

Carbon structure and enzyme activities in alpine and forest ecosystems

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Abstract

The chemical structure of soil organic matter fractions and its relationship to biological processes remains uncertain. We used pyrolysis-gas chromatography/mass spectrometry to analyze the molecular structure of light and heavy fraction C from soils in the San Juan Mountains, Colorado. The soil samples, each replicated three times, were from two elevations (alpine and low forest) within two geochemically distinct basins (igneous and sedimentary). We also analyzed whether variation in the activity of nine enzymes that mediate soil organic matter turnover and nutrient cycling could explain differences in C structure. We found that, across basins and elevation, light fraction and heavy fraction C had distinct chemistries. The light fraction was characterized by an abundance of plant lignin biomarkers, including phenol, 2-methoxy-4-vinyl-(vinylguaiacol) and phenol, 2-methoxy-(guaiacol); in contrast heavy fraction had very little unaltered lignin but an abundance of polysaccharides, such as furfural, and proteins such as pyrrole. In alpine sites, light fraction was less abundant (4.27 versus 31.79 g kg⁻¹) and had a lower C/N ratio (17.25 versus 32.01) than in forests. The alpine sites also had higher activities of phosphatase, β -D-1,4-cellobiosidase, β -1,4-glucosidase, L-leucine aminopeptidase, and β -1,4-xylosidase. Protein abundance in the heavy fraction was correlated with peptidase, β -1,4-glucosidase, and phosphatase activities; in the light fraction, protein abundance was correlated with peptidase, xylosidase, and β -D-1,4-cellobiosidase activities. β -1,4-N-acetyl-glucosaminidase was negatively correlated with polysaccharides in the light and heavy fractions and positively correlated with lignin in the light fraction. However, there were not always significant correlations between enzymes and substrates. We suggest that this is likely because soil organic matter chemistry reflects long-term decomposition processes while enzyme dynamics fluctuate with current conditions or due to the presence of a pool of sorbed enzymes in the heavy fraction. While alpine and forest ecosystem C distribution and enzyme activities varied, substantial depletion of lignin derivatives in the heavy fraction across sites suggest that these compounds do not persist in stable soil C pools.

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1. Introduction

The physical and chemical changes that occur in soils during decomposition are central to understanding soil carbon stabilization and nutrient cycling. There are multiple physical and chemical methods available to separate soils into fractions with different functional characteristics in order to better understand the transformations that occur during C decomposition and stabilization (e.g. Cambardella and Elliott, 1993; Sollins et al., 1999; Marx et al., 2005). Density separation uses a high-density liquid

(typically 1.6–2.0 g cm⁻³) to separate soils into mineral associated and uncomplexed soil C (Strickland and Sollins, 1987; Gregorich et al., 2006). The uncomplexed fraction, or light fraction (LF), consists of partially decomposed plant residues, animal remains, and microorganisms, along with some charcoal (Molloy and Speir, 1977; Golchin et al., 1994), and is in an intermediate stage of decomposition between plant residues and physically stabilized C.

The general chemical characteristics of LF and heavy fraction (HF) have been reported for multiple ecosystems and soil types. In general, LF is dominated by plant materials and has a wider C:N ratio (~20:1) than does HF, which contains organic matter in more advanced stages of decomposition with a lower C:N ratio. LF may represent

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as much as 25–35% of total soil C, and slightly less N, in native ecosystems but is rapidly depleted in agricultural soils and is frequently less than 10% of the total C (Grandy and Robertson 2007). Because of its abundance, high C:N ratio, age, and general similarity to plant residues LF typically has a fairly rapid turnover time (<5 years) and is considered an important source of plant nutrients in native ecosystems and many agricultural soils (Wander et al., 1994; Swanston et al., 2002; Yamashita et al., 2006).

During decomposition of LF, there is a reduction in particle size and an increase in SOM density resulting in formation of microbially processed, mineral-associated HF C (Olk and Gregorich, 2006; Sollins et al., 2006). LF and HF are thus assumed to be at different points along the decomposition continuum and their chemical characteristics can be used to understand decomposition sequences (Baldock et al., 1992). Comparisons between HF and LF molecular structure can thus reveal the preservation, loss and transformation of organic molecules during decomposition (Sollins et al., 2006). To date, however, generalizations about SOM decomposition sequences based on LF and HF chemistry remain contentious. This is due in part to the fact that results generated using different methods can be difficult to compare. For example, lignin derivatives can be directly determined using pyrolysis-gas chromatography/mass spectrometry or CuO oxidation but these results can be hard to collate and cannot be quantitatively compared to acid-resistant fractions or aromatic groups from NMR spectroscopy (Preston and Trofymow, 2000; Preston et al., 2006). Inherent variation in chemistry among sites coupled with relatively few data points also make generalizations difficult.

Mahieu et al. (1999) found that in silt and clay fractions, which are analogous to the HF, *O*-alkyls were the most abundant group but they were diminished relative to concentrations found in whole soils and in the sand fraction. Alkyls and aromatics, in contrast, were enriched in the silt and clay fractions. Comparably, Schnitzer et al. (2006) found that long-term cultivation increases the relative abundance of aromatic compounds, likely due to the preservation of lignin monomers and phenols, and decreases in aliphatics. Similarly, Martin et al. (1980) suggested that polysaccharides are rapidly decomposed while lignin and its derivatives are incorporated into stable soil C pools.

The preservation of lignin and its ability to constrain SOM decomposition rates is consistent with litter decomposition theories in which lignin concentration is an important predictor of C mineralization potential (Melillo et al., 1982; Berg and Laskowski, 2006). The conservation of lignin is due to its complex and random molecular structure, which limits the enzyme systems that can break it down (Kirk and Farrell, 1987; Ekschmitt et al., 2005). Recently, however, there have been questions raised about the long-term persistence of lignin during decomposition in soils. Kiem and Kögel-Knabner (2003) studied the molecular structure of soil organic matter (SOM) following

years of intensive tillage designed to deplete soil C pools. They found selective decomposition of lignin following disturbance and concluded that lignin contributes to labile soil C pools but not refractory C pools. Similarly, Gleixner et al. (2002) and Grandy and Neff (2007) proposed that while highly modified, unrecognizable lignin decomposition products, such as phenol, may accumulate in clay fractions, distinct, unmodified lignin macromolecules do not. Stable soil C pools are dominated instead by lipids and carbohydrates, likely produced during microbial turnover, that are protected by their association with mineral surfaces.

Soil enzymes catalyze the transformation of organic matter and several studies have found correlations between enzymes and the molecular structure or turnover dynamics of SOM (Orwin et al., 2006; Zak and Kling 2006). Grandy and Neff (2007) reported using pyrolysis-gas chromatography/mass spectrometry that changes in lignin abundance and structure are associated with decreasing phenol oxidase activity following N fertilization of a Michigan Forest. These results were consistent with those of Gallo et al. (2005) who found using Fourier transform infrared spectroscopy that changes in C structure were linked to changing enzyme activities. Several studies have also reported that accelerated hydrolase enzyme activity associated with N fertilization is associated with carbohydrate breakdown (Sinsabaugh et al., 2004, 2005). Because enzymes are directly linked to the turnover rates of different organic functional groups they may provide key insights into the preservation or loss of specific compounds during the transformation of LF to HF. For example, is there an accumulation of lignin in LF and subsequent transfer of unaltered lignin into the HF where oxidase enzyme activity is low? Where oxidative enzyme activity is high do we expect to see more advanced lignin alteration in LF and little lignin in the HF?

Our objectives in this study are: (1) to determine using replicated soil samples whether the molecular structure of LF and HF varies with elevation or bedrock geochemistry in the San Juan Mountains of Colorado; and (2) determine how the activity of enzymes that mediate decomposition and nutrient cycling varies in these soils and whether this variation is correlated to differences in the molecular structure of LF and HF.

2. Materials and methods

2.1. Site description

The San Juan Mountains are located in southwestern Colorado directly adjacent to the Colorado Plateau. Our samples come from two distinct basins in this geologically diverse region: (1) Sultan, a sedimentary basin consisting of alternating layers of sandstone, shale, and limestone that is capped by a thick layer of Telluride conglomerate; (2) Columbine, a Tertiary igneous basin. The mixed forests are dominated by Engelmann spruce, subalpine fir, and an

occasional corkbark fir. The alpine sites are dominated by shrubs and lush herbaceous plants including alpine avens, cinquefoils, Indian paintbrush and patches of bistort (Jamieson et al., 1996). The San Juan Mountains receive an average of 77 cm precipitation annually, about half as snow between November and May. Average temperatures in the winter are -9°C and in the summer are 11°C . Soils in the region are heterogeneous and include co-occurring entisols, inceptisols, and alfisols.

2.2. Soil sampling and processing

We sampled over a 3-day period starting 01 October 2005 from both high alpine (above 3600 m) and low forest (3200) sites in both basins. At each basin and elevation we collected three replicate sample soils to 10 cm after removing the litter layer. Samples were immediately stored on ice in a cooler and then placed in a freezer at -20°C 48 h after collection.

2.3. Soil C and N fractions

LF and HF were separated in sodium polytungstate at a density of 1.8 g cm^{-3} . We placed 12 g of soil in a 50 ml centrifuge tube with 40 ml of sodium polytungstate. Soil structure was broken down to release LF from within aggregates by placing the centrifuge tubes on a rotary shaker for 30 min at 50 rpm. The HF and LF were then stratified by centrifugation at $2250g$ for 20 min and LF was collected by aspiration with a vacuum pump. LF was rinsed with >600 ml of deionized water and dried at 60°C . In order to rinse the HF, we aspirated as much sodium polytungstate as possible from it, added ca. 40 ml of deionized water, and brought the soil pellet back into suspension by shaking for 10 min as described above. The soil suspension was then centrifuged as described previously and the water aspirated off. This was repeated 4 times before the HF was collected and dried at 60°C . Carbon and N concentrations of LF and whole soils were determined using a Carlo Erba high-temperature combustion instrument.

2.4. Pyrolysis-gas chromatography/mass spectrometry

Pyrolysis-gas chromatography/mass spectrometry (py-GC/MS) provides a molecular fingerprint of SOM (Hempfling and Schulden, 1990; White et al., 2004) and it is not uncommon to generate 100 or more distinct peaks in a single soil sample. Because of this, identifying pyrolysis products can be one of the greatest challenges with this method. Recently, however, coupling Curie-point pyrolysis-gas chromatography with mass spectrometry and advancement of software for peak identification—along with improved understanding of the structure of commonly occurring compounds in soils—have facilitated peak identification. The adoption of the Curie-point pyrolyzer, where the thermal degradation temperature is dependent

on properties of the pyrofoil, has greatly improved reproducibility (Samukawa 1996; Gleixner et al., 2002). Although, the method is generally only semi-quantitative it affords a good opportunity to directly link microbial processes with organic matter chemistry.

We examined the chemical structure of LF and HF using py-GC/MS at the University of Colorado at Boulder using previously described methods (Neff et al., 2006). Samples were pyrolyzed at 590°C in pyrofoils (Pyrofoil F590, Japan Analytical Company, Tokyo, Japan) within a Curie-point pyrolyzer (Pyromat, Brechbühler Scientific Analytical Solutions, Houston, TX). Pyrolysis products were transferred online to a gas chromatograph (ThermoQuest Trace GC, Thermo Finnigan, San Jose, CA). We used an interface temperature of 250°C with a split injection (split ratio 50:1, He flow rate 1.0 ml min^{-1}). Separation of pyrolysis products was done on a BPX 5 column ($60 \times 0.25\text{ mm}$, film thickness $0.25\text{ }\mu\text{m}$) using a temperature program of 40°C for 5 min, $5^{\circ}\text{C min}^{-1}$ to 270°C followed by a jump ($30^{\circ}\text{C min}^{-1}$) to a final temperature of 300°C . The column outlet was coupled to a Thermo Polaris-Q ion-trap mass spectrometer (Polaris Q, Thermo Finnigan, San Jose, CA) operated at 70 eV in the EI mode. The transfer line was heated to 270°C and the source temperature was held at 200°C .

2.5. Enzyme assays

We analyzed the activity of nine enzymes involved in C cycling and nutrient turnover (Supplemental Materials) using the methods reported in Saiya-Cork et al. (2002) and Weintraub et al. (2007). Sample suspensions were prepared by homogenizing 1.0 g soil in 125 ml 50 mM sodium acetate buffer, pH 6 (a typical pH for these soils) using a Virtex 45 tissue homogenizer (Virtex Inc., Yonkers, NY). The suspensions were continuously stirred on a magnetic stir plate while $200\text{ }\mu\text{l}$ aliquots were pipetted into 96-well microplates; 16 replicate wells were created for each enzyme assay and sample. All of the enzyme assays were fluorimetric except peroxidase and phenol oxidase, which were colorimetric (see Supplemental Materials). The fluorimetric assays were conducted in black 96-well microplates and the colorimetric assays in clear microplates.

In the fluorimetric assays $50\text{ }\mu\text{l}$ of 200 mM substrate solution was added to each sample well (see Supplemental Materials). Eight blanks wells per sample were created with $50\text{ }\mu\text{l}$ of buffer and $200\text{ }\mu\text{l}$ of sample suspension. Eight quench control wells per sample were created with $50\text{ }\mu\text{l}$ of fluorescent standard (10 mM 4-methylumbelliferone, or 7-amino-4-methylcoumarin in the case of leucine aminopeptidase) and $200\text{ }\mu\text{l}$ of the sample suspension. Eight substrate controls wells per plate were created with $50\text{ }\mu\text{l}$ substrate solution and $200\text{ }\mu\text{l}$ of buffer. Eight reference standards per plate were created with $50\text{ }\mu\text{l}$ of fluorescent standard and $200\text{ }\mu\text{l}$ of buffer. The assay plates were incubated in the dark at 13°C for up to 24 h and the

reactions terminated adding by 10 μl of 1.0 M NaOH to each well to raise the pH to 9, the optimal pH for the fluorescence of these substrates. Fluorescence was measured with 365 nm excitation and 460 nm emission filters using a microplate fluorometer (Fluoroskan II, Thermo Labsystems, Waltham, MA).

For the phenol oxidase and peroxidase assays each sample well received 200 μl of sample suspension and 50 μl of 25 mM L-3,4-dihydroxyphenylalanine (L-DOPA) as the substrate. For the phenol oxidase assays the negative control wells contained 200 μl of acetate buffer and 50 μl of L-DOPA; the blank wells contained 200 μl of sample suspension and 50 μl of acetate buffer. The wells used for peroxidase assays were the same except they received 10 μl of 0.3% H_2O_2 , including the negative control and blank wells. Sixteen replicate wells were created for each sample and assay and 8 replicate wells were set up for the blanks and controls. The colorimetric assays plates were incubated for the same duration and at the same temperature as the fluorimetric plates. Enzyme activity was determined spectrophotometrically by measuring absorbance at 460 nm (Spectramax Microplate Spectrophotometer, Molecular Devices Inc., Sunnyvale, CA). Phenol oxidase activity was subtracted from peroxidase activity to calculate the net peroxidase activities reported here. Enzyme activities were expressed on a per unit C basis after correcting for the percent C in each sample. This removes the potential for differences in C quantity among ecosystems to be the primary control over enzyme activity so that the effects of C quality and environmental factors can be determined.

2.6. Data analysis

Peaks corresponding to pyrolysis products were compared to reference spectra after deconvolution and extraction using AMDIS v 2.64 and National Institute of Standards and Technology mass spectral libraries and published literature (Pouwels et al., 1989; Schulten and Schnitzer, 1997). Results were expressed as the relative abundance of total detectable compounds and grouped into 8 chemical classes: benzene (e.g. benzene; toluene), benzofuran (e.g. benzofuran, 2-methyl; 6-methoxy-3-methylbenzofuran), lignin derivative (e.g. benzene, 1,2-dimethoxy; phenol, 2-methoxy), phenol of unknown origin (e.g. phenol; phenol, 4-methyl), polysaccharide (e.g. furfural; furfural, 5-methyl), lipid (e.g. decanal), protein (e.g. pyrrole; pyridine) and unknown. Compounds classified as unknown were either ones that could not be identified or compounds that could not be definitively grouped into any of the above categories.

All data analysis was done using Statistica Vs 7 (Statsoft Inc, Tulsa, OK). Statistical comparisons of SOM chemistry were done using a 3-way factorial completely random design with three replications. The factors included two basins (Sultan and Columbine), 2 elevations (forest and alpine) and 2 soil fractions (LF and HF). For enzymes and

other variables that were measured on whole soils the statistical design was similar but did not contain a term for soil fractions. Post-hoc comparisons (Tukey's HSD) were used to compare the chemical structure of different sites. Principal components analysis (PCA) of the compound classes was used to simplify and reduce the dimensionality of the molecular carbon data set. We used simple correlations to look at relationships between enzyme activities and chemical structure separately for forest and alpine ecosystems ($n = 6$) and also with these ecosystems combined ($n = 12$).

3. Results

There was a trend ($p < 0.085$) towards higher whole soil percent C in the forest sites, which also had a higher C/N ratio than the alpine sites (Table 1). There was also a trend towards higher clay concentrations in Columbine soils. Otherwise the whole soil total C and N concentrations, C/N ratios and sand, silt, and clay distribution of the sites was similar. LF abundance ranged from 9.84 to 117.5 g kg^{-1} soil and LF C concentration ranged from 26.39% to 34.07% (Table 2). The C/N ratio of LF was higher in forests than in alpine sites but in both systems the C/N ratio of LF was greater than that of the whole soil. All LF parameters were significantly different in forest and alpine systems but only LF N concentration was influenced by basin. LF C as a percentage of total C was highest in the Sultan forest soils (69.14%) and lowest in the Sultan alpine site (12.15%). The Columbine sites similarly showed a high percentage of the total C in the LF of forest sites (49.78%) but a much lower proportion in the alpine sites (15.69%).

The 20 most abundant soil compounds, their chemical class and distribution in LF and HF are reported in Table 3. All but one of the 20 most abundant compounds was classified as polysaccharide, protein, or lignin. There were significant basin, elevation, and fraction main effects for the abundance of lignin and polysaccharides and significant elevation effects for the abundance of unknown compounds (Table 4). Sultan soils had higher lignin content than Columbine soils (35.90 versus 23.20%) and forest soils had considerably more lignin than alpine soils (40.18 versus 18.92%).

Perhaps the most striking pattern relating to soil chemical structure was the difference in lignin abundance between LF (45.46%) and HF (13.64%). In contrast to lignin, polysaccharides were far more abundant in the HF (49.63%) than in LF (19.86%). Proteins were also more abundant in HF than in LF (8.93% versus 4.24%) and in alpine (8.51%) than forest (4.66%) ecosystems. Polysaccharides were more abundant in Columbine (40.89%) than in Sultan (28.60%) and more abundant in alpine (41.86%) than in forest (27.63%) systems (Fig. 1; Table 4).

Principal components analysis showed the distinct difference in structure between LF and HF C (Fig. 2). Factor one, which explained 85.65% of the variability in the data, was primarily a contrast between samples with a

Table 1
Effects of basin and elevation on total soil C, N, and soil texture^{a,b}

Basin	Elevation	Total C (g kg ⁻¹)	Total N (g kg ⁻¹)	C/N ratio	Sand (g kg ⁻¹)	Silt (g kg ⁻¹)	Clay (g kg ⁻¹)
Columbine	Alpine	38.65 (6.11)	3.98 (0.45)	9.62 (0.49)	326.6 (65.64)	443.7 (45.14)	229.7 (20.59)
Columbine	Forest	56.10 (24.94)	3.09 (1.47)	18.55 (0.99)	261.6 (54.24)	496.8 (37.40)	241.6 (18.38)
Sultan	Alpine	24.03 (7.38)	2.48 (0.68)	9.51 (0.66)	376.0 (53.14)	428.8 (39.24)	195.2 (17.43)
Sultan	Forest	55.11 (4.15)	2.91 (0.33)	19.15 (1.10)	475.2 (125.0)	364.4 (81.86)	160.4 (44.20)
ANOVA <i>F</i> -tests							
	<i>p</i> -Values						
	Basin	0.537	0.356	0.779	0.139	0.210	0.068
	Elevation	0.085	0.796	0.000	0.836	0.919	0.688
	Basin × elevation	0.241	0.462	0.683	0.336	0.309	0.421

^aMeans with standard errors in parentheses.

^bAnalysis of variance results for basin, elevation, and basin by elevation were not significant ($p < 0.05$) for all variables other than soil C/N ratio which had a significant elevation effect ($p < 0.000$).

Table 2
Effects of basin and elevation on the distribution of carbon and nitrogen in light fraction organic matter of the San Juan Mountains, CO^a

Basin	Elevation	LF dry weight (g kg ⁻¹ soil)	LF N (%)	LF C (%)	C/N ratio	LF N (g kg ⁻¹ soil)	LFC (g kg ⁻¹ soil)	LF N (%) total	LF C (%) total
Columbine	Alpine	21.73 (2.19)	1.64 (0.02)	27.33 (0.81)	16.66 (0.43)	0.36 (0.04)	5.92 (0.49)	8.99 (0.15)	15.69 (1.24)
Columbine	Forest	72.30 (22.10)	1.08 (0.04)	34.07 (0.68)	31.62 (1.03)	0.80 (0.26)	24.69 (7.51)	29.85 (8.70)	49.78 (11.78)
Sultan	Alpine	9.84 (1.18)	1.48 (0.04)	26.39 (0.45)	17.83 (0.38)	0.15 (0.02)	2.61 (0.35)	6.38 (1.06)	12.15 (2.23)
Sultan	Forest	117.47 (28.48)	1.05 (0.03)	34.06 (2.05)	32.40 (2.17)	1.23 (0.30)	38.88 (7.68)	41.31 (7.22)	69.14 (9.41)
ANOVA <i>F</i> -tests									
	<i>p</i> -Values								
	Source								
	Basin	0.384	0.021	0.695	0.455	0.586	0.342	0.458	0.331
	Elevation	0.002	0.000	0.000	0.000	0.005	0.001	0.001	0.000
	Basin × elevation	0.153	0.073	0.703	0.876	0.145	0.143	0.250	0.172

^aMeans with standard errors in parentheses.

Table 3
Twenty most abundant compounds and their distribution in light and heavy fraction C^a

Compound	Class	Heavy fraction (%)	Light fraction (%)
Furfural	Polysaccharide	20.22 (3.24)	7.00 (1.90)
Furfural, 5-methyl-	Polysaccharide	10.01 (2.08)	3.25 (0.67)
Acetic acid	Polysaccharide	3.34 (1.07)	1.07 (0.240)
Levoglucosenone	Polysaccharide	3.02 (0.70)	2.39 (0.94)
Furan, 3-methyl-	Polysaccharide	2.61 (0.48)	0.40 (0.12)
3-Furaldehyde	Polysaccharide	1.74 (0.29)	0.52 (0.18)
Furan	Polysaccharide	1.43 (0.48)	0.43 (0.220)
Pyrrole	Protein	1.45 (0.23)	0.31 (0.09)
Isobutyl nitrate	Protein	1.15 (0.28)	0.42 (0.130)
Pyridine	Protein	1.04 (0.21)	0.18 (0.07)
Phenol	Phenol	4.67 (1.57)	6.07 (1.51)
Phenol, 4-methyl-	Phenol	1.32 (0.48)	4.15 (0.91)
Phenol, 3-ethyl-	Phenol	1.16 (0.40)	1.06 (0.21)
Phenol, 2-methoxy-4-vinyl-(vinylguaiaicol)	Lignin	3.50 (1.10)	14.31 (1.50)
Phenol, 2-methoxy-(Guaiacol)	Lignin	2.52 (0.78)	6.4 (2.59)
(E)-Isoeugenol	Lignin	1.76 (0.89)	2.76 (1.04)
Acetovanillone	Lignin	1.42 (0.80)	3.08 (0.94)
Vanillin	Lignin	1.22 (0.74)	4.18 (1.22)
Phenol, 2-methoxy-methyl-(methylguaiaicol)	Lignin	1.21 (0.50)	2.71 (0.61)
Toluene	Benzene	2.58 (0.47)	1.85 (0.47)

^aMeans with standard errors in parentheses.

Table 4

Main effect means and analysis of variance results for the effects of basin, elevation, and C fraction on the distribution of organic matter in soils of the San Juan Mountains, Co.^a

		Benzene	Benzofuran	Lignin	Lipid	Phenol	Polysaccharide	Protein	Unknown
Basin	Columbine	7.29	3.69	23.20	1.44	9.95	40.89	6.87	6.56
	Sultan	5.19	5.60	35.90	1.62	11.02	28.60	6.29	5.61
Elevation	Alpine	5.28	3.62	18.92	1.69	12.80	41.86	8.51	7.21
	Forest	7.19	5.67	40.18	1.38	8.17	27.63	4.66	4.96
Fraction	Light fraction	5.06	4.92	45.46	1.68	12.63	19.86	4.24	5.97
	Heavy fraction	7.41	4.38	13.64	1.39	8.35	49.63	8.93	6.20
ANOVA <i>F</i> -tests									
<i>p</i> -Values									
Basin		0.210	0.093	0.021	0.696	0.772	0.035	0.595	0.279
Elevation		0.252	0.074	0.001	0.485	0.222	0.017	0.002	0.018
Fraction		0.163	0.623	0.000	0.502	0.258	0.000	0.000	0.791
Basin × elevation		0.968	0.958	0.096	0.614	0.796	0.090	0.715	0.377
Basin × fraction		0.554	0.793	0.514	0.362	0.798	0.603	0.619	0.881
Elevation × fraction		0.618	0.010	0.944	0.149	0.363	0.060	0.897	0.002
Basin × elevation × fraction		0.231	0.059	0.107	0.597	0.601	0.077	0.707	0.959

^aMeans with standard errors in parentheses.

high lignin content (factor score coefficient of -2.22) or high polysaccharide content (factor score coefficient of 2.13). The clustering of HF samples where principal component one is greater than zero indicates the predominance of polysaccharides; the clustering of LF samples where principal component one is negative indicates that lignin is dominant in LF. There were exceptions, however. The HF in the Sultan forest soils tended to have high lignin content and LF in one of the alpine soils from columbine had high polysaccharide content. The differences in chemical structure between LF and HF and their relationship to soil C/N ratio is shown in Fig. 3. As the C/N ratio decreases from the LF to the HF the polysaccharide/lignin ratio increases.

Enzyme activities were statistically indistinguishable between basins, other than a proportionally small difference in α G, and there were no basin by elevation interactions (Table 5). The activities of phosphatase and four other enzymes (CBH, β G, β X, and LAP) were significantly greater in alpine than forest sites. No enzymes had statistically higher activities in the forest than in the alpine systems. We further found that enzyme activities expressed per g dry soil rather than per g soil C were generally similar or greater in the alpine soils (data not shown). The one exception to this was Nag, which showed statistically greater activity in the forest soil ($0.15 \mu\text{mol h}^{-1} \text{g}^{-1}$) than in the alpine soil ($0.06 \mu\text{mol h}^{-1} \text{g}^{-1}$).

There were several significant correlations between enzyme activities and the chemical composition of SOM in LF and HF (Table 6), but relationships were fundamentally different between forest and alpine ecosystems and LF and HF. In the forest systems, α -1,4-glucosidase was negatively correlated with lipids in the HF and peptidase was correlated positively with benzofuran and negatively

with phenol in the HF. There were no significant correlations between HF chemistry and enzymes in alpine systems. When combined across forest and alpine ecosystems, protein abundance in the HF was correlated with peptidase, β -1,4-glucosidase, and phosphatase activities while in the LF protein abundance was correlated with peptidase, xylosidase, and β -D-1,4-cellobiosidase activities. Polysaccharides were negatively correlated with β -1,4-*N*-acetyl-glucosaminidase in HF and LF and positively correlated with β -1,4-xylosidase in the HF. Lignin in LF was positively correlated with β -1,4-*N*-acetyl-glucosaminidase and negatively correlated with xylosidase.

4. Discussion

We found large differences in the distribution and structure of SOM along an elevation gradient in the San Juan Mountains, CO (Figs. 1–3). Across sites and elevations, we found that molecules derived from lignin generally dominated LF but were substantially depleted in HF, suggesting that during decomposition lignin is rapidly transformed and its relative abundance decreases compared to polysaccharides, proteins and other structures. This pattern was apparent across both basins and at both elevations, despite large differences in environmental conditions and LF concentration between the alpine ecosystem and sub-alpine forests. These results are consistent with those of other researchers who have recently found evidence for lignin oxidation and depletion during SOM transformations from younger, more labile C pools to more stable organo-mineral fractions (e.g. Nierop et al., 2001; Dignac et al., 2002, 2005; Gleixner et al., 2002; Lobe et al., 2002; Schmidt and Kögel-Knabner, 2002). The relative degradability of lignin is further demonstrated

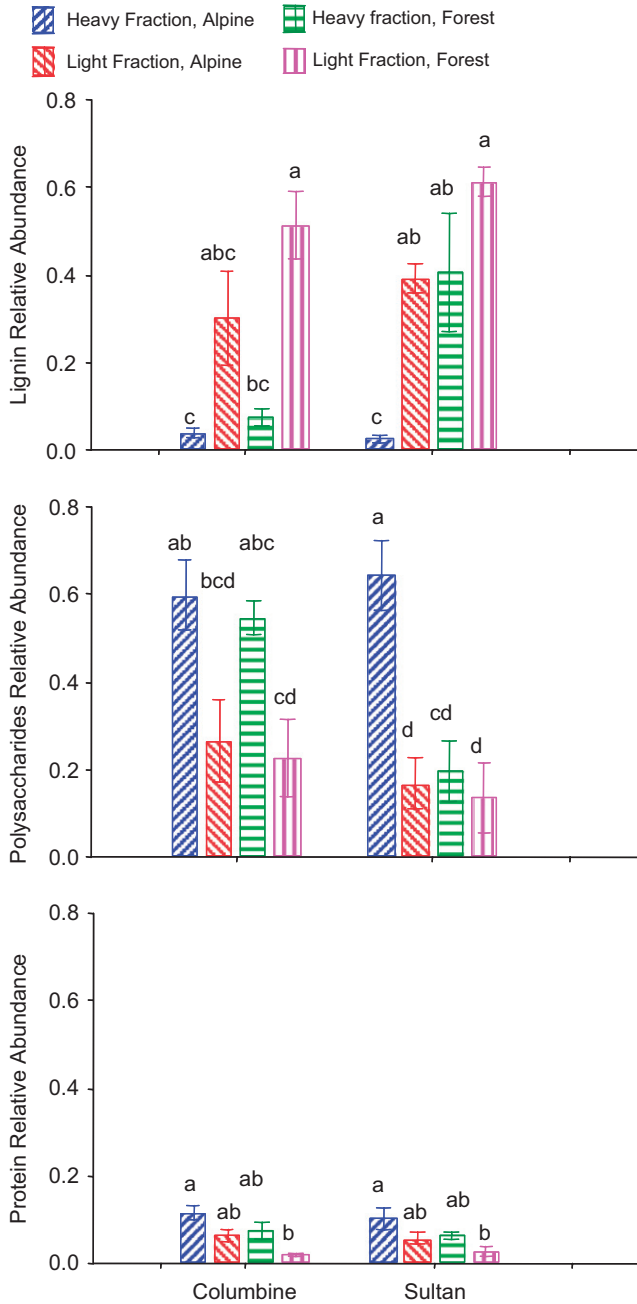


Fig. 1. Relative abundance of polysaccharides, lignin, and protein in San Juan Mountain Ecosystems. Sites included alpine and forest ecosystems in sedimentary (Sultan) and igneous (Columbine) basins. Bars show means with standard errors. Bars with different letters within a compound type are significantly different ($p < 0.05$, Tukey's HSD).

by its rapid loss following disturbance. Kiem and Kögel-Knabner (2003) examined the chemical structure of SOM in conventionally managed (receiving mineral and organic fertilizers) and C-depleted (unfertilized crops or bare fallow) agricultural systems. They found lower lignin concentrations and greater structural alteration of lignin in C depleted plots. In soil fractions $< 63 \mu\text{m}$ they also found lower relative abundance of lignin and concluded that refractory C pools are dominated by microbial polysaccharides.

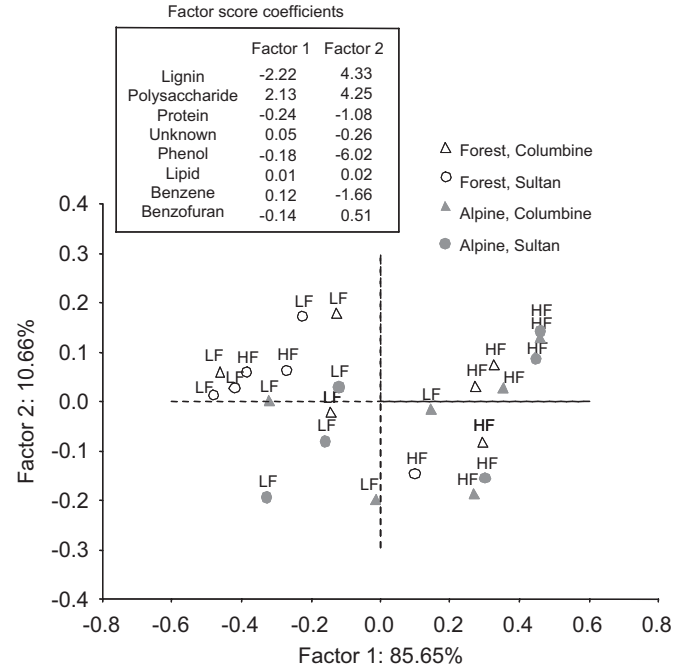


Fig. 2. Principal components analysis (PCA) of the distribution of organic matter in different chemical classes by soil fraction (light fraction and heavy fraction), elevation (high alpine and low forest), and basin (Sultan, sedimentary, and Columbine, igneous).

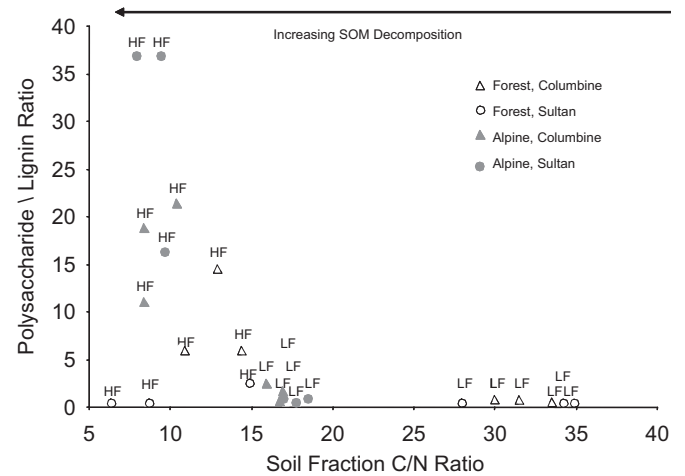


Fig. 3. Polysaccharide/lignin ratio of light- and heavy-fraction organic matter as a function of C/N ratio. The results show that as the fraction C/N ratio decreases, an indicator of more advanced decomposition, polysaccharides increase in relative abundance compared to lignin.

In soils, physical C stabilization by aggregation and sorption of organic matter onto mineral surfaces primarily accounts for recalcitrant C pools that persist for hundreds to thousands of years (Zimmerman et al., 2004; Wiseman and Püttmann, 2005; Mikutta et al., 2006). Using model calibrations, Rasse et al. (2006) estimated that 92% of undecomposed plant residue lignin in an agricultural soil is lost as CO_2 or transformed during its first year after soil-incorporation; 8% of lignin reaches soil pools where it persists long-term only because it is physically

Table 5
Means and analysis of variance results for the effects of basin and elevation on soil enzyme activity^a

Basin	Elevation	NAG ($\mu\text{mol h}^{-1} \text{g}^{-1}$ C)	CBH ($\mu\text{mol h}^{-1} \text{g}^{-1}$ C)	α G ($\mu\text{mol h}^{-1} \text{g}^{-1}$ C)	β G ($\mu\text{mol h}^{-1} \text{g}^{-1}$ C)	LAP ($\mu\text{mol h}^{-1} \text{g}^{-1}$ C)	β X ($\mu\text{mol h}^{-1} \text{g}^{-1}$ C)	PhOx ($\mu\text{mol h}^{-1} \text{g}^{-1}$ C)	Perox ($\mu\text{mol h}^{-1} \text{g}^{-1}$ C)	aP ($\mu\text{mol h}^{-1} \text{g}^{-1}$ C)
Columbine	Alpine	1.81 (0.12)	2.61 (0.44)	0.21 (0.01)	8.55 (0.47)	0.16 (0.03)	1.88 (0.25)	3.52 (3.52)	13.83 (13.83)	9.70 (0.67)
	Forest	2.63 (0.43)	0.93 (0.20)	0.22 (0.05)	3.64 (0.34)	0.06 (0.01)	0.36 (0.07)	3.09 (3.09)	27.80 (21.45)	5.14 (0.77)
Sultan	Alpine	2.10 (0.72)	2.57 (0.16)	0.23 (0.02)	9.90 (2.01)	0.11 (0.02)	1.63 (0.04)	2.08 (2.08)	11.06 (7.39)	10.91 (1.53)
	Forest	3.03 (0.44)	1.69 (0.22)	0.19 (0.02)	4.11 (0.14)	0.06 (0.02)	0.52 (0.02)	5.46 (3.46)	19.48 (10.13)	6.48 (0.26)
ANOVA <i>F</i> -tests										
<i>p</i> -Values										
	Basin	0.485	0.232	0.027	0.411	0.291	0.746	0.885	0.707	0.206
	Elevation	0.105	0.002	0.393	0.001	0.006	0.000	0.645	0.454	0.001
	Basin \times elev	0.912	0.192	0.668	0.689	0.205	0.160	0.554	0.850	0.949

^aEnzyme abbreviations: Nag (β -1,4-*N*-acetylglucosaminidase); CBH (β -D-1,4-cellobiosidase); α G (α -1,4-glucosidase); β G (β -1,4-glucosidase); LAP (*L*-leucine aminopeptidase); β X (β -1,4-xylosidase); PhOx (phenol oxidase); Perox (peroxidase); aP (acid phosphatase).

Table 6
Correlations between enzymes and light and heavy fraction compounds in forest and alpine systems and combined across systems^{a,b}

	Heavy fraction								Light fraction							
	Lip	Bf	Lig	Unk	Benz	Prot	Phen	Poly	Lip	Bf	Lig	Unk	Benz	Prot	Phen	Poly
<i>Forest</i>																
β -1,4- <i>N</i> -acetyl-glucosamin.	0.21	-0.03	0.42	-0.71	-0.27	0.04	0.36	-0.44	0.12	0.54	0.55	0.36	0.19	0.62	0.43	-0.82
β -D-1,4-cellobiosidase	0.32	0.46	0.44	-0.47	0.06	-0.07	0.39	-0.65	0.26	0.35	0.25	-0.81	-0.42	0.47	-0.50	0.09
α -1,4-glucosidase	-0.81	0.03	-0.26	0.37	0.35	0.73	0.11	0.07	-0.18	0.64	0.35	0.04	-0.64	-0.17	-0.63	0.09
β -1,4-glucosidase	0.01	0.23	0.16	-0.11	0.24	0.67	0.64	-0.52	0.29	0.45	0.79	-0.33	-0.83	-0.27	-0.23	-0.33
Leucine amino peptidase	0.21	0.82	0.56	0.61	-0.77	-0.35	-0.84	-0.37	-0.92	-0.63	0.20	-0.35	0.30	-0.20	0.35	-0.17
β -1,4-xylosidase	0.39	0.63	0.46	-0.16	-0.07	-0.25	0.07	-0.59	0.04	-0.04	0.07	-0.97	-0.31	0.26	-0.46	0.29
Phosphatase	0.80	0.15	0.51	-0.56	-0.35	-0.24	0.32	-0.54	0.28	-0.17	0.39	-0.06	0.19	0.14	0.67	-0.63
Phenol oxidase	-0.14	0.43	0.36	-0.18	-0.16	-0.15	-0.13	-0.37	-0.27	0.41	0.10	-0.41	-0.05	0.65	-0.46	0.08
Peroxidase	0.26	-0.50	-0.22	-0.49	0.13	-0.62	0.01	0.41	0.41	-0.16	-0.80	0.18	0.59	0.50	0.06	0.37
<i>Alpine</i>																
β -1,4- <i>N</i> -acetyl-glucosamin.	-0.72	-0.26	-0.21	-0.64	0.31	0.71	0.52	-0.52	-0.14	-0.44	0.43	-0.60	-0.01	-0.59	0.77	-0.65
β -D-1,4-cellobiosidase	-0.08	0.80	0.68	0.20	-0.41	0.15	-0.24	0.09	-0.84	-0.02	0.81	0.32	-0.17	0.47	-0.07	-0.78
α -1,4-glucosidase	-0.48	-0.07	0.16	-0.18	-0.28	0.11	-0.03	0.10	-0.26	-0.01	0.25	-0.19	0.04	-0.41	-0.75	-0.69
β -1,4-glucosidase	-0.44	-0.39	-0.46	-0.52	0.41	0.60	0.43	-0.42	-0.16	-0.53	0.44	-0.83	0.09	-0.77	0.83	-0.61
Leucine amino peptidase	-0.73	0.22	0.35	-0.42	0.14	0.61	0.54	-0.59	0.17	-0.32	0.00	0.14	0.05	0.25	-0.14	0.08
β -1,4-xylosidase	-0.01	0.38	0.55	0.65	-0.73	-0.38	-0.54	0.55	-0.29	-0.37	-0.11	-0.05	0.82	-0.07	0.37	-0.24
Phosphatase	-0.23	-0.31	-0.45	-0.33	0.32	0.44	0.25	-0.26	-0.28	-0.51	0.48	-0.81	0.14	-0.73	0.78	-0.63
Phenol oxidase	0.08	-0.44	-0.52	-0.34	0.63	0.16	0.47	-0.44	0.72	0.05	-0.52	-0.01	-0.19	0.06	-0.51	0.87
Peroxidase	-0.30	-0.28	-0.05	0.12	-0.27	-0.09	-0.12	0.23	0.07	-0.47	-0.25	-0.57	0.73	-0.70	0.86	-0.23
<i>Forest and alpine</i>																
β -1,4- <i>N</i> -acetyl-glucosamin.	-0.22	0.36	0.49	-0.33	0.16	-0.03	0.34	-0.63	-0.25	-0.19	0.66	-0.59	0.15	-0.52	0.22	-0.68
β -D-1,4-cellobiosidase	-0.19	-0.41	-0.28	-0.40	-0.34	0.51	0.06	0.33	0.09	0.21	-0.32	0.63	-0.30	0.78	0.26	-0.08
α -1,4-glucosidase	-0.53	-0.15	-0.30	0.18	0.11	0.47	0.05	0.19	-0.08	0.30	0.05	0.12	-0.37	0.01	0.11	-0.13
β -1,4-glucosidase	-0.42	-0.68	-0.49	-0.38	-0.17	0.74	0.29	0.41	0.29	-0.04	-0.44	0.43	-0.16	0.42	0.71	-0.11
Leucine amino peptidase	-0.55	-0.38	-0.27	-0.17	-0.40	0.63	0.22	0.24	0.25	-0.12	-0.49	0.61	-0.04	0.63	0.34	0.10
β -1,4-xylosidase	-0.24	-0.65	-0.48	-0.24	-0.44	0.49	-0.06	0.61	0.31	0.08	-0.68	0.71	0.00	0.72	0.51	0.11
Phosphatase	-0.22	-0.64	-0.37	-0.46	-0.29	0.60	0.21	0.37	0.27	-0.07	-0.41	0.43	-0.05	0.45	0.76	-0.21
Phenol oxidase	0.05	0.26	0.30	-0.15	0.17	-0.09	0.21	-0.40	0.22	0.15	-0.05	0.20	-0.09	0.04	-0.48	0.41
Peroxidase	0.02	-0.08	0.00	-0.23	0.10	-0.44	-0.07	0.10	0.08	-0.32	-0.17	-0.35	0.64	-0.30	0.23	0.09

^aCorrelation coefficients in bold are significant ($p < 0.05$).

^bCompound abbreviations: Lip (lipids); Bf (benzofurans); Lig (Lignin); Unk (unknown); Benz (Benzene); Prot (Protein); Phen (phenol); Poly (polysaccharides).

protected from decomposition. Those lignin-derived molecules that do persist are likely oxidized phenols that sorb to mineral surfaces via ligand exchange reactions (Sollins

et al., 2006). Although studies using NMR spectroscopy have often found an abundance of aromatic structures in humic materials or that cultivation increases the relative

abundance of aromatics in soils (Lessa et al., 1996; Schnitzer et al., 2006), aromatic compounds originating from plant polysaccharides or proteins, as well as microorganisms, may be contributing to these pools (Guggenberger et al., 1995).

4.1. Soil chemistry interactions with enzyme activity

The forest soils contained more total LF and a higher relative concentration of LF, which we anticipated would result in higher enzyme activities. Enzyme activity should parallel the concentration of LF because this fraction has a high C concentration and is largely free from association with soil minerals. Accordingly, LF generally turns over rapidly and typically exhibits high microbial cell counts and enzyme activities (Kandeler et al., 1999; Blackwood and Paul, 2003; Marx et al., 2005). Allison and Jastrow (2006), for example, found that the activities of chitinase and cellulase were significantly higher in particulate organic matter fractions, which correspond to LF, than silt and clay-sized fractions, which correspond to HF, and suggested that these enzymes may contribute to the rapid turnover of particulate C. Contrary to our expectations, we found that alpine ecosystems had higher enzyme activities per unit C than did the forest systems.

One explanation for the higher enzyme activity in the alpine ecosystems is that they support more rapid turnover rates of C compared to the subalpine forest. This could be the case if environmental conditions such as temperature and soil moisture differ between the sites or if decomposition dynamics were being driven not by the amount of LF but by its chemical structure. The relative abundance of LF polysaccharides, protein, and lignin was statistically indistinguishable between the forest and alpine sites (Fig. 1) but the C/N ratio of the forest soil LF was higher (Table 2). Another explanation may be that there is a sorbed pool of enzymes in the HF, the predominant fraction in the alpine systems, which contributed to potential activities in the lab but which are not active in situ. The activity of some enzymes, such as polyphenol oxidase, can be higher in clay and microaggregate fractions than in whole soils (e.g. Stemmer et al., 1997; Allison and Jastrow, 2006) but due to enzyme sorption and stabilization there may be a disconnect between potential activities and in situ C turnover times in this fraction.

Correlations show that there were fundamental differences in the relationships between soil chemistry and enzyme activities in LF and HF (Table 6). LF was more strongly correlated with enzyme activities than HF, likely because this SOM pool is more physically accessible and is less processed than HF C. Correlations also suggest a direct enzymatic influence over chemical structure. Phenols, along with lignin, were the most abundant compounds in LF and peroxidase is expected to play a central role in their breakdown. The strong correlation between

peroxidase and phenols in the alpine LF supports the notion that this enzyme is a particularly important control over LF decomposition. Similarly, across sites peptidase was significantly correlated with protein abundance suggesting that chemical structure and enzyme activities are directly related.

Many of the relationships between enzymes and chemical structure were more difficult to explain than those discussed above. This was also the case when we correlated the twenty most abundant compounds listed in Table 3 with enzyme activities (data not shown). One possible explanation for this is that the time scales over which enzymes and SOM chemistry change and interact varies. SOM chemistry reflects long-term decomposition processes while enzyme dynamics fluctuate seasonally or with current environmental conditions (Weintraub et al., 2007). There may also be a sorbed pool of enzymes in the HF, creating a disconnect between enzyme potentials in the lab and their in situ activities, as discussed above. Additional insights into the relationship between enzymes and C chemistry at these sites may require measuring enzyme activity separately for LF and HF.

5. Conclusions

We found no consistent differences in soil chemistry among geochemically distinct basins but there were differences in LF C content and distribution between forest and alpine ecosystems, likely due to differences in the quality and quantity of plant inputs and decomposition rates. Consistent across basins and elevations was a depletion of lignin and accumulation of polysaccharides in the HF. This suggests a common decomposition sequence in these soils, irrespective of ecosystem characteristics and their effects on LF chemistry, where plant-derived lignin and other LF constituents are broken down and lipids, polysaccharides, proteins and other microbially derived C compounds accumulate in the HF. Although enzyme kinetics and rates of SOM turnover can be altered by plant inputs and LF chemistry, the production of microbial by-products and their stabilization on mineral surfaces is the primary mechanism that stabilizes C long-term.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.soilbio.2007.05.009.

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