ABSTRACT

BURGER, JULIA CAMILLE. **Long-term Impacts of Silvicultural Treatments on Soil Microbial Biomass, Community Composition, and N Mineralization**
(Under the direction of Jennifer N. Bennett)

This study was conducted in a late-rotation loblolly pine (*Pinus taeda* L.) plantation to assess the effects of silvicultural treatments (site preparation and vegetation control) on (1) microbial biomass, (2) microbial community composition, and (3) nitrogen (N) mineralization in the humus layer (Oa horizon) and upper mineral soil (0-5 cm). An additional objective was to determine relationships among stand characteristics, substrate properties, and the microbial community. The study was established in 1981 in a 22-year-old loblolly pine plantation. Site preparation treatments were chopping and burning and shearing, piling and disking and the vegetation control treatments were no control and complete control with herbicide for the first five years. For this experiment, humus layer and mineral soil samples were collected three times in 2004. To determine microbial biomass, samples were analyzed using the chloroform fumigation extraction method, microbial community composition was evaluated using phospholipid fatty acid (PLFA) analysis and an aerobic incubation was conducted to determine net N mineralization. After 22 years, site preparation and vegetation control still had an effect on microbial biomass, microbial community composition and net N mineralization. Both microbial biomass and microbial community composition were affected by the different plant communities that formed over the life of the rotation and resulted from the interaction of site preparation and vegetation control. The addition of hardwood basal area in plots with no vegetation control was strongly correlated with higher pH and lower C:N ratios which affected the substrate

environment and therefore the soil microbial community. Microbial biomass in the mineral soil was affected by the vegetation control treatment; plots with no vegetation control had higher MBC than plots with no vegetation control (0.63 mg g^{-1} and 0.56 mg g^{-1}, respectively). In the humus layer, bacteria and arbuscular mycorrhizae PLFAs were greatest in plots with no vegetation control, and in the mineral soil, fungi were most abundant in the chop and burn plots with no vegetation control. Microbial biomass carbon and bacteria and arbuscular mycorrhizae PLFA mole percentages were positively correlated with pH and negatively correlated with the C:N ratio of plots with no vegetation control and greater hardwood biomass. In both the mineral soil and the humus layer, the concentration of net mineralized N was affected by site preparation, with the highest rates occurring on the chop and burn plots. The effects of site preparation on net N mineralization may be attributed to the lasting effects of the removal of around 600 kg N ha^{-1} at study establishment from the shear, pile and disked plots. Site preparation and vegetation control influence directly or indirectly, through plant community composition, microbial biomass, the composition of the microbial community, and net N mineralization. These results suggest that the impacts of intensive silvicultural practices do have an impact on the complex interrelationship of plant communities and substrate properties with microbial community characteristics and function.

LONG-TERM IMPACTS OF SILVICULTURAL TREATMENTS ON SOIL MICROBIAL BIOMASS, COMMUNITY COMPOSITION, AND N MINERALIZATION

By

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To James Burger who has taught me more than he knows and to Ben Corl who can always make me smile.

BIOGRAPHY

Julia Camille Burger was born in Lafayette, Indiana but spent most of her formative years in Blacksburg, Virginia. A lifetime Hokie, Julie attended Virginia Tech and received a Bachelor of Science degree in Crop and Soil Environmental Science. She was accepted into the Peace Corps and, shortly after graduation from Virginia Tech, went off to save the world and sample Eastern European pastries. She spent two years in Kardjali, Bulgaria working as an environmental consultant writing grants and teaching both environmental science and English as a second language. After traveling the world she decided to brave the winters of New York State and settled down in the beautiful Finger Lakes region. She found a job at the Schuyler County Soil and Water Conservation District where she worked as a Watershed Resources Specialist. Her job involved many facets of natural resources and she was involved in different projects from installing Best Management Practices on farms, to stream bank restoration, to environmental education. After three years, one wedding, and 10 weeks of road trip across the United States, Julie was ready for graduate school. She was guided through her program by Lee Allen and Jennifer Bennett and along the way even learned a thing or two.

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INTRODUCTION

In the southeastern United States, much of the forest economy is based on intensively managed loblolly pine (*Pinus taeda* L.) plantations (Allen et al., 2005) that supply a large part of the nation's wood and paper. Increasing plantation growth and yield, and sustaining productivity are long-term goals of forest managers. Intensive management encompasses a gradient of silvicultural practices that range from allowing stands to regenerate naturally to treatments that actively manage the target crop, other vegetation, and soil to optimize value (Allen et al., 2005). As yet, we do not fully understand how intensive management influences forest ecosystem processes. Knowledge of nutrient cycling processes and the role of soil microorganisms in those processes, particularly in nitrogen (N) cycling, may improve the scientific basis of forest management decisions, which in turn will improve the productivity and sustainability of forest resources (Salamanca et al., 2002).

Nitrogen has been identified as one of the primary limiting resources in forest systems in the southeastern United States (Allen et al., 1990; Ohtonen et al., 1992; Allen et al., 2001). Studies have shown that intensive forestry practices may decrease (Morris et al., 1983; Fox et al., 1986; Tew et al., 1986), increase (Vitousek and Matson, 1984) or have no effect on N mineralization (Piatek and Allen, 1999). A series of studies were established across the southeastern US to examine the impact of intensive management on forest productivity. Management practices that were tested in these studies included harvesting (whole tree and stem only), site preparation (chop and burn and shear, pile and disk), and herbicide application (no control and complete control with herbicide for the first five years) to control competing vegetation. At one location in the Piedmont of North Carolina, removal of N with

site preparation surpassed the removals associated with harvesting. Shearing, piling and disking removed almost 600 kg N ha^{-1} (Tew et al., 1986) and subsequent studies examining N at the site showed that surface soil net N mineralization rates peaked during the first two years following treatments and have declined since (Vitousek and Matson, 1984; 1985; Vitousek et al., 1992; Piatek and Allen, 1999). Nitrogen in forest soils is found largely in the humus layers of the forest floor and in the top few centimeters of mineral soil (Pritchett and Fisher, 1987). Site preparation practices like shearing and piling lead to the removal of the forest floor and topsoil, in effect removing organic matter and N as well. This N loss may exceed the potential for atmospheric replacement over the complete rotation (Wells and Morris, 1983).

Many studies have described the effects of site management practices on N availability early in the rotation. Net N mineralization has been shown to increase directly after harvesting and/or site preparation (Burger and Pritchett, 1984; Vitousek and Matson, 1985, Vitousek et al., 1992, Knoepp and Swank, 1993; Carter et al., 2002). This increase was attributed to incorporation of organic matter (Burger and Kluender, 1982; Carter et al., 2002), increased soil temperatures and aeration (Vitousek and Matson, 1985), and decreased plant uptake (Smethurst and Nambiar, 1990). Measurements at mid-rotation suggest that net N mineralization rates are lower than shortly after site treatment and still show effects of harvesting and site preparation. Piatek and Allen (1999) determined that in a mid-rotation, 15-year-old, southeastern loblolly pine stand, N mineralization was affected by harvest intensity but not site preparation, and N mineralization rates were almost three times lower than shortly after treatment. This reduction in net N mineralization was attributed to low

phosphorous availability. In a 10-year-old clear-cut stand, Hassett and Zak (2005) also found that the method of harvest negatively affected net N mineralization. Brais et al. (2002) had similar conclusions in northwestern Quebec; at 15 years, net N mineralization was affected by harvesting but not by windrowing. Both groups credited the results to changes in ecosystem characteristics such as litter inputs and microclimate. The majority of studies focus on early- and mid-rotation net N mineralization. A key question when determining site preparation effects on soil properties is the length of time such treatments impact site processes and characteristics, and few studies have documented the long-term impacts of early disturbance on net N mineralization in late-rotation stands (Jurgensen et al., 1997; Goodale and Aber, 2001).

Integral to N cycling in forest ecosystems, soil microorganisms comprise a potential source of plant-available N and they can increase nutrient availability through mineralization of soil organic matter and the solubilization of soil minerals (Jenkinson and Ladd, 1981; Lee and Pankhurst, 1992; Ross and Sparling, 1993). Little is known about the long-term effects of silvicultural practices on microbial communities and their functions. In forest ecosystems, microbial decomposition of organic material is a vital process in N cycling and is fundamental to the long-term functioning of ecosystems (Zak et al., 1994; Pennanen et al., 1999). Most nutrients used by plants, other than those derived directly from mineral weathering, are from the decomposition of organic matter (Marshall, 2000). Site preparation practices like shearing and windrowing remove organic matter and much of the topsoil, which contain a large portion of site nutrients and are the center of microbial activity. Such removals could potentially have a negative effect on the soil microbial community (Pritchett

and Fisher, 1987). Manipulation of soil physical and chemical properties through intensive silvicultural practices has been shown to either decrease (Baath, 1980; Hendrickson et al., 1985; Li et al., 2004; Hassett and Zak, 2005) or have no effect (Ohtonen et al., 1992; Pennanen et al., 1999; Myers et al., 2001; Ponder and Tadros, 2002; Wright and Coleman, 2002) on soil microbial community size. Studies have shown that the composition of the community, that is the representation of bacteria, fungi, and other microorganisms, can also be affected (Ohtonen et al., 1992; Baath et al., 1995; Pennanen et al., 1999; Donegan et al., 2001; Ponder and Tadros, 2001; Li et al., 2004) or not affected (Carter et al., 2002; Hassett and Zak, 2005) by silvicultural treatments.

Intensive management can also indirectly influence belowground ecosystem processes and characteristics by changing the composition of the plant community. The plant community affects nutrient cycling in a variety of ways such as, differences in nutrient uptake, litter quality, and associations with microbes (Hobbie, 1992; Pennanen et al., 1999). Additionally, the soil microbial community is affected by aboveground stand biomass by the light and temperature conditions trees create in the stand as well as root activities and exudates (Nüsslein and Tiedje, 1999; Priha et al., 1999). Roots and leaf litter differ chemically within and among tree species, and differences in plant community structure can influence microbial community composition (Myers et al., 2001; Hackl et al., 2004). Boettcher et al. (1990) found that the effect of even a single tree on soil properties is detectable. In intensively managed loblolly pine plantations, the addition of hardwood species in the stand results in different carbon and N inputs into the belowground environment. In general, slow-decomposing pine litter contains compounds such as lignin in higher quantities than those

found in hardwood leaf litter, which tends to decompose more quickly (McClaugherty et al., 1985). Additionally, root exudates and litter from hardwoods typically have higher contents of water-soluble sugars, organic acids and amino acids (Priha and Smolander, 1997). Within pine and mixed pine/hardwood stands, different substrates could give rise to microbial communities that differ in composition and function (Myers et al., 2001).

Numerous studies have been conducted to investigate early-rotation impacts of silvicultural management on microbial community size and composition (Baath, 1980; Hendrickson et al., 1985; Ohtonen et al., 1992; Baath et al., 1995; Pennanen et al., 1999; Donegan et al., 2001; Myers et al., 2001; Carter et al., 2002; Ponder and Tadros, 2002; Wright and Coleman, 2002; Li et al., 2004; Hassett and Zak, 2005) and N mineralization (Burger and Pritchett, 1984; Vitousek and Matson, 1985; Vitousek et al., 1992; Knoepp and Swank, 1993; Piatek and Allen, 1999; Carter et al., 2002). Additional studies have determined the influence of individual plant species in pure stands on soil microbial biomass, community structure and N mineralization (Graystone and Campbell, 1996; Priha et al., 1999; Myers et al., 2001). There is a lack of information on the impacts of silvicultural treatments and stand composition on microbial biomass, microbial community composition, and net N mineralization in a late-rotation stand.

The objectives of this study were to: (i) determine the influence of post-harvest site preparation and vegetation control on microbial biomass, community composition and net N mineralization in a 22-year-old loblolly pine plantation; and (ii) determine the influence of plant community composition and characteristics on forest floor and mineral soil properties

and microbial community characteristics (biomass, community composition and net N mineralization) as well as the relationships between variables.

METHODS

Site Description and Study

This study was conducted in Vance County, in the lower Piedmont of North Carolina (36˚25'N, 78˚30'W), as a part of a larger long-term site productivity study; a project designed to investigate the effects of plantation management on pine productivity. The study was established in 1981 following the clear-cutting of a 22-year-old loblolly pine plantation. It is located on gently sloping (2 to 10%) Cecil soils (fine, kaolinitic, thermic Typic Kanhapludult). Average annual precipitation is 114 cm and average temperatures range from 2°C in January to 26°C in July. The study consisted of three experimental blocks, each with eight 42 m x 27 m plots established in a 2 x 2 x 2 factorial, randomized split-plot design. Each block had four main plots with the factorial combination of two levels of harvest utilization (stem only: only the bole of the tree was removed and limbs and leaf litter were left on site, and whole tree removal: the entire above-ground tree biomass was removed from the site), and two levels of site preparation (chop and burn (**CH**): a drum roller was used to break up large organic debris followed by a light burn of the site, and shear, pile and disk (**DI**): a blade was used to shear stumps, the organic material was pushed into windrows and the site disked to a depth of 7-12 cm). The chop and burn treatment resulted in the estimated removal of 46 kg N ha^{-1} and the shear, pile and disk treatment removed 591 kg N ha^{-1} (Tew et al., 1986). These two treatments were divided into two subplots and two levels of vegetation control were applied (no control (**NO**) and complete vegetation control (**VC**) using glyphosate once a year for the first five years). One-year-old loblolly pine seedlings were planted at 2 x 3 m spacing in March of 1982.

Stand Characterization

Diameters at breast height of all planted pines within each plot were measured in December, 2003, and all non-planted pine and hardwoods were measured in June of 2004. Stand characteristics and measurements are detailed in Allen et al. (2005). Briefly, chop and burn plots with no vegetation control (CHNO) had the least pine basal area, 26 $m^2 ha^{-1}$, and the greatest hardwood basal area, 16 $m^2 ha^{-1}$. Shear, pile and disk plots with no vegetation control (DINO) also had a fair amount of hardwood basal area (5 $m^2 ha^{-1}$), and a pine basal area of 42 $m^2 ha^{-1}$. Chop and burn and shear, pile, and disk plots with vegetation control (CHVC and DIVC) had the least amount of hardwood basal area (0.2 and 0.3 $m^2 ha^{-1}$, respectively), and the greatest amount of pine basal area (50 and 51 $m^2 ha^{-1}$, respectively) (Figure 1, Table 1).

Mineral Soil and Humus Layer Sampling

In each of the 24 subplots, 10 mineral soil and 10 forest floor (humus (Oa) layer) samples were collected within the first two weeks of June, August, and October, 2004. Plots were sampled three times to capture the extent of any seasonally dynamic effects, not to determine seasonal trends. In each plot, samples were collected from 10 randomly selected locations and composited by plot. To collect samples from the humus layer, the litter (Oi) and fragmented (Oe) layers were removed and discarded from a circular area 23 cm in diameter. Only the humus layer (Oa) was collected with a trowel and placed in a bucket for compositing with the other samples from the plot. The 10 samples were well mixed by hand and one sub-sample per plot was bagged and stored in a cooler. Within each of the 10 areas cleared of organic material, a 5 cm diameter x 5 cm depth (volume = 39.3 cm^3) core of

mineral soil was collected with a bulk density slide hammer and placed in a bucket, and the samples from the plot were mixed and bagged to produce one composite sample per plot. To prevent microbial cross-contamination from one plot to another, after sampling, all equipment was wiped with paper towels and then rubbed with forest floor material or mineral soil (which was then discarded) from the subsequent plot prior to sampling it. All samples were put on ice and transported back to the laboratory at North Carolina State University. In the laboratory, humus layer and mineral soil samples were sieved (2 mm) and stored at 4°C until further preparation and analysis.

In January 2005, 5 randomly selected areas within each of the 24 plots were sampled to determine the total amount of forest floor humus layer (kg ha^{-1}). Using an aluminum sampling ring with an internal area of 730 cm^2, the litter (Oi) and fragmented (Oe) layers were removed and the humus layer (Oa) collected and placed in a paper bag for transport back to the laboratory. Samples were air-dried to constant weight and then weighed. Bulk density cores from the upper 5 cm of mineral soil (volume = 39.3 cm^3) were also collected from 5 randomly selected locations using a hammer-driven core sampler (AMS, Inc.) (Grossman and Reinsch, 2002). Intact cores were placed in separate plastic bags for transport to the lab. In the lab, the cores were weighed and a sub-sample of each was weighed and dried at 105°C for 24 hours. Based on the moisture content, core weight and volume, bulk density was calculated as: weight of oven dry soil (g)/ volume of soil (cm^3). Averaged plot bulk densities were used to scale values to kg ha^{-1}.

Laboratory Analyses

Humus layer and mineral soil sample dry weights were determined by oven drying for 24 hours at 105°C. Volumetric water content was determined by multiplying sample gravimetric weights by the average plot-specific estimates of bulk density.

Soil reaction (pH) of a 1:1 mineral soil: water suspension (1:4 humus layer: water) was determined using a glass electrode (Mettler Toledo DL12 Titrator, Schwerzenbach, Switzerland 1997).

Total carbon and total N in the humus layer and mineral soil were determined on air-dried sub-samples (50 mg of mineral soil and 8 mg humus layer) that were taken from samples collected in June. They were ground and sieved (1 mm) placed in tin capsules, and analyzed using a NC 2100 Elemental Analyzer (Analyzer, 1997, Milan, Italy).

Dissolved organic carbon (DOC) and nitrogen (DON) and microbial biomass carbon (MBC) and nitrogen (MBN) were determined based on the chloroform fumigation extraction method outlined by Brookes et al. (1985). Duplicate sub-samples of each mineral soil (20 g) and humus layer (5 g) sample were weighed into 125 ml Erlenmeyer flasks. One sub-sample of each was placed along with a beaker of 50 ml chloroform in a vacuum dessicator, which was lined with damp paper towels to reduce evaporate moisture loss from the samples. The dessicator was sealed, attached to a vacuum pump and evacuated for 10 minutes to boil the chloroform, and the samples were fumigated for 48 hours. The other sample of each duplicate was extracted immediately with 50 ml of 0.5 *M* K_2SO_4. After shaking for 30

minutes, the samples were filtered gravimetrically with Whatman No. 42 filter paper pre-rinsed with supernatant. Fumigated samples were processed in the same manner after releasing the vacuum and venting for 30 minutes to remove all chloroform. The extracts were stored in the freezer until analysis. Carbon concentrations in the extractions were determined using an automated TOC analyzer (TOC 5050A, Shimadzu Corporation, Kyoto, Japan). Microbial biomass carbon (MBC) was estimated as the difference between carbon in the fumigated and non-fumigated sample extracts divided by the K_2SO_4 extract ion efficiency factor for carbon (K_c= 0.33, Sparling and West, 1988). To determine total nitrogen in the extract, the samples were first processed using the alkaline persulfate oxidation method to convert all N to nitrate (NO_3^-) (Cabrera and Beare, 1993). Briefly, 5 ml of persulfate reagent (in 1000 ml deionized water: 50 g $K_2S_2O_8$, 30 g H_3BO_3 and 15 g NaOH) was added to 5 ml of extract and the mixture was autoclaved at 121°C for 30 minutes. Samples were weighed before and after autoclaving to ensure that there was no significant moisture loss. The oxidized samples were analyzed colormetrically using a Latchet flow injection analyzer (Method 13-107-06-2-D, Quickchem, Latchet Instruments, Mequon, WI). Microbial biomass nitrogen (MBN) was estimated as the difference between the nitrogen content in the extracts from the fumigated and non-fumigated samples divided by the K_2SO_4 extract ion efficiency factor for nitrogen (K_n= 0.45, Jenkinson, 1988).

To determine microbial community composition, phospholipid fatty acid (PLFA) profiles of the microbes in the humus layer and mineral soil were obtained using methods based on Frostegard et al. (1993), as modified by Bossio et al. (1998). After sieving (2 mm), sub-samples of each mineral soil and humus layer sample were freeze-dried for 24 hours.

Phosphate buffer (4.5 ml), chloroform (5 ml), and methanol (10 ml) were added sequentially to the freeze-dried samples (7 g mineral soil and 2 g humus layer), which were then shaken for one hour and centrifuged at 2000 rpm for 30 minutes. The supernatants were decanted into 40 ml glass tubes and additional chloroform (5 ml) and phosphate buffer (4.5 ml) were added sequentially to each tube. Samples were capped and shaken for two minutes. The tubes were then vented and covered and the phases of the supernatant were allowed to separate overnight. The next day, the top layer was aspirated off and the bottom chloroform layer evaporated with nitrogen gas. Solid phase extraction columns (500 mg silica, Fisher Scientific) were used to separate phospholipids from neutral lipids and glycolipids. The column was conditioned with 5 ml chloroform and lipids were transferred to the column with 2 x 3 ml aliquots of chloroform. Neutral lipids and glycolipids were filtered out using 10 ml acetone and discarded. The columns were then positioned over 10 ml glass collection tubes and polar lipids were eluted with 6 ml methanol and then dried with nitrogen gas. Samples were saponified using 1 ml of saponification reagent (45 g sodium hydroxide/150 ml methanol/150 ml deionized water) and heated to 100°C for 25 minutes. They were then methylated with 2 ml methylation reagent (325 ml hydrochloric acid and 275 ml methanol) and heated for 10 minutes at 80°C. Next, samples were extracted with 1.25 ml of extraction solvent (200 ml hexane and 200 ml methyl tert-butyl ether) and the top phase retained. Three milliliters of base wash (10.8 g sodium hydroxide and 900 ml deionized water) were added, the sample rotated, and the top layer transferred to a gas chromotography vial. Lastly, samples were concentrated by evaporation with nitrogen gas and collected with 200 µl of extraction solvent. The vial was capped and analyzed for mole percent fatty acid peaks using a gas chromatograph (Hewlett-Packard 5890A, Hewlett-Packard Inc., Avondale, PA, USA).

PLFA nomenclature is as follows: total carbon atoms: number of double bonds, followed by the position (ω) of the double bond from the methyl end of the molecule. "Cis" and "trans" configurations are indicated by *c* and *t*, and "cy" refers to cyclopropyl fatty acids. The prefixes "a" and "i" indicate anteiso- and iso-branching and 10Me indicates a methyl group on the tenth carbon atom from the carboxyl end of the molecule (Baath et al., 1995). The sum of the fatty acids i15:0, a15:0, 15:0, i16:0, i17:0, a17:0, 17:0, cy17:0, 18:1 ω7 and cy19:0 were used to represent the bacterial PLFAs (Frostegard et al., 1993). Fungal PLFAs were represented with the fatty acid signatures 18:2 ω6, 9c and 18:1 ω7c (Frostegard et al., 1993; Hill et al., 2000). The fungi to bacteria ratio was determined by dividing the sums of fungal and bacterial PLFAs (Frostegård and Bååth, 1996). Actinomycetes were represented with 10Me 18:0, and arbuscular mycorrhizae (AM) with 16:1 ω5c (Olsson et al., 1995; Zogg et al., 1997).

Net N mineralization was determined in the laboratory using an aerobic incubation (Hart et al., 1994). Duplicate sub-samples of each mineral soil (10 g, fresh weight) and humus layer (2.5 g, fresh weight) sample were weighed into centrifuge tubes. One sample of each duplicate was lightly covered with plastic wrap and incubated for 28 days at 25ºC. A beaker of deionized water was placed in the incubator with the samples to maintain humidity and reduce water loss from samples. Once a week, samples were weighed to ensure that moisture content did not change by more than 5%. The other sample of each pair was immediately extracted (to determine extractable nitrogen) with 25 ml of 2*M* KCl, and the samples were shaken on a mechanized shaker and then centrifuged at 4000 rpm for 15 minutes. The supernatant was vacuum filtered with Whatman No. 42 filter paper, pre-soaked in 2*M* KCl

and rinsed with deionized water. After 28 days, the incubated samples were extracted and filtered using the same method. Total ammonium and NO_3^- concentrations in the sample extracts were determined colorimetrically (Methods 12-107-04-1-B and 12-10-7062-A, Quickchem, Latchet Instruments). Both extractable N and net N mineralization were reported as concentration and on a kilogram per hectare basis to discuss the amount of available and potentially available N.

Statistical Analyses

Treatment effects on variables sampled only once (total humus mass and total carbon and total N) were tested with analysis of variance (ANOVA) for split-plot design (SAS Procedures Guide, 1988). Data analysis involved a split-plot ANOVA with whole plots of the harvest x site preparation factorial, and sub-plots of vegetation control (n=24). Initial analyses indicated no significant harvest effects so the harvest effect was removed from the model and pooled into the main plot error. For variables measured three times (pH, moisture content, DOC and DON, MBC and MBN, and mol% of bacteria, fungi, actinomycetes, and AM, and extractable and mineralized N), treatment effects were analyzed using the general linear model procedure (GLM) for split-split plot design (SAS Institute, 1985). Sampling date was considered a random variable as seasonal trends were not of interest in this study. Analysis of the data was conducted as a split-split plot with the study's regular split-plot design nested within sampling date (n=72). Standard errors were calculated as described in Steel and Torrie (1980). Correlations among the measured characteristics were analyzed to evaluate relationships among microbial community characteristics and other substrate and stand properties. Significance was accepted at $p \leq 0.10$.

RESULTS

Block effects were more pronounced in the mineral soil than in the humus layer. In the humus layer only total carbon and total N (both on a percent and per hectare basis) and pH showed any block effect (Table 2). Total carbon and total N were highest in block C, and pH was lowest in block C and highest in block A. In contrast, most of the variables in the mineral soil differed significantly among blocks. Moisture content, pH, MBC and MBN were all lowest in block C and highest in block A. The C:N ratio was opposite with the highest values occurring in block C and lowest values in block A. Total N was lowest in block C and the other two blocks were not different. Conversely, DOC was highest in block C, and the other two blocks were not different. Dissolved organic nitrogen was lowest in block B and the other two blocks were not different.

Humus Layer

Various factors in the humus layer were not affected by treatment (Table 2) including percent total carbon and total N whose values ranged from 24 to 27% and 0.82 to 0.88%, respectively (Table 1). However, the ratio of total carbon to total N did differ among treatments (Table 2); with the lowest values (29:1) occurring in plots with no vegetation control (CHNO and DINO) and plots with chop and burn (CHNO and CHVC) site preparation (Table 1). Many variables followed the same pattern of response, with the chop and burn plots with no vegetation control (CHNO) differing significantly from the other treatments as indicated by a significant site preparation x vegetation control interaction. Total humus layer mass accumulation was lowest (8,500 kg ha^{-1}) in CHNO plots, half as much mass as the other three treatments (≈ 17,000 kg ha^{-1}) (Table 1). Similarly, on a per hectare basis, total N differed by

the treatment interaction and was lowest in the CHNO plots (75 kg ha^{-1}) compared to the other three treatments (≈ 140 kg ha^{-1}). Total carbon on a per hectare basis ranged from 2100 to 4700 kg ha^{-1} and was affected by both site preparation and vegetation control. The highest values of total carbon occurred in the shear, pile and disk plots (4350 kg ha^{-1}) and plots with vegetation control (4450 kg ha^{-1}). Moisture content was lowest in CHNO plots (38%), and the two vegetation control treatments (CHVC and DIVC) were not significantly different (both, 43%) (Table 1). On the other hand, the highest pH values were found on CHNO plots (4.64), and the most intensively managed plots, DIVC, were the most acidic (4.09) (Table 1). Dissolved organic carbon was also highest on the CHNO plots (1.3 mg g^{-1}), and the lowest values occurred in DIVC plots (0.81 mg g^{-1}) (Table 1).

Unlike DOC, DON was significantly affected by vegetation control but did not have a site preparation x vegetation control interaction (Table 2). Plots with no vegetation control had higher DON (0.11 mg g^{-1}) than those with vegetation control (0.08 mg g^{-1}) (Table 1).

Microbial biomass carbon and N did not differ by treatment (Table 2). However, the relative content of individual microbial groups within the community did show a treatment influence (Table 4). Bacteria and AM both had larger representation in the plots with no vegetation control (31.9% and 2.2%, respectively) than in plots with vegetation control (28.9% and 1.6%, respectively) (Table 3). Actinomycetes represented the smallest group and differed by site preparation. Higher values were found in the chop and burn plots (0.51%) than in the shear, pile and disk sites (0.37%) (Table 3). The PLFA mol % values for fungi

did not differ by treatment (Table 4), and the ratio of fungi to bacteria was highest in plots with vegetation control (0.57) and lowest in plots with no vegetation control (0.51) (Table 3).

Hardwood basal area was correlated with many of the humus layer characteristics (Table 5). Humus mass, moisture content, and C:N ratio were negatively correlated with hardwood basal area (r = -0.64, -0.49, and -0.75, respectively), and pH, DOC and DON were positively correlated (r = 0.48, 0.77, and 0.57, respectively) (Table 5). Soil activity (pH) also showed a significant correlation to the C:N ratio, DOC and DON (r = -0.58, 0.37, and 0.78, respectively) (Table 5).

Microbial community characteristics also showed strong correlations to stand and site factors (Table 5). Microbial biomass carbon was positively correlated with hardwood basal area, pH, DOC and DON (r = 0.48, 0.44, 0.40, and 0.45) and negatively correlated to humus mass and the C:N ratio (r = -0.45 and -0.54) (Table5). The relative amounts of bacteria and AM were both positively correlated with hardwood basal area (r = 0.56 and 0.37), pH (r = 0.85 and 0.84), DON (r = 0.65 and 0.63) and MBC (r = 0.65 and 0.41), and were negatively correlated with the C:N ratio (r = -0.71 and -0.54) (Table 5). Bacteria were also positively correlated with DOC (r = 0.41) (Table 5). Actinomycetes were positively correlated with hardwood basal area (r = 0.46) and MBC (r = 0.36), and unlike the other community constituents, were negatively correlated with humus mass (r = -0.36) and moisture content (r = -0.46) (Table 5). Unlike bacteria and AM, fungi were negatively correlated with pH (r = -0.36) and positively correlated with DOC (r = 0.34) (Table 5). Lastly, the fungi to bacteria

ratio was positively correlated with the C:N ratio (r = 0.41) and negatively correlated with pH, DON, and MBC (r = -0.71, -0.45, and -0.34) (Table 5).

Extractable N concentration ranged from 14 to 39 μg g^{-1} and had a site preparation x vegetation control interaction (Tables 1 and 2); chop and burn plots with no vegetation control (CHNO) had the highest concentration of extractable N (39 μg g^{-1}), the shear, pile, and disk plots with no vegetation control (DINO) had less (21 μg g^{-1}), and the two treatments with vegetation control (CHVC and DIVC) had the least amount and were not different from each other (both, 14 μg g^{-1}) (Table 1). On a kilogram per hectare basis, extractable N differed by vegetation control (Table 2). Plots with no vegetation control had more extractable N (0.3 kg ha^{-1}) than those with vegetation control (0.2 kg ha^{-1}) (Table 1). Net mineralized N concentrations ranged from 73.2 to 91.9 μg g^{-1}, and chop and burn plots had higher concentrations (89.4 μg g^{-1}) than the shear, pile and disk plots (67.1 μg g^{-1}) (Table 1). On a per hectare basis, net mineralized N ranged from 0.7 to 1.4 kg ha^{-1}, and within site preparation, vegetation control behaved differently; CHVC plots had twice as much net N mineralization per hectare (1.4 kg ha^{-1}) as CHNO plots (0.7 kg ha^{-1}), and DIVC plots had more N than DINO plots (1.2 and 1.0 kg ha^{-1}, respectively) (Table 1).

Extractable N concentrations were negatively correlated with total humus mass (r = -0.50) and the C:N ratio (r = -0.52) and positively correlated with hardwood basal area (r = 0.63), pH (r = 0.65), DOC and DON (r = 0.59 and 0.46) (Table 7). Extractable N on a kilogram per hectare basis was positively correlated with pH (r = 0.59) and DON (r = 0.45) (Table 7). Net N mineralization concentration was not positively correlated with any of the stand or humus

layer characteristics, however, on a per hectare basis net N mineralization was negatively correlated with DOC (r = -0.46) (Table 7).

Mineral Soil

Unlike the humus layer, the mineral soil factors did not show consistent responses to treatment. Soil moisture content was lower in chop and burn plots (14%) than shear, pile and disk plots (15%) (Table 1). Percent total carbon showed an opposite trend. Plots that were chopped and burned had a higher percent total carbon than the shear, pile and disk plots (3 and 2%, respectively) (Table 1). Percent total N was different by vegetation control (Table 2). Plots with vegetation control had less (14%) N than the plots with no vegetation control (16%) (Table 1). The C:N ratio was opposite, with higher values in plots with vegetation control (20:1) than those with no vegetation control (18:1) (Table 1). On a per hectare basis, both total carbon and total N were affected by site preparation. Chopped and burned plots had more total carbon and total N (20,050 and 1045 kg ha^{-1}) than shear, pile and disk plots (15,300 and 865 kg ha^{-1}). Total N on a per hectare basis was also affected by vegetation control. Plots with no vegetation control had higher total N (1035 kg ha^{-1}) than plots with vegetation control (875 kg ha^{-1}). Dissolved organic carbon and DON concentrations behaved similarly to one another. Both had higher values in the chop and burn plots (1.11 and 0.10 mg g^{-1}) than in shear pile and disk plots (0.88 and 0.09 mg g^{-1}) (Table 1). Plots with no vegetation control had more DOC and DON (1.14 and 0.11 mg g^{-1}) than plots with vegetation control (0.85 and 0.08 mg g^{-1}) (Table 1). In the mineral soil, pH did not differ by treatment (Table 2).

The microbial community behaved differently in the mineral soil than in the humus layer. In the mineral soil, MBC and MBN were affected by vegetation control (Table 2). Plots with no vegetation control had more MBC (0.63 mg g^{-1}) and MBN (0.05 mg g^{-1}) than plots with vegetation control (0.56 mg g^{-1} and 0.04 mg g^{-1}, respectively) (Table 1).

The relative contents of the individual microbial groups were also different in the mineral soil. Fungi were affected by the site preparation x vegetation control interaction (Table 4). The mol% of fungal PLFAs was greatest in the least intensively managed plots (11.8%) (CHNO), and the two treatments with vegetation control (CHVC and DIVC) were not different (Table 3). Arbuscular mycorrhizae had higher representation (3.1%) in shear, pile and disk plots than chop and burn plots (2.7%) and plots with no vegetation control had higher values (3.0%) than plots with vegetation control (2.8%) (Table 3). Unlike the other groups, bacteria and actinomycetes values were not affected by treatment (Table 4). The fungi to bacteria ratios were overall much lower in the mineral soil than in the humus layer. Among the treatments, the highest ratio (0.29) occurred in plots with no vegetation control and the lowest ratio (0.27) occurred in the plots with vegetation control (Table 3).

Unlike the strong relationship with humus layer characteristics, hardwood basal area was correlated only with DOC and DON in the mineral soil ($r = 0.44$ and 0.41) (Table 6). In contrast, soil organic matter and moisture contents were strongly correlated with many of the other mineral soil characteristics (Table 6). Organic matter content was positively correlated with moisture content, pH, total carbon and total N, and DON ($r = 0.70$, 0.57, 0.84, 0.91, and 0.62) and negatively correlated to the C:N ratio ($r = -0.34$) (Table 6). Moisture content was

also positively correlated to pH, total carbon, total N, and DON (r = 0.90, 0.35, 0.80, and 0.47) and like organic matter, negatively correlated to the C:N ratio and DOC (r = -0.68 and -0.40) (Table 6).

Microbial community characteristics showed very strong relationships to stand and site characteristics (Table 6). Microbial biomass carbon was positively correlated to organic matter content (r = 0.83), moisture content (r = 0.83), total carbon (r = 0.55), total N (r = 0.87), pH (r = 0.80), and DON (r = 0.41) and was negatively correlated with the C:N ratio (r = -0.66) (Table 6). Individual components of the community, bacteria and AM, acted similarly. Both were positively correlated to organic matter content (r = 0.41 and 0.35), moisture content (r = 0.68 and 0.82), pH (r = 0.75 and 0.87), total N (r = 0.56 and 0.56), MBC (r = 0.60 and 0.66), and MBN (r = 0.63 and 0.71), and negatively correlated to both the C:N ratio (r = -0.67 and -0.80) and DOC (r = -0.50 and -0.50) (Table 6). The mol% of AM was also positively correlated to DON (r = 0.36) (Table 6). Similar to bacteria and AM, actinomycetes were positively correlated to organic matter content, total carbon, total N, DON, and MBN (r = 0.38, 0.37, 0.40, 0.39, and 0.34) (Table 6). In contrast, the mol % of fungi was negatively correlated with organic matter content and moisture content (r = -0.61 and -0.72), total N (r = -0.62), and pH (r = -0.80) and positively correlated to hardwood basal area (r = 0.39), the C:N ratio (r = 0.47), and DOC (r = 0.56) (Table 6). The fungi to bacteria ratio was negatively correlated with organic matter content, moisture content, pH, total N, MBC and MBN (r = -0.59, -0.79, -0.82, -0.64, -0.69, and -0.72) and was positively correlated to the C:N ratio and DOC (r = 0.54 and 0.56) (Table 6).

Extractable N concentrations (values ranged from 2.0 to 4.6 µg g^{-1}) and extractable N on a per hectare basis (1.3 to 2.8 kg ha^{-1}) were similar (Table 1). Both were higher in plots with no vegetation control (3.8 µg g^{-1} and 2.4 kg ha^{-1}) than in plots with vegetation control (2.7 µg g^{-1} and 1.5 kg ha^{-1}) (Table 1). Net mineralized N concentration (values ranged from 4.8 to 7.8 µg g^{-1}) and on a per hectare basis (2.9 to 5.2 kg ha^{-1}) were also similar to one another (Table 1). Chop and burn plots had higher concentrations of net N mineralization (7.3 µg g^{-1} and 4.9 kg ha^{-1}) than shear pile and disk plots (5.0 µg g^{-1} and 3.1 kg ha^{-1}) (Table 1).

Extractable N concentration and extractable N on a per hectare basis were both positively correlated with moisture content (both, $r = 0.62$), pH (both, $r = 0.60$), and total N ($r = 0.59$ and 0.58) and both were negatively correlated with the C:N ratio ($r = -0.47$ and -0.49) (Table 8). Net mineralized N concentration and net mineralized N on a per hectare basis were not correlated with any of the stand or mineral soil characteristics (Table 8).

DISCUSSION

After 22 years, imposed silvicultural treatments, site preparation and vegetation control, still had significant effects on the properties and processes of both the humus layer and surface mineral soil (Table 2). Numerous early-rotation studies at the same site showed treatment effects on properties such as net N mineralization, soil physical properties, and vegetation composition and abundance (Vitousek and Matson 1984, 1985; Gent et al., 1984; Vitousek et al., 1992; Tew et al., 1986; Piatek and Allen, 1999; Jefferies, 2002). Unlike the earlier studies, this late-rotation evaluation of the site found that harvest was not a significant factor most likely because the disturbance associated with harvesting was minor compared to site preparation (Tew et al., 1986) and any residual effects were too small to detect.

In addition to the apparent effects of site preparation and vegetation control and their interaction on the properties and processes of the humus layer and upper mineral soil, the aboveground biomass and plant community composition that developed over the rotation was also very influential. Pine and hardwood basal areas were both significantly affected by the interaction of site preparation and vegetation (Allen et al., 2005) (Figure 1), and the plant community that formed as a result of the initial treatments has had a significant influence on the microbial community. The CHVC and DIVC plots were almost pure pine stands with the highest pine basal area (50 m^2ha^{-1}) of the four treatments and negligible hardwood basal area ($\approx$ 0.3 m^2ha^{-1}) (Table 1). The DINO plots had an intermediate level of hardwood and pine basal area (5 and 42 m^2ha^{-1}), and the CHNO plots had the least total basal area with the most hardwood and least amount of pine (16 and 26 m^2ha^{-1}) (Table 1). Similar to aboveground biomass, almost three-quarters of the factors measured in the humus layer and mineral soil

were affected by vegetation control and/or the interaction of site preparation and vegetation control. Of the site properties that showed an interaction effect, plots with the CHNO treatment were often the most different from the other treatment interactions.

One prominent effect of plant community composition on site properties was total humus mass. As the substrate for microbial communities, the quantity and quality of the humus layer is important. It was not surprising that the humus layer itself was strongly influenced by aboveground biomass since it developed with the plant community over the term of the rotation. As noted above, the CHNO plots tended to be different than the other treatments and, in this case, had half as much humus mass accumulation as the others (Figure 1). This was the result of the quality of the litter on the CHNO plots. More hardwood basal area would suggest that the litter layer was subject to faster decomposition and different litter properties. At the same study site at year 15, Piatek (1997) found that the hardwood litter decomposed faster than pine litter. Specifically, hardwood litter on the CHNO plots had the fastest rate of decay, and pine litter on the plots with vegetation control (CHVC and DIVC) decayed at the slowest rate. Prescott et al. (2000) found similar trends in mixed wood forests in British Columbia; litter decomposed faster in broadleaf forests compared to coniferous forests and was attributed to more active soil fauna. McClaugherty et al. (1995) state that, in general, pine litter decomposes slowly because it contains higher quantities of lignin than hardwood leaf litter, which tends to break down more quickly.

The properties of the humus layer and mineral soil also differed with vegetation control treatments and were influenced by the differences in the plant community. In both the humus

layer and mineral soil, moisture content was lowest and DOC highest in the CHNO plots (Table 1). Additionally, humus layer pH was also highest in the CHNO plots (Table 1). Changes in humus layer and mineral soil properties, such as higher DOC and pH, and lower C:N ratio (in plots with no vegetation control), were strongly correlated with hardwood basal area (Tables 5 and 6). Similar trends are also seen in various other studies (Thomas and Prescott, 2000; Smolander and Kittunen, 2002; Templer et al., 2003). These differences in humus layer and mineral soil properties across treatments suggest that there were variations in the microbial environment that influenced microbial biomass, community composition and net N mineralization.

Microbial Biomass

Although MBC in the humus layer was not affected by treatment, the mean concentrations followed the significant effects found in the mineral soil, where MBC concentrations were higher in plots with no vegetation control and greater hardwood basal area (Table 1). In the mineral soil, MBC was negatively correlated with the C:N ratio and positively correlated with pH (Table 6), confirming that MBC is affected by the environment created by the presence of hardwoods. Priha et al. (2001) found that MBC was highest under a hardwood stand (silver birch) compared to a coniferous stand (Norway spruce). They associated the stimulatory effects of birch on soil microbes to a faster litter decomposition rate, thermal and light conditions under the canopy, and greater amounts of labile C from root exudates. In a similar study, Smolander and Kittunen (2002) also found higher MBC in hardwood stands than in conifer stands and credited the differences to either the chemical composition of leaf litter or root distribution and activity.

Microbial Community Composition

The microbial community composition differed between the humus layer and mineral soil, but in both cases was influenced by vegetation. The ratio of fungi to bacteria was twice as high in the humus layer as the mineral soil (Table 3). The differences in the ratios in the humus layer and mineral soil can be attributed, in part, to the differences in the properties of the two strata. Individual components of the community also behaved differently between the humus layer and mineral soil. Fungi had greater representation in the humus layer while bacteria and actinomycetes had greater representation in the mineral soil (Table 3). The behavior among the four community constituents was also most likely due to the fact that each constituent has different environmental requirements and the two substrates, the humus layer and mineral soil, had dissimilar pH and C:N ratios. The humus layer was, on average, more acidic and had a higher C:N ratio than the mineral soil and that environment tends to favor fungi (Killham 1995; Paul and Clark 1996). In fact, fungi had a negative correlation to pH ($r = -0.36$) (Table 5) suggesting that as pH decreased, fungi increased. The opposite was true for bacteria and actinomycetes which tend to favor the conditions that were found in the mineral soil, such as higher pH levels and lower C:N ratios (Killham 1995). Arbuscular mycorrhizae in the humus layer and mineral soil had the highest representation in plots with no vegetation control (Table 3). This was almost certainly due to greater hardwood biomass on those plots, as most hardwood species are predominantly associated with AM (Olsson et al., 1995; Sumner, 1999). Grayston and Prescott (2005) attributed the differences in the total biomass and composition of microbial communities in forest floor layers (F and H) under different tree species to pH, moisture content and fine root exudates. They postulated that

the higher pH levels in cedar plots favored the growth of bacteria compared to Douglas-fir and spruce sites. Likewise, Leckie at al. (2004) found significant differences in the abundance and proportion of constituents of the microbial community under two different mixed stands (cedar-hemlock and hemlock-amabilis) and greater fungal biomass was credited to higher C:N ratios in the cedar-hemlock stands. Brady and Weil (1996) have also stated that bacterial competition is favored in hardwood leaf litter which has a lower C:N ratio and higher pH and, in contrast, fungi dominate the microbial activity in acidic, higher C:N coniferous forest litter.

Nitrogen Availability

Effects of site preparation and vegetation control on extractable N and net N mineralization were still apparent 22 years after treatment implementation. Similar to microbial biomass and community composition, extractable N concentrations in the humus layer and mineral soil were also affected by the differences in the plant communities. In contrast, net N mineralization in both layers showed a site preparation effect (Table 2) that may be attributed to organic matter removals during the installation of the treatments.

Extractable N concentration, indicative of the present condition of the substrate, was highest in the plots with no vegetation control in both the humus layer and mineral soil (Table 1). Again, as with the microbial factors, this may be attributed to the presence of greater hardwood biomass on the plots with no vegetation control which, in turn, produce more decomposable litter with a lower C:N ratio. Lovett et al. (2004) who looked at N cycling in northern hardwood forests found that the substrate under eastern hemlock had a high C:N

ratio and low extractable N, and sugar maple plots had the lowest C:N ratio with the highest levels of extractable N. Thomas and Prescott (2000) also showed that extractable N concentrations were the lowest and C:N ratios highest under Douglas-fir compared to other tree species (lodgepole pine and paper birch).

Although net N mineralization concentrations in the humus layer were highest in chop and burn plots, these same plots had the lowest N mineralization per hectare because they had half as much humus mass accumulation as the other treatments (Table 1). In contrast, N mineralization concentrations and amounts on a per hectare basis in the mineral soil were similar due to the similarity of bulk density values across plots (Table 1). In both the mineral soil and the humus layer, net N mineralization concentrations were affected by site preparation with the highest rates occurring on the chop and burn plots (Table 1). Also, in the mineral soil total carbon and DOC were higher in the chop and burn plots and were positively correlated to net N mineralization (Table 6). The effects of site preparation on net N mineralization may be attributed to the lasting effects of the removal of 30 t ha^{-1} of forest floor and almost 180 t ha^{-1} of topsoil which resulted in the loss of around 600 kg N ha^{-1} from the shear, pile and disked plots during site preparation (Tew et al., 1986). In fact, after 22 years, those plots still have less total nitrogen in the top 5 cm of mineral soil than the chop and burn plots (865 versus 1045 kg N ha^{-1}). These results provide the first direct evidence to support the hypothesis proposed by many researchers that losses of organic matter and nitrogen during site preparation may result in long-term nitrogen limitations (Burger and Kluender, 1982; Morris and Lowery, 1988; Allen et al., 1990; Thornley and Cannell, 1992; Attiwell and Adams, 1993).

Net N mineralization estimated at the same site early in the rotation (years 1, 2, and 5) by Vitousek et al. (1984, 1985, 1992) and at mid-rotation (year 15) by Piatek and Allen (1999) show both similarities and differences with the results measured 22 years after study establishment. The significance of harvest intensity effects varied; in years 1 and 2 and at 15 years there was a significant effect, but not in years 3-5 or at 22 years. The effects of harvest in the first two years were attributed to the removal of N-rich foliage and branches (Vitousek and Matson, 1985), and those effects converged in years 3-5 due to the decomposition of the organic matter that was left on the site (Vitousek et al., 1992). The harvest effects observed at year 15 by Piatek and Allen (1999) were attributed to a possible phosphorous deficiency. Significant site preparation and vegetation control effects were found across all dates. While Vitousek and Matson (1984, 1985) showed an increase in net N mineralization on DI plots in the first two years, by year 5, the highest rates occurred on CHNO plots. Although the methods used to determine net N mineralization in mineral soil were different in each study, the same trend has continued from years 5, 15 and 22 with CHNO plots showing the highest rates of net N mineralization.

Site preparation and vegetation control influence directly or indirectly, through plant community composition, microbial biomass, the composition of the microbial community, and nitrogen cycling. These results suggest that intensive silvicultural practices do have an impact on the complex interrelationship of plant communities and substrate properties with microbial community characteristics and functions.

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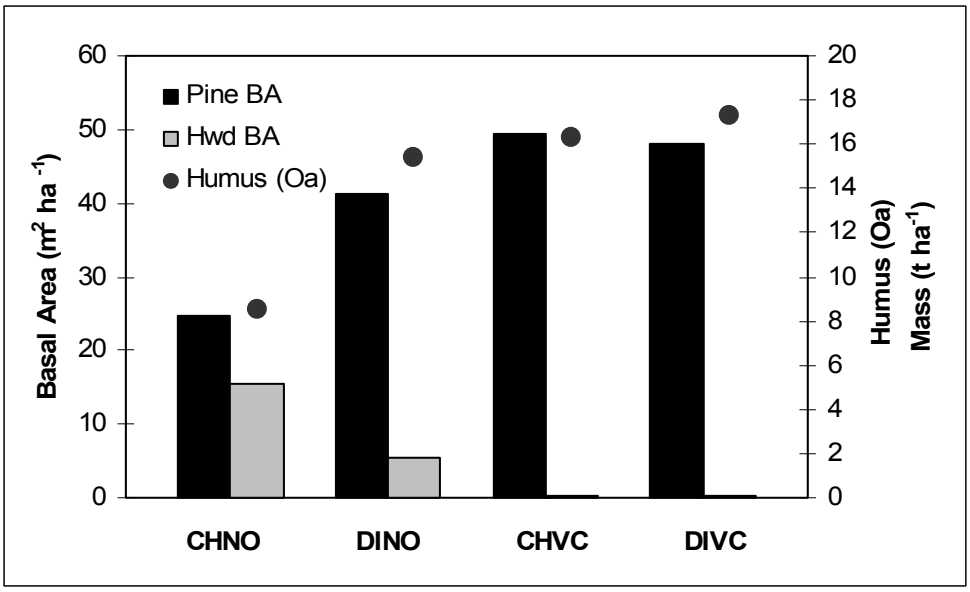

Figure 1: Pine and hardwood basal area and humus layer (Oa horizon) mass accumulation.

Table 1: Treatment means for site preparation and vegetation control effects on stand, humus layer and mineral soil characteristics in a 23-year old loblolly pine plantation.

	CH		DI		*SE*	*Significant Effects*
STAND CHARACTERISTICS	**NO**	**VC**	**NO**	**VC**		
Pine Basal Area ($m^2\ ha^{-1}$)	25.6	50.5	42.3	49.6	5.5	P, C, PxC
Hardwood Basal Area ($m^2\ ha^{-1}$)	15.6	0.2	5.4	0.3	2.6	P, C, PxC
HUMUS LAYER (Oa horizon)						
Humus Accumulation (kg ha^{-1})	8500	16000	15000	17000	3000	P, C, PxC
Total Carbon (%)	24.4	27.0	26.2	27.3	4.0	
Total Nitrogen (%)	0.87	0.88	0.86	0.82	0.12	
Total Carbon (kg ha^{-1})	2100	4200	4000	4700	900	P, C
Total Nitrogen (kg ha^{-1})	75	139	132	143	27	P, C, PxC
C:N Ratio	28	31	30	30	1.3	P, C
pHw	4.64	4.16	4.40	4.09	0.16	P, C, PxC
Moisture Content (%)	38	43	41	43	0.03	P, PxC
Dissolved Organic Carbon (mg g^{-1})	1.33	0.90	0.94	0.81	0.22	P, C, PxC
Microbial Biomass Carbon (mg g^{-1})	5.01	4.22	4.38	4.04	0.90	
Dissolved Organic Nitrogen (mg g^{-1})	0.11	0.08	0.10	0.08	0.03	C
Microbial Biomass Nitrogen (mg g^{-1})	0.30	0.27	0.27	0.25	0.07	
Extractable N ($\mu g\ g^{-1}$)	39.5	14.0	20.9	13.7	9.0	P, C, P x C
Extractable N (kg ha^{-1})	0.34	0.23	0.32	0.22	0.13	C
Mineralized N ($\mu g\ g^{-1}$)	69.7	70.6	66.3	73.2	17.3	P
Mineralized N (kg ha^{-1})	0.74	1.38	0.96	1.23	0.41	C, P x C

Values are means ± SE, n=72
Significant effects are indicated by: P (Site preparation), C (vegetation control) or P x C (site preparation x vegetation control interaction) ($p \leq 0.10$)

Table 1 (continued)

	CH		DI		*SE*	*Significant Effects*
MINERAL SOIL (0-5 cm)	**NO**	**VC**	**NO**	**VC**		
Total Carbon (%)	3.0	3.1	2.6	2.3	0.5	P
Total Nitrogen (%)	0.2	0.2	0.2	0.1	0.0	C
Total Carbon (kg ha^{-1})	19900	20200	16000	14600	2800	P
Total Nitrogen (kg ha^{-1})	1100	990	970	760	150	P, C
C:N Ratio	19	21	18	20	1.5	C
pHw	4.8	4.8	4.8	4.8	0.1	
Moisture Content (%)	0.14	0.14	0.16	0.14	0.0	P, PxC
Dissolved Organic Carbon (mg g^{-1})	0.17	0.15	0.14	0.13	0.02	P, C
Microbial Biomass Carbon (mg g^{-1})	0.64	0.59	0.63	0.52	0.11	P, C
Dissolved Organic Nitrogen (mg g^{-1})	0.03	0.02	0.03	0.02	0.00	P, C
Microbial Biomass Nitrogen (mg g^{-1})	0.04	0.04	0.05	0.04	0.01	C
Extractable N (μg g^{-1})	2.9	3.4	4.6	2.0	1.9	C
Extractable N (kg ha^{-1})	1.9	1.6	2.8	1.3	1.1	C
Mineralized N (μg g^{-1})	7.8	6.9	4.8	5.2	3.0	P
Mineralized N (kg ha^{-1})	5.2	4.6	2.9	3.3	1.9	P

Values are means ± SE, n=72
Significant effects are indicated by: P (Site preparation), C (vegetation control) or P x C (site preparation x vegetation control interaction) ($p \leq 0.10$)

Table 2: ANOVA p-values for date, block and treatment effects on stand, humus layer and mineral soil characteristics in a 23-year old loblolly pine plantation.

STAND CHARACTERISTICS	Date	Block	Site Preparation	Vegetation Control	Site Preparation x Vegetation Control
Pine Basal Area	***	0.002	0.002	0.003	0.008
Hwd Basal Area	***	0.597	0.009	0.000	0.004
HUMUS LAYER (Oa horizon)					
Humus Accumulation	***	0.220	0.072	0.001	0.040
Total Carbon (%)	***	0.056	0.550	0.445	0.737
Total Nitrogen (%)	***	0.064	0.554	0.859	0.680
Total Carbon (kg ha^{-1})	***	0.089	0.031	0.013	0.168
Total Nitrogen (kg ha^{-1})	***	0.063	0.055	0.017	0.066
C:N ratio	***	0.492	0.010	0.001	0.827
pHw	0.108	0.014	0.011	<0.0001	0.089
Moisture Content	0.222	0.596	0.006	0.190	0.050
DOC	0.090	0.613	0.001	0.001	0.058
MBC	0.019	0.819	0.182	0.279	0.380
DON	0.003	0.297	0.570	0.000	0.523
MBN	0.450	0.698	0.234	0.486	0.956
Extractable N (μg g^{-1})	0.120	0.609	0.007	<0.0001	0.001
Extractable N (kg ha^{-1})	0.073	0.195	0.772	0.005	0.986
Mineralized N (μg g^{-1})	0.011	0.173	0.008	0.964	0.238
Mineralized N (kg ha^{-1})	0.007	0.209	0.776	<0.0001	0.075

*** indicates samples that were measured at only one date

Table 2 (continued)

MINERAL SOIL (0-5 cm)	Date	Block	Site Preparation	Vegetation Control	Site Preparation x Vegetation Control
Bulk Density	***	0.451	0.163	0.920	0.439
Total Carbon	***	0.226	0.067	0.457	0.219
Total Nitrogen	***	0.019	0.278	0.032	0.186
Total Carbon (kg ha^{-1})	***	0.252	0.026	0.522	0.342
Total Nitrogen (kg ha^{-1})	***	0.109	0.012	0.043	0.295
C:N Ratio	***	0.000	0.128	0.028	0.792
pHw	0.117	0.000	0.202	0.271	0.128
Moisture Content	0.026	0.010	0.090	0.300	0.110
DOC	0.001	0.002	0.000	0.030	0.971
MBC	0.066	0.002	0.106	0.034	0.659
DON	<0.0001	0.026	0.028	0.002	0.316
MBN	0.001	0.001	0.778	0.085	0.412
Extractable N (μg g^{-1})	0.140	0.055	0.496	0.007	0.106
Extractable N (kg ha^{-1})	0.129	0.055	0.610	0.007	0.130
Mineralized N (μg g^{-1})	0.002	0.359	0.040	0.825	0.431
Mineralized N (kg ha^{-1})	0.001	0.358	0.014	0.850	0.336

*** indicates samples that were measured at only one date

Table 3: Treatment means for site preparation and vegetation control for mole percentages of bacteria, fungi, actinomycetes, and arbuscular mycorrhizae in the humus layer and mineral soil of a 23-year old loblolly pine plantation.

	CH		DI		*SE*	*Significant Effects*
HUMUS LAYER (Oa horizon)	**NO**	**VC**	**NO**	**VC**		
Bacteria (mol %)	32.5	28.9	31.3	28.9	2.3	C
Fungi (mol %)	16.5	16.3	15.8	16.1	1.5	
Arbuscular Mycorrhizae (mol %)	2.2	1.6	2.2	1.6	0.5	C
Actinomycetes (mol %)	0.56	0.46	0.41	0.32	0.24	P
Fungi: Bacteria	0.51	0.57	0.51	0.56	0.07	C
MINERAL SOIL (0-5 cm)						
Bacteria (mol %)	38.1	37.9	39.4	39.2	2.2	
Fungi (mol %)	11.8	10.4	10.4	10.4	0.8	C, P x C
Arbuscular Mycorrhizae (mol %)	2.8	2.6	3.2	2.9	0.3	P, C
Actinomycetes (mol %)	2.8	2.9	2.9	2.8	0.8	
Fungi: Bacteria	0.31	0.27	0.26	0.27	0.03	C

Values are means ± SE, n=72
Significant effects are indicated by: P (Site preparation), C (vegetation control) or P x C (site preparation x vegetation control interaction) ($p < 0.10$)

Table 4: ANOVA p-values for block and treatment effects on mole percentages of bacteria, fungi, arbuscular mycorrhizae, and actinomycetes in the humus layer and mineral soil of a 23-year old loblolly pine plantation.

HUMUS LAYER (Oa horizon)	Date	Block	Site Preparation	Vegetation Control	Site Preparation x Vegetation Control
Bacteria	0.184	0.146	0.467	<0.0001	0.275
Fungi	0.272	0.556	0.308	0.816	0.612
Arbuscular Mycorrhizae	0.765	0.009	0.945	<0.0001	0.991
Actinomycetes	0.036	0.274	0.085	0.255	0.886
Fungi:Bacteria	0.030	0.057	0.913	0.010	0.953
MINERAL SOIL (0-5 cm)	Date	Block	Site Preparation	Vegetation Control	Site Preparation x Vegetation Control
Bacteria	0.639	0.186	0.213	0.657	0.476
Fungi	0.102	0.011	0.133	0.002	0.073
Arbuscular Mycorrhizae	0.037	0.000	0.016	0.003	0.796
Actinomycetes	0.082	0.194	0.479	0.779	0.198
Fungi:Bacteria	0.221	0.012	0.159	0.004	0.260

Table 5: Correlations of stand and humus layer (Oa) properties with microbial community measurements. Correlations are between stand attributes (hardwood basal area (HBA)), humus layer properties (organic matter content (OM), moisture content (MC), pHw, total carbon (TC), total nitrogen (TN), C:N ratio (C:N), and dissolved organic carbon and nitrogen (DOC and DON)) and microbial community characteristics (microbial biomass (MBC and MBN) and individual microbial community constituents (bacteria (BAC), fungi (FUN), arbuscular mycorrhizae (AM) and actinomycetes (ACT)). If r is greater than 0.71 then $p < 0.0001$, if $r = 0.41$ then $p = 0.05$, and if r is less than 0.34 then $p > 0.10$ (n=24).

HUMUS LAYER	HBA	OM	MC	pHw	TC	TN	C:N	DOC	DON	MBC	MBN	BAC	FUN	AM	ACT	F:B
HBA	*															
OM	-0.64	*														
MC	-0.49	0.19	*													
pHw	0.48	-0.24	0.05	*												
TC	-0.27	0.04	0.05	-0.24	*											
TN	0.02	-0.15	-0.09	-0.01	0.92	*										
C:N	-0.75	0.41	0.36	-0.58	0.44	0.06	*									
DOC	0.77	-0.68	-0.30	0.37	-0.23	0.01	-0.64	*								
DON	0.57	-0.28	0.13	0.78	-0.19	0.05	-0.58	0.49	*							
MBC	0.48	-0.45	-0.08	0.44	0.09	0.32	-0.54	0.40	0.45	*						
MBN	0.14	-0.32	-0.28	0.11	-0.19	0.22	-0.18	0.20	-0.33	0.18	*					
BAC	0.56	-0.17	-0.14	0.85	-0.31	-0.04	-0.71	0.41	0.65	0.52	0.22	*				
FUN	0.15	-0.26	-0.17	-0.36	-0.04	-0.06	0.00	0.34	-0.12	-0.08	-0.15	-0.40	*			
AM	0.37	0.01	0.01	0.84	-0.14	0.07	-0.54	0.18	0.63	0.41	0.12	0.89	-0.57	*		
ACT	0.46	-0.36	-0.46	0.02	0.07	0.19	-0.30	0.19	0.10	0.35	0.13	0.02	0.14	0.02	*	
F:B	-0.22	-0.08	-0.02	-0.71	0.15	-0.02	0.41	-0.02	-0.45	-0.34	-0.21	-0.80	0.85	-0.88	0.06	*

Table 6: Correlations of stand and mineral soil (0-5 cm) properties with microbial community measurements. Correlations are between stand attributes (hardwood basal area (HBA)), mineral soil properties (organic matter content (OM), moisture content (MC), pHw, total carbon (TC), total nitrogen (TN), C:N ratio (C:N), and dissolved organic carbon and nitrogen (DOC and DON)) and microbial community characteristics (microbial biomass (MBC and MBN) and individual microbial community constituents (bacteria (BAC), fungi (FUN), arbuscular mycorrhizae (AM) and actinomycetes (ACT)). If r is greater than 0.71 then $p < 0.0001$, if $r = 0.41$ then $p = 0.05$, and if r is less than 0.34 then $p > 0.10$ (n=24).

MINERAL SOIL	HBA	OM	MC	pHw	TC	TN	C:N	DOC	DON	MBC	MBN	BAC	FUN	AM	ACT	F:B
HBA	*															
OM	0.13	*														
MC	-0.06	0.70	*													
pHw	-0.13	0.57	0.90	*												
TC	0.21	0.84	0.35	0.16	*											
TN	0.22	0.91	0.80	0.71	0.75	*										
C:N	-0.14	-0.34	-0.68	-0.83	0.07	-0.57	*									
DOC	0.44	0.12	-0.40	-0.52	0.41	0.02	0.38	*								
DON	0.41	0.62	0.47	0.38	0.53	0.71	-0.41	0.48	*							
MBC	0.14	0.83	0.83	0.80	0.55	0.87	-0.66	-0.24	0.41	*						
MBN	0.10	0.68	0.83	0.40	0.40	0.77	-0.69	-0.35	0.29	0.93	*					
BAC	-0.03	0.41	0.68	0.75	0.16	0.57	-0.67	-0.50	0.26	0.60	0.63	*				
FUN	0.39	-0.61	-0.79	-0.80	-0.32	-0.62	0.47	0.56	-0.16	-0.68	-0.70	-0.68	*			
AM	0.03	0.35	0.82	0.87	0.01	0.56	-0.80	-0.50	0.36	0.66	0.71	0.80	-0.56	*		
ACT	0.20	0.38	0.29	0.25	0.37	0.40	-0.11	0.19	0.39	0.29	0.34	0.16	-0.28	0.06	*	
F:B	0.32	-0.59	-0.79	-0.82	-0.30	-0.64	0.54	0.56	-0.18	-0.69	-0.72	-0.83	0.98	-0.63	0.06	*

Table 7: Correlations of stand and humus layer (Oa) properties with nitrogen measurements. Correlations are between stand attributes (hardwood basal area (HBA)), humus layer properties (organic matter content (OM), moisture content (MC), pHw, total carbon (TC), total nitrogen (TN), C:N ratio (C:N), and dissolved organic carbon and nitrogen (DOC and DON)) and nitrogen values (extractable N concentration (EN), extractable N on a per hectare basis (ENHA), net N mineralization concentration (NM) and net N mineralization on a per hectare basis (NMHA)). If r is greater than 0.71 then $p < 0.0001$, if $r = 0.41$ then $p = 0.05$, and if r is less than 0.34 then $p > 0.10$ ($n=24$).

HUMUS LAYER	HBA	OM	MC	pHw	TC	TN	C:N	DOC	DON	EN	ENHA	NM	NMHA
HBA	*												
OM	-0.64	*											
MC	-0.49	0.19	*										
pHw	0.48	-0.24	0.05	*									
TC	-0.27	0.04	0.05	-0.24	*								
TN	0.02	-0.15	-0.09	-0.01	0.92	*							
C:N	-0.75	0.41	0.36	-0.58	0.44	0.06	*						
DOC	0.77	-0.68	-0.30	0.37	-0.23	0.01	-0.64	*					
DON	0.57	-0.28	0.13	0.78	-0.19	0.05	-0.58	0.49	*				
EN	0.81	-0.64	-0.21	0.67	-0.19	0.08	-0.67	0.78	0.63	*			
ENHA	0.29	0.08	-0.01	0.68	-0.07	0.09	-0.38	0.19	0.48	0.62	*		
NM	0.31	-0.34	-0.40	-0.17	-0.07	0.05	-0.31	0.24	-0.18	0.31	-0.06	*	
NMHA	-0.48	0.63	-0.12	-0.42	0.13	0.03	0.23	-0.55	-0.53	-0.45	-0.03	0.46	*

Table 8: Correlations of stand and mineral soil (0-5 cm) properties with nitrogen measurements. Correlations are between stand attributes (hardwood basal area (HBA)), mineral soil properties (organic matter content (OM), moisture content (MC), pHw, total carbon (TC), total nitrogen (TN), C:N ratio (C:N), and dissolved organic carbon and nitrogen (DOC and DON)) and nitrogen values (extractable N concentration (EN), extractable N on a per hectare basis (ENHA), net N mineralization concentration (NM) and net N mineralization on a per hectare basis (NMHA)). If r is greater than 0.71 then $p < 0.0001$, if $r = 0.41$ then $p = 0.05$, and if r is less than 0.34 then $p > 0.10$ ($n=24$).

MINERAL SOIL	HBA	OM	MC	pHw	TC	TN	C:N	DOC	DON	EN	ENHA	NM	NMHA
HBA	*												
OM	0.13	*											
MC	-0.06	0.70	*										
pHw	-0.13	0.57	0.90	*									
TC	0.21	0.84	0.35	0.16	*								
TN	0.22	0.91	0.80	0.71	0.75	*							
C:N	-0.14	-0.34	-0.68	-0.83	0.07	-0.57	*						
DOC	0.44	0.12	-0.40	-0.52	0.41	0.02	0.38	*					
DON	0.41	0.62	0.47	0.38	0.53	0.71	-0.41	0.48	*				
EN	0.15	0.62	0.84	0.79	0.34	0.75	-0.60	-0.23	0.64	*			
ENHA	0.16	0.62	0.84	0.80	0.32	0.75	-0.63	-0.23	0.65	1.00	*		
NM	0.43	0.31	-0.01	-0.20	0.40	0.17	0.22	0.50	0.44	0.27	0.28	*	
NMHA	0.46	0.23	-0.08	-0.23	0.32	0.11	0.19	0.49	0.39	0.19	0.21	0.98	*