted in the depletion of soil Ca, Al mobilization, and reductions in forest health (Likens et al. 1998, Driscoll et al. 2001). In 1999, a watershed at the HBEF was amended with wollastonite ($CaSiO_3$), at a dosage designed to return the base saturation of the soil to estimated preindustrial levels (Peters et al. 2004). Researchers at the HBEF found significant increases in soil and stream pH, cation exchange capacity, and base saturation, a reduction in winter-injury/dieback of forest vegetation, and an increase in aboveground net primary productivity after the wollastonite treatment (Peters et al. 2004; Hawley et al. 2006; Juice et al. 2006; Cho et al. 2010; Battles et al. 2014; Johnson et al. 2014). However, unlike many liming studies, Ca addition at the HBEF did not enhance soil microbial biomass or activity (Groffman et al. 2006). This unexpected result raises questions about our fundamental understanding of the effect of Ca treatment on soil biology.

One important factor underlying the surprising lack of microbial response to Ca amendment at the HBEF is the interaction of Ca with soil organic matter (SOM). Hobbie et al. (2002) found greater *in situ* soil respiration, greater laboratory soil respiration, and greater dissolved organic carbon (DOC) production in acidic tundra soils, compared to nonacidic soils

with high concentrations of Ca. They speculated that Ca bridging of the labile SOM rendered it less accessible to microbes. Melvin et al. (2013) reported large increases in C and N pools in the forest floor 19 years after liming in a northern hardwood forest in the Adirondack region of New York. They suggested that this increase was at least partly the result of decreased microbial activity after liming, possibly due to the binding of SOM by the added Ca. These results suggest that Ca may affect the fundamental structure and/or bioavailability of SOM, which serves as a principal energy source for soil microbes.

Soil acidification and associated depletion of available Ca in the 20th century appears to have reduced P availability in HBEF soils (Fiorentino et al. 2003). An alternative explanation for the lack of a microbial response to Ca treatment at the HBEF is therefore that P limitation may have prevented microbes from responding to more favorable pH and Ca conditions after the treatment. To explore interactions between Ca, P and organic matter in Hubbard Brook soils, we analyzed the effect of Ca application, with and without P, on the structural chemistry of SOM and its water-extractable fraction. Hot-water-extractable organic matter (HWEOM) has been proposed to be an indicator of SOM bioavailability (Ghani et al. 2003; Chen et al. 2004). Since we are examining the factors that potentially influence soil microbial activity, differences in the amount and/or composition of HWEOM between treatments may provide insight on the effects of the treatments on bioavailable organic matter. We hypothesized that: (**H1**) Ca treatment decreases the amount of hot-water-extractable organic carbon (HWEOC) in the soil; and (**H2**) Ca treatment alters the structure of HWEOM. We explored hypothesis **H1** by determining the dissolved organic carbon (DOC) concentration in hot-water extracts from treated and control soils in a plot-level manipulation experiment. To examine the bioavailability of HWEOM and test treatment effects, we analyzed the mineralization of the organic C in this fraction in

laboratory incubations. To test hypothesis **H2**, we used nuclear magnetic resonance (NMR) spectroscopy and elemental analysis to quantify the structural variation in soils subjected to the various amendments. Solid-state NMR spectroscopy is a non-destructive technique that provides information on the chemical bonds associated with a target nucleus – ^{13}C in this study. Because of the chemical heterogeneity of natural organic matter, ^{13}C NMR is generally used to characterize the distribution of C among broad classes of organic structures, including alkyl C, O-alkyl and di-O-alkyl C, aryl and O-aryl C, and carbonyl C (e.g., Nelson and Baldock 2005). Together, ^{13}C NMR spectroscopy and elemental analysis can be used to provide insight into the relative abundance of carbohydrates, lignin, lipids and protein (Nelson and Baldock 2005). We focused our work on organic horizons since these horizons showed the most significant chemical effects in the watershed-scale Ca application (Cho et al. 2010).

Methods

Site description

We established four blocks of 5 plots each (twenty plots total) in July 2006. The four blocks were all located in an area of approximately 3 ha, and separated by less than 100 m. The individual plots were 5-m × 5-m each and separated from one another by at least 2 m (Minick et al. 2011). The study site was in an area dominated by sugar maple (*Acer saccharum*) at an elevation of approximately 500 m (Minick et al. 2011). The soils in the study area are mostly well-drained, acidic Spodosols (Haplorthods) formed in glacial till. The average pH for these soils is 3.4 to 4.0 in the surface organic horizons due to the acidic character of SOM and long-term inputs of acid rain (Johnson et al. 1991).

Experimental treatments

In each of the four blocks, one plot was randomly assigned to each of the following treatments: (1) Control, (2) High Ca (4250 kg Ca/ha), (3) Low Ca (850 kg Ca/ha), (4) P (50 kg P/ha; as $Na_2HPO_4 \cdot 2H_2O$), (5) Low Ca + P (amounts as indicated in (3) and (4)). The amount of P was selected to be more than triple the amount of P required by plants, to obviate the potential microbial limitation of P because of uptake by vegetation and adsorption by soils (Minick et al. 2011). Calcium was manually applied to the plots in the form of wollastonite ($CaSiO_3$) powder – from the same source of material used in the watershed-scale manipulation (Peters et al. 2004). The plots were treated in October 2006 and Oie (Oi + Oe) and Oa horizon soils were sampled in July 2006 (pre-treatment), October 2006, and then May, July, and October of 2007 and 2008.

Sample compositing and analytical methods

We performed hot-water extractions on every individual sample (5 treatments × 4 replicates × 2 horizons = 40 samples per collection date) collected in July 2006 (pretreatment), October 2006, July 2007, and July 2008 to determine sampling variability. For the remaining dates, we composited all the field-moist replicates from each treatment on an equal dry-weight basis before performing hot-water extractions and subsequent analyses. We also pooled the corresponding dried soil samples in equal amounts for the same sampling dates.

The procedural and analytical details for hot-water extraction, DOC analyses, freeze-drying, elemental analyses, and NMR spectroscopy are detailed in Balaria et al. (2009). Details of the biomolecular modeling approach may be found in Balaria and Johnson (2013). These methods will only be summarized briefly here.

Subsamples of the field-moist samples were dried at 80 ^{o}C to determine moisture content. After drying, these subsamples were ground and used for elemental and NMR analyses. Hot-

water extractions were performed by adding 10 or 30 g (dry equivalent) of field-moist Oie or Oa soil, respectively, to 150 mL of deionized water in acid-washed HDPE bottles. After 30 min shaking, the bottles were incubated for 18 h in a 70 ^{o}C water bath. The extracts were centrifuged (3500 rpm for 20 min), passed through glass-fiber filters, then vacuum-filtered through MF-Millipore mixed esters of cellulose membrane filters. An aliquot of the filtered extract was set aside for DOC analysis, and the remaining extract was freeze-dried into solid HWEOM for elemental and NMR analysis.

The DOC concentrations of the HWEOM extracts were determined using persulfate-ultraviolet oxidation and CO_2 detection. The concentrations of C and N in soil and freeze-dried HWEOM samples were determined by dry combustion – gas chromatography using a Costech ECS 4010 elemental analyzer. Organic matter content of soil and HWEOM samples was estimated by loss-on-ignition at 500 ^{o}C overnight in a muffle furnace.

Solid-state ^{13}C NMR experiments were conducted using the cross polarization with magic angle spinning (CPMAS) technique on a Bruker AVANCE 300 spectrometer. The NMR analyses were performed using a 1-ms contact time, 3-s recycle delay, 17.5-ms acquisition time, and 7000 Hz spinning speed. Samples were packed in 7-mm zirconia rotors with Kel-F caps. All spectra were processed with 50-Hz Gaussian line broadening and integrated using the following chemical shift assignments (Nelson and Baldock 2005): 0-45 ppm, alkyl C; 45-60 ppm, N-alkyl/methoxyl C; 60-95 ppm, O-alkyl C; 95-110 ppm, di-O-alkyl C; 110-145 ppm, aromatic (aryl) C; 145-165 ppm, phenolic C; 165-215 ppm amide/carboxyl C. We did not correct for spinning sidebands, which were negligible in our spectra. The integrated intensities of the O-alkyl and di-O-alkyl C fractions, which largely represent C in carbohydrate structures, were combined. This sum is referred to as ‘O-alkyl C’ in this paper.

We used the molecular mixing model (MMM) of Nelson and Baldock (2005) to estimate the content of four major biomolecular groups (carbohydrates, proteins, lipids, lignin) in SOM and HWEOM. The MMM is a linear deconvolution routine that uses the NMR spectra and elemental composition of model compounds for each biomolecular group to determine the relative proportions of the biomolecule groups that provide the best statistical fit to the NMR spectrum and C:N ratio of the sample. Balaria and Johnson (2013) showed that the spectral distributions estimated by the fitted MMM results closely matched the spectra of soils and HWEOM from the HBEF.

To examine the biodegradation of HWEOM, we used the Oie and Oa horizon samples for all treatments from the October 2008 sampling event. After hot-water extraction, we incubated triplicate samples of the liquid HWEOM in brown bottles with loose caps to maintain dark conditions but allow aeration for microbial activity. Results from a pilot study indicated that re-inoculation was not necessary. We analyzed the biodegradability of HWEOM by measuring the DOC concentration at 0, 1, 2, 3, 7, 14, 21, 28, 35, and 42 days. At each sampling date, we collected a 1-ml subsample from each sample using a pipette. These subsamples were kept frozen until the day of DOC analysis to prevent any further microbial degradation during storage. The evaporation loss during the experiment was minimal, yet we accounted for it in the biodegradation calculation using the volume of a blank sample (V_b) which had the 1-ml subsample taken out at each sampling date. The amount of biodegraded C was calculated as follows:

$$\text{Biodegraded C (mg)} = (M_i - M_t) \times V_o \qquad [1]$$

where:

M_i = initial DOC concentration, mg L^{-1}

M_t = DOC concentration after t days × [$V_{b,t}$ /(V_o-n)], mg L^{-1}

V_o = initial volume (100 mL or 0.1 L)

n = number of 1-mL aliquots removed at time t

We also compared the concentration of HWEOC in our samples to microbial biomass carbon (MBC) and microbial C respiration measured on the same samples. Methods and results for MBC and C respiration are detailed in Groffman and Fisk (2011a,b).

Data analysis

To test hypothesis **H1**, we analyzed whether or not the Ca treatments significantly affected the amount of organic carbon extracted by hot-water using DOC analysis of the hot water extracts. In the NMR study conducted to test hypothesis **H2**, we analyzed soil samples for the October events of 2006, 2007, and 2008, and the pretreatment July 2006 event. On both composited and individual samples, we performed elemental analysis on both the bulk soil (referred to here as "whole-soil") and freeze-dried HWEOM. Thus, the amount of hot-water extractable carbon (HWEOC) could be expressed on an absolute (mg C per gram soil) or relative (mg C per gram soil C) basis.

We analyzed the HWEOC amounts for both Oie and Oa horizons using two different approaches. First, we performed an analysis of variance (ANOVA) using Tukey's honestly significant difference at the 95% confidence level on the data from the three sampling dates for which individual replicate samples were analyzed (July 2006, 2007, 2008). In order to analyze the whole data set, we also analyzed how the mean values of HWEOC changed over the course

of three years in the various treatments. We used a similar approach for elemental analyses on both the whole soil and HWEOM data. For the biodegradation study, we performed ANOVA on the cumulative biodegradation (after 6 weeks), also using Tukey's honestly significant difference at the 95% confidence level.

We conducted ^{13}C CPMAS NMR analyses on soil samples collected in October 2006, 2007, and 2008, to study the structural chemistry of organic matter immediately after, one year after, and two years after the treatment. We performed these analyses on both the whole-soil and the HWEOM from Oie and Oa horizons. For October 2006, all the replicates were analyzed individually, while for 2007 and 2008, the samples were composited for NMR analysis. For these NMR analyses, we first compared the spectral intensity for different functional groups obtained through CPMAS analysis to quantify the differences between treatments. For October 2006, we performed ANOVA for the O-alkyl C component for both whole soil and HWEOM for the Oie horizon as this group largely represents the biodegradable structures contributed from carbohydrates. Since we did not have estimates of variability for 2007 and 2008 due to sample compositing, we used the standard deviation values from October 2006 to compare the mean values obtained for those years with individual control values using a two-sample t test.

Results

Hot-water extractable organic carbon

While the Low-Ca treatment did not affect HWEOC over time in the Oie horizon, the High-Ca treatment significantly ($P < 0.05$) reduced HWEOC values in July 2008 (3.86 mg g^{-1} soil) compared to July 2006 (6.91 mg g^{-1}) (Table 1). Phosphorus treatment also significantly reduced HWEOC over time when applied with Ca. However, P alone did not affect HWEOC. The mean

HWEOC in control plots ranged from 8.25 mg g^{-1} in July 2006 to 6.35 mg g^{-1} in July 2008, but this difference was not significant at either the 90% or 95% confidence level, suggesting that the treatment effects were genuine. The HWEOC concentration in the Oie horizon decreased for the first year for all treatments including the control (Fig. 1), but the decrease was greatest for the Ca-treated plots. The HWEOC in Oie horizons of the control plots remained approximately constant from July 2007 to July 2008 while HWEOC in the High-Ca plots continued to decline. By the last sampling date, October 2008, the HWEOC concentration in the High-Ca Oie horizons was less than half of the concentration in the control plots (Fig. 1).

Analysis of variance for the three sampling dates on which replicate samples were analyzed did not detect significant differences in Oie-horizon HWEOC between treatments for either pretreatment (July 2006) or post-treatment (July 2007 and July 2008) data. This is likely attributable to high variability among soil samples and the relatively small (N = 4) within-treatment sample sizes. The pre-treatment HWEOC values for the Oie horizon were consistent with the Oi and Oe horizon values reported in Balaria et al. (2009) for Hubbard Brook soils: 16.7 mg g^{-1} in the Oi horizon, and 5.2 mg g^{-1} in the Oe horizon.

No significant differences in HWEOC were observed in the Oa horizons for any of the dates when replicates were analyzed. However, the mean HWEOC values were lower in High-Ca plots than in control plots on every sampling date after the treatments were applied (Fig. 1). In a parallel study of microbial activity and nitrogen mineralization in these soils, Minick et al. (2011) also observed few statistically significant treatment effects for these treatments in the Oa horizon. The HWEOC values we measured were lower than the Oa horizon values reported in Balaria et al. (2009), which varied from 5.2 mg g^{-1} in the top 1 cm of the Oa horizon to 3.1 mg g^{-1} at the bottom of the Oa horizon. These lower values probably reflect the fact that the samples

collected in this plot study also included some soil from A horizons. The HWEOC values for upper mineral horizons (top 10-cm) in the plots were determined for July 2006, and the average HWEOC content was 0.54 ± 0.04 mg g^{-1}. We did not conduct further analyses on mineral soil since the treatments were not likely to result in significant effects that deep into the soil. Cho et al. (2010) found no significant treatment effect on the chemical properties of mineral horizons in the first three years after wollastonite application in watershed 1 at Hubbard Brook.

Elemental analysis

The C:N ratios in Oie and Oa horizons for both SOM and HWEOM for the four treatments and control plots are presented in Fig. 2. The values of the C:N ratio ranged from approximately 18 to 24 for both Oie and Oa horizon soils, similar to values for Hubbard Brook soils published in Balaria et al. (2009). An ANOVA revealed no significant treatment effects in either Oie or Oa horizons on those dates for which we had replicate samples, and there were no consistent patterns in the rank order of the various treatments (Fig. 2).

The C:N ratios were lower in HWEOM than the whole soil, suggesting a higher relative contribution from microbial sources. The HWEOM C:N ratios in Oie horizons of the High-Ca plots increased significantly ($P < 0.05$) for the first year (July 2007) after treatment and then remained high the following year (July 2008) (Fig. 2). The C:N ratio of HWEOM in control plots did not change significantly over time, suggesting that Ca treatment indeed led to higher C:N ratios. We observed slight increases in C:N ratios in Low-Ca and Ca + P plots as well, but these differences were not statistically significant. The P-only treatment did not induce any significant changes in the C:N ratios of HWEOM and tracked the control values closely (Fig. 2). The ANOVA revealed that the C:N ratio of HWEOM in the Oie horizon was significantly higher in High-Ca and Low-Ca plots, compared to control plots, in July 2007. In July 2008, however, only

the High-Ca treated plots had significantly higher C:N ratio compared to the control plots. In Oa horizon HWEOM, the C:N ratio in High-Ca plots increased after treatment, while the C:N ratio in control plots decreased (Fig. 2). However, after one year there were no differences among treatments.

Molecular-scale structural assessment

NMR spectroscopic analysis

An example comparison of ^{13}C CPMAS NMR spectra for various treatments for the whole soil and the corresponding HWEOM fraction of Oie horizons is presented in Fig. 3. In general, whole-soil from the Oie horizons had a significantly higher O-alkyl C percentage than Oa horizons (Fig. 4). While O-alkyl C represented 40-50% of total spectral intensity in Oie horizons, in Oa horizons O-alkyl C represented 35-45% and alkyl C accounted for approximately 30% of total intensity. Thus, SOM in the Oa horizon contained a comparatively higher percentage of the more recalcitrant alkyl C fraction compared to the Oie horizon. The third largest constituent was aryl C (8-12% of total spectral intensity in the Oie horizon, 5-14% in the Oa). Phenolic (O-aryl) C contributed an average of 2.8% of spectral intensity in Oie-horizon SOM and 2.9% in the Oa horizon.

The HWEOM spectra were dominated by the peaks in the O-alkyl C region (60-95 ppm, Fig. 3), and O-alkyl C accounted for up to 77% of the total spectral intensity, much higher than SOM (Fig. 4). The O-alkyl C content in HWEOM was greater in Oa horizons than in Oie horizons (Fig. 4). This observation is consistent with a previous study conducted on other Hubbard Brook soils (Balaria et al. 2009), where an increase in HWEOM O-alkyl C content with depth within the organic horizons was observed. The second largest component of HWEOM was alkyl C, which was only 20% and 15% of spectral intensity in Oie and Oa horizons, respectively.

The contributions of aryl and O-aryl C were small (< 5%) for both Oie and Oa horizons, suggesting that lignin is not a major component in HWEOM (Balaria and Johnson 2013).

In whole soils, no treatment effects were observed in NMR spectral properties among the five treatments in either Oie or Oa horizons for any year. For HWEOM collected in October 2006, shortly after the treatments were applied, there were no differences in spectral properties between control and treated plots in either Oie or Oa horizons. However, HWEOM spectra indicated a lower percentage of O-alkyl C in Oie horizons of the High-Ca treated plots compared to control plots ($P < 0.1$) in October 2007 (Fig. 4). O-alkyl C in HWEOM from the Oie horizon was also reduced by Ca + P treatment ($P < 0.1$). However, O-alkyl C in Oie-horizon HWEOM was not significantly lower in Low-Ca than control plots. In October 2008, the differences between treatments became more subtle (and statistically insignificant), as shown in Fig. 4. Phosphorous treatment did not show any significant patterns, suggesting that microbial processing of organic matter in these soils was not limited by P-related effects on organic matter composition. A lack of a P-effect on microbial biomass and activity was also observed by Groffman and Fisk (2011a) in a parallel study for the same plots, suggesting that microbial growth and decomposition of labile organic matter are likely not limited by P availability in these soils.

Biomolecular modeling

The carbohydrate content, estimated with the molecular mixing model, was about 50% of SOM and 60-80% of HWEOM (Fig. 5). Estimated carbohydrate content of whole-soil samples did not differ significantly among treatments (Fig. 5). However, the hot-water extracts from the High-Ca treatment had somewhat lower carbohydrate content than control plots (Fig. 5). Protein content was higher in HWEOM compared to the whole soil from which it was extracted (Fig. 4 and 5).

We found a higher protein content in Ca-treated soils for whole-soil samples, but the HWEOM samples showed no effects of Ca treatment on protein content.

The whole-soil samples exhibited slightly lower lignin and lipid contents in Ca-treated plots, compared to controls. However, for HWEOM, these values were higher in Ca-treated soils than in the control plots. Overall, these modeling analyses suggest that the HWEOM in Ca-treated soils has a lower content of energy-rich biomolecules (carbohydrates and proteins) compared to control soils.

Biodegradation Analysis

The analysis of HWEOM indicated that Ca treatment leads to decreases in HWEOC in the Oie horizon. We carried out biodegradation experiments to understand whether only the extractability of organic C was affected by these treatments, or whether there was also a shift in the bioavailability of the organic matter in the soil. The biodegradation study showed that the organic matter extracted from the Oie horizon of the High-Ca plots was the most degradable (~60% C loss over 6 weeks) out of the five treatments, while the P-treated plots showed the least biodegradation (~25%) by indigenous microbes (Fig. 6). This pattern suggests that while the extractability of organic matter decreases with Ca treatment, as evidenced by reduced HWEOC concentration, the biodegradability of the extracted components increases. Most of the biodegradation occurred within the first seven days of incubation, followed by a plateau to six weeks. However, the experiments exhibited high variability and with only three replicate experiments per treatment, the cumulative biodegradation for the different treatments was not significantly different at a 95% confidence level. The only statistically significant difference at the end of the six-week incubation period was between the High-Ca treated plots and P-treated plots, the highest and the lowest values, respectively. Treatment effects on biodegradation were

only visible for Oie-horizon soils. The Oa horizon hot-water extracts showed lower biodegradation levels with very high variability and inconclusive results (Fig. 6).

We observed significant positive correlations between HWEOC concentrations and microbial biomass C (MBC; Fig. 7a) and C respiration (Fig. 7b) in Oie horizons across all treatments. Although there is considerable variability, both MBC and C respiration approached zero at the lowest HWEOC concentrations.

Discussion

Effects of $CaSiO_3$ treatment on SOM

This study was performed at two different levels of $CaSiO_3$ treatment. The Low-Ca treatment was an amount estimated to bring the base status of Hubbard Brook soils back to its estimated pre-acid rain value. The High-Ca treatment was five times that amount, similar in magnitude to Ca additions used in liming experiments in other forest ecosystems (Kreutzer 1995; Melvin et al. 2013). The Low-Ca treatment did not induce a significant change in HWEOC concentration in the soil. Consistent with hypothesis **H1**, the HWEOC content of the soil treated with a high dosage of Ca decreased significantly over the two-year study period. These treatment effects were limited to the Oie horizon, where the observed short-term chemical effects of wollastonite addition at the watershed scale were also the greatest (Cho et al. 2010). While this study is the first work on the influence of Ca treatment on HWEOM, Belkacem and Nys (1995) observed no significant change in soil-solution DOC concentration in a Mull humus layer over a 20-month experimental period after a relatively small addition of lime.

The decrease in the concentration of HWEOC after high-level Ca treatment could be attributed to two possible mechanisms. First, there may have been a decrease in the content of water-soluble structures in the SOM due to the treatment. Second, the extractability of water-soluble carbon may have been reduced due to the chemical binding of SOM by Ca or Si released in the dissolution of the wollastonite. If the first mechanism were true, the composition of the whole-soil organic matter would have been impacted with regard to water-soluble structures such as carbohydrates and proteins. If the second mechanism were true, the composition of SOM would not show significant changes – only the water-extractable fraction would be affected. We explored these alternatives by studying the differences in the structural chemistry of SOM in control and wollastonite-treated soils and HWEOM fractions.

For Oie-horizon soils, whole-soil samples showed no significant differences in the O-alkyl C fraction between control and Ca-treated plots for 2006, 2007 or 2008 (Fig. 4). The HWEOM fraction, however, showed a lower percentage of O-alkyl C in October 2007 in High-Ca plots compared to control plots, consistent with the second hypothesis, **H2**, of this study (Fig. 4). The molecular mixing model corroborated these predictions by showing a decrease in the amount of carbohydrates in HWEOM upon wollastonite treatment, but not in the whole soil. If the soil were being depleted in degradable organic fractions such as carbohydrates, this should have been reflected in the whole-soil NMR spectra, and not just the HWEOM spectra. The lower O-alkyl C content in HWEOM from Ca-treated plots suggests that wollastonite treatment affects the extractability of organic matter as opposed to causing a shift in the overall SOM composition.

Wollastonite dissolution releases both ionic Ca^{2+} and dissolved Si to the soil solution. Binding with either or both of these weathering products could explain lower solubility of SOM. The binding of Ca^{2+} with negatively charged functional groups is known to reduce the solubility

of SOM and has been suggested as an explanation for lower C respiration in high-Ca tundra soils (Hobbie et al. 2002). Ionic Ca^{2+} may bind to deprotonated hydroxyl (–OH) and carboxyl (–COOH) structures in carbohydrates and proteins, reducing their solubility and causing lower O-alkyl C content in HWEOM. High dissolved Si concentrations may result in the precipitation of SiO_2 and/or kaolinite ($Al_2Si_2O_5(OH)_4$). Indeed, Cho et al. (2012) observed that O-horizon soil solutions were highly oversaturated with respect to kaolinite after the watershed-scale wollastonite amendment at the HBEF. The soil solutions were undersaturated with respect to amorphous SiO_2 and near equilibrium with quartz. The watershed wollastonite addition was equivalent to our Low-Ca treatment, so it is likely that our High-Ca treatment produced even higher dissolved Si concentrations and more oversaturated conditions with respect to kaolinite and/or SiO_2. Precipitation of either kaolinite or SiO_2 would take place on surfaces including SOM and organic coatings on soil minerals, potentially making those materials less soluble.

Microbial Activity in Ca-Treated Soil

To investigate the relationship between HWEOM and microbial processes, we examined the relationships between HWEOC and microbial biomass and respiration in Oie horizons for all treatments. Considering the heterogeneity of the soil and the various treatments, we found remarkably strong positive correlations for both of these microbial parameters with HWEOC content (Fig. 7). We found that both microbial biomass C and C respiration increase as HWEOC increases, suggesting that HWEOC is an indicator of microbial activity in this forest soil. Furthermore, both MBC and C respiration approach zero as HWEOC decreases, suggesting a linkage between water-soluble C and microbial process rates.

We also studied how the biodegradation of HWEOM was affected by the treatment of the soils from which it was extracted. Our biodegradation study showed that even though the High-

Ca treated plots had the least amount of extractable organic matter, there was no significant effect on the biodegradation of the extracted material. This observation suggests that the primary effect of Ca treatment is to decrease the solubility of SOM, perhaps through organic matter binding with weathering products. In this way, binding by Ca and/or Si derived from the added wollastonite may ultimately reduce the accessibility of labile C to soil microbes.

The lowest biodegradation was found in the HWEOM extracted from the P-treated plots, suggesting a possible inhibitory effect of phosphorus on biodegradation. Phosphorus treatment has been found to have both positive (Haynes and Swift 1988; Poozesh et al. 2010) and negative (Cleveland et al. 2002; Hobbie and Vitousek 2000) effects on soil microbial activity. Phosphate addition has been found to inhibit activity of some important enzymes for microbial degradation including phosphatase, protease and sulphatase (Nannipierie et al. 1978; Speir and Ross, 1978). In a parallel study conducted on our plots, Minick et al. (2011) found a decrease in N mineralization after P treatment when added with Ca. We found that the HWEOC content in soils from Low-Ca treated plots was significantly lower than in control plots only when the Ca was applied with P. Together, these results suggest an additive effect of P on the solubility of organic matter and soil microbial processes.

Soil C:N ratios are known to influence microbial biomass and processes. At Hubbard Brook, the C:N ratios of microbial biomass are much lower than SOM derived from plant material (Bohlen et al. 2001). Also, the C:N ratio of SOM decreases during decomposition. For the whole soil, we found no significant differences between the C:N ratios in control and Ca- or P-treated plots, suggesting little or no microbial response to the treatments. Persson et al. (1989) predicted that for C:N ratios less than 30, like Hubbard Brook soils, microbial stimulation due to liming would increase N mineralization, whereas liming soils with greater C:N ratios would

reduce N mineralization. Minick et al. (2011) did not detect significant effects of Ca treatment on net N mineralization in Oe horizons in our study plots, but did find a significant negative relationship between net N mineralization and pH. Thus, microbial activity was not stimulated, and was possibly depressed due to the pH increase caused by Ca amendment.

In contrast to the whole soil, C:N ratios of HWEOM were significantly higher in the High-Ca treated plots compared to the control plots in the Oie horizon. In comparison to SOM, HWEOM is enriched in proteinaceous matter probably derived from microbial biomass and microbial residues (Balaria et al. 2009; Fig. 5). A significant increase in the C:N ratio of HWEOM after Ca treatment therefore may reflect a decrease in microbially derived material, an observation consistent with results from the watershed-level study at Hubbard Brook (Groffman et al. 2006), but in contrast to a number of studies that observed an enhanced microbial response to liming treatment (Haynes and Swift, 1988; Persson et al. 1989, Badalucco et al. 1992; Filep and Szili-Kovacs, 2010). The absence of a microbial response in this study may be partly due to the fact that wollastonite was used instead of lime. The dissolution of wollastonite, a silicate mineral, results in a more modest pH increase than calcium carbonate dissolution.

Another possible factor contributing to an increase in the C:N ratio of HWEOM after wollastonite application could be a shift in the type of microbial biomass. Fungi normally have higher C:N ratios than bacteria (Griffin 1985) and the increase in C:N may be an indication of an increased contribution from fungal biomass. Soil acidity has been found to decrease fungal hyphae (Baath et al. 1980) and increased pH due to Ca application (Minick et al. 2011) may have stimulated fungal growth to a greater extent than bacterial growth. However, Groffman and Fisk (2011b) found a higher prokaryote:eukaryote ratio of 2.10 (±0.08) in the High-Ca treated soils, compared to 1.88 (±0.24) in the control plots, indicating a lower contribution from fungal (or

other eukaryotic) sources upon High-Ca treatment. An analysis of microbial diversity in HWEOM may be useful in exploring this issue further.

Conclusions

High-dose wollastonite treatment resulted in decreased solubility of SOM in surface Oie horizons in this base-poor northern hardwood forest. This decreased solubility was not the result of changes in SOM composition. While Ca treatment had no significant effect on SOM composition, HWEOM from Ca-treated soils had lower O-alkyl C content than HWEOM from control soils, indicative of lower carbohydrate content. Binding of Ca^{2+} to hydroxyl or carboxyl functional groups, and/or precipitation of Si-bearing mineral forms on SOM surfaces, may explain the reduced solubility of SOM. While high-dose wollastonite addition reduced the solubility of SOM in the forest floor, it did not reduce the biodegradation of the water-soluble fraction. Thus, it appears that bioavailability of SOM after $CaSiO_3$ treatment may be controlled by physic-chemical rather than biological factors.

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References

Adams SN, Cooper JE, Dickson DA, Dickson EL, Seaby DA (1978) Some effects of lime and fertilizer on a Sitka spruce plantation. Forestry 51:57–65

Baath E, Berg B, Lohm U, Lundgren B, Lundkvist H, Rosswall T, Soderstrom B, Wiren A (1980). Effects of experimental acidification and liming on soil organisms and decomposition in a Scots pine forest. Pedobiologia 28:85–100

Badalucco L, Grego S, Dell'Orco S, Nannipieri P (1992) Effect of liming on some chemical, biochemical, and microbiological properties of acid soils under spruce (Picea abies L.) Biol Fert Soils 14:76–83

Balaria A, Johnson CE (2013) Compositional characterization of soil organic matter and hot-water-extractable organic matter in organic horizons using a molecular mixing model. J Soils Sediments 13:1032–1042

Balaria A, Johnson CE, Xu Z (2009) Molecular-scale characterization of hot-water extractable organic matter in a northeastern forest soil. Soil Sci Soc Am J 73:812–821

Battles JJ, Fahey TJ, Driscoll CT, Blum JD, Johnson CE (2014) Restoring soil calcium reverses forest decline. Environ. Sci. Technol. Letters 1:15-19

Belkacem S, Nys C (1995) Consequences of liming and gypsum top-dressing on nitrogen and carbon dynamics in acid forest soils with different humus forms. Plant Soil 173:79–88

Bohlen PJ, Groffman PG, Driscoll CT, Fahey TJ, Siccama TG (2001) Plant–soil–microbial interactions in a northern hardwood forest. Ecology 82:965–978

Chen CR, Xu Z, Mathers NJ (2004) Soil carbon pools in adjacent natural and plantation forests of subtropical Australia. Soil Sci Soc Am J 68:282–291

Cho Y, Driscoll CT, Johnson CE, Siccama TG (2010) Chemical changes in soil and soil solution from calcium silicate addition to a northern hardwood forest. Biogeochemistry 100:3–20

Cho Y, Driscoll CT, Johnson CE, Blum JD, Fahey TJ (2012) Watershed-level responses to calcium silicate treatment in a northern hardwood forest. Ecosystems 15:416–434

Cleveland CC, Townsend AR, Schmidt SK (2002) Phosphorus limitation of microbial processes in moist tropical forests; evidence from short-term laboratory incubations and field studies. Ecosystems 5:680–691

Driscoll CT, Lawrence GB, Bulger TJ, Butler CS, Cornan CS, Eager C, Lambert KF, Likens GE, Stoddard JL, Weathers KC (2001) Acidic deposition in the northeastern United States: sources and inputs, ecosystem effects, and management strategies. BioScience 51:180–198

Filep T, Szili-Kovács T (2010) Effect of liming on microbial biomass carbon of acidic arenosols in pot experiments. Plant Soil Environ 56:268–273

Fiorentino I, Fahey TJ, Groffman PM, Driscoll CT, Eager C, Siccama TG (2003) Initial response of phosphorous biogeochemistry to calcium addition in a northern hardwood forest ecosystem. Can J For Res 33:1864–1873

Frostegard A, Baath E, Tunlid A (1993) Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analyses. Soil Biol Biochem 25:723–730

Ghani A, Dexter M, Perrott KW (2003) Hot-water extractable carbon in soils: A sensitive measurement for determining impacts of fertilization, grazing and cultivation. Soil Biol Biochem 35:1231–1243

Griffin EM (1985) A comparison of the roles of bacteria and fungi. In: Leadbetter, E.R., Poindexter, J.S (eds) Bacteria in Nature, vol. 1. Bacterial activities in perspective. Plenum Press, New York, pp. 221–255

Groffman PM, Fisk MC (2011a) Phosphate additions have no effect on microbial biomass and activity in a northern hardwood forest. Soil Biol Biochem 43:2441–2449

Groffman PM, Fisk MC (2011b). Calcium constrains plant control over forest ecosystem nitrogen cycling. Ecology 92:2035–2042

Groffman PM, Fisk CF, Driscoll CT, Likens GE, Fahey TJ, Eager C, Pardo LH (2006) Calcium additions and microbial nitrogen cycle processes in a northern hardwood forest. Ecosystems 9:1289–1305

Hawley GJ, Schaberg PG, Eager C, Borer CH (2006) Calcium addition at the Hubbard Brook Experimental Forest reduced winter injury to red spruce in a high-injury year. Can J For Res 36:1–6

Haynes RJ, Swift RS (1988) Effect of lime and phosphate additions on changes in enzyme activities, microbial biomass and levels of extractable nitrogen, sulphur and phosphorous in an acid soil. Biol Fert Soils 6:153–158

Hobbie SE, Miley TA, Weiss MS (2002) Carbon and nitrogen cycling in soils from acidic and nonacidic tundra with different glacial histories in northern Alaska. Ecosystems 5:761–775

Hobbie SE, Vitousek PM (2000) Nutrient limitation of decomposition in Hawaiian forests. Ecology 81:1867–1877

Illmer P, Schinner F (1991) Effects of lime and nutrient salts on the microbiological activities of forest soils. Biol Fert Soils 11:261–266

Ivarson KC (1977) Changes in decomposition rate, microbial population and carbohydrate content of an acid peat bog after liming and reclamation. Can J Soil Sci 57:129–137

Johnson CE, Driscoll CT, Blum JD, Fahey TJ, Battles JJ (2014) Soil chemical dynamics after calcium silicate addition to a northern hardwood forest. Soil Sci Soc Am J 78:1458-1468

Johnson CE, Johnson AH, Siccama TG (1991) Whole-tree clearcutting effects on exchangeable cations and soil acidity. Soil Sci Soc Am J 55:502–508

Juice SM, Fahey TJ, Siccama TG, Driscoll CT, Denny EG, Eager C, Cleavitt NL, Minocha R, Richardson AD (2006) Response of sugar maple to calcium addition to northeastern hardwood forest. Ecology 87:1267–1280

Kreutzer K (1995) Effects of forest liming on soil processes. Plant Soil 168-169:447-470

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

Lohm U, Larsson K, Nömmik H (1984) Acidification and liming of coniferous forest soil: long-term effects on turnover rates of carbon and nitrogen during an incubation experiment. Soil Biol Biochem 16:343–346

Melvin AM, Lichstein JW, Goodale CL (2013) Forest liming increases forest floor carbon and nitrogen stocks in a mixed hardwood forest. Ecol Applic 23:1962-1975

Minick KJ, Fisk MC, Groffman PM (2011) Calcium and phosphorus interact to reduce mid-growing season net nitrogen mineralization potential in organic horizons in a northeastern hardwood forest. Soil Biol Biochem 43:271–279

Nannipieri P, Johnson RL, Paul EAB (1978) Criteria for measurement of microbial growth and activity in soil. Soil Biol Biochem 10:223–228

Neale SP, Shah Z, Adams WA (1997) Changes in microbial biomass and nitrogen turnover in acidic organic soils following liming. Soil Biol Biochem 29:1463–1474

Nelson PN, Baldock JA (2005) Estimating the molecular composition of a diverse range of natural organic materials from solid-state ^{13}C NMR and elemental analyses. Biogeochemistry 72:1–34

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

Peters SC, Blum JD, Driscoll CT, Likens GE (2004) Dissolution of wollastonite during the experimental manipulation of Hubbard Brook Watershed 1. Biogeochemistry 67:309–329

Poozesh V, Castillon P, Cruz P, Bertoni G (2010) Re-evaluation of the liming fertilization interaction in grassland on poor and acid soils. Grass Forage Sci 65:260–272

Speir TW, Ross DJ (1978) Soil phosphatase and sulphatase. In: Burns, R.G., (ed) Soil Enzymes. Academic Press, London, pp. 197–250

Yavitt JB, Newton RM (1990) Liming effects on some chemical and biological parameters of soil (spodosols and histosols) in a hardwood forest watershed. Water Air Soil Pollut 54:529–544

Zelles L, Scheunert I, Kreutzer K (1978) Bioactivity in limed soil of a spruce forest. Biol Fert Soils 3:211–216

Table 1 Hot-water extractable organic carbon (HWEOC) values for Oie and Oa horizons for Ca and P treated plots (mean ± standard error, n=4)

		Control (mg C/g soil)	High Ca (mg C/g soil)	Low Ca (mg C/g soil)	Low Ca + P (mg C/g soil)	P (mg C/g soil)
Oie	July 2006	8.3 ± 0.1 a[†]	6.9 ± 0.8 a	8.2 ± 1.0 a	9.5 ± 0.4 a	8.2 ± 0.7 a
	July 2007	6.5 ± 1.0 a	4.9 ± 0.5 a b	5.8 ± 0.4 a	5.9 ± 0.8 b	4.7 ± 0.6 b
	July 2008	6.3 ± 1.1 a	3.9 ± 0.4 b	6.5 ± 0.2 a	5.0 ± 0.5 b	6.1 ± 1.1 a b
Oa	July 2006	1.9± 0.1 a	1.9 ± 0.0 a	2.4 ± 0.3 a	2.1 ± 0.5 a	1.9 ± 0.4 a
	July 2007	2.1 ± 0.3 a	1.2 ± 0.5 a	1.9 ± 0.3 a	1.7 ± 0.3 a	1.2 ± 0.2 a
	July 2008	1.8 ± 0.3 a	1.5 ± 0.4 a	1.9 ± 0.2 a	1.3 ± 0.2 a	1.7 ± 0.2 a

[†]Different letters (a, b) represent statistically significant differences among different years, based on Tukey's method for each treatment, and are to be read vertically. We did not detect any significant differences between treatments on any of these sampling dates.

Figure Captions

Fig. 1 Hot-water extractable organic carbon (HWEOC) concentrations in soils subjected to five treatments at different sampling dates. Filled symbols represent Oie horizons, while open symbols depict Oa horizons. The symbol shapes are assigned to the same treatments in both horizons. Error bars are shown for sampling dates on which individual samples were analyzed. Samples were composited on other dates.

Fig. 2 Changes in C:N ratios in soil organic matter (SOM, filled symbols) and hot-water-extractable organic matter (HWEOM, open symbols) in control and treated plots. Error bars are shown for sampling dates on which individual samples were analyzed. Samples were composited on other dates. The symbol shapes are assigned to the same treatments in both SOM and HWEOM.

Fig. 3 Solid-state cross polarization with magic-angle spinning (CPMAS) ^{13}C nuclear magnetic resonance spectra of (a) whole soil and (b) hot-water-extractable organic matter (HWEOM) for Oie horizons for various calcium and phosphorus treatments (October 2007).

Fig. 4 Total O Alkyl C content of soil organic matter (SOM) and hot-water-extractable organic matter (HWEOM) based on ^{13}C CPMAS NMR spectroscopy. Plotted values are the percent of total spectral intensity in the O-Alkyl and di-O-Alkyl C regions combined. Error bars represent one standard error and are not shown for October 2007 and 2008 because samples were composited prior to analysis on those dates. The symbol shapes are assigned to the same treatments in both SOM and HWEOM panels.

Fig. 5 Composition of organic matter in soils and hot-water extracts of Oie horizons control and treated plots in October 2007. Values were predicted using the molecular mixing model, and are shown with standard errors computed based on replicate analyses of samples from the High-Ca treatment in October 2006.

Fig. 6 Biodegradation (%C loss) of hot-water extractable organic matter in Oie and Oa horizons computed using a dissolved organic carbon consumption method. Filled symbols represent Oie horizons, while open symbols depict Oa horizons. The symbol shapes are assigned to the same treatments in both horizons.

Fig. 7 Correlations between hot-water extractable organic carbon (HWEOC) and microbial activity in Oie horizons of all treatments, including control plots: (a) microbial biomass carbon and (b) microbial C respiration.

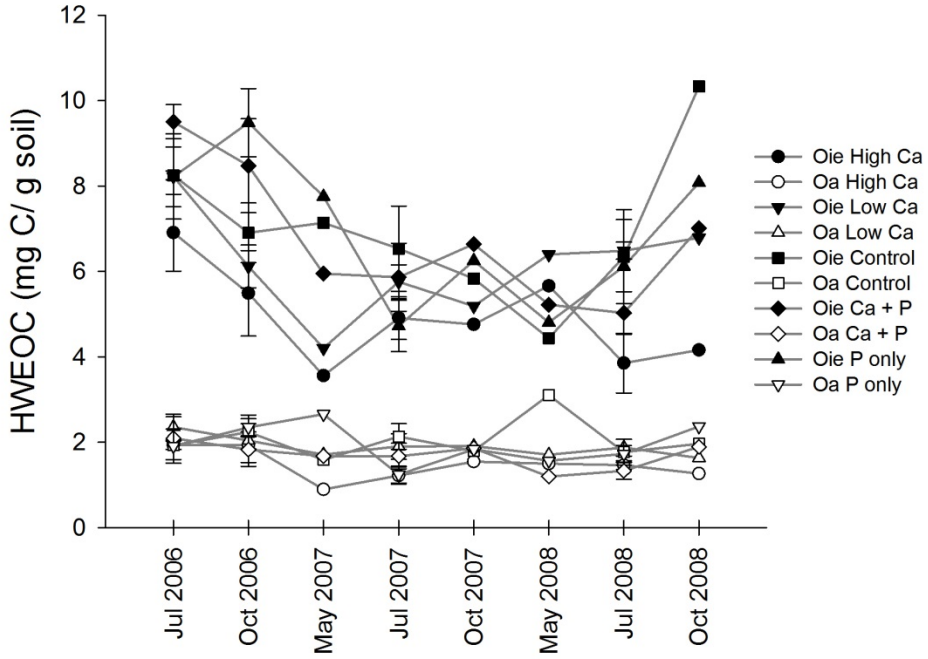

HWEOC (mg C/ g soil)
12
10
8
6
4
2
0
Jul 2006
Oct 2006
May 2007
Jul 2007
Oct 2007
May 2008
Jul 2008
Oct 2008
Oie High Ca
Oa High Ca
Oie Low Ca
Oa Low Ca
Oie Control
Oa Control
Oie Ca + P
Oa Ca + P
Oie P only
Oa P only

Figure 1

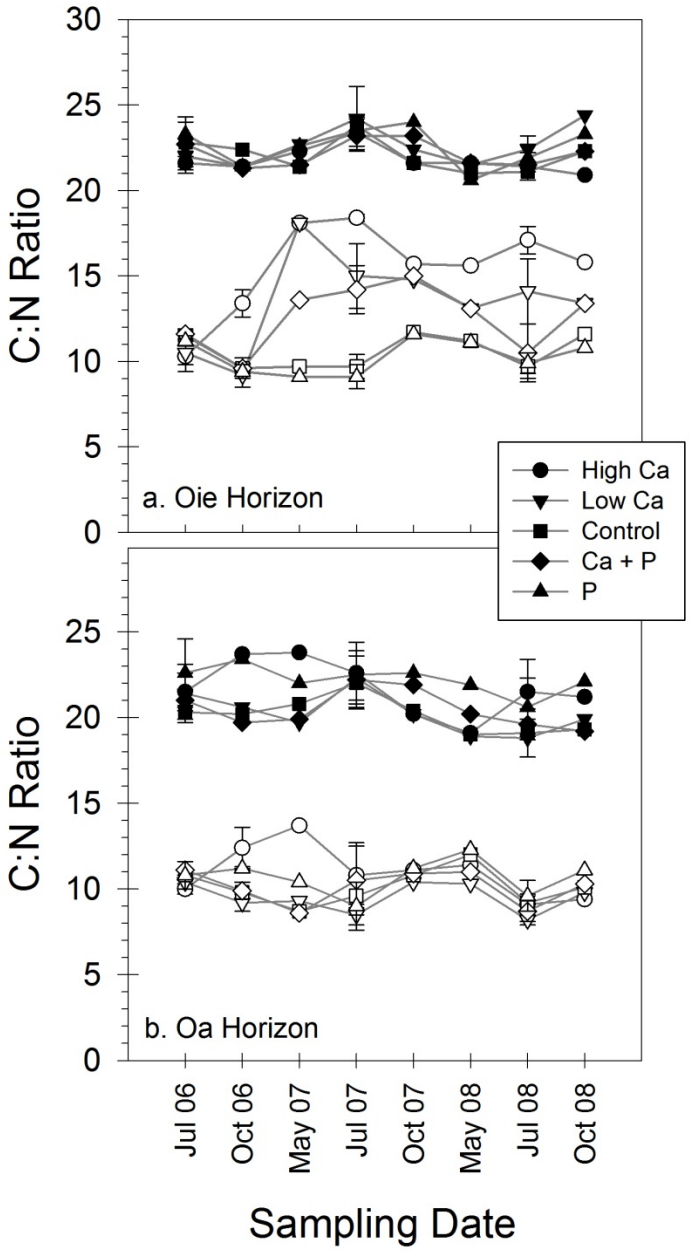

30
25
20
15
10
5
0
C:N Ratio
a. Oie Horizon
High Ca
Low Ca
Control
Ca + P
P
b. Oa Horizon
Jul 06
Oct 06
May 07
Jul 07
Oct 07
May 08
Jul 08
Oct 08
Sampling Date

Figure 2

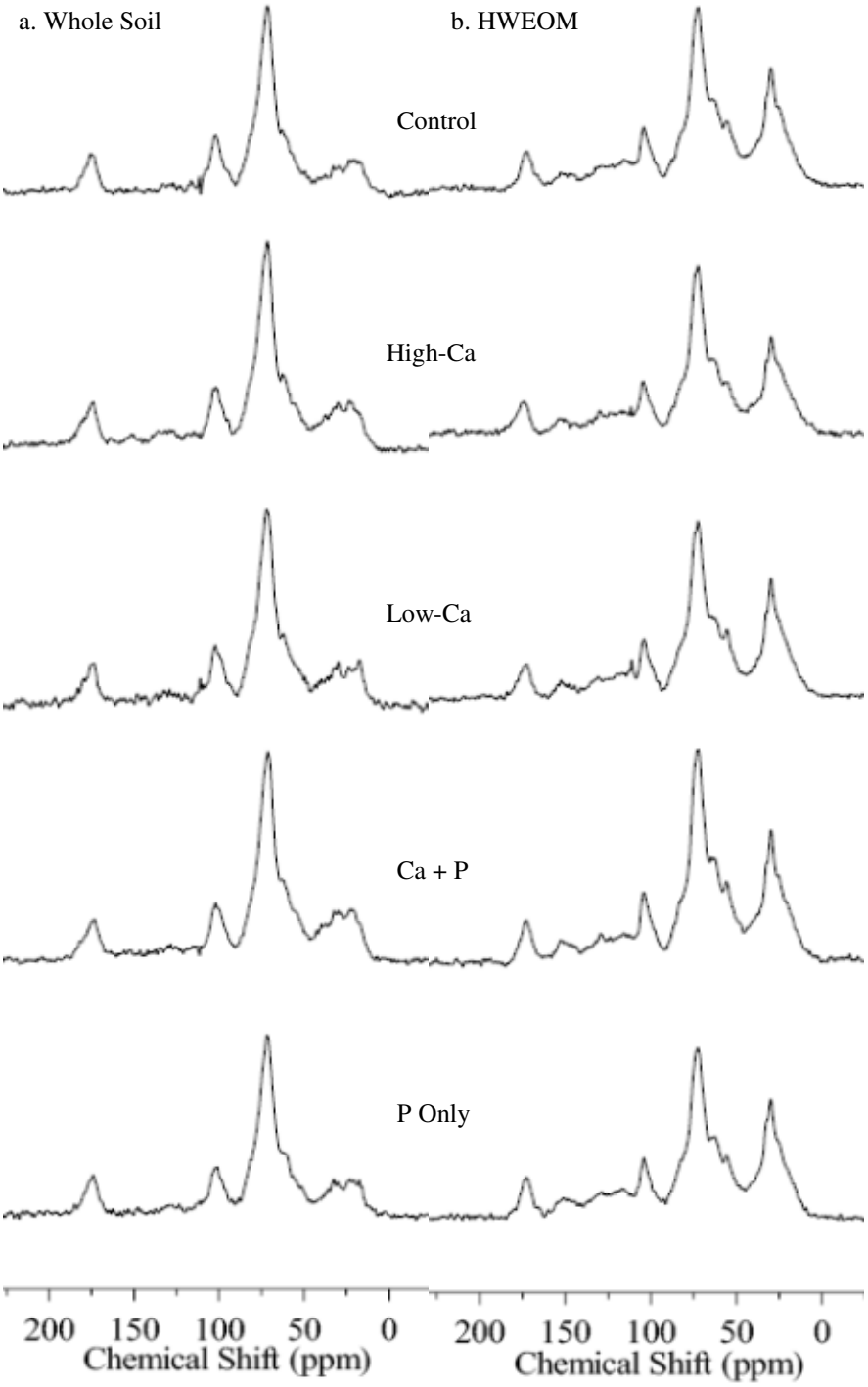

a. Whole Soil
b. HWEOM
Control
High-Ca
Low-Ca
Ca + P
P Only
200 150 100 50 0
Chemical Shift (ppm)
200 150 100 50 0
Chemical Shift (ppm)

Figure 3

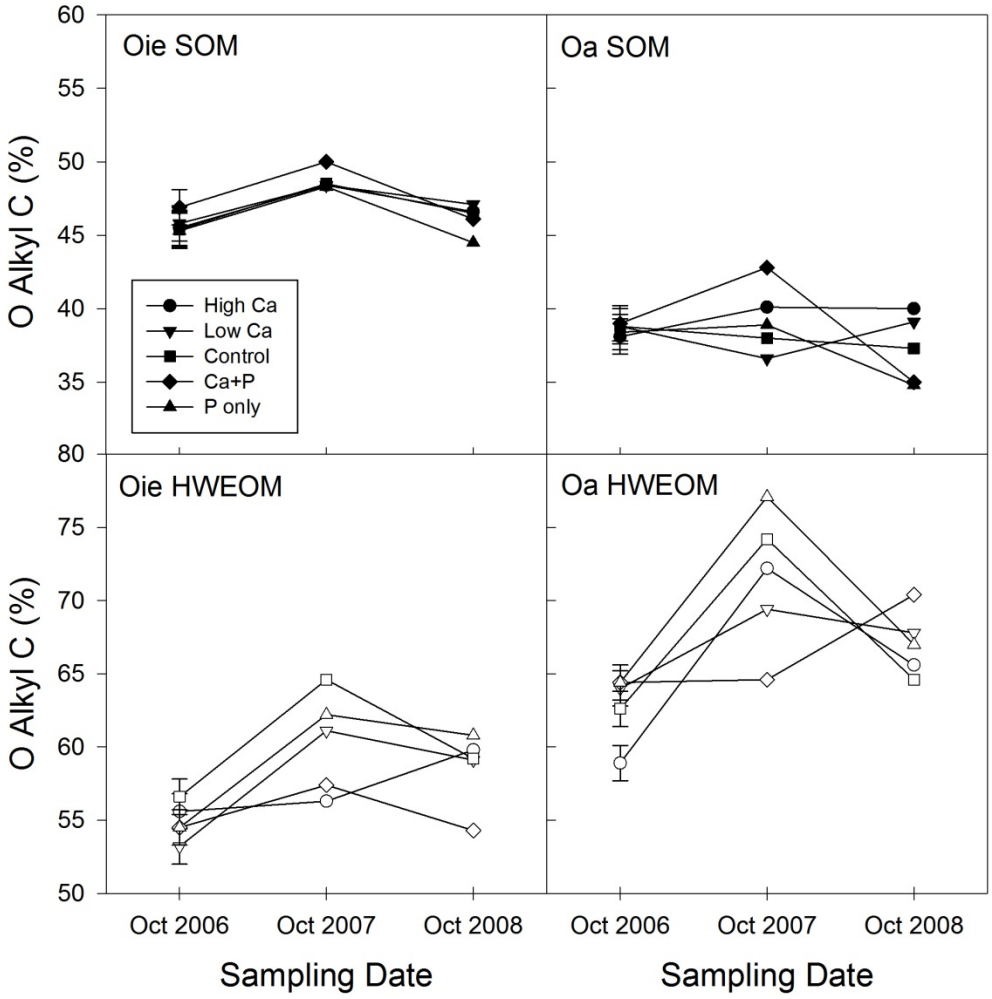

Oie SOM
Oa SOM
Oie HWEOM
Oa HWEOM
O Alkyl C (%)
O Alkyl C (%)
High Ca
Low Ca
Control
Ca+P
P only
Oct 2006
Oct 2007
Oct 2008
Oct 2006
Oct 2007
Oct 2008
Sampling Date
Sampling Date

Figure 4

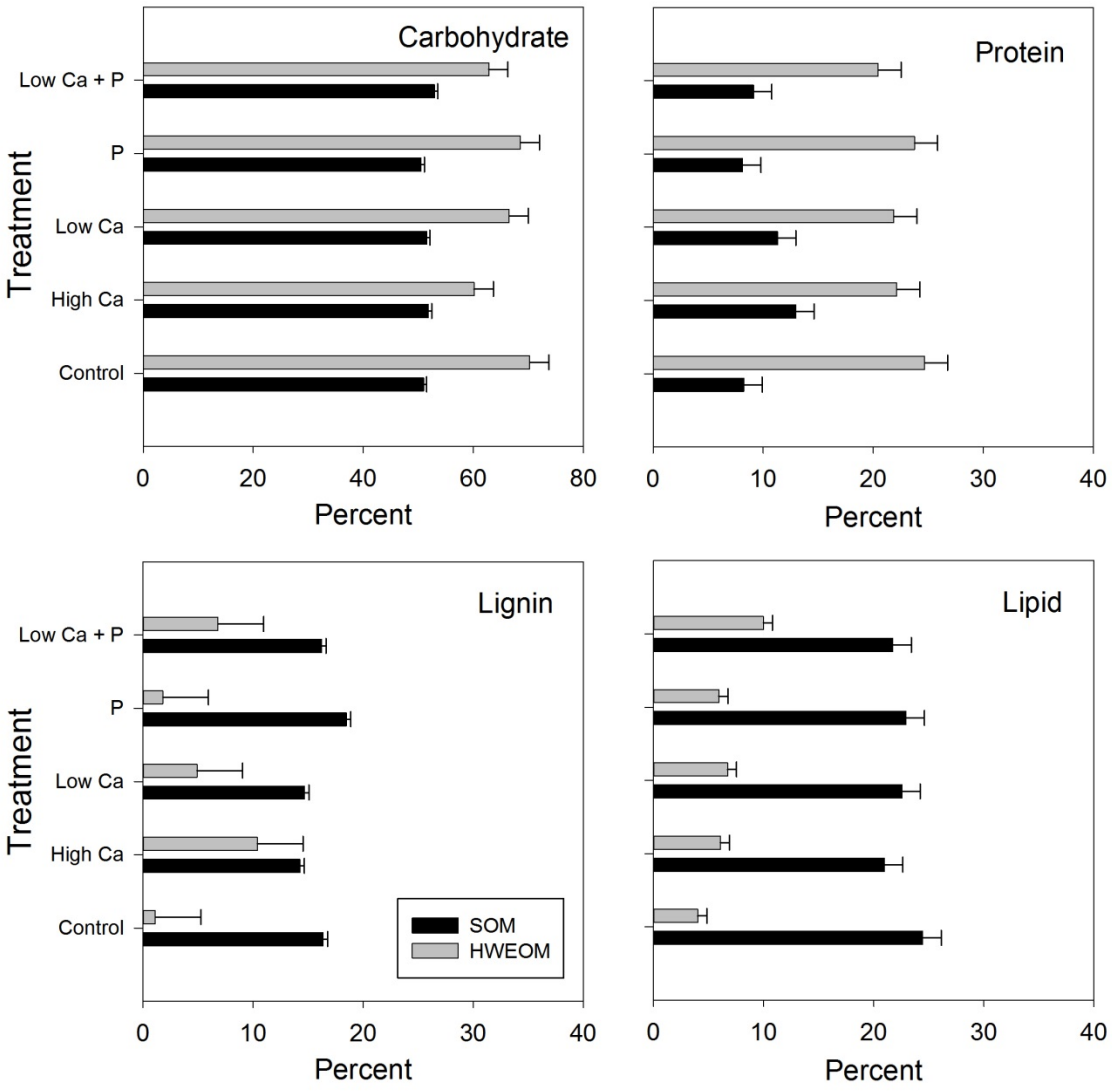

Carbohydrate
Protein
Lignin
Lipid
Treatment
Low Ca + P
P
Low Ca
High Ca
Control
Percent
0
20
40
60
80
10
30
SOM
HWEOM

Figure 5

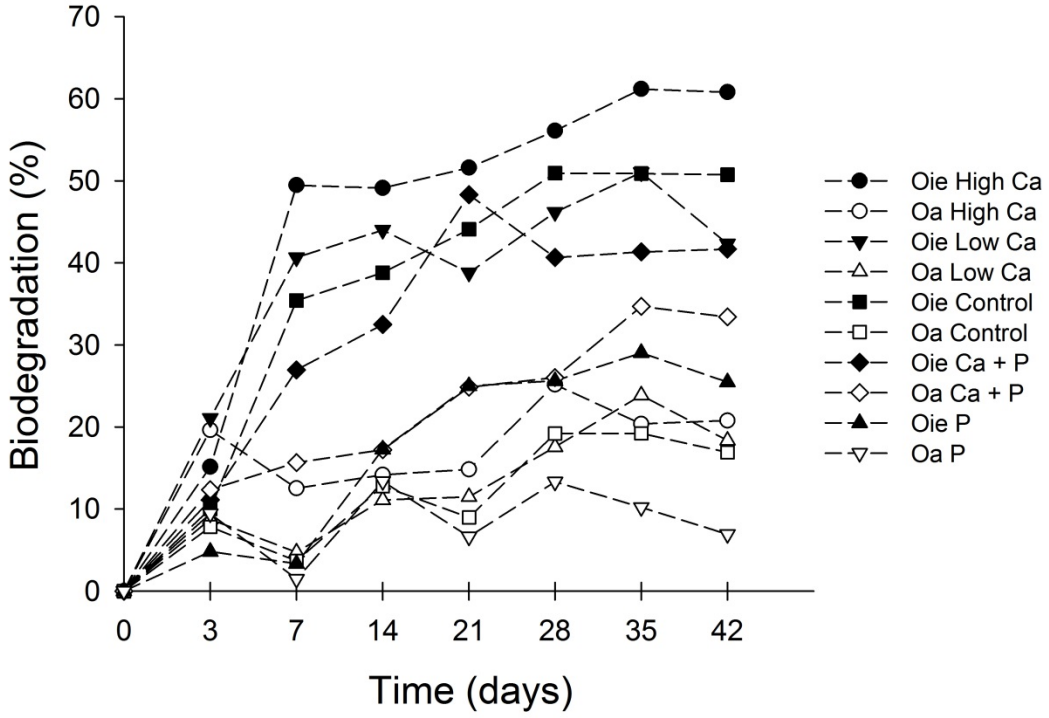
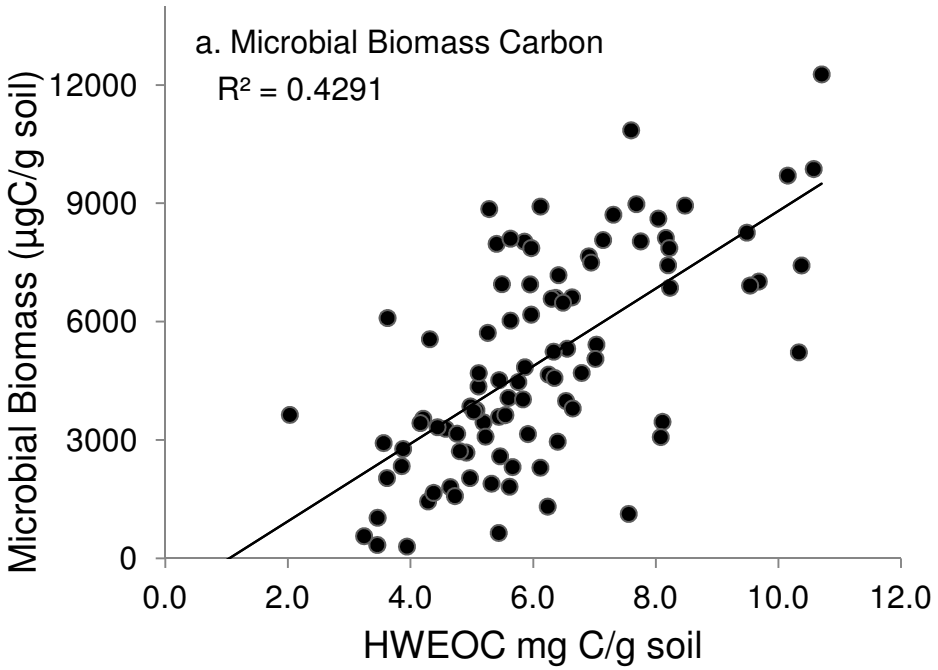

Biodegradation (%)
70
60
50
40
30
20
10
0
0
3
7
14
21
28
35
42
Time (days)
Oie High Ca
Oa High Ca
Oie Low Ca
Oa Low Ca
Oie Control
Oa Control
Oie Ca + P
Oa Ca + P
Oie P
Oa P

Figure 6


a. Microbial Biomass Carbon
R² = 0.4291
Microbial Biomass (µgC/g soil)
12000
9000
6000
3000
0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
HWEOC mg C/g soil

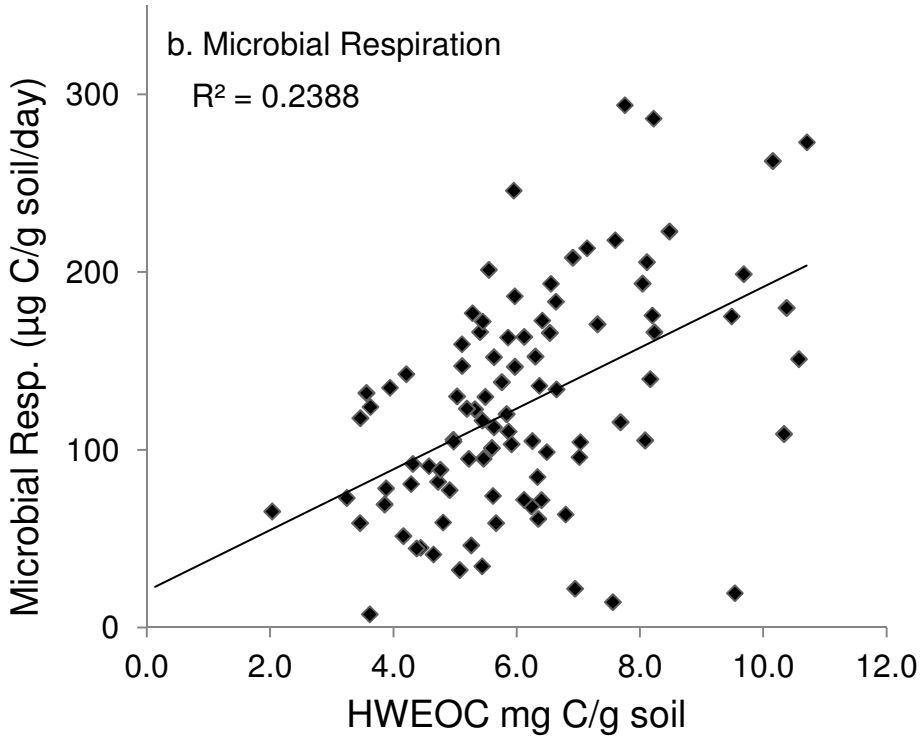

b. Microbial Respiration
R² = 0.2388
Microbial Resp. (µg C/g soil/day)
300
200
100
0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
HWEOC mg C/g soil

Figure 7