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# Effects of Calcium Treatment on Forest Floor Organic Matter Composition Along an Elevation Gradient

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# Effects of calcium treatment on forest floor organic matter composition along an elevation gradient

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**Abstract:** Calcium amendment is a restorative option for nutrient-depleted, acidic soils in the forests of the northeastern United States. We studied the effects of watershed-scale wollastonite ($CaSiO_3$) application on the structural composition of soil organic matter (SOM) and hot-water extractable organic matter (HWEOM) at the Hubbard Brook Experiment Forest in New Hampshire 7-9 years after treatment, along an elevation gradient. Soils in the high-elevation spruce/fir/birch (SFB) zone contained significantly greater amounts of HWEOM compared to lower elevation hardwood soils, likely due to differences in litter quality and slower decomposition rates in colder soils at higher elevation. The only significant difference in hot-water extractable organic carbon (HWEOC) concentration between reference and calcium-treated watersheds was in Oie horizons of the SFB zone, which also exhibited the greatest degree of soil chemical change after treatment. The ^{13}C nuclear magnetic resonance (NMR) spectra showed no significant patterns in O-alkyl C abundance for either soil or HWEOM along the elevation gradient, suggesting that there were no elevation-related patterns in carbohydrate concentration. The general absence of long-term effects in this study suggests that effects of Ca amendment at this dosage on the composition of soil organic matter were small or short-lived.

**Introduction**

In glaciated environments of New England, many higher elevation forests are characterized by shallow soils with small pools of weatherable minerals, resulting in higher soil acidity, lower stream calcium, lower acid-neutralizing capacity, lower cation exchange capacity, and shallow flow paths compared to lower-elevation stands (Likens et al. 1996; Johnson et al. 2000). Many of these acid-sensitive soils in the northeastern USA have deteriorated in overall quality and available calcium content due to a long history of acid rain (Driscoll et al. 2001). Liming or other forms of calcium amendment may help restore these acid-impacted systems to their pre-acidification state. At the Hubbard Brook Experimental Forest (HBEF) in New Hampshire, a watershed-scale study was initiated in 1999, in which wollastonite ($CaSiO_3$) was applied to replace soil Ca estimated to have been leached by acid rain (Peters et al. 2004; Groffman et al. 2006; Cho et al. 2010). Wollastonite dissolves more slowly than lime, resulting in slower release of Ca and a less dramatic 'pulse' response in soils and drainage waters (Peters et al. 2004). The wollastonite treatment on W1 resulted in increases in soil pH, exchangeable Ca, solution pH, acid neutralizing capacity, and dissolved Si after the wollastonite addition (Cho et al. 2010; 2012). The greatest chemical changes were found at higher elevations in the treated watershed.

In coarse-grained soils, soil organic matter is crucial to soil structure, water transport, and acid-base chemistry. Soil organic matter (SOM) also serves as an important microbial energy source (Metting 1993). Within the soil organic matter, the hot-water extractable fraction is highly correlated with soil microbial biomass and has been found to be a sensitive indicator for the effects of soil fertilization (Sparling et al. 1998; Ghani et al. 2003). The chemical composition of both SOM and hot-water extractable organic matter (HWEOM) has been found to vary with elevation and vegetation patterns (e.g., Bu et al. 2010; Djukic et al. 2010). The transition from

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hardwood vegetation at lower elevations to conifers at higher elevations results in strikingly different litter composition and, ultimately, SOM composition (Berg and Laskowski 2006). Higher elevations are associated with cooler and moister climates with larger pools of soil C and N, lower rates of organic matter decomposition, and longer turnover times (Simmons et al. 1996; Garten and Hanson 2006). Bohlen et al. (2001) investigated the microbial activity in soils at the Hubbard Brook Experimental Forest (HBEF) in New Hampshire and found higher elevation soils to be associated with greater nitrification rates and denitrification enzyme activity. Cooler soil temperatures have been found to result in SOM richer in labile structures such as O-alkyl C (Faz Cano et al. 2002; Dalmolin et al. 2006) and poorer in less degradable aromatic C (Faz Cano et al. 2002).

The purpose of this study was to examine the long-term impact of wollastonite ($CaSiO_3$) treatment on SOM, with an emphasis on elevation patterns. Calcium application has been found to influence soil microbial activity with contradicting results. While laboratory studies have shown that rates of microbial C and N cycle processes in acid soils increase with pH and/or Ca amendment, results from field studies have been mixed (Groffman and Fisk 2011). Some researchers have suggested that binding with Ca may limit the bioavailability of natural organic matter and, consequently, reduce the rates of microbial processes (e.g., Hobbie et al. 2002; Groffman et al. 2006). Furthermore, there is very limited knowledge concerning the effects of Ca treatment on soil organic matter quality, especially in the long term (Lorenz et al. 2001; Rosenberg et al. 2003). Melvin et al. (2013) documented large increases in C and N stocks in limed soils in the watershed of Woods Lake in the Adirondack Mountains of New York 19 years after liming treatment, along with significantly lower basal respiration rates in organic-horizon

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soils, suggesting that Ca treatment may have substantial long-term impacts on organic matter quality and quantity.

We investigated the composition of SOM and its hot-water extractable fraction 7 to 9 years after the wollastonite treatment at Hubbard Brook. We analyzed these effects at four different elevations within both a calcium-treated and a reference watershed with similar elevation ranges. The lowest three elevation zones in this study had predominantly hardwood vegetation while the highest elevation plots were characterized by spruce, fir, and white birch vegetation.

We anticipated that organic matter decomposition would be greater at lower elevations, and that Ca amendment would enhance organic matter decomposition by providing a more favorable soil environment for microbial activity. The specific hypotheses examined in the study were: (1) SOM and its hot-water extractable fraction are richer in structures related to carbohydrates (O-alkyl C) and lignin (O-aryl C) at higher elevations; (2) wollastonite treatment results in organic matter containing less O-alkyl C than organic matter in the reference watershed. We performed this work for Oie and Oa horizons separately, since the structure of both SOM and HWEOM varies with soil depth (Balaria et al. 2009; Balaria et al. 2013), as did the inorganic chemical response to wollastonite treatment (Cho et al. 2010).

**Methods**

**Site Description**

The HBEF is located in the southern portion of the White Mountain National Forest in central New Hampshire, USA (43°56'N, 71°45'W). The HBEF has a humid-continental climate, with long, cold winters (average January temperature: -9°C) and short, cool summers (average July

temperature: 19°C). Average annual precipitation at the site is 139.5 cm, with 30% falling as snow and a snowpack depth of up to 1.5 m (Federer et al. 1990). The HBEF is a second-growth forest with an average age of about 90 years. Boreal vegetation including red spruce (*Picea rubens* Sarg.), balsam fir (*Abies balsamea* [L.] Mill.), and white birch (*Betula papyrifera* Marsh.) occurs at higher elevations, and the northern hardwood species sugar maple (*Acer saccharum* Marsh.), American beech (*Fagus grandifolia* Ehrh.), and yellow birch (*Betula alleghaniensis* Britt.) dominate at lower elevations (Juice et al. 2006).

The soils of the HBEF are diverse. Most common are coarse-textured, well-drained Spodosols (Typic Haplorthods) originated from glacial till of largely local origin. The average depth of the O horizons is 6.9 cm (Johnson et al. 1991). The average pH in these surface organic horizon ranges from 3.4 to 4.0 (Johnson et al. 1991). Soil samples for this study were collected from sites within watershed 1 (W1) and just outside of watershed 6 (W6), at elevations ranging from 488 m to 747 m and 549 to 792 m, respectively. The partially decomposed litter and the fibrous layer (the Oi and Oe horizons) were collected as one sample and are referred to here as the Oie horizon. The highly decomposed humic layer (Oa horizon) was also sampled. These samples were collected from four different elevation zones, the highest in the boreal Spruce/Fir/Birch (SFB) zone (~790 m), and the others in High (~750 m), Mid (~600 m), and Low (~525 m) elevation hardwood zones.

Watersheds W1 and W6 are similar in climatic conditions (Federer et al. 1990); however, they differ in their treatment history. W6 is the biogeochemical reference watershed at the HBEF, while W1 was treated with wollastonite ($CaSiO_3$) in October 1999. An amount equivalent to 1028 kg Ca/ha was added by helicopter in an attempt to bring the base saturation from the then-current value of 10% to the estimated pre-acid-rain value of 19%. The average time for

wollastonite to dissolve was estimated to be approximately 7 years (Peters et al. 2004). The soil samples for this study were collected in July and October of 2006, and May, July, and October of 2007 and 2008. For each sampling event, five forest floor samples were collected from each elevation in each watershed. The samples collected near W6 were taken from a 15-m radius plot around an existing litter trap at each of the four elevations. Each sampling plot in W1 was a $2 \times 3$ m rectangle on moderately sloping ground near lysimeter installations at each elevation. Forest floor samples were collected using a 10-cm diameter PVC corer. The five samples from each elevation were separated by horizon, then pooled together for each site and sampling date for laboratory analyses. The samples were stored moist at 4 °C until extraction and then dried at 80 °C and stored for loss-on-ignition, elemental analyses, and solid-state nuclear magnetic resonance (NMR) spectroscopy. We found no significant differences among the three years for any of the response variables. We therefore treated the years as replicates for each season for statistical purposes.

**Hot-water Extraction and Chemical Analyses**

The Oie and Oa horizons for this study were subjected to hot-water extraction, as described in detail in Balaria et al. (2009). Hot-water extraction was performed by pooling the five replicates on an equal dry-mass basis. An aliquot of the extracted organic matter was analyzed for dissolved organic C. The remaining extract was freeze-dried. The freeze-dried hot-water extractable organic matter (HWEOM) was analyzed for loss-on-ignition, elemental composition, and NMR spectral properties following the procedures outlined in Balaria et al. (2009). The percentages of spectral intensity attributable to alkyl C, O-alkyl C (methoxyl, O-alkyl, and di-O-alkyl C), aryl C, O-aryl C, and carbonyl C (carboxyl and amide C) were computed using the chemical shift assignments in Nelson and Baldock (2005). For soil analyses, each replicate was

dried and ground separately and then combined on an equal mass basis. The dried soil samples were also analyzed for loss-on-ignition and elemental composition for all sampling dates. The $^{13}C$ NMR spectra were acquired using the cross polarization with magic angle spinning (CPMAS) and dipolar dephasing (DD) techniques (Balaria et al. 2009). The NMR spectra were obtained for all HWEOM samples, but only for the October soil samples.

**Statistical Analyses and Data Representation**

For quality control and quality assurance, blank and control standards were used after every ten samples for dissolved organic C and elemental analyses. Duplicate samples were analyzed at random to ascertain the reproducibility of the data. We used analysis of variance with Tukey's honestly significant difference at a confidence level of 95% to compare elevations or watersheds for each of the analyses performed. Since we composited five replicates for each sampling event to get a representative sample for each elevation, watershed, and horizon, and then combined the three years based on each season, we were left with two data points for May (2007 and 2008) and three data points for July and October (2006, 2007, and 2008). We used the standard errors based on these two or three data points in constructing the figures in this paper.

**Results**

**Soil organic matter and hot-water extractable organic matter**

The results of the hot-water extractable organic C (HWEOC) measurements are presented in Fig. 1. The Oie horizon had significantly greater HWEOC than the Oa horizon in both reference (W6) and wollastonite-treated (W1) watersheds at each elevation. There was a general decreasing

pattern in HWEOC with decreasing elevation for all watersheds and horizons. Soils in the Spruce/Fir/Birch (SFB) elevation zone of the reference watershed (W6) had HWEOC concentrations that were significantly higher than the three lower elevations in the Oie horizon, and significantly higher than the lowest two elevations in the Oa horizon. In W1, HWEOC concentration was significantly higher in SFB Oa horizons than those in mid and low elevations, but no significant differences were observed between elevations in Oie horizons. The long-term treatment effects of wollastonite were only visible in Oie horizons of the SFB zone, which showed significantly lower HWEOC concentration compared to the reference watershed (W6).

Organic matter concentration for the soil and the HWEOM fraction was determined using loss-on-ignition and is presented for the October samples in Fig. 2a and 2b, respectively. For soils, the Oie horizon had significantly greater organic matter concentration than the Oa horizon in both treated and reference watersheds at all elevations (Fig. 2a). Reference (W6) and calcium-treated (W1) watersheds had significantly different organic matter concentration only in Oa horizons in the low-elevation zone. Soils from both Oie and Oa horizons in the SFB zone in treated W1 had significantly higher SOM concentration than mid and low elevations. For reference W6, no significant elevational differences in organic matter concentration were observed for Oie horizons, but in the Oa horizon, the organic matter concentration decreased significantly from the SFB zone to the high-elevation hardwood zone, and from high to mid elevation hardwood sites.

Loss-on-ignition was high (> 85%) in all HWEOM samples (Fig. 2b), indicating that HWEOM was, indeed, primarily organic matter. There were no significant differences in the loss-on-ignition of HWEOM between Oa and Oie horizons in either watershed (Fig. 2b). No elevation patterns were observed in the loss-on-ignition of HWEOM for W1 in either horizon or in the Oie

horizon for W6. However, similar to the soil, HWEOM in the Oa horizon in W6 had significantly higher loss-on-ignition in the SFB zone compared to high and mid elevations.

**Chemical and structural composition of SOM**

The H:C and O:C ratios of soil and HWEOM were compared to model biomolecular compounds present in forest soils using a van Krevelen diagram (see Balaria et al. 2009 for details on the composition of the model compounds). Figures 3 and 4 show the treatment effect of Ca amendment and the effect of elevation, respectively, on the composition of whole soils and HWEOM.

Soil organic matter samples plot in a space generally bounded by carbohydrates, proteins and lipids, lignin, and humic substances (humic and fulvic acids) (Fig. 3a, 4a). The HWEOM samples lie in a narrower range, closer to carbohydrates and chitin, indicative of high contributions of sugars and microbial residues in this highly bioavailable fraction (Balaria and Johnson 2013). The SOM composition showed a great deal of variability, but no differences were found between the treated and reference watersheds (Fig. 3a) or between SFB and low elevations (Fig. 4a). The HWEOM samples appeared to occupy slightly different positions for W1 compared to the reference watershed, indicating a possible treatment effect even seven years after the treatment (Fig. 3b). On the whole, HWEOM samples collected from W6 had somewhat higher H:C ratios and lower O:C ratios compared to W1. The HWEOM present at low elevation generally had higher O:C and H:C ratios than HWEOM from the SFB zone, plotting closer to soil polysaccharides and carbohydrates, while that from the SFB zone lies more toward the lignin composition (Fig. 4b).

Figure 5 presents examples of solid-state $^{13}$C CPMAS NMR spectra of soils and HWEOM from the reference watershed (W6) for the Oie horizon. All spectra were integrated based on the following regions (Nelson and Baldock 2005): alkyl C (0-45 ppm), O-alkyl C (45-110 ppm), aryl C (110-144 ppm), O-aryl C (144-165 ppm), and carbonyl C (165-190 ppm). The integrated signal intensities provide estimates of the percent contributions from these major functional groups. The results are given in Table 1 for the reference and calcium-treated watersheds for soil and HWEOM in both Oie and Oa horizons.

The alkyl C region in the soil spectra contained a large peak at 30 ppm (Fig. 5), indicating the presence of mobile methylene (-CH$_2$-) groups in aliphatic rings or chains as well as terminal methyl groups (Rosenberg et al., 2003; Balaria et al., 2009). This peak appeared to decrease in relative intensity with decreasing elevation for Oie as well as Oa horizon soils for both control and Ca treated watersheds (Fig. 5). These elevation-related differences, however, were not statistically significant. Alkyl C accounted for 30-40% of the signal intensity of SOM. While similar in value for Oie and Oa horizon of the reference watershed, Oa horizons in the calcium-treated watershed had a significantly greater percentage of alkyl C than Oie horizons (Table 1).

The HWEOM spectra contain a very small and broad peak in the alkyl C region, as opposed to the large peak in the spectra of the soils from which this organic matter was extracted (Fig. 5). We found no significant effect of elevation or Ca treatment on the alkyl C concentration of HWEOM samples.

The O-alkyl C region includes the peaks for methoxyl/N-alkyl C (45-60 ppm), non-aromatic ring O-alkyl C (60-90 ppm), and di-O-alkyl anomeric C (90-110 ppm). All spectra included a 55 ppm shoulder, indicative of methoxyl C. This region also exhibited no patterns with elevation in either

Oie or Oa horizons for SOM. For HWEOM, the O-alkyl C region was the largest contributor to the CPMAS spectra, suggesting their richness in carbohydrates and more biodegradable molecules (Balaria et al. 2009). O-alkyl C represented 60%-80% of the C in HWEOM (Table 1). There was no elevation-related pattern for O-alkyl C, although the Oa horizon had a higher percentage than the Oie horizon, as observed previously (Balaria et al. 2009). The percent distribution values for W1 and W6 were similar in these regions, indicating an absence of treatment effects 7-9 years after the Ca treatment.

The aryl C (110-144 ppm) and O-aryl C (144-165 ppm) regions represent aromatic C-H/C-R (e.g., in guaiacyl lignin) and C-OR (e.g., in syringyl lignin), respectively, suggesting the contributions from conifer and hardwood litter. These peaks were very small for all CPMAS spectra in both Oie and Oa horizons, indicating minor contributions from lignin or lignin derivatives in our soils. The O-aryl peak was, however, a little larger in the lower elevations than the SFB zone. These peaks accounted for a very small proportion of the total signal in the soil spectra, with values around 8-12% and 3-6% in the aryl and O-aryl C regions, respectively (Table 1). These values were similar for Oie and Oa horizons, and had no specific pattern with elevation in the reference watershed. The Ca-treated watershed appeared to have an increasing pattern with decreasing elevation, but it was statistically insignificant. For HWEOM, both aryl and O-aryl C contributed less than 8% of the signal intensity in spectra from both watersheds (Table 1). No significant elevation or Ca treatment effect was observed for these regions for HWEOM either.

The carbonyl C region (165-190 ppm) includes contributions from carboxylated and amide structures in proteins, fatty acids, and degraded lignins (Rosenberg et al. 2003). This peak was very small compared to the alkyl and O-alkyl C peaks for the CPMAS spectra of the soil

samples, suggesting minimal contributions from these biomolecules. Carbonyl C values were slightly higher for W6 compared to W1, but W6 also had a high variability, making it difficult to draw any conclusions. These values were approximately 6-8% for the HWEOM from these two watersheds. There was no elevation-related pattern in the carbonyl C region.

Elemental analyses were performed to estimate the C:N ratios of the soils in Ca-treated and reference watersheds at all four elevations. Figure 6 presents the C:N ratios of Oie and Oa horizon soils and HWEOM for samples collected in October 2006-2008. We found no significant difference in C:N ratios between the reference and treated watershed at any elevation in either the Oie or Oa horizon. The Oie and Oa horizons also had similar C:N ratios in both soils and HWEOM for all elevations and treatments.

The C:N ratios for the reference watershed (W6) differed at different elevations for both the whole soil and HWEOM (Fig. 6). We observed higher C:N ratios in the SFB zone compared to the upper deciduous zone soils: significantly greater than high elevation for Oie and significantly greater than high and mid elevation for Oa horizons. Within the deciduous region, high and mid elevations had lower C:N ratios than the low elevation zone for both Oie and Oa horizons in soil as well as HWEOM. W1, however, showed no significant differences among different elevations for either Oie or Oa horizons.

**Discussion**

**Influence of Elevation on SOM Composition**

In temperate forest soils, higher elevations have been associated with greater soil organic C stocks (Johnson et al. 2000; Garten and Hanson 2006), but not necessarily higher microbial activity (Bohlen et al. 2001). We expected to find an increasing pattern in SOM concentration with increasing elevation as well as increasing HWEOC yield and loss-on-ignition. For reference W6, Oie horizons in the SFB zone soils were significantly higher in HWEOC yield than the three lower elevations, and SFB Oa horizons had significantly greater HWEOC than the two lowest elevations (Fig. 1). The lower three elevations, all in hardwood zones, had no significant differences amongst each other in organic matter or HWEOC concentration. This indicates an important link between vegetation patterns and soil organic C for these soils, with conifer vegetation supporting higher levels of SOM and HWEOC than deciduous vegetation. In W1, similar results were obtained for Oa horizons but no significant differences were observed for the Oie horizon, suggesting that Ca treatment may have lowered HWEOC in SFB Oie horizons.

Total organic matter concentration, as shown by loss-on-ignition, was also significantly higher in SFB-zone soils compared to lower elevations, further suggesting that Hubbard Brook soils are richer in soil organic matter in the SFB zone (Fig. 2). Soil temperature and litter quality are likely the most important factors in this pattern. Soils at higher elevations are relatively cooler, perhaps resulting in lower rate of organic matter decomposition. Lower soil temperatures have been associated with higher SOM concentration in numerous studies (Hart and Perry 1999; Kane et al. 2005). Similarly, litter quality is known to be a dominant factor in decomposition processes, with coniferous litter exhibiting slower decomposition rates than deciduous litter

(McClaugherty et al. 1985). Interesting, though, Bohlen et al. (2001) found greater microbial biomass C and N at higher elevations in W6, indicating the complex nature of soil-microbial-vegetation interactions.

Interactions between elevation, Ca treatment and vegetation type were further explored by a structural evaluation of the SOM and HWEOM using elemental analyses and NMR spectroscopy. We found no significant differences in H:C and O:C ratios with elevation for either HWEOM or the soil (Figs. 3 and 4). The patterns for C:N ratios were different between the two watersheds. While Ca-treated W1 showed no significant elevational effects, C:N ratios were higher in the SFB zone compared to the upper deciduous region in the reference watershed (Fig. 6). Microbial activity generally results in the preferential consumption of soil organic C, resulting in a lower C:N ratio of SOM. Lower microbial activity in the SFB zone, perhaps due to lower bioavailable organic C content of the soil or other climatic factors (e.g. soil temperature, litter quality), may be responsible for the higher C:N ratios we observed. Therefore a study of these structures using advanced tools such as NMR spectroscopy may be helpful in understanding these interactions.

The NMR spectra of soils showed an increase in aryl and O-aryl C in Oa horizons with decreasing elevation, suggesting that soils at lower elevations might be richer in lignin content, which is rather recalcitrant towards microbial consumption (Table 1). Warmer soils have been found to be richer in aromatic C, indicative of the accumulation of lignin and lignin derivatives during decomposition (Faz Cano et al. 2002). However, the elemental composition of soils from SFB and low-elevation sites plotted in similar positions in the van Krevelen diagram (Fig. 4), suggesting that there were no substantial differences in biomolecular composition.

In contrast, HWEOM from the SFB and low-elevation sites occupied different positions in the van Krevelen diagram (Fig. 4). The HWEOM obtained from soils at low elevation was closer to the soil polysaccharide region, while that from SFB zone soils was generally closer in position toward the lignin region. Bu et al. (2010) also found that HWEOM from higher elevations was rich in aromatic moieties compared to lower-elevation soils. Thus, it appears that the lignin in the conifer-rich SFB zone was more water-soluble than the lignin in the lower, hardwood-dominated zones.

The NMR spectra of HWEOM in this study showed no significant pattern for aryl C and O-aryl C in either watershed. Moreover, we did not find any differences with elevation in alkyl C or O-alkyl C for either the whole soil or HWEOM. Thus, there is no direct elevational pattern in the content of the most biodegradable compounds such as carbohydrates. This relative uniformity in SOM structure with elevation may help explain why C mineralization was found to be similar at different elevation in the soils of reference watershed (W6) for Hubbard Brook (Bohlen et al. 2001).

**Long-term Effects of Ca Treatment**

The effects of wollastonite treatment on HWEOC concentration seven years after the application were only visible at high elevations (Fig. 1). Cho et al. (2010) also observed that wollastonite treatment had the greatest effects on soil chemistry in the SFB zone of W1. We found a significantly higher concentration of HWEOC in the SFB zone in W6 compared to W1, suggesting that Ca treatment may have reduced the solubility of soil organic C. The overall organic matter concentration was greater for W1 compared to W6 at higher elevations, which may be due to a slower rate of biodegradation of organic matter in W1, suggesting a possible

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negative effect of Ca treatment on microbial activity (Groffman et al. 2006; Melvin et al. 2013). These results are consistent with recent studies suggesting that binding between Ca and labile organic matter structures may limit the bioavailability of those compounds (e.g., Hobbie et al. 2002; Groffman et al. 2011). In a particularly dramatic case, Melvin et al. (2013) found that C and N stocks in the forest floor of limed soils were double the stocks in unlimed soils in the watershed of Woods Lake, New York, 19 years after treatment.

In this study, C:N ratios were not significantly different between the two watersheds at any elevation (Fig. 6). Belkacem and Nys (1995) observed a decrease in C:N for O and A horizon after liming of a Moder humus soil. They also found a slight increase in C:N in the O horizon of a mull humus soil after liming. A lower C:N ratio suggests enhanced microbial activity since during decomposition of organic matter, C is respired as $CO_2$ while N may be assimilated in the SOM structure (Neale et al. 1997; Rosenberg et al. 2003). The similar C:N ratios between the two watersheds 7-9 years after the wollastonite treatment indicates that any treatment effects were small or short-lived. Moore et al. (2012) also did not observe significant changes in the C:N ratio of the humus (O) layer in a northern hardwood stand 15 years after liming with 0.5 to 50 Mg ha$^{-1}$ of dolomitic lime at a site in Québec. Ingerslev (1997) also observed no effect on C:N ratios in conifer forests in Denmark eight years after liming.

Long-term effects of liming on organic matter functional groups using $^{13}$C CPMAS NMR spectroscopy have previously been studied for German soils in spruce forests. Lorenz et al. (2001) found no significant differences in NMR spectra between limed and control soils in three of the four soil types studied 7-13 years after treatment. However, in the fourth soil (a Dystric Luvisol), they found lower O-alkyl C intensity in the limed plots. A decrease in the alkyl C/O-alkyl C ratio and in alkyl C concentration was observed by Rosenberg et al. (2003) 4-11 years

after liming, and was attributed to an increase in fine root input in Oe and Oa horizons after liming, causing O-alkyl C to increase. We found no significant differences between the organic structures of control (W6) and Ca-treated (W1) watersheds for any elevation (Table 1). The absence of significant differences in the structural chemistry of SOM between W1 and W6 suggests that the binding of labile organic matter with Ca, if it is occurring, does not alter the fundamental structure of the SOM.

In a short-term, plot-based study described in Balaria (2011), some influence of Ca treatment on O-alkyl C in HWEOM was observed, which was only significant at a substantially higher dose of Ca, five times the dose that was used on W1. The lack of response after 7-9 years of low-dosage Ca treatment is therefore not surprising. The SOM showed no significant changes in either this study or the plot experiments (Balaria 2011). Therefore, any Ca-related effects on the structure of the SOM, as well as its bioavailable fraction, do not appear to have persisted in the long-term on W1.

The relative absence of Ca-treatment effects observed in this study may be the result of three factors: material, dose and time. Wollastonite, a silicate mineral, dissolves more slowly and produces a smaller pH effect than lime. Thus, one would expect a smaller effect on the microbial environment at a particular Ca dose. Furthermore, the Ca dose used in this study was lower than the liming studies discussed above. The wollastonite application on W1 added 1028 kg Ca ha^{-1}. The liming studies with which we compared our results featured Ca additions ranging from 1200 kg ha^{-1} (Lorenz et al. 2001) to 2760 kg ha^{-1} (Melvin et al. 2013). Finally, it is possible that we missed the period in which significant effects may have occurred by focusing our efforts 7-9 years post-treatment.

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**Table 1.** Distribution of NMR spectral intensity among major C resonance regions in soil organic matter and hot-water-extractable organic matter (HWEOM) from organic horizons at different elevations in reference W6 (Ref) and wollastonite-treated W1 (Trt). Values shown are percentages of total spectral intensity, mean ± standard error (N=3). SFB: Spruce/Fir/Birch zone; High, Mid, and Low refer to the corresponding hardwood zones.

| Horizon | Zone | Watershed | Alkyl C | O-Alkyl C | Aryl C | O-Aryl C | Carbonyl C |
|---------|------|-----------|---------|-----------|--------|----------|------------|
| | | | | Soil Organic Matter | | | |
| Oie | SFB | Ref | 32.4 ± 1.3 | 47.1 ± 2.5 | 11.2 ± 0.7 | 4.7 ± 0.9 | 4.7 ± 0.5 |
| | | Trt | 29.7 ± 1.6 | 51.3 ± 1.6 | 10.6 ± 0.5 | 3.4 ± 0.7 | 5.0 ± 0.9 |
| Oie | High | Ref | 29.7 ± 0.4 | 50.0 ± 1.6 | 11.1 ± 0.7 | 4.4 ± 0.9 | 4.8 ± 1.6 |
| | | Trt | 28.5 ± 0.9 | 49.0 ± 1.6 | 11.4 ± 0.3 | 4.7 ± 0.6 | 6.5 ± 0.4 |
| Oie | Mid | Ref | 30.0 ± 4.4 | 47.8 ± 4.6 | 11.0 ± 0.5 | 4.3 ± 0.9 | 6.9 ± 1.2 |
| | | Trt | 25.7 ± 1.5 | 49.1 ± 0.3 | 12.4 ± 1.1 | 5.6 ± 0.4 | 7.3 ± 0.2 |
| Oie | Low | Ref | 27.9 ± 0.2 | 49.6 ± 1.5 | 11.6 ± 0.5 | 4.7 ± 0.6 | 6.3 ± 0.6 |
| | | Trt | 27.7 ± 0.7 | 51.0 ± 1.3 | 11.1 ± 0.4 | 4.3 ± 0.8 | 5.9 ± 0.9 |
| Oa | SFB | Ref | 35.2 ± 0.8 | 44.7 ± 0.8 | 10.3 ± 0.6 | 3.8 ± 0.5 | 6.0 ± 0.5 |
| | | Trt | 37.2 ± 0.4 | 43.5 ± 1.5 | 9.1 ± 0.3 | 3.4 ± 0.6 | 6.8 ± 1.1 |
| Oa | High | Ref | 35.1 ± 2.9 | 44.7 ± 1.4 | 10.5 ± 0.4 | 3.8 ± 0.8 | 6.0 ± 0.5 |
| | | Trt | 40.8 ± 1.4 | 39.9 ± 2.0 | 10.0 ± 0.1 | 3.4 ± 0.2 | 5.9 ± 0.4 |
| Oa | Mid | Ref | 30.8 ± 0.7 | 44.1 ± 1.2 | 12.8 ± 0.3 | 4.7 ± 0.4 | 7.6 ± 0.9 |
| | | Trt | 38.3 ± 1.7 | 40.4 ± 0.6 | 11.5 ± 0.8 | 3.8 ± 0.7 | 6.1 ± 0.9 |
| Oa | Low | Ref | 31.2 ± 3.2 | 49.3 ± 0.6 | 11.9 ± 1.1 | 3.9 ± 0.7 | 3.7 ± 1.3 |
| | | Trt | 35.1 ± 1.4 | 43.2 ± 1.1 | 11.4 ± 1.0 | 3.9 ± 0.7 | 6.4 ± 0.8 |

| | | | —————————————— HWEOM —————————————— | | | | |
|---|---|---|---|---|---|---|---|
| Oie | SFB | Ref | 20.1 ± 1.0 | 62.1 ± 1.7 | 5.8 ± 0.6 | 3.6 ± 0.6 | 8.5 ± 2.2 |
| | | Trt | 20.2 ± 1.1 | 64.2 ± 1.4 | 5.7 ± 1.0 | 3.8 ± 0.6 | 6.2 ± 1.0 |
| Oie | High | Ref | 17.6 ± 0.2 | 66.5 ± 2.4 | 5.0 ± 0.7 | 4.2 ± 1.2 | 6.7 ± 1.5 |
| | | Trt | 19.1 ± 0.6 | 61.7 ± 1.7 | 8.2 ± 0.7 | 6.4 ± 0.3 | 4.7 ± 1.5 |
| Oie | Mid | Ref | 20.9 ± 1.0 | 60.9 ± 2.5 | 6.2 ± 0.2 | 4.1 ± 0.2 | 7.9 ± 2.3 |
| | | Trt | 19.8 ± 0.3 | 63.5 ± 0.7 | 6.5 ± 0.5 | 5.2 ± 0.5 | 5.1 ± 0.8 |
| Oie | Low | Ref | 16.8 ± 2.4 | 66.4 ± 5.8 | 6.1 ± 0.3 | 5.5 ± 0.5 | 5.0 ± 0.8 |
| | | Trt | 21.7 ± 0.4 | 61.7 ± 0.3 | 8.2 ± 0.7 | 6.4 ± 0.3 | 4.7 ± 1.5 |
| Oa | SFB | Ref | 16.1 ± 1.6 | 69.1 ± 2.6 | 4.6 ± 0.6 | 3.0 ± 1.9 | 7.3 ± 1.7 |
| | | Trt | 16.5 ± 1.1 | 71.1 ± 1.2 | 2.8 ± 0.03 | 3.5 ± 1.0 | 6.1 ± 1.0 |
| Oa | High | Ref | 12.8 ± 2.3 | 75.0 ± 2.2 | 3.8 ± 0.7 | 4.2 ± 1.3 | 4.2 ± 0.9 |
| | | Trt | 14.9 ± 1.1 | 72.3 ± 2.3 | 3.7 ± 0.1 | 4.5 ± 0.2 | 3.9 ± 1.1 |
| Oa | Mid | Ref | 14.6 ± 4.1 | 68.3 ± 4.3 | 5.7 ± 0.2 | 4.4 ± 1.3 | 7.0 ± 1.5 |
| | | Trt | 15.6 ± 0.4 | 72.7 ± 1.4 | 3.3 ± 0.4 | 4.5 ± 0.5 | 4.0 ± 1.0 |
| Oa | Low | Ref | 13.9 ± 0.9 | 72.0 ± 2.3 | 4.2 ± 0.9 | 2.6 ± 1.5 | 7.3 ± 2.0 |
| | | Trt | 16.8 ± 0.6 | 68.8 ± 1.0 | 3.9 ± 0.5 | 5.2 ± 0.8 | 5.3 ± 0.9 |

**Figure Captions**

**Fig. 1.** Hot-water extractable organic carbon in Oie and Oa horizons of calcium-treated (W1) and reference (W6) forests along an elevation gradient (mean ± standard error, N=3).

**Fig. 2.** Organic matter concentration of (a) soil and (b) hot-water extractable organic matter (HWEOM) for Ca-treated W1 and reference W6 in four different elevation zones for Oie and Oa horizons (mean ± standard error, N=3).

**Fig. 3.** van Krevelen diagrams presenting a comparison of H:C and O:C ratios in (a) soil and (b) hot-water-extractable organic matter (HWEOM) from Ca-treated W1 and reference W6.

**Fig. 4.** van Krevelen diagrams presenting a comparison of H:C and O:C ratios in the Spruce/Fir/Birch (SFB) zone and low-elevation hardwood zone for (a) soil and (b) hot-water-extractable organic matter (HWEOM), and selected model compounds.

**Fig. 5.** Solid-state cross polarization with magic-angle spinning (CPMAS) $^{13}$C nuclear magnetic resonance spectra of an Oie horizon sample from reference W6 for (a) soil and (b) HWEOM at four different elevations: Spruce/fir/birch (SFB), High, Mid, and Low.

**Fig. 6.** C:N ratios of (a) soil and (b) hot-water extractable organic matter (HWEOM) for Ca-treated W1 and reference W6 in four different elevation zones for Oie and Oa horizons (mean ± standard error, N=3).
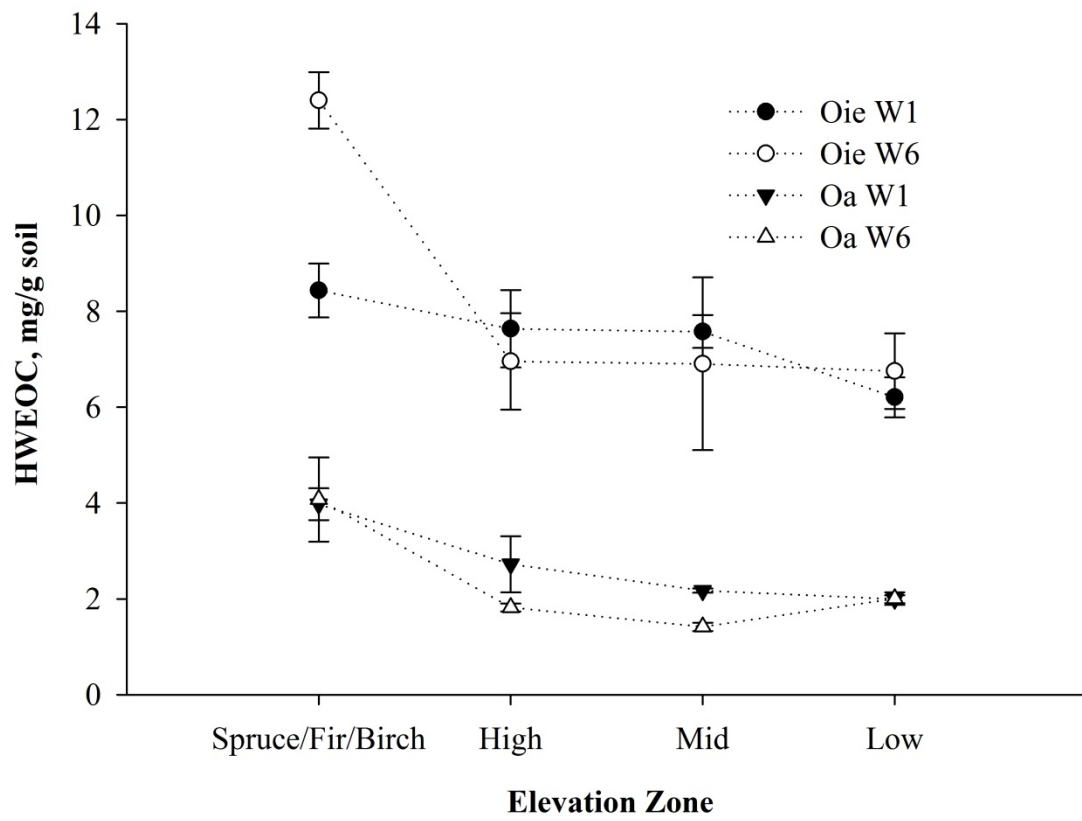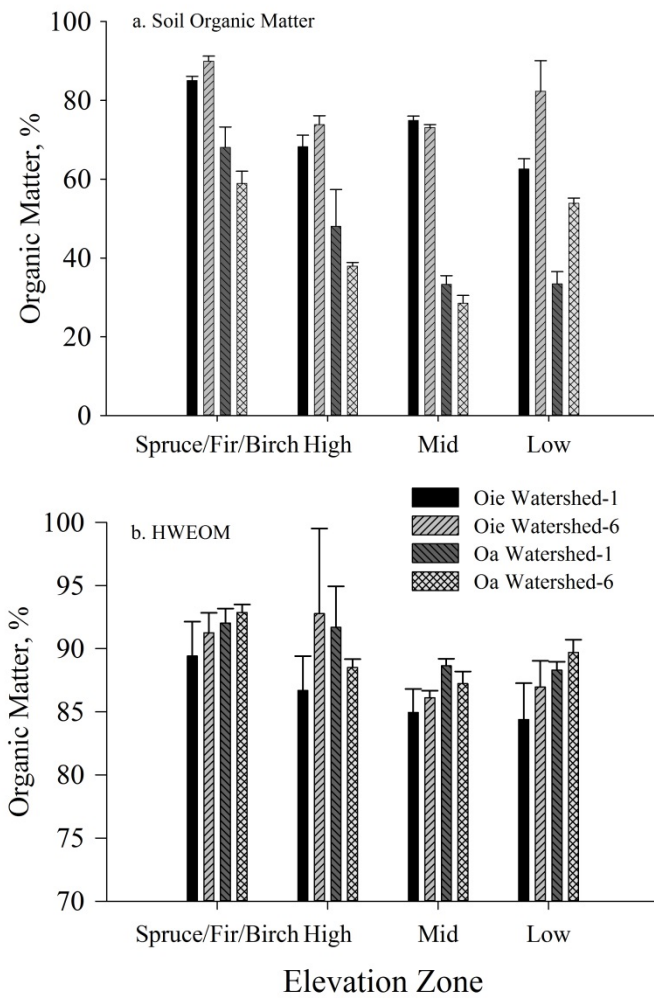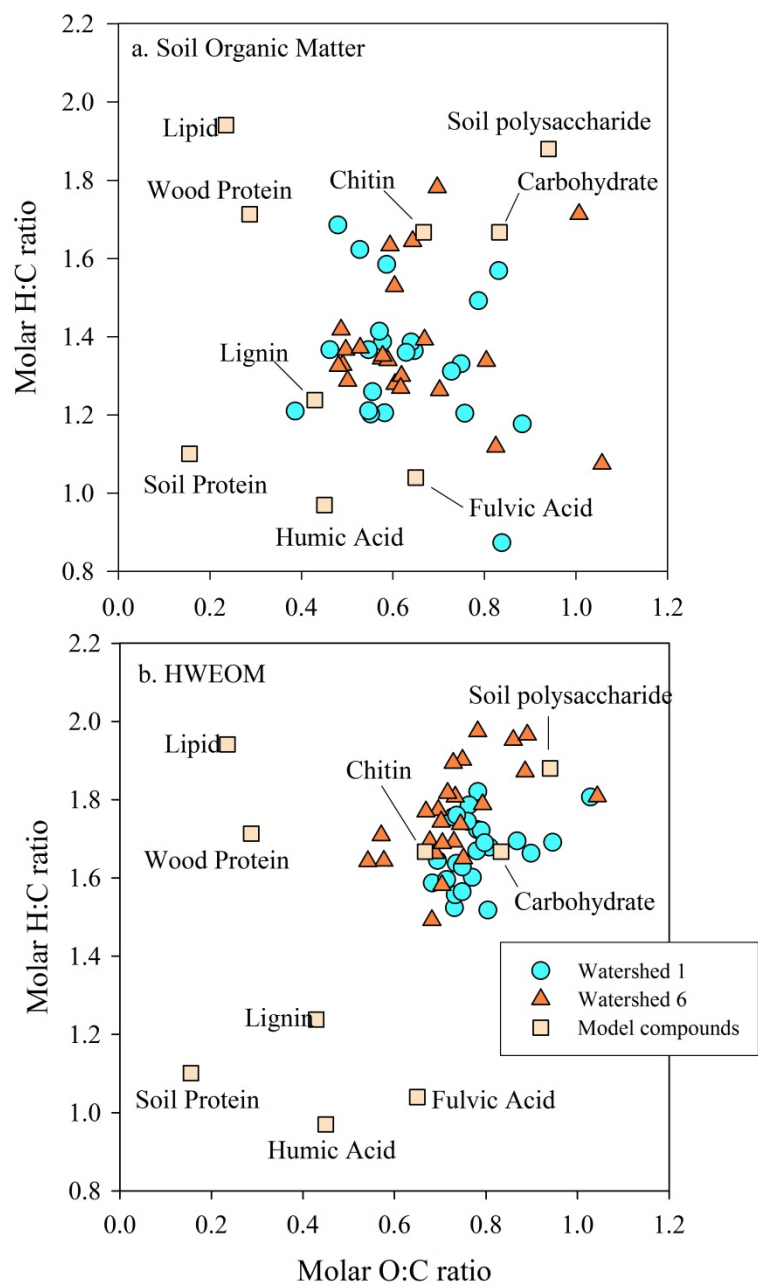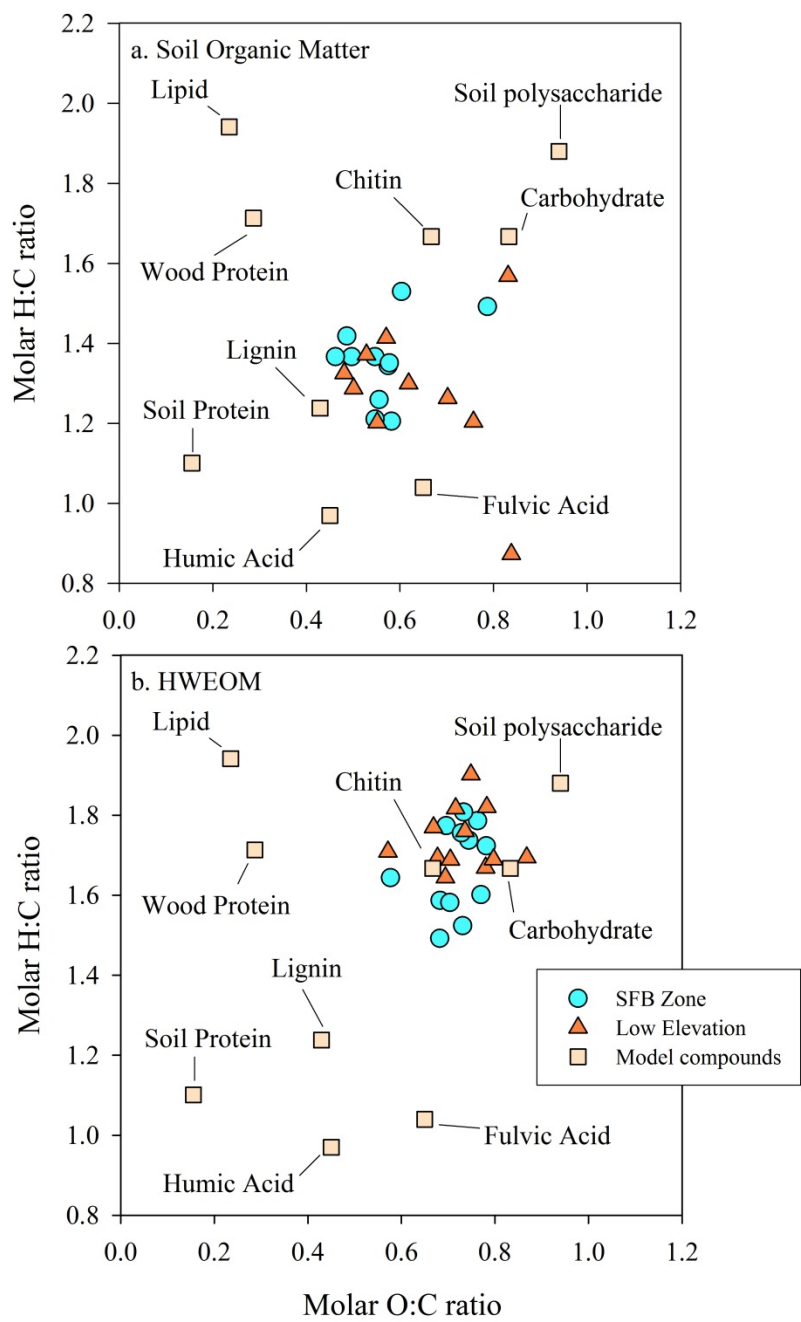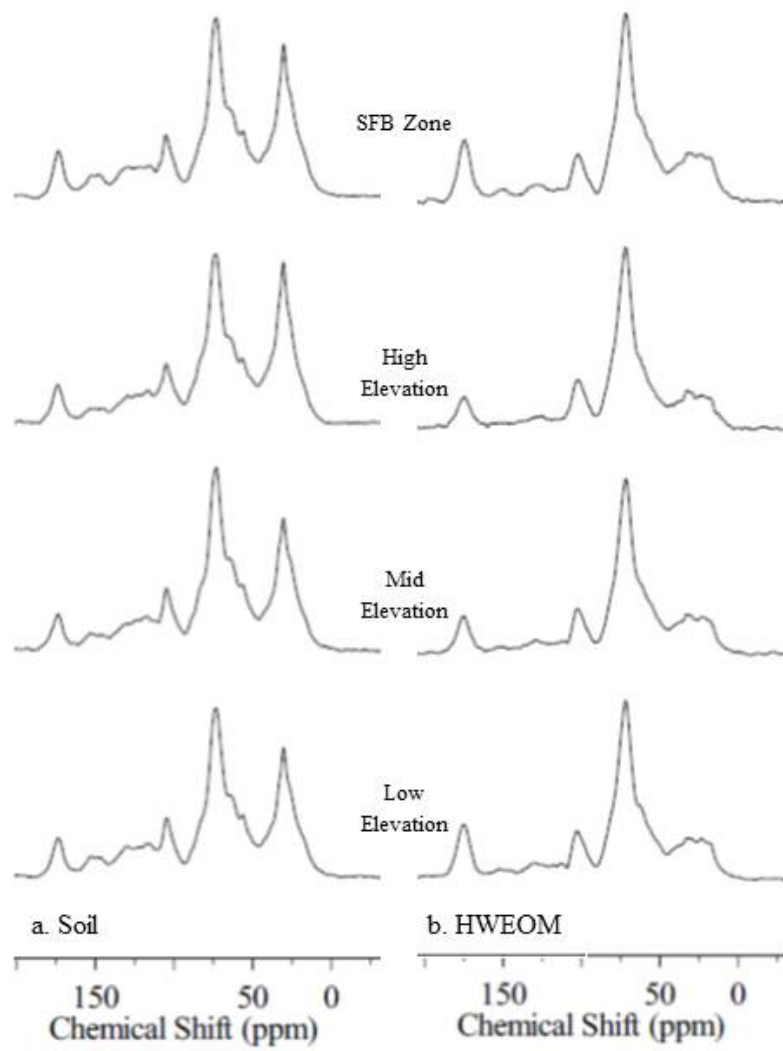
Figure 1

Figure 2

Figure 3

Figure 4

a. Soil

b. HWEOM

Figure 5

Figure 6