AN ABSTRACT OF THE DISSERTATION OF

Elizabeth Ann Brewer for the degree of Doctor of Philosophy in Soil Science presented on November 29, 2010.
Title: Response of Soil Microbial Communities and Nitrogen Cycling Processes to Changes in Vegetation Input.

Abstract approved:

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David D. Myrold

Changes in the type and amount of plant inputs can occur gradually, as with succession, or rapidly, as with harvesting or wildfire. With global change it is anticipated that both gradual and immediate scenarios will occur at increasing frequency. Changes in vegetation inputs alter the quality and quantity of soil organic matter inputs, thus influencing the composition of soil microbial communities and the nutrient cycles they mediate. Understanding the relationship of soil organic matter inputs on soil microbial communities and nutrient cycles will be beneficial in predicting responses to changes in vegetation inputs.

During the last 100-150 years, the vegetation of the Rio Grande Plains of the United States has been shifting from grasslands/savannas to woodlands as the result of encroachment of N_2-fixing trees and their associated plant communities. The structure and diversity of soil microbial communities were examined under woody species and remnant grasslands. In addition, relationships between soil microbial communities and

soil physical and chemical characteristics were explored. Soil microbial communities differed in soils under N_2-fixing trees and associated vegetation compared to remnant grasslands. Differences in both fungal and bacterial communities were anticipated with vegetation shifts; however, only fungal communities correlated with vegetation, whereas bacterial communities were influenced by spatial heterogeneity.

Soil microbial N cycling was investigated in long-term (>10 years) organic matter manipulations in an old-growth forest, dominated by large *Pseudotsuga menziesii* (Mirb.) Franco (Douglas-fir). The objectives of this research were to: 1) determine if long-term organic matter manipulations in old-growth forests altered microbial N cycling, 2) determine the contribution of litter to N cycling, and 3) determine if litter quality (low C/N red alder and high C/N Douglas-fir) affected the contribution of litter-derived N to N transformations. Long-term organic matter manipulations were found to affect microbial C and N cycling, but to a lesser degree than anticipated. After 10 years of organic matter exclusions and additions, microbial communities in all treatments remained N limited, although N limitation was less severe in organic matter exclusion treatments. Adding leached litter to control and organic matter exclusion soils initially altered N processes but differences dissipated during a 151-day incubation. Litter quality had little impact on the N cycling and litter made modest contributions to N mineralization and nitrification. The exclusion of organic matter altered the functionality of the microbial community to access litter-derived N.

Both the gradual establishment of woody clusters on grassland and abrupt manipulations of old-growth vegetation inputs elicited responses in microbial communities and N cycling. Although some responses were subtle, they nonetheless support the responsiveness and importance of microbial communities to soil processes. Understanding feedbacks among plant inputs, microbial communities and nutrient cycles will aid in predicting microbial, ecosystem, and global responses to vegetation changes.

Response of Soil Microbial Communities and Nitrogen Cycling Processes to Changes in Vegetation Inputs

by

Elizabeth Ann Brewer

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

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Elizabeth Ann Brewer, Author

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CONTRIBUTION OF AUTHORS

Dr. Thomas W. Boutton contributed with the experimental design, additional background site data, data interpretation, and the preparation of the manuscript for Chapter 2. Dr. Kate Lajtha maintains the Detritus Inputs and Removal Treatments used in Chapters 3 and 4. Dr. Lajtha also assisted with sampling, experimental design, data interpretation and manuscript preparation for both Chapters 3 and 4. Dr. Stephanie A. Yarwood contributed to the experimental design, and data collection and interpretation of Chapter 4. Dr. Rockie R. Yarwood contributed with data collection and analysis Chapter 4. Dr. Peter J. Bottomley assisted with experimental design and data interpretation on Chapter 4.

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Chapter 1: General Introduction

Elizabeth A. Brewer

Introduction

Below- and above-ground plant inputs correspond to the rate and quality of soil organic matter in an ecosystem. Anthropogenic manipulations or land used changes can alter root an litter inputs and in some cases change in inputs occurs on relatively short time scales. Changes in below- and above- ground inputs as are often associated with shifts in microbial communities (Brant et al., 2006; Kageyama, 2005; Nemergut et al., 2010; Yarwood et al., 2009) and alter the cycling of C and N (Hannam and Prescott, 2003; Zhong and Makeschin, 2003) .

Shifts in vegetation often occur over more gradual time scale but ultimately result in a change of above- and below-ground inputs (Hibbard et al., 2001; Liao et al., 2006a; Liao et al., 2006b; Smith and Johnson, 2003). In many grassland systems, there has been a increase of woody encroachment, resulting in change from grassland/savanna vegetation to woodlands (Archer et al., 2001; Asner et al., 2003; Bork and Burkinshaw, 2009; Knapp et al., 2008; Smith and Johnson, 2003; Van Auken, 2000). Shifts from herbaceous to woody, or relatively labile to relatively more recalcitrant litter, also influences microbial communities (Biederman and Boutton, 2009; Hollister et al., 2010; Waldrop and Firestone, 2006) and C and N cycling (Hughes et al., 2006; Jackson et al., 2002; Liao et al., 2006a; Smith and Johnson, 2003)

The quality and quantity of organic matter, as well as the presence of plant competition, influences the N availability for microbial communities. Increase in the C/N ratio, which can also be related to decreased lability, of organic matter is often associated with a decrease in the rate of gross mineralization (Booth et al., 2005) and microbial assimilation (net immobilization) (Cabrera et al., 2005; Hart et al., 1994a; Hart et al., 1994b). When C/N ratios decrease or plant competition is removed, N mineralization increases and if there is a nitrifier population present nitrification will occur (Booth et al., 2005).

In many ecosystems, soluble organic N pools from throughfall, leaching of litter, root exudation and microbial cell-constituents. This soluble pool contains a wide variety of chemical structures but contains short monomers and proteins readily available for N mineralization (Guggenberger et al., 1994; Jones and Murphy, 2007; Neff et al., 2003; Qualls, 2000). It is the insoluble pool that is proposed to be the rate limiting step in the N cycle (Schimel and Bennett, 2004). Releasing this insoluble or litter-derived N, is largely dependent on the functionality of the innate microbial communities (Caldwell, 2005; Romani et al., 2006). Understanding the contribution of the insoluble N pool to the N cycle as well as the functionality of the microbial community will increase the knowledge regarding the regulation of N cycling processes.

The first study presented here examines how microbial communities respond to vegetation shifts and how they correlate to chemical properties. In the subsequent

studies, I examined the effects of long-term exclusion of organic matter inputs on N cycling processes and the functionality of microbial communities in cycling litter-derived N. I also examined the contribution of litter-derived N to old-growth forest soils and the influence of litter quality contributions to N cycling processes.

Literature Cited

Archer, S.R., T.W. Boutton, and K.A. Hibbard. 2001. Trees in grassland: Biogeochemical consequences of woody plant expansion, p. 115-137, *In* E.-D. Schulze, et al., eds. Global Biogeochemical Cycles in the Climate System. Academic Press, San Diego.

Asner, G.P., S. Archer, R.F. Hughes, R.J. Ansley, and C.A. Wessman. 2003. Net changes in regional woody vegetation cover and carbon storage in Texas rangelands, 1937-1999. Global Change Biology 9:1-20.

Biederman, L.A., and T.W. Boutton. 2009. Biodiversity and trophic structure of soil nematode communities are altered following woody plant invasion of grassland. Soil Biology & Biochemistry 41:1943-1950.

Booth, M.S., J.M. Stark, and E. Rastetter. 2005. Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data. Ecological Monographs 75:139-157.

Bork, E.W., and A.M. Burkinshaw. 2009. Cool-Season Floodplain Meadow Responses to Shrub Encroachment in Alberta. Rangeland Ecology & Management 62:44-52.

Brant, J.B., D.D. Myrold, and E.W. Sulzman. 2006. Root controls on soil microbial community structure in forest soils. Oecologia 148:650-659.

Cabrera, M.L., D.E. Kissel, and M.F. Vigil. 2005. Nitrogen mineralization from organic residues: Research opportunities. Journal of Environmental Quality 34:75-79.

Caldwell, B.A. 2005. Enzyme activities as a component of soil biodiversity: A review. Pedobiologia 49:637-644.

Guggenberger, G., W. Zech, and H.R. Schulten. 1994. Formation and mobilization pathways of dissolved organic matter: evidence from chemical structural studies of organic matter fractions in acid forest floor solutions. Organic Geochemistry 21:51-66.

Hannam, K.D., and C.E. Prescott. 2003. Soluble organic nitrogen in forests and adjacent clearcuts in British Columbia, Canada. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 33:1709-1718.

Hart, S.C., J.M. Stark, E.A. Davidson, and M.K. Firestone. 1994a. Nitrogen mineralization, immobilization, and nitrification, *In* R. W. Weaver, et al., eds. Methods of soil analysis, Part 2. Microbiological and biochemical properties. Soil Science Society of America, Madison, WI.

Hart, S.C., G.E. Nason, D.D. Myrold, and D.A. Perry. 1994b. Dynamics of gross nitrogen transformations in an old-growth forest: The carbon connection. Ecology 75:880-891.

Hibbard, K.A., S. Archer, D.S. Schimel, and D.W. Valentine. 2001. Biogeochemical changes accompanying woody plant encroachment in a subtropical savanna. Ecology 82:1999-2011.

Hollister, E.B., C.W. Schadt, A.V. Palumbo, R.J. Ansley, and T.W. Boutton. 2010. Structural and functional diversity of soil bacterial and fungal communities following woody plant encroachment in the southern Great Plains. Soil Biology & Biochemistry 42:1816-1824.

Hughes, R.F., S.R. Archer, G.P. Asner, C.A. Wessman, C. McMurtry, J. Nelson, and R.J. Ansley. 2006. Changes in aboveground primary production and carbon and nitrogen pools accompanying woody plant encroachment in a temperate savanna. Global Change Biology 12:1733-1747.

Jackson, R.B., J.L. Banner, E.G. Jobbagy, W.T. Pockman, and D.H. Wall. 2002. Ecosystem carbon loss with woody plant invasion of grasslands. Nature 418:623-626.

Jones, D.L., and D.V. Murphy. 2007. Microbial response time to sugar and amino acid additions to soil. Soil Biology and Biochemistry 39:2178-2182.

Kageyama, S.A. 2005. Effects of vegetation and disturbance on fungal communities in the western Cascades of Oregon. Ph.D. dissertation, Oregon State University, Corvallis.

Knapp, A.K., J.M. Briggs, S.L. Collins, S.R. Archer, M.S. Bret-Harte, B.E. Ewers, D.P. Peters, D.R. Young, G.R. Shaver, E. Pendall, and M.B. Cleary. 2008. Shrub encroachment in North American grasslands: shifts in growth form dominance rapidly alters control of ecosystem carbon inputs. Global Change Biology 14:615-623.

Liao, J.D., T.W. Boutton, and J.D. Jastrow. 2006a. Storage and dynamics of carbon and nitrogen in soil physical fractions following woody plant invasion of grassland. Soil Biology & Biochemistry 38:3184-3196.

Liao, J.D., T.W. Boutton, and J.D. Jastrow. 2006b. Organic matter turnover in soil physical fractions following woody plant invasion of grassland: Evidence from natural C-13 and N-15. Soil Biology & Biochemistry 38:3197-3210.

Neff, J.C., F.S. Chapin, and P.M. Vitousek. 2003. Breaks in the cycle: dissolved organic nitrogen in terrestrial ecosystems. Frontiers in Ecology and the Environment 1:205-211.

Nemergut, D.R., C.C. Cleveland, W.R. Wieder, C.L. Washenberger, and A.R. Townsend. 2010. Plot-scale manipulations of organic matter inputs to soils correlate with shifts in microbial community composition in a lowland tropical rain forest. Soil Biology & Biochemistry 42:2153-2160.

Qualls, R.G. 2000. Comparison of the behavior of soluble organic and inorganic nutrients in forest soils. Forest Ecology and Management 138:29-50.

Romani, A.M., H. Fischer, C. Mille-Lindblom, and L.J. Tranvik. 2006. Interactions of bacteria and fungi on decomposing litter: Differential extracellular enzyme activities. Ecology 87:2559-2569.

Schimel, J.P., and J. Bennett. 2004. Nitrogen mineralization: Challenges of a changing paradigm. Ecology 85:591-602.

Smith, D.L., and L.C. Johnson. 2003. Expansion of *Juniperus virginiana L*. in the Great Plains: Changes in soil organic carbon dynamics. Global Biogeochemical Cycles 17:12.

Van Auken, O.W. 2000. Shrub invasions of North American semiarid grasslands. Annual Review of Ecology and Systematics 31:197-215.

Waldrop, M.P., and M.K. Firestone. 2006. Seasonal dynamics of microbial community composition and function in oak canopy and open grassland soils. Microbial Ecology 52:470-479.

Yarwood, S.A., D.D. Myrold, and M.N. Hogberg. 2009. Termination of belowground C allocation by trees alters soil fungal and bacterial communities in a boreal forest. FEMS Microbiology Ecology 70:151-162.

Zhong, Z.K., and F. Makeschin. 2003. Soluble organic nitrogen in temperate forest soils. Soil Biology & Biochemistry 35:333-338.

Chapter 2: Leguminous Woody Plant Encroachment Alters the Soil Microbial Community Composition of a Sub-tropical Grassland

Elizabeth A. Brewer

Abstract

Encroachment of woody plants into grasslands alters the quantity and quality of litter and root inputs to soil, and often modifies the storage and dynamics of soil organic matter. These shifts in ecosystem processes may result in, or be the result of, changes in soil microbial communities. Our objective was to examine changes in soil microbial communities in the subtropical grasslands of southern Texas that have been encroached by trees and shrubs. Soils from two depths (0-15 cm and 15-30 cm) were collected at four positions along transects extending from the centers of woody patches dominated by *Prosopis glandulosa* out into open grassland. Soil communities were characterized by phospholipid fatty acid (PLFA), and terminal restriction fragment length polymorphisms (T-RFLP) of bacterial 16S rRNA genes and length heterogeneity polymerase chain reaction (LH-PCR) of the fungal internal transcribed spacer (ITS) regions. All methods revealed significant differences in microbial communities between the two depths. At both depths, no differences in microbial communities of woody clusters were observed, with the exception of fungi communities at 0-15 cm. Differences across transect positions were found only for the fungal community at 0-15 cm depth. Taxonomic groupings based on bacterial PLFAs showed location and depth effects but no interactions between depths and transect location, whereas the fungal group showed only an effect of location. These results indicate that soil microbial communities were altered following woody plant encroachment; however, we were only able to detect differences among fungi across transects. This suggests that in this ecosystem, fungi are more strongly correlated with

the vegetation type and their roots, whereas bacterial communities may be more influenced by spatial heterogeneity associated with the clusters.

Introduction

One of the most prevalent changes to ecosystems is a shift in plant communities resulting from non-indigenous plant invasions and/or a proliferation of a native species outside normal vegetation succession patterns. The proliferation of woody plants onto grassland and savanna ecosystems has been documented worldwide (Archer et al., 2001; Asner et al., 2003; Bork and Burkinshaw, 2009; Knapp et al., 2008; Smith and Johnson, 2003; Van Auken, 2000). Shifts from an herbaceous dominated ecosystem to a woody plant system are associated with changes in the quantity and/or quality of root and litter inputs to the soil (Hibbard et al., 2001; Liao et al., 2006a; Liao et al., 2006b; Smith and Johnson, 2003).

Shifts in quality and quantity of SOM have implications for nutrient turnover, in particular C and N cycling (Hibbard et al., 2001; Hughes et al., 2006; Liao et al., 2006a; Liao et al., 2006b; McCulley et al., 2004; Throop and Archer, 2008). Plant inputs, below- and above-ground can influence microbial communities as they are determinants of not only substrate/nutrient availability but can also influence soil physical and chemical characteristics. Changes in the structure and diversity of soil communities with shifting of vegetation have been documented frequently in a variety of climatic conditions (e.g.,(Biederman and Boutton, 2009; Eskelinen et al., 2009; Goberna et al., 2005; Kourtev et al., 2003; Lauber et al., 2008; MacDonald et al.,

2009; Mitchell et al., 2010; Mummey et al., 2010; Strickland et al., 2010; Waldrop and Firestone, 2006).

Microbial community shifts following vegetation change are common, but determining what drives these shifts is complex. In addition to the quality and quantity of organic matter inputs, the location of these inputs in the soil profile in combination with natural soil physical and chemical gradients (i.e., moisture, O_2, bulk density) influence microbial communities. Studies of soil profiles have shown decreases in microbial biomass and shifts in microbial communities with increasing depth (Fierer et al., 2003; Holden and Fierer, 2005; LaMontagne et al., 2003; Steenwerth et al., 2008; Zhou et al., 2004).

The Rio Grande Plains historically were open subtropical grasslands or savannas dominated by C_4 grasses (Boutton et al., 1999). During last 100-150 years, the area has shifted to subtropical thorn woodlands, with *Prosopis glandulosa* (honey mesquite) being the dominant colonizer (Zitzer et al., 1996). *Prosopis glandulosa*, a N_2-fixing tree, facilitates the recruitment of other woody species beneath its canopy. Under woody vegetation, soil organic C (SOC) and total N pools increased in comparison to remnant grasslands (McCulley et al., 2004) and the rates of accumulation in these pools also increased (Liao et al., 2006a). In addition, potential N and C mineralization rates (Hibbard et al., 2001; McCulley et al., 2004) and microbial biomass C and N (Liao and Boutton, 2008; McCulley et al., 2004) were greater under woody vegetation.

Changes in C and N dynamics led us to hypothesize that soil microbial communities under woody species and remnant grasslands would be different in structure (e.g., ratio of fungi to bacteria) and diversity. Our objective was to examine soil microbial community composition of bacterial and fungal communities of sub-tropical grassland following woody encroachment. We characterized the soil microbial community structure and the composition of bacterial and fungal communities. The communities were examined at two depths across transects extending from the centers of woody patches dominated by *P. glandulosa* out into open grassland. We predicted that microbial community structure and composition would differ between the two soils depths and across transect locations. We also anticipated that examining correlations with soil and site variables (e.g., C and N content, microbial biomass, root biomass) would aid in identifying key variables responding to and influencing microbial communities.

Methods

Site Description

The Texas AgriLife La Copita Research Area (27° 40'N; 98° 12'W) is located in the Rio Grande Plains of Texas. The site has a mean annual temperature of 22.4°C and receives a mean annual precipitation of 716 mm with major fluxes in May-June and September (McCulley et al., 2004; Liao et al., 2006b). The sandy loam soils are classified as Typic and Pachic Argiustolls (Boutton et al., 1998; Liao et al., 2006a). Livestock have grazed the site since the late 1800's. Studies of soil $\delta^{13}C$, (Boutton et

al., 1998; Boutton et al., 1999) and aerial photos (Archer et al. 2004) have suggested the shift from grassland to woodlands has occurred during the last 100 -150 years.

In August 2005, soil samples were collected from five woody clusters located within a grassland matrix. Clusters are characterized by a single *P. glandulosa* (a N_2-fixing tree legume) with an understory comprised of shrub species such as *Condalia hookeri* (M.C. Johnst.), *Berberis trifoliolata* (Moric.), and *Zanthoxylum fagara* (L.) (Boutton et al., 1998; Archer et al., 2001; Liao et al., 2006a). Each of the five clusters, estimated to be 40-60 years old, developed as the result of *P. glandulosa* colonization and the establishment of a woody shrub understory typically containing 10-15 plant species (Table 2.1).

In each woody cluster, soils were collected from two depths (0-15 and 15-30 cm) at four positions along three radial transects (one core/per position/per depth/per transect) extending from the center of shrub/tree clusters out into the surrounding open grassland. Samples taken within 0.5 m of the bole of the primary *P. glandulosa* tree and approximately 2 m from the primary *P. glandulosa*, are referred to as the bole and mid-canopy positions, respectively. Samples were also taken at the woody canopy drip line of the cluster, and 2 m from the woody canopy drip line in the remnant grassland. These positions are referred to as the drip line and grassland, respectively, throughout the paper. For each cluster, soil samples from the three transects were composited by position and depth, providing a total of 40 samples for analysis.

Microbial Communities

PLFA

Phospholipid fatty acid (PLFA) analysis was used to characterize the composition of the soil microbial communities. A single-phase extraction method was used, with PLFAs being converted to fatty acid methyl esters, which were separated by capillary chromatography using an Agilent 6890 (Agilent Inc., Palo Alto, CA) capillary gas chromatograph equipped with a 30-m Hewlett-Packard Ultra 2 column (Hewlett Packard, Palo Alto, CA; 0.20 mm i.d., 0.33 μm film) and flame ionization detector (Butler et al., 2004; McMahon et al., 2005). Phospholipid fatty acids were identified using standard nomenclature and assigned to taxonomic groups based on previous literature (Table 2.1) (Zelles et al., 1994; Stahl et al., 1996; Myers et al., 2001; McMahon et al., 2005). Fungal to bacterial ratios were calculated from the designated taxonomic groupings of bacteria and fungi (Waldrop et al., 2004; McMahon et al., 2005; Boyle et al., 2008). Taxonomical results are reported as a relative abundance, mol%, to account for variation in extraction efficiencies across samples

T-RFLP and LH-PCR

DNA-based fingerprinting techniques were used to characterize bacterial and fungal communities. DNA was extracted in duplicate from soil (0.5 g) using the PowerSoil kit (MOBio Laboratories, Carlsbad, CA) according to the manufacturer's instructions with the modification of using a Bio101 FastPrep (Bio 101, Carlsbad, CA)

instrument to lyse cells (Yarwood et al., 2010). DNA was quantified using a NanoDrop™ ND-1000 UV–Visible Spectrophotometer (Nanodrop Technologies, Wilmington, DE). PCR templates were composites of the duplicate DNA extractions. For bacteria, extracted DNA was amplified by PCR using 16S rRNA gene primers 16S 8-F (Edwards et al., 1989) and 16S 907-R (Muyzer et al., 1995) under conditions described by Hackl et al. (2004). For fungi, the ribosomal Internal Transcribed Spacer (ITS) region was amplified using ITS1-F and ITS4-B (Bruns et al., 1998) as described by Anderson et al. (2003). All PCR products were purified using Qiagen DNA clean-up kit (Qiagen Inc., Valencia, CA).

Bacterial communities were analyzed using the 16S rRNA gene for terminal restriction fragment length polymorphisms (T-RFLP) (Hackl et al., 2004) by digesting PCR products with AluI and MspI (3 hours, 37°C) followed by heat inactivation (15 min, 65°C). Fungal communities were not restricted but analyzed using length heterogeneity PCR (LH-PCR).

For both fungi and bacteria, approximately 5 ng of DNA from the restriction digest was analyzed using an ABI 3100 capillary sequencer (Applied Biosystems, Foster City, CA) at the Oregon State University's Center of Genome Research and Biocomputing. The length and fluorescence of length heterogeneity and terminal restriction fragments were determined using GeneScan version 3.5 and Genotyper version 2.5 software (Applied Biosystems, Foster City, CA). Methods for analyzing T-RFLP and LH-PCR profiles have been described in detail previously (Rich et al.,

2003; Boyle et al., 2006). In brief, each peak for an individual sample was relativized as a fraction of the total fluorescence of all the peaks. Peaks were aligned by base pair size and differences between subsequent base pairs were calculated. When differences between adjacent peaks were greater than 0.5 base pairs, peaks were considered to be different. For the bacterial community, restriction enzyme profiles were combined to create one composite T-RFLP profile.

Statistical Analyses

Nonmetric multidimensional scaling (NMS) (McCune et al., 2006) ordination techniques were used with PLFA, LH-PCR, and T-RFLP data. NMS, using Sørensen distance, was selected based on the premise that it avoids normality and linearity assumptions (McCune et al., 2002), which are often absent from community data sets. Ordinations were initiated with random configurations and 250 runs with real data with a final instability criterion of 0.0001. All NMS configuration had a stress level under 13.5. To assess whether the k-dimensional solution was significant, randomization tests were performed. Potential outliers, using Sørensen distance measure, were identified as any sample that was greater than two standard deviations from the mean. Outliers for fungal and bacterial communities were not removed as they did not alter any results or conclusions; however, two outliers from PLFA communities were deleted. Fungal communities at depth 15-30 cm could not reach a final ordination configuration. A power transform of 0.5 was applied to this data set to obtain a stable configuration.

To determine if there were differences among groupings (e.g., depth, transect location), multi-response blocked permutation procedures (MRBPP) were used. Poor lipid extraction and removal of outliers prevented the use of a complete randomized block design in the PLFA analyses, therefore multi-response permutation procedures (MRPP) were used to test for differences between the two depths as well as among locations at each depth. In both MRPP and MRBPP, Sørensen distance measure was selected to calculate differences. For each group comparison a chance-corrected within group agreement statistic is provided (A) along with a p-value. The A-statistic indicates the homogeneity within a group, while the p-value evaluates how likely the observed difference is due to chance. If all samples within a group are identical then A=1, whereas A=0 if heterogeneity within a group is the same as would be expected by chance (McCune et al., 2002). It has been suggested that A>0.3 is high for community data (McCune et al., 2002). Unless otherwise noted, p-values less than or equal to 0.05 were considered significant.

To aid understanding of what may be driving the differences among groups, indicated by MRPP results, an overlay of explanatory/environmental variables are used as a joint plot in the NMS ordinations. The explanatory variables used in analyses were age of colonizing *P. glandulosa*, microbial biomass, root biomass, soil bulk density, taxonomic groupings calculated from PLFAs, presence and absence of plant species, and soil and root $\delta^{15}N$, %N, $\delta^{13}C$, %C, and C/N ratio (Table 2.1).

To test if there were significant correlations among microbial community data sets, Mantel tests were used (McCune et al., 2002). The Mantel test assesses if two communities are more correlated than could occur by chance, by testing calculated distance matrices against a random distribution.

Statistical significance of PLFA-based taxonomic groupings between transect locations by depth were analyzed with randomized block analysis of variance (ANOVA), with woody clusters serving as random blocks, using PROC GLM (SAS version, 9.2, SAS Institute, Inc. Cary, NC).

Results

All methods of community analysis showed a significant difference between the microbial communities of the 0-15 cm and 15-30 cm depths (Figure 2.1): PLFA ($A = 0.07$, $p = 0.00$), fungal LH-PCR ($A = 0.08$, $p = 0.00$), and bacterial T-RFLP ($A = 0.13$, $p = 0.00$). NMS analyses of PLFA's yielded a two-dimensional solution with 97% of variance explained and showed no significant differences among woody clusters ($A = 0.025$, $p = 0.205$) or location ($A = 0.003$, $p = 0.38$) (Figure 2.1A).

When taxonomic groups based on PLFA biomarkers were examined we found greater percentages of bacteria, actinomycetes and gram-negative bacteria at 0-15 cm than 15-30 cm (Table 2.3). There was a significant location effect for the fungal taxonomical group ($p = 0.09$) and marginal effect for the fungal to bacterial ratio (Table 2.4; $p=0.09$). The bole position had a greater percentage of fungi than any other

transect position, while the fungal to bacterial ratio was lowest at the grassland location. Actinomycetes and gram-negative bacteria were different across transect locations with no depth interaction. Actinomycete groups were greater at the bole position than all other transect positions. Gram-negative bacteria percentages at the bole and mid-canopy were greater than those of the drip line and grassland positions (Table 2.4).

Fungal communities showed no differences among clusters (Figure 2.1B; A = 0.01, p = 0.26) but did show a marginal difference among transect locations (Figure 2.1B; A = 0.02, p = 0.06). Communities of the bole and grassland positions were different (A = 0.05, p = 0.01), as were the mid-canopy and grassland positions (A = 0.04, p = 0.04). In contrast, bacterial communities were different among clusters (Figure 2.1C; A = 0.05, p = 0.01) but not among transect locations (A = -0.01, p = 0.76). Woody cluster five was significantly different from all other clusters. Bacterial communities were also significantly correlated to fungal communities (Table 2.5).

Because depth was a significant factor for all three community measurements, communities at each depth were also analyzed separately to determine if cluster and location were determinants of soil communities. At the shallower depth, PLFA communities did not show differences among woody clusters (A = -0.02, p = 0.57) or transect locations (A = -0.00, p = 0.44). The fungal mol% percentage was greater at the bole and mid-canopy and there was a trend for the fungal to bacteria ratio to be the lowest at the grassland location (Table 2.4).

Fungal communities at the 0-15 cm depth did not differ among woody clusters (Fig. 2; A = 0.00, p = 0.44) but did show significant differences among locations (A = 0.09, p = 0.01). The grassland differed from the bole (A = 0.12, p = 0.01) and from the mid-canopy (A = 0.16, p = 0.01). The drip line position was also different from the mid-canopy (A = 0.07, p = 0.05), however was not different from the bole positions.

Bacterial communities in the surface 0-15 cm were different among woody clusters (Figure 2.3; A = 0.10, p = 0.00) but showed no differences among location (A = -0.03, p = 0.94). Woody cluster two was not different from cluster one nor cluster three, but all other cluster comparisons were significantly different from one another. The 0-15 cm bacterial communities correlated with the fungal communities (Table 2.5).

PLFAs from 15-30 cm did not show differences in cluster (A= 0.03, p = 0.33) or locations (A=0.04, p = 0.26). In the deeper soil (15-30 cm), differences in bacterial and fungal communities were found among clusters (bacteria: A = 0.17, p = 0.000, fungi: A = 0.12, p = 0.00) but not transect position (bacteria: A = -0.01, p = 0.73, fungi: A = -0.02, p = 0.80). The bacterial community of woody cluster two was not different from clusters one and three; however, all other clusters were different from one another. Most clusters did not show differences in fungal communities, but cluster five did differ from clusters one and two (Figure 2.4). There were no correlations among the bacterial and fungal communities at the deeper depth (Table 2.5).

Discussion

As expected, depth was a strong determinant of microbial community composition. All three community analyses revealed significant differences between the two depths. Similar to other depth comparison studies (Allison et al., 2007; Fierer et al., 2003; LaMontagne et al., 2003), decreases in biomass (total PLFA) and changes

in relative contributions of taxonomic groups were observed. Shifts of bacterial and of fungal communities between depths did not correlate with any environmental factors but rather with individual fragments (i.e., taxa or groups of taxa). Separation of PLFA communities by depth correlated with the relative abundance of gram-positive and gram-negative bacteria (Figure 2.1). The decrease in gram-negative bacteria with depth (Table 2.3; Figure 2.1) is similar to other studies (Fierer et al., 2003; Steenwerth et al., 2008; Zelles and Bai, 1994) as is the association of shallow soils with gram-positive bacteria (Blume et al., 2002; Zogg et al., 1997).

Community changes with depth have been attributed to soil moisture (Fierer et al., 2003) as well as C substrate availability (Fierer et al., 2003; Goberna et al., 2005). Within woody clusters, C and water are also likely drivers of community shifts. *Prosopis glandulosa* can have rooting depths as deep as 15 m (Canadell et al., 1996) and are capable of hydraulically lifting water from deeper in the profile up to the surface soil (Zou et al., 2005) thereby moderating gradients of C and soil moisture. Grasses of these systems tend to have shallower roots (Boutton et al., 1999; Scholes and Archer, 1997). And, gradients of chemical and physical properties that we did not measure may also drive community shifts.

Differences in transect locations for fungi (mol%) and the ratio of fungi to bacteria (Table 2.4), in addition differences of fungal communities across transect positions at the 0-15 cm (Figure 2.2), suggest that vegetation type and potentially depth are important determinants for fungal communities. Increases in relative

abundance and differences between fungal communities at the bole and mid-canopy positions is likely the result of more recalcitrant organic matter inputs from the woody trees and shrubs (Fierer et al., 2003; Waldrop et al., 2006) and potentially increased root biomass. Higher fungal biomass under woody vegetations compared to grasslands have been found in other *Prosopis* systems (Purohit et al., 2002).

The soil $\delta^{13}C$ also correlated with the separation of grassland and drip line communities from those of the mid-canopy and bole (Figure 2.2). The grassland soils are more enriched in $\delta^{13}C$, due to the organic matter inputs from C_4 grasses, than soils from the C_3 woody clusters (Archer et al., 2004; Boutton et al., 1998). This correlation with the plant-derived C signature supports the potential role of altered rhizosphere and litter influences on community composition and structure.

In our study, PLFA profiles did not differ across the vegetation transect at either depth. The lack of changes in microbial communities may suggest a spatially heterogeneous distribution of the communities; however, taxonomic groupings based on PLFAs did show vegetation differences (Table 2.5). Gram-negative bacteria and actinomycetes were greater at the *P. glandulosa* bole than any other transect location. Actinomycete populations have been associated with deeper soil horizons, with the hypothesis that it is associated with the more recalcitrant organic matter (Fierer et al., 2003). We did not observe an increase with depth but rather a decrease from the bole to the other locations, which may suggest a shift towards to relatively more recalcitrant organic matter inputs.

Shifts in bacterial communities across locations were anticipated, particularly as a result of the N_2-fixing rhizobia associated with the *P. glandulosa*; however, no differences were observed. The lower proportions of gram-negative bacteria in the drip line and grassland positions relative to the bole and mid-canopy positions (Table 2.4) would be consistent with higher rhizobia populations associated with *P. glandulosa*. It is also feasible that the high variation among clusters mask transect position differences.

The strong cluster effect for bacteria communities (Figure 2.3) suggests that there are other drivers to the microbial communities other than the presence of the *P.glandulos*a. Differences in bacterial communities associated with an individual tree cluster correlated with shrub/tree species and root biomass. The species *Lantana horrida* and *Ephedra antisyphilitica* corresponded to the clusters one and four.

There were no significant bacterial community changes across transect location at 15-30 cm and did not strongly correlate with any environmental factor, but again showed strong cluster effects. The fungal did show also showed a cluster effect, with the youngest cluster differing from among any other clusters. In addition there was a correlation of age and diameter of the *P. glandulosa*, suggesting a relationship between microbial communities and plant establishment. Age of dominant plant species has been recognized as influencing microbial community dynamics among vegetation transects (Allison et al., 2007; Tarlera et al., 2008).

Savanna and grassland ecosystems are estimated to cover approximately 40% of terrestrial ecosystems and store more than 30% of global soil organic carbon (Schlesinger, 1997). The shift of grasslands into woody grasslands has been shown to have significant effect on nutrient turnover. Differences among fungal communities at shallow depths suggest that these communities may be driving nutrient dynamics. Bacterial differences were lacking across vegetation transects suggesting that spatial heterogeneity and/or other edaphic characteristics may be determining community composition; however their correlation with fungal communities also suggests that the two communities likely share some environmental drivers. Understanding how microbial communities correspond to these vegetation shifts may offer insights into how soil communities influence larger scale nutrient cycling processes.

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References

Allison, V.J., Z. Yermakov, R.M. Miller, J.D. Jastrow, and R. Matamala. 2007. Using landscape and depth gradients to decouple the impact of correlated environmental

variables on soil microbial community composition. Soil Biology & Biochemistry 39:505-516.

Anderson, I.C., C.D. Campbell, and J.I. Prosser. 2003. Diversity of fungi in organic soils under a moorland - Scots pine (*Pinus sylvestris L.*) gradient. Environmental Microbiology 5:1121-1132.

Archer, S.R., T.W. Boutton, and K.A. Hibbard. 2001. Trees in grassland: Biogeochemical consequences of woody plant expansion, p. 115-137, *In* E.-D. Schulze, et al., eds. Global Biogeochemical Cycles in the Climate System. Academic Press, San Diego.

Archer, S.R., T.W. Boutton, and C.R. McMurtry. 2004. Carbon and nitrogen accumulation in a savanna landscape: field and modeling perspectives, p. 359-373, *In* M. Shiyomi, et al., eds. Global Environmental Change in the Ocean and on Land. Terra Scientific Publ., Toyko.

Asner, G.P., S. Archer, R.F. Hughes, R.J. Ansley, and C.A. Wessman. 2003. Net changes in regional woody vegetation cover and carbon storage in Texas rangelands, 1937-1999. Global Change Biology 9:1-20.

Biederman, L.A., and T.W. Boutton. 2009. Biodiversity and trophic structure of soil nematode communities are altered following woody plant invasion of grassland. Soil Biology & Biochemistry 41:1943-1950.

Blume, E., M. Bischoff, J.M. Reichert, T. Moorman, A. Konopka, and R.F. Turco. 2002. Surface and subsurface microbial biomass, community structure and metabolic activity as a function of soil depth and season. Applied Soil Ecology 20:171-181.

Bork, E.W., and A.M. Burkinshaw. 2009. Cool-Season Floodplain Meadow Responses to Shrub Encroachment in Alberta. Rangeland Ecology & Management 62:44-52.

Boutton, T.W., S.R. Archer, and A.J. Midwood. 1999. Stable isotopes in ecosystem science: Structure, function and dynamics of a subtropical savanna. Rapid Communications in Mass Spectrometry 13:1263-1277.

Boutton, T.W., S.R. Archer, A.J. Midwood, S.F. Zitzer, and R. Bol. 1998. $\delta^{13}C$ values of soil organic carbon and their use in documenting vegetation change in a subtropical savanna ecosystem. Geoderma 82:5-41.

Boyle, S.A., R.R. Yarwood, P.J. Bottomley, and D.D. Myrold. 2008. Bacterial and fungal contributions to soil nitrogen cycling under Douglas fir and red alder at two sites in Oregon. Soil Biology & Biochemistry 40:443-451.

Boyle, S.A., J.J. Rich, P.J. Bottomley, K. Cromack, and D.D. Myrold. 2006. Reciprocal transfer effects on denitrifying community composition and activity at forest and meadow sites in the Cascade Mountains of Oregon. Soil Biology & Biochemistry 38:870-878.

Bruns, T.D., T.D. Szaro, M. Gardes, K.W. Cullings, J.J. Pan, D.L. Taylor, T.R. Horton, A. Kretzer, M. Garbelotto, and Y. Li. 1998 A sequence database for the identification of ectomycorrhizal Basidiomycetes by phylogenetic analysis. . Molecular Ecology 7 257-272.

Butler, J.L., P.J. Bottomley, S.M. Griffith, and D.D. Myrold. 2004. Distribution and turnover of recently fixed photosynthate in ryegrass rhizospheres. Soil Biology & Biochemistry 36:371-382.

Canadell, J., R.B. Jackson, J.R. Ehleringer, H.A. Mooney, O.E. Sala, and E.D. Schulze. 1996. Maximum rooting depth of vegetation types at the global scale. Oecologia 108:583-595.

Edwards, U., T. Rogall, H. Blöcker, M. Emde, and E.C. Böttger. 1989. Isolation and direct complete nucleotide determination of entire genes:characterization of a gene coding 16s ribosomal RNA. Nucleic Acids Research 17:7843-7853.

Eskelinen, A., S. Stark, and M. Mannisto. 2009. Links between plant community composition, soil organic matter quality and microbial communities in contrasting tundra habitats. Oecologia 161:113-123.

Fierer, N., J.P. Schimel, and P.A. Holden. 2003. Variations in microbial community composition through two soil depth profiles. Soil Biology & Biochemistry 35:167-176.

Goberna, M., H. Insam, S. Klammer, J.A. Pascual, and J. Sanchez. 2005. Microbial community structure at different depths in disturbed and undisturbed semiarid Mediterranean forest soils. Microbial Ecology 50:315-326.

Hackl, E., S. Zechmiester-Boltenstern, L. Bodrossy, and A. Sessitsch. 2004. Comparison of diversities and compositions of bacterial populations inhabiting natural forest soils Applied & Environmental Microbiology 5057-5065

Hibbard, K.A., S. Archer, D.S. Schimel, and D.W. Valentine. 2001. Biogeochemical changes accompanying woody plant encroachment in a subtropical savanna. Ecology 82:1999-2011.

Holden, P.A., and N. Fierer. 2005. Microbial processes in the vadose zone. Vadose Zone Journal 4:1-21.

Hughes, R.F., S.R. Archer, G.P. Asner, C.A. Wessman, C. McMurtry, J. Nelson, and R.J. Ansley. 2006. Changes in aboveground primary production and carbon and nitrogen pools accompanying woody plant encroachment in a temperate savanna. Global Change Biology 12:1733-1747.

Knapp, A.K., J.M. Briggs, S.L. Collins, S.R. Archer, M.S. Bret-Harte, B.E. Ewers, D.P. Peters, D.R. Young, G.R. Shaver, E. Pendall, and M.B. Cleary. 2008. Shrub encroachment in North American grasslands: shifts in growth form dominance rapidly alters control of ecosystem carbon inputs. Global Change Biology 14:615-623.

Kourtev, P.S., J.G. Ehrenfeld, and M. Haggblom. 2003. Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. Soil Biology & Biochemistry 35:895-905.

LaMontagne, M.G., J.P. Schimel, and P.A. Holden. 2003. Comparison of subsurface and surface soil bacterial communities in california grassland as assessed by terminal restriction fragment length polymorphisms of PCR-amplified 16S rRNA genes. Microbial Ecology 46:216-227.

Lauber, C.L., M.S. Strickland, M.A. Bradford, and N. Fierer. 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biology & Biochemistry 40:2407-2415.

Liao, J.D., and T.W. Boutton. 2008. Soil microbial biomass response to woody plant invasion of grassland. Soil Biology & Biochemistry 40:1207-1216.

Liao, J.D., T.W. Boutton, and J.D. Jastrow. 2006a. Storage and dynamics of carbon and nitrogen in soil physical fractions following woody plant invasion of grassland. Soil Biology & Biochemistry 38:3184-3196.

Liao, J.D., T.W. Boutton, and J.D. Jastrow. 2006b. Organic matter turnover in soil physical fractions following woody plant invasion of grassland: Evidence from natural C-13 and N-15. Soil Biology & Biochemistry 38:3197-3210.

MacDonald, C.A., N. Thomas, L. Robinson, K.R. Tate, D.J. Ross, J. Dando, and B.K. Singh. 2009. Physiological, biochemical and molecular responses of the soil microbial community after afforestation of pastures with *Pinus radiata*. Soil Biology & Biochemistry 41:1642-1651.

McCulley, R.L., S.R. Archer, T.W. Boutton, F.M. Hons, and D.A. Zuberer. 2004. Soil respiration and nutrient cycling in wooded communities developing in grassland. Ecology 85:2804-2817.

McCune, B., and J.B. Grace. 2002. Analysis of Ecological Communities MjM Software Design, Glenden Beach, OR.

MjM Software. 2006. PC-ORD. Multivariate Analysis of Ecological Data. Release 5.05. MjM Software, Gleneden Beach, Oregon, U.S.A.

McMahon, S.K., M.A. Williams, P.J. Bottomley, and D.D. Myrold. 2005. Dynamics of microbial communities during decomposition of carbon-13 labeled ryegrass fractions in soil. Soil Science Society of America Journal 69:1238-1247.

Mitchell, R.J., A.J. Hester, C.D. Campbell, S.J. Chapman, C.M. Cameron, R.L. Hewison, and J.M. Potts. 2010. Is vegetation composition or soil chemistry the best predictor of the soil microbial community? Plant and Soil 333:417-430.

Mummey, D.L., J.T. Clarke, C.A. Cole, B.G. O'Connor, J.E. Gannon, and P.W. Ramsey. 2010. Spatial analysis reveals differences in soil microbial community interactions between adjacent coniferous forest and clearcut ecosystems. Soil Biology & Biochemistry 42:1138-1147.

Muyzer, G., A. Teske, and C.O. Wirsen. 1995. Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturating gradient gel electrophoresis of 16s rDNA fragments. Archives of Microbiology 164:165-172.

Myers, R.T., D.R. Zak, D.C. White, and A. Peacock. 2001. Landscape-level patterns of microbial community composition and substrate use in upland forest ecosystems. Soil Science Society of America Journal 65:359-367.

Purohit, U., S. Mehar, and S. Sundaramoorthy. 2002. Role of *Prosopis cineraria* on the ecology of soil fungi in Indian desert. Journal of Arid Environments 52:17-27.

Rich, J.J., R.S. Heichen, P.J. Bottomley, K. Cromack, and D.D. Myrold. 2003. Community composition and functioning of denitrifying bacteria from adjacent meadow and forest soils. Applied and Environmental Microbiology 69:5974-5982.

Schlesinger, W.H. 1997. Biogeochemistry: An Analysis of Global Change 2nd Edition ed. Academic Press. , San Deigo, CA.

Scholes, R.J., and S.R. Archer. 1997. Tree-Grass Interactions in Savannas. Annual Review of Ecology and Systematics 28:517-544.

Smith, D.L., and L.C. Johnson. 2003. Expansion of *Juniperus virginiana L*. in the Great Plains: Changes in soil organic carbon dynamics. Global Biogeochemical Cycles 17:12.

Stahl, P.D., and T.B. Parkin. 1996. Relationship of soil ergosterol concentration and fungal biomass. Soil Biology & Biochemistry 28:847-855.

Steenwerth, K.L., R.E. Drenovsky, J.J. Lambert, D.A. Kluepfel, K.M. Scow, and D.R. Smart. 2008. Soil morphology, depth and grapevine root frequency influence microbial communities in a Pinot noir vineyard. Soil Biology & Biochemistry 40:1330-1340.

Strickland, M.S., J.L. Devore, J.C. Maerz, and M.A. Bradford. 2010. Grass invasion of a hardwood forest is associated with declines in belowground carbon pools. Global Change Biology 16:1338-1350.

Tarlera, S., K. Jangid, A.H. Ivester, W.B. Whitman, and M.A. Williams. 2008. Microbial community succession and bacterial diversity in soils during 77,000 years of ecosystem development. FEMS Microbiology Ecology 64:129-140.

Throop, H.L., and S.R. Archer. 2008. Shrub (*Prosopis velutina*) encroachment in a semidesert grassland: spatial-temporal changes in soil organic carbon and nitrogen pools. Global Change Biology 14:2420-2431.

Van Auken, O.W. 2000. Shrub invasions of North American semiarid grasslands. Annual Review of Ecology and Systematics 31:197-215.

Waldrop, M.P., and M.K. Firestone. 2006. Seasonal dynamics of microbial community composition and function in oak canopy and open grassland soils. Microbial Ecology 52:470-479.

Waldrop, M.P., D.R. Zak, and R.L. Sinsabaugh. 2004. Microbial community response to nitrogen deposition in northern forest ecosystems. Soil Biology and Biochemistry 36:1443-1451.

Waldrop, M.P., D.R. Zak, C.B. Blackwood, C.D. Curtis, and D. Tilman. 2006. Resource availability controls fungal diversity across a plant diversity gradient. Ecology Letters 9:1127-1135.

Yarwood, S., P. Bottomley, and D. Myrold. 2010. Soil Microbial Communities Associated with Douglas-fir and Red Alder Stands at High- and Low-Productivity Forest Sites in Oregon, USA. Microbial Ecology:1-12.

Zelles, L., and Q.Y. Bai. 1994. Fatty-acid patterns of phospholipids and lipopolysaccharaides in environmental samples. Chemosphere 28:391-411.

Zhou, J.Z., B.C. Xia, H. Huang, A.V. Palumbo, and J.M. Tiedje. 2004. Microbial diversity and heterogeneity in sandy subsurface soils. Applied and Environmental Microbiology 70:1723-1734.

Zitzer, S.F., S.R. Archer, and T.W. Boutton. 1996. Spatial variability in the potential for symbiotic N_2 fixation by woody plants in a subtropical savanna ecosystem. Journal of Applied Ecology 33:1125-1136.

Zogg, G.P., D.R. Zak, D.B. Ringelberg, N.W. MacDonald, K.S. Pregitzer, and D.C. White. 1997. Compositional and functional shifts in microbial communities due to soil warming. Soil Science Society of America Journal 61:475-481.

Zou, C.B., P.W. Barnes, S. Archer, and C.R. McMurtry. 2005. Soil moisture redistribution as a mechanism of facilitation in savanna tree-shrub clusters. Oecologia 145:32-40.

Table 2.1 Summary of environmental characteristics of the five woody clusters located at the Agricultural Experimental Station, La Copita Research Area in Texas. For plant species, + indicates the presence of the plant species within the woody cluster, while – indicates the absence of the species.

Cluster	*Prosopis* Age (yr)	Circumference of Entire Cluster (m)	Height of *Prosopis* (m)	Height of Understory Shrub Canopy (m)	Basal Diameter of *Prosopis* (cm)	Transect Position	Microbial Biomass (mg kg^{-1} soil)	Soil $\delta^{15}N$	Soil N (g N kg^{-1} dry soil)	Soil $\delta^{13}C$	Soil N (g C kg^{-1} dry soil)	Soil C/N
1	57.1	28	5	2	21.7	Bole	712.2	7.2	1.0	-22.9	11.3	11.2
						Mid-Canopy	460.3	7.4	1.0	-21.5	9.8	10.3
						Drip Line	352.1	8.0	0.6	-19.7	5.6	10.3
						Grassland	380.9	8.4	0.5	-17.0	5.5	11.0
2	40.6	17.4	2.6	1.5	14	Bole	497.5	6.9	0.8	-21.8	8.2	11.0
						Mid-Canopy	648.9	7.0	0.8	-22.4	8.9	11.1
						Drip Line	409.8	7.6	0.6	-18.7	3.0	5.8
						Grassland	472.8	8.1	0.7	-18.3	7.5	10.7
3	48.3	23.4	6	1.8	17.6	Bole	456.9	7.1	0.8	-21.5	9.0	11.3
						Mid-Canopy	636.7	7.5	0.7	-20.1	7.6	10.8
						Drip Line	295.6	8.1	0.6	-18.0	6.2	10.5
						Grassland	344.4	8.1	0.6	-16.8	5.3	9.7
4	40.6	24	3.5	2.2	14	Bole	330.1	7.9	0.7	-20.4	6.6	10.1
						Mid-Canopy	491.4	8.0	0.7	-20.0	7.2	10.3
						Drip Line	337.4	7.9	0.6	-17.4	6.0	9.9
						Grassland	324.7	8.9	0.7	-15.6	6.4	9.8
5	34.1	13.8	2.3	1.6	11	Bole	635.2	6.1	1.1	-20.1	10.9	10.3
						Mid-Canopy	568.1	6.6	1.0	-19.0	11.4	11.4
						Drip Line	459.4	6.7	0.8	-18.8	7.5	9.9
						Grassland	440.6	6.9	0.8	-16.7	7.7	10.3

Table 2.1 cont.

Cluster	Transect Position	Prosopis glandulosa	Zanthoxylum fagara	Mahonia trifoliolata	Karwinskia humboldtiana	Diospyros texana	Opuntia lindheimeri	Ziziphus obtusifolia	Condalia hookeri
1	Bole	+	+	+	+	+	+	+	+
	Mid-Canopy	+	+	+	+	+	+	+	+
	Drip Line	+	+	+	+	+	+	+	+
	Grassland	+	+	+	+	+	+	+	+
2	Bole	+	+	+	-	+	+	-	+
	Mid-Canopy	+	+	+	-	+	+	-	+
	Drip Line	+	+	+	-	+	+	-	+
	Grassland	+	+	+	-	+	+	-	+
3	Bole	+	+	+	+	+	+	-	+
	Mid-Canopy	+	+	+	+	+	+	-	+
	Drip Line	+	+	+	+	+	+	-	+
	Grassland	+	+	+	+	+	+	-	+
4	Bole	+	+	+	+	+	+	-	-
	Mid-Canopy	+	+	+	+	+	+	-	-
	Drip Line	+	+	+	+	+	+	-	-
	Grassland	+	+	+	+	+	+	-	-
5	Bole	+	+	+	-	+	+	-	+
	Mid-Canopy	+	+	+	-	+	+	-	+
	Drip Line	+	+	+	-	+	+	-	+
	Grassland	+	+	+	-	+	+	-	+

Cluster	Transect Position	Lantana horrida	Castela texana	Celtis pallida	Opuntia leptocaulis	Ephedra antisyphilitica	Acacia farnesiana	Sophora secundiflora	Gaillardia sp.	Acacia greggii
1	Bole	+	+	+	+	+	+	-	-	-
	Mid-Canopy	+	+	+	+	+	+	-	-	-
	Drip Line	+	+	+	+	+	+	-	-	-
	Grassland	+	+	+	+	+	+	-	-	-
2	Bole	-	+	-	-	-	-	+	-	-
	Mid-Canopy	-	+	-	-	-	-	+	-	-
	Drip Line	-	+	-	-	-	-	+	-	-
	Grassland	-	+	-	-	-	-	+	-	-
3	Bole	-	-	-	-	-	-	-	-	+
	Mid-Canopy	-	-	-	-	-	-	-	-	+
	Drip Line	-	-	-	-	-	-	-	-	+
	Grassland	-	-	-	-	-	-	-	-	+
4	Bole	+	-	-	-	+	-	+	-	+
	Mid-Canopy	+	-	-	-	+	-	+	-	+
	Drip Line	+	-	-	-	+	-	+	-	+
	Grassland	+	-	-	-	+	-	+	-	+
5	Bole	-	-	-	-	-	-	+	-	+
	Mid-Canopy	-	-	-	-	-	-	+	-	+
	Drip Line	-	-	-	-	-	-	+	-	+
	Grassland	-	-	-	-	-	-	+	-	+

Table 2.2 Taxonomic groupings of phospholipid fatty acids.

Taxonomical Group	Phospholipid Fatty Acids
Actinomycetes	10Me16:0, 10Me17:0; 10Me18:0
Bacteria	i14:0,i15:0, a15:0, i16:0, 16:1ω9, 16:1ω5, 17:1ω9, i17:0, a17:0, cy17:0, 18:1ω7, cy19:0
Fungi	18:2ω6,9
Gram-positive bacteria	i14:0, i15:0, a15:0, i16:0, i17:0, a17:0
Gram-negative bacteria	16:1ω9, 17:1ω9, cy17:0, 18:1ω7, cy19:0

Table 2.3 Relative abundance of bacteria, actinomycetes, and gram-negative bacteria, using mol% PLFA, and total PLFA, nmol g^{-1} soil, between depths in a subtropical grassland ecosystem. All values are means ± one standard error. Significant differences ($p \leq 0.05$) among depths are indicated by different letters.

Depth	Bacteria (mol%)	Fungi (mol%)	Actinomycetes (mol%)	Gram-Negative Bacteria (mol%)	Gram-Positive Bacteria (mol%)	Total PLFA (nmol g^{-1} soil)
0-15 cm	39.7 ± 0.9^{a}	2.12 ± 0.14^{a}	14.9 ± 0.5^{a}	12.7 ± 0.3^{a}	25.41 ± 0.85^{a}	99.1 ± 22.1^{a}
15-30 cm	37.4 ± 0.9^{a}	1.97 ± 0.15^{a}	12.4 ± 0.5^{b}	11.5 ± 0.3^{b}	24.57 ± 0.91^{a}	50.6 ± 23.7^{a}

Table 2.4 Taxonomic characterization of bacteria, actinomycetes, gram-positive bacteria, and gram-negative bacteria, using mol% PLFA across transect positions in a subtropical grassland ecosystem. All values are means ± one standard error. Significant differences ($p \leq 0.05$) across transect positions are indicated by different letters.

Transect Position	Actinomycetes (mol %)	Gram Negative Bacteria (mol %)	Gram Positive Bacteria (mol %)	Fungi (mol%)	Fungi:Bacterial (mol%)
Bole	15.4 ± 0.7^{a}	8.5 ± 0.4^{a}	15.1 ± 0.6^{a}	2.5 ± 0.2^{a}	0.060 ± 0.005^{a}
Mid-Canopy	12.9 ± 0.8^{b}	9.1 ± 0.5^{a}	12.8 ± 0.7^{b}	2.2 ± 0.2^{a}	0.058 ± 0.005^{a}
Drip Line	13.6 ± 0.6^{b}	7.65 ± 0.4^{b}	14.3 ± 0.6^{a}	1.9 ± 0.2^{b}	0.052 ± 0.004^{b}
Grassland	13.2 ± 0.7^{b}	7.67 ± 0.5^{b}	15.1 ± 0.7^{b}	7.7 ± 0.2^{b}	0.043 ± 0.005^{b}

Table 2.5 Correlations between fungal and bacteria communities, using Mantel tests. For profile comparisons, distance matrices were calculated for each community profile. Significance was determined via Monte Carlo tests.

Comparison	**R^2**	**p value**
Fungal ITS x bacterial 16S (both depths)	0.37	0.00
Fungal ITS x bacterial 16S (0-15 cm)	0.17	0.03
Fungal ITS x bacterial 16S (15-30 cm)	0.22	0.19

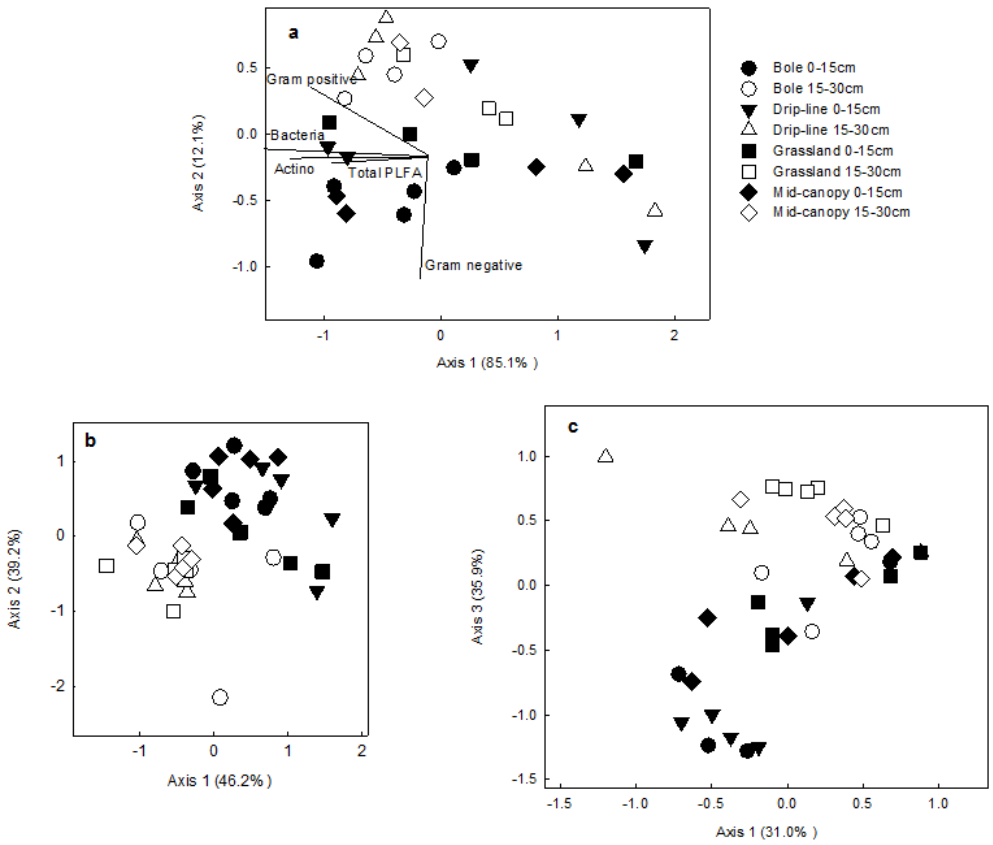


Figure 2.1 NMS ordination of: (a) phospholipid fatty acid profiles (PLFAs), (b) bacterial communities (T-RFLP of 16S rRNA gene), and (c) fungal communities (LH-PCR of ITS region) of soil samples taken at the Texas AgriLife, La Copita Research Area in Texas. Samples were taken at two depths, 0-15 cm (closed symbols) and 15-30 cm (open symbols). Samples were collected from four transect locations: the bole (adjacent to *Prosposis glandulosa*, mid-canopy, drip line and grassland) from five tree clusters. Percent variance explained on the axes appear in parentheses. Differences ($p<0.05$) in transect position (fungal ITS) and woody cluster (bacterial 16S) are indicated by different superscript letters.

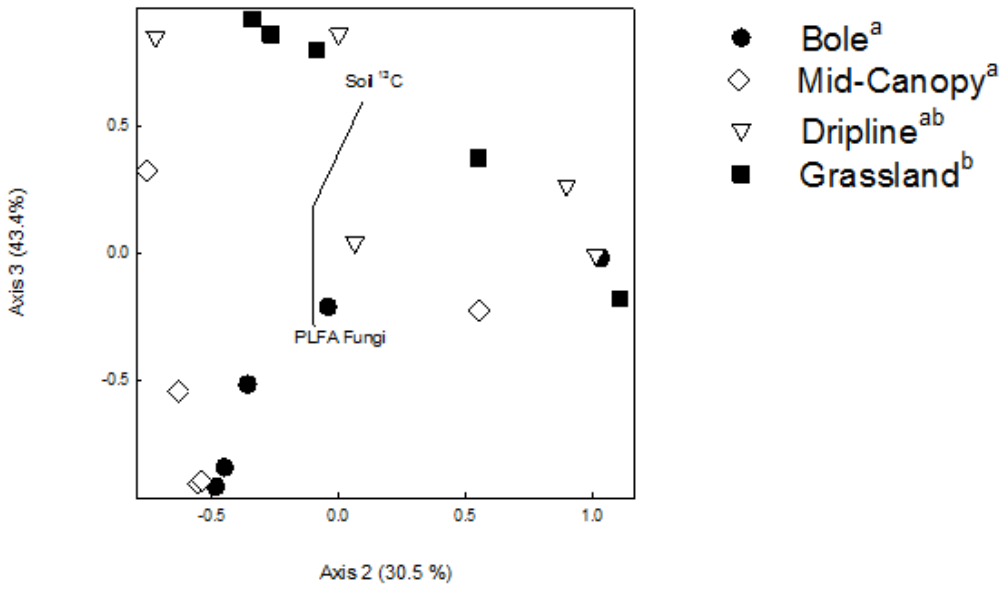
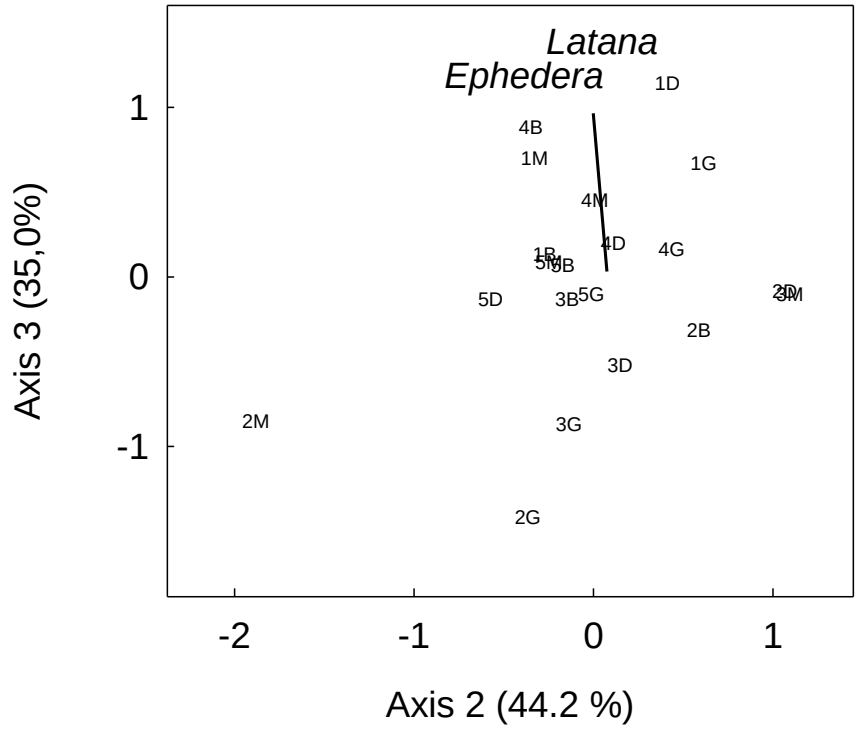


Figure 2.2 NMS ordination of fungal communities (LH-PCR of ITS region) at 0-15 cm depth of soil samples taken at the Texas AgriLife, La Copita Research Area in Texas. Samples were collected from four transect locations: the bole (adjacent to primary *Prosposis glandulosa*, mid-canopy, drip line and grassland) from five tree clusters at the station. Percent variance explained for two of the three axes appear in parentheses. Differences ($p<0.05$) in transect position are indicated by different superscript letters.



1B	Bole C1	1D	Drip line C1	1M	Mid-Canopy C1	1G	Grassland C1
2B	Bole C2	2D	Drip line C2	2M	Mid-Canopy C2	2G	Grassland C2
3B	Bole C3	3D	Drip line C3	3M	Mid-Canopy C3	3G	Grassland C3
4B	Bole C4	4D	Drip line C4	4M	Mid-Canopy C4	4G	Grassland C4
5B	Bole C5	5D	Drip line C5	5M	Mid-Canopy C5	5G	Grassland C5

Figure 2.3 NMS ordination of bacterial communities (T-RFLP of 16S rRNA gene) at 0-15 cm depth of soil samples taken at the Texas AgriLife, La Copita Research Area in Texas. Samples were collected from four transect locations: the bole (B) (adjacent to primary *Prosposis glandulosa*), mid-canopy (M), drip line (D) and grassland (G) from five tree clusters at the station. Clusters (C) are respresented as numbers Percent variances explained for the two of the three axes appear in parentheses. Differences ($p<0.05$) in clusters are indicated by different superscript letters.

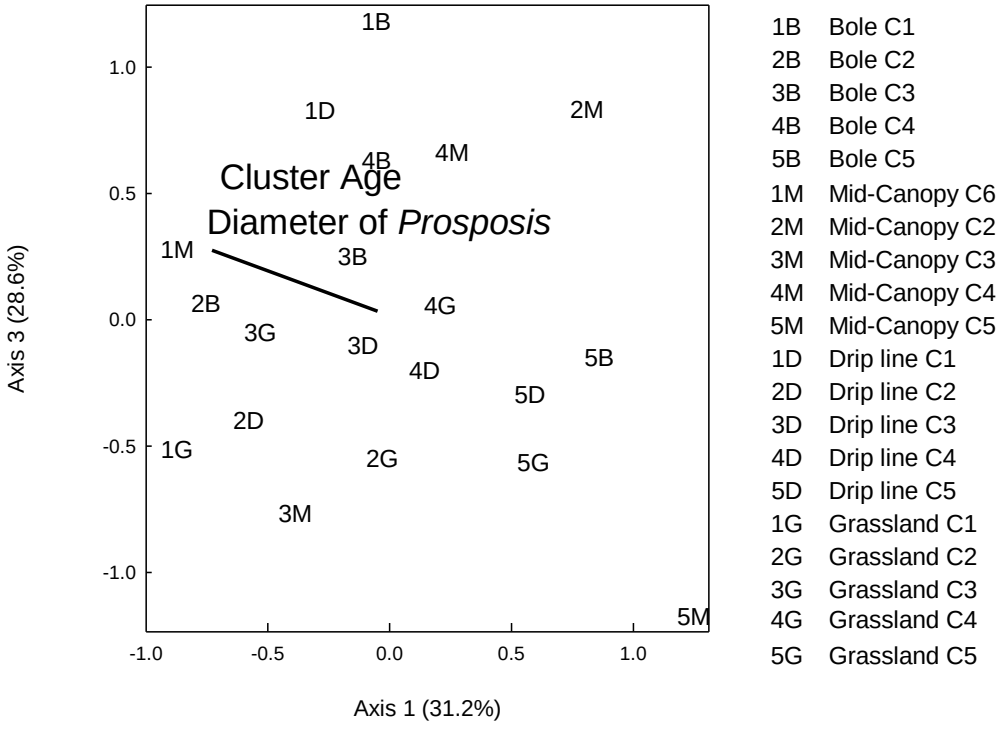


Figure 2.4 NMS ordination of fungal communities (LH-PCR of ITS region) at 15-30 cm depth of soil samples taken at the Texas AgriLife, La Copita Research Area in Texas. Samples were collected from four transect locations: the bole (B) (adjacent to primary *Prosposis glandulosa*), mid-canopy (M), drip line (D) and grassland (G) from five tree clusters at the station. Clusters (C) are represented as numbers. Percent variances explained for two axis of three axes appear in parentheses. Differences ($p<0.05$) in clusters are indicated by different superscript letters.

Chapter 3: **Impacts of Long-Term Organic Matter Manipulations on Nitrogen Cycling in an Old-Growth Forest**

Elizabeth A. Brewer

Abstract

The Detritus Inputs and Removal Treatments (DIRT) were established at the H.J. Andrews Experimental Forest in Oregon to examine the effects of organic matter manipulations on soil organic matter chemistry and nutrient cycling. In 2007, after ten years of manipulations, isotope dilution methods were used to estimate gross N mineralization and nitrification rates among the six treatments that vary in the amount and quality of organic matter (control, double wood, double litter, no roots, no litter, and no inputs). We hypothesized that long-term soil organic matter manipulations would have altered the rate and dynamics of microbial N turnover, with organic matter exclusion treatments having greater nitrification and organic matter input treatments having greater N immobilization. Respiration was lower from organic matter exclusion plots relative to the control, double litter and double wood treatments. Ammonium production and consumption as well as NO_3^- production did not significantly differ among any of the treatments. A greater rate of NH_4^+ consumption relative to NO_3^- production suggested immobilization was the primary pathway for NH_4^+ consumption in all but the double wood treatment. Further support for microbial assimilation came from ^{15}N remaining in soils after extraction of exchangeable N. Rates of NH_4^+ consumption were greater than NO_3^- consumption suggesting preferential microbial usage of NH_4^+. My results suggested that NH_4^+ was a significant N source for microbial assimilation and NO_3^- was also important N source in

woody debris addition and potentially control treatments. Following 10 years of altering soil organic matter inputs, the microbial communities of these systems remain N limited, although less so in the organic matter exclusion plots.

Introduction

Carbon and N cycles are complexly intertwined and numerous papers (e.g., Booth et al., 2005; Geisseler et al., 2009; Hart et al., 1994a; Melillo et al., 1989; Michalzik et al., 2001; Schimel and Weintraub, 2003) have been devoted to understanding the interactions between the two cycles. Changes in soil organic matter inputs from either additions or removals caused by natural disturbances or management have been used to enhance our understanding of these two cycles and their interactions. It has been observed that the form (e.g., rate, type of application, relative quality) of soil organic matter manipulations influence the rate and manner in which C and N are cycled by microbial communities (e.g., Compton and Boone, 2000; Flavel and Murphy, 2006; Hart and Firestone, 1991; Holub et al., 2005; Park and Matzner, 2003; Smolander et al., 2008; Zeller et al., 2008).

The lability of soil organic matter, as well as the size and composition of the microbial community, determines the relative magnitude of rates in the prevalent N pathways (e.g., immobilization vs. nitrification). Gross N mineralization has been found to be positively correlated with soil microbial

biomass, in addition to soil C and N content (Barrett and Burke, 2000; Bengtsson et al., 2003; Booth et al., 2005) and negatively correlated with increasing C:N ratios (Booth et al., 2005). The relationship with soil C:N ratios relates to organic matter inputs, as deciduous forests have faster gross N mineralization rates than coniferous forests, with comparably higher C:N inputs (Booth et al., 2005).

Gross immobilization of NH_4^+ has been found to correlate with C respiration (Barrett and Burke, 2000; Bengtsson et al., 2003; Flavel and Murphy, 2006; Hart et al., 1994a; Luxhoi et al., 2006). Ammonium and NO_3^- assimilation have been found to relate to soil C content as this often determines microbial N needs (Booth et al., 2005; Hart et al., 1994a; Hart et al., 1994b). Nitrification has been found to be dependent on NH_4^+ pool sizes as well as N needs of the microbial population (Booth et al., 2005), as nitrifiers tend to be poor competitors for acquiring NH_4^+ (Roberston and Groffman, 2007)

Additions or removals of soil organic matter alter both C and N availability and have shown varying results on N dynamics. The removal of roots has resulted in increases of gross nitrification (Ross et al., 2001), while the removal of above-ground litter may not impact N cycling (Fisk and Fahey, 2001). The addition of organic matter inputs alters rates depending on the quality of addition. In some cases increases in N mineralization have been attributed to available C and growth of microbial biomass (Castells et al.,

2003; Stark et al., 2008). In cases where manipulations have shown little or no differences, it was hypothesized that large C or N pool size and relatively short times scales of the manipulations masked treatment effects (Fisk and Fahey, 2001; Holub et al., 2005).

The time required to detect differences at the microbial scale may not be the same as at the ecosystem level. Long-term manipulations of above- and below-ground inputs offer the opportunity to understand how vegetation inputs can influence C and N availability to soil microbial communities. In 1997, the Detritus Input and Removal Treatments (DIRT) experiment was established at the H.J. Andrews Experimental Forest (Andrews). Treatments included wood debris additions, litter additions, and exclusion of above- and below-ground inputs (Table 3.1). Previous studies at these sites have shown differences in quality of dissolved organic matter (Lajtha et al., 2005; Yano et al., 2005); C and N storage (Crow et al., 2009), microbial communities (Brant et al., 2006b; Kageyama, 2005) and shifts in respiration (Sulzman et al., 2005) among treatments. In 2002, Holub et al. (2005) examined N cycling dynamics in the plots and no treatment effects were found for net N mineralization, net nitrification, and gross NH_4^+ production and consumption. The lack of significant differences among treatments was hypothesized to be in part due to the relatively short duration of the manipulations.

In 2007, ten years after the experiment's establishment, we examined the influence of organic matter manipulations on microbial N dynamics. We expected that treatments with greater woody inputs, control and double wood, would have the greatest rates of N immobilization, with double wood being the highest, and have no nitrification. We also anticipated that organic matter exclusion plots (no litter, no roots and no inputs) would have relatively low rates of N immobilization relative to the control and would show evidence of nitrification. We hypothesized that double litter would have intermediate rates to the control and organic matter exclusion plots, primarily due to having a higher proportion of litter relative to wood debris.

Methods

Site Description

The DIRT plots at the Andrews' (OR, USA; 44°13′N, 122°13′W, 531 m elevation) are a part of inter-site network that manipulate above- and below-ground plant inputs to examine relationships between plants, SOM, and soil microbial communities (Nadelhoffer et al., 2005). Manipulations at the Andrews' plots have been ongoing since their establishment in 1997 and are located in an old-growth Douglas-fir (*Pseudotsuga menziesii*) – western hemlock (*Tsuga heterophylla*) stand. The soils are classified at coarse loamy mixed mesic Typic Hapulands (Dixon, 2003). At the nearby headquarters of the Andrews' mean annual temperature is 8.7°C and mean annual precipitation

is 2370 mm $year^{-1}$, with the majority of inputs occurring between September and May. Additional details regarding the soil and site management can be found at (Dixon, 2003; Keirstead, 2004; Sulzman et al., 2005).

Six litter input/exclusion treatments were established at the Andrews'. Each treatment (Table 3.1) was replicated three times and treatment plots were randomly located at the site. Plot sizes were approximately 10 m x 15 m; however some plots were slightly smaller due to obstacles. Litter exclusion plots had a 1-mm mesh screen on the surface to prevent any above-ground inputs. The litter from the surface of these plots was removed twice a year and added to the double litter plots. Root exclusion plots were trenched to a 1 m depth and root exclusion barriers were installed to prevent new root growth. Double wood plots receive woody debris additions at a rate approximately double to field rates (2.7 Mg ha^{-1} yr^{-1}) every other year (Holub et al., 2005). The additions contain both intact and decomposed woody debris at a ratio of 4:1 (Lajtha et al., 2005).

Sampling and Background Characteristics

In July 2007, 20 soil cores (2 cm diameter, 0-10 cm depth) were taken randomly from each treatment replication. The soil for each replicate plot was homogenized and sieved through a 4 mm mesh.

Gravimetric soil water contents were measured prior to incubations. Exchangeable NH_4^+, NO_3, soluble organic N and soluble organic C were

extracted with 0.05 M K_2SO_4 (20 dry grams: 50 ml). Extracts were shaken for 1 h and filtered through pre-rinsed Whatman #2 filter papers. Extract concentrations of NH_4^+ and NO_3^- were measured by automated colorimetry (Astoria-Pacific 300 series autoanalyzer, Clackamas, OR). Soluble N and C were measured using combustion oxidation (TOC-V CSH with a TNM-1 module, Tokyo, Japan). Soluble organic N was calculated as the difference between total N and the sum of NH_4^+ NO_3^-. Extracted soils were air-dried and ground in a roller mill for C and N analysis (Roboprep elemental analyzer).

Isotope Dilution (Gross N -Mineralization and Nitrification)

Two sets of incubations were set-up in the laboratory, one for gross N mineralization and one for gross nitrification. Gross NH_4^+ production and consumption were estimated using 99 atom % $(^{15}NH_4)_2SO_4$; gross nitrification and NO_3^- consumption were estimated using 99 atom % $K^{15}NO_3$. The ^{15}N label was evenly added to the equivalent of 80 g of dry soil from each field replicate. A solution of water and the respective form ^{15}N was added to the field moist soil at a rate of 2 ug N g^{-1} dry soil. The label addition increased gravimetric soil water content by 5%. The equivalent of 20 g of dry soil was weighed into Mason jars, which were sealed with lids containing septa. There were four jars per treatment: two laboratory replicates for Time 0 (destructively sampled after 2 h) and two laboratory replicates for Time 24 (destructively sampled after 26 h). Respiration (CO_2 evolved in the headspace) was measured on the $^{15}NO_3^-$

Time 24 incubations just prior to the Time 0 and Time 24 harvests using gas chromatography (HACH CARLE Series 100, Loveland, CO).

Exchangeable N pools were extracted with 50 ml of 0.05 *M* K_2SO_4 and measured as described above. Soils on the filters were rinsed with three, 25-ml aliquots of 0.05 *M* K_2SO_4, air-dried and ground in a roller mill for ^{15}N analysis. Sequential diffusion of NH_4^+ and NO_3^- was used to prepare soil extracts for ^{15}N analysis (Brooks et al., 1989; Hart et al., 1994b). A Europa 20-20 mass spectrometer (PDZ Europa, England) interfaced with a Roboprep elemental analyzer was used for all ^{15}N analyses.

The equations of Kirkham and Bartholomew (1954) were used to calculate gross rates of production and consumption. In cases where there no significant changes in NH_4^+ or NO_3^- pool sizes between Time 0 and Time 24, the mean concentration of the respective inorganic N pool was used in the equation for equal rates of production and consumption. In instances where pool sizes did change significantly, the mean of the two lab replications from each time point were used in the unequal rate equations.

Nitrification Potentials

In August 2009, we sampled control, double wood and no input treatments. Five soil cores (2 cm diameter) from each treatment replication were taken to a depth of 10 cm and sieved to 4 mm and homogenized. Nitrification potentials were run in at 30°C in 30 mmol TES buffer with 1

mmol of NH_4^+ with an initial pH of 7.2 (Schmidt et al., 1994; Taylor et al., *In Press*). Three lab replicates and one acetylene control, to block autotrophic ammonia oxidation, were set-up and measured for each field replicate. Accumulations of NO_2^- and NO_3^- were measured at 24 and 48 h. Methods used to determine NO_2^- and NO_3^- are described by Taylor et al. (*In Press*)

Statistical Analyses

Data was examined using a PROC GLM ANOVA in SAS Software (SAS Institute Inc., Cary, NC). Due to non-constant variances, CO_2 respiration rates, ratios of CO_2 respired to N mineralized, and NO_3^- production rates were natural ln transformed. Transformations were unable to resolve non-constant variances of gross rates of NH_4^+ production/consumption; therefore the data were not transformed. All results are presented on original (non-transformed) scales.

When an ANOVA provided a significant p value (<0.05), multiple comparisons using the Tukey-Kramer procedure were used. Furthermore, because of high variability we used planned comparisons, using orthogonal contrasts, examining the effects of litter type removal and litter type addition were done.

Results

Site Description

The DIRT plots at the Andrews (OR, USA; 44°13′N, 122°13′W, 531 m elevation) are a part of inter-site network that manipulates above- and below-ground plant inputs to examine relationships detrital inputs and soil organic matter quality and quantity (Nadelhoffer et al., 2005). Manipulations at the Andrews plots have been ongoing since their establishment in 1997 and are located in an old-growth Douglas-fir (*Pseudotsuga menziesii*) – western hemlock (*Tsuga heterophylla*) stand. The soils are classified at coarse loamy mixed mesic Typic Hapulands (Dixon, 2003). At the nearby headquarters of the Andrews mean annual temperature is 8.7°C and mean annual precipitation is 2370 mm $year^{-1}$, with the majority of inputs occurring between September and May. Additional details regarding the soil and site management can be found at Dixon (2003) , Keirstead (2004) and Sulzman (2005).

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additions, as wood chips, at a rate approximately double to field rates (2.7 Mg ha^{-1} yr^{-1}) every other year (Holub et al., 2005). The additions contain both intact and decomposed woody debris at a ratio of 4:1 (Lajtha et al., 2005).

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Gravimetric soil water contents were measured prior to incubations by drying 10 g of field moist soil at 105°C for 24 hours. Exchangeable NH_4^+, NO_3, soluble organic N and soluble organic C were extracted with 0.05 M K_2SO_4 (20 dry grams: 50 ml). Extracts were shaken for 1 h and filtered through pre-rinsed (5 mL of 0.05 M K_2SO_4) Whatman #2 filter papers. Extract concentrations of NH_4^+ and NO_3^- were measured by automated colorimetry (Astoria-Pacific 300 series autoanalyzer, Clackamas, OR). Soluble N and C were measured using combustion oxidation (Shimadzu TOC-V CSH with a TNM-1 module, Tokyo, Japan). Soluble organic N was calculated as the difference between total N and the sum of NH_4^+ and NO_3^-. Extracted soils were air-dried and ground in a roller mill for C and N analysis (Roboprep elemental analyzer).

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Statistical Analyses

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When an ANOVA provided a significant p value (<0.05), multiple comparisons using the Tukey-Kramer procedure were used. Furthermore, because of high variability, we used planned comparisons, using orthogonal contrasts to examine the effects of litter type removal and litter type addition.

Discussion

Within the variability of the data, the rates of NH_4^+ production and consumption that we observed were comparable to those observed five years earlier by Holub et al. (2005). When individual treatments are examined, rates measured after 10 years of organic matter manipulation tended to be lower than those measured by Holub et al. (2005) in all but the control and no input treatments. Rates of the no litter, no roots, and double litter were about one-half, and the double wood treatment about one-fourth as great as measured five years earlier. The lower NH_4^+ rates were not unexpected for organic matter exclusion plots as they have received less organic matter inputs, thus likely

have smaller pools of organic N. It is unclear why double wood and double litter rates decreased when they there would potentially be a supply of organic N.

Increased microbial assimilation of NH_4^+ has been linked to SOM content and quality (e.g., C:N ratios) (Barrett et al., 2000; Accoe et al., 2004; Luxhoi et al., 2006). Respiration, directly related to SOM labiality, has also been found to direct correlation with greater rates of NH_4^+ immobilization and mineralization (Hart et al., 1994a; Bengtsson et al., 2003; Arnold et al., 2009). The Andrew's DIRT plots did not show a significant correlation with the amount of C respired to NH_4^+ mineralized (Figure 3.5b), but there was a marginal correlation with the NH_4^+ immobilized (Figure 3.5a). The wide range of C:N ratios found within a treatment may result in a lack of correlation (Luxhoi et al., 2006). The data did show a general trend for more C to be respired relative to N mineralized with increasing amount of organic matter additions. Ratios of C respired to N mineralized (Figure 3.5a) were six-fold greater than Hart et al. 1994, who also worked in an old-growth forest at the Andrews'; however, this could be due differences C content and organic matter qualities associated with each treatment, as previous studies have found non-linear relationship between N concentration and decomposition (Berg, 2000), and differences in incubation pre-treatment.

Comparisons of NH_4^+ consumption and NO_3^- production were used to examine whether there was a prevalence of N immobilization or nitrification in each treatment (Figure 3.4). If NH_4^+ consumption exceeds NO_3^- production it can be assumed that NH_4^+ consumed was via immobilization. If NH_4^+ consumption equals NO_3^- production, than nitrification is the dominant pathway. Similar to other old-growth studies (Davidson et al., 1992; Perakis et al., 2001; Fisk et al., 2002), there was a trend for the old-growth soil to have greater NH_4^+ assimilation and little nitrification. Double litter showed a similar trend as the control. In organic matter exclusion treatments, the amount of NH_4^+ consumed was greater than the amount of NO_3^- being produced suggesting microbial assimilation of N rather than nitrification. In double wood, NO_3^- production approached NH_4^+ consumption suggesting nitrification was occurring in this treatment.

Nitrification in double wood was counter to our original hypothesis in which NH_4^+ immobilization would be prevalent. Nitrification potentials revealed nitrification in one of the three double wood field replications. Woody forests have been associated with heterotrophic nitrification (Schimel et al., 1984; Barraclough et al., 1995); however, the lack of nitrification potential in the presence of acetylene and similar values of NH_4^+consumption and NO_3^- production eliminated the possibility of heterotrophic nitrification. The higher rates of NO_3^- consumption in the double wood and control plots (Figure 3.3b)

were not as surprising. In old-growth or mature forests where N is limited, NO_3^- may be an important for N source for microbial assimilation (Davidson et al., 1992; Hart et al., 1994a; Myrold et al., 2007) and the assimilation of NO_3^- correlates to C content (Booth et al., 2005).

The lack of significant nitrification in the organic matter removal plots (Figure 3.3a) was unexpected as Lajtha et al. (2005) found large amounts of NO_3^- in lysimeter water suggesting active nitrification and NO_3^- leaching. A decrease in N mineralization in response to girdling/root removal (Zeller et al., 2008) and lack of gross nitrification in response to litter removal (Fisk et al., 2001) have been observed previously in other forests. Measurement of nitrification under optimal conditions in the no input plots revealed nitrification at two of the three field plots under optimal conditions (Table 3.3). These nitrification potentials were relatively low to other ecosystems (Griffiths et al., 1998; Mintie et al., 2003) but comparable to other Douglas-fir systems of the Pacific Northwest (Griffiths et al., 1998).

The lack of expected nitrification in these plots may be a result of disturbance associated with the soil preparation (e.g., sieving, moisture addition) (Booth et al., 2006; Arnold et al., 2008); however, other studies have found increases in nitrification with disturbance (Booth et al., 2006; Kaur et al.). It is possible that intact soil core gross measurements may yield different results. Conversely our results of low nitrification may be justifiable for micro-scale processes and

these results could potentially correlate to macro-scale observations when scaled up.

A potential scenario may be that the microbial communities of the organic matter exclusion plots have acclimated to the reduced C inputs, in addition to reduction of other nutrients associated with vegetation inputs. Suggestions of decreased microbial biomass (Brant et al., 2006b) (Figure 3.6a,b), changes in microbial community members (Kageyama, 2005) may have shifted the once relatively N-rich systems to N-limited systems. This is supported by the higher rates of immobilization compared to nitrification (Figure 3.4). The lack of microbial assimilation of NO_3^- (NO_3^- consumption) (Figure 3.3b) would then suggest that the microbial community may be short on electron donors/C. Lower C respiration (Figure 3.1), (Sulzman et al., 2005) and differences in C substrate utilization (Brant et al., 2006a) support C limitation in these treatments.

Alternatively, lysimeters may result in preferential flow paths (Asano et al., 2006) leading to collections greater than the soil column immediately surrounding it, thus leading to NO_3^- concentration differences. Lysimeter data from years 5-10 of the experiment have not been completely synthesized (Lajtha et al., 2005), but will provide additional insights on the N dynamics of these systems.

The amount of ^{15}N remaining in soil post extraction can be considered a surrogate for a measurement of immobilization, as the microbial biomass will remain in the soil following exchangeable N extraction. In $^{15}NH_4^+$ incubations no litter, no roots and no inputs treatments all had significantly less ^{15}N remaining than control (Figure 3.6a). In $^{15}NO_3^-$ incubations the ^{15}N in the soil was lower in the no litter and no inputs compared to the double wood (Figure 3.6b). Lower ^{15}N remaining in organic matter exclusion soils may be indicative of lower microbial biomass. There has been a trend of lower microbial biomass in organic matter exclusion treatments, based on PLFAs at the Andrews' and other DIRT sites (Brant et al., 2006b).

There is some support for our biomass hypothesis as there was a strong trend of increasing ^{15}N retention with time (Figure 3.6c,d). Comparison of changes in ^{15}N retention and the calculated rates of which $^{15}NH_4^+$ and NO_3^- are consumed do show correlation. Nitrate consumption in the control and double wood were comparable to the differences in the amount of ^{15}N retained at Time 0 and 24. Ammonium consumption did not correlate as well, suggesting that there may be differences in sorption/retentions of NH_4^+ due to differences in organic matter and clay content differences (Fitch et al., 1986; Drury et al., 1991; Trehan, 1996).

Mass balances of both incubation sets reveal a mass recovery ranging 67-84% recovery of the added label. The ^{15}N content of the soluble organic N

pool was not analyzed and thus the missing mass could be retained in this pool. Based on soluble organic N pool sizes (Table 2), the unaccounted for ^{15}N (0.5 to 0.6 mg ^{15}N kg^{-1} soil) would represent 23-38% of the pool of the soluble organic N pool in the $^{15}NH_4^+$ experiment. For the $^{15}NO_3^-$ experiment the unaccounted for ^{15}N (0.3 to 0.6 ^{15}N kg^{-1} soil) would comprise 10-25% of the pool. Thus, in both experiments, the soluble organic N pools could reasonably account for the missing ^{15}N, which may represent^{15}N that had been incorporated into cell constituents subsequently released upon microbial turnover or excreted as exoenzymes. These pools may turn over quite quickly (Cookson et al., 2004; Jones et al., 2007) and have significant contributions to N turnover in this relatively N poor system (Schimel et al., 2004; Cookson et al., 2007).

In conclusion, we have found that organic matter manipulations have not statistically altered the NH_4^+ production and consumption dynamics even after 10 years; however, differences in C respiration were apparent. Nitrification was relatively small across all treatments but added $^{15}NO_3^-$ was an N source for microbial communities in treatments with woody inputs. The low level of gross and potential nitrification, as well as ^{15}N soil retention from the organic matter exclusion plots, suggests that the microbial communities are still limited by N. Further research on the role of soluble organic N and its source (e.g. above versus below-ground) in this N-limited system, would be

beneficial in understanding it contributions to microbial N cycling The high spatial variability, in addition to the hundreds years of organic matter inputs that Pacific Northwest old-growth forests have received should be kept in mind and likely contribute to the difficulty in detecting differences as result of manipulations at relatively short time scales. Within the variability of the data, the rates of NH_4^+ production and consumption that we observed were comparable to those observed five years earlier by Holub et al. (2005). When individual treatments are examined, rates measured after 10 years of organic matter manipulation tended to be lower than those measured by Holub et al. (2005) in all but the control and no input treatments. Rates of the no litter, no roots, and double litter were about one-half, and the double wood treatment about one-fourth as great as measured five years earlier. The lower NH_4^+ rates were not unexpected for organic matter exclusion plots as they have received less organic matter inputs, thus likely have smaller pools of labile organic N to serve as substrate for ammonification. It is unclear why double wood and double litter rates decreased when they there would potentially be a supply of organic N.

Increased microbial assimilation of NH_4^+ has been linked to SOM content and quality (e.g., C:N ratios) (Accoe et al., 2004; Barrett and Burke, 2000; Luxhoi et al., 2006). Respiration, directly related to SOM lability, has also been found to correlate directly with greater rates of NH_4^+ immobilization

and mineralization (Arnold et al., 2009; Bengtsson et al., 2003; Hart et al., 1994a). The Andrews' DIRT plots did not show a significant correlation with the amount of C respired to NH_4^+ mineralized (Figure 3.5b), but there was a marginal correlation with the NH_4^+ immobilized (Figure 3.5a). The wide range of C:N ratios found within a treatment may result in a lack of correlation (Luxhoi et al., 2006). The data did show a general trend for more C to be respired relative to N mineralized with increasing amount of organic matter additions. Ratios of C respired to N mineralized (Figure 3.5a) were six-fold greater than Hart et al. 1994, who also worked in an old-growth forest at the Andrews; however, this could be due to differences in C content and organic matter qualities associated with each treatment, as previous studies have found non-linear relationship between N concentration and decomposition (Berg, 2000), and differences in incubation pre-treatment.

Comparisons of NH_4^+ consumption and NO_3^- production were used to examine whether there was a prevalence of N immobilization or nitrification in each treatment (Figure 3.4). If NH_4^+ consumption exceeds NO_3^- production it can be assumed that NH_4^+ consumed was via immobilization. If NH_4^+ consumption equals NO_3^- production, then nitrification is the dominant pathway. Similar to other old-growth studies (Davidson et al., 1992; Fisk et al., 2002; Perakis and Hedin, 2001), there was a trend for the old-growth soil to have greater NH_4^+ assimilation and little nitrification. Double litter showed a

trend similar to that of the control. In organic matter exclusion treatments, the amount of NH_4^+ consumed was greater than the amount of NO_3^- being produced suggesting microbial assimilation of N rather than nitrification. In double wood, NO_3^- production approached NH_4^+ consumption suggesting nitrification was occurring in this treatment.

Nitrification in double wood was counter to our original hypothesis in which NH_4^+ immobilization would be prevalent. Nitrification potentials revealed nitrification in one of the three double wood field replications. Other forests have been associated with heterotrophic nitrification (Barraclough and Puri, 1995; Schimel et al., 1984); however, the lack of nitrification potential in the presence of acetylene and similar values of NH_4^+consumption and NO_3^- production eliminated the possibility of heterotrophic nitrification. The higher rates of NO_3^- consumption in the double wood and control plots (Figure 3.3b) were not as surprising. In old-growth or mature forests where N is limited, NO_3^- may be an important N source for microbial assimilation (Davidson et al., 1992; Hart et al., 1994a; Myrold and Posavatz, 2007) and the assimilation of NO_3^- correlates to C content (Booth et al., 2005).

The lack of significant nitrification in the organic matter removal plots (Figure 3.3a) was unexpected as Lajtha et al. (2005) found large amounts of NO_3^- in lysimeter water (30 cm and 100 cm depth) suggesting active nitrification and NO_3^- leaching. A decrease in N mineralization in response to

girdling/root removal (Zeller et al., 2008) and lack of gross nitrification in response to litter removal (Fisk and Fahey, 2001) have been observed previously in other forests. Measurement of nitrification under optimal conditions in the no input plots revealed nitrification at two of the three field plots under optimal conditions (Table 3.3). These nitrification potentials were low relative to other ecosystems (Griffiths et al., 1998; Mintie et al., 2003), but comparable to other Douglas-fir systems of the Pacific Northwest (Griffiths et al., 1998).

The lack of expected nitrification in these plots may be a result of disturbance associated with the soil preparation (e.g., sieving, moisture addition) (Arnold et al., 2008; Booth et al., 2006); however, other studies have found increases in nitrification with disturbance (Booth et al., 2006; Kaur et al., 2010). It is possible that intact soil core gross measurements may yield different results. Conversely, our results of low nitrification may be justifiable for micro-scale processes and these results could potentially correlate to macro-scale observations when scaled up.

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have shifted the once relatively N-rich systems to N-limited systems. This is supported by the higher rates of immobilization compared to nitrification (Figure 3.4). The lack of microbial assimilation of NO_3^- (NO_3^- consumption) (Figure 3.3b) would then suggest that the microbial community may be C limited. Lower C respiration (Figure 3.1) (Sulzman et al., 2005) and differences in C substrate utilization (Brant et al., 2006a) in organic matter exclusion plots support C limitation in these treatments.

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lower microbial biomass in organic matter exclusion treatments, based on PLFAs at the Andrews' and other DIRT sites (Brant et al., 2006b).

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In conclusion, we have found that organic matter manipulations have not statistically altered the NH_4^+ production and consumption dynamics even after 10 years; however, differences in C respiration were apparent. Nitrification was relatively small across all treatments, but added $^{15}NO_3^-$ was an N source for microbial communities in treatments with woody inputs. The low level of gross and potential nitrification, as well as ^{15}N soil retention from the organic matter exclusion plots, suggests that the microbial communities are still limited by N. Further research on the role of soluble organic N and its source (e.g. above versus below-ground) in this N-limited system, would be beneficial in understanding it contributions to microbial N cycling. The high spatial variability, in addition to the hundreds of years of organic matter inputs that Pacific Northwest old-growth forests have received should be kept in mind and likely contribute to the difficulty in detecting differences as result of manipulations of relatively short time scales.

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Literature Cited

Accoe, F., P. Boeckx, J. Busschaert, G. Hofman, and O. Van Cleemput. 2004. Gross N transformation rates and net N mineralisation rates related to the C and N contents of soil organic matter fractions in grassland soils of different age. Soil Biology & Biochemistry 36:2075-2087.

Arnold, J., M.D. Corre, and E. Veldkamp. 2008. Cold storage and laboratory incubation of intact soil cores do not reflect in-situ nitrogen cycling rates of tropical forest soils. Soil Biology & Biochemistry 40:2480-2483.

Arnold, J., M.D. Corre, and E. Veldkamp. 2009. Soil N cycling in old-growth forests across an Andosol toposequence in Ecuador. Forest Ecology and Management 257:2079-2087.

Asano, Y., J.E. Compton, and M.R. Church. 2006. Hydrologic flowpaths influence inorganic and organic nutrient leaching in a forest soil. Biogeochemistry 81:191-204.

Barraclough, D., and G. Puri. 1995. The use of N-15 pool dilution and enrichment to separate the heterotrophic and autotrophic pathways of nitrification. Soil Biology & Biochemistry 27:17-22.

Barrett, J.E., and I.C. Burke. 2000. Potential nitrogen immobilization in grassland soils across a soil organic matter gradient. Soil Biology & Biochemistry 32:1707-1716.

Bengtsson, G., P. Bengtson, and K.F. Mansson. 2003. Gross nitrogen mineralization-, immobilization-, and nitrification rates as a function of soil C/N ratio and microbial activity. Soil Biology & Biochemistry 35:143-154.

Berg, B. 2000. Litter decomposition and organic matter turnover in northern forest soils. Forest Ecology and Management 133:13-22.

Booth, M.S., J.M. Stark, and E. Rastetter. 2005. Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data. Ecological Monographs 75:139-157.

Booth, M.S., J.M. Stark, and S.C. Hart. 2006. Soil-mixing effects on inorganic nitrogen production and consumption in forest and shrubland soils. Plant and Soil 289:5-15.

Brant, J.B., E.W. Sulzman, and D.D. Myrold. 2006a. Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation. Soil Biology & Biochemistry 38:2219-2232.

Brant, J.B., D.D. Myrold, and E.W. Sulzman. 2006b. Root controls on soil microbial community structure in forest soils. Oecologia 148:650-659.

Brooks, P.D., J.M. Stark, B.B. McInteer, and T. Preston. 1989. Diffusion method to prepare soil extracts for automated N-15 analysis. Soil Science Society of America Journal 53:1707-1711.

Castells, E., J. Penuelas, and D.W. Valentine. 2003. Influence of the phenolic compound bearing species Ledum palustre on soil N cycling in a boreal hardwood forest. Plant and Soil 251:155-166.

Compton, J.E., and R.D. Boone. 2000. Long-term impacts of agriculture on soil carbon and nitrogen in New England forests. Ecology 81:2314-2330.

Cookson, W.R., and D.V. Murphy. 2004. Quantifying the contribution of dissolved organic matter to soil nitrogen cycling using N-15 isotopic pool dilution. Soil Biology & Biochemistry 36:2097-2100.

Cookson, W.R., M. Osman, P. Marschner, D.A. Abaye, I. Clark, D.V. Murphy, E.A. Stockdale, and C.A. Watson. 2007. Controls on soil nitrogen cycling and microbial community composition across land use and incubation temperature. Soil Biology & Biochemistry 39:744-756.

Crow, S.E., K. Lajtha, T.R. Filley, C.W. Swanston, R.D. Bowden, and B.A. Caldwell. 2009. Sources of plant-derived carbon and stability of organic matter in soil: implications for global change. Global Change Biology 15:2003-2019.

Davidson, E.A., S.C. Hart, and M.K. Firestone. 1992. Internal cycling of nitrate in soils of a mature coniferous forest. Ecology 73:1148-1156.

Dixon, J.J. 2003. Applying GIS to soil-geomorphic landscape mapping in the Lookout Creek Valley, western Cascades, Oregon. M.S. thesis Oregon State University, Corvallis.

Drury, C.F., and E.G. Beauchamp. 1991. Ammonium fixation, release, nitrification and immobilization in high- and low-fixing soils. Soil Science Society of America Journal 55:125-129.

Fisk, M.C., and T.J. Fahey. 2001. Microbial biomass and nitrogen cycling responses to fertilization and litter removal in young northern hardwood forests. Biogeochemistry 53:201-223.

Fisk, M.C., D.R. Zak, and T.R. Crow. 2002. Nitrogen storage and cycling in old- and second-growth northern hardwood forests. Ecology 83:73-87.

Fitch, A., F.J. Stevenson, and Y. Chen. 1986. Complexation of Cu(II) with a soil humic-acid- Reponse characterists of the Cu(II) ion-selective electrod and ligan concentration effects Organic Geochemistry 9:109-116.

Flavel, T.C., and D.V. Murphy. 2006. Carbon and nitrogen mineralization rates after application of organic amendments to soil. Journal of Environmental Quality 35:183-193.

Geisseler, D., W.R. Horwath, and T.A. Doane. 2009. Significance of organic nitrogen uptake from plant residues by soil microorganisms as affected by carbon and nitrogen availability. Soil Biology & Biochemistry 41:1281-1288.

Griffiths, R.P., P.S. Homann, and R. Riley. 1998. Denitrification enzyme activity of Douglas-fir and red alder forest soils of the Pacific Northwest. Soil Biology & Biochemistry 30:1147-1157.

Hart, S.C., and M.K. Firestone. 1991. Forest-floor mineral-soil interactions in the internal nitrogen-cycle of an old-growth forest. Biogeochemistry 12:103-127.

Hart, S.C., G.E. Nason, D.D. Myrold, and D.A. Perry. 1994a. Dynamics of gross nitrogen transformations in an old-growth forest: The carbon connection. Ecology 75:880-891.

Hart, S.C., J.M. Stark, E.A. Davidson, and M.K. Firestone. 1994b. Nitrogen mineralization, immobilization, and nitrification, *In* R. W. Weaver, et al., eds. Methods of soil analysis, Part 2. Microbiological and biochemical properties. Soil Science Society of America, Madison, WI.

Holub, S.M., K. Lajtha, J.D.H. Spears, J.A. Toth, S.E. Crow, B.A. Caldwell, M. Papp, and M.T. Nagy. 2005. Organic matter manipulations have little effect on gross

and net nitrogen transformations in two temperate forest mineral soils in the USA and central Europe. Forest Ecology and Management 214:320-330.

Jones, D.L., and D.V. Murphy. 2007. Microbial response time to sugar and amino acid additions to soil. Soil Biology and Biochemistry 39:2178-2182.

Kageyama, S.A. 2005. Effects of vegetation and disturbance on fungal communities in the western Cascades of Oregon. Ph.D. dissertation, Oregon State University, Corvallis.

Kaur, A.J., D.S. Ross, and G. Fredriksen. 2010. Effect of soil mixing on nitrification rates in soils of two deciduous forests of Vermont, USA. Plant and Soil 331:289-298.

Keirstead, H. 2004. Quantifying C and N contents and isotope signatures of SOM pools in the HJ Andrews DIRT plots. M.S. thesis, Oregon State University, Corvallis.

Kirkham, D., and W.V. Bartholomew. 1954. Equations for following nutrient transformations in soil, utilizing tracer data. Soil Science Society of America Proceedings 18:33-34.

Lajtha, K., S.E. Crow, Y. Yano, S.S. Kaushal, E. Sulzman, P. Sollins, and J.D.H. Spears. 2005. Detrital controls on soil solution N and dissolved organic matter in soils: a field experiment. Biogeochemistry 76:261-281.

Luxhoi, J., S. Bruun, B. Stenberg, T.A. Breland, and L.S. Jensen. 2006. Prediction of gross and net nitrogen mineralization-immobilization turnover from respiration. Soil Science Society of America Journal 70:1121-1128.

Melillo, J., J. Aber, A. Linkins, A. Ricca, B. Fry, and K. Nadelhoffer. 1989. Carbon and nitrogen dynamics along the decay continuum: Plant litter to soil organic matter. Plant and Soil 115:189-198.

Michalzik, B., K. Kalbitz, J.H. Park, S. Solinger, and E. Matzner. 2001. Fluxes and concentrations of dissolved organic carbon and nitrogen - a synthesis for temperate forests. Biogeochemistry 52:173-205.

Mintie, A.T., R.S. Heichen, K. Cromack, D.D. Myrold, and P.J. Bottomley. 2003. Ammonia-oxidizing bacteria along meadow-to-forest transects in the oregon cascade mountains. Applied and Environmental Microbiology 69:3129-3136.

Myrold, D.D., and N.R. Posavatz. 2007. Potential importance of bacteria and fungi in nitrate assimilation in soil. Soil Biology & Biochemistry 39:1737-1743.

Nadelhoffer, K.J., R.D. Boone, R.D. Bowden, J. Canary, J. Kaye, K. Lajtha, W. McDowell, P. Micks, and A. Ricca. 2005. The DIRT experiment: litter and root influences on forest soil organic matter stocks and function, *In* F. D and A. J, eds. Forests in time: the environmental consequences of 1000 years of change in New England. Yale University Press, New Haven, CT.

Park, J.-H., and E. Matzner. 2003. Controls on the release of dissolved organic carbon and nitrogen from a deciduous forest floor investigated by manipulations of aboveground litter inputs and water flux. Biogeochemistry 66:265-286.

Perakis, S.S., and L.O. Hedin. 2001. Fluxes and Fates of Nitrogen in Soil of an Unpolluted Old-Growth Temperate Forest, Southern Chile. Ecology 82:2245-2260.

Roberston, G.P., and P.M. Groffman. 2007. Nitrogen Transformations, p. 341-364, *In* E. A. Paul, ed. Soil Microbiology, Ecology, and Biochemistry, Third ed. Academic Press, Burlington.

Ross, D.J., N.A. Scott, K.R. Tate, N.J. Rodda, and J.A. Townsend. 2001. Root effects on soil carbon and nitrogen cycling in a *Pinus radiata* D. Don plantation on a coastal sand. Australian Journal of Soil Research 39:1027-1039.

Schimel, J.P., and M.N. Weintraub. 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biology & Biochemistry 35:549-563.

Schimel, J.P., and J. Bennett. 2004. Nitrogen mineralization: Challenges of a changing paradigm. Ecology 85:591-602.

Schimel, J.P., M.K. Firestone, and K.S. Killham. 1984. Identification of heterotrophic nitrification in a sierran forest soil. Applied and Environmental Microbiology 48:802-806.

Schmidt, E.L., and L.W. Bulterm. 1994. Autotrophic nitrifying bacteria, p. 159-177, *In* R. W. Weaver, et al., eds. Methods of Soil Analysis Part 2- Microbiological and biochemical properties. Soil Science Society of America, Madison, WI.

Smolander, A., T. Levula, and V. Kitunen. 2008. Response of litter decomposition and soil C and N transformations in a Norway spruce thinning stand to removal of logging residue. Forest Ecology and Management 256:1080-1086.

Stark, C.H., L.M. Condron, M. O'Callaghan, A. Stewart, and H.J. Di. 2008. Differences in soil enzyme activities, microbial community structure and short-term nitrogen mineralisation resulting from farm management history and organic matter amendments. Soil Biology & Biochemistry 40:1352-1363.

Sulzman, E.W., J.B. Brant, R.D. Bowden, and K. Lajtha. 2005. Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO_2 efflux in an old growth coniferous forest. Biogeochemistry 73:231-256.

Taylor, A.E., S. Dooley, L.H. Zeglin, D.D. Myrold, and P.J. Bottomley. *In Press*. Evidence for different contributions of archaea and bacteria to ammonia oxidizing potential of diverse Oregon soils Applied and Environmental Microbiology.

Trehan, S.P. 1996. Immobilisation of (NH_4^+)-N-15 in three soils by chemical and biological processes. Soil Biology & Biochemistry 28:1021-1027.

Yano, Y., K. Lajtha, P. Sollins, and B.A. Caldwell. 2005. Chemistry and dynamics of dissolved organic matter in a temperate coniferous forest on Andic soils: Effects of litter quality. Ecosystems 8:286-300.

Zeller, B., J.X. Liu, N. Buchmann, and A. Richter. 2008. Tree girdling increases soil N mineralisation in two spruce stands. Soil Biology & Biochemistry 40:1155-1166.

Table 3.1 Description of treatment manipulations of the H.J. Andrews Experimental Forest's Detritus Inputs and Removal Treatments (DIRT).

Treatment	Description
Control (CO)	No input or removal of organic matter
No Litter (NL)	Covered with screen to exclude aboveground litter inputs
No Roots (NR)	Trenched to 1 m depth and lined to exclude roots. Trees girdled to prevent new root growth.
No Inputs (NI)	Combined NL and NR manipulations
Double Litter (DL)	Non-woody aboveground litter doubled
Double Wood (DW)	Aboveground inputs of woody debris doubled

Table 3.2 Soil properties of the H.J. Andrews DIRT plots in 2007. Total C and Total N numbers are after 0.05 K_2SO_4 extraction. Treatment abbreviations can be found in Table 1. All values are means (n=3) with standard errors for a 0-10 cm soil depth.

Treatment	Soil moisture (g H_2O g^{-1} soil)	Soil organic C (g C kg^{-1} soil)	Soil total N (g N kg^{-1} soil)	Total soil C/N	NH_4^+ (mg N kg^{-1} soil)	NO_3^- (mg N kg^{-1} soil)	Soluble organic C (mg C kg^{-1} soil)	Soluble organic N (mg N kg^{-1} soil)	Soluble organic C/N
CO	0.49 ± 0.06	56.36 ± 13.69	2.22 ± 0.43	24.75 ± 1.71	0.73 ± 0.25	0.60 ± 0.08	35.11 ± 4.38	2.43 ± 0.24	14.65 ± 2.10
NL	0.43 ± 0.01	34.79 ± 0.62	1.72 ± 0.16	20.54 ± 1.56	0.91 ± 0.42	0.79 ± 0.20	24.60 ± 5.80	1.78 ± 0.47	14.47 ± 1.84
NR	0.42 ± 0.03	41.31 ± 1.59	1.96 ± 0.02	21.10 ± 0.86	0.57 ± 0.21	0.61 ± 0.09	31.23 ± 3.67	2.47 ± 0.28	12.64 ± 0.22
NI	0.44 ± 0.01	40.36 ± 4.71	1.82 ± 0.12	22.02 ± 1.19	0.87 ± 0.27	0.85 ± 0.31	25.44 ± 4.36	1.70 ± 0.39	15.53 ± 1.34
DL	0.42 ± 0.02	44.68 ± 5.94	2.07 ± 0.17	21.37 ± 1.05	0.39 ± 0.16	0.42 ± 0.03	53.52 ± 18.85	2.99 ± 0.40	17.21 ± 4.65
DW	0.48 ± 0.04	53.74 ± 4.04	2.19 ± 0.11	24.45 ± 0.92	0.39 ± 0.07	0.63 ± 0.11	54.91 ± 12.69	2.46 ± 0.11	22.30 ± 4.72

Table 3.3 Residence times of NH_4^+ and NO_3^- pools and nitrification potentials for the H.J. Andrews DIRT plots. Treatment abbreviations can be found in Table 1. Residence times were calculated by dividing the pool size by the respective gross rate. Data that was not measured is noted with NM. Data are means ± one standard error (n=3).

Treatment	**NH_4^+ (days)**	**NO_3^- (days)**	**Nitrification Potential (mg NO_3^--N kg^{-1} soil day^{-1})**
CO	0.70 ± 0.38	0.55 ± 0.19	0.00 ± 0.00
NL	1.51 ± 0.66	1.32 ± 0.29	NM
NR	0.76 ± 0.05	1.11 ± 0.50	NM
NI	1.12 ± 0.20	1.09 ± 0.28	0.23 ± 0.23
DL	0.40 ± 0.10	0.55 ± 0.18	NM
DW	4.00 ± 3.13	6.61 ± 5.37	0.76 ± 0.38

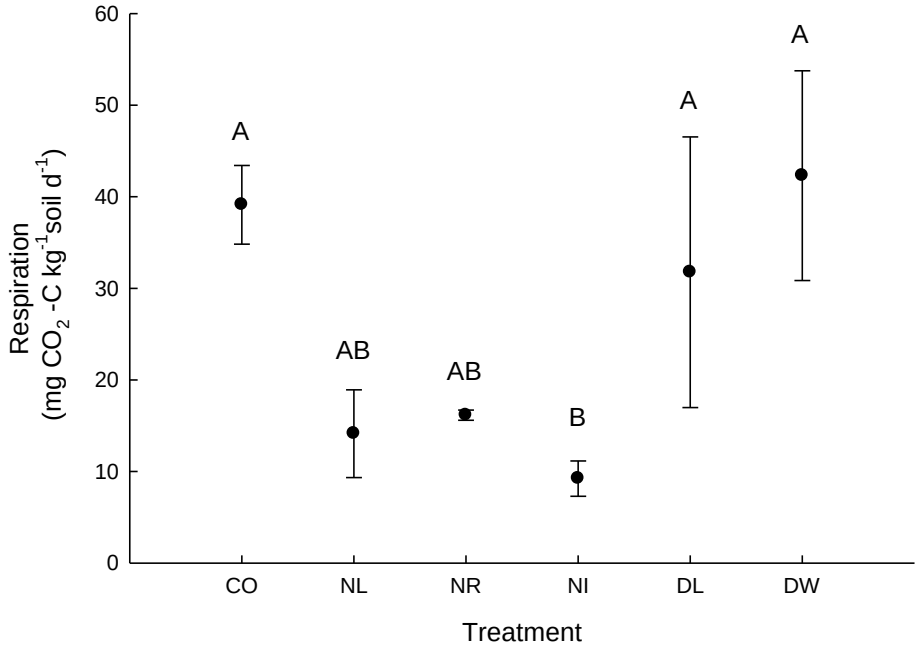

Figure 3.1 Rates of CO_2 respired during gross $^{15}NO_3^-$ mineralization incubations using the Andrews' DIRT soils. Treatment abbreviations can be found in Table 1. Points represent treatment means (n=3) with error bars representing one standard error. Different letters signifies differences among treatments using Tukey-Kramer procedure ($p<0.05$). Specific treatment contrasts are discussed in the text.

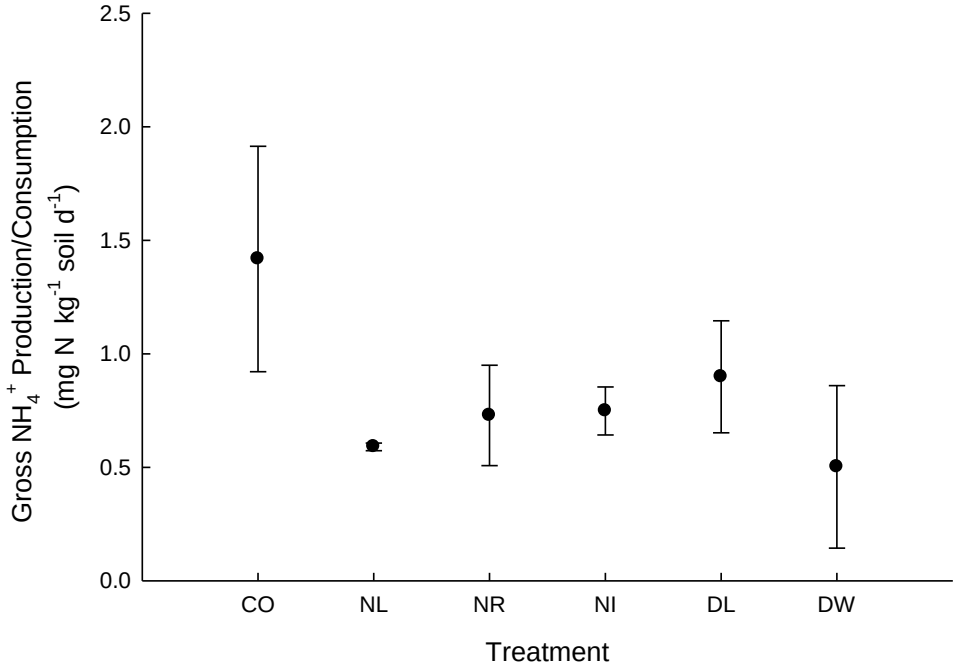

Figure 3.2 Gross NH_4^+ production and consumption rates for the Andrews' DIRT plots. Treatment abbreviations can be found in Table 1. Each point represents treatment means (n=3) with error bars representing one stand error.

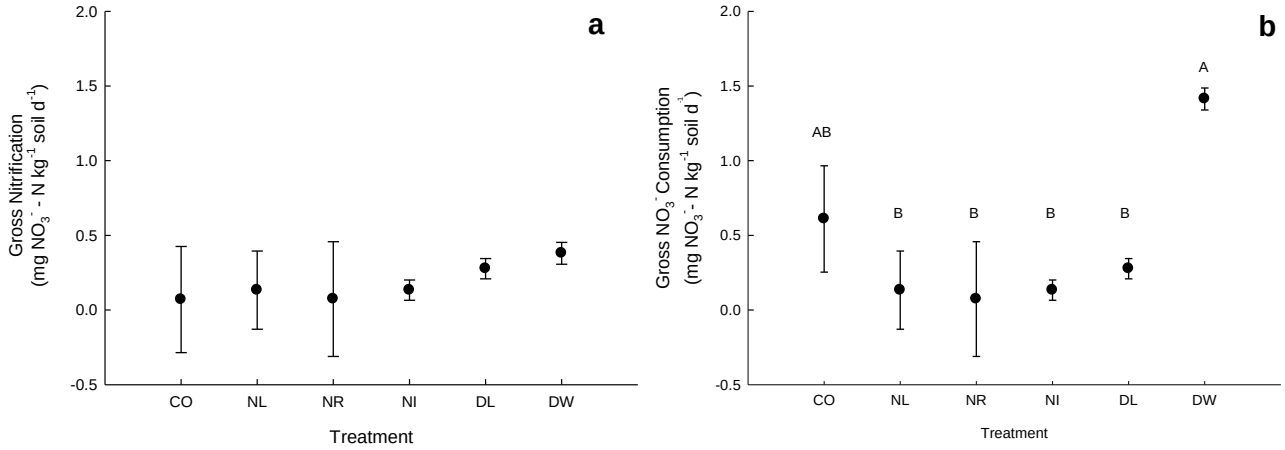

Figure 3.3 Gross nitrification (a) and gross NO_3^- consumption (b) means for the Andrews' DIRT plots. Treatment abbreviations can be found in Table 1. Points are treatment means (n=3) and error bars represents one standard error. Different letters in (b) signify differences ($p<0.05$) among treatments.

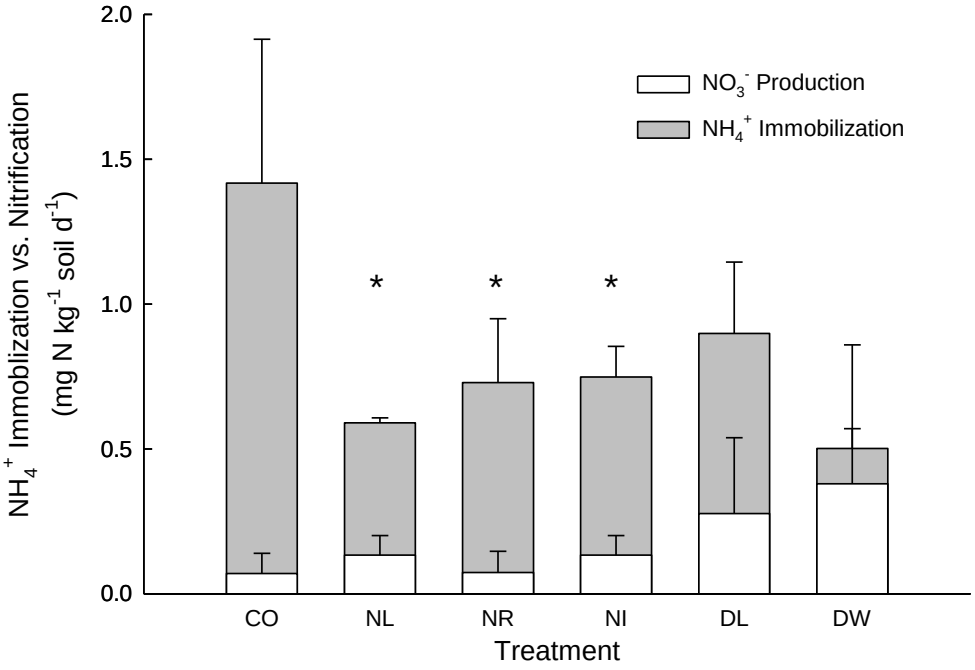

Figure 3.4 Comparison of gross NH_4^+ immobilization/consumption and gross NO_3^- production (rates. Treatment abbreviations can be found in Table 1. All bars represent means (n-3) with error bars representing one standard error. Asterisks (*) represent significant differences ($p<0.05$) between the two rates.

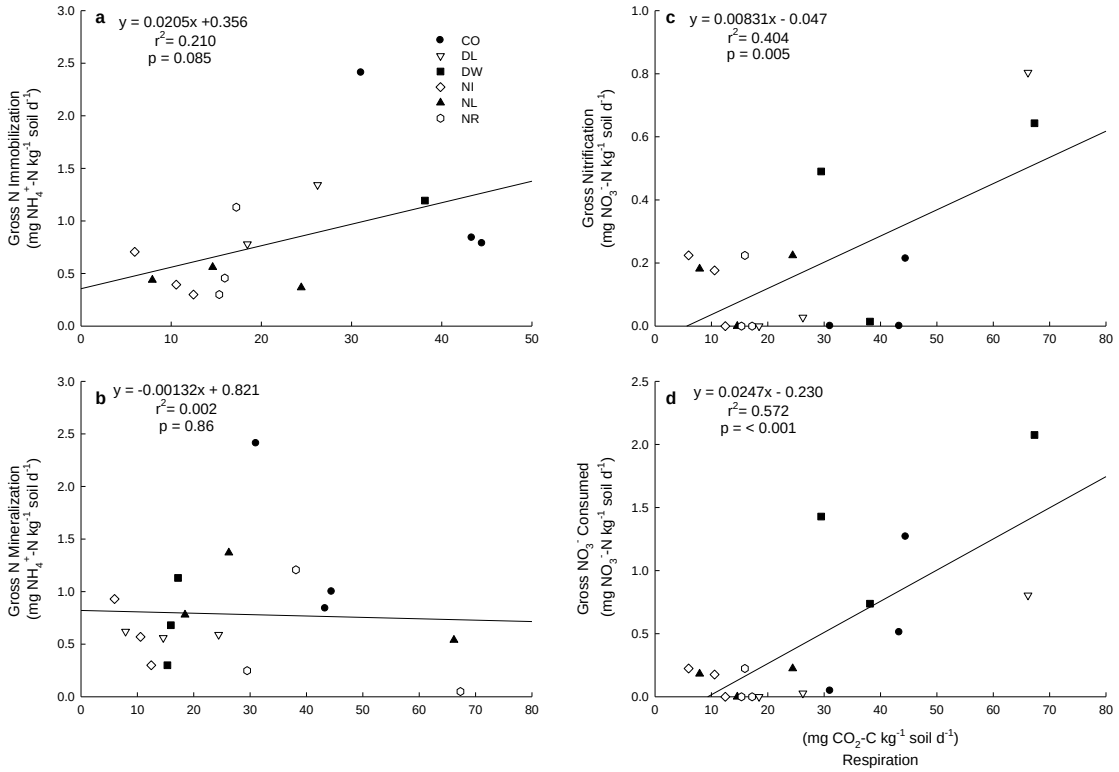

Figure 3.5 Correlations of gross NH_4^+ immobilization (a), N mineralization (b), NO_3^- production (c), and NO_3^- consumption. Treatment abbreviations can be found in Table 1. Each point represents one field replication.

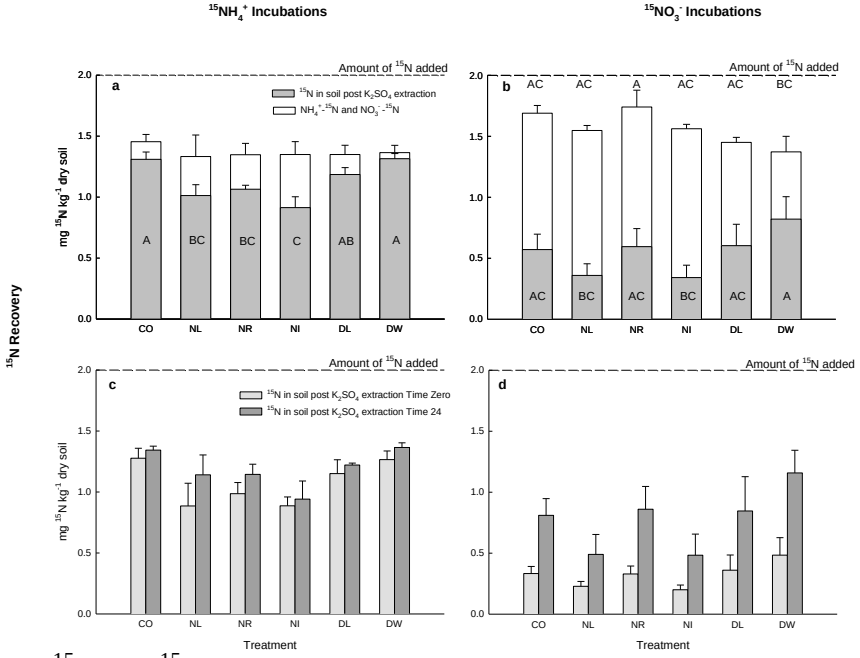

Figure 3.6 Mass balance of ^{15}N and ^{15}N remaining in after K_2SO_4 extraction soil for exchangeable N pools of Time 0 and Time 24 incubations. (a) Mass balance and ^{15}N are remaining post exchangeable N extraction of $^{15}NH_4^+$ incubations (b) Mass balance and ^{15}N remaining post exchangeable N extraction of $^{15}NO_3^-$ incubations (c) ^{15}N remaining post exchangeable N extraction for Time 0 and Time 24 $^{15}NH_4^+$ incubations (d) ^{15}N remaining post exchangeable N extraction for Time 0 and Time 24 $^{15}NO_3^-$ incubations. Treatment abbreviations can be found in Table 1. All bars represent means (n=6, 3 values per treatment per time point) with errors bars representing one standard error. Different letters signifies differences among treatments (Total ^{15}N recovery and ^{15}N remaining in soil) using Tukey-Kramer procedure ($p<0.05$).

Chapter 4: Contributions of litter-derived N to N turnover in old-growth forest soils with long-term organic matter exclusions

Elizabeth A. Brewer

Abstract

In old-growth forests of Oregon, N availability is often considered limiting, and is dependent on soil microorganisms and organic matter inputs to build and replenish organic N and NH_4^+ pools. Changes in the quantity and quality of organic matter can alter the availability of C and N in a system, thus potentially shifting the dominant processes occurring in the ecosystem N cycle. To examine these shifts in N cycling processes, particularly the rate of depolymerization and transfer from insoluble to soluble N, we added leached, ^{15}N-labeled litter to old-growth soils, considered to be N limited, and to soils that have experienced 12 years of litter and root exclusions (i.e., No Inputs). Two litter types, red alder and Douglas-fir, and no litter addition controls, were incubated in the laboratory, and harvested at four times (3, 10, 56, and 151 days). During the first 10 days of the experiment, only the No Inputs-no litter treatment showed net N mineralization; all other treatments showed net N immobilization. By 151 days, all treatments clearly showed net N mineralization and nitrification but there were no differences among treatments. The contribution of litter-N to inorganic N pools was greater in Control soil than No Input soil, and red alder litter contribution was greater than Douglas-fir. Respiration from No Input soil was lower than Control soil. The addition of litter increased respiration. Throughout the incubation, we observed that litter type was inconsequential to net N mineralization in both the Control and No Input soil, but was a factor when it came to C utilization.

Introduction

Nitrogen is generally considered the most limiting nutrient in most ecosystems. In the wake of the changing conditions in ecosystems (e.g., changing climates, elevated carbon dioxide (CO_2), atmospheric nutrient deposition, and land use changes) there is an increasing number of studies focusing on the response of the N cycle and the feedback mechanisms that control it (Rennenberg et al., 2009; Wang and Houlton, 2009; Zaehle et al., 2010) .

Most anthropogenic influences (e.g., land management, N deposition) on ecosystems result in changes in vegetation inputs and/or nutrient availability to soils and microbes. Vegetation inputs are the primary sources of soil organic matter (Kogel-Knabner, 2002) and changes in the quantity and/or quality of organic matter inputs (Fisk and Fahey, 2001; Lajtha et al., 2005), as well as the inherent microbial communities (Brant et al., 2006b; McMahon et al., 2005; Strickland et al., 2009b; Waldrop and Firestone, 2006), determine the rate at which nutrients are recycled or lost from a system.

In N cycling, the quality of organic matter is often described in terms of C:N ratio. When the C:N ratio is below 20 (Cabrera et al., 2005), N availability exceeds microbial N needs and an increase in ammonium (NH_4^+) and/or nitrate (NO_3^-) pools results (net N mineralization). When the C:N ratio is greater than 40, microbial demands exceed N availability resulting in

decreases in the inorganic (NH_4^+ and/or NO_3^-) pools (net N immobilization) (Cabrera et al., 2005). Other chemical and structural components of organic matter (tannins, lignin), can also influence the release and cycling of N (Berg, 2000; Kanerva et al., 2006; Scott and Binkley, 1997).

Several studies have focused on how the quality of litter (e.g., C:N ratio, lignin content) can influence decomposition (e.g., Aerts, 1997; Berg, 2000; Cabrera et al., 2005; McClaugherty et al., 1985; Scott and Binkley, 1997; Silver and Miya, 2001). Other work has shown that although the quality of the residue is important in decomposition dynamics, the ability of the microbial communitiy to break down litter is partially determined by the legacy of organic matter inputs into the soil (Ayres et al., 2009; Gholz et al., 2000; Strickland et al., 2009b; Vivanco and Austin, 2008). These studies have found differences in microbial communities (Strickland et al., 2009b), metabalomes (Wallenstein et al., 2010), respiration (Strickland et al., 2009a); edaphic characteristics (Strickland et al., 2009b), and mass loss (Ayres et al., 2009; Gholz et al., 2000), but few have addressed N mineralization (Kooijman and Smit, 2009).

The leachable/soluble pool from plant material can vary in chemical composition but often contains short monomers and proteins (Kalbitz et al., 2003; Schulze, 2005), that can contribute to the C and N availability to microbial communities (Amon and Benner, 1996; Cleveland et al., 2004;

Geisseler et al., 2009; Park and Matzner, 2003). In the field, dissolved or soluble organic matter may contribute greatly to microbial nutrient supplies (McMahon et al., 2005) but the contribution of older, leached litter to N cycling processes is less understood. In order for insoluble organic matter to contribute to the N cycle there has to be sufficient depolymerization. Throughout this manuscript when litter is referred to as "unsoluble" litter we are defining this as litter that has lost or has substantially decreased its leached/extractable soluble organic pool compared to unleached litter.

In old-growth forests of Oregon, N availability is generally considered limiting. These microbial communities of these soils may have the functional capability of breaking down insoluble organic matter. In old-growth Douglas-fir (*Pseudotsuga menziesii*) – western hemlock (*Tsuga heterophylla*) stands there was little evidence of nitrification (Chapter 3), suggesting that microbial communities are N limited and short term incubations would likely yield net N immobilization.

Removal of plant nutrient demand (e.g., harvesting, wind-falls, or fire) often leads to a shift from a microbial N-limited system to a N-replete system. Nitrification rates increase and NO_3^- losses from the system have been observed. A long-term organic matter manipulation study, Detritus Inputs and Removal Treatments (DIRT) at the H.J. Andrews, showed similar results with higher NO_3^- in lysimeters of root removal plots during the first five years

(Lajtha et al., 2005). At ten years, there was no evidence of gross or potential nitrification (Chapter 3) and a decrease in the amount of NO_3^- in lysimeters compared to the first five years (K. Lajtha, 2010, personal communication).

The exclusion of roots and litter prevent the addition of new, relatively labile organic matter, particularly soluble organic matter into the system. Field and lab N measurements, particularly those related to nitrification, led us to question how N dynamics in old-growth and organic matter exclusion plots are differing. We had three specific research questions: (a) What is the contribution of "insoluble" litter-derived N to old-growth forest soils? (b) Would the microbial communities of long-term organic-matter exclusion soils maintain, enhance, or lose functionality to breakdown "insoluble" litter to obtain N? (c) Is the relative quality of the "insoluble" litter important to microbial communities in these systems?

Methods

The DIRT experiment at the H.J. Andrews Experimental Forest (Andrews), established in 1997, is part of an inter-site network that manipulates above- and below-ground vegetation inputs (Nadelhoffer et al., 2005). The Andrews' DIRT plots are located in an old-growth Douglas-fir – western hemlock stand and consist of six litter input/exclusion treatments. Each treatment is replicated three times. Plot sizes are approximately 10 m x 15 m; however, some plots were slightly smaller due to trees and their debris

(Lajtha et al., 2005; Sulzman et al., 2005). The soils at the site are classified as coarse loamy mixed mesic Typic Hapulands (Dixon, 2003). Additional details regarding site properties have been presented elsewhere (Holub et al., 2005; Lajtha et al., 2005; Sulzman et al., 2005; Yano et al., 2005).

This study focused on two DIRT treatments: Control and No Inputs (Lajtha et al., 2005; Nadelhoffer et al., 2005; Sulzman et al., 2005). The No Inputs treatment excludes both above- and below-ground vegetation inputs. Above-ground inputs were excluded with 1-mm mesh screen on the surface and below-ground inputs were excluding by trenching to a 1 m depth and installing root exclusion barriers.

In August 2009, soil cores (0-10 cm depth) were collected from Control and No Inputs plots. Thirty, 2.2 cm diameter cores, were taken from each field plot replicate and sieved to 4 mm. For each treatment, soils from the three plots were composited and allowed to rest at 25°C for ten days. Two days prior to incubation soil from both treatments were brought up to 0.5 g/g gravimetric water potential. Background properties for each of the organic matter manipulated soils can be found in Table 4.1.

Ninety-six incubations were set up in Ball jars, 48 jars per field manipulation. Three litter addition treatments were applied to each field manipulation soil: Douglas-fir (*Pseudotsuga menziesii*), red alder (*Alnus*

rubra), and a no litter control. Each incubation jar contained the equivalent of 30 g dry soil and 0.078 g litter (Douglas-fir or red alder). Litter was incorporated into the soil, at a rate of double to average field inputs (1.3 Mg ha^{-1} y^{-1}) (Holub et al., 2005).

The litter was obtained from trees grown from seed in a greenhouse. The trees were grown in sand-perlite mixtures and fertilized with a ^{15}N-labeled Hoagland's solution. After a year of growth, the saplings were forced to senesce by withholding water. Ten grams of each litter type was finely chopped (approximately ≤ 0.5 cm). The litter types were each divided into seven containers and leached with 1 L of water. Because a large proportion of the soluble pool has been leached away, this will be referred to as "insoluble" litter. Details regarding the litter can be found in Table 4.2.

At random, four replicate jars were destructively sampled at 3, 10, 56, and 151 days after litter addition. On day 42 of the incubation, the Day 56 and Day 151 incubations were leached with 20 ml of water. On day 143, the Day 151 incubations were leached again with 20 ml of water. Leaching was done to prevent inhibition of N cycling processes as a result of NH_4^+ and NO_3^- accumulation.

Respiration, CO_2 evolved in the headspace, was measured on the Day 151 jars throughout the incubation period. Gas chromatography was used to

determine CO_2 concentrations (Hach Carle Series 100, Loveland, CO). Jars were flushed regularly to prevent an anoxic headspace.

At each harvest, exchangeable N and C pools were extracted using an equivalent of 15 g dry soil to 50 ml of 0.05 *M* K_2SO_4. Extracts were shaken for 1 h and filtered through pre-rinsed (5 ml of 0.05 *M* K_2SO_4) Whatman #2 filter papers. Concentrations of NH_4^+ and NO_3^- were measured by automated colorimetry (Astoria-Pacific 300 series autoanalyzer, Clackamas, OR). Combustion oxidation was used to determine total soluble C and N (Shimadzu TOC-V CSH with a TNM-1 module, Tokyo, Japan). Inorganic N was subtracted from total soluble N to yield soluble organic N; because of the low pH of the soil total soluble C was assumed to be equivalent to soluble organic C.

The ^{15}N abundance of NH_4^+ and NO_3^- in soil extracts were determined using sequential diffusion (Brooks et al., 1989; Hart et al., 1994). All ^{15}N analyses were done on a Europa 20-20 mass spectrometer (PDZ Europa, England) interfaced with a Roboprep elemental analyzer. For each soil type, contributions of litter-N to inorganic N pools were calculated by taking the difference between the atom% ^{15}N of a given pool from litter treatments and the mean atom% ^{15}N of the same pool in the no litter treatment and dividing the result by the atom% ^{15}N of the respective litter at that harvest.

Microbial biomass C and N are based on a 24-hour chloroform fumigation. Microbial biomass C and N were determined using a Shimadzu TOC-V CSH with a TNM-1 module (Tokyo, Japan). A correction factor of 0.45 was used for biomass C (Joergensen, 1996) and a correction factor of 0.54 was used for biomass N (Brookes et al., 1985; Joergensen and Mueller, 1996).

Data analyses were done using SAS ver. 9.2 software (SAS Institute Inc., Cary, NC). PROC GLM ANOVA was used to determine main effects and interactions of soil, litter type, and harvest date for N pool sizes, ^{15}N and microbial biomass. Net N mineralization rates were analyzed for each time interval, to determine main effects and interactions of soil and litter type. Cumulative respiration was evaluated using a repeated measures approach, as the same individuals jars were used throughout the incubation. All normality and residual variances were checked to ensure ANOVA assumptions were met. When an ANOVA provided a significant p-value (<0.05), multiple comparisons were made using the Tukey-Kramer procedure.

The exclusion of organic matter inputs resulted in a decrease of respiration compared to the Control (Figure 4.1). Although the addition of litter tended to increase the amount of soil respired, there was no effect of litter type on respiration.

During the first week of incubation, net N mineralization in the no litter-No Inputs treatment was significantly greater than all other treatments (Figure 4.2a). Accumulation of NO_3^- showed the same pattern with no litter-No Inputs having the greatest rate (Figure 4.2b). During days 10 through 56, individual treatments did not differ from one another, but both field manipulated soils and litter type had a significant effect on net N mineralization and nitrification (Figure 4.2). Control soils had lower net rates as did the addition of litter, regardless of litter quality (Figure 4.2). During the last portion of the incubation period, net N mineralization and nitrification rates did not show any difference among treatments (Figure 4.2).

At the end of the incubation red alder litter N had a greater contribution to soil NH_4^+ and NO_3^- pools than Douglas-fir litter (Figure 4.3). Although the pattern was not consistent throughout the incubation, at the end of the incubation Control soils had a tendency to have greater litter-N contributions than the No Input soils (Figure 4.3).

Control soils had greater microbial biomass C (Figure 4.4) and N (Figure 4.5) than No Input soils. Microbial biomass C (Figure 4.4) and N (Figure 4.5) decreased at the Day 56 harvest but increased again by Day 151. There was no effect of litter type on microbial biomass. There was a significant interaction between soil type, litter type, and harvest date for the

microbial C:N ratio (Figure 4.6). No Input soils with No Litter had an increase in C:N at the last two harvest dates and overall had the highest microbial C:N.

Discussion

Long-term exclusion of organic matter influenced C and N cycling processes. Soils after ten years of organic matter exclusion had lower respiration than Control soils (Figure 4.1). The addition of litter caused net N immobilization (Figure 4.2), suggesting that the microbial communities assimilated the inorganic N pools during litter decomposition. Litter addition in Control soils did not alter net N mineralization during days 3 to 10 (Figure 4.2), likely the result of the soils still receiving litter inputs in the field.

Higher net N mineralization and nitrification rates in the No Input soils from Day 10 to 56 suggest that the microbial N needs were lower than those of Control soils (Figure 4.2). Changes in rates could be attributed to changes in microbial biomass. Microbial biomass C and N did decrease over time (Figure 4.4). A smaller microbial population would reduce the N need of the community, thus decreasing overall N assimilation. This could explain the increasing net N mineralization rates at the end of the incubation (Figure 4.2).

The addition of "insoluble" litter influenced the net N cycling rates during the first portion of the incubation, but differences dissipated. From Day 10 to 56, the addition of litter in the lab decreased net N mineralization and

nitrification rates (Figure 4.2). The greater microbial N needs could be attributed to changes in microbial biomass; however, no differences in microbial biomass C and N were observed among litter type additions (Figure 4.4). There were differences in microbial C:N hinting perhaps of a microbial community shift (Figure 4.5).

The lack of differences among litter types from Day 10 to 56 and Day 56 to151 were contrary to what we originally hypothesized. The two litter types had drastically different C:N ratios both prior to and following leaching. Previous studies examining litter additions varying in quality did not show differences in net N mineralization in mineral soil layers but small differences in organic layers (Kooijman and Smit, 2009).

Increases in net N mineralization rates were primarily driven by increases in the NO_3^- pool, particularly in No Input soils. Microbial NO_3^- assimilation can be inhibited by large NH_4^+ pool sizes (McCarty and Bremner, 1992), reducing competition for ammonia oxidizing communities. Although there was a small increase in NH_4^+ pool sizes of the Control soils with litter additions, it is unlikely the increase was enough to inhibit NO_3^- assimilation. A concurrent shift in microbial community composition could have also occurred. The lack of a measurable nitrification potential in the Control soils and very low rates in No Input soils by Brewer (Chapter 3), suggests there was a growth of a nitrifier population throughout the incubation.

Examination of the ^{15}N recovered in the NO_3^- pool showed that the red alder litter contributed a greater proportion of N to inorganic N pools than Douglas-fir litter in both field manipulated soil (Figure 4.3). The increased contribution of litter-derived N from the red alder is approximately four times that of Douglas-fir, this is about the same difference in the original N concentrations (Table 4.2). This may suggest that the same amount of litter was decomposed, but the greater N contained in the red alder releases more N into the system. Although there were differences in the litter N contribution a not net N cycling rates. With both litter types, Control soils had a greater contribution of litter-N to inorganic N pools. These differences hint of the functionality of the microbial community to break down this relatively recalcitrant litter. Previous studies by Brant et al. (2006a; 2006b) have shown a decrease in fungal to bacterial ratios and shifts in functional capabilities of the microbial communities of No Inputs soils relative to Control soils. Differences in fungal to bacterial ratios in the No Inputs plots are a result of the loss of fungal, particularly mycorrhizal, community members (Brant et al., 2006a), which can be involved in organic matter decomposition (Dix and Webster, 1995). Ongoing work is examining the ^{15}N microbial biomass and these results may provide additional insights to the functional dynamics.

Lower respiration rates and lower contribution of litter-N to the NH_4^+ and NO_3^- in the No Inputs soils, demonstrates that the long-term organic matter

input exclusions have altered the ability of the soil to cycle C and N. The addition of the "insoluble" litter initially increased N immobilization but differences in rates dissipated over time. Despite lack of differences in rates, both litter types made modest contributions to N mineralization and nitrification. Ongoing work examining microbial ^{15}N assimilation and active microbial communities will aid in teasing apart the contributions of relatively recalcitrant organic matter and the functionality of the community to N cycling process.

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Literature Cited

Aerts, R. 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: A triangular relationship. Oikos 79:439-449.

Amon, R.M.W., and R. Benner. 1996. Bacterial utilization of different size classes of dissolved organic matter. Limnology and Oceanography 41:41-51.

Ayres, E., H. Steltzer, B.L. Simmons, R.T. Simpson, J.M. Steinweg, M.D. Wallenstein, N. Mellor, W.J. Parton, J.C. Moore, and D.H. Wall. 2009. Home-field advantage accelerates leaf litter decomposition in forests. Soil Biology & Biochemistry 41:606-610.

Berg, B. 2000. Litter decomposition and organic matter turnover in northern forest soils. Forest Ecology and Management 133:13-22.

Brant, J.B., D.D. Myrold, and E.W. Sulzman. 2006a. Root controls on soil microbial community structure in forest soils. Oecologia 148:650-659.

Brant, J.B., E.W. Sulzman, and D.D. Myrold. 2006b. Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation. Soil Biology & Biochemistry 38:2219-2232.

Brookes, P.C., J.F. Kragt, D.S. Powlson, and D.S. Jenkinson. 1985. Chloroform fumigation and the release of soil-nitrogen -The effects of fumigation time and temperature Soil Biology & Biochemistry 17:831-835.

Brooks, P.D., J.M. Stark, B.B. McInteer, and T. Preston. 1989. Diffusion method to prepare soil extracts for automated N-15 analysis. Soil Science Society of America Journal 53:1707-1711.

Cabrera, M.L., D.E. Kissel, and M.F. Vigil. 2005. Nitrogen mineralization from organic residues: Research opportunities. Journal of Environmental Quality 34:75-79.

Cleveland, C.C., J.C. Neff, A.R. Townsend, and E. Hood. 2004. Composition, dynamics, and fate of leached dissolved organic matter in terrestrial ecosystems: Results from a decomposition experiment. Ecosystems 7:275-285.

Dix, N.J., and J. Webster. 1995. Fungal Ecology University Press, Cambridge, U.K.

Dixon, J.J. 2003. Applying GIS to soil-geomorphic landscape mapping in the Lookout Creek Valley, western Cascades, Oregon. M.S. thesis Oregon State University, Corvallis.

Fisk, M.C., and T.J. Fahey. 2001. Microbial biomass and nitrogen cycling responses to fertilization and litter removal in young northern hardwood forests. Biogeochemistry 53:201-223.

Geisseler, D., W.R. Horwath, and T.A. Doane. 2009. Significance of organic nitrogen uptake from plant residues by soil microorganisms as affected by carbon and nitrogen availability. Soil Biology & Biochemistry 41:1281-1288.

Gholz, H.L., D.A. Wedin, S.M. Smitherman, M.E. Harmon, and W.J. Parton. 2000. Long-term dynamics of pine and hardwood litter in contrasting environments: toward a global model of decomposition. Global Change Biology 6:751-765.

Hart, S.C., J.M. Stark, E.A. Davidson, and M.K. Firestone. 1994. Nitrogen mineralization, immobilization, and nitrification, *In* R. W. Weaver, et al., eds. Methods of soil analysis, Part 2. Microbiological and biochemical properties. Soil Science Society of America, Madison, WI.

Holub, S.M., K. Lajtha, J.D.H. Spears, J.A. Toth, S.E. Crow, B.A. Caldwell, M. Papp, and M.T. Nagy. 2005. Organic matter manipulations have little effect on gross and net nitrogen transformations in two temperate forest mineral soils in the USA and central Europe. Forest Ecology and Management 214:320-330.

Joergensen, R.G. 1996. The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k(EC) value. Soil Biology & Biochemistry 28:25-31.

Joergensen, R.G., and T. Mueller. 1996. The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k(EN) value. Soil Biology & Biochemistry 28:33-37.

Kalbitz, K., J. Schmerwitz, D. Schwesig, and E. Matzner. 2003. Biodegradation of soil-derived dissolved organic matter as related to its properties. Geoderma 113:273-291.

Kanerva, S., V. Kitunen, O. Kiikkila, J. Loponen, and A. Smolander. 2006. Response of soil C and N transformations to tannin fractions originating from Scots pine and Norway spruce needles. Soil Biology & Biochemistry 38:1364-1374.

Kogel-Knabner, I. 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biology & Biochemistry 34:139-162.

Kooijman, A.M., and A. Smit. 2009. Paradoxical differences in N-dynamics between Luxembourg soils: litter quality or parent material? European Journal of Forest Research 128:555-565.

Lajtha, K., S.E. Crow, Y. Yano, S.S. Kaushal, E. Sulzman, P. Sollins, and J.D.H. Spears. 2005. Detrital controls on soil solution N and dissolved organic matter in soils: a field experiment. Biogeochemistry 76:261-281.

McCarty, G.W., and J.M. Bremner. 1992. Regulation of assimilatory nitrate reductase activity in siol by microbial assimilation of ammonium. Proceedings of the National Academy of Sciences of the United States of America 89:453-456.

McClaugherty, C.A., J. Pastor, J.D. Aber, and J.M. Melillo. 1985. Forest litter decomposition in relation to soil nitrogen dynamics and litter quality Ecology 66:266-275.

McMahon, S.K., M.A. Williams, P.J. Bottomley, and D.D. Myrold. 2005. Dynamics of microbial communities during decomposition of carbon-13 labeled ryegrass fractions in soil. Soil Science Society of America Journal 69:1238-1247.

Nadelhoffer, K.J., R.D. Boone, R.D. Bowden, J. Canary, J. Kaye, K. Lajtha, W. McDowell, P. Micks, and A. Ricca. 2005. The DIRT experiment: litter and root influences on forest soil organic matter stocks and function, *In* F. D and A. J, eds. Forests in time: the environmental consequences of 1000 years of change in New England. Yale University Press, New Haven, CT.

Park, J.-H., and E. Matzner. 2003. Controls on the release of dissolved organic carbon and nitrogen from a deciduous forest floor investigated by manipulations of aboveground litter inputs and water flux. Biogeochemistry 66:265-286.

Rennenberg, H., M. Dannenmann, A. Gessler, J. Kreuzwieser, J. Simon, and H. Papen. 2009. Nitrogen balance in forest soils: nutritional limitation of plants under climate change stresses. Plant Biology 11:4-23.

Schulze, W.X. 2005. Protein analysis in dissolved organic matter: What proteins from organic debris, soil leachate and surface water can tell us - a perspective. Biogeosciences 2:75-86.

Scott, N.A., and D. Binkley. 1997. Foliage litter quality and annual net N mineralization: Comparison across North American forest sites. Oecologia 111:151-159.

Silver, W.L., and R.K. Miya. 2001. Global patterns in root decomposition: comparisons of climate and litter quality effects. Oecologia 129:407-419.

Strickland, M.S., C. Lauber, N. Fierer, and M.A. Bradford. 2009a. Testing the functional significance of microbial community composition. Ecology 90:441-451.

Strickland, M.S., E. Osburn, C. Lauber, N. Fierer, and M.A. Bradford. 2009b. Litter quality is in the eye of the beholder: initial decomposition rates as a function of inoculum characteristics. Functional Ecology 23:627-636.

Sulzman, E.W., J.B. Brant, R.D. Bowden, and K. Lajtha. 2005. Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO_2 efflux in an old growth coniferous forest. Biogeochemistry 73:231-256.

Vivanco, L., and A.T. Austin. 2008. Tree species identity alters forest litter decomposition through long-term plant and soil interactions in Patagonia, Argentina. Journal of Ecology 96:727-736.

Waldrop, M.P., and M.K. Firestone. 2006. Seasonal dynamics of microbial community composition and function in oak canopy and open grassland soils. Microbial Ecology 52:470-479.

Wallenstein, M.D., A.M. Hess, M.R. Lewis, H. Steltzerae, and E. Ayres. 2010. Decomposition of aspen leaf litter results in unique metabolomes when decomposed under different tree species. Soil Biology & Biochemistry 42:484-490.

Wang, Y.P., and B.Z. Houlton. 2009. Nitrogen constraints on terrestrial carbon uptake: Implications for the global carbon-climate feedback. Geophysical Research Letters 36:L24403.

Yano, Y., K. Lajtha, P. Sollins, and B.A. Caldwell. 2005. Chemistry and dynamics of dissolved organic matter in a temperate coniferous forest on Andic soils: Effects of litter quality. Ecosystems 8:286-300.

Zaehle, S., P. Friedlingstein, and A.D. Friend. 2010. Terrestrial nitrogen feedbacks may accelerate future climate change. Geophysical Research Letters 37:L01401.

Table 4.1 Background soil characteristics for the Control and No Input soils from the Detritus Inputs and Removals Treatments (DIRT) at the H.J. Andrews Experimental Forest. Soil C and N numbers were measured following a 0.05M K_2SO_4 extraction

Soil Type	Total C (mg C kg^{-1} dry soil)	Total N (mg N kg^{-1} dry soil)	C/N ratio	^{15}N Atom%	Fungal: Bacterial Ratio[b]
Control	56.36	2.22	25.4	0.3676	0.16
No input	40.36	1.82	22.2	0.3677	0.05

[b] Data from (Brant et al., 2006b)

Table 4.2 Background litter characteristics for the Douglas-fir and red alder added to the laboratory incubations.

Litter Type	Total C (mg C g^{-1} litter)	Total N (mg N g^{-1} litter)	C/N ratio	^{15}N Atom%	C Added (mg C g^{-1} soil	N Added (mg N g^{-1} soil)
Douglas-fir	480	6.69	71.9	7.96	1.25	0.02
Red alder	488	27.1	18.2	1.01	1.27	0.07

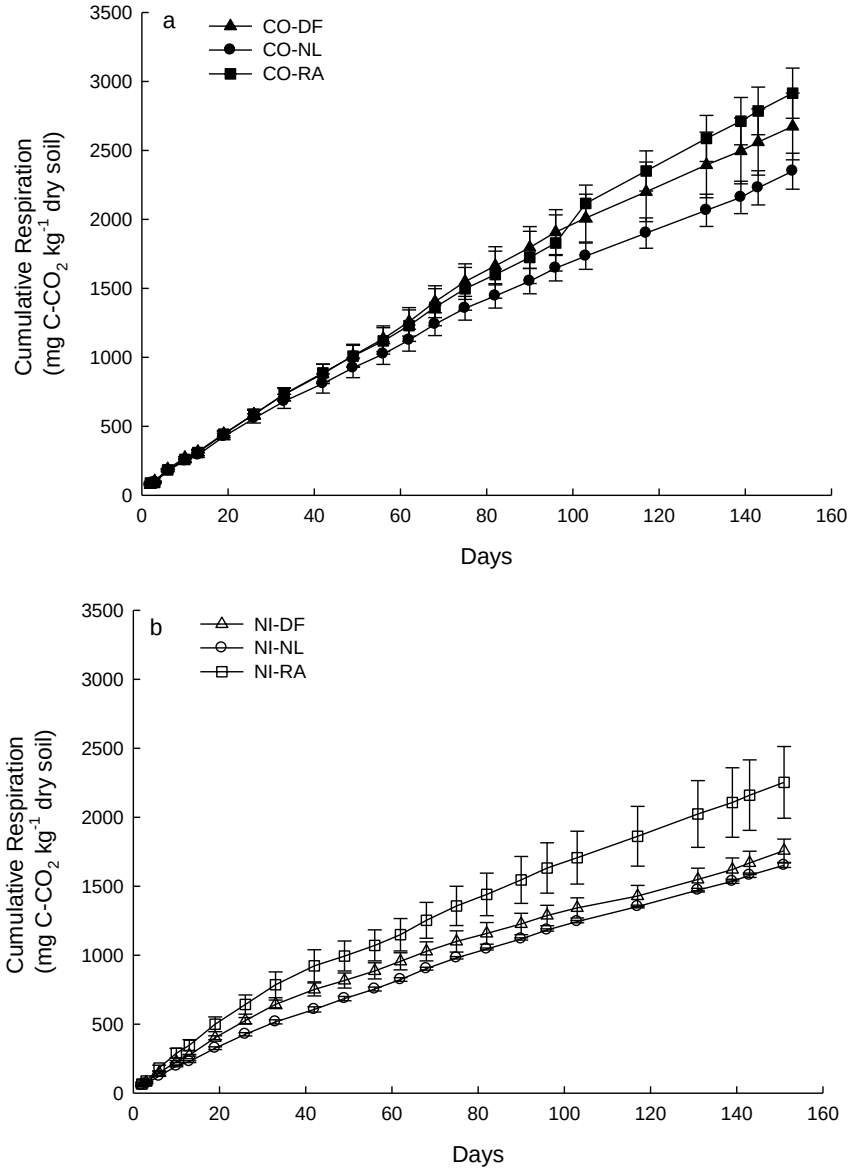

Figure 4.1 Cumulative respiration (mg CO_2-C kg^{-1} soil) of litter amended (a) Control (CO) and (b) No Inputs (NI) soils from the H.J. Andrews Experimental Forest DIRT plots. Soils amended with Douglas-fir litter are symbolized as DF, whereas soils amended with red alder litter are symbolized as RA. Each point represents a treatment mean with error bars representing one standard error.

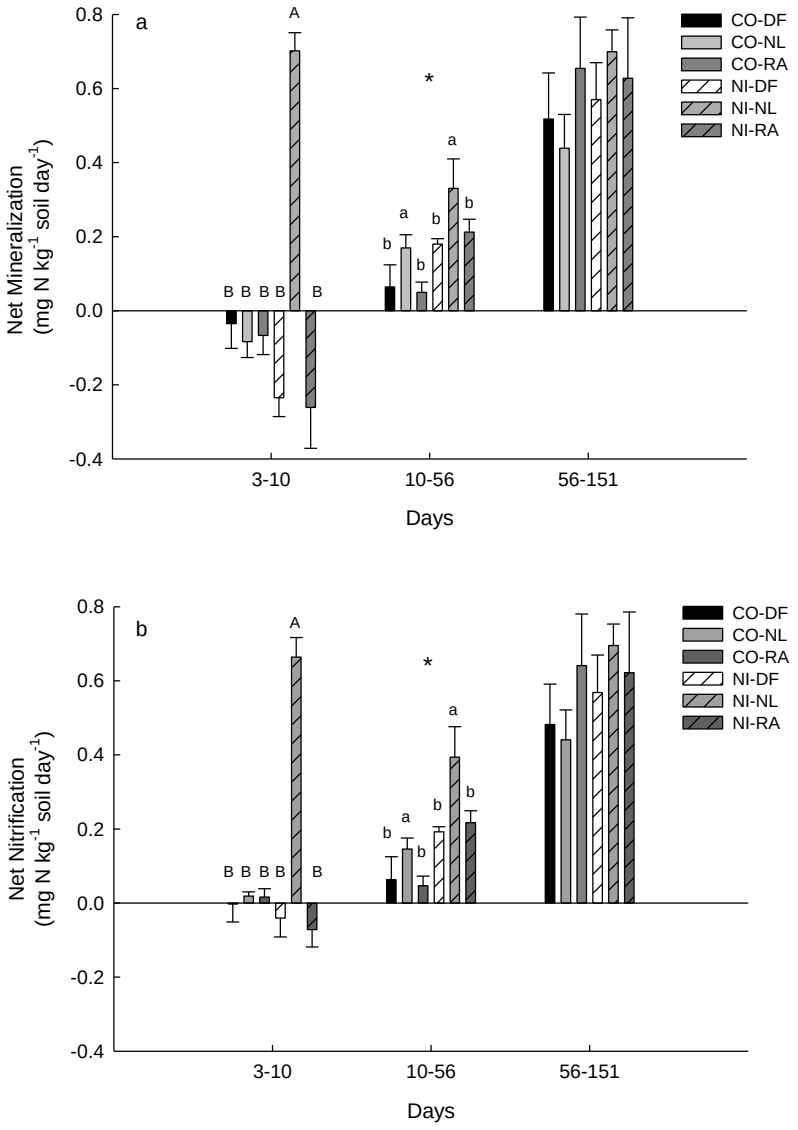

Figure 4.2 Net (a) N mineralization and (b) nitrification (mg N kg^{-1} soil d^{-1}) for Control (CO) and No Inputs (NI) soils amended with litter (DF-Douglas-fir, RA-red alder). Each bar represents a treatment mean with error bars representing one standard error. Capital letters signify differences among individual treatments using the Tukey-Kramer procedure ($p<0.05$). Asterisks (*) signify differences between soil types and small letters signify a litter main effect, with Litter addition soils being different from No Litter addition soils, using the Tukey-Kramer procedure ($p<0.05$).

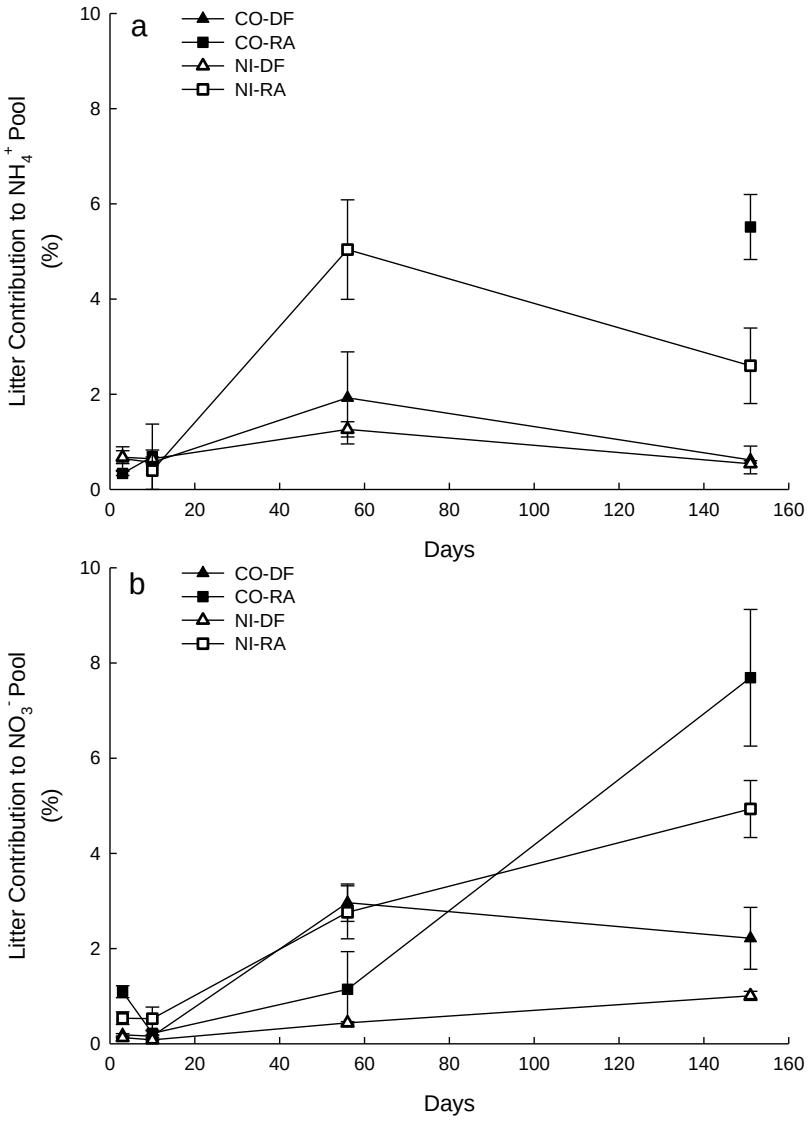

Figure 4.3 Contribution (%) of litter-N to the NH_4^+ and NO_3^- pools for Douglas-fir (DF) or red alder (RA) litter in Control (CO) or No Inputs (NI) soils at the four harvest time points. Each bar represents a treatment mean with error bars representing one standard error.

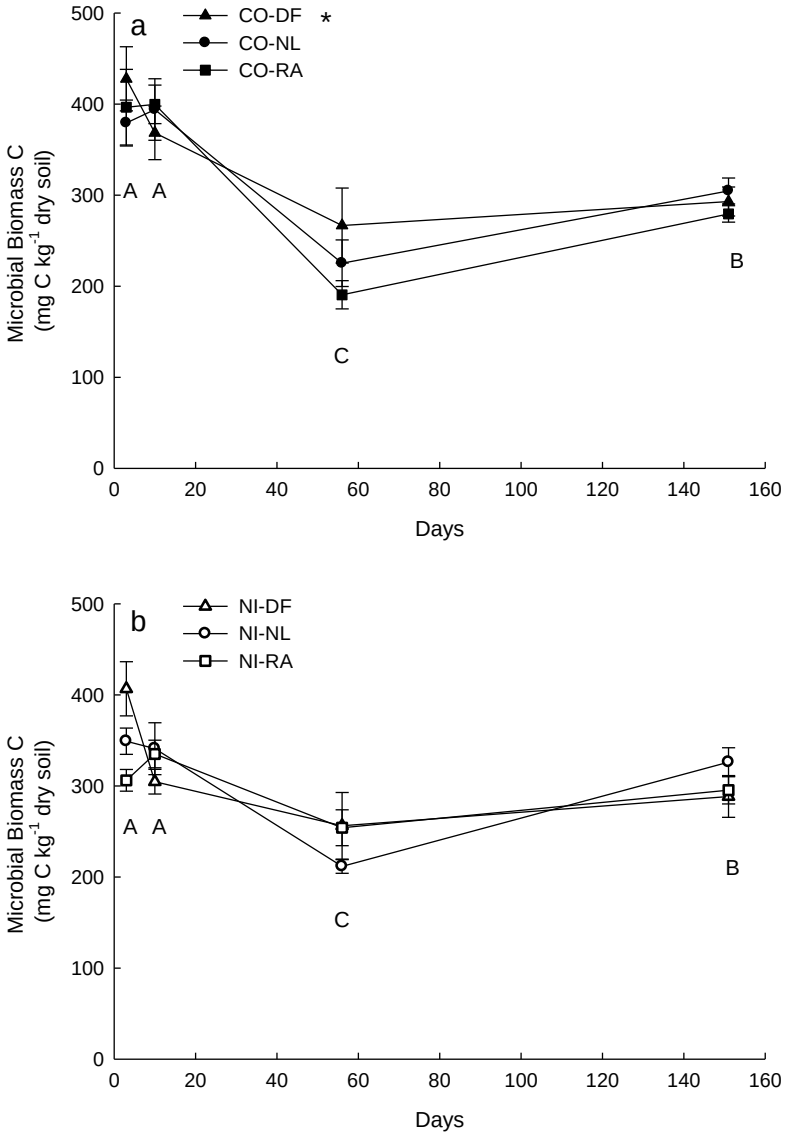

Figure 4.4 Microbial Biomass C (mg C kg^{-1} dry soil) for (a) Control (CO) or (b) No Input (NI) soils with no litter (NL), Douglas-fir (DF), or red alder (RA) litter additions at the four harvest time points. Each point represents a treatment mean with error bars representing one standard error. The asterisks (*) signify there a significant soil type effect on microbial biomass C. Capital letters signify differences among harvest time points using the Tukey-Kramer procedure ($p<0.05$).

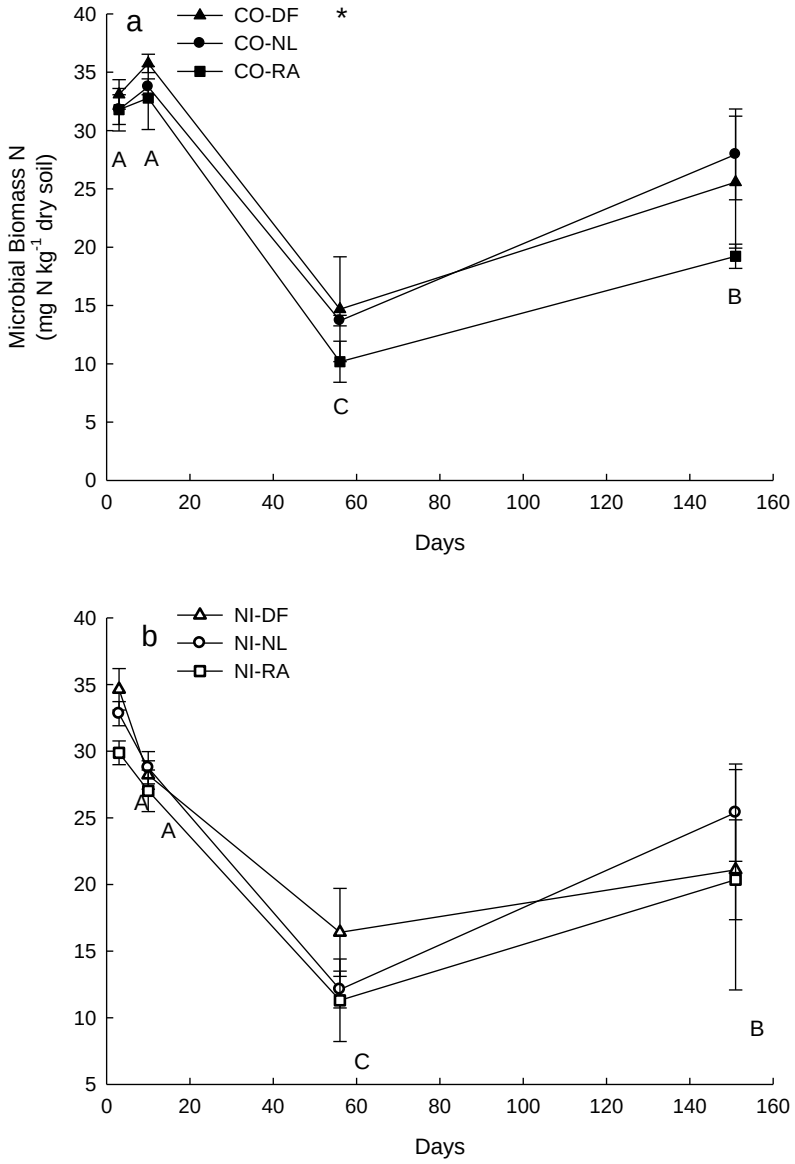

Figure 4.5 Microbial Biomass N (mg N kg^{-1} dry soil) for (a) Control (CO) or (b) No Input (NI) soils with no litter (NL), Douglas-fir (DF), or red alder (RA) litter additions at the four harvest time points. Each point represents a treatment mean with error bars representing one standard error. The asterisks (*) signify there a significant soil type effect on microbial biomass C. Capital letters signify differences among harvest time points using the Tukey-Kramer procedure ($p<0.05$).

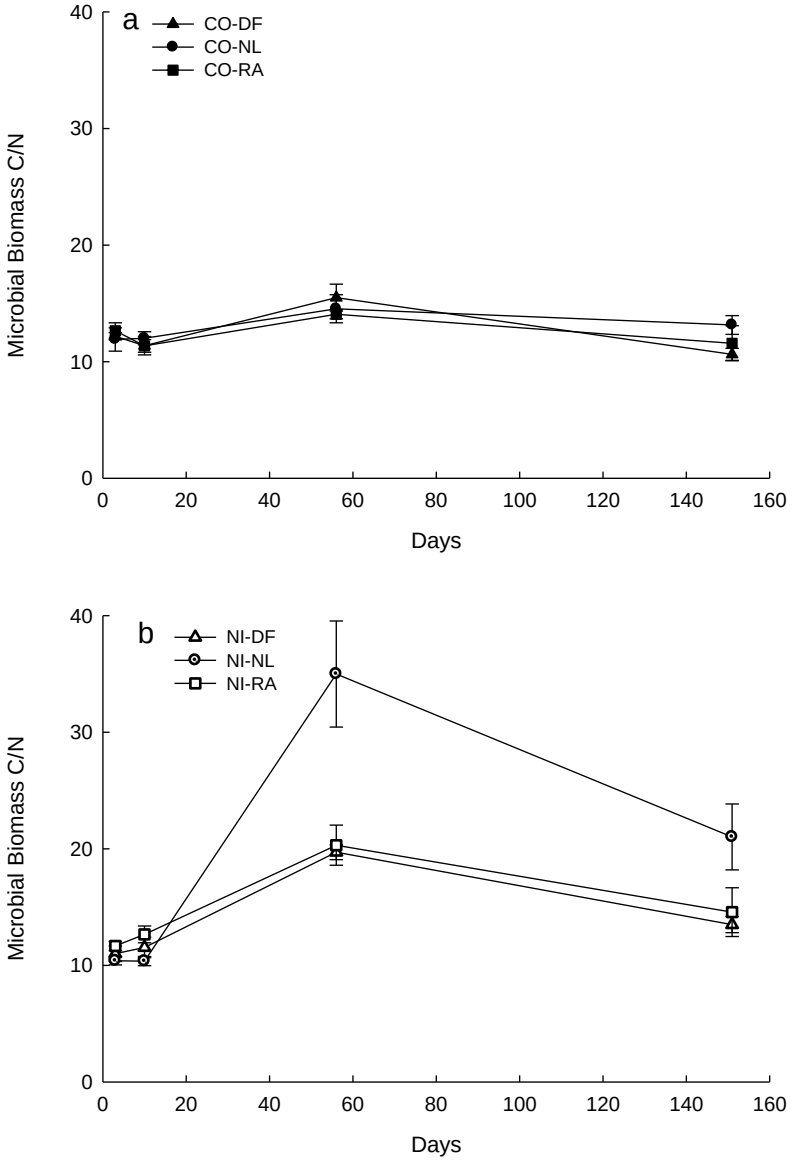

Figure 4.6 Microbial Biomass C:N for (a) Control (CO) or (b) No Input (NI) soils with no litter (NL), Douglas-fir (DF), or red alder (RA) litter additions at the four harvest time points. Each point represents a treatment mean with error bars representing one standard error. The asterisks (*) signify there a significant soil type effect on microbial biomass C. Capital letters signify differences among harvest time points using the Tukey-Kramer procedure ($p<0.05$).

Chapter 5: Conclusions

Elizabeth Brewer

Conclusions

Differences and changes in the quality and quantity of organic matter influence soil microbial communities and C and N cycling. Both ecosystems examined had undergone changes in organic matter inputs for extended periods of time. In Chapter 2, the woody clusters that had invaded the grasslands were approximately 50 years old but the shift in vegetation was a gradual process. In Chapters 3 and 4 the organic matter manipulations imposed were more rapid; however, changes have not been drastic over the last 12 years.

Although I examined what could be considered to be varying extremes in organic matter shifts, both systems have shown differences in microbial communities (Chapter 2; (Kageyama, 2005; Brant et al., 2006; Liao et al., 2008; Hollister et al., 2010) and C and N cycling processes (Chapter 3 and 4; (Martin et al., 2003; Lajtha et al., 2005; Hughes et al., 2006; Liao et al., 2006a; Liao et al., 2006b; Crow et al., 2009).

In the Rio Grande Plains, the more gradual vegetation shift from grassland to woodland revealed differences in fungal communities at the 0-15 cm depth but not at 15-30 cm. Bacterial community differences were lacking across vegetation transects suggesting that spatial heterogeneity and/or other edaphic characteristics were more influential. Other work in a similar ecosystem has also shown greater differences among fungal communities than bacterial communities in this ecosystem (Hollister et al., 2010). There was a

significant correlation of fungal and bacterial communities suggesting some vegetation influence on bacterial community composition.

In the Pacific Northwest I found long-term manipulations of organic matter inputs did alter respiration but did not significantly alter gross NH_4^+ production and consumption. A previous field study has suggested that organic matter exclusion treatments (Lajtha et al., 2005) had nitrification occurring; however, the gross nitrification and nitrification potential measurements did not support this.

Addition of insoluble/leached litter showed contributions to the N cycle in both Control and No Inputs treatments. The addition of the leached litter initially increased N immobilization, but following 151 days of incubations net N mineralization and nitrification rated litter addition treatments did not differ from no litter addition treatments. Also the quality of the litter did not appear to make a large difference the contribution of litter-derived N. Previous studies have suggested that legacies of organic matter inputs can influence how microbial communities perceived litter quality (Gholz et al., 2000; Strickland et al., 2009). My work suggests that in N-limited, old-growth forests of the Pacific Northwest the quality of the less labile, insoluble, or "older" organic matter may not matter. My work does not address it, but it would be of interest to investigate the role of the more labile, easily leached, soluble pools of litter to N dynamics.

The differences in contributions of litter-derived N between the two soil types, suggests that the No Inputs plots have lost some of their functionality to degrade litter. Previous work at these sites have shown differences in fungal communities (Kageyama, 2005; Brant et al., 2006) and perhaps the change or loss in fungal biomass and/or diversity, which are known for their ability to degrade less labile organic matter (Dix et al., 1995), are driving differences in N harvesting. Ongoing work investigating the dynamics of microbial communities among the two soils and throughout the incubation will increase our knowledge.

Perhaps one of the most interesting points of Chapters 3 and 4 was that nitrification and NO_3^- accumulation based on gross N laboratory incubations in root exclusions treatments did not match results based on the field measurements during the first five years of manipulation. The disturbance of soil sampling and sieving may have allowed N-limited microorganisms that had been contained within aggregates, access to available N sources, thus leading to gross N immobilization. Contrary, recently analyzed lysimeter data (K. Lajtha, personal communication, 2010) showed decreased NO_3^- concentration in 2008, suggests that N processes may have shifted. Longer incubation revealed greater nitrification in No Inputs soils, similar to what has been observed in the field. Differences among the data suggest that caution

should be taken when making inferences about ecosystem dynamics based upon laboratory measurements.

In conclusion, changes in the quality and quantity of organic matter inputs into ecosystems influence soil microbial communities and N cycling processes. Although different questions were addressed in each ecosystem, both revealed that fungal communities are related to the vegetation and quality of the organic matter inputs. Further work addressing the importance of the functionality of the fungal community in the N cycle will aid in our understanding of ecosystem processes. To maximize our knowledge it will be important to further address the diversity, functionality of all microbial communities in N cycling and their responses to changing environments.

Literature Cited

Brant, J.B., Myrold, D.D., Sulzman, E.W., 2006. Root controls on soil microbial community structure in forest soils. Oecologia 148, 650-659.

Crow, S.E., Lajtha, K., Bowden, R.D., Yano, Y., Brant, J.B., Caldwell, B.A., Sulzman, E.W., 2009. Increased coniferous needle inputs accelerate decomposition of soil carbon in an old-growth forest. Forest Ecology and Management 258, 2224-2232.

Dix, N.J., and J. Webster. 1995. Fungal Ecology University Press, Cambridge, U.K.

Gholz, H.L., Wedin, D.A., Smitherman, S.M., Harmon, M.E., Parton, W.J., 2000. Long-term dynamics of pine and hardwood litter in contrasting environments: toward a global model of decomposition. Global Change Biology 6, 751-765.

Hollister, E.B., Schadt, C.W., Palumbo, A.V., Ansley, R.J., Boutton, T.W., 2010. Structural and functional diversity of soil bacterial and fungal communities following woody plant encroachment in the southern Great Plains. Soil Biology & Biochemistry 42, 1816-1824.

Hughes, R.F., Archer, S.R., Asner, G.P., Wessman, C.A., McMurtry, C., Nelson, J., Ansley, R.J., 2006. Changes in aboveground primary production and carbon and nitrogen pools accompanying woody plant encroachment in a temperate savanna. Global Change Biology 12, 1733-1747.

Kageyama, S.A. 2005. Effects of vegetation and disturbance on fungal communities in the western Cascades of Oregon. Ph.D. dissertation, Oregon State University, Corvallis.

Lajtha, K., Crow, S.E., Yano, Y., Kaushal, S.S., Sulzman, E., Sollins, P., Spears, J.D.H., 2005. Detrital controls on soil solution N and dissolved organic matter in soils: a field experiment. Biogeochemistry 76, 261-281.

Liao, J.D., Boutton, T.W., 2008. Soil microbial biomass response to woody plant invasion of grassland. Soil Biology & Biochemistry 40, 1207-1216.

Liao, J.D., Boutton, T.W., Jastrow, J.D., 2006a. Storage and dynamics of carbon and nitrogen in soil physical fractions following woody plant invasion of grassland. Soil Biology & Biochemistry 38, 3184-3196.

Liao, J.D., Boutton, T.W., Jastrow, J.D., 2006b. Organic matter turnover in soil physical fractions following woody plant invasion of grassland: Evidence from natural C-13 and N-15. Soil Biology & Biochemistry 38, 3197-3210.

Martin, R.E., Asner, G.P., Ansley, R.J., Mosier, A.R., 2003. Effects of woody vegetation encroachment on soil nitrogen oxide emissions in a temperate savanna. Ecological Applications 13, 897-910.

Strickland, M.S., Osburn, E., Lauber, C., Fierer, N., Bradford, M.A., 2009. Litter quality is in the eye of the beholder: initial decomposition rates as a function of inoculum characteristics. Functional Ecology 23, 627-636.