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MICROBIAL COMMUNITY VARIATION AND ITS RELATIONSHIP WITH NITROGEN MINERALIZATION IN HISTORICALLY ALTERED FORESTS

JENNIFER M. FRATERRIGO,[1,3] TERI C. BALSER,[2] AND MONICA G. TURNER[1]

[1]*Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706 USA*
[2]*Department of Soil Science, University of Wisconsin, Madison, Wisconsin 53706 USA*

Abstract. Past land use can impart soil legacies that have important implications for ecosystem function. Although these legacies have been linked with microbially mediated processes, little is known about the long-term influence of land use on soil microbial communities themselves. We examined whether historical land use affected soil microbial community composition (lipid profiles) and whether community composition was related to potential net nitrogen (N) mineralization rates in southern Appalachian (USA) forest stands abandoned from agriculture or logging and reforested >50 yr ago. Microbial community composition was determined by a hybrid procedure of phospholipid fatty acid (PLFA) and fatty acid methyl ester (FAME) analysis. We found that community composition varied significantly with past land use. Communities in formerly farmed stands had a higher relative abundance of markers for gram-negative bacteria and a lower abundance of markers for fungi compared with previously logged and reference (i.e., no disturbance history) stands. Potential net N mineralization rates were negatively correlated with fungal and gram-negative bacterial markers in both farmed and reference stands, and fungal abundance and soil bulk density effectively predicted mineralization rates in all stands. Our results indicate that the alteration of microbial communities by historical land use may influence the ecosystem processes they mediate. This is in contrast to typical expectations about microbial community resilience to change. Here, the decrease in fungal abundance observed from disturbance appeared to result in decreased nitrogen mineralization over the long term.

Key words: bacteria; FAME; fungi; land-use history and ecosystem function; PLFA; soil microbial communities; southern Appalachians (USA).

INTRODUCTION

Imprints of past land use on soil properties have been widely documented in native ecosystems developing on abandoned lands. Reduced soil carbon (C) and nitrogen (N) and enriched soil phosphorus (P) can still be detected for decades after the cessation of agriculture (Post and Kwon 2000, Richter et al. 2000, Dupouey et al. 2002, DeGryze et al. 2004), while logging can decrease forest-floor nutrients and redistribute soil resources for at least 50 years following the removal of timber (Johnson et al. 1991, Goodale and Aber 2001). Although there is a well-established physical basis for such enduring effects (reviewed in Foster et al. [2003]), the biological underpinnings of altered soil properties remain unclear. Some evidence suggests that the alteration of microbially mediated processes may play a role maintaining soil legacies. For example, Richter et al. (1999) found that rapid decomposition limited C sequestration, and Compton and Boone (2000) observed elevated nitrification rates and nitrifier populations in historically cultivated forests. However, few studies have examined the impacts of historical activities on microbial communities or the functional consequences of microbial community variation in abandoned and subsequently revegetated areas.

Former human practices may persistently affect microbial communities and the processes they mediate both directly and indirectly. Direct effects of past land use may occur via the long-term (>50 yr) physical alteration of the rhizosphere caused by historic practices. Soil compaction is an enduring consequence of cultivation, grazing, and logging that can cause increased bulk density and reduced pore space (Johnson et al. 1991). These changes may affect the abundance of aerobic and anaerobic microorganisms and subsequently reduce the cycling of several elements, including C, N, and P (Waldrop et al. 2000, Cleveland et al. 2003). The depletion of soil organic matter, combined with the gradual re-accumulation of organic-matter pools, may also lead to lasting changes in microbial community composition and activity. For example, the reduction and slow accrual of light-fraction organic matter following former cultivation has been shown to account for declines in microbial immobilization of N (Compton and Boone 2002). In previously logged areas, the redistribution of organic matter in the surface

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[3] Present address: Department of Ecology, Evolution and Organismal Biology, 253 Bessey Hall, Iowa State University, Ames, Iowa 50011-3221 USA. E-mail: jmfrater@iastate.edu

soil may influence soil fungi by altering substrate quantity (Bååth et al. 1995).

Past land use may also engender changes in vegetation that influence microbial community composition by altering contemporary substrate pools. Disturbance by humans can favor a shift from long-lived and shade-tolerant late-successional forest species to more rapidly growing species that aggressively invade open sites (Foster et al. 2003). Consequently, current root biomass and litter composition may vary with former activities. If microbial communities respond solely to such changes, the effect of land-use history on microbial composition would be largely indirect.

Here we investigate the influence of past land use on microbial community composition by comparing microbial community profiles from southern Appalachian forests farmed or logged at least 50–75 years ago with those from stands without a history of intensive human disturbance. We tested the competing hypotheses of direct and indirect effects using tree-community and litter-composition data determined in other studies (S. Pearson and B. Ball, respectively, *unpublished data*). We also examined the functional consequences of variation in the microbial community by relating community composition and N mineralization rates.

Methods

Study area and sampling

Eight north-facing sites that had been farmed or logged in the past, or showed little or no evidence of disturbance (hereafter referred to as ''reference''), were located in closed-canopy cove-hardwood forests of the French Broad River Basin (35°81′ N, 82°35′ W) in western North Carolina, USA (Table 1). Mean annual precipitation and temperature in the area are 1120 mm and 13°C, respectively. Soils are well drained, upland mountain soils derived from high-grade metamorphic or igneous rock. All series are classified as Typic Dystrudepts, except the Toecane series which is a Humic Hapludult (Table 1). In the southern Appalachians, historical farming was practiced as subsistence agriculture, with fields typically converted to pastures after about five years of cultivation (Otto 1987). Most farms were abandoned by 1930 due to socioeconomic changes in the region. Historical logging utilized clear-cutting techniques and lasted until about 1950. Farmed and logged sites naturally reforested following the cessation of those practices and have been forested for at least 50 years. The present vegetation of the sites is similar (Table 1) and characteristic of the mixed mesophytic forest community of the Southern Blue Ridge Province (Braun 1950).

Despite efforts to find sites that occurred at similar elevations and slopes, former farms occurred only at lower elevations and reference sites occurred only at higher elevations. Previous work indicated that microclimate variation in air temperature and soil moisture were minimal across this elevation gradient: in June 2002, air temperature ranged from 19.7 to 19.4°C and soil water content at 0–30 cm ranged from 0.247 to 0.127 cm between sites located at 795 m and 1001 m (L. Swift, *unpublished data*). Nonetheless, to provide a means for comparison we selected logged stands that were adjacent to farmed and reference sites and occurred at similar elevations and slopes (Table 1).

Depending on site size, one or three 20 × 20 m plots were established in each set of adjacent sites ($n = 16$ plots total). Plots within sites were considered independent because they were located at least 200 m apart. Plots were divided into four, 10 × 10 m quadrants. In the center of each quadrant and from the center of the plot, a pair of adjacent samples were collected from the upper 15 cm of mineral soil during June 2002 with a 5.2-cm diameter cylindrical PVC corer ($n = 10$ samples/plot). Samples were transferred to plastic bags and immediately cooled for transport to the laboratory. Litter was collected in August and September 2004 from five adjacent locations in each plot. Samples were sorted into categories (woody, mast, flower parts, invertebrates, miscellaneous) or tree species, oven-dried at 60°C, and weighed (B. Ball, *personal communication*). The tree community was assessed at the site scale using four 20-m-diameter circular plots located at each corner of a 1-ha area centered on the plots. All trees >2 m tall were censused in June 2001 and the diameter of stems ≥10 cm in diameter at breast height were used to compute total basal area for each species (S. Pearson, *personal communication*).

Analysis of soil properties and microbial communities

One sample from each pair was sieved to <4 mm to remove roots and coarse fragments and to homogenize the soil. Subsamples were taken to measure general soil characteristics, including soil moisture, texture, pH, bulk density, and elemental concentrations as described in Fraterrigo et al. (2005). Subsamples were also collected for a 28-d, aerobic, laboratory incubation to determine potential net N mineralization rates (sensu Robertson 1999) as described in Fraterrigo et al. (2005). The remaining samples from each pair were shipped overnight to the University of Wisconsin (Madison, Wisconsin, USA) where they were homogenized, frozen, and freeze-dried pending analysis.

A hybrid procedure of phospholipid fatty acid (PLFA) and fatty acid methyl ester (FAME) analysis was used to assess microbial community composition at each sampling location ($n = 80$ points). The procedure is based on the extraction of ''signature'' lipid biomarkers from the cell membrane and wall of microorganisms (White and Ringelberg 1998) and is described in Appendix A. In all subsequent analyses we used fatty acids that were identifiable and present at >0.5 mole percent. We included one ''summed'' fatty acid that could not be uniquely identified due to its

TABLE 1. Description of the eight north-facing study plots located in closed-canopy cove-hardwood forests in western North Carolina, USA.

Site	LUH†	Last used	Dominant tree species‡	Soil series§
Staire Branch, SB	F	1930	*Liriodendron tulipifera, Tsuga canadensis, Betula lenta*	T-T
Staire Branch, SB	L	1950	*Quercus prinus, B. lenta, L. tulipifera, T. canadensis, Acer saccharum, Tilia Americana, Carya cordiformis, Quercus rubra*	P-U
Picnic Area, PA	F	1930	*L. tulipifera, B. lenta, Pinus strobus, Q. rubra*	PUT
Little Andy, LA	L	1950	*L. tulipifera, A. saccharum, B. lenta*	P-U
Corner Rock, CR	L	1950	*L. tulipifera, Q. rubra, Acer rubrum*	T-T
Glassmine, GM	R	N/A	*L. tulipifera, Q. rubra*	P-U
Big Andy, BA	L	1950	*L. tulipifera, A. saccharum, B. lenta*	P-U
Walker Cove, WC	R	N/A	*A. saccharum, Fagus grandifolia*	P-U

Note: N/A indicates not applicable.

† Land-use history: F, farmed; L, logged; R, reference (showing little or no history of disturbance). See Fraterrigo et al. (2005) for a list of information sources.

‡ Total basal area for species listed was ~330 000 cm^2 (data courtesy of S. Pearson).

§ Soil series: P-U, Porters-Unaka complex; T-T, Toecane-Tusquitee complex; PUT, Porters-Unaka and Toecane-Tusquitee complex.

high relative abundance; we refer to this marker as ''unknown,'' although it represents two overlapping response peaks. Terminology to describe fatty acids is given as ''A:BωC'' where ''A'' indicates the total number of carbon atoms, ''B'' the number of double bonds (unsaturations), and ''C'' indicates the position of the double bond from the methyl end of the molecule. Prefixes ''*i*'' and ''*a*'' refer to iso and anti-iso methyl branching. Hydroxy groups are indicated by ''OH'' Cyclopropyl groups are denoted by ''*cy*'' (Steenwerth et al. 2003, Balser and Firestone 2005).

Lipids cannot confidently be used to represent particular species but are more commonly assigned functional groups. Bacterial abundance was estimated by taking the average of the following fatty acids: *a*15:0, *i*15:0, 15:1ω9, *i*16:0, 16:1ω7, 16:1ω9, *a*17:0, *cy*17:0, *i*17:0, 17:1ω7, 17:1ω8, 18:0 2OH, 18:1ω5, and 18:1ω9c (Frostegård and Bååth 1996). Fungal abundance was estimated from concentrations of the marker 18:2ω6, and marker 16:1ω5 was used to estimate arbuscular mycorrhizal fungi (AMF) abundance (Balser et al. 2005). The monounsaturated group was represented by unbranched lipids, such as 15:1ω9 and 16:1ω7; the hydroxy group was represented by lipids with an OH group; and the branched group was represented by lipids with *iso* or *ante-iso* methyl branching, such as *i*15:0, *a*15:0. Gram-positive bacteria were represented by *i*14:0, *a*15:0, *i*15:0, *i*16:0, *a*17:0, and *i*17:0, whereas gram-negative bacteria were represented by 16:0 2OH, 16:1ω9, *cy*17:0, 17:1ω7, 17:1ω8, 18:0 2OH, 18:1ω5, and 18:1ω9c. We also calculated the ratios of fungal/bacterial markers (18:2ω6 only) and Gm+/Gm− bacterial markers. Total microbial biomass was estimated by summing the concentration of all lipids.

Statistics

Lipids were averaged by plot and ordinated using nonmetric multidimensional scaling (NMS) to summarize microbial community composition. NMS is a robust ordination method that avoids assumptions of linear relationships among variables, preserving the rank order of among-sample dissimilarities in the rank order of distances (Clarke 1993). A matrix of Bray-Curtis dissimilarities was calculated from the relative mole fraction of individual lipids ($n = 32$ lipid samples) and subjected to NMS. Fifty runs starting from random configurations were performed with the real data and checked against the same number of runs done with randomized data to ensure a better-than-random solution. Diagnostic (scree) plots suggested a two-dimensional solution was optimal. NMS was thus rerun, specifying two dimensions and the best starting configuration. The final stress (i.e., departure from monotonicity between the distance measure and the distance in ordination space) for this solution was 5.84. (Note: stress values <10 are considered satisfactory [McCune and Grace 2002]).

The ordination scores of plots were then used in a multivariate analysis of covariance with elevation as a covariate to determine whether microbial community

TABLE 1. Extended.

Replicate	Elevation (m)	Soil				
		Sand (%)	Silt (%)	Clay (%)	P (mg/kg)	Soil C:N
A	861	63	27	10	43	25.0
A	906	56	34	10	25	31.9
A	812	60	29	11	82	26.1
B	836	56	31	13	38	34.8
C	812	63	25	12	29	28.1
A	894	66	24	10	36	24.2
B	921	64	27	9	26	26.1
C	924	74	19	7	24	31.7
A	1024	71	21	8	24	26.2
A	1076	74	18	8	27	26.0
A	1148	64	25	11	38	22.7
B	1152	69	23	8	39	20.6
C	1124	61	27	12	48	25.6
A	1182	60	29	11	48	29.4
B	1139	60	28	12	43	26.6
C	1182	72	20	8	49	23.0

composition differed with past land use. This modeling approach allowed for the entire constellation of points to be compared simultaneously while controlling for differences in elevation among plots. Similarly, differences with past land use in microbial biomass, specific lipids, microbial functional groups and ratios, litter, and site-level total basal area of dominant trees were tested with analysis of covariance. Past land use was included as a fixed effect and elevation as a covariate in all models. Orthogonal contrasts were employed to evaluate planned comparisons among past land-use categories and differences with a P value <0.05 were considered significant.

To evaluate the association between microbial community composition and other variables, we calculated partial Spearman rank correlations, controlling for the effect of elevation. Correlations were computed between the NMS axes and total extracted lipids, pH, bulk density, soil moisture, soil organic-matter content, mineral-soil characteristics, and soil texture, as well as between microbial markers and substrate pools (litter). Relationships between microbial markers and total basal area of dominant trees were not examined because plot-level tree data were not available.

We also computed Spearman rank correlations between N mineralization rate, soil P, fungal markers 18:2ω6 and 16:1ω5, bacterial groups, and ratios. Significantly correlated microbial variables (after Bonferroni correction), as well as elevation and soil chemical and physical characteristics, were regressed against N mineralization rate to examine how well they predicted potential N transformation. The final model included only those terms that explained a significant amount of variation in N mineralization rates.

RESULTS

The two-dimensional nonmetric multidimensional scaling (NMS) solution explained 97% (axis 1 = 34%, axis 2 = 63%) of the variation in the microbial community and indicated that the profiles of historically farmed, logged, and reference plots were distinctly different (Fig. 1; Pillai's trace $F_{4,24} = 4.06$, $P = 0.01$). Along axis 1, microbial profiles in reference stands stood out from those of farmed and logged stands ($P = 0.02$, $P = 0.03$, respectively), while along axis 2, logged and reference plots were found to differ from farmed plots ($P = 0.01$, $P < 0.05$, respectively). Several lipids were correlated with the axes. Those most highly correlated ($r > |0.65|$) with axis 1 included some indicators for gram-positive (Gm+) (*i*15:0, *i*16:0) and gram-negative (Gm−) (16:1ω7c, 15:1ω9c, 18:0 2OH) bacteria, 9:0, *i*17:1, and 19:1ω8t. Those most highly correlated ($r > |0.80|$) with axis 2 included the markers for arbuscular mycorrhizal fungi (AMF) (16:1ω5c), Gm+ (*i*14:0) and Gm− (15:1ω9c, 16:0 2OH) bacteria, several saturated lipids (12:0, 14:0, 16:0), 19:1ω8t, and unknown 1 (Fig. 1). Few variables were significantly associated with the axes. Axis 2 was negatively correlated with soil P concentration ($P = 0.01$) and clay content ($P = 0.003$), whereas axis 1 was not correlated with any of the measured variables.

Several functional groups and individual lipid biomarkers also varied with historical human activity (Appendix B). There was a marginal difference ($P < 0.06$) in the relative abundance of bacterially related lipids in the microbial communities of old farms (48%) compared with logged and reference stands (~44%). In contrast, the relative abundances of fungal-originating lipids in formerly logged areas were significantly high-

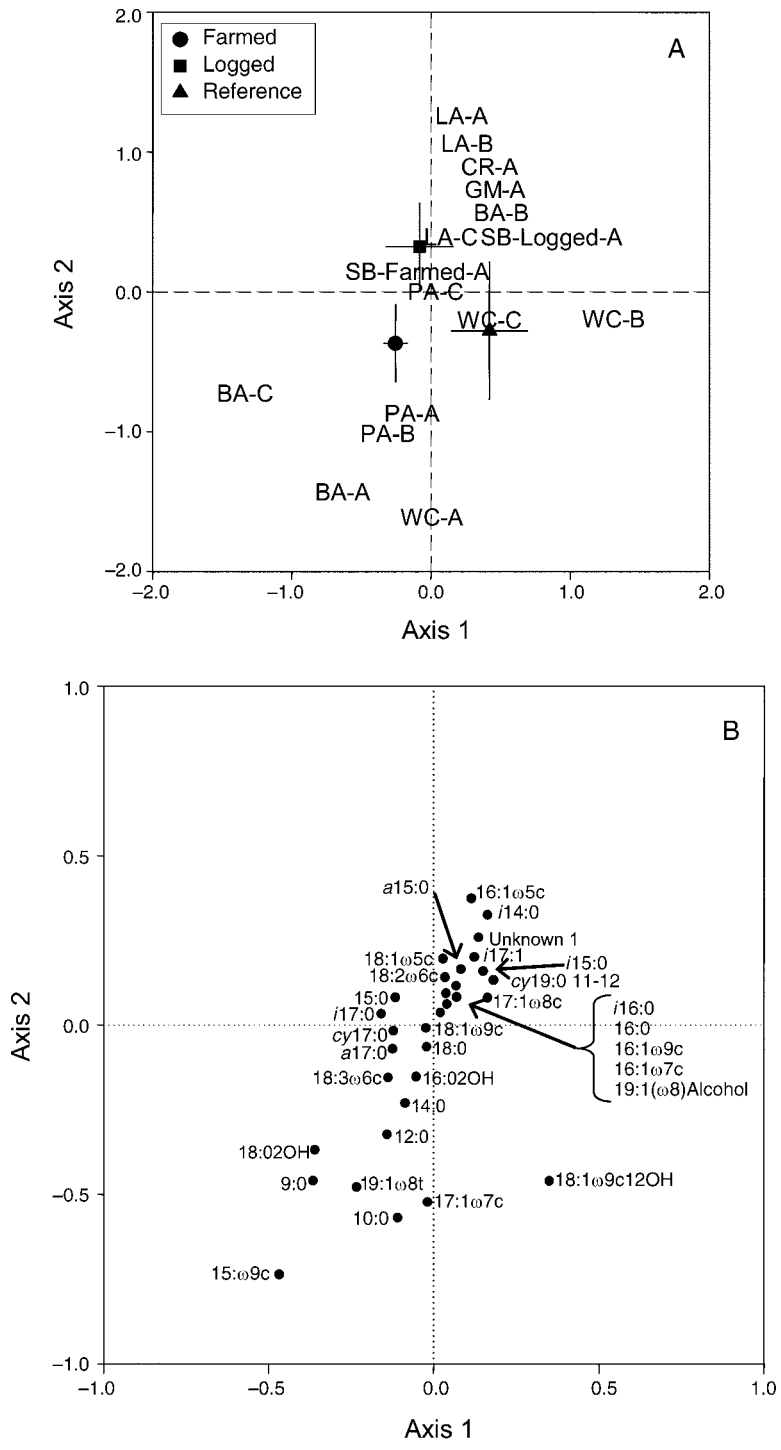

FIG. 1. (A) Scores (means ± SE) by past land use based on the nonmetric multidimensional scaling (NMS) ordination of plots and relative mole percentage of lipid biomarkers, and (B) the association of lipid biomarkers with NMS ordination axes. Refer to Table 1 for site names and descriptions.

er than those in former farms (11% vs. 9%), but did not differ from reference stands. The relative abundance of 16:1ω5c (an AMF indicator), 18:2ω6c (a fungal indicator), and Gm+ bacterial markers were also markedly higher in previously logged stands than in old farms, whereas Gm− bacterial markers were higher in historically farmed stands (4.0%) than in logged (2.5%) and reference stands (2.3%) (Appendix B). Ac-

TABLE 2. Variation in plot-level substrate pools and site-level total basal area of dominant trees with past land use.

Component	Past land use		
	Farmed	Logged	Reference
Litter (g)			
Total	1.13 (0.08)	2.03 (1.15)	1.45 (0.49)
Leaf	0.62 (0.09)	0.30 (0.08)	0.56 (0.21)
Woody	0.26 (0.08)	0.55 (0.21)	0.25 (0.11)
Acer rubrum	0.02 (0.01)	0.03 (0.01)	0.03 (0.02)
A. saccharum	0	0.03 (0.02)	0.01 (0.01)
Betula lenta	0.16 (0.09)	0.03 (0.01)	0.01 (0.004)
Fagus grandifolia	0.002 (0.002)	0.01 (0.01)	0
Liriodendron tulipifera	0.30[a] (0.08)	0.03[b] (0.01)	0.004[b] (0.003)
Quercus spp.	0.04 (0.01)	0.08 (0.06)	0.45 (0.17)
Tsuga canadensis	0.001	0.001 (0.001)	0.002 (0.002)
Total basal area (cm^2)			
Acer saccharum	840 (71)	11 803 (2363)	46 992 (12 164)
Betula lenta	9261 (264)	8882 (1871)	5493 (1412)
Liriodendron tulipifera	61 860 (2971)	47 496 (8897)	37 520 (5153)
Quercus rubra	5065 (801)	9452 (5494)	26 004 (2780)

Notes: Data are means with SE in parentheses. Data with the same lowercase superscript letters are not significantly different at $P < 0.05$.

cordingly, monounsaturated lipids were higher in former farms than in logged and reference stands, whereas branched lipids were significantly higher in logged stands than in farmed stands. Total extracted lipid abundance, representing total microbial biomass, ranged from 6.59 ± 0.39 nmol/g in previously farmed stands to 7.82 ± 1.52 nmol/g in reference stands, but did not differ among past land uses.

Differences in functional-group ratios further suggested a relationship between past land use and contemporary microbial community patterns. The ratio of Gm+/Gm− bacteria markers was significantly lower in old farms (0.52) than in logged (0.75) and reference stands (0.66), reflecting the relative dominance of Gm− bacteria markers in these areas. However, after the effect of elevation was removed, farmed stands had a significantly higher fungal/bacterial ratio (1.25) than logged stands (low-elevation plots: 1.18, high-elevation plots: 1.85) and reference stands (1.18).

Microbial community variation with past land use stood in contrast to the similarities among substrate pools and total basal area of dominant trees in historically heterogeneous stands. Total, woody and leaf litter did not differ among plots, and total basal area of the dominant tree species did not differ among sites (Table 2). Generally, the mean abundance of litter from individual species was also comparable among plots. *Acer rubrum*, *A. saccharum*, *Betula lenta*, *Fagus grandifolia*, *Quercus* spp., and *Tsuga canadensis* were similarly represented in the litterfall of each plot (Table 2). Only the abundance of *Liriodendron tulipifera* litter varied with past land use ($P = 0.03$), with the average dry mass being significantly higher in farmed plots than in logged and reference plots. However, none of the fungal or bacterial biomarkers were correlated with substrate pools, including *L. tulipifera* litter.

Correlations between microbial community indices and potential N mineralization rate varied with past land use. In previously farmed stands, potential net N mineralization (9.68 mg·kg dry $soil^{-1}$·28 d^{-1}) was negatively associated with the abundance of the Gm+ bacteria marker ($P < 0.001$) and 18:2ω6c ($P < 0.001$), while in reference stands mineralization rates (87.8 mg·kg dry $soil^{-1}$·28 d^{-1}) were negatively associated with the abundance of the AMF marker (16:1ω5c) ($P < 0.001$) and the ratio of Gm+/Gm− markers ($P < 0.001$). None of the microbial parameters tested were correlated with N mineralization in logged stands (43.4 mg·kg dry $soil^{-1}$·28 d^{-1}). Soil P was negatively associated with the abundance of the AMF (16:1ω5c: $r = -0.49$, $P = 0.05$) and fungi (18:2ω6c: $r = 0.64$, $P < 0.01$) biomarkers regardless of stand history (Fig. 2).

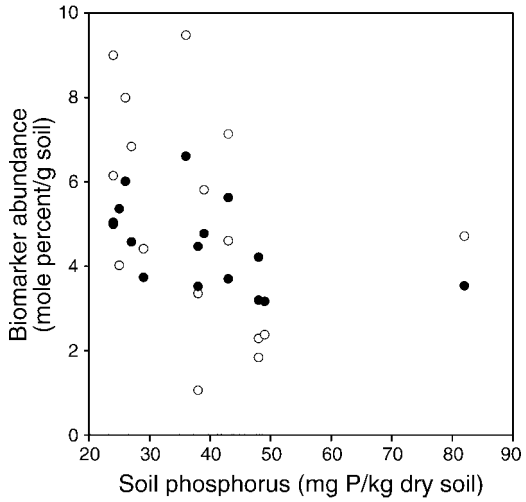

FIG. 2. The association between fungal biomarkers and soil phosphorus concentration. Solid circles indicate lipid 16:1ω5c (AMF marker) and open circles indicate lipid 18:2ω6c.

Regressed together against N mineralization rates, the individual fungal markers and markers for Gm+ bacteria, as well as the ratio of Gm+/Gm− bacterial markers, had low predictive power (adjusted $R^2 = 0.11$, $P = 0.29$). However, when the abundance of 18:2ω6c and the AMF marker were summed, they accounted for a significant amount of variation in potential net N mineralization (Fig. 3). Soil bulk density and soil moisture also showed a strong negative relationship with N mineralization rate (Fig. 3). However, when both terms were included in a model with the fungal biomarkers, soil moisture was no longer a significant factor ($P = 0.85$). Jointly, soil bulk density and the relative abundance of fungal biomarkers 18:2ω6c and 16:1ω5c explained 91% (adjusted R^2) of the variation in potential net N mineralization rate ($P < 0.0001$). Soil C:N did not account for a significant amount of variation in N mineralization rate ($P = 0.23$).

Discussion

To our knowledge, this is the only study to investigate the long-term (>50 years) legacies of land use in microbial community composition in temperate deciduous forests, and one of the first to relate microbial legacies to ecosystem processes. Previous work examining the influence of past management on soil microbial communities has focused on grassland communities (Steenwerth et al. 2003, Allison et al. 2005), where vegetation recovery and organic-matter accumulation may proceed slowly. In southern Appalachian cove-hardwood communities, conditions are highly conducive to vegetation reestablishment and nutrient re-accumulation following disturbance. The warm and moist climate, prolific sprouting ability of hardwood species, and abundance of N-fixing species, as well as the high productivity of vegetation once it is established, all contribute to a relatively rapid invasion of abandoned lands and subsequent accrual of nutrients in live biomass (Elliott et al. 2002), and suggest that legacies of land use in belowground soil communities should be minimal.

Despite favorable conditions for quick forest recovery, we observed appreciable differences among microbial communities in stands that varied in land-use history. Nonmetric multidimensional scaling (NMS) ordination showed that the microbial composition of farmed stands were unique, distinguishable by their high abundance of Gm− (gram-negative) bacterial markers (15:1ω9c and 18:0 2OH) and low abundance of fungal indicators. In contrast, logged and reference stands were characterized by their high levels of fungal (16:1ω5c) and Gm+ bacterial (*i*14:0) markers. Reference stands could be further differentiated by their positive association with lipids *i*15:0, *i*16:0, which are indicators for Gm+ bacteria. These compositional trends were supported by functional-group analyses. Although all the microbial communities studied were bacterially dominated, bacterial markers were more numerous in previously farmed stands due to an abundance of Gm− bacterial markers, whereas logged and reference stands harbored a greater abundance of Gm+ markers.

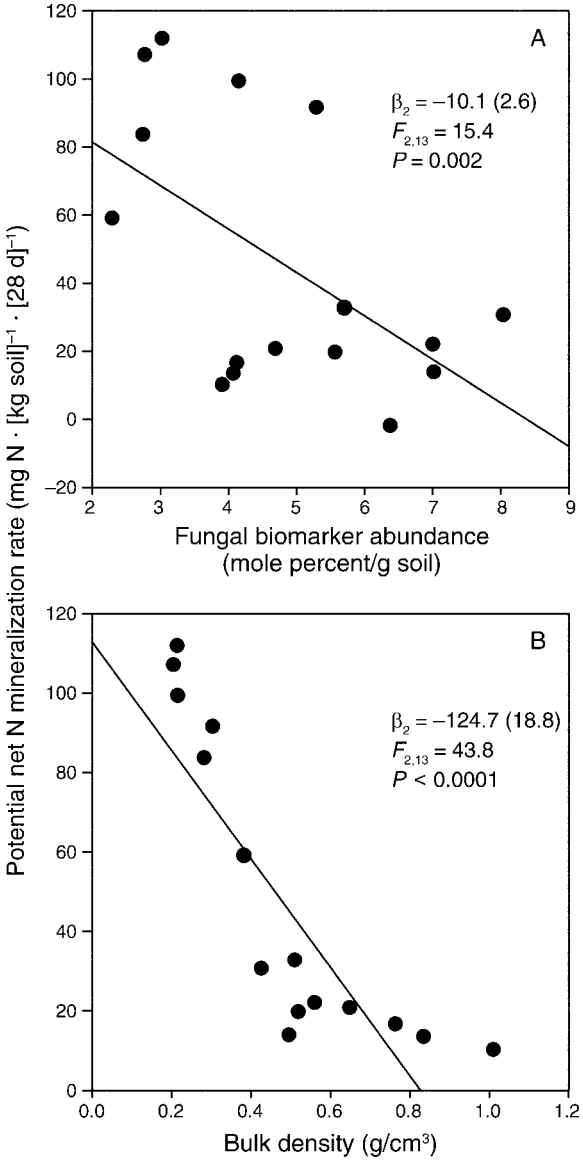

FIG. 3. The relationship between potential net nitrogen mineralization rate and (A) the summed abundance of fungal biomarkers 18:2ω6c and 16:1ω5c and (B) soil bulk density. Model parameters (with SE in parentheses) and *F* and *P* values are from the least-squares regression of fungal biomarkers and soil bulk density against the natural log of potential net nitrogen mineralization.

Of the two alternative hypotheses that might explain these patterns, our data suggest that past land use directly influences microbial communities through its physical alteration of the rhizosphere. We found no evidence that differences in current substrate pools account for the observed microbial variation. Total litter abundance, as well as the woody and leaf components, were similar among plots and were not statistically as-

sociated with the abundance of fungal or bacterial markers. Although the litter of *Liriodendron tulipifera* was appreciably more abundant in former farms, it also was not associated with microbial markers. Moreover, because past land use did not account for variation in the basal area of dominant trees, there is little support for the alternative hypothesis that differences in the relative live biomass (i.e., roots) were driving the relationship between land-use history and microbial community composition. Rather, the observed patterns are consistent with the results of previous work investigating the short-term effects of land management on soil microbial communities. Cultivation and tillage substantially disrupt the soil by breaking up soil aggregates, increasing soil compaction, exposing previously protected organic matter, and mixing soil horizons (Beare 1997, Allison et al. 2005). These changes cause significant declines in microbial biomass, reducing the abundance of fungi, aerobic microorganisms, and facultative anaerobes, while increasing the relative abundance of Gm− bacteria (Doran 1980, Beare 1997). By extracting microbial RNA, Buckley and Schmidt (2001) found that the abundance of groups comprised of Gm+ bacteria (aerobes) and fungi were significantly lower in fields abandoned from agriculture seven years earlier. In our study, old farms exhibited similar compositional patterns, despite having experienced relatively little tillage and having been abandoned since 1930. The high bulk density of former farms (Fraterrigo et al. 2005) and the elevated abundance of Gm− bacterial markers, the most abundant of which indicate anaerobic organisms, suggest that these enduring microbial patterns may be partly due to a continued state of soil hypoxia. This is a common condition in highly managed soils (Hill 1990). Early manure inputs also may have contributed to the long-term effect of farming on microbial community composition by promoting microorganisms that can exploit labile C. Manure application has been found to enrich the soil bacterial community and increase the dominance of Gm− bacteria in fields currently under cultivation (Peacock et al. 2001).

Steenwerth et al. (2003) found that old-fields in the California (USA) coastal valley were associated with 18:2ω6 (a fungal marker) and Gm+ indicators (*i*14:0, *i*15:1), and perennial grasslands were related to Gm− markers (*cy*19:0 and hydroxys). This suggests that microbial communities in grasslands and forests do not respond similarly to land abandonment. The composition of grassland communities appeared to be strongly influenced by differences in soil clay and moisture content (Steenwerth et al. 2003), whereas the forest communities we sampled were only somewhat related to clay content and not associated with soil moisture. More work is needed to understand how abiotic and biotic factors influence microbial community response to land-use change along environmental and vegetation gradients.

In contrast to bacterial patterns, the relatively low abundance of fungal markers in old farms may indicate that fungi need more time to recover from the changes imposed by agriculture. Unlike logging, farming in southern Appalachia entailed the removal of all woody biomass from fields, including brush and tree trunks. This practice may have negatively affected the survival and long-term development of saprophytic fungi by reducing cellulose and lignin sources. Mycorrhizal fungi may have been further impacted both by the reduction in mycorrhizal root tips (Bååth 1980) and the increased availability of P that resulted from manure inputs (Fraterrigo et al. 2005). This latter relationship was alluded to by the negative associations we found between fungal biomarkers (16:1ω5c and 18:2ω6) and soil P (Fig. 2) and agrees with the findings of others who have observed a direct negative effect of soil P availability on fungi, especially mycorrhizal colonization (Amijee et al. 1993, Treseder and Allen 2002, Balser and Firestone 2005).

Microbial communities in logged areas were generally similar to those in reference stands. Although the composition of the microbial community differed among logged and reference stands along one axis of variation (NMS analysis), the major functional groups (fungi, gram-positive and gram-negative bacteria) and ratios (fungi/bacteria ratio, Gm+/Gm− ratio) did not vary between these stands. These conflicting patterns suggest that logged and reference stands are similar with respect to the most important microbial groups but somewhat unique in terms of their entire lipid profile. Given that not all lipids can be meaningfully associated with a functional group at this time, the most sensible interpretation of the evidence is that logged and reference stands are generally equivalent.

Logging produces few long-term physical changes in the rhizosphere compared with cultivation (Compton and Boone 2000, Fraterrigo et al. 2005), which may explain the absence of a microbial legacy in stands subjected to harvesting. Although others have shown that clear-cutting appreciably affects lipid patterns through its influence on soil organic-matter quantity and mycorrhizal roots (Harvey 1980, Bååth et al. 1995), it is unlikely that these mechanisms for microbial alteration would persistently affect logged stands in the southern Appalachians. Here, clear-cut areas rapidly reforest, resulting in tree communities that resemble the preexisting community within 20 years (Table 2; Elliott et al. 2002). This may favor the early development of microbial communities that are compositionally comparable to those present prior to logging, particularly with respect to C and N requirements. In addition, resprouting by trees may facilitate the survival of ectomycorrhizal fungi following logging because root tissues remain viable (Harvey 1980). These factors may reduce long-term differences between logged and reference stands and explain why logging

did not lead to enduring changes in community composition in this study.

The association between microbial community composition and N mineralization suggests that the impacts of historic practices on belowground communities can influence ecosystem function, yet not necessarily in straightforward ways. Bulk density, which can be augmented by past land use (Fraterrigo et al. 2005), explained a considerable amount of variation in N mineralization rates (Fig. 3) and superseded the role of bacterial markers in predicting N mineralization. Thus, while bacterial composition (especially the abundance of Gm+ markers) was correlated with N mineralization, the underlying cause of this relationship may have been due to the influence of bulk density on bacterial activity, particularly in former farms. Conversely, the abundance of fungal markers was an important predictor of N mineralization regardless of whether bulk density was included in the predictive model (Fig. 3). This suggests that there is a direct relationship between past land use, fungal abundance, and N mineralization rates. Fungi generally metabolize substrates more efficiently than bacteria, resulting in biomass with a higher C:N (Griffith and Bardgett 2000, Allison et al. 2005). The resultant lower requirement for biomass nitrogen may therefore lead to altered mineralization in soils dominated by fungi. The negative relationship between the abundance of the AMF marker and N mineralization in reference stands suggests that other mechanisms may be operating as well. There is strong evidence that our references stands are P limited; relative plant growth is positively correlated with P availability in reference stands, and P concentration is positively correlated with potential N cycling rates (J. Fraterrigo, *unpublished data*). Phosphorus limitation is consistent with the increase in AMF abundance observed in reference stands. By constraining energy production and microbial growth, low P availability can impede microbial activity and the turnover of organic N (Olander and Vitousek 2000). Thus, it is possible that a lack of available P is inhibiting the capacity of the microbial community to utilize C supplies in reference stands and is ultimately regulating N transformations. The enhancement of P may have prevented similar constraints on microbial activity in farmed areas.

Differences in community composition as a result of past land use may have important implications for ecosystem function aside from those associated with N cycling. Waldrop et al. (2000) found that land-use-driven changes in the structure of microbial communities altered their capacity to degrade macromolecular C compounds. Others have shown that microbial changes due to land use can directly affect the P cycle (Cleveland et al. 2003). Clearly, the potential for changes in microbial composition as a result of past land use to influence biogeochemical transformations needs further investigation. Such information may be critical for the accurate prediction of ecosystem response to global change, and will be necessary if land-use change is to be effectively integrated into broad-scale biogeochemical models.

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APPENDIX A

Methods for the hybrid procedure of phospholipid fatty acid (PLFA) and fatty acid methyl ester (FAME) analysis used to characterize microbial community composition (*Ecological Archives* E087-032-A1).

APPENDIX B

Relative mole percent of lipids by past land use and functional guild (*Ecological Archives* E087-032-A2).