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Microbial biomass and bacterial functional diversity in forest soils: effects of organic matter removal, compaction, and vegetation control

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Abstract

The effects of organic matter removal, soil compaction, and vegetation control on soil microbial biomass carbon, nitrogen, C-to-N ratio, and functional diversity were examined in a 6-year loblolly pine plantation on a Coastal Plain site in eastern North Carolina, USA. This experimental plantation was established as part of the US Forest Service's Long Term Soil Productivity Study. Sampling was undertaken on eight treatments within each of three blocks. Treatments sampled included main 2 × 2 factorial treatments of organic matter removal (stem-only or complete tree plus forest floor) and compaction (none or severe) with split-plot treatment of vegetation control (none or total vegetation control). Two blocks were located on a somewhat poorly drained, fine-loamy, siliceous, thermic aeric Paleaquult (Lynchburg soil) and one on a moderately well drained, fine-loamy, siliceous, thermic aquic Paleudult (Goldsboro soil). Soil microbial C and N were positively related with soil C and N, respectively. Microbial C and N on the Lynchburg soil were higher than those on the Goldsboro soil. Organic matter removal decreased microbial N. Compaction reduced microbial C-to-N ratio. Vegetation control decreased microbial C and C-to-N ratio. The number of C compounds utilized by bacteria was not affected by soil type or treatment. However, soil types and treatments changed bacterial selections for a few C compounds on BIOLOG plates. Soil microbial properties varied more due to the natural soil differences (soil type) as compared with treatment-induced differences.
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Keywords: Microbial biomass; Bacterial diversity; Carbon; Nitrogen; Odds ratio; Detrended correspondence analysis; Loblolly pine; BIOLOG; Forest soils; Compaction; Organic matter removal

1. Introduction

Quantifying the effects of forest management activities, e.g. harvesting, soil preparation, vegetation control on forest soil properties is an important step in accessing the potential for these activities to have long-term effects on forest productivity and other forest values. Five soil attributes have been identified as critical for maintaining productivity including a soil's capacity to promote root growth; accept, hold, and supply water; hold, supply, and transform nutrients; promote optimum gas exchange; and promote soil biological activity (Larson and Pierce, 1994; Kelting et al., 1999). Organic matter is frequently suggested as an indicator of forest soil quality and sustainability as its presence contributes positively to each of these five attributes (Powers et al., 1990; Nambiar, 1996; Burger and Kelting, 1998). However, the effects of forest management on soil organic matter (SOM) content and composition have generally been small and difficult to detect (Johnson et al., 1991; Chappell et al., 1999; Piatek and Allen, 1999; Li et al., 2003).

Because of the importance of biological activity as an attribute for soil sustainability, Srivastava and Singh (1991), Fauci and Dick (1994) and Chang et al. (1995) proposed the use of soil microbial biomass and composition as indicators. Microbial biomass and activity can be affected by changing substrate quality, quantity and environmental conditions and it is generally accepted that microbial biomass is positively related to organic matter content (Vance and Nadkarni, 1990; Sparling, 1992; Sparling et al., 1994). Microbial biomass may respond more rapidly to changes induced by forest management activities than SOM and consequently may be an early and sensitive indicator of

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change (Wolters and Jöergensen, 1991; Sparling, 1992; Bosatta and Ågren, 1993).

Harvesting may affect microbial biomass through its effects on the quantity and type of organic matter and soil environmental conditions. Reports of harvesting effects on microbial biomass are varied with reductions (Regina et al., 1992; Fritze et al., 1994; Ross et al., 1995), increases (Pietikäien and Fritze, 1993), and no effects (Hossain et al., 1995). Soil may be compacted during harvesting resulting in reduced soil aeration and a decrease in microbial biomass (Šantrůčková et al., 1993; Breland and Hansen, 1995). The presence of hardwood vegetation in conifer plantations is considered as highly favourable for microbial activity because root exudates and litter from hardwoods typically have higher contents of water-soluble sugars, organic acids and amino acids (Qian and Doran, 1996; Priha and Smolander, 1997). Henrot and Robertson (1993) reported that microbial biomass had decreased by 50%, 6 months after hardwood vegetation removal.

Reports of seasonal changes in microbial biomass are varied. Several studies have shown high amounts of microbial biomass in the fall and lesser amounts during summer months (Díaz-Raviña et al., 1994; Smith and Paul, 1990). Others, Chang et al. (1995) and Holmes and Zak (1994), reported no change with season. Entry et al. (1986), Srivastava (1992) and Hossain et al. (1995) were able to correlate seasonal changes with soil water availability. Clearly, the seasonal dynamics of available C, temperature, and moisture need to be understood before progress can be made in predicting the seasonal dynamics of microbial biomass and activity.

Microbial functional biodiversity has been widely used to describe soil microbial composition and relative abundance, because both methodological difficulties of classification and species abundance limit our understanding of microbial communities. Since the early 1990s, the Biolog Microplate identification system (BIOLOG Inc., 1993) has been used to measure bacterial diversity for a variety of soils and water samples (Garland and Mills, 1991; Zak et al., 1994; Grayston and Campbell, 1996). The BIOLOG technique has the potential to produce a rich data set that is ideal to detect site-specific differences in soil bacteria and evaluate the relationship between biodiversity and site conditions.

Sundman et al. (1978), Entry et al. (1986), Pietikäien and Fritze (1994), and Ross et al. (1995) imposed harvest and site preparation treatments such that the effects of organic matter removal and compaction on soil microbial characteristics were confounded. Our objective was to investigate the effects of organic matter removal, compaction, and vegetation control applied separately and in combination on microbial biomass and functional diversity. Our previous assessments of N availability on this site (Li et al., 2003) indicated strong effects of soil type, vegetation control, and compaction on net N mineralization, consequently we expected that microbial characteristics would be affected by these same treatments. Specifically, we hypothesized that microbial biomass and functional diversity would be significantly greater on treatments where more organic matter was retained, where the soil were not compacted, and where the non-pine vegetation community was not controlled.

2. Materials and methods

2.1. Study site and treatments

Our study was made at one of the Long Term Soil Productivity Study (LTSP) sites established in the early 1990s across the United States and Canada to examine the effects of organic matter removal and compaction on tree growth and soil processes (Powers et al., 1990). This LTSP site is located in the lower coastal plain of North Carolina where annual temperature averages 17.0 °C with monthly averages of 26.7 °C in July and 7.0 °C in January. Annual precipitation averages 1440 mm and is generally well distributed throughout the year.

Prior to harvest, three blocks of 10, 0.4-ha plots were established in a 60-year old natural pine-hardwood stand. One block was located on a Goldsboro soil (fine-loamy, siliceous, thermic aquic Paleudult) and two blocks were located on a Lynchburg series soil (fine-loamy, siliceous, thermic aeric Paleaquult). Soil C, N and C-to-N ratio in the top 0–10 cm averaged 32, 1.2 mg ha^{-1} and 27, respectively, for the Goldsboro soil and 49, 1.4 mg ha^{-1} and 34, respectively, for the Lynchburg soil. Nine plots within each block were randomly assigned a 3×3 factorial combination of organic matter removal (stem-only, whole-tree, or whole-tree plus forest floor), and compaction (none, medium or severe) treatments. Three organic matter removal treatments were imposed by removing merchantable stems (stem-only), all stems, branches and foliage (whole-tree), and all stems, branches, foliage and forest floor material (whole-tree plus forest floor). On plots selected for the no-compaction treatment, tree boles were removed by cranes working from outside the plot and depending on the organic matter removal treatment, remaining branches (including foliage) and forest floors (L, F and H layers) were removed by hand. On plots selected for medium or severe compaction, tree boles were removed by skidder, and depending on the organic matter removal treatment remaining branches were removed by hand, and forest floor was removed by a bulldozer equipped with a shear blade. The mineral soil was compacted with one pass of a smooth drum vibrator roller without vibration for medium compaction and two passes with full vibration for severe compaction. To facilitate comparable compaction of the mineral soil on stem-only and whole-tree removal plots, branches (including foliage) and forest floor materials were removed by hand raking prior to compaction and then redistributed after compaction.

Harvesting took place between July and September 1991. Forest floor removal, soil compaction, and forest floor

replacement (if required) were completed by April 1992. The biomass removals were 37, 168 and 218 mg ha^{-1} for stem-only, whole-tree, and whole-tree plus forest floor treatments, respectively. Corresponding N removals were 46, 146 and 500 kg ha^{-1}, respectively. Bulk density in the surface 10 cm averaged 1.28 g cm^{-3} with no compaction, 1.40 with medium compaction, and 1.48 g cm^{-3} with severe compaction (Wilson, unpub., MSc, North Carolina State University, 1994).

Following organic matter removal and compaction treatments, each 0.4 ha plot was split into 0.2 ha plots with vegetation control treatments (no control or complete control) randomly assigned. Complete vegetation control, including all non-pine woody and herbaceous plants, was accomplished by repeated applications of Glyphosate (Accord), Imazapyr (Arsenal), or Sulfometuron Methyl (Oust) in combination with manual cutting of resprouting or germinating vegetation.

Only four of the original nine organic matter removal × compaction treatment combinations were sampled—the 2 × 2 factorial of organic matter removal (stem-only or whole-tree plus forest floor) and compaction (none or severe). Within these main plots, both vegetation control treatment sub-plots were sampled.

2.2. Soil sampling

Five cores (2 cm-dia.) were randomly taken to a depth of 10 cm on each plot. One core was taken within a 5 m radius of each plot corner and a fifth core was taken within a 5 m-dia. circle in the center of each plot. These five cores were immediately composited, cooled to 4 °C, returned to the laboratory, and processed within 2 days. Samples were collected in early March, May, July and September. Because the soils were sampled by depth and not by weight, it was possible that soils that were slightly deeper than 10 cm prior to compaction were included in the 0–10 cm sample after compaction. Given the amount of compaction, we estimated that it was possible that an additional 1 cm (based on pre-compaction soil) was included in the post-compaction sampling.

2.3. Microbial and soil C and N determinations

Microbial C and N were determined by using the chloroform fumigation extraction method (Brookes et al., 1985). Two 15 g sub-samples from each plot were fumigated with alcohol-free $CHCl_3$ for 24 h. These samples were then extracted by adding 30 ml of 0.5 M K_2SO_4, shaking for 60 min and filtered through Whatman No. 2 paper. Two non-fumigated soil samples from each plot were processed in the same manner. The filtered extracts were stored at 4 °C for 2 days until microbial C and N were measured. Microbial biomass C was measured using digestion–titration method (Nelson and Sommers, 1982). After transferring 4 ml of extract to a fresh beaker, 1 ml of 66 mM $K_2Cr_2O_7$ and 5 ml of conc. H_2SO_4 were added. The samples were digested for 30 min at 150 °C and titrated using 0.033 N $Fe(NH_4)_2(SO_4)_2$ with 1,10-phenanthrobine ferrous sulfate as the indicator. Microbial biomass C was estimated as the difference between fumigated and non-fumigated samples divided by the K_2SO_4 extract efficiency factor for microbial C ($K_c = 0.379$, Vance et al., 1987). Nitrogen content in the extract was measured colorimetrically (Keeney and Nelson, 1982) using a Lachat flow injection autoanalyzer (QuikChem method No. 10-107-06-2-E, QuickChem, Lachat Instruments, Mequon, WI). Microbial N content was calculated by dividing the total N difference between fumigated and non-fumigated samples with K_2SO_4 extract efficiency factor for microbial N ($K_n = 0.54$, Brookes et al., 1985).

The composite soil samples collected in September were analyzed for total C and N using a PE 2400 CHN Elemental Analyzer (Perkin–Elmer Corporation, 1988).

2.4. Bacterial functional diversity determinations

Bacterial functional diversity was assessed using GN Microplate identification system (BIOLOG Inc., 1993). This method tests for the utilization of 95 different C compounds classified into six different groups: carbohydrates, carboxylic acid, polymer, amines/amides, amino acid and miscellaneous. Functional diversity is practically defined as the numbers and types of substrate utilization.

Thoroughly mixed soil samples (2.00 g) were diluted to 10^{-4} soil solution in sterile saline (0.85 N NaCl). Then, 150 μl aliquots of this dilution were inoculated to each well of GN Microplates within 24 h after sampling. The inoculated plates were kept at 25 °C for 72 h. Bacterial utilization of substrates was detected by the color changes of wells. If the substrate in a cell was utilized, its color turned pink, otherwise, remained colorless. The color development of each plate was examined at 48 h; the time needed for microbial function to reach a maximum value, but before any contamination (Zak et al., 1994). The GN Microplate results were recorded as binary data with each cell being recorded either as utilized (1) or not (0).

2.5. Statistical analyses

The generalized linear model procedure for mixed models (Littell et al., 1996) was used to test the effects of soil type, organic matter removal, compaction, and vegetation control on microbial C, N and C-to-N ratio across the four dates (repeated measures analysis). Linear regression analysis was used to explore the relationships of microbial C, N and C-to-N ratio with soil C, N and C-to-N ratio (SAS Institute Inc., 1994).

Two methods were used to test for soil type and treatment effects on bacterial functional diversity. The first method used logistic regression (Agresti, 1990) to estimate the probability that a C compounds would be utilized by

bacteria presented in a soil sample from a given soil type and treatment combination. The non-utilized and utilized probabilities were $P_{i00} = (Y_{i00}/N_i)$ and $P_{i01=}(Y_{i01}/N_i)$, respectively, for level 0 of treatment i, and $P_{i10} = (Y_{i10}/N_i)$ and $P_{i11=}(Y_{i11}/N_i)$, respectively, for level 1 of treatment i, where Y_{i01} and Y_{i11} were the number of C compounds utilized for levels 0 and 1 of treatment i, respectively, and Y_{i00} and Y_{i10} were the number of C compounds not utilized for levels 0 and 1 of treatment i, respectively, and N_i was the total number of C compounds for each group. Probabilities were calculated separately for each of the six groups.

The odds ratio (Agresti, 1990) was then used to compare the number of C compounds utilized by bacteria from the two levels of treatments

$$\text{Odds ratio} = (P_{i00}/P_{i01})/(P_{i10}/P_{i11})$$

If the upper bound (UB) of 95% confidence interval (CI) of the odds ratio was less than 1, then bacteria from level 0 of treatment i utilized significantly fewer C compounds as compared with bacteria from the level 1. If the lower bound (LB) of 95% CI of the odds ratio was greater than 1, then bacteria from level 0 utilized more of C compounds compared with level 1. If the 95% CI of the odds ratio contained 1, then both levels utilized the same number, but possibly different C compounds. Odds ratios were calculated for every group of C compounds and each treatment, including soil type, organic matter removal, compaction, and vegetation control.

The second method used detrended correspondence analysis (DCA) (Hill and Gauch, 1980). DCA was selected over principal component analyses (PCA) because DCA is not constrained by PCA assumption of linear treatment effects (Jongman et al., 1995). For each sampling date, the utilization data for all 95 C compounds were arrayed along the first two principal factor axes. Separations between treatments within this two-dimensional space were used to indicate treatment-induced differences in C compound utilization. The associations of treatments with principle factors provided information for the effects of treatments on the possibility of utilization for each C compound.

Statistical significance was accepted at $P \leq 0.05$ for all analyses.

3. Results

3.1. Microbial C, N, and C-to-N ratios

Microbial C, N, and C-to-N ratios significantly differed over the four sampling dates with the highest means of C (559 mg kg^{-1}) in May, N (47 mg kg^{-1}) in September, and C-to-N ratios (14) in July and lowest means of C (440 mg kg^{-1}) in September, N (38 mg kg^{-1}) in July, and C-to-N ratios (10) in September (Tables 1 and 2). Microbial C, N, and C-to-N ratios differed significantly by soil type with higher values in the moister Lynchburg soil. Greater organic matter removal associated with whole-tree plus forest floor removal significantly decreased microbial N but did not affect microbial C or C-to-N ratios. Compaction did not significantly affect microbial C or N but the trend for lower C with compaction resulted in significantly lower C-to-N ratios in the compacted areas. Vegetation control resulted in significantly less microbial C and also lower C-to-N ratios. No interactions were observed among the treatments.

Table 1
Statistical significances (P-values) of soil type (ST), organic matter removal (OM), compaction (CP), vegetation control (VC), and sampling date (time) effects on soil microbial C, N, and C-to-N ratio

Effects	C	N	C-to-N ratio
ST	0.01	0.04	0.04
OM	0.11	0.04	0.41
CP	0.22	0.22	0.03
VC	0.03	0.99	0.05
OM × CP	0.28	0.08	0.91
OM × VC	0.12	0.40	0.79
CP × VC	0.42	0.77	0.24
ST × OM × CP	0.41	0.91	0.84
OM × CP × VC	0.43	0.80	0.38
Time	0.80	0.07	0.35
$Time^2$	0.52	0.15	0.06
$Time^3$	0.09	0.21	0.24

Time, $time^2$, and $time^3$ are the linear, quadratic, and cubic effects of sampling date on microbial C, N, and C-to-N ratio.

Microbial C was positively related to soil C ($r = 0.46$, $P < 0.02$) and microbial N was positively related to soil C ($r = 0.53$, $P < 0.007$) and N ($r = 0.52$, $P < 0.009$). Microbial C-to-N ratios were not related with soil C ($r = 0.20$, $P < 0.36$), N ($r = 0.04$, $P < 0.87$) or C-to-N ratios ($r = 0.13$, $P < 0.54$).

3.2. Bacterial functional diversity

Soil type, organic matter removal, compaction and vegetation control did not significantly affect the number of C compounds utilized by soil bacteria, because the CIs of the odds ratios included 1. However, organic matter removal significantly reduced utilization of carbohydrates in May, compaction reduced the bacteria utilization of amino acids in May and of carbohydrates in September, and vegetation control significantly reduced utilization of carbohydrates and amino acids (Table 3).

The first two principal factors of DCA accounted for 70, 87, and 81% of total variances for the May, July and September C compound utilization data. However, the associations between treatments and C compound utilization were not consistent across dates (Figs. 1–3). In May, the Lynchburg soil scored higher than the Goldsboro soil on axis 1 (Fig. 1). The complete tree plus forest floor removal plots scored higher on axis 1 and the vegetation control plots scored higher on axis 2. In July, the Goldsboro soil scored

Table 2
Soil type, organic matter removal, compaction, and vegetation control treatment means for microbial C, N, and C-to-N ratio by sampling dates

Treatments	March			May			July			September			Average		
	C mg kg^{-1}	N mg kg^{-1}	C-to-N ratio	C mg kg^{-1}	N mg kg^{-1}	C-to-N ratio	C mg kg^{-1}	N mg kg^{-1}	C-to-N ratio	C mg kg^{-1}	N mg kg^{-1}	C-to-N ratio	C mg kg^{-1}	N mg kg^{-1}	C-to-N ratio
Soil															
Goldsboro	330 (170)	30.0 (3.4)	11.4 (5.1)	537 (143)	41.4 (9.2)	13.4 (3.9)	288 (93)	40.2 (12.6)	8.7 (7.8)	328 (146)	40.9 (15.8)	9.4 (5.4)	371 (166)	38 (11.7)	10.7 (6.0)
Lynchburg	548 (229)	44.9 (10.7)	12.4 (5.9)	570 (190)	45.6 (11.0)	12.9 (4.6)	586 (176)	36.3 (7.3)	16.6 (5.5)	497 (339)	49.8 (19.2)	10.3 (7.2)	550 (239)	44 (13.4)	13.1 (5.9)
Organic matter removal															
Stem	436 (160)	44.7 (11.8)	9.7 (7.9)	645 (179)	44.9 (10.9)	15.1 (3.8)	544 (199)	34.6 (7.4)	16.5 (6.8)	483 (283)	54.2 (21.1)	9.4 (6.3)	527 (251)	44.7 (15.0)	12.6 (6.3)
Whole-tree plus FF	515 (290)	35.3 (9.3)	14.5 (2.7)	473 (121)	43.4 (10.3)	11.2 (5.1)	430 (210)	40.6 (10.3)	11.5 (6.9)	398 (185)	39.4 (11.6)	10.7 (5.9)	454 (209)	40 (10.5)	11.9 (5.8)
Compaction															
None	469 (273)	39.4 (10.2)	12.5 (2.4)	582 (206)	42.3 (10.6)	13.6 (4.8)	498 (196)	34.9 (9.0)	15.4 (6.7)	521 (339)	46.5 (19.7)	11.9 (7.0)	517 (255)	40.8 (13.4)	13.4 (6.4)
Compacted	482 (196)	40.6 (12.9)	11.7 (7.2)	536 (139)	46.0 (10.4)	12.5 (3.2)	475 (228)	40.3 (9.1)	12.6 (6.3)	360 (234)	47.1 (17.6)	8.2 (5.6)	463 (206)	43.6 (12.9)	11.2 (5.5)
Vegetation control															
None	538 (156)	40.5 (13.2)	14.1 (6.7)	600 (156)	41.7 (7.0)	14.6 (4.0)	526 (238)	36.0 (10.1)	15.9 (6.3)	492 (344)	50.4 (21.1)	10.2 (6.5)	539 (232)	42.2 (14.5)	13.7 (5.9)
Complete	413 (283)	39.4 (9.9)	10.2 (5.0)	517 (187)	46.6 (12.8)	11.6 (4.8)	448 (175)	39.2 (8.5)	12.1 (5.5)	389 (245)	43.2 (14.7)	9.9 (6.3)	442 (225)	42.2 (11.8)	10.9 (5.8)
Average	475 (232)	40.1 (11.4)	12.1 (5.8)	559 (174)	44.3 (13.1)	13.1 (4.5)	487 (208)	38 (9.3)	13.9 (6.9)	440 (297)	47 (18.3)	10.0 (6.2)	490 (233)	42.3 (13.1)	12.3 (6.0)

Standard deviation given in parentheses.

Table 3
Lower (LB) and upper (UB) bounds (95% CI) for odds ratios for carbon compound utilization by soil type, organic matter removal, compaction, vegetation control and sampling date

Carbon compound	Soil type		Organic matter removal		Compaction		Vegetation control	
	LB	UB	LB	UB	LB	UB	LB	UB
May								
Carbohydrates	0.33	1.33	1.01	1.08	0.70	1.55	1.01	2.25
Carboxylic acids	0.76	1.74	0.63	1.40	0.80	1.79	0.65	1.46
Amines/amides	0.42	2.37	0.62	3.20	0.86	4.58	0.37	1.90
Amino acids	0.47	1.35	0.95	2.69	1.02	2.88	1.17	3.33
Polymers	0.33	2.36	0.88	5.00	0.74	4.13	0.74	4.13
Miscellaneous	0.40	1.82	0.48	2.04	0.63	2.68	0.48	2.04
July								
Carbohydrates	0.82	1.61	0.82	1.53	0.59	1.10	0.62	1.15
Carboxylic acids	0.84	1.85	0.74	1.54	0.67	1.39	0.52	1.09
Amines/amides	0.76	4.21	0.50	2.29	0.67	3.10	0.32	1.47
Amino acids	0.96	2.20	0.64	1.38	0.57	1.23	0.55	1.19
Polymers	0.73	3.52	0.31	1.37	0.36	1.57	0.31	1.37
Miscellaneous	0.76	2.72	0.43	1.37	0.43	1.37	0.39	1.26
September								
Carbohydrates	0.77	1.84	0.69	1.55	1.10	2.48	0.91	1.10
Carboxylic acids	0.90	2.14	0.57	1.26	0.82	1.81	0.62	1.36
Amines/amides	0.53	3.26	0.36	1.91	0.30	1.06	0.36	1.91
Amino acids	0.93	2.60	0.61	1.53	0.68	1.71	0.42	1.05
Polymers	0.54	4.99	0.29	2.08	0.97	7.83	0.29	2.08
Miscellaneous	0.68	3.29	0.38	1.56	0.56	2.29	0.43	1.77

higher than the Lynchburg soil on axis 2, and vegetation control scored higher than no-vegetation control on axis 1 (Fig. 2). Similarly, in September, the Lynchburg soil scored higher on axis 2 (Fig. 3).

In May, the association of treatments with the scores of C compounds on axes 1 and 2 showed that the bacteria in the Lynchburg soil and the complete tree plus forest floor removal plots were more likely to use lactulose, D-raffinose, χ-hydroxybutyric acid, itaconic acid, alaninamide, and 2,3-butanediol than from bacteria in the Goldsboro soil and stem-only removal plots. Similarly, bacteria in vegetation control plots were more likely to utilize lactulose, α-D-lactose, β-methyl-D-glucoside, formic acid, α-hydroxybutyric acid, α-ketobutyric acid than bacteria in no-vegetation control plots. In July, the bacteria in the Goldsboro soil were

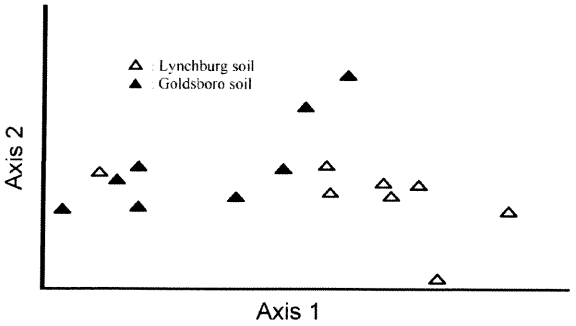

Fig. 1. Soil type distribution within the space of axes 1 and 2 in May.

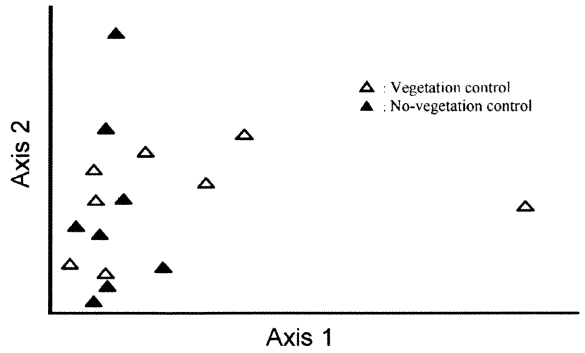

Fig. 2. Vegetation control treatment distribution within the space of axes 1 and 2 in July.

more likely to use α-ketoglutaric acid, xylitol, phenylethylamine, 2-amino-ethanol, and L-ornithine, and maltose than the bacteria in the Lynchburg soil. Similarly, bacteria with vegetation control were more likely to use α-cyclodextrin, glycogen, α-D-lactose, lactuose, D-raffinose, α-hydroxybutyric acid, alaninamide, glycyl-L-aspartic acid, L-leucine, D-serine, L-serine, uridine, and thymidine than the bacteria in the no-vegetation control plots. In September, the bacteria in the Lynchburg soil were more likely to use xylitol, α-ketobutyric acid, phenyl-ethyamine, 2-amino-ethanol, L-leucine, and D-raffinose than bacteria in the Goldsboro soil. The bacteria with vegetation control treatment were more likely to metabolize α-cyclodextrin, *i*-erythritol, α-D-lactose, *m*-inositol, succinamic acid, L-ornithine, uridine, and thymidine than the bacteria in the no-vegetation control plots.

4. Discussion

4.1. Microbial C, N, and C-to-N ratios

Differences of microbial C, N and C-to-N ratios among blocks (soil type) were greater than the differences caused by any treatments (Tables 1 and 2). Blocking was based on modest differences in drainage (somewhat poorly

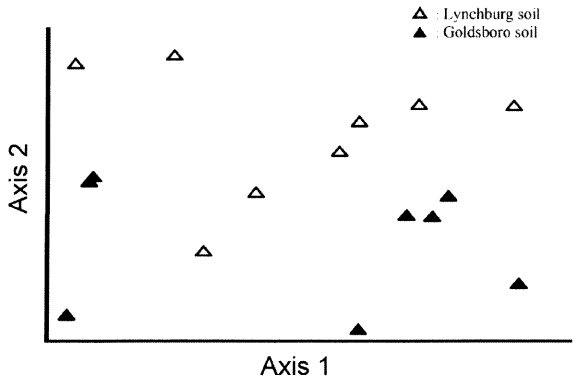

Fig. 3. Soil type distribution within the space of axes 1 and 2 in September.

and moderately well drained) associated with less than 100 cm differences in elevation and landscape position. The variation that was found on this site is typical of most stands in the study area. Li et al. (2003) working at the same site reported strong soil effects on N mineralization. The overriding effect that within site soil differences can have on soil N availability had been noted by Piatek and Allen (1999) for a well-drained clayey Piedmont site with a similar set of treatments. Apparently, substrate quantity and quality differences between the soil types contributed to the differences in microbial biomass C and N as indicated by the positive correlations between microbial C and N and soil C and N. Similar strong correlations between microbial C and N and soil C and N have been reported by Vance and Nadkarni (1990), Sparling (1992) and Sparling et al. (1994).

However, the microbial C-to-N ratio was not correlated with soil C, N or C-to-N ratio. Srivastava et al. (1989) found that microbial C-to-N ratio was only negatively correlated with mineral N. The microbial C-to-N ratio of the Lynchburg soil (13.1) was greater than that of the Goldsboro soil (11.7). This may suggest greater fungal biomass in the Lynchburg soil, because the C-to-N ratios of fungi are typically higher than those of bacteria (Marumoto et al., 1982). The microbial C-to-N ratios were similar to the ratios reported by Srivastava and Singh (1991) and Srivastava (1992), less than ratios reported by Bauhus et al. (1998).

In September, microbial C and N averaged 328 and 41 mg kg^{-1}, representing 1.4 and 4.1%, respectively, of the total C and N found in the top 10 cm of the Goldsboro soil. Similarly, microbial C and N averaged 497 and 50 mg kg^{-1}, representing 1.3 and 4.3%, respectively, of the total C and N found in the top 10 cm of the Lynchburg soil. These percentages were slightly less than microbial C and N fractions reported by Scott et al. (1998), similar to the levels reported by Bauhus et al. (1998), Anderson and Domsch (1989), Brookes et al. (1985), and Zak et al. (1990), and slightly higher than the levels reported by Srivastava and Singh (1991).

Vegetation control had the greatest effect of any of the treatments imposed on microbial C and C-to-N ratio (Tables 1 and 2). The significantly lower microbial C (442 versus 539 mg kg^{-1}—an 18% reduction) and unchanged microbial N resulted in a lower microbial C-to-N ratio (10.5 versus 12.8) on plots with complete vegetation control. We believe that the negative effects of vegetation control on microbial biomass were mainly due to the loss of vegetation, rather than toxicity of herbicides to microorganisms (Domsch et al., 1983; Busse et al., 2001). Vegetation control completely eliminated the development of herbaceous and hardwood biomass. This reduction in annual production of 'competing' vegetation would have also reduced the input of labile C from root exudates, fine root mortality, and leaf and litterfall leachates with a subsequent reduction in microbial productivity and C on plots with vegetation control. The reduction in microbial C-to-N ratio may have also resulted from differences in the composition

of the microbial community (e.g. fewer fungi) in response to a reduction in total fine root production on plots where the vegetation was controlled (Bauhus et al., 1998).

The only effect of organic matter removal was the reduction in microbial N associated with whole-tree plus forest floor removal. Clearly, inputs of organic matter from harvest residues and the previous stand's forest floor into mineral soil were greatly reduced. However, by age 6 following the treatment, this reduction apparently did not adversely affect the C supply that was being used for microbial growth since microbial C was not affected. Microbial C-to-N ratio, which is influenced by the composition of the microbial communities, remained the same, probably because organic matter removal also did not change soil moisture, soil aeration, or available N supply (Li et al., 2003).

The only effect of compaction was a consistent reduction in microbial C-to-N ratio. Possible explanations include a change in the microbial population with fewer fungi due to lack of aeration (Pritchett and Fisher, 1987) and reduced soluble carbohydrates resulting from decreased fine root production (USDA, unpublished data). Compaction might also have reduced soil bacteria production, resulted in the lower microbial C-to-N ratio, because it created anaerobic conditions by increasing soil moisture (Li et al., 2003). Aerobes make up the majority of the total bacteria in a soil system; only obligate anaerobes and facultative anaerobes (Sylvia et al., 1998) can grow under anaerobic conditions. The aerobes would decline in number as the soil becomes more anaerobic; however, the anaerobes would not increase in number to compensate for the loss in overall quantity. Compaction may have introduced a limited bias in the results, because sampling was based on a constant depth rather than on a constant mass of soil.

4.2. Bacterial functional diversity

Interestingly, soil type and the treatments had little to no detectable effects on bacterial functional diversity as assessed by the total number of C compounds utilized by soil bacteria using the BIOLOG GN plate technique. This result might be caused by the bacteria multiple selections for the C compounds on BIOLOG GN plates. Microbial functional diversity differences, in terms of C compounds utilization numbers, have mostly been reported where comparison have been made between significantly distinctive different biological systems, such as among freshwater, coastal lagoon, and soil (Garland and Mills, 1991) and different soil and plant communities (Zak et al., 1994; Garland, 1996; Grayston and Campbell, 1996). Soil bacteria from two different soil types and silvicultural treatments did have different selection for a few C compounds on BIOLOG GN plates (Figs. 1–3). These findings coupled with the change in microbial C-to-N ratio with soil type and treatment suggests that the composition of the microbial communities had changed. However, with current BIOLOG GN plate techniques, it is very difficult to detect any changes in the total numbers of C compounds utilized and even more difficult to predict the effect that these changes may have on long-term soil sustainability. Strong seasonal dynamics of microbial biomass and inconsistency of microbial selections for C compounds will limit the use of microbial assessments as criteria for long-term soil sustainability assessments.

4.3. Conclusions

The lack of strong treatment effects on soil bacterial properties contrasted with the significant differences found between soil types and sampling dates. The imposed treatments did not significantly influence soil microbial properties during the sixth year after planting whereas inherited soil differences and season did. If we believe that soil microbial properties are integrative measures of a range of soil quality factors, then our results indicate that the type of severe treatments imposed at this site may have little to no adverse effects on long-term soil sustainability under similar soil and climatic conditions.

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