AN ABSTRACT OF THE THESIS OF

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Abstract Approved:

David D. Myrold

Forests are one of the largest repositories of terrestrial C. Understanding factors that drive organic matter transformations and nutrient efflux from these systems is therefore highly important. Temperate forests are of particular significance due to the large fraction of C that is stored below ground in the soil. Characterizing nutrient transformations, especially for C and N, and factors that influence their mineralization is critical in managing these ecosystems sustainably. The objective of this study was to characterize the metabolic function of the soil microbial community as it relates to C and N cycling in managed second-growth Douglas-fir forests of the Pacific Northwest.

In the first study, extracellular enzyme profiles were characterized and correlated with a wide range of environmental variables present at nine sites located in western Oregon and Washington. Enzyme profiles were also correlated with measures of microbial biomass and with C and N mineralization rates obtained from a two-month incubation. Sites varied significantly from one another in all enzymes. C-cycling enzymes were correlated with respired C, and N-cycling enzymes, in addition to phosphatase and oxidative enzymes, were correlated with released N. Enzyme profiles grouped similarly for sites of the same soil type. Abiotic factors such as CEC,

EC, and cation concentrations were correlated strongly with many enzymes, suggesting that soil physical and chemical properties influence extracellular enzyme function.

In the second study, a year-long soil microcosm incubation measured respired C as well as total released N: NO_3^-, NH_4^+, and dissolved organic N (DON). These cumulative mineralization measurements were fitted with kinetic models to characterize C and N cycling in the same nine soils. Mineralization rates, and their descriptive parameters derived from the models, were correlated with the same set of environmental characteristics and enzyme activities from the previous study. Total soil C and N, as well as microbial biomass, were strongly positively correlated with both C and N mineralization. However, abiotic factors such as soil chemical components also had significant effects. This, coupled with anomalous behavior noted in C mineralization in response to leaching treatments, may suggest substrate supply to microorganisms constrains much of C mineralization. N mineralization seemed strongly tied to biotic factors in addition to abiotic factors. Often in studies of N dynamics in soil, only mineralized N (NO_3^- and NH_4^+) are examined; our study revealed that DON was the largest fraction of released N in these systems, and dynamics of DON in N cycle warrant further research.

Together, these studies provide insights into factors that drive soil microbial community function across a broad range of site conditions for Douglas-fir dominated forests of the Pacific Northwest region and could serve as a baseline for future research.

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Functional Profiles of Soil Microbial Communities in Second-growth Douglas-fir Forests of the Pacific Northwest

by

Megan L. McGinnis

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Megan L. McGinnis, Author

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Chapter 1:
General Introduction

Megan L. McGinnis

Introduction

Soils have a large global capacity to store and cycle carbon (C). However, predicting the fluxes of C into and out of soil is difficult because of the mechanistic complexity governing these processes. Furthermore, ecosystem disturbances have the potential to dramatically influence these C-cycling processes, and the microbial communities that control them, in poorly understood ways. The goal of this thesis was to examine forest soils from the Pacific Northwest and to assess their C and nitrogen (N) cycling and extracellular enzyme activities in relation to soil microbial communities and environmental conditions. The relationship between C and N cycling, extracellular enzymes, and site conditions was examined in the first experiment; in the second experiment, changes C and N mineralization rates obtained from a year-long soil microcosm incubation were examined to better understand the processes acting on organic matter decomposition. These soils, analyzed prior to logging, will serve as the baseline for assessing logging impacts on C-cycling and community structure in future years.

Soils and Carbon Cycling

Atmospheric CO_2 continues to rise, impacting global temperatures and becoming a source of growing international concern (IPCC, 2001). Thus, understanding mechanisms influencing the global C cycle and C pools is critical to predicting and moderating the effects of climate change.

The soil C pool is the largest terrestrial reservoir of C that cycles on a non-geological time scale. It is estimated that soils globally contain 1500 Pg C in the top

meter, and 2300 Pg C in the top 3 m, which is over twice the amount of C as in the atmosphere (Post et al., 1982; Jobbagy and Jackson, 2000). Because of this, small changes in soil respiration can have dramatic effects on atmospheric CO_2 levels (Schlesinger and Andrews, 2000). Forests and forest soils play a particularly important role in mediating the global C cycle. Recent studies have suggested that nearly all of the global terrestrial C sink can be attributed to forests, and changes in soil C stock account for more than 10% of that total forest sink, averaging about 477 Tg C $year^{-1}$ over the period from 1990 to 2007 (Pan et al., 2011). However, forests and forest soils' ability to act as a C sink can be dramatically altered with changes in land use and management (Lal, 2005). For example, it has been suggested that loss of soil organic C can be as great as 50% in the 20 years following clear cutting in hardwood forests (Covington, 1981). Because of the crucial and sensitive role of forest soils in mediating atmospheric CO_2 levels, understanding the processes that influence C storage and turnover is central to modeling and mitigating the effects of climate change. Unfortunately, many aspects of soil C cycling remain poorly understood.

Carbon is stored in soils in the form of soil organic matter. Soil organic matter often enters the soil through plant inputs, either above or below ground, or through microbial biomass, and consists of a wide variety of organic polymers in varying states of decomposition. Soil C is often understood as consisting of two or three main pools; the fast-cycling pool is believed to consist of labile inputs such as sugars and lipids, and turns over in a matter of less than a year, while the slower cycling pools consist of residues and more complex macromolecules like lignin that turn over on

decadal, centurial, or even millennial time scales (Baisden and Parfitt, 2007; Post et al., 1990). The degradation of these C inputs is carried out by a wide host of soil microbes.

As microbes degrade these plant inputs, they either assimilate it into their bodies to grow, respire it as CO_2, or re-release a portion of it in the form of enzymes or other products. Some of the C is incompletely degraded and becomes incorporated as soil organic matter. The amount retained versus respired varies depending on environmental conditions, land management, and the soil microbial community. Thus, the microbial community and its activity provide a direct control on terrestrial CO_2 fluxes into the atmosphere, and alteration to microbial communities and processes will determine soil's ability to act as a C sink.

Predicting mineralization and activity: Extracellular Enzymes

Extracellular enzymes are released by soil microbes into the soil matrix to mediate the breakdown of organic constituents, and to aid in the acquisition of nutrients such as C, N, and P (Henry, 2013; Sinsabaugh and Shah, 2011). Thus, measuring the activity of these enzymes may help indicate rates of decomposition of organic matter, determine nutrient limitations in the soil environment, and provide a broad overview of microbial community activity and function as a whole. For example, extracellular enzymes catalyze the rate-limiting step in soil organic matter decomposition (Caldwell, 2005). Similarly, understanding environmental factors that alter extracellular enzyme activities can help elucidate what factors influence community size, activity, and function.

Changes in enzyme activities have been observed in response to substrate changes, nutrient limitation, and other factors (Hernandez and Hobbie, 2010; Allison and Vitousek, 2005). Ratios of enzyme activities often are useful for providing information on the nutrient status of a given soil (Sinsabaugh et al., 2009). However, there remains a great deal of uncertainty when attempting to use enzyme activity as an indicator of nutrient status or as a predictor of future activity. Competing hypotheses exist stating that enzymes are either produced as a response to nutrient limitation, or are produced constitutively and reflect the size and activity of the soil microbial population. The combination of these somewhat competing hypotheses creates a dilemma when it comes to interpreting a soil's extracellular enzyme activity status, as high or low enzyme activity status may be reflect either abundant microbial population or increased nutrient stress. Furthermore, each hypothesis is correct for specific enzymes, under specific circumstances. For example, globally, phosphatase activity has been correlated with P stress, whereas other enzymes, such as β-1-4-glucosidase, have been strongly correlated with microbial metabolism (Sinsabaugh et al., 2008; Sinsabaugh and Shah, 2012). Untangling the intricacies of extracellular enzymes remains the topic of ongoing research, and is an important aspect of this study.

Despite the complicated nature of interpreting extracellular enzyme data, measures of extracellular enzyme activity remain insightful for assessing nutrient status and reflecting the active microbial community. For this reason, they are able to provide a sort of functional fingerprint of microbial community status, and while their

predictive utility is still debatable, they can be critical indicators and tools for assessing pre- and post-disturbance changes in nutrient cycling function.

Although the techniques utilized vary depending on the specific study objective and examined enzymes, the concept under which extracellular enzyme assays operate remains the same (German et al., 2011). Labeled substrates are added to a soil slurry. These labeled substrates may be either fluorometric or colorimetric. In fluorometric assays, when an enzyme present in the soil slurry encounters the labeled substrate, it cleaves a fluorescent tag from the substrate molecule. The greater the enzyme concentration in the soil solution or the activity of the targeted enzyme, the more fluorescent tag is released. This corresponds to greater fluorescence when the assay is then measured in a fluorometer, and thus provides a measure of potential activity for the substrate-targeted enzyme in the soil sample. A similar process occurs with enzymes which are measured colorimetrically, except that the resulting compound, once acted on by the enzyme, absorbs light at a different wavelength than the initial substrate, and the absorption at the target wavelength provides a similar measure of potential enzyme activity.

For both of these methods, a non-limiting substrate concentration is required in order to measure the maximum potential activity. Additionally, the conditions (temperature, pH, soil moisture, etc.) under which the assay is carried out may not perfectly reflect field conditions (German et al., 2011). Thus, due consideration is required when interpreting extracellular enzyme results. Under the described methods, all measured activity reflects the maximum potential activity of the system, which may

not be representative of typical activity in the field. However, it remains useful as it represents a maximum potential, and this potential can be further correlated with other activity and community measures to provide meaningful results. Additionally, a system's maximum capacity for activity may be directly affected by disturbance. For assessing both of these, extracellular enzyme assays remain a useful tool.

Quantifying mineralization

In addition to extracellular enzymes, soil activity and rates of C and N mineralization can be measured directly through the use of soil incubations. Soil microcosm incubations are a well-established method of measuring the respired C and mineralized N from soils under various conditions (Stanford and Smith, 1972). Mathematical models have been fitted to C or N accumulation curves derived from incubations to describe meaningful properties of the soil nutrient pools, such as the size of fast and slow cycling pools and their turnover rates (Sleutel et al., 2005; Hess and Schmidt, 1995). Many studies examine changes in CO_2 efflux associated with increased temperature, moisture, substrate additions or losses, and other factors (Wagai et al., 2013; Curtin et al., 2012; Conant et al., 2011; Crow and Lajtha, 2009). It has generally been noted that soil respiration tends to increase with increased temperature, and that different types of soil organic matter respond with differing sensitivities to increased temperature (Conant et al., 2008). This trend has strong implications in predicting soil CO_2 efflux from soils in response to climate change. Thus, quantifying the size and mineralization rates of both fast and slow cycling pools in different environments is critical to better understand ecosystem responses.

In addition to temperature and properties of the organic mater itself, the availability of other nutrients impacts the amount of C assimilated and respired. The ratio of C:N often influences the growth rates of microbes, and thus rates of C respiration and sequestration (Swanston et al., 2009). Soils shift towards retaining more C when there is a greater proportion of N (Lal, 2005). Swanston et al. (2009) demonstrated that in sites with elevated N stabilized the organic matter in both the whole soil and in separated soil fractions. Thus, quantifying and monitoring changes in N mineralization over time are also important, from a nutrient balance perspective as well as from a C cycling perspective.

Effects of disturbance

Logging and other forest management activities can create many changes in the soil environment and the soil microbial community. Changes in above and below ground plant inputs, soil compaction, and soil temperature, and moisture have been noted to cause changes in forest soil's ability to store and cycle C and N (Churchland et al., 2013; Nave et al., 2010). Covington (1981) found that logging reduced a hardwood forest's floor 50% in the first 15 yr after timber harvest. Trends like these illustrate how important it is to understand how logging affects nutrient turnover and microbial function in a variety of ecosystems. A thorough characterization of soil functional properties in managed forests is the first step in this process.

Objectives

The objectives of this thesis are: to determine how soil microbial community function differs between and within study sites covering a broad geographical range; quantify changes in C and N mineralization in relation to enzymatic activity and community size; determine which environmental variables are most indicative of differences in microbial community function. Furthermore, these baseline data will be instrumental in understanding the short-term effects of timber harvest on microbial community function and C and N-cycling dynamics when the sites are sampled again post-harvest, yielding critical details about the time scales at which microbial community structural and functional changes are evident. Insights gained from this study will be critical in assessing changes in response to logging in future years, and assessing land management impacts on C cycling and microbial community dynamics.

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Chapter 2:
Regional assessment of soil microbial functional diversity of Douglas-fir forests

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Abstract

Extracellular enzyme activity (EEA) provides a functional fingerprint of soil microbial communities and can be an indicator of change following disturbance. This study examined the EEA of nine managed, Douglas-fir (*Pseudotsuga menziesii*) dominated forests in the Pacific Northwest of the United States. It assessed the variability of EEA both within and between sites, and correlated EEA with environmental variables covered by the broad geographical range of the study sites. This characterization was linked with measures of community size, C and N cycling ability, and other soil properties, such as texture and nutrient status, to provide insights into extracellular enzyme production and microbial function. Principal components analysis (PCA) and multiple response permutation procedure (MRPP) results indicated that EEA is highly variable among sites, but much less variable within sites. It also revealed that the functional fingerprint of the soil tended to be more similar for soils within the same soil order. Additionally, results implied that phosphatase, and the C-cycling enzymes β-glusosidase and cellobiohydrolase are constitutive in these systems, whereas N-scavenging enzymes N-acetylglucosaminidase and leucine aminopepdidase are facultative. Furthermore, the N-cycling EEA suggests N limitation in these sites despite N fertilization. This characterization will be critical in assessing microbial community changes following timber harvest.

List of Abbreviations

EEA – Extracellular enzyme activity, PCA – principal components analysis. MRPP-multiple response permutation procedure. LAP – Leucine-aminopeptidase, NAG – N-acetyl-b-glucosaminidase, PHOS – phosphatase, CBH – cellobiohydrolase, BG – β-1-4-glucosidase, PHENOX – phenoloxidase, PEROX – peroxidase, qPCR – quantitative PCR

Introduction

Soil microbial communities are responsible for performing most of the biogeochemical transformations on Earth and driving the global C and N cycles. Extracellular enzymes, which are produced by microorganisms and released into the soil matrix, catalyze the break down of polymeric molecules of soil organic matter, and thus are seen as the rate-limiting step for the decomposition and turnover of nutrients (Henry, 2013; Sinsabaugh and Shah, 2011). Extracellular enzymes reflect both the size and activity of the microbial community, as well as the nutrient status of the soil environment (Caldwell, 2005). For these reasons, characterizing extracellular enzymes in natural systems is critical in understanding nutrient cycling dynamics.

A great deal of work has been done on enzyme production and activity. Studies have examined how enzymes and microbial community functions change in response to various substrate inputs (Hernandez and Hobbie, 2010; Allison and Vitousek, 2005). A series of recent review and synthesis articles have characterized global trends in extracellular enzyme activity (EEA) (Sinsabaugh and Shah, 2012; Sinsabaugh et al., 2011, 2008; Wallenstein et al., 2011). These studies have covered a wide range of ecosystem types and spanned large ranges of abiotic factors. Their results indicated that globally, phosphatase activity is inversely proportional to environmental P availability, the ratio of N-scavenging enzymes to β-glucosidase activity is an indicator of N limitation, hydrolytic C-metabolizing enzymes are well correlated with microbial metabolism, and that oxidative enzyme activity is controlled by a variety of factors such as pH, O_2 levels, and Mn and Fe concentrations. However, less work has

been done characterizing microbial function within forest ecosystems, while still spanning large ranges in other characteristics.

Forest soils are huge repositories of C. This is particularly true of temperate forests, which store a greater fraction of their C below ground than tropical forests (Pan et al., 2011). Because they are so critical in global C and N transformations, small changes in forests' nutrient cycling activities can have potentially large effects on total fluxes from these systems (Lal, 2005). Furthermore, a significant proportion of forest soils are managed systems (FAO, 2010), and the effects of management practices and environmental change on EEA and microbial community function are only beginning to be elucidated.

Nevertheless, EEA in temperate conifer forests of the Pacific Northwest have had considerably less characterization. Selmants and colleagues (2005) examined differences between conifer stands and alder (*Alnus rubra*) dominated forests in Oregon. Their work demonstrated distinct differences between alder-dominated stands and conifer stands, in that alder stands had greater enzyme activities for all their measured enzymes, and emphasized the relationship between plant species composition and microbial community function. Although their study examined variation in plant communities in a single forest, it did not capture a broad range of other environmental factors present in the region. A geographically robust study in western Canada examined enzyme profiles across seven different forest types and found distinct EEA profiles for soil microbial communities in the different forests. In most cases, factors such as soil moisture and soil organic matter tended to drive these

among-site differences in microbial community function more than plant community differences (Brockett et al., 2012). The insights gleaned from these studies are relevant, but Selmants et al. (2005) examined a single soil environment under different tree species, while Brockett et al. (2012) examined different soil environments as well as different tree species. Our study examines functional profiles of microbial communities in differing soil environment conditions among forests of the same dominant tree species. Other studies have examined soil physical properties in relation to C and N cycling in the region, but have been less comprehensive in their analysis of EEA in these Pacific Northwest forest ecosystems (Homann et al., 2001; Spears et al., 2001).

A more thorough characterization of EEA in temperate coniferous forests is needed both to assess C and N cycling function, as well as changes following disturbance such as logging. By characterizing the variability in ecosystem function within the same eco-region, while still covering a broad range of other environmental variables such as precipitation, elevation, latitude, and soil type, it was anticipated that this study would yield geographic level insights into how these forests respond to differing environmental conditions. This study sought to provide such a regional assessment of microbial functional diversity by characterizing EEA in nine Douglas-fir (*Pseudotsuga menziesii*) dominated forests in the Pacific Northwest of the United States. This assessment of EEA variability was examined both within and between sites, and correlated with environmental variables covered by the broad geographical range of the study sites. This characterization was linked with measures of community

size (via quantitative PCR (qPCR) of bacterial and fungal markers), and C and N cycling ability (derived from a laboratory incubation), and soil nutrient status to provide insights into extracellular enzyme production and function.

Materials and methods

Site descriptions

Nine commercial-harvest-age, second-growth forest sites dominated by Douglas-fir (*Pseudotsuga menziesii*) were randomly selected from within Weyerhaeuser Company ownership in western Oregon and Washington, USA. Excessively rocky sites were rejected from consideration, to accommodate soil collection with corers rather than quantitative pits. These sites, while having similar plant-species composition, cover a wide range of environmental, climactic, and soil conditions (Table 1.1). Sites were fertilized with varying frequencies (from 0 to 6 times) and application rates (370 to 560 kg ha^{-1}, average 463 kg ha^{-1}) of urea. Because of the random selection procedure, these sites represent a reasonable overview of harvest-age commercial timberland in the region.

Soil Sampling

Each of the nine, 2.5 to 8.1-ha study sites was subdivided into 25 plots of approximately equal area. From the 25 plots at each site, five were selected to sample in the summer of 2011. The five plots were nearby the soil pits used for soil classification. Within each of these five plots, a grid pattern was established of 12

sampling points, ranging from 9 to 12 m apart, depending on the site. A composite sample for the plot was created from cores obtained at these 12 sampling points. Cores of 2 cm diameter were taken from 0-15 cm depth, with two adjacent cores were taken very near each of the 12 designated sampling points. This formed a composite sample of 24 cores for each plot. In total 45 composite surface soil samples were taken, five from each of the nine sites.

Samples were brought back to the lab, sieved to <4 mm, measured for water content, and frozen at -4°C until further analysis. Gravimetric moisture content was determined by oven drying a subsample at 105°C for 24 h. Total C and N content was determined via dry combustion on those samples.

Microbial biomass was determined by the chloroform fumigation-extraction method (Brookes et al., 1985) using 0.05 M K_2SO_4. Fumigated and unfumigated extracts were measured on a Shimadzu TC/TN analyzer (Shimadzu Corp, Kyoto, Japan) to determine dissolved C and N. Microbial biomass C and N were calculating using conversion factors of 0.45 for C and 0.56 for N (Brookes et al., 1985; Vance et al., 1987).

DNA extraction and quantification

DNA was extracted from field moist soil (0.25 g dry weight equivalent) using the MoBio PowerSoil ® DNA Isolation kit. Three replicate extractions were made for each soil sample. Extraction was conducted according to the manufacturer's protocol.

Quantitative PCR was performed using the Applied Biosystems 7500/7500 Fast Real-Time PCR System (Life Technologies, Grand Island, NY). Amplification

was carried out in 25-µl reactions. Fungal amplification was done as follows: 7.62 µl of water; 2 µl of 0.4% bovine serum albumin (BSA); 12.5 µl of the 2x SYBR green master mix (containing Taq polymerase); 0.25 µl of the 10 mM forward primer ITS1F, and 0.25 µl of the 10 mM reverse primer, ITS 5.8 and 0.38 µl of a 1:50 dilution of ROX dye. For bacteria, twice the primer amount was used (0.5 µl of 10 mM Eub 338F, and 0.5 µl of 10 mM Eub 518) to ensure efficient amplification. Thermal cycler profiles for amplification were adapted from Boyle et al. (2008) and consisted of an initial step of 50°C for 2 min followed by 95°C for 10 min. Thirty-five amplification cycles followed: 95°C for 30 s, 53°C for 1 min, 72°C for 1 min. Dissociation consisted of a single cycle of 95°C for 15 s, 60°C for 1 min, and 95°C for a final 15 s. All amplifications for both bacteria and fungi were > 93% efficient and had R^2-values >97%. Dissociation curves contained a single large peak.

Enzyme activities

A suite of enzymes was measured on each soil sample with techniques adapted from Sinsabaugh et al. (1993), with consideration given to optimization techniques described by German et al. (2011). The hydrolytic enzymes: phosphatase (PHOS), β-glucosidase (BG), N-acetylglucosaminidase (NAG), cellobiohydrolase (CBH), and leucine aminopeptidase (LAP), were measured fluorometrically in black 96-well plates. Oxidative enzymes, phenoloxidase (PHENOX) and peroxidase (PEROX), were measured colorimetrically in clear 96-well plates. All assays used 50 mM sodium acetate (NaOAc) buffer solution adjusted to pH 5, as the bulk of our soils had pH values close to 5.

A soil slurry was prepared using 1 g field moist soil in 100 ml of the NaOAc buffer solution. The slurry was homogenized on a stir plate for approximately 3 min before being loaded into the 96-well plates. For the fluorometric enzymes, assays were carried out in 250-µl reactions (200 µl of soil slurry and 50 µl of either substrate, standard, or blank). Oxidative enzyme assays were carried out similarly; however, for the peroxidase assay, 10 µl of 0.3% H_2O_2 were added, bringing the well total to 260 µl.

Assay plates were incubated in the dark at 25°C for varying lengths of time, depending on the enzyme: PHOS and BG were incubated for 2 hr, NAG and CBH for 4 hr, and LAP, PHENOX, and PEROX for 26 hr. These times were determined experimentally by finding the optimum absorbance or fluorescence for each enzyme. Following incubation, fluorometric reactions were terminated by raising the pH with 10 µl of 0.5 N NaOH. Colorimetric assays were read at ambient pH, but 200 µl of supernatant was transferred to a clean, clear 96-well plate prior to measurement. Samples were then measured using a Spectramax Pro spectrophotometer (Spectramax 190, Molecular Devices, Sunnyvale, CA, USA). PHOS, BG, CBH, and NAG were measured fluorometrically at a 365 nm wavelength, and LAP at 385 nm. Maximum absorption for oxidative enzymes was measured at 450 nm. Examination of correlation coefficients indicated that many enzymes were correlated with biomass C, therefore activities were calculated and reported as nmol absorbance or fluorescence per gram of microbial biomass C per hour for all subsequent analysis.

Soil microcosm incubation

A soil microcosm incubation was conducted to quantify soil C and N mineralization in a similar manner as described by Stanford and Smith (1972). Field moist soil (50 g dry weight equivalent) was measured into filter-towers with grade 1 Whatman glass microfiber filters on bottom—to prevent soil loss during the incubation, and top—to prevent disturbance to soil aggregate structure. Soils were first wet with 25 ml of 0.01 M $CaCl_2$, and then pulled to 0.33 bar vacuum (field capacity). This followed by another 25 ml of Nanopure water once no $CaCl_2$ solution remained on the surface of the soil. The vacuum remained constant until all samples had ceased to lose water (approximately 20 min). Leachates were frozen for storage, and measured later for organic and inorganic N.

The soil samples in their filter towers, uniformly at field capacity, were then placed into 450-ml glass canning jars. The jars were covered with thin polyethylene plastic film to allow for gas exchange, but minimize evaporation during incubation. Soils were kept in the dark at 25°C for the duration of the incubation.

Respiration rates were measured with a Picarro gas analyzer equipped with a multiport-valve sampler (Picarro Inc., Santa Clara, CA). For measurement, microcosm samples were capped with canning jar lids fitted with airtight septa. Samples were measured for 2 min followed by a 58 min waiting period, before being measured again for a second 2 min period. The increase in CO_2 gave the respiration rate per hour. As the respiration rates decreased over time, the length of the waiting period was lengthened to allow for accurate measurement. Samples were measured on days 0, 1,

3, 6, 11, 18, 28, 43, and concluded on day 63 of the incubation. This final respiration measurement was taken after the first leaching event, and was the first time point for which C and N data were available. Respiration was averaged over this time period and reported as μg CO_2-C g^{-1} dry soil d^{-1}.

At the end of the two-month incubation period, the leaching process was repeated to track changes in ammonium, nitrate, and soluble organic N and C.

Ammonium and Nitrate Assays

Ammonium was quantified using the colorimetric assay of Qiu et al. (1987) modified as follows for a 96-well format. Leachate collected from the incubation was aliquoted (178 μl per well) into the colorimetric 96-well plates. A salicylate mixture was prepared which consisted of 10 g of trisodium citrate and 10 g of salicylic acid combined with 35 ml of 2 M NaOH and diluted to 200 ml with water. A subsequent 1:10 mixture of sodium nitroprusside (1% m/V) to the salicylate mixture was then aliquoted (22 μl per well), followed by 8 μl of a sodium hypochlorite solution, for a well total of 208 μl. The sodium hypochlorite solution was prepared fresh daily by mixing 0.35% available chlorine in 2 M NaOH and deionized water. All readings were measured in reference to a standard curve prepared from 1:2 serial dilutions of ammonium sulfate (10 to 0.3125 mg NH_4^+-N l^{-1}). The assay plate was incubated for 1 h at room temperature before measuring absorbance at 660 nm with a plate reader (Spectramax 190, Molecular Devices, Sunnyvale, CA).

Nitrate was quantified using a colorimetric assay in 96-well format (Inselbacher et al., 2011; Hood-Nowotny et al., 2010). The assay was carried out in

150 µl reactions: 50 µl of the leachate sample, 50 µl of a vanadium chloride solution, 25 µl of Griess reagent 1, and 25 µl of Greiss reagent 2. The Griess reagents were mixed in equal volumes immediately before being added to the assay mixtures. All readings were measured in reference to a standard curve prepared from 1:2 serial dilution of potassium nitrite. The plates were incubated at room temperature for 1 hr and the absorbance was measured at 540 nm using a plate reader (Spectramax 190, Molecular Devices, Sunnyvale, CA).

The leachates analyzed using these assays were collected the day prior to the corresponding respiration measurement (i.e., day 62 of the incubation).

Soil nutrient analysis

To characterize other nutrient availability parameters, <2 mm fraction composites were analyzed for Bray extractable P; ammonium acetate extractable K, Ca, Mg, and Na; and DTPA (diethylenetriamine pentaacetic acid—a chelator) extractable S, Fe, B, Mn, Cu, and Zn. Electrical conductivity (EC) and pH were measured in a 1:1 soil to water ratio. Cation exchange capacity (CEC) was also determined. Soil texture was determined on the <2 mm soils using the hydrometer method and reported as percent sand, silt, and clay.

Statistical methods

For detecting differences in activity between sites for individual enzymes, one-way ANOVA was used. Data was analyzed and graphs for individual enzymes were generated in SigmaPlot version 12 (Systat Software, Inc., San Jose, CA).

PC-ORD version 6.06 (McCune & Mefford, 2011) was used for multivariate statistical analysis. Matrices were prepared in Microsoft Excel 2007 and imported into PC-ORD. The activity matrix (45 soil samples x 9 variables) contained measures of enzyme activities and C and N mineralization for each composite soil sample. All values in the activity matrix represented absolute, quantitative measurements. The environmental matrix consisted of 45 soil samples x 24 environmental variables. This matrix included both categorical variables (site membership, Coast vs. Cascade locale, soil type, and fertilization frequency) and quantitative variables (e.g., site latitude, annual precipitation, pH) at each site from which the sample was taken.

A third matrix was generated containing the geographical distances among all sites. This geographical distance matrix was generated using the Geographic Distance Matrix Generator (Ersts, 2013). This distance matrix was relativized so that scales between the two matrices would be the same, and prevent any possible skew that may occur from having numbers that spanned a broad range of values. A Mantel test comparing the similarity in the distance matrix to the log transformed, relativized activity matrix (with activities averaged by site) served as an indication of the amount of variability that could be explained by spatial separation among sites.

A few of the enzyme activities had slightly negative values because of subtracting soil and substrate blanks from the assay values. This was accounted for by adding the absolute value of the lowest measured value to all measurements of that enzyme. This maintained the relative distances between each value, while correcting the negatives so that they could be subsequently log transformed and analyzed.

Because activity data spanned many orders of magnitude and variance tended to increase with higher values in many of the enzyme activities, data in the activity matrix was modified using the general log transformation by column (activity variable). This transformation also improved the linearity for many variables. Additionally, data were also general relativized by activity variable. This allowed for comparison among samples because the activity data was expressed in various units.

Outlier analysis identified three samples with greater than two standard deviations from the grand mean. However, because there was a much broader range of total values, these outliers were retained because of the meaningful information they contributed to the data set.

Correlations are reported in the text at an absolute r-value at or above 0.372, which is the critical value corresponding with a 0.01 p-value based on a Pearson correlation coefficient (for 43 degrees of freedom). Significance at the 0.05 level occurred at an r-value of 0.288, and 0.474 at the 0.001 significance level.

Principal components analysis (PCA) was chosen as the ordination technique and for visual analysis. PCA is an ordination technique which seeks to maximize the amount of variation explained by sequentially added orthogonal axes. It was chosen over other ordination methods, primarily non-metric scaling, because a scatter plot matrix showed many strong linear trends among the variables with few "dust bunny" patterns (McCune and Grace, 2002).

Cluster analysis and testing with multiple response permutation procedure (MRPP) were also conducted to determine if groups based on site, soil type, and other

categorical variables were stronger than what could be expected by chance. All cluster analyses were performed using Sørensen distance measures and a flexible-beta value of 0.25. The A statistic ranges from 0 (the expectation based on random distribution) to 1 (perfect group separation), and indicates how strong a relationship is between elements which are grouped together. A strong relationship has an A value greater than 0.1.

Results

In general, at least ten-fold differences in EEA were observed (Table 1.2) as a result of both within and among site variability. Significant site differences were observed for each EEA, suggesting that variation between sites was greater than within sites, and at least one difference among sites occurred for every enzyme (Fig. 1.1). No one study site consistently had higher or lower activities for all enzymes or other activity measures, however. The relative rankings of sites showed similar patterns for the hydrolytic C-cycling enzymes BG and CBH, with OR 1 and OR 2 being high and WA 1 and WA 4 being low. A consistent pattern was also seen for the hydrolytic N-cycling enzymes NAG and LAP. Sites were also different in terms of the amount of mineralized C ($F=4.69$, $p<0.001$) and N ($F=6.72$, $p<0.001$), total C ($F=37.67$, $p<0.001$) and N ($F=37.20$, $p<0.001$), and microbial biomass C ($F=13.08$, $p<0.001$) and N ($F=13.76$, $p<0.001$). There was no difference in either the log numbers of bacteria or the log numbers of fungi among sites.

There were also many correlations between variables (Table 1.3). Notable positive correlations for respired C were microbial biomass C (r= 0.470) and N (r= 0.525). When enzyme activities were adjusted according to microbial biomass C, BG (r= 0.746) and CBH (r= 0.735) were strongly positively correlated with respiration. PHENOX (r= 0.569), LAP (r= 0.388), NAG (r= 0.312), and PHOS (r = 0.360) activities were all significantly correlated with N mineralization, in addition to microbial biomass C and N. Activity for PHENOX was correlated with NAG activity. Microbial biomass C was strongly correlated with the total C and N content of the soil.

Two-way cluster analysis demonstrated many samples grouping together according to site membership, and also revealed patterns across all enzymes (Fig 1.2). MRPP analysis was performed to determine if differences existed between categorical variables of site, soil type, forest establishment method, and locale (Coast vs. Cascade mountains). The strongest grouping was by site ($A=0.422$, $p<0.0001$), indicating that soils were much more similar in activity to samples of the same site than with samples from other sites. Strong correlation also existed with fertilization frequency ($A=0.166$, $p<0.0001$). Somewhat weaker grouping existed for soil type ($A=0.099$, $p<0.0001$). There was no significant group based on locale.

PCA of the samples yielded an ordination in which the first three axes represented 34.6%, 22.8%, and 11.5% of the variance. A two-dimensional representation was chosen as suggested by the Rnd-lambda criterion, and because it illustrated clear differences among samples, while still representing more than half of the total variation. An overlay of environmental variables demonstrated strong

groupings based on the sample's soil type (Fig. 1.3). Other overlays such as by site and fertilization also had some visual separation, but not as visually appreciable as that for soil type.

Like microbial functional characteristic, environmental variables ranged widely across the sites (Table 1.4). Total C was the environmental variable that was most strongly correlated with either of the first two ordination axes (r=0.718). This was followed by total N content (r=0.680) and precipitation (r=0.622). CEC (r=0.573) and bacterial abundance (r=-0.551) were the next most strongly correlated environmental variables followed by Na, Mn, temperature, and clay content (Fig. 1.3). Weaker correlations existed for EC, pH, and Ca, Cu, and Fe contents. No notable correlations were observed for latitude, elevation, Bray extractable P, Zn, S, or B content, fungal abundance, or for percent sand (Table 1.5).

A Mantel test comparing the activity matrix to a geographic distance matrix showed no significant correlation between differences in microbial activity with distance among sites (Mantel statistic r=0.152, p-value = 0.364).

Discussion

The objectives of this study were to better characterize the range of EEA in Douglas-fir forests of the Pacific Northwest, and to correlate these measures of activity with measures of mineralization, microbial community, and the wide range of environmental variables encompassed by the study. When examining results with

respect to individual enzymes, trends based on site differences were not always consistent. By this we mean two things: first, within-site differences were smaller than among-site differences for nearly all enzymes; second, there was a lack of consistent EEA patterns of high or low activity for individual enzymes across sites. For example, PHOS activity for all samples across sites had a coefficient of variation (CV) considerably higher than the CV for individual sites (0.56 for the grand mean, and an average of 0.26 for sites), indicating that PHOS activity within sites was more similar than it was across among sites. However, sites that were high in PHOS were not consistently higher in other enzyme activities (Fig. 1.1). Although high within-site variability may have limited our ability to measure more statistically significant differences in EEA among sites, we nevertheless found some differences. Combined with multivariate analysis, the collective EEA profile does allow sites to be better distinguished. Furthermore, the different rankings among sites for individual EEA suggest different degrees of potential C, N, and P limitation at different sites.

Multivariate analysis

When enzyme activities were examined with multivariate techniques that examine all activities simultaneously, differences among sites were more readily apparent. Samples from the same site tended consistently to be more similar to each other than they are to samples from different sites. Furthermore, differences between sites did not correlate well with their geographical distance from each another. This suggests that on regional scales within the same ecosystem type, there is little spatial correlation in functional diversity of surface soils.

Several environmental factors were correlated with EEA among sites, such as soil type. It is possible that site-level differences are simply representative of finer scale distinctions of soil type. Differences in soil microbial community structure and function with soil type have been observed in several studies (Yao et al., 2006; Zeglin et al., 2007; Wakelin et al., 2008; Katsalirou et al., 2010; Brockett et al., 2012). This is supported in part by the fact that the clay content of our soils was a significant environmental explanatory variable, in addition to MRPP groupings based on soil type. The fact that so many other strongly correlated variables were for abiotic factors (clay content, base cation concentrations, soil type, precipitation, and temperature) supports the conclusions reached by McDaniel and colleagues (2013) that abiotic factors are important controls on EEA variance. Factors such as pH, CEC, EC, and base cation concentrations all affect the electrochemical environment in which soil enzymes operate, so it follows that these factors would have a notable contribution to influencing EEA.

Furthermore, the availability of metal cations also had some correlation with enzyme activities. Given that phenol oxidases have Cu atoms in their active site, peroxidases use Fe, and both often use Mn^{2+} and Mn^{3+} to mediate the oxidation of lignin compounds (Burns et al., 2013), it implies that the significant correlations for Cu, Mn, and Fe in this study were important for the function of oxidative enzymes in these forests.

Another important aspect of this study is how enzyme activities correlate with each other, as well as other measures of functional diversity in soil microbial

communities, and how, in turn, the microbial communities interact with nutrient status of forests. The EEA levels we observed were very similar to those reported by Holden et al. (2013) in Alaskan forests seven years after wildfire. Our activities were two to ten times higher than those reported by Rietl et al. (2012), who were working in temperate oak forests in the southern U.S. Their observed decreases in nearly all enzyme activities in response to prescribed burning and thinning demonstrated that management effects can have substantial impacts on EEA.

One of the forest types examined by Brockett et al. (2012), a Douglas-fir dominated forest with some cedar and western hemlock, had a unique microbial community structure consisting of relatively higher bacteria numbers over fungi, which they attributed the high available N which characterizes Douglas-fir dominated forests. In Douglas-fir forests in Oregon, Chaer et al. (2009) also found that soil organic C was strongly correlated with PHOS, PHENOX, and BG activities. Unlike our study, they did not find N mineralization to be correlated with soil C; however, they worked in undisturbed old-growth forests assumed to be at equilibrium, whereas we studied much younger forests.

Selmants and colleagues (2005) analyzed EEA in coastal conifer forests in Oregon for several of the enzymes analyzed in our study. Comparison of our data (adjusted to reflect activities per gram of dry soil) to theirs showed similar activities for PHOS and NAG, but somewhat lower activities in our soils for BG and CBH. Examination of ectomycorrhizal root tips EEA in Douglas-fir saplings also demonstrated correlation in activity between PHOS and NAG, as well as similar

correlation with PHOS, NAG, and BG in a multivariate ordination (Jones et al., 2010), which is similar to trends noted in our study. Taken together with the context provided by these studies, the activities we measured are representative of EEA in similar systems throughout the Pacific Northwest region.

Nutrient turnover and limitation

An examination of activity variables with each other yielded several interesting insights. Foremost among these is the close relationship between the hydrolytic C cycling enzymes BG, CBH, and NAG (adjusted for microbial biomass, data not shown) with respired C. It is important to note that these trends existed having already taken into account differences in microbial biomass, which reinforces the direct effects these enzymes have in substrate availability to microbes, and thus their effect on respiration. Similar trends have been observed before (Sinsabaugh et al., 1993; Sinsabaugh and Shah, 2012; Allison and Vitousek, 2005). These data reinforce the importance of these enzymes in affecting C turnover. Peroxidase activity adjusted for biomass was also significantly correlated with respired C, which suggests that oxidative enzymes also play an important role in mineralizing C.

In regards to N cycling, it was somewhat surprising to observe that PHENOX activity was the enzyme most strongly correlated with mineralized N, followed by LAP activity, and then finally NAG and PHOS activity. Ratios of combined N-scavenging enzymes, NAG and LAP, to BG and PHOS can give an indication of what nutrients are most limiting in a given environment. For example, higher production of phosphatase activity compared to N-scavenging enzymes would provide a ratio

indicating P limitation in that environment. Using data from a variety of ecosystems globally, Sinsabaugh et al. (2009) derived ratios against which other soils can be compared to assess nutrient limitation (BG: NAG+LAP <1.41 suggests N limitation compared to C; NAG+LAP: PHOS <0.44 suggests P limitation with respect to N). Compared to soils globally, our enzyme ratios suggest that these soils are N limited with respect to C (BG: NAG+LAP = 0.83 < 1.41) and P (LAP+NAG: PHOS = 0.46 > 0.44) (Sinsabaugh et al., 2009; Zeglin et al., 2013). This may suggest that PHENOX and PEROX production serves largely to scavenge N from chemically complex macromolecules, in addition to NAG and LAP, in these N limited systems. Why N mineralization is also correlated with PHOS activity remains unclear.

Given that PHOS activity was strongly correlated with biomass, but not with Bray extractable P, it is likely that P is not a limiting nutrient, that PHOS production is controlled by other factors, or it is expressed constitutively.

Conclusion

Even among the similar forest types involved in this study, enzyme activities varied among sites. Enzyme activities were correlated with both biotic factors such as microbial biomass C, and abiotic factors, such as soil type, clay content, base cation concentrations, and precipitation. Samples that shared similar soil properties (such as soil order) tended to be more similar to one another than with samples from a different soil order. This may reflect the importance of abiotic controls on EEA, perhaps linked with soil mineralogy. Stoichiometric ratios implied that these forests tended to be N-

limited rather than C- or P-limited. Further research is needed to determine how abiotic soil factors such as binding with the soil mineral surfaces can impact overall measured activity, and how soil mineralogy in turn impact nutrient cycling and community structure. In the future, these data will provide the baseline data against which post-harvest samples will be compared.

Table 1.1. Site characteristics. Sites named "OR" are located in Oregon and "WA" are located in Washington.

Site name	Location	Latitude	Longitude	Elevation (m)	Annual precipitation (mm)	Average daily temperature (°C)	Soil classification	Years fertilized	Locale
OR1	Lebanon	44.5580	-122.5424	700	2017	11.3	Typic Dystrudepts	1991, 1998	Cascade
OR2	Vernonia	45.9010	-123.2215	260	1715	12.1	Aquic Palehumults	N/A	Coast
OR3	Roseburg	43.2490	-123.5670	750	2062	11.2	Typic Palehumults	1972, 1980, 1986, 1991, 1997, 2003	Coast
OR4	Leaburg	44.0845	-122.6628	600	1668	12.6	Humic Dystrudepts	1986, 1999, 2003	Cascade
OR5	Yamhill	45.3858	-123.3924	535	2173	10.9	Typic Haplohumults	1984, 1992	Coast
WA1	Aberdeen	46.9058	-123.7458	130	2483	11.9	Typic Fulvudands	1992, 2000	Coast
WA2	Castle Rock	46.2638	-123.0502	430	2416	11.0	Andic Dystroxerepts	2004	Coast
WA3	Vail	46.8595	-122.7576	195	1377	13.0	Typic Haploxerands	1985, 1996, 2004	Cascade
WA4	Mt. St.Helens	46.3168	-122.5713	795	2567	9.5	Typic Fulvicryands	1973, 1985, 1992	Cascade

Daymet model values were used for precipitation and temperature.

Table 1.2. Activity measures and value ranges

Activity measure	Abbreviation	Unit	Range of values
Peroxidase	PEROX	nmol μg^{-1} biomass C hr^{-1}	0.2 to 12.5
Phenol Oxidase	PHENOX	nmol μg^{-1} biomass C hr^{-1}	0.47 to 4.7
β-glucosidase	BG	nmol μg^{-1} biomass C hr^{-1}	0.15 to 2.0
Phosphatase	PHOS	nmol μg^{-1} biomass C hr^{-1}	0.5 to 4.8
Cellobiohydrolase	CBH	nmol μg^{-1} biomass C hr^{-1}	0.02 to 0.81
N-acetyl-glucosaminidase	NAG	nmol μg^{-1} biomass C hr^{-1}	0.16 to 1.4
Leucine aminopeptidase	LAP	nmol μg^{-1} biomass C hr^{-1}	0.02 to 0.34
Biomass C	Biom C	μg C g^{-1} dry soil	75 to 477
Biomass N	Biom M	μg N g^{-1} dry soil	14 to 54
Respired C	Resp C	μg C g^{-1} dry soil d^{-1}	18 to 103
Mineralized N	Min. N	μg NO_3^-+NH_4^+ g^{-1} dry soil d^{-1}	0.01 to 0.29

Table 1.3. Table of correlation between activity variables. Bold designates significance at the 0.001 level.

	PHOS	BG	NAG	CBH	LAP	PHENOX	PEROX	Biom C	Biom N	Resp C	Min. N
PHOS	1										
BG	-0.38**	1									
NAG	0.40**	0.13	1								
CBH	-0.46**	0.43**	-0.34*	1							
LAP	0.25	0.13	**0.55***	-0.33*	1						
PHENOX	0.35*	-0.24	0.29*	-0.32*	0.22	1					
PEROX	0.10	-0.07	-0.02	-0.32*	-0.15	0.02	1				
Biom C	**0.65***	-0.15	0.37**	-0.33*	**0.48***	0.25	-0.03	1			
Biom N	0.44**	0.02	0.27	-0.25	0.40**	0.24	0.05	**0.87***	1		
Resp C	0.08	0.26	0.07	0.26	0.16	-0.14	-0.44**	0.47**	**0.53***	1	
Min. N	0.36*	-0.03	0.31*	-0.33*	0.39**	**0.57***	0.11	0.47**	**0.57***	0.11	1

Correlations for EEA reported above were for enzyme activity per gram of dry soil. Because of the relationship between many enzyme activities and microbial biomass C, enzyme activities are reported per gram of μg biomass C in all subsequent analyses. Significance is designated * = 0.05, ** = 0.01, and *** = 0.001.

Table 1.4. Environmental variables and value ranges.

Environmental variable	Abbreviation	Unit	Range of values
Latitude	Lat	degrees	43.25 to 46.90
Elevation	Elev	m	132 to 796
Temperature	Temp	°C	9.52 to 13
Precipitation	Precip	mm	1323 to 2468
Bray Extractable P	Bray P	mg kg^{-1}	4 to 103
K	K	mg kg^{-1}	101 to 348
Ca	Ca	$cmol_c$ kg^{-1}	0.8 to 11.5
Mg	Mg	$cmol_c$ kg^{-1}	0.3 t0 3.9
Na	Na	$cmol_c$ kg^{-1}	0.12 to 0.34
Zn	Zn	mg kg^{-1}	1.0 to 6.2
Mn	Mn	mg kg^{-1}	35 to 165
Cu	Cu	mg kg^{-1}	1.8 to 6.2
Fe	Fe	mg kg^{-1}	73 to 543
S	S	mg kg^{-1}	13 to 80
B	B	mg kg^{-1}	0.04 to 0.26
pH	pH	pH	4.4 to 5.8
EC	EC	mmhos cm^{-1}	0.08 to 0.38
CEC	CEC	$cmol_c$ kg^{-1}	18.9 to 45.7
Percent Sand	SAND	percent	15 to 53
Percent Clay	CLAY	percent	6 to 30
Bacteria	Bact	gene copy number g^{-1} dry soil	$2.76x10^5$ to $8.47x10^9$
Fungi	Fungi	gene copy number g^{-1} dry soil	$2.46x10^3$ to $1.7x10^9$
Total C	Total C	g C kg^{-1} dry soil	27 to 152
Total N	Total N	g N kg^{-1} dry soil	1.34 to 7.78

Table 1.5. Correlations of environmental variables with PCA ordination of activity. Bold designates significance at the 0.001 level.

	Pearson Correlation (r)	
Environmental Variable	Axis 1	Axis 2
Lat	0.23	-0.22
Elev	0.14	0.20
Temp	-0.45**	-0.07
Precip	**0.62****	0.09
Bray P	-0.22	-0.02
K	-0.25	0.22
Ca	-0.37*	0.22
Mg	-0.26	0.29*
Na	0.45**	-0.05
Zn	0.20	0.00
Mn	-0.01	0.44**
Cu	0.32*	0.25
Fe	0.32*	-0.21
S	0.12	-0.02
B	0.26	-0.04
pH	-0.40**	0.15
EC	0.42**	-0.36*
CEC	**0.57****	0.10
SAND	0.03	-0.08
CLAY	-0.20	0.44**
Bact	0.19	**-0.55****
Fungi	-0.24	-0.13
Total C	**0.72****	-0.14
Total N	**0.68****	-0.22

Significance is designated * = 0.05, ** = 0.01, and *** = 0.001.

Figure 1.1. Mean enzyme activities by site: A—β-glucosidase (BG), B—cellobiohydrolase (CBH), C—N-acetylglucosaminidase (NAG), D—leucine aminopeptidase (LAP), E—phenol oxidase (PHENOX) and peroxidase (PEROX), and F—phosphatase (PHOS). Error bars indicate the standard error for activity of a given site. Lower case letters designate significant differences among sites at the 0.05 level. In E, letters represent only differences among sites not between oxidative enzyme activities.

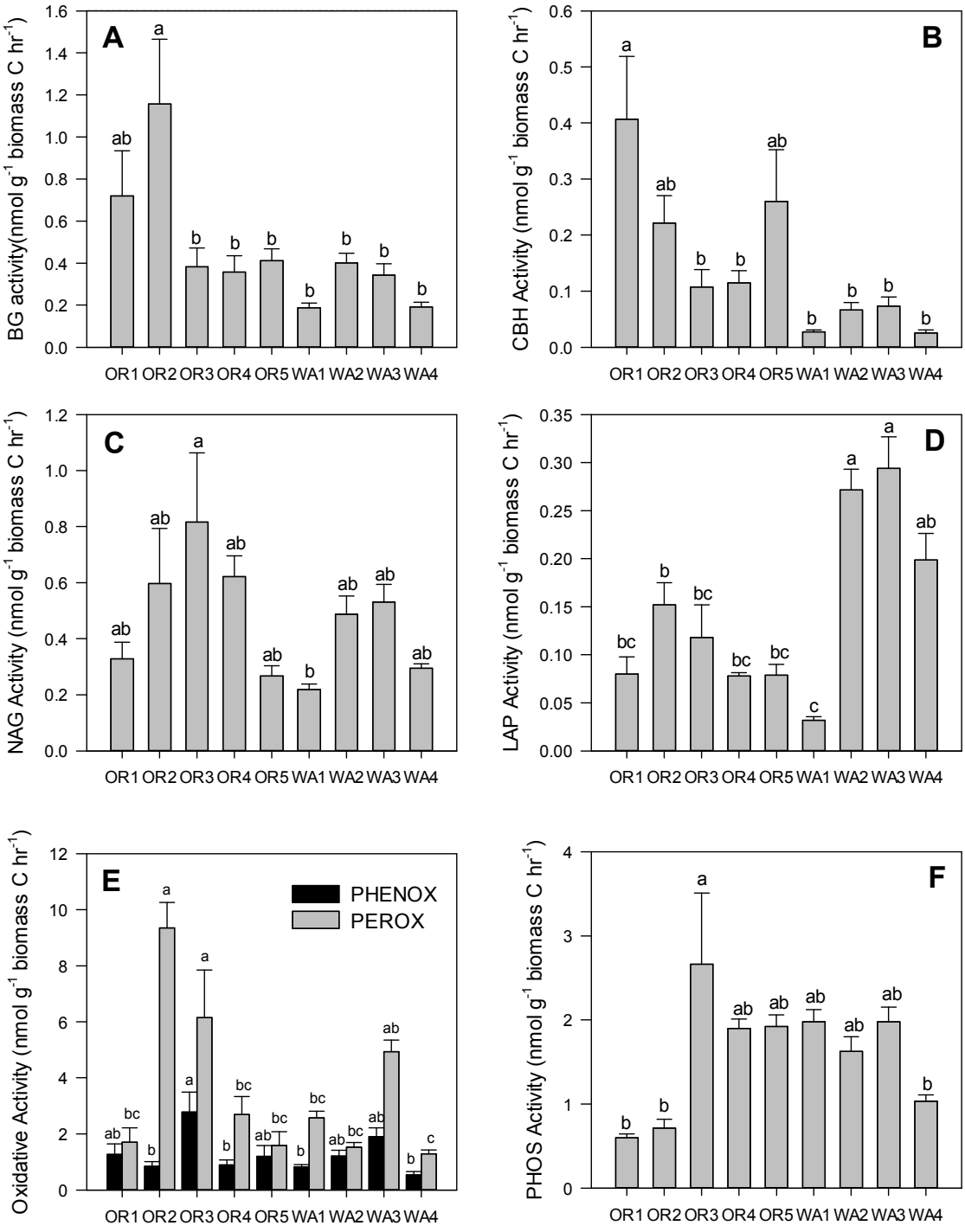
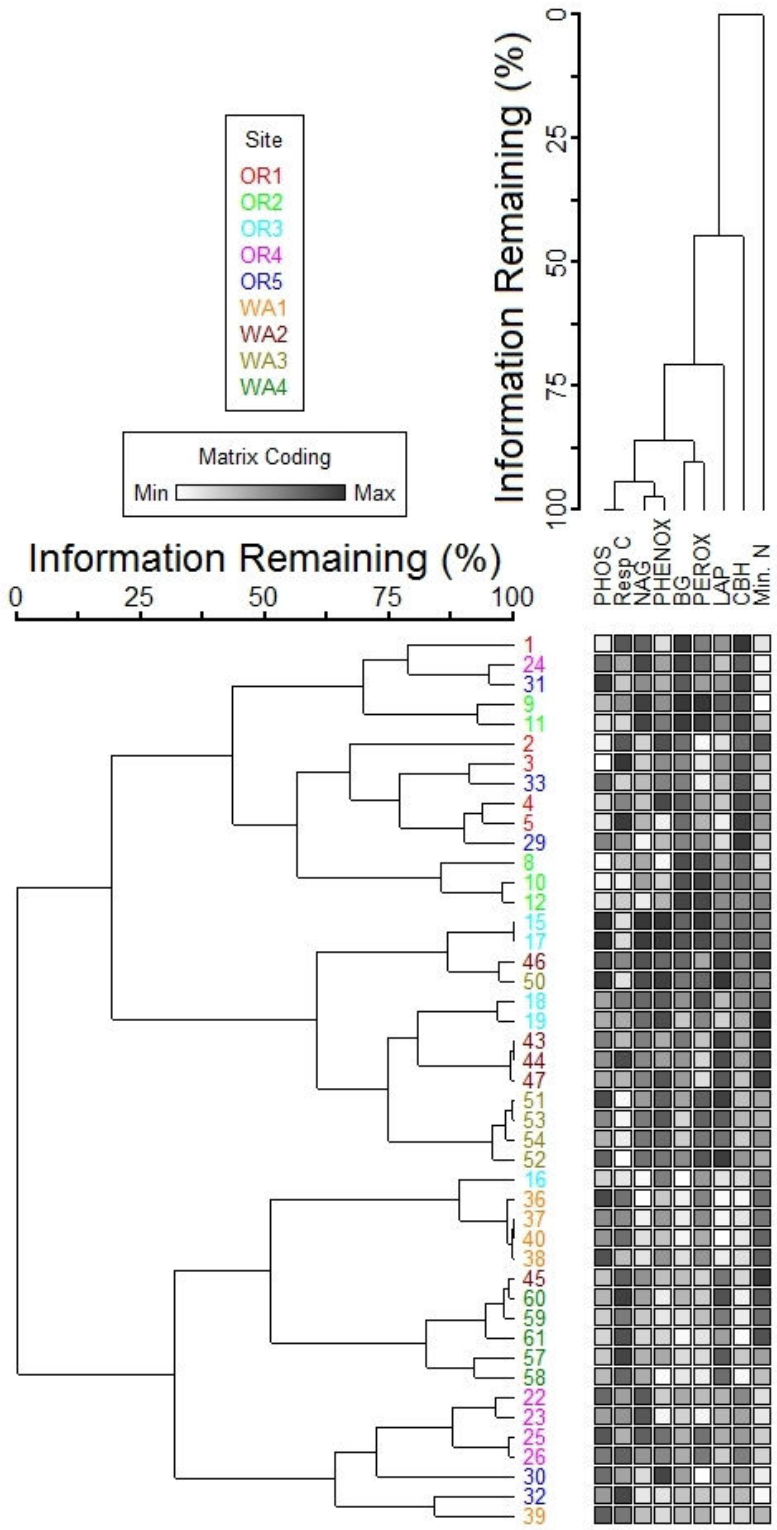

A
BG activity(nmol g-1 biomass C hr-1)
B
CBH Activity (nmol g-1 biomass C hr-1)
C
NAG Activity (nmol g-1 biomass C hr-1)
D
LAP Activity (nmol g-1 biomass C hr-1)
E
PHENOX
PEROX
Oxidative Activity (nmol g-1 biomass C hr-1)
F
PHOS Activity (nmol g-1 biomass C hr-1)
OR1 OR2 OR3 OR4 OR5 WA1 WA2 WA3 WA4

Figure 1.1

Figure 1.2. Two-way cluster analysis. Soil samples, color coded by site, are arrayed by similarity in the left side of the figure. Activity variables are arrayed on the top. The heat map represents the activity of each enzyme or activity measure for each sample. Darkly shaded boxes indicate relatively high activity, whereas lightly shaded boxes indicate lower activity. Patterns are visible indicating similar activity for samples within sites.


Site
OR1
OR2
OR3
OR4
OR5
WA1
WA2
WA3
WA4
Matrix Coding
Min
Max
Information Remaining (%)
0
25
50
75
100
PHOS
Resp C
NAG
PHENOX
BG
PEROX
LAP
CBH
Min. N
1
24
31
9
11
2
3
33
4
5
29
8
10
12
15
17
46
50
18
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32
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Figure 1.2

Figure 1.3. PCA ordination using Sørensen distances, displaying soil samples coded in color and symbol by soil type, with vectors representing activity measures. Vectors in panel A represent activity measures; environmental variables are shown as vectors in panel B. Oxidative enzymes and N-scavenging enzymes lie together towards the lower left of the ordination. Correlations with activity show respired C and BG and CBH enzymes grouping closely. Biomass C and N, and total C and N also group closely.

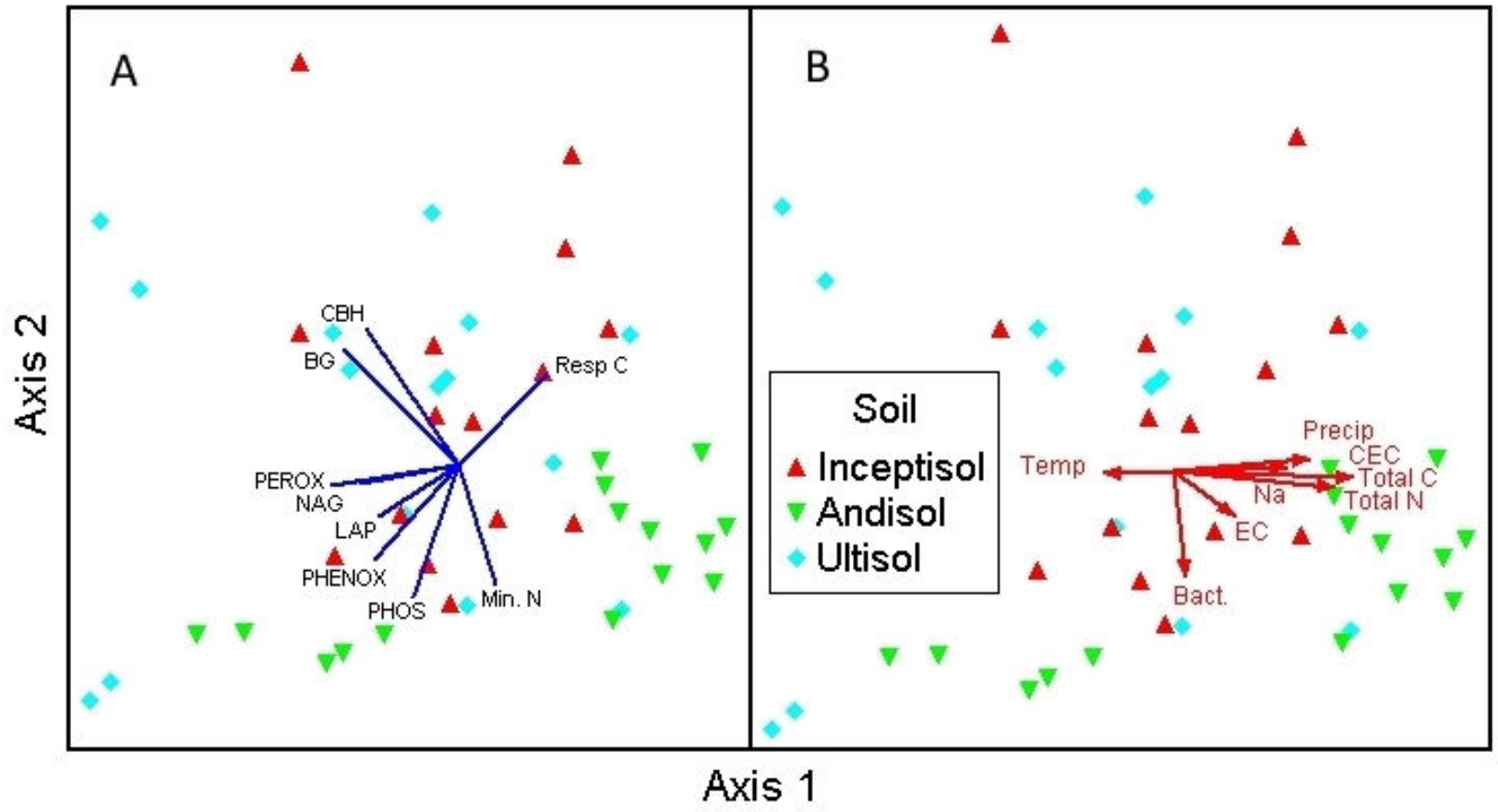
A
B
Axis 2
Axis 1
CBH
BG
Resp C
PEROX
NAG
LAP
PHENOX
PHOS
Min. N
Soil
Inceptisol
Andisol
Ultisol
Temp
Precip
CEC
Total C
Total N
Na
EC
Bact.

Figure 1.3

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Chapter 3:
Carbon and nitrogen mineralization rates in managed forest soils of the Pacific Northwest.

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Abstract

Forest soils play a central role in the global carbon (C) cycle. Their C cycling function is sensitive to a wide variety of environmental factors and management practices. The objective of this study was to characterize differences in C and nitrogen (N) mineralization in soils from managed forests throughout western Oregon and Washington. Soils were collected from nine second-growth Douglas-fir (*Pseudotsuga menziesii*) stands and incubated for one year in the laboratory. Mineralization activities were correlated with extracellular enzyme activities measured prior to incubation to link extracellular enzyme status to mineralization rates and to site environmental variables. Mineralization rates for both C and N were highly variable among sites. Soils mineralized about 13.5% of their C and 7.4% N during the incubation. However, sites varied significantly in total mineralization, and with respect to parameters predicted by kinetic models. Mineralization pool sizes and rate constants were strongly correlated with total soil C and N, microbial biomass, and a variety of abiotic factors, such as CEC and soil cation concentrations. These correlations suggest the importance of substrate supply, heavily influenced by macromolecule depolymerization and diffusion, in controlling mineralization rates. These results indicate wide functional variation even among sites of similar management and plant species composition, and emphasize how abiotic soil properties impact mineralization across geographic scales.

Introduction

Soil microbial communities are responsible for performing most of the biogeochemical transformations on Earth and driving the global carbon (C) and nitrogen (N) cycles. Because of this, decades of research have examined the interplay of microbial communities, environmental factors, and C and N mineralization rates from soils. Mineralization rates are frequently characterized through the use of laboratory soil incubations. These methods were developed in the 1970s, and have been employed and optimized through the years (Stanford and Smith, 1972). Many mathematical models have been used to condense incubation results into meaningful parameters, including the size estimates of discrete soil nutrient pools and their respective rate constants. Common models are first-order and parallel first-order kinetic models, but parallel first- and zero-order kinetic models, second-order kinetic, and equations that include measures of microbial growth, such as Monod kinetic models have also been used (Sleutel et al., 2005). These studies and models have helped to elucidate key drivers in the global C cycle, including factors influencing C storage in the soil, C turnover rates, and characteristics of soil organic matter. Characterizing these factors within and across ecosystems remains an important focus of C cycling studies.

Temperate and boreal forests play a particularly important role for C storage globally, storing most of the C belowground in the soil (Lal, 2005). Because they are so critical in global C and N transformations, small changes in forests' nutrient cycling activities can have potentially large effects on total fluxes from these systems.

Furthermore, a significant proportion of forest soils are managed systems (FAO, 2010). As soil microbial communities are responsible for carrying out the bulk of the chemical transformations influencing C and N cycling, understanding the details of microbial function in managed temperate and boreal forest systems is central to our understanding the global C cycle, nutrient cycles and transformations, and to sustainably managing forest systems.

In recent years, much research effort has been directed at elucidating the relationship between C and N cycling and the soil microbial communities in temperate and boreal forest ecosystems (Jassal et al., 2010; Woldesselassie et al., 2011; Swanston et al., 2002). It has been determined that timber harvest can have significant impacts on soil C storage (Nave et al., 2010). Thus, characterizing C and N mineralization and storage rates at the ecosystem level both prior to and following disturbance is critical in assessing nutrient cycling changes and predicting ecosystem response. Perakis and colleagues (2011) examined N mineralization along a natural N gradient in Douglas-fir (*Pseudotsuga menziesii*) plantations of the Oregon Coast Range, studying N saturation in coniferous forests. Their results indicated that N mineralization was highly variable, and variations in mineralization exceeded variation in soil N. They also concluded that in these systems, microbial N uptake saturates before plant uptake. This emphasizes the importance of soil microbial controls on N dynamics in forest systems. Although Perakis et al. (2011) provided important insights into the N-cycling patterns in Douglas-fir forests of the Pacific Northwest, their research objectives necessitated limiting their study sites to a narrow

range of environmental variables. There is still a need for more work to be done characterizing C and N mineralization across the entire ecotype, spanning the range of environmental and soil conditions found in the region.

By characterizing the variability in ecosystem function within the same eco-region, while still covering a broad range of other environmental variables such as precipitation, elevation, latitude, and soil type, it was anticipated that this study would yield geographic level insights into how these forests respond under differing environmental conditions. This study sought to provide such a regional assessment of microbial functional diversity by characterizing C and N cycling in nine Douglas-fir (*Pseudotsuga menziesii*) dominated forests in the Pacific Northwest of the United States. This assessment of nutrient cycling variability was examined both within and between sites, and correlated with environmental variables covered by the broad geographical range of the study sites. This characterization was linked with measures of community size (via quantitative PCR (qPCR) of bacterial and fungal markers), extracellular enzyme activities (EEA), and soil nutrient status to provide insights into extracellular enzyme production and function.

Materials and methods

Site descriptions

Nine commercial-harvest-age, second-growth forest sites (designated OR1-OR5 and WA1-WA4), dominated by Douglas-fir (*Pseudotsuga menziesii*), were

randomly selected from within Weyerhaeuser Company ownership in western Oregon and Washington, USA. Excessively rocky sites were rejected from consideration, to accommodate soil collection with corers rather than quantitative pits. These sites, while having similar plant-species composition, cover a wide range of environmental, climactic, and soil conditions (Table 2.1). Sites were fertilized with urea at varying frequencies (from 0 to 6 times) and application rates (370 to 560 kg ha^{-1}, average 463 kg ha^{-1}). Because of the random selection procedure, these sites comprise a representative survey of harvest-age commercial timberland in the region.

Soil Sampling

Each of the nine, 2.5 to 8.1-ha study sites was subdivided into 25 plots of approximately equal area. From the 25 plots at each site, five were selected to sample in the summer of 2011. The five plots were nearby the soil pits used for soil classification. Within each of these five plots, a grid pattern was established of 12 sampling points, ranging from 9 to 12 m apart, depending on the site. A composite sample for the plot was created from cores obtained at these 12 sampling points. Cores of 2-cm diameter were taken from 0-15 cm depth, with two adjacent cores taken near each of the 12 designated sampling points. This formed a composite sample of 24 cores for each plot. In total 45 composite surface soil samples were taken, five from each of the nine sites.

Samples were brought back to the laboratory, sieved to <4 mm, measured for water content, and frozen at -4°C until further analysis. Gravimetric moisture content

was determined by oven drying a subsample at 105°C for 24 h. Total C and N content was determined via dry combustion on those samples.

Microbial biomass was determined by the chloroform fumigation-extraction method (Brookes et al., 1985) using 0.05 M K_2SO_4. Fumigated and unfumigated extracts were measured on a Shimadzu TC/TN analyzer (Shimadzu Corp, Kyoto, Japan) to determine dissolved C and N. Microbial biomass C and N were calculating using conversion factors of 0.45 for C and 0.56 for N (Brookes et al., 1985; Vance et al., 1987).

Soil nutrient analysis

To characterize other nutrient availability parameters, <2 mm fraction composites were analyzed for Bray extractable P; ammonium acetate extractable K, Ca, Mg, and Na; and DTPA (diethylenetriamine pentaacetic acid—a chelator) extractable S, Fe, B, Mn, Cu, and Zn. Electrical conductivity (EC) and pH were measured in a 1:1 soil to water ratio. Cation exchange capacity (CEC) was also determined. Soil texture was determined on the <2 mm soils using the hydrometer method and reported as percent sand, silt, and clay.

DNA extraction and quantification

DNA was extracted from field moist soil (0.25 g dry weight equivalent) using the MoBio PowerSoil ® DNA Isolation kit. Three replicate extractions were made for each soil sample. Extraction was conducted according to the manufacturer's protocol.

Quantitative PCR was performed using the Applied Biosystems 7500/7500 Fast Real-Time PCR System (Life Technologies, Grand Island, NY). Amplification was carried out in 25-µl reactions. Fungal amplification was done as follows: 7.62 µl of water; 2 µl of 0.4% bovine serum albumin (BSA); 12.5 µl of the 2x SYBR green master mix (containing Taq polymerase); 0.25 µl of the 10 mM forward primer ITS1F, and 0.25 µl of the 10 mM reverse primer, ITS 5.8; and 0.38 µl of a 1:50 dilution of ROX dye. For bacteria, twice the primer amount was used (0.5 µl of 10 mM Eub 338F, and 0.5 µl of 10 mM Eub 518) to ensure efficient amplification. Thermal cycler profiles for amplification were adapted from Boyle et al. (2008) and consisted of an initial step of 50°C for 2 min followed by 95°C for 10 min. Thirty-five amplification cycles followed: 95°C for 30 s, 53°C for 1 min, 72°C for 1 min. Dissociation consisted of a single cycle of 95°C for 15 s, 60°C for 1 min, and 95°C for a final 15 s. All amplifications for both bacteria and fungi were > 93% efficient and had R^2-values >97%. Dissociation curves contained a single large peak.

Enzyme activities

A suite of enzymes was measured on each soil sample with techniques adapted from Sinsabaugh et al. (1993), with consideration given to optimization techniques described by German et al. (2011). The hydrolytic enzymes: phosphatase (PHOS), β-glucosidase (BG), N-acetylglucosaminidase (NAG), cellobiohydrolase (CBH), and leucine aminopeptidase (LAP), were measured fluorometrically in black 96-well plates. Oxidative enzymes, phenoloxidase (PHENOX) and peroxidase (PEROX), were measured colorimetrically in clear 96-well plates. All assays used 50 mM sodium

acetate (NaOAc) buffer solution adjusted to pH 5, as the bulk of our soils had pH values close to 5.

A soil slurry was prepared using 1 g field moist soil in 100 ml of the NaOAc buffer solution. The slurry was homogenized on a stir plate for approximately 3 min before being loaded into the 96-well plates. For the fluorometric enzymes, assays were carried out in 250-µl reactions (200 µl of soil slurry and 50 µl of either substrate, standard, or blank). Oxidative enzyme assays were carried out similarly; however, for the peroxidase assay, 10 µl of 0.3% H_2O_2 were added, bringing the well total to 260 µl.

Assay plates were incubated in the dark at 25°C for varying lengths of time, depending on the enzyme: PHOS and BG were incubated for 2 hr, NAG and CBH for 4 hr, and LAP, PHENOX, and PEROX for 26 hr. These times were determined experimentally by finding the optimum absorbance or fluorescence for each enzyme. Following incubation, fluorometric reactions were terminated by raising the pH with 10 µl of 0.5 N NaOH. Colorimetric assays were read at ambient pH, but 200 µl of supernatant was transferred to a clean, clear 96-well plate prior to measurement. Samples were then measured using a Spectramax Pro spectrophotometer (Spectramax 190, Molecular Devices, Sunnyvale, CA, USA). PHOS, BG, CBH, and NAG were measured fluorometrically at a 365 nm wavelength, and LAP at 385 nm. Maximum absorption for oxidative enzymes was measured at 450 nm. Examination of correlation coefficients indicated that many enzymes were correlated with biomass C, therefore

activities were calculated and reported as nmol absorbance or fluorescence per gram of microbial biomass C per hour for all subsequent analysis.

Soil microcosm incubation

A soil microcosm incubation was conducted to quantify soil C and N mineralization in a similar manner as described by Stanford and Smith (1972). Frozen soils were measured into small plastic bags and allowed to thaw overnight at 4°C. Field moist soil (50 g dry weight equivalent) was measured into filter-towers with grade 1 Whatman glass microfiber filters on bottom—to prevent soil loss during the incubation, and top—to prevent disturbance to soil aggregate structure. Soils were first wet with 25 ml of 0.01 M $CaCl_2$, and then pulled to 0.33 bar vacuum (field capacity). This followed by another 25 ml of Nanopure water once no $CaCl_2$ solution remained on the surface of the soil. The vacuum remained constant until all samples had ceased to lose water (approximately 20 min). Leachates were frozen for storage, and measured later for organic and inorganic N.

The soil samples in their filter towers, uniformly at field capacity, were then placed into 450-ml glass canning jars. The jars were covered with thin polyethylene plastic film to allow for gas exchange, but minimize evaporation during incubation. Soils were kept in the dark at 25°C for the duration of the incubation.

Respiration rates were measured with a Picarro gas analyzer equipped with a multiport-valve sampler (Picarro Inc., Santa Clara, CA). For measurement, microcosm samples were capped with canning jar lids fitted with airtight septa. Samples were measured for 2 min followed by a 58 min waiting period, before being measured again

for a second 2 min period. The increase in CO_2 gave the respiration rate per hour. As the respiration rates decreased over time, the length of the waiting period was lengthened to allow for accurate measurement. Samples were measured on days 1, 2, 4, 7, 12, 19, 29, 44, 64, 91, 119, 147, 175, 203, 238, 266, 294, 322, and concluded on day 350 of the incubation.

The leaching process was repeated every 2 months to track changes in ammonium (NH_4^+), nitrate (NO_3^-), and soluble organic N and C. Ammonium was quantified using the colorimetric assay of Qiu et al. (1987) modified for a 96-well format. All readings were measured in reference to a standard curve prepared from 1:2 serial dilutions of ammonium sulfate (10 to 0.3125 mg NH_4^+-N l^{-1}). The assay plate was incubated for 1 h at room temperature before measuring absorbance at 660 nm with a plate reader (Spectramax 190, Molecular Devices, Sunnyvale, CA).

Nitrate was quantified using a colorimetric assay in 96-well format (Inselbacher et al., 2011; Hood-Nowotny et al., 2010). All readings were measured in reference to a standard curve prepared from 1:2 serial dilution of potassium nitrite. The plates were incubated at room temperature for 1 hr and the absorbance was measured at 540 nm using a plate reader (Spectramax 190, Molecular Devices, Sunnyvale, CA). The leachates analyzed using these assays were collected the day prior to the corresponding respiration measurement.

Statistical methods

For analysis of C cycling parameters, two models were fitted: a parallel first-order kinetic model (hereafter referred to as the double-exponential model), and a

parallel first- and zero-order kinetic model (hereafter referred to as the linear-exponential model). The double-exponential model estimates two discrete C pools, a fast and slow cycling pool, with individual rate constants. It is described in the integrated form by the equation:

$$y = c_f(1-\exp(-k_f*t)) + c_s(1-\exp(-k_s t))$$

where y is the cumulative CO_2, c_f represents the size of the fast cycling pool, c_s represents the size of the slow cycling pool, t represents the time of incubation, k_f is the rate constant of the fast cycling pool, and k_s is the rate constant of the slow cycling pool.

The linear-exponential model describes two pools as well, with a fast cycling pool with a first order rate constant, and a second pool which mineralizes linearly over time. The linear-exponential model is described by the equation:

$$y = c_f(1-\exp(-k_f*t)) + k_s*t.$$

The parameters of this equation are similar, but not identical, to those of the double-exponential model, as this model does not define the size of the slow cycling pool. These models were then compared by analyzing the fit of the model (R^2-value, mean sum of squares value, and F-value), the standard error of the model parameters, and the biological significance of the parameters (Sleutel et al., 2005; Hess and Schmidt, 1995). After comparison based on these criteria, the linear-exponential model was chosen for further analysis. For sites OR5 and WA2, C mineralization increased considerably during the last four measurement points of the incubation. This is assumed to have been the result of microbial community changes, or alterations in

substrate availability following a leaching event. Because this change does not likely reflect a change in C pool properties, the last four data points were removed for model fitting for these sites. However, the data was retained for calculating total mineralization due to its biological significance.

For analysis of N cycling parameters, a first-order kinetic model was fitted. The equation for this model is described as follows:

$$y= n(1-\exp(-k*t))$$

where y is the total N leached, n is the pool size, t is the incubation time, and k is the rate constant. There was more variation in N mineralization patterns than in C mineralization patterns, but a single exponential model described N mineralization for nearly all samples with an R^2-value >0.98. Therefore, for uniform comparison between samples, this model was chosen. Samples that had non-significant parameter values were excluded in calculations and comparisons of parameter values, but were included in graphs and calculations of measured N quantities because of their biological significance.

ANOVA was used to determine differences among sites. Correlations between variables and nutrient cycling parameters are reported in the text at an absolute r-value at or above 0.372, which is the critical value corresponding with a 0.01 p-value based on a Pearson correlation coefficient (for 43 degrees of freedom). Significance at the 0.05 level occurred at an r-value of 0.288, and 0.474 at the 0.001 significance level. Data was analyzed and graphs were generated in SigmaPlot version 12 (Systat Software, Inc., San Jose, CA).

Results

Soils varied considerably in the amounts of total C and N among sites. Total C ranged from site averages of 31.2 to 123.3 g C kg^{-1} soil, with and average of 62.8 g C kg^{-1} for all samples. Total N ranged from 1.5 to 5.8 g N kg^{-1} soil, with an average of 3.0 g N kg^{-1} soil. The average C:N ratio was 21.5.

Both double-exponential and linear-exponential models of C cycling data provided extremely good fits to observed mineralization rates. Based on the R^2 criterion alone, the double-exponential model could have been chosen (average R^2=0.998); however, in about one fourth of samples, the standard error associated with parameter estimates was greater than 50% of the parameter value, and in some cases even exceeded the parameter value (Hess and Schimdt, 1995). Furthermore, in some cases the slow-cycling pool size estimated by the double-exponential model exceeded the amount of total C, and had improbably slow turnover rates. The linear-exponential model had an average R^2 value of 0.996, had parameter standard errors that were always smaller than 50% of the parameter value, and did not estimate impossibly large pool sizes or miniscule turnover rates. Therefore, despite the double-exponential model providing very good fits and meaningful parameters for many samples, the linear-exponential model provided a better overall fit while avoiding unlikely parameter values. Linear-exponential parameter values are outlined by site in Table 2.2.

Carbon mineralization

On average, soils mineralized 8.4 g C kg^{-1} dry soil, or 13.4 % of the total soil C. The fast cycling pool was estimated to be of 2.9 g C kg^{-1} dry soil, or about 4.7% of the total C pool, with a turnover time of 29.3 days (Table 2.2). Carbon mineralization was different among sites, both for cumulative mineralization values, as well as parameter values. DOC lost due to leaching was always less than or equal to 1% of respired C, so it was considered negligible and excluded from further analysis.

Cumulative C mineralization was highly correlated with several factors, the strongest of which were total C and precipitation (r=0.635 and r=0.632 respectively). Microbial biomass, soil N, Na concentrations, and CEC were also had strong positive correlations. Significant, but somewhat weaker negative correlations existed with site temperature, soil pH, Mg, and Ca concentrations.

Fewer significant correlations existed for individual C cycling model parameters. Many correlations that existed for the fast cycling pool (c_f) and k_s were common to those of cumulative C mineralization, such as soil C and N, precipitation, microbial biomass C and N, and Na concentrations. This is reasonable given that the fast cycling pool size and k_s were highly correlated with cumulative respired C (fast pool size r=0.734, $p<0.001$; k_s r=0.862, $p<0.001$. Correlations not shown in table). The correlations unique to just one model parameters were a strong negative correlation for k_s with site temperature, and a positive correlation of k_f with fungal gene copy numbers (Table 2.3).

For some samples, an increase in mineralized C was observed late in the incubation (Figure 2.1). This increase occurred strongly for some sites, but also occurred to lesser degrees for individual samples within a site. This change could not be attributed to changes in incubation conditions or methodological error, but was noted to have occurred following a leaching event. Furthermore, respiration measurements immediately following a leaching event tended to be slightly higher than respiration measurements in between leaching events, despite the fact that soils did not dry significantly during the 2-month incubation periods. Thus, leaching contributed to variation observed in the data. Although not examined directly, it is assumed that these variations are the result of altered biological activity as a result of leaching.

Nitrogen mineralization and organic N release

On average, soils released about 0.22 g N kg^{-1} dry soil over the course of the incubation, or about 7.4% of the total soil N. This amount is comprised of NH_4^+, NO_3^-, and dissolved organic N (DON), of which DON was a strikingly large fraction. On average, about 0.035 g N kg^{-1} were released as NO_3^- (equaling 16% of cumulative released N), though this percentage was higher for soils that released smaller amounts of cumulative N (Table 2.4). The amount of NO_3^- released increased throughout the incubation in a sigmoidal fashion, likely reflecting the growth of nitrifiers (Figure 2.2). Ammonium accumulation tended to be small and relatively constant for most sites with most of the accumulation occurring in the first two-month period followed by

minimal accumulation thereafter. OR3 had relatively high amounts of NH_4^+ (25% of mineralized N). On average, 93% of all mineralized N was nitrified.

Released N, nitrified N, and predicted pool size were strongly correlated with many environmental factors (Table 2.5). The strongest of these correlations existed for total soil C and N, as well as initial biomass C and N. After these, the strongest correlations tended to be for soil chemical properties, with positive correlations for EC and CEC, and negative correlations with pH and K, Mg, and Ca concentrations. Negative correlations also existed for site temperature, and positive correlations existed for site precipitation. Other positive correlations also existed for several metal ions and other nutrients.

Fewer correlations existed for biological variables. However, all parameters were always negatively correlated with fungal abundance and positively correlated with bacteria. PHOS and NAG activity were correlated with released N and predicted pool size, and LAP activity was strongly positively correlated with all N parameters. PHENOX activity was positively correlated with released N.

Correlations for the rate constant diverged somewhat from correlations with pool sizes in that it had fewer significant correlations over all. Additionally, the correlations that existed sometimes were with unique factors. For example, the rate constant was strongly negatively correlated with soil sand content whereas pool size parameters were weakly correlated, if at all. The rate constant was also negatively correlated with soil C:N ratio, as is commonly observed. Unlike other N cycling parameters, the rate constant was not significantly correlated with soil C. It was also

less strongly correlated with microbial biomass than other parameters were. This suggests that rate constant for N mineralization is controlled by fewer and different factors than those that affect pool size.

Discussion

The objectives of this study were to characterize C and N mineralization rates in managed Douglas-fir forests of the Pacific Northwest, and to correlate mineralization with the wide range of environmental conditions encompassed by the study. Sites differed significantly for both C and N mineralization for all examined parameters. However, many of these differences were correlated with environmental and biological variations among soils. Soils likely varied in the percent of C and N mineralized due to differences in soil physical (percent sand positively correlated with N cycling rate constant) and chemical properties (CEC was positively correlated with nearly all C and N cycling parameters), and due to dominance of either bacteria or fungi at a given site (N cycling was positively correlated with bacterial abundance and negatively correlated with fungal abundance; the fast pool rate constant for C was positively correlated with fungal abundance).

Overall, soil C and N, and mineralization rates tended to be comparable to observations of others working in temperate conifer forests (Chaer et al., 2009; Perakis et al., 2011; Chatterjee et al., 2008). A long term (108 d) incubation of pine forest soil found slightly lower rates of C mineralization in the top 15 cm (Chatterjee et al., 2008). They observed an active pool that ranged from 0.2 to 1.01 g C kg^{-1}soil, with

turnover times from 1 to 11 d; our study found a larger active pool that turned over about 16.6 to 45.7 d. A longer incubation (588 d) comparison of soils from varying soil types, land uses, and ecosystem types fit a 2-pool model across all their study sites, which predicted an active pool of 1.06 g C kg^{-1} soil that turned over approximately every 24 days, which is similar to our predicted turnover times (Haddix et al., 2011). The active pools for their sites comprised between 0.1 and 14% of the soil organic C. Our observed range of approximately 2 to 8% for the active pool falls well within the range they observed, as does our range of total respired C values (Haddix observed 2 to 17% of total soil C respired in 588 d; we observed 4 to 11.5% respired in 350 d). Differences we observed with these studies are likely the result of differing ecosystem types and soil properties associated with the Douglas-fir forests of our study. A year-long incubation of soil from the control plot of the Detritus Input Removal and Transfer (DIRT) experiment in the H.J Andrews experimental forest in Oregon had cumulative respiration of slightly over 6% of the soil organic C (Crow et al., 2009). This is lower than our rates of 9.5 to 20% of soil C, but may reflect the old-growth nature of the H.J. Andrews stand compared to our younger, managed forests, most of which were fertilized at least once.

Rates of N mineralization (NO_3^- and NH_4^+) were similar to rates reported by Chaer et al. (2009) in the top 5 cm of soil also taken from the H.J. Andrews forest. They observed N mineralization rates from 0 to 0.5 μg N g^{-1} soil d^{-1}, with an average of 0.2. Adjusted to reflect cumulative N nitrified per day, our sites ranged from 0.06 to 0.16, with an average of 0.1 μg N g^{-1} soil d^{-1}. It is reasonable that their rates were

somewhat higher than our values as they examined soils closer to the surface. Over all, our observed values were similar to those derived from other incubations, and characteristic of rates found throughout the region. It is important to note that many studies examining N dynamics tend to only include measures of inorganic N, however DON was the largest fraction of total released N in our study. Other studies of DON in forest soils in the region have found similar trends. Cairns et al. (2009) found that soil solution DON averaged 64% of total soil dissolved N, with NO_3^- making up the next largest fraction in Douglas-fir forest in the Cascades of Oregon. These results suggest that measures of DON should be more routinely taken and reported, as DON likely constitutes a significant factor affecting N cycling dynamics.

Two factors that have been extensively examined in relation to C and N mineralization rates are moisture and temperature (Wagai et al., 2013; Conant et al., 2011; Curtin et al., 2012). All of our samples were incubated at a single temperature, and at a uniform matric potential. However, both precipitation and temperature of the sites were significantly correlated with C mineralization and k_s, as well as for N pool sizes, with temperature being negatively correlated with all parameters and precipitation being positively correlated with all parameters. These correlations likely reflected the effect that temperature and precipitation have on the amount and quality of the C and N at the study sites.

Despite not directly testing for mechanistic controls on C and N mineralization, our results suggest that supply of organic substrate to microbes drives mineralization, especially for slow cycling pools. It has been hypothesized that higher

water contents facilitate the diffusion of organic molecules, increasing the accessible substrate supply to soil microbes (Curtin et al., 2012). The concept of mineralization rates being controlled first and primarily by abiotic factors governing substrate availability is referred to as the regulatory-gate hypothesis (Kemmitt et al., 2008). Our observed increases in C mineralization following leaching events support this hypothesis, especially in relation to C mineralization. Even though soils did not dry out appreciably between leaching events, and thus moisture content can be assumed to be relatively constant, the leaching event would provide a flush of redistributed nutrients, which may account for the increased C mineralization we observed following leaching. This redistribution of nutrients may also account for the bumps in mineralization that were observed for some samples late into the incubation period. However, the late increase in respiration may also be a result of changes in the composition or activity of the microbial community. Unfortunately, a more in-depth analysis of the samples that demonstrated the unusual behavior was beyond the scope of this study. Nevertheless, it provides evidence that wetter soils have increased substrate diffusion, which may stimulate microbial activity and drive higher rates of C mineralization.

Furthermore, in the linear-exponential model we derived, the fast cycling pool appears to be related to microbial turnover. However, as the slow pool mineralized linearly, this implies that mineralization rates were decoupled from biotic factors, and are therefore governed by factors that operate at a more or less constant rate. This could be the result of the slow depolymerization of organic macromolecules, and the

diffusion of DOC and DON to microbes. The strong correlations we observed with soil C and N support this hypothesis. It is also supported by several correlations we observed with soil chemical factors. CEC was nearly always significantly and positively correlated with C, and especially N parameters, while EC and pH were also correlated for N pool sizes (EC positively correlated, and pH negatively correlated). Additionally, all parameters with the exception of the rate constant of the fast C pool had negative relationships many of the base cations, Ca, Mg, and K. Together, these data possibly suggest that the ability for soils to adsorb organic molecules plays a significant role in affecting diffusion, and thus C and N mineralization.

Other notable correlations demonstrate that the bacterial community is the driver of nitrification. Nitrate accumulation followed a sigmoidal curve, likely following the growth of nitrifiers during the incubation. It was notable that LAP activity rates were strongly correlated with all N parameters. NAG also appears to affect the amount of quickly cycling N. Thus, these N-cycling enzymes have a strong impact on the pool size and the total released N, probably due to their ability to produce DON, which was a major fraction of soluble N in this and other studies. Why PHOS is so strongly correlated to N cycling parameters is unclear, however. Similarly, it is not immediately obvious why Na concentrations would be positively correlated with both C and N cycling. These relationships, along with leaching related increases in C mineralization, warrant further investigation.

Conclusions

Soils in Douglas-fir dominated forests of the Pacific Northwest, despite being of similar management and plant species composition were variable in soil C and N content, mineralizable pool sizes, and turnover rates. Although microbial biomass was positively correlated with both C and N mineralization, abiotic factors also were highly correlated with mineralized C and N. This, together with changes in C mineralization following leaching, provides evidence that substrate supply is the main limiting factors on C mineralization. A combined first- and zero-order kinetic model effectively described C mineralization over the course of our laboratory incubation while avoiding non-significant parameter values. Nitrogen released was more difficult to describe with a single model due to variability in the data, but was adequately described by a single-exponential kinetic model. Our results imply that the contribution of organic N will be important to monitor in follow-up, and other related studies. Furthermore, these results suggest that factors impacting substrate supply to microbes could have significant effects on long term C and N cycling. This characterization, and insights derived from it will be instrumental in assessing changes in C and N mineralization following timber harvest.

Table 2.1. Site characteristics. Sites named "OR" are located in Oregon and "WA" are located in Washington.

Site name	Location	Latitude	Longitude	Elevation (m)	Annual precipitation (mm)	Average daily temperature (°C)	Soil classification	Years fertilized	Locale
OR1	Lebanon	44.5580	-122.5424	700	2017	11.3	Typic Dystrudepts	1991, 1998	Cascade
OR2	Vernonia	45.9010	-123.2215	260	1715	12.1	Aquic Palehumults	N/A	Coast
OR3	Roseburg	43.2490	-123.5670	750	2062	11.2	Typic Palehumults	1972, 1980, 1986, 1991, 1997, 2003	Coast
OR4	Leaburg	44.0845	-122.6628	600	1668	12.6	Humic Dystrudepts	1986, 1999, 2003	Cascade
OR5	Yamhill	45.3858	-123.3924	535	2173	10.9	Typic Haplohumults	1984, 1992	Coast
WA1	Aberdeen	46.9058	-123.7458	130	2483	11.9	Typic Fulvudands	1992, 2000	Coast
WA2	Castle Rock	46.2638	-123.0502	430	2416	11.0	Andic Dystroxerepts	2004	Coast
WA3	Vail	46.8595	-122.7576	195	1377	13.0	Typic Haploxerands	1985, 1996, 2004	Cascade
WA4	Mt. St.Helens	46.3168	-122.5713	795	2567	9.5	Typic Fulvicryands	1973, 1985, 1992	Cascade

Daymet model values were used for precipitation and temperature.

Table 2.2. Carbon mineralization parameters. Site averages are reported, with standard errors in parentheses. Statistical differences among sites are indicated by superscript letters. Mean residence time was calculated as $1/k_f$.

Site	Total C ($g\ C\ kg^{-1}$ soil)	Respired C ($g\ C\ kg^{-1}$ soil)	Percent of total	Fast pool size ($g\ C\ kg^{-1}$ soil)	Percent of total	Mean residence time (d^{-1})	k_s ($g\ C\ kg^{-1}$ soil d^{-1})	R^2
OR 1	60.61 (2.38)^{c}	10.77 (0.30)^{a}	17.87	4.07 (0.20)^{a}	6.76	25.44 (0.87)^{a}	19.96 (0.48)^{ab}	0.997 (<0.0001)
OR 2	31.20 (0.51)^{d}	6.25 (0.31)^{bc}	20.05	2.38 (0.12)^{b}	7.64	38.62 (0.94)^{a}	11.20 (0.76)^{bc}	0.998 (<0.0001)
OR 3	37.30 (1.85)^{d}	7.15 (0.52)^{bc}	19.18	1.89 (0.09)^{bc}	5.08	24.47 (1.25)^{a}	15.13 (1.29)^{abc}	0.995 (<0.0001)
OR 4	61.70 (3.04)^{c}	7.94 (0.36)^{abc}	12.87	4.05 (0.23)^{a}	6.56	45.66 (4.85)^{a}	11.51 (0.47)^{bc}	0.998 (<0.0001)
OR 5	40.45 (1.06)^{cd}	8.14 (0.69)^{abc}	20.12	2.30 (0.13)^{bc}	5.69	16.61 (1.15)^{b}	16.96 (1.56)^{abc}	0.994 (<0.0001)
WA 1	98.44 (2.68)^{b}	9.30 (0.26)^{a}	9.45	3.27 (0.11)^{ab}	3.32	30.73 (2.31)^{a}	17.54 (0.75)^{abc}	0.994 (<0.0001)
WA 2	73.77 (2.50)^{bc}	10.04 (0.26)^{a}	13.61	2.17 (0.22)^{bc}	2.94	27.27 (3.48)^{c}	23.79 (0.74)^{a}	0.992 (<0.0001)
WA 3	35.41 (0.76)^{d}	4.06 (0.14)^{c}	11.46	1.18 (0.06)^{c}	3.34	26.20 (0.88)^{a}	8.21 (0.30)^{c}	0.999 (<0.0001)
WA 4	123.27 (3.73)^{a}	11.50 (0.35)^{a}	9.33	3.85 (0.15)^{a}	3.12	28.64 (1.79)^{a}	22.49 (1.00)^{a}	0.998 (0.0001)

Table 2.3. Carbon parameter correlations with environmental variables. Bold designates significance at the 0.001 level.

	Respiration total	Fast pool (c_f)	k_f	k_s
Temp	**-0.60*****	-0.28	-0.23	**-0.63*****
Precip	**0.63*****	**0.50*****	0.21	**0.54*****
Bray P	-0.19	-0.26	-0.09	-0.08
K	-0.26	-0.13	0.08	-0.24
Ca	-0.41**	-0.26	0.01	-0.33*
Mg	-0.35*	-0.25	0.05	-0.23
Na	**0.47*****	**0.49*****	-0.13	0.29*
Zn	0.07	-0.06	0.02	0.13
Mn	0.26	0.30*	0.13	0.19
Cu	0.38**	0.19	0.33*	0.37**
Fe	0.09	0.00	0.03	0.15
S	0.36*	0.21	0.09	0.18
B	0.25	0.16	-0.12	0.18
pH	-0.33*	-0.2	0.18	-0.28
EC	0.22	0.03	0.07	0.27
CEC	**0.51*****	**0.54*****	-0.12	0.39**
SAND	0.02	0.08	0.01	0.11
CLAY	0.08	0.03	0.17	0.02
Bact.	-0.05	-0.02	-0.01	-0.03
Fungi	-0.13	-0.03	0.30*	0.04
Total C	**0.64*****	**0.54*****	-0.12	**0.53*****
Total N	**0.55*****	0.41**	-0.12	0.46**
soil C:N	0.28	0.39**	0.08	0.25
PHOS	0.21	0.09	0.04	0.16
BG	0.25	0.19	-0.05	0.16
NAG	0.16	0.12	-0.14	0.05
CBH	0.11	0.20	0.17	0.07
LAP	0.25	0.06	0.00	0.17
PHENOX	0.02	-0.21	-0.02	0.05
PEROX	-0.36*	-0.28	-0.33*	-0.29*
Biomass C	**0.53*****	0.40**	-0.15	0.43**
Biomass N	**0.58*****	0.39**	-0.18	**0.53*****

Significance is designated * = 0.05, ** = 0.01, and *** = 0.001.

Table 2.4. Nitrogen cycling parameters. Site averages are reported with units designated in the table heading. Standard errors located in parentheses. Statistical differences among sites are indicated by superscript letters.

Site	Total N (g N kg^{-1} soil)	Released N (g N kg^{-1} soil)	Percent of total	Nitrified N (mg N kg^{-1} soil)	Percent of released N	Pool size (g N kg^{-1} soil)	Percent of total	Turnover time (d^{-1})	R^2
OR 1	2.57 (0.07)^{d}	0.21 (0.01)^{b}	8.24	32.41 (1.73)^{b}	15.33	0.23 (0.01)^{b}	9.10	84.74 (18.28)^{ab}	0.995 (0.001)
OR 2	1.71 (0.05)^{ef}	0.15 (0.01)^{c}	8.57	24.77 (1.41)^{bc}	16.87	0.17 (0.01)^{bc}	9.81	76.59 (13.53)^{ab}	0.993 (0.002)
OR 3	1.46 (0.05)^{f}	0.14 (0.01)^{c}	9.33	27.65 (0.93)^{bc}	20.23	0.17 (0.02)^{bc}	11.65	117.88 (16.69)^{ab}	0.984 (0.001)
OR 4	2.65 (0.02)^{d}	0.11 (0.02)^{c}	4.13	25.15 (2.54)^{bc}	23.02	0.22 (0.05)^{bc}	8.25	247.55 (45.13)^{a}	0.992 (0.002)
OR 5	1.84 (0.08)^{ef}	0.10 (0.01)^{c}	5.18	24.25 (2.12)^{c}	25.41	0.14 (0.02)^{c}	7.37	119.51 (33.07)^{ab}	0.982 (0.004)
WA 1	4.90 (0.12)^{b}	0.37 (0.02)^{a}	7.56	51.23 (2.54)^{a}	13.82	0.37 (0.01)^{a}	7.46	65.76 (3.67)^{b}	0.991 (0.001)
WA 2	4.17 (0.13)^{c}	0.40 (0.01)^{a}	9.57	52.15 (1.35)^{a}	13.08	0.39 (0.01)^{a}	9.35	40.07 (3.38)^{c}	0.995 (0.000)
WA 3	1.98 (0.05)^{e}	0.16 (0.00)^{bc}	7.86	22.20 (0.18)^{c}	14.29	0.15 (0.01)^{c}	7.81	63.24 (3.01)^{b}	0.986 (0.001)
WA 4	5.80 (0.25)^{a}	0.36 (0.02)^{a}	6.13	55.69 (1.28)^{a}	15.66	0.37 (0.02)^{a}	6.34	64.02 (9.05)^{b}	0.992 (0.001)

Table 2.5. Nitrogen parameter correlations with environmental variables. Bold designates significance at the 0.001 level.

	Released N	Nitrified N	Pool size	Rate constant
Temp	**-0.56*****	-0.42**	**-0.49*****	-0.15
Precip	**0.54*****	**0.49*****	**0.61*****	0.14
Bray P	-0.16	-0.20	-0.27	-0.19
K	**-0.48*****	**-0.58*****	**-0.46*****	**-0.42*****
Ca	**-0.64*****	**-0.71*****	**-0.67*****	**-0.46*****
Mg	**-0.55*****	**-0.61*****	**-0.60*****	**-0.47*****
Na	**0.47*****	0.39**	**0.56*****	0.09
Zn	0.34**	0.34*	0.27	-0.02
Mn	-0.12	-0.18	-0.08	-0.26
Cu	0.33**	0.33*	0.43**	0.11
Fe	0.35**	0.39**	0.38**	0.06
S	0.44**	**0.54*****	**0.60*****	**0.47*****
B	**0.53*****	**0.61*****	**0.57*****	0.39**
pH	**-0.66*****	**-0.73*****	**-0.67*****	-0.35*
EC	**0.63*****	**0.60*****	**0.62*****	0.23
CEC	**0.55*****	**0.52*****	**0.55*****	0.05
SAND	-0.22	-0.35*	-0.29*	**-0.49*****
CLAY	-0.17	-0.09	-0.13	0.10
Bact.	0.29*	0.32*	0.31*	0.28
Fungi	-0.36**	-0.42**	-0.38**	-0.26
Total C	**0.80*****	**0.72*****	**0.76*****	0.25
Total N	**0.85*****	**0.81*****	**0.82*****	0.39**
soil C:N	-0.26	-0.39**	-0.21	**-0.51*****
PHOS	**0.55*****	**0.53*****	0.59**	0.24
BG	0.02	0.09	0.14	0.21
NAG	0.29*	0.25	0.38**	0.23
CBH	-0.26	-0.26	-0.27	-0.23
LAP	**0.47*****	**0.50*****	**0.44*****	**0.50*****
PHENOX	0.31*	0.28	0.15	0.25
PEROX	-0.10	-0.01	-0.18	-0.03
start Biom C	**0.79*****	**0.72*****	**0.75*****	0.32*
start Biom N	**0.81*****	**0.77*****	**0.77*****	0.29*

Significance is designated * = 0.05, ** = 0.01, and *** = 0.001.

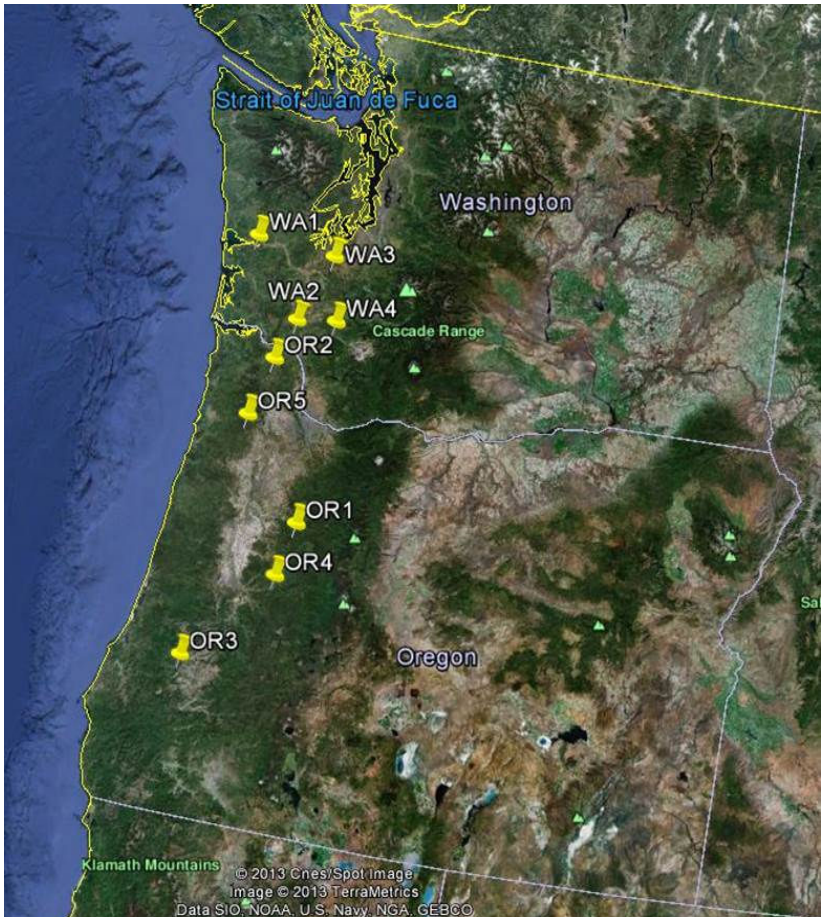

Figure 2.1. A map of Oregon and Washington, showing the location of the nine study sites in the Coast and Cascade ranges throughout the states.

Figure 2.2. Cumulative C mineralization during 350 days of incubation. Plots A though E show sites OR1 through 5, plots F through I show sites WA1 through 4. Symbols represent cumulative respiration on the corresponding day for individual plots within sites. Lines represent the kinetic model which best fits the average for the site.

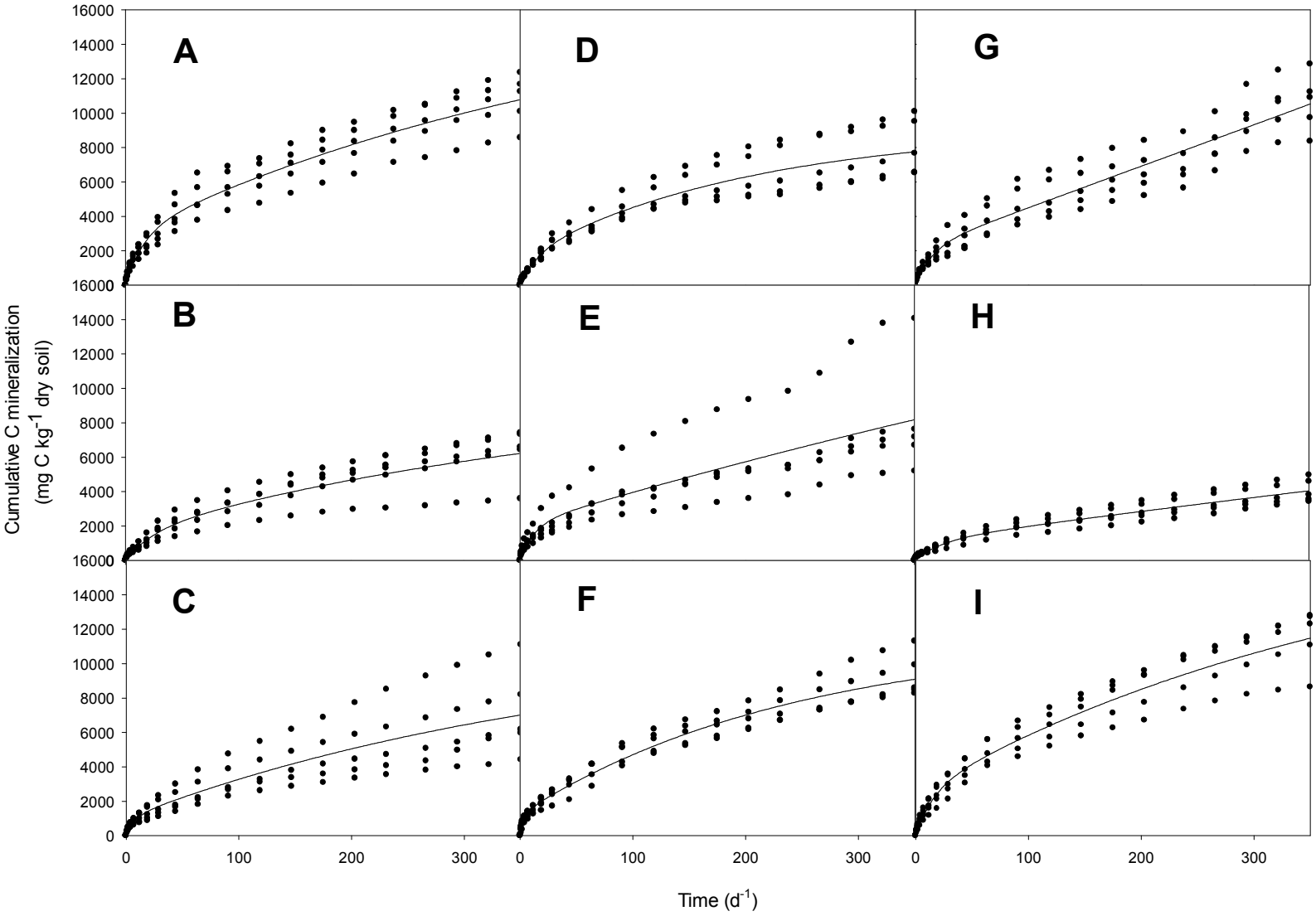
A
B
C
D
E
F
G
H
I
Cumulative C mineralization
(mg C kg-1 dry soil)
Time (d-1)
0
2000
4000
6000
8000
10000
12000
14000
16000
0
100
200
300

Figure 2.2

Figure 2.3. Cumulative N released during 350 days. Plots A though E show sites OR1 through 5, plots F through I show sites WA1 through 4. Graphs show cumulative DON (in dark gray), NH_4^+ (in light gray), and NO_3^- (in black).

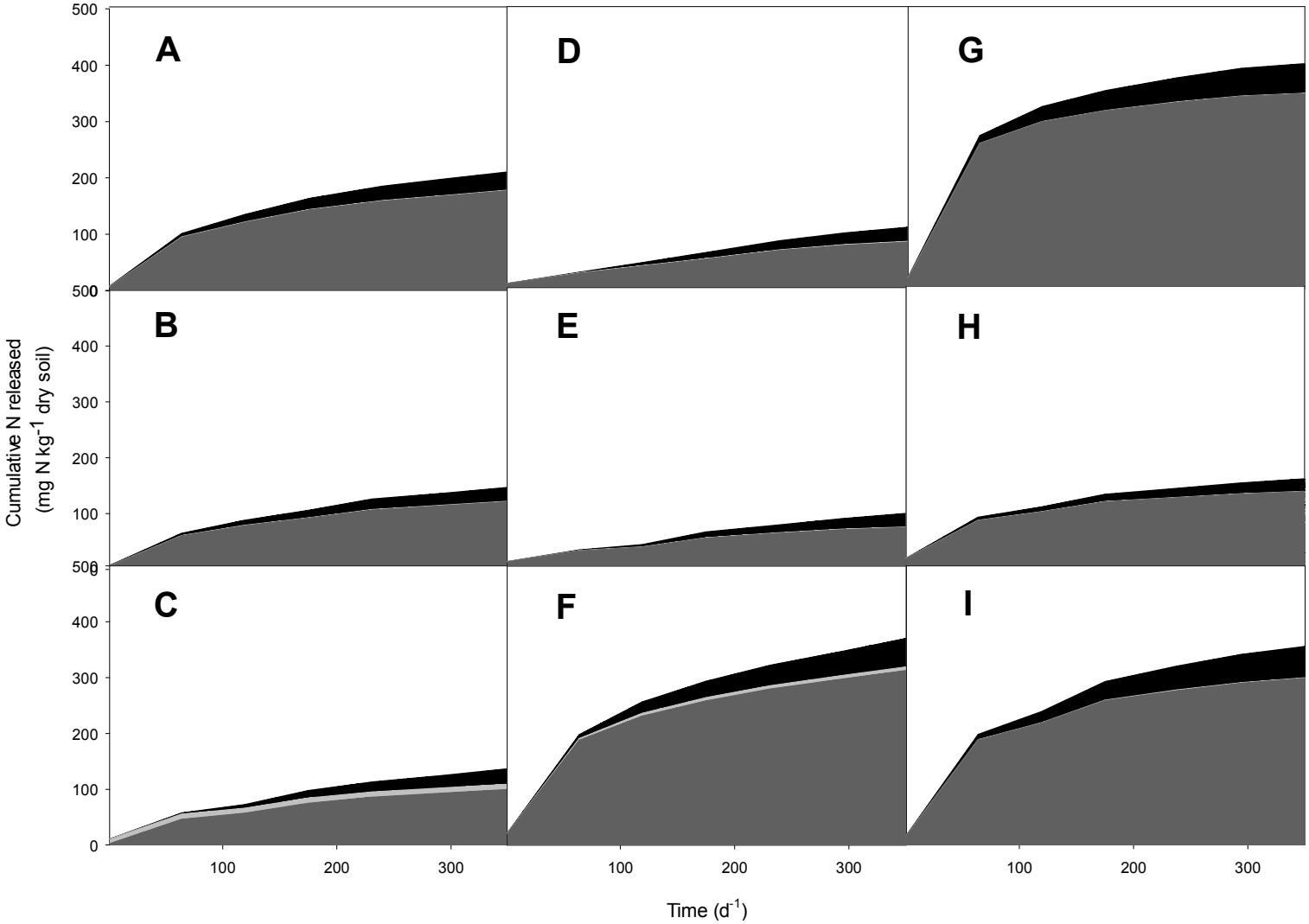
A
D
G
B
E
H
C
F
I
Cumulative N released
(mg N kg-1 dry soil)
Time (d-1)
0
100
200
300
400
500

Figure 2.3

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Chapter 4:
General Conclusion

Megan L. McGinnis

Small changes in C and N mineralization of forest soils can have potentially large impacts on atmospheric levels of CO_2 and aquatic N levels. Temperate forests are particularly important due to the relatively large fractions of C that are stored in the soil organic matter. Thus, understanding the factors that influence C and N cycling over broad geographic scales is critical both for land management, predicting ecosystem responses to disturbance, and for understanding nutrient fluxes through ecosystems.

This thesis sought to characterize soil microbial function in relation to C and N cycling in managed Douglas-fir forests of the Pacific Northwest. This characterization included a variety of environmental conditions such as soil type, elevation, precipitation, and fertilization.

The first study examined profiles of extracellular enzymes, and correlated them with C and N mineralization rates obtained from a short-term incubation as well as a wide range of environmental variables, including soil nutrients, texture, and site characteristics. Key findings illustrated clear differences among sites in all of the enzymes we measured. Multivariate analysis was an important tool in drawing meaning out of these differences and distinguishing site differences as a whole. C-cycling enzymes were correlated with respired C, and oxidative enzymes, in addition to N-cycling enzymes and PHOS, were correlated with leached N. Stoichiometric ratios between enzymes revealed N limitation in these soils. Soils tended to have more similar functional profiles with soils of the same soil type. Abiotic factors such as physical and chemical properties were also strongly correlated with enzyme activity.

This suggests that abiotic soil properties are key influencers of EEA in these forest systems.

The second study examined C and N mineralization rates derived from a year-long soil incubation. These measures of mineralization were also correlated with environmental factors and the previously obtained enzyme activities to elucidate relationships in nutrient cycling with the environment. Carbon and N mineralization were both fitted with kinetic curves to establish pool sizes and rate constants. A combined first- and zero-order kinetic model best fit C mineralization, whereas a single first-order kinetic model fit N mineralization. Mineralization, and parameters describing their characteristics, also differed significantly among sites. On average, soils respired 13.5% of the soil C, and released 7.4% of the soil N. Biotic factors such as biomass and enzyme activities had some influence on mineralization rates, particularly for N, however abiotic factors also were highly influential. Soil chemical factors such as base cation concentrations, pH, and CEC, coupled with increased soil respiration following leaching, lend support to the regulatory gate hypothesis and suggest that substrate supply limitation is one of the main rate-limiting factors controlling mineralization.

Future Research

Coupling the functional diversity of soils from these sites with microbial community structure would lend critical insights into the roles of various microbial community members in C and N processing. Understanding the link between the two would be fundamental in characterizing the soil microbial ecology of the region.

One of the main goals of this study was to establish baseline activity for soils in the region, so that changes following logging can be measured. Thus, in the future, tracking changes in both enzymatic activity and potential mineralization following timber harvest will yield insights into how land management impacts soil microbial activity and C and N pool sizes and turnover times.

Investigating the dynamics in DON in relation to N cycling is another major area of nutrient cycling dynamics that requires further research. Much attention is generally placed on inorganic N forms, and amounts of DON often go unmeasured and unreported. However, it appeared in our study that the DON pool was a large and dynamic pool of N.

Finally, the mechanistic elements governing the relationship between soil abiotic properties, enzyme activities, and nutrient mineralization needs to be further elucidated. This study's results suggest that availability of organic substrates may be a main driver of mineralization. Further studies are needed to directly test this hypothesis. Additionally, incubation studies including leaching events such as this one should be repeated to determine if the increased respiration response we observed was anomalous. If not, the mechanisms driving the flush of respiration late into the incubation need to be tested further to see if changes in the microbial community structure could account for this increase.

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