

## The influence of soil properties on the structure of bacterial and fungal communities across land-use types

Christian L. Lauber<sup>a,\*</sup>, Michael S. Strickland<sup>b</sup>, Mark A. Bradford<sup>b</sup>, Noah Fierer<sup>a,c</sup>

<sup>a</sup> Cooperative Institute for Research in the Environmental Sciences, University of Colorado at Boulder, Attn Christian Lauber UCB 216, Boulder, CO 80309-0216, USA

<sup>b</sup> Odum School of Ecology, University of Georgia, Athens, GA 30602, USA

<sup>c</sup> Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309, USA

### ARTICLE INFO

#### Article history:

Received 30 January 2008

Received in revised form 27 May 2008

Accepted 30 May 2008

Available online 25 June 2008

#### Keywords:

Biogeography

Bacteria

Fungi

Land-use change

Soil pH

Quantitative PCR

16S rRNA, 18S rRNA

### ABSTRACT

Land-use change can have significant impacts on soil conditions and microbial communities are likely to respond to these changes. However, such responses are poorly characterized as few studies have examined how specific changes in edaphic characteristics do, or do not, influence the composition of soil bacterial and fungal communities across land-use types. Soil samples were collected from four replicated ( $n=3$ ) land-use types (hardwood and pine forests, cultivated and livestock pasture lands) in the southeastern US to assess the effects of land-use change on microbial community structure and distribution. We used quantitative PCR to estimate bacterial–fungal ratios and clone libraries targeting small-subunit rRNA genes to independently characterize the bacterial and fungal communities. Although some soil properties (soil texture and nutrient status) did significantly differ across land-use types, other edaphic factors (e.g., pH) did not vary consistently with land-use. Bacterial–fungal ratios were not significantly different across the land-uses and distinct land-use types did not necessarily harbor distinct soil fungal or bacterial communities. Rather, the composition of bacterial and fungal communities was most strongly correlated with specific soil properties. Soil pH was the best predictor of bacterial community composition across this landscape while fungal community composition was most closely associated with changes in soil nutrient status. Together these results suggest that specific changes in edaphic properties, not necessarily land-use type itself, may best predict shifts in microbial community composition across a given landscape. In addition, our results demonstrate the utility of using sequence-based approaches to concurrently analyze bacterial and fungal communities as such analyses provide detailed phylogenetic information on individual communities and permit the robust assessment of the biogeographical patterns exhibited by soil microbial communities.

© 2008 Elsevier Ltd. All rights reserved.

### 1. Introduction

Land-use change, particularly the conversion of forests to pasture or cultivated fields and vice versa, is a common occurrence in many landscapes and is one of the dominant factors affecting the biodiversity and functioning of terrestrial ecosystems (Sala et al., 2000). The effects of alteration in land-use on the physical and chemical properties of soils have been well studied. We know that land-use change can have significant and long-lasting effects on soil carbon and nutrient contents, soil texture, and pH (Post and Mann, 1990; Murty et al., 2002), effects that largely arise from changes in plant species composition and associated management practices across land-use types.

Although numerous studies have shown that changes in land-use also have a significant effect on microbial communities (Bossio et al., 1998; Johnson et al., 2003; Ovreas and Torsvik, 1998; Steenwerth et al., 2002; Zelles et al., 1992), we have a poor understanding of how land-use (and the associated changes in plant and soil properties) may affect the abundance of specific taxonomic groups. For instance, culture-independent techniques such as PLFA and FAME have proven useful in identifying edaphic properties which influence microbial community structure (Bååth and Anderson, 2003; Bååth et al., 1995; Frostegård et al., 1993) and how shifts from forest to agriculture may influence litter decomposers (Carlile et al., 2001). However, few studies have simultaneously examined both bacterial and fungal biogeographical patterns across multiple land-use types in a given landscape. Moreover, most molecular surveys of microbial communities are often based on an individual sample collected from a given land-use type and this strategy does not allow for a statistical assessment of the specific effects of land-use or edaphic properties on microbial biogeography.

\* Corresponding author. Tel.: +1 303 492 2099; fax: +1 303 492 1149.

E-mail address: [chris.lauber@gmail.com](mailto:chris.lauber@gmail.com) (C.L. Lauber).

We expect that any shifts in bacterial and fungal communities associated with land-use differences will be directly related to the effects of that land-use change on edaphic properties. Previous studies have shown that shifts in the structure of bacterial and fungal communities can be associated with changes in a number of soil properties including soil texture (Girvan et al., 2003), soil pH (Blagodatskaya and Anderson, 1998; Fierer and Jackson, 2006) and soil nitrogen availability (Frey et al., 2004). All of these edaphic factors may be altered by changes in land-use type, but it is not clear which of these factors has the dominant influence on the biogeographical patterns exhibited by soil microbial communities. As a result, we cannot currently use information on soil environmental characteristics to predict how soil microbial communities may change across multiple land-use types.

Fundamental differences in bacterial and fungal physiology and ecology would suggest that the biogeography of each group would be controlled by separate edaphic factors which may vary among land-uses (van der Wal et al., 2006). For example, fungi may be more sensitive to shifts in vegetation type than soil bacteria, particularly mycorrhizal fungi that form symbiotic associations with specific plant types (Heinemeyer et al., 2004) or Basidiomycota which are involved in decomposing lignified plant detritus (Bardgett and McAlister, 1999). Likewise, bacteria and fungi may not mineralize the same types of carbon substrates in soil, and therefore, shifts in carbon pools may have different effects on these two groups of microorganisms (Bossuyt et al., 2001; Six et al., 2006). The tendency of edaphic factors to shift with land-use would suggest that similar land-uses would have similar edaphic characteristics and thus, similar microbial communities. However, within land-use variation of edaphic factors may obscure the more obvious differences between land-uses and limit our ability to predict the biogeography of microbes based on a straightforward visual assessment of the environment. As bacteria and fungi are likely to have distinct functional roles in soil (Hendrix et al., 1986), a more robust understanding of the specific effects of land-use and edaphic factors on these two microbial groups will improve our ability to predict the specific effects of land-use change on soil biotic processes.

In this study, we examined differences in bacterial and fungal communities across a series of replicated land-use types in and around the Calhoun experimental forest in South Carolina, USA. We used a sequence-based approach to quantify phylogenetic differences in the bacterial and fungal communities across four land-use types and related these differences to measured edaphic properties. Our objective was to use these land-use types to examine how soil bacteria and fungi may respond to land-use change and, more specifically, to examine how shifts in the abundance and composition of soil bacterial and fungal communities correspond to changes in specific edaphic properties.

## 2. Materials and methods

### 2.1. Site description

Soils were collected from a series of plots (12 total) in and around the Calhoun Experimental Forest (CEF), which is managed by the US Department of Agriculture and located in the Piedmont region (approximately 34.5°N, 82°W) of northwestern South Carolina (Callaham et al., 2006; Gaudinski et al., 2001). The CEF was established in 1947 to study soil and watershed dynamics typical of the Piedmont region of the southeastern US and the history of land-use change in this landscape is well-documented (Richter and Markewitz, 2001).

In September 2006, we sampled from three plots per land-use type (Table 1). The four land-use types (cultivated fields, pasture land, pine forest plantation, and mixed hardwood forest) are

common in the coastal plain and Piedmont regions of the southeastern US (Callaham et al., 2006) and the land-management practices at these plots reflect current practices used in the region. The cultivated plots are managed by the South Carolina Department of Natural Resources to support wildlife populations with annual crop rotation between corn, millet, wheat, sorghum, sunflowers and fallow using conventional tillage practices (Callaham et al., 2006). Pasture sites are continuously grazed and dominated by rye grass. The cultivated and pasture land-uses have been under their respective management regimes for at least 40 years (Callaham et al., 2006). Pine forest sites 1 and 3 consist of loblolly pine (*Pinus taeda*) with varying amounts of oak and hickory species in the understory (*Quercus* spp., *Carya* spp.), while the pine plot 2 is a loblolly pine monoculture. Pine plots 1 and 3 are approximately 50 years old, while plot 2 is 10 years in age (Callaham et al., 2006). The hardwood sites are mature oak–hickory stands at least 75 years of age. Plot 2 of the hardwood land-use is grazed by cattle and has minimal understory.

Our goal was to select plots representative of the landscape and land-use types in this region. As a result, the plots within a given land-use type are not necessarily identical with respect to land-management practices and soil characteristics. The plots are located within 30 km of each other and the soils are classified as fine, kaolinitic, thermic Typic Kanhapludults of the Appling, Cecil, Hiwassee, and Madison series (Callaham et al., 2006), except for hardwood plot 1 which is an active, thermic, shallow Typic Hapludalf of the Wilkes series (Callaham, M., personal communication). The region receives an average of 1250 mm of rainfall annually with a mean annual temperature of 15.9 °C (Fierer and Jackson, 2006). Detailed site and soil characteristics for all of the plots are provided in Supplementary material Tables 1 and 2.

### 2.2. Soil collection and DNA extraction

We used a stratified random sampling approach to collect 10 individual A horizon soil cores (8 cm diameter, 0–7.5 cm depth) from a 100 m<sup>2</sup> plot within each land-use replicate; sampling locations were marked to avoid re-sampling from the same exact location. Soils were sieved on-site through 4 mm mesh and then all 10 of the cores from a given plot were homogenized by hand into a single bulked sample per plot. The bulked samples were then divided into two sub-samples, one of which was transported to the laboratory on ice for chemical analysis and the other on dry-ice for DNA extraction. Soils transported on dry-ice were then stored at –80 °C until DNA extraction; the other soils were stored at +5 °C until chemical analysis.

DNA was isolated from soil using the MOBIO Power Soil DNA Extraction kit (MOBIO Laboratories, Carlsbad, CA, USA) with modifications. Specifically, soil samples (3 g) were ground in a mortar with pestle in liquid nitrogen and then sub-samples of 0.3–0.5 g of soil were placed into a bead tube for extraction. Bead tubes were heated to 65 °C for 10 min, and then shaken horizontally for 2 min at maximum speed with the MOBIO vortex adapter. The remaining steps were performed as directed by the manufacturer. DNA was extracted from three replicate sub-samples per plot and all DNA samples were stored at –20 °C until needed.

### 2.3. Edaphic soil properties

Nutrient pools, pH, soil texture, bulk density and moisture were calculated from three analytical replicates per homogenized soil sample from each plot. Following air-drying and then ball-milling, total carbon (C) and nitrogen (N) concentrations were quantified on a Carlo Erba NA1500 C/H/N Analyzer. Extractable phosphorus (P) was quantified using the method described by Kuo (1998) with Mehlich I extract (0.05 M HCl + 0.0125 M H<sub>2</sub>SO<sub>4</sub>) and run on an

**Table 1**  
Land-use edaphic properties

Land-use	pH	%Silt* + clay	%Soil moisture	Bulk density, g cm <sup>-3</sup>	C:N	C*, kg <sup>-1</sup>	N*, g kg <sup>-1</sup>	P*, mg kg <sup>-1</sup>
Cultivated	4.7 (0.8)	39 (2.9)	13 (1.0)	1.2 (0.1)	15 (1.2)	12 (1.7)	0.8 (0.1)	17 (0.6)
Pasture	4.2 (0.2)	21 (4.2)	10 (0.7)	1.7 (0.1)	14 (0.4)	10 (0.7)	0.7 (0.04)	16 (3.0)
Hardwood	4.4 (0.5)	30 (3.2)	14 (2.3)	1.2 (0.2)	22 (2.9)	18 (3.4)	0.8 (0.04)	1.8 (0.9)
Pine	3.6 (0.4)	18 (3.1)	8 (1.6)	1.3 (0.1)	24 (5.0)	9 (0.4)	0.4 (0.1)	5.4 (3.9)

Mean values within land-use for measured soil properties. Variables that were significantly ( $n = 4$ ,  $P < 0.05$ ) related to land-use are indicated with an asterisk. Standard error of the means (SEM) are indicated in parentheses. Complete soil and site information for all 12 plots is provided in Supplementary material Tables 1 and 2.

Alpkem auto-analyzer (OIAAnalytical, College Station, TX, USA) using a 1:4 mass:volume ratio. Soil pH was measured on supernatants of 0.01 M CaCl<sub>2</sub> soil slurries, 1:1 (w/v), after soil particles had settled from 10 min of vigorous shaking (Thomas, 1996). Silt and clay contents were measured using a simplified version of the hydrometer method as described by Gee and Orr (2002). Soil bulk density was calculated after correcting for the mass and volume of roots and stones (Culley, 1993). The mean of the analytical replicates was used for data analysis and the calculations of land-use effects on edaphic properties.

#### 2.4. Amplification, cloning and sequencing of partial 16S and 18S rRNA genes

Small-subunit ribosomal genes were amplified using universal 515F (5'-GTGCCAGCMGCCGCGTAA-3') and 1391R (5'-GACGG GCGGTGWGTRCA-3') primers for bacterial 16S rRNA genes (Lane, 1991). Fungal 18S rRNA genes were amplified with EF4 (5'-GGA AGGRTGATTATTAG-3') and fung5 (5'-GTAAAGTCCTGG TTCCC-3') (Smit et al., 1999). The PCR reaction contained 1× PCR Buffer (Invitrogen, Carlsbad, CA, USA), 2.5 mM MgCl<sub>2</sub>, 0.2 μM of each primer, 0.2 μM dNTPs, 0.5 U Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA) and 1.0 μl template DNA. Amplification was accomplished by initial denaturation at 94 °C for 3 min followed by 25 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 30 s with a final extension at 72 °C for 10 min. Each DNA sample was amplified in triplicate and the amplicons were pooled by plot ( $n = 12$ ) and run on a 1.5% agarose gel. The bands were purified using the Promega Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) followed by ligation of the amplicons into the pCR4 TOPO TA cloning vector (Invitrogen, Carlsbad, CA, USA) and transformation into TOP10 chemically competent *Escherichia coli* cells according to the manufacturer's directions. Transformations were plated onto L-agar (Miller) with 50 μg ml<sup>-1</sup> ampicillin and grown overnight at 37 °C. Fungal 18S rRNA genes were amplified directly from transformants using M13 forward and reverse primers (1× PCR Buffer, 1.5 mM Mg-acetate, 0.2 μM dNTPs, 0.4 μM each primer and 0.75 U Taq DNA polymerase) at 94 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 45 s with a final extension at 72 °C for 10 min. Agar lawns (bacterial 16S rRNA genes) or M13 PCR products (fungal 18S rRNA genes) were submitted to Agencourt Biosciences (Beverly, MA, USA) for single pass sequencing using the 515F or EF4-F primers, respectively. The non-redundant sequences from this study have been deposited in the GenBank database and have accession numbers EU656144–EU657021 and EU657022–EU657365 for bacteria and fungi, respectively.

#### 2.5. Taxonomic classification and phylogenetic distance

Bacterial sequences were aligned against the Greengenes database (<http://greengenes.lbl.gov/cgi-bin/nph-index.cgi>) using the NAST alignment utility (DeSantis et al., 2006a). The sequences were classified using the RDP taxonomy and chimera-checked using utilities available on the Greengenes website (DeSantis et al.,

2006b). Sequences which were of poor quality or suspected to be chimeric were eliminated from the analysis (less than 9% of the sequences). Fungal sequences were aligned using MUSCLE 3.6 (Edgar, 2004) and compared to an in house database of 100 fungal 18S sequences derived from the AFTOL data set (Lutzoni et al., 2004) using the BLAST algorithm (Altschul et al., 1990) to determine taxonomic classification. Sequences with <90% similarity to the in house database or presumed to be chimeric were discarded (less than 3% of the fungal sequences were discarded). The number of unique operational taxonomic units (OTU) in a given library was determined using Fastgroup II (Yu et al., 2006), with an OTU defined as those sequences that are 97% similar.

Land-use effects on microbial community composition were determined using the UniFrac algorithm (Lozupone and Knight, 2005) and the phylogenetic (P) test (Martin, 2002). UniFrac quantifies the fraction of unique branch lengths against the total branch length between pairs of communities from one phylogenetic dendrogram, giving an estimate of the phylogenetic distance between those communities. Separate neighbor-joining phylogenetic trees containing either all of the bacterial or all of the fungal sequences were generated with PHYLIP 3.6 (Felsenstein, 2005) and rooted with partial *Sulfolobus acidocaldarius* 16S or *Arabidopsis thaliana* 18S genes, respectively. Phylogenetic distances between the bacterial and fungal communities for each plot were generated using the weighted-normalized option available through UniFrac.

#### 2.6. Quantitative PCR analysis

Relative abundances of bacteria and fungi were quantified according to Fierer et al. (2005). Standard curves to estimate bacterial and fungal small-subunit rRNA gene abundances consisted of a 10-fold serial dilution of a plasmid containing a full-length copy of either the *E. coli* 16S rRNA gene or the *Saccharomyces cerevisiae* 18S rRNA gene. Soil DNA concentrations were normalized to 10 ng μl<sup>-1</sup>. The 25 μl qPCR reactions contained 12.5 μl ABgene SYBR Master Mix (ABgene, Rochester, NY, USA), 0.5 μl each 10 μM forward and reverse primers, and 10.5 μl sterile, DNA free water. Standard and environmental DNA samples were added at 1.0 μl per reaction. The reaction was carried out on a Eppendorf Mastercycler<sup>®</sup> ep Realplex thermocycler (Eppendorf North America, Westbury, NY, USA) using the program of 94 °C for 3 min followed by 40 cycles of 94 °C for 30 s, 50 °C for 45 s and 72 °C for 30 s. Melting curve and gel electrophoresis analysis were performed to confirm that the amplified products were the appropriate size. Ratios of fungal to bacterial gene copy numbers were generated using a regression equation for each assay relating the cycle threshold (Ct) value to the known number of copies in the standards. All qPCR reactions were run in quadruplicate with the pooled DNA sample from each plot.

#### 2.7. Statistical analyses

Relationships between microbial community phylogenetic distances, land-use, and edaphic properties were assessed using Plymouth Routines In Multivariate Ecological Research v5 (Primer

v5 – Lutton, UK) software. Using land-use as the main factor, an analysis of similarity (ANOSIM) procedure was used to relate shifts in the phylogenetic distance of bacterial and fungal communities between plots to land-use. Non-metric multidimensional scaling (NMDS) was used to visualize the phylogenetic distance between the bacterial and fungal communities in each pair of plots as calculated using UniFrac (see above). The RELATE function (a Mantel-type test) was used to determine if there were significant correlations between community-level phylogenetic distances and measured soil properties. Effects of land-use or correlations between soil properties and microbial community distances were considered significant when  $P < 0.05$ .

Phylogenetic independent analyses were performed by recording the distribution of taxa for each plot and normalizing the values to represent the percentage of each taxon within a plot. Land-use effects on the distribution of taxa, fungi:bacteria ratios as well as edaphic properties were tested using the Mann–Whitney  $U$  test on untransformed data. Relationships between edaphic properties were also assessed by correlation analysis. Linear regression analysis was performed on the three most abundant bacterial or fungal taxa with soil properties that had significant correlations to the phylogenetic distances identified through ANOSIM analysis of the distance matrices, to link changes in the soil environment with the microbial community. Mann–Whitney  $U$  Tests, correlations (between edaphic factors) and regression analyses were performed with SYSTAT 11.0 (San Jose, CA, USA).

### 3. Results

#### 3.1. Land-use and edaphic soil properties

Soil texture, total C, total N, and extractable P differed significantly between land-use types ( $P \leq 0.5$  in all cases), while soil pH, moisture, and bulk density did not vary significantly across the landscape ( $P > 0.07$  in all cases) (Table 1). Total N in the pine plots was approximately 50% less than in the hardwood, cultivated or pasture soils, and total C in the hardwood soils was approximately twice that of the other soils. The C:N ratio did not vary significantly between land-uses ( $P = 0.07$ ), but the agricultural (cultivated and pasture) land-use types tended to have lower soil C:N ratios than the forest (hardwood and pine) soils (Table 1, Supplementary material Table 2). Likewise, extractable P varied between forest and agricultural soils, with cultivated and pasture land-uses having approximately 10 times greater phosphorus concentrations than hardwood and pine soils. Notable exceptions are hardwood plot 2, which had approximately four times greater P than hardwood 1 and 3, and pine plot 2, which had a phosphorus concentration similar to that of the agricultural soils (Supplementary material Table 2). Soil pH was not different between the land-uses and was fairly consistent (ranging from 3.6 to 4.7) across all plots except cultivated 3, which had a pH of 6.2. Significant correlations exist between soil pH, texture, and moisture as well as between extractable P and the soil C:N ratio (Supplementary material Table 3).

#### 3.2. Taxonomic classification and abundance

At the phylum level there were 11 major bacterial taxa present within most of the soils and, averaged across all soils, the most abundant bacterial groups were the Acidobacteria (28%), Actinobacteria (16%) and  $\alpha$ -Proteobacteria (15%) (Table 2, Supplementary material Table 4). The relative abundances of the different taxa varied between and within land-uses, but only acidobacterial abundances were significantly greater in forest soils than in agricultural (cultivated and pasture) land-use types ( $P = 0.048$ ). Actinobacteria were most abundant in pasture soils, but not significantly so, whereas  $\alpha$ -Proteobacteria were found in roughly

the same proportion across all plots and land-uses (Supplementary material Table 4). The remaining individual taxa of bacteria represented less than 8% of the sequences in the data set (Table 2). A complete description of the bacterial communities in each of the plots is provided in Supplementary material Table 4.

The Ascomycota and Basidiomycota represented the majority of the fungal sequences in this study (27% and 70%, respectively) (Table 3). The remaining taxa included Chytridiomycota (<1%), Glomeromycota ( $\approx 1.5\%$ ), and the Zygomycota (<1%). Of the Ascomycota, the Sordariomycetes were the most abundant (38%), followed by the Chaetothyriomycetes (12%), Dothideomycetes (11%) and the Leotiomyces (8.2%). The Agaricales (23%) and the Aphyllophorales (2.9%) were the most abundant groups of Basidiomycota. The abundance of Ascomycota (mainly Sordariomycetes) was greater in the cultivated and pasture soils, while most of the forest soils had greater abundances of the basidiomycete Agaricales. Hardwood 2 and pine 2 plots were exceptions where Chaetothyriomycetes and Sordariomycetes (both Ascomycota) were particularly abundant and Agaricales were relatively rare (Table 3). None of the fungal taxa varied significantly between land-uses ( $P > 0.09$ ). Taxonomic details on the fungal communities are provided in Supplementary material Table 4.

The ratio of fungi to bacteria ranged from 0.02 to 0.10 across all plots (Table 3). These low ratios indicate that fungal rRNA genes were less abundant than the bacterial rRNA genes in the extracted samples. There was no significant overall effect of land-use type on the ratio of fungi to bacteria ( $P = 0.09$ ). However, the ratio of fungi to bacteria was typically lower in the agricultural soils than in the hardwood and pine forest soils (Table 3).

#### 3.3. Phylogenetic analysis

The UniFrac distance matrices were tested to determine if land-use had a significant effect on the bacterial and fungal communities. Using land-use as the main effect, no significant differences between the four land-use types were observed for either the fungal or the bacterial communities. However, bacterial community phylogenetic distances were significantly correlated with soil pH ( $r = 0.47$ ) and soil texture ( $r = 0.42$ ) ( $P < 0.01$  in both cases). Analyses of the fungal communities showed that C:N ratio ( $r = 0.73$ ,  $P < 0.001$ ) total N ( $r = 0.39$ ,  $P = 0.001$ ), and extractable P ( $r = 0.54$ ,  $P < 0.001$ ) were correlated with the phylogenetic distances between fungal communities (Table 4). NMDS ordination of the bacterial and fungal phylogenetic distance matrices qualitatively confirm these correlations; that is, soils with similar pH tend to have similar bacterial communities and soils with similar extractable P concentrations (and C:N ratios, data not shown) harbor similar fungal communities within this landscape (Fig. 1).

#### 3.4. Linking edaphic properties and taxonomic distribution

The relationships between the relative abundances of specific microbial taxa and edaphic soil properties were examined by regression analyses of the most abundant bacterial and fungal taxa to determine which taxa drove the overall patterns described above. For the most part, only those edaphic soil properties found to be correlated with the phylogenetic distances had significant regression coefficients with the abundances of specific bacterial and fungal taxa (Table 4, Figs. 2 and 3). Specifically, the changes in bacterial community composition with soil pH appear to be largely driven by the abundances of Acidobacteria and  $\alpha$ -Proteobacteria, which increased and decreased, respectively, with increasing soil pH. There were no significant correlations between pH and the relative abundances of any of the other bacterial phyla.

We regressed the relative abundances of the dominant fungal taxa (Ascomycota, Sordariomycetes and Leotiomyces, and the

**Table 2**  
Distribution of bacterial phyla

Plot	N	OTU	Acido	Actino	Bact	Chloro	Firm	Gemma	Proteo	Verruco	Unclass	Other
Cultivated 1	72	34	14	29	2.8	9.7	2.8	0.0	25	1.4	9.7	5.6
Cultivated 2	74	51	23	11	4.1	22	9.5	0.0	20	2.7	4.1	4.1
Cultivated 3	84	59	9.5	9.5	13	14	2.4	9.5	25	7.1	2.4	7.1
Pasture 1	83	39	14	41	4.8	1.2	4.8	3.6	12	16	1.2	1.2
Pasture 2	58	30	16	12	1.7	3.4	26	3.4	33	3.4	0.0	1.7
Pasture 3	71	43	24	21	2.8	1.4	13	4.2	24	1.4	7.0	1.4
Hardwood 1	82	45	27	9.8	8.5	2.4	0.0	2.4	24	20	0.0	6.1
Hardwood 2	82	42	35	11	0.0	3.7	3.7	1.2	29	6.1	4.9	4.9
Hardwood 3	77	47	21	17	5.2	10.4	0.0	1.3	27	7.8	3.9	6.5
Pine 1	79	38	35	3.8	2.5	3.8	0.0	2.5	29	14	0.0	8.9
Pine 2	81	32	51	17	0.0	0.0	3.7	0.0	20	3.7	1.2	3.7
Pine 3	79	44	57	5.1	0.0	1.3	2.5	0.0	19	1.3	5.1	8.9
Total	922		28	16	3.9	6.1	5.1	2.4	24	7.3	3.3	5.1
Mean (SEM)												
Cultivated	230		15 (4.0)	17 (6.3)	6.6 (3.2)	15 (3.5)	4.9 (2.3)	3.2 (3.2)	23 (1.6)	3.7 (1.7)	5.4 (2.2)	5.6 (0.9)
Pasture	212		18 (3.0)	25 (8.5)	3.1 (0.9)	2.0 (0.7)	15 (6.1)	3.8 (0.2)	23 (6.0)	6.8 (4.5)	2.7 (2.2)	1.4 (0.2)
Hardwood	241		28 (4.2)	13 (2.2)	4.6 (2.5)	5.5 (2.5)	1.2 (1.2)	1.7 (0.4)	27 (1.4)	11 (4.2)	2.9 (1.5)	5.8 (0.5)
Pine	239		48 (6.4)	8.7(4.3)	0.8 (0.8)	1.7 (1.1)	2.1 (1.1)	0.8 (0.8)	23 (3.3)	6.3 (3.9)	2.1 (1.5)	7.1 (1.7)

The relative abundance of bacterial phyla in each of the plots. Values represent the percentage of each group in the respective library of size 'N'. OTU, or 'operational taxonomic unit', describes the number of unique groups at the 97% sequence similarity level. "Other" includes Aquifex, Chlamydiae, Chlorobi, Genera incertae sedis WS3, Nitrospira, OP10, OP3, Planctomycetes, SPAM, and TM6. Means were calculated for each land-use with 1 SEM in parentheses. Abbreviations: Acido, Acidobacteria; Actino, Actinobacteria; Bact, Bacteroides; Chloro, Chloroflexi; Firm, Firmicutes; Gemma, Gemmatimonadetes; Proteo, Proteobacteria; Verruco, Verrucomicrobia; Unclass, unclassified.

Basidiomycete, Agaricales), against those edaphic factors (soil C:N ratio and extractable P) that were significantly correlated with the phylogenetic distance between fungal communities. The abundances of the Sordariomycetes and Agaricales were significantly correlated with both the soil C:N ratio and extractable P concentrations (Fig. 3). The abundance of Agaricales was positively associated with C:N but decreased as extractable P increased, while Sordariomycetes were most prevalent in low C:N plots with high extractable P. The abundances of the other dominant fungal group, the Leotiomycetes, was not linked with either soil C:N ( $r^2 = 0.0038$ ,  $P > 0.1$ ) or extractable P ( $r^2 = 0.053$ ,  $P > 0.1$ ). This suggests that the correlations between nutrient availabilities and fungal community

composition are largely driven by shifts in the relative abundances of the Sordariomycetes and the Agaricales.

#### 4. Discussion

##### 4.1. Land-use effects on edaphic properties

Edaphic properties are often shown to be consistent among similar land-use types where management practices have appreciable effects on nutrient concentrations and soil texture (Girvan et al., 2003; Johnson et al., 2003; Murty et al., 2002). Though we observed significant differences in nutrient concentrations (C, N

**Table 3**  
Distribution of fungal taxa

	N	OTU	Agaricales	Aphylo	Dothideo	Leotio	Chaetothyrio	Sordario	Other	Fungi:bacteria
Cultivated 1	20	10	0	0	15	10	0	70	5	0.0417
Cultivated 2	32	12	0	3	34	16	3	38	6	0.0216
Cultivated 3	29	10	0	0	0	41	0	55	3	0.0381
Pasture 1	26	16	27	12	4	0	15	38	4	0.0355
Pasture 2	30	14	0	0	3	7	27	60	3	0.0238
Pasture 3	31	12	0	0	0	19	3	74	3	0.0395
Hardwood 1	32	21	44	6	9	0	13	13	16	0.0611
Hardwood 2	31	14	35	0	45	0	6	3	10	0.0461
Hardwood 3	24	13	17	4	0	4	21	42	13	0.0352
Pine 1	27	10	63	0	0	0	26	4	7	0.0692
Pine 2	31	10	0	10	10	0	19	58	3	0.0562
Pine 3	31	6	84	0	3	0	10	3	0	0.1018
Total	344		23	2.9	11	8.2	12	38	6.2	
Mean (SEM)										
Cultivated	81		0 (0)	1.0 (1.0)	16 (10)	22 (9.7)	1.0 (1.0)	54 (9.4)	4.9 (0.8)	0.034 (0.01)
Pasture	88		9.0 (9.0)	3.8 (3.8)	2.4 (1.2)	8.7 (5.7)	15 (6.8)	58 (10)	3.5 (0.2)	0.030 (0.00)
Hardwood	86		32 (8.0)	3.5 (1.8)	18 (14)	1.4 (1.4)	13 (4.1)	19 (12)	13 (1.7)	0.047 (0.01)
Pine	89		49 (25)	3.2 (3.2)	4.3 (2.8)	0 (0)	18 (4.7)	22 (18)	3.5 (2.1)	0.076 (0.01)

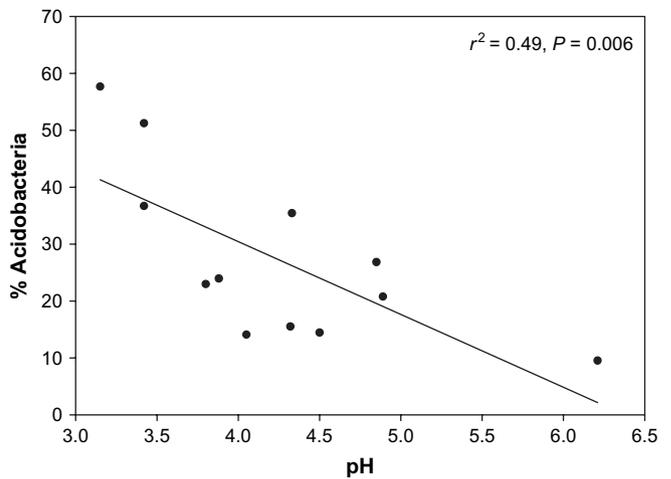
Distribution of fungal taxa across the plots. Values represent the percentage of each group in each library. OTU, or 'operational taxonomic unit', describes the number of unique groups at the 97% sequence similarity level. 'Other' includes Boletales, Chytridiomycota, Eurotiomycetes, Glomeromycota, Hymenochaetales, Pezizomycetes, and Zygomycota. Means were calculated for each land-use with 1 SEM in parentheses. Fungal:bacterial ratios represent ratios of fungal and bacterial small-subunit rRNA gene copy numbers as determined by quantitative PCR with four replicate reactions per assay. Abbreviations: Aphylo, Aphylophorales; Dothideo, Dothideomycetes; Leotio, Leotiomycetes; Chaetothyrio, Chaetothyriomycetes; Sordario, Sordariomycetes.

**Table 4**  
Land-use effects on edaphic properties and correlations between microbial communities and edaphic properties

	Land-use	Correlation coefficients	
	Main effect	Bacteria	Fungi
pH	2.2	<b>0.47</b>	0.15
%Silt + clay	<b>8.2</b>	<b>0.42</b>	0.00
%Soil moisture	6.9	0.18	0.00
Bulk density, g cm <sup>-3</sup>	6.7	0.00	0.00
C:N	6.9	0.16	<b>0.73</b>
C, kg <sup>-1</sup>	<b>8.2</b>	0.00	0.07
N, g kg <sup>-1</sup>	<b>8.1</b>	0.13	<b>0.39</b>
P, mg kg <sup>-1</sup>	<b>7.7</b>	0.33	<b>0.54</b>

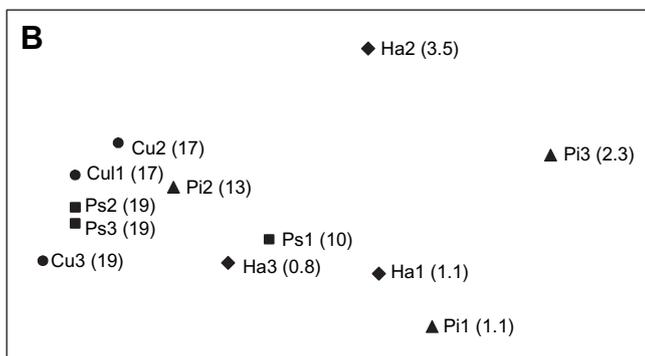
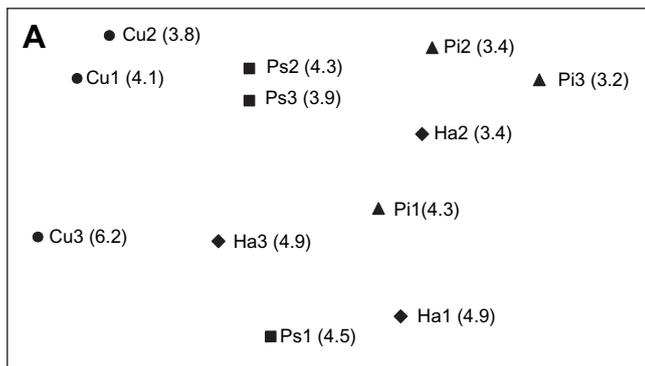
Significant effects of land-use on soil properties were determined using the Mann–Whitney *U* test for non-parametric data with land-use as the main factor. The Mann–Whitney *U* test statistic is reported. Pearson correlation coefficients relate the calculated UniFrac community distance between each pair of bacterial and fungal communities to the measured soil properties. Significant land-use effects and correlation coefficients are noted as bold text where  $P < 0.05$ .

and P) and soil texture between land-use types (Table 1), we also saw a high degree of spatial variability within land-use types for a number of edaphic factors including soil pH (Supplementary material Table 2) (Table 1). Since the land-use types are clearly distinct with regards to vegetation type and the land-use histories are well-established, the lack of a consistent land-use effect on soil edaphic factors is most likely to be a result of the variability in land-management practices (e.g., fertilization and liming) within a given land-use or an inherent variability in soil types across the landscape. Regardless of the causes, it is clear that different land-use types in this landscape are not necessarily distinct with regards to

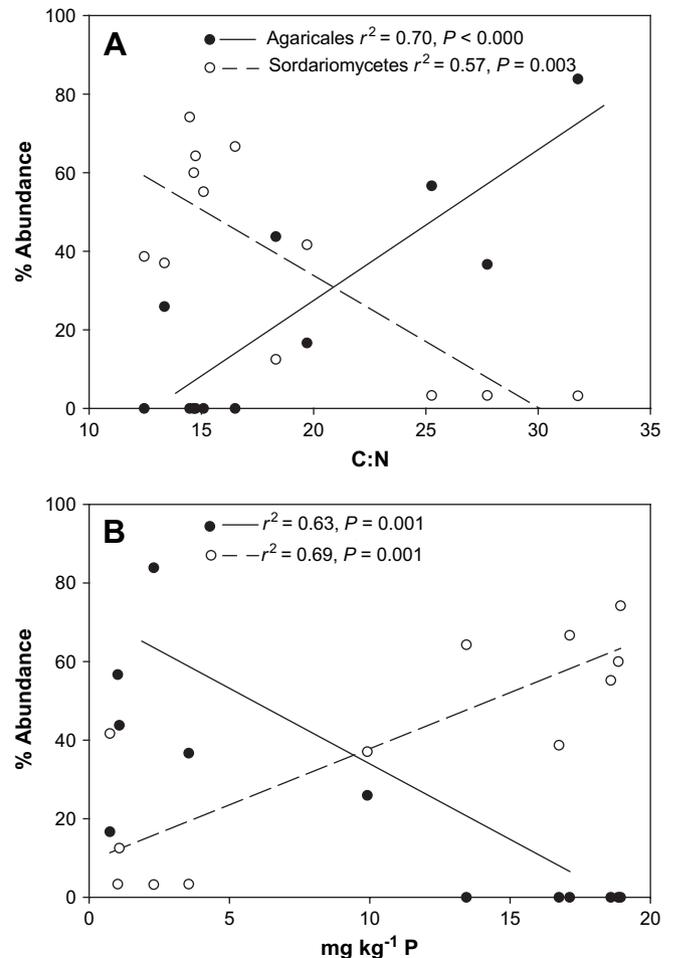


**Fig. 2.** Linear regression showing the relationship between acidobacterial abundances and soil pH across the 12 sampled plots.

their soil characteristics. Since the composition of soil microbial communities should be closely associated with soil characteristics, information on land-use type alone cannot be used to predict bacterial and fungal community composition across this landscape given that the edaphic properties are not necessarily consistent within individual land-use types.



**Fig. 1.** NMDS ordination of bacterial and fungal community phylogenetic distances. Panel A: bacterial community phylogenetic distance between plots with soil pH in parentheses. Panel B: fungal community phylogenetic distances between plots with extractable P (mg kg<sup>-1</sup> soil) in parentheses. The axes on these types of plots are unitless. The stress values for both plots were <0.11 which indicate that these data were well-represented by the two-dimensional representation (Clarke and Warwick, 2001). ● Cu, cultivated; ■ Ps, pasture; ◆ Ha, hardwood; ▲ Pi, pine.



**Fig. 3.** Linear regressions showing the relationship between (A) soil C:N and Agaricales abundance, and (B) extractable P concentration and Sordariomycete abundance across the 12 sampled plots.

#### 4.2. Bacterial community response to edaphic factors

The importance of edaphic factors in shaping microbial communities has been established by a number of studies (Bååth and Anderson, 2003; Buckley et al., 2006; Singh et al., 2006). Across this landscape, we found that the phylogenetic distance between bacterial communities was significantly correlated with soil pH and soil texture (Table 4). Our observations are concomitant with other studies which have demonstrated that soil pH is often correlated with bacterial community composition at multiple scales of geographic resolution (Högberg et al., 2007; Singh et al., 2008). A number of mechanisms may account for this association between pH and bacterial community composition. Soil pH may impose a direct stress on bacterial cells with certain pH levels selecting for some bacterial taxa over others (Kowalchuk et al., 1997; McCaig et al., 1999), a pattern demonstrated by the apparent changes in acidobacterial abundances with soil pH across this landscape (Fig. 2). Differences in soil pH can arise from many factors, including vegetation type, soil type, and management regime. Thus, pH may serve as a convenient integrating variable representing the physicochemical characteristics of a particular soil. Regardless of the mechanism, our results demonstrate that, just as pH is a reasonably good predictor of soil bacterial community composition at the continental scale (Fierer and Jackson, 2006), it is also a good predictor of the phylogenetic distance between bacterial communities across a much narrower range of soil types and land-uses in this landscape.

Soil texture was also correlated with bacterial community composition (Table 4) (Supplementary material Table 3), a pattern consistent with previous studies (Girvan et al., 2003; Johnson et al., 2003). More specifically, our results are broadly consistent with work by Girvan et al. (2003) which indicated that spatially diverse soils with the same soil texture had nearly identical bacterial communities based on DNA fingerprinting analyses. In our study, we cannot determine which factor, pH or texture, has a more important influence on bacterial community composition. While pH may represent the cumulative effects of many chemical species, soil texture is important in determining soil moisture and nutrient status, both of which may have a significant impact on bacterial communities.

#### 4.3. Fungal community response to edaphic factors

While bacterial community distances were correlated with texture and pH, the phylogenetic distance between fungal communities was correlated with soil nutrient pools, namely extractable P concentrations and the soil C:N ratio. For instance, soils with similar extractable P concentrations (pasture and cultivated) tended to cluster together when compared to the more broadly dispersed low P soils in the NMDS ordination of phylogenetic distance (Fig. 1B). Analyses of the relative abundances of specific fungal taxa indicated that these differences are largely attributable to the relatively P-rich soils containing more Ascomycota (mainly Sordariomycetes) and fewer Basidiomycota (mainly Agaricales) than the soils with lower P concentrations (Tables 1 and 3). Although extractable P concentrations were significantly different among land-uses, the changes in fungal communities were more closely associated with P concentrations than land-use type. For example, the P concentration in pine plot 2 was comparable to that of the cultivated and pasture soils and had a similar percentage of Sordariomycete sequences (Fig. 1B, Supplementary material Table 5). This suggests that extractable soil P may be an important regulator of the biogeographical patterns exhibited by fungal communities in this landscape.

Fluctuations in the abundance of Ascomycota and Basidiomycota could not be wholly ascribed to extractable P as soil C:N ratio was also significantly correlated with phylogenetic distance

(Table 2). Shifts in substrate quality (high vs low C:N) and availability (nitrogen fertilization) have previously been linked to shifts in fungal abundance (Allison et al., 2007; Bossuyt et al., 2001; Christensen, 1989; Frey et al., 2004). Likewise, some basidiomycete groups, particularly white rot fungi, mediate the decomposition of low quality (high C:N) lignified, and aromatic substrates commonly found in coniferous and deciduous litters (Bardgett and McAlister, 1999; Six et al., 2006; Swift, 1982) and this may explain why those soils with higher C:N ratios (e.g., hardwood and pine forest soils) may also have a higher prevalence of basidiomycetes than those soils with lower C:N ratios (e.g., the cultivated and pasture soils).

#### 4.4. Fungal to bacterial ratios

The relative abundances of fungi and bacteria across the plots were estimated by quantitative PCR of ribosomal genes (Table 3). Quantitative PCR does not provide an estimate of biomass ratios because ribosomal gene copy number and cellular nucleic acid concentrations can vary between taxa. However, it does provide a reproducible metric to track shifts in the relative abundances of bacteria and fungi across a landscape (Fierer et al., 2005). In accordance with multiple surveys of fungal to bacterial biomass, we observed that fungal:bacterial ratios were greater in forest soils than in agricultural or grassland soils (Bailey et al., 2002; Bossuyt et al., 2001; Högberg et al., 2007; Treseder, 2004). This is likely to be related to shifts in nutrient concentration and quality as the forest land-uses receive litter which is relatively more recalcitrant than the plant detritus in the cultivated and grassland soils. Moreover, these data correspond to shifts in the overall distribution of fungi across these land-uses, suggesting there may be significant differences in fungal community function between the land-uses (van der Wal et al., 2006).

#### 4.5. Using sequence-based approaches for microbial community analyses

In recent years, the analysis of microbial communities using sequence-based methods has greatly advanced our knowledge of soil microbial ecology (Pace, 1997). However, a number of challenges associated with sequence-based surveys of microbial communities have hindered their widespread use. For instance, the cost associated with generating sequence data has limited the number of sequences which can be analyzed. As sequence costs continue to drop, replicated surveys of microbial communities will become the norm, and studies that simultaneously assess the composition of both bacterial and fungal communities will become more common. A second difficulty associated with sequence-based approaches is deciding on the appropriate level of phylogenetic resolution for comparing microbial communities. Many studies use arbitrarily high sequence similarities (e.g., 97% or above) to compare microbial communities at the 'species' level. However, a single clone library, even a very large library, is not likely to capture all the 'species'-level diversity in a given soil sample, which may contain many thousands of 'species' per gram of soil (Roesch et al., 2007). For this study, we chose to analyze the microbial communities at a fairly coarse level of taxonomic resolution to minimize biases against individual species and focus on the overall shifts in the phylogenetic composition of bacterial and fungal communities across the landscape. Using this 'phylogenetic' approach across replicated land-uses, we were able to show that shifts in the dominant bacterial and fungal taxa could be linked with shifts in specific edaphic characteristics.

The last and perhaps most significant challenge associated with sequence-based approaches pertain to how sequence data are interpreted. Currently, most studies compare community composition qualitatively (as demonstrated in Tables 2 and 3) or they

compare communities by estimating diversity at a specific level of taxonomic resolution (e.g., rarefaction curves, Chao I estimates) (Hughes et al., 2001). Such approaches invariably ignore much of the phylogenetic information contained within the sequences. We used the web-based program UniFrac (Lozupone and Knight, 2005) to estimate the phylogenetic distance between each pair of samples based on the fraction of unique branch lengths between each sample. UniFrac has a number of distinct advantages over more traditional analytical approaches including the fact that entire phylogenies can be compared, generating data that can be used to quantify the phylogenetic variation between microbial communities and link the phylogenetic variation to specific edaphic factors.

#### 4.6. Conclusions

Many studies have examined the distribution and diversity of microorganisms in soil, but often such studies do not directly link the microbial information with information on edaphic properties even though such properties are increasingly being shown to have an important influence on the biogeographical patterns exhibited by soil bacteria and fungi (Fierer and Jackson, 2006). Here we have shown that variability in edaphic factors within and across land-use types can have a significant effect on microbial community structure and should not be ignored. Although land-use provides a convenient, broad description of a field site, our findings suggest that more detailed analyses of soil properties will enable identification of significant predictors of soil microbial distribution. Across this landscape, bacterial community composition was largely driven by changes in pH and soil texture, while fungal community composition was most strongly related to changes in soil nutrient availability.

#### Acknowledgements

N.F. and M.A.B. gratefully acknowledge the Andrew W. Mellon Foundation for funding. Thanks to Dan Richter and Mac Callahan for assistance in establishing the work. Special thanks to landowner J.F. Burnett for graciously allowing us to sample his property.

#### Appendix. Supplementary material

Supplementary material for this article may be found, in the online version, at doi: 10.1016/j.soilbio.2008.05.021.

#### References

- Allison, S.D., Hanson, C.A., Treseder, K.K., 2007. Nitrogen fertilization reduces diversity and alters community structure of active fungi in boreal ecosystems. *Soil Biology and Biochemistry* 39, 1878–1887.
- Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215, 403–410.
- Bååth, E., Anderson, T.H., 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. *Soil Biology and Biochemistry* 35, 955–963.
- Bååth, E., Frostegård, A., Pennanen, T., Fritze, H., 1995. Microbial community structure and pH response in relation to soil organic-matter quality in wood-ash fertilized, clear-cut or burned coniferous forest soils. *Soil Biology and Biochemistry* 27, 229–240.
- Bailey, V.L., Smith, J.L., Bolton, H., 2002. Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. *Soil Biology and Biochemistry* 34, 997–1007.
- Bardgett, R.D., McAlister, E., 1999. The measurement of soil fungal:bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. *Biology and Fertility of Soils* 29, 282–290.
- Blagodatskaya, E.V., Anderson, T.H., 1998. Interactive effects of pH and substrate quality on the fungal-to-bacterial ratio and QCO<sub>2</sub> of microbial communities in forest soils. *Soil Biology and Biochemistry* 30, 1269–1274.
- Bossio, D.A., Scow, K.M., Gunapala, N., Graham, K.J., 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. *Microbial Ecology* 36, 1–12.
- Bossuyt, H., Deneff, K., Six, J., Frey, S.D., Merckx, R., Paustian, K., 2001. Influence of microbial populations and residue quality on aggregate stability. *Applied Soil Ecology* 16, 195–208.
- Buckley, D.H., Huangyutham, V., Nelson, T.A., Rumberger, A., Thies, J.E., 2006. Diversity of planctomycetes in soil in relation to soil history and environmental heterogeneity. *Applied and Environmental Microbiology* 72, 4522–4531.
- Callahan, M.A., Richter, D.D., Coleman, D.C., Hofmockel, M., 2006. Long-term land-use effects on soil invertebrate communities in southern piedmont soils, USA. *European Journal of Soil Biology* 42, S150–S156.
- Carlile, M.J., Watkinson, S.C., Gooday, G.W., 2001. *The Fungi*. Academic Press, San Diego.
- Christensen, M., 1989. A view of fungal ecology. *Mycologia* 81, 1–19.
- Clarke, K.R., Warwick, R.M., 2001. A further biodiversity index applicable to species lists: variation in taxonomic distinctness. *Marine Ecology Progress Series* 216, 265–278.
- Culley, J.L.B., 1993. Density and compressibility. In: Carter, M.R. (Ed.), *Soil Sampling and Methods of Analysis*. Lewis Publishers, Boca Raton, Florida, USA, pp. 529–539.
- DeSantis, T.Z., Hugenholtz, P., Keller, K., Brodie, E.L., Larsen, N., Piceno, Y.M., Phan, R., Andersen, G.L., 2006a. NAST: a multiple sequence alignment server for comparative analysis of 16S rRNA genes. *Nucleic Acids Research* 34, W394–W399.
- DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006b. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology* 72, 5069–5072.
- Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32, 1792–1797.
- Felsenstein, J., 2005. PHYLIP: Phylogeny Inference Package Version 3.6 (Distributed by the author). Department of Genome Sciences, University of Washington, Seattle.
- Fierer, N., Jackson, J.A., Vilgalys, R., Jackson, R.B., 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Applied and Environmental Microbiology* 71, 4117–4120.
- Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America* 103, 626–631.
- Frey, S.D., Knorr, M., Parrent, J.L., Simpson, R.T., 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. *Forest Ecology and Management* 196, 159–171.
- Frostegård, A., Bååth, E., Tunlio, A., 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. *Soil Biology and Biochemistry* 25, 723–730.
- Gaudinski, J.B., Trumbore, S.E., Davidson, E.A., Cook, A.C., Markewitz, D., Richter, D.D., 2001. The age of fine-root carbon in three forests of the eastern United States measured by radiocarbon. *Oecologia* 129, 420–429.
- Gee, G.W., Orr, D., 2002. Particle-size analysis. In: Dame, J.H., Topp, G.C. (Eds.), *Methods of Soil Analysis, Part 4 – Physical Methods*. Soil Science Society of America, Madison, WI, pp. 255–293.
- Girvan, M.S., Bullimore, J., Pretty, J.N., Osborn, A.M., Ball, A.S., 2003. Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. *Applied and Environmental Microbiology* 69, 1800–1809.
- Heinemeyer, A., Ridgway, K.P., Edwards, E.J., Benham, D.G., Young, J.P.W., Fitter, A.H., 2004. Impact of soil warming and shading on colonization and community structure of arbuscular mycorrhizal fungi in roots of a native grassland community. *Global Change Biology* 10, 52–64.
- Hendrix, P.F., Parmelee, R.W., Crossley, D.A., Coleman, D.C., Odum, E.P., Groffman, P.M., 1986. Detritus food webs in conventional and no-tillage agroecosystems. *Bioscience* 36, 374–380.
- Högberg, M.N., Högberg, P., Myrold, D.D., 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? *Oecologia* 150, 590–601.
- Hughes, J.B., Hellmann, J.J., Ricketts, T.H., Bohannon, B.J.M., 2001. Counting the uncountable: statistical approaches to estimating microbial diversity. *Applied and Environmental Microbiology* 67, 4399–4406.
- Johnson, M.J., Lee, K.Y., Scow, K.M., 2003. DNA fingerprinting reveals links among agricultural crops, soil properties, and the composition of soil microbial communities. *Geoderma* 114, 279–303.
- Kowalchuk, G.A., Stephen, J.R., DeBoer, W., Prosser, J.I., Embley, T.M., Woldendorp, J.W., 1997. Analysis of ammonia-oxidizing bacteria of the beta subdivision of the class Proteobacteria in coastal sand dunes by denaturing gradient gel electrophoresis and sequencing of PCR-amplified 16S ribosomal DNA fragments. *Applied and Environmental Microbiology* 63, 1489–1497.
- Kuo, S., 1998. Phosphorus. In: Sparks, D.L. (Ed.), *Methods of Soil Analysis, Part 3 – Chemical Methods*. Soil Science Society of America, Madison, WI, USA.
- Lane, D.J., 1991. 16S/23S rRNA sequencing. In: Stackebrandt, E., Goodfellow, M. (Eds.), *Nucleic Acid Techniques in Bacterial Systematics*. John Wiley and Sons, New York, NY, pp. 115–175.
- Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology* 71, 8228–8235.
- Lutzoni, F., Kauff, F., Cox, C.J., McLaughlin, D., Celio, G., Dentinger, B., Padamsee, M., Hibbett, D., James, T.Y., Baloch, E., Grube, M., Reeb, V., Hofstetter, V., Schoch, C., Arnold, A.E., Miadlikowska, J., Spatafora, J., Johnson, D., Hambleton, S., Crockett, M., Shoemaker, R., Hambleton, S., Crockett, M., Shoemaker, R., Sung, G. H., Lücking, R., Lumbsch, T., O'Donnell, K., Binder, M., Diederich, P., Ertz, D., Gueidan, C., Hansen, K., Harris, R.C., Hosaka, K., Lim, Y.W., Matheny, B., Nishida, H., Pfister, D., Rogers, J., Rossman, A., Schmitt, I., Sipman, H., Stone, J., Sugiyama, J., Yahr, R., Vilgalys, R., 2004. Assembling the fungal tree of life:

- progress, classification and evolution of subcellular traits. *American Journal of Botany* 91, 1446–1480.
- Martin, A.P., 2002. Phylogenetic approaches for describing and comparing the diversity of microbial communities. *Applied and Environmental Microbiology* 68, 3673–3682.
- McCaig, A.E., Glover, L.A., Prosser, J.I., 1999. Molecular analysis of bacterial community structure and diversity in unimproved and improved upland grass pastures. *Applied and Environmental Microbiology* 65, 1721–1730.
- Murty, D., Kirschbaum, M.U.F., McMurtrie, R.E., McGilvray, A., 2002. Does conversion of forest to agricultural land change soil carbon and nitrogen? A review of the literature. *Global Change Biology* 8, 105–123.
- Ovreas, L., Torsvik, V., 1998. Microbial diversity and community structure in two different agricultural soil communities. *Microbial Ecology* 36, 303–315.
- Pace, N.R., 1997. A molecular view of microbial diversity and the biosphere. *Science* 276, 734–740.
- Post, W.M., Mann, L.K., 1990. Changes in soil organic carbon and nitrogen as a result of cultivation. In: Bowman, A.F. (Ed.), *Soil and the Greenhouse Effect*. Wiley, New York, pp. 401–407.
- Richter, D.D., Markewitz, D., 2001. *Understanding Soil Change: Sustainability Over Millennia, Centuries, and Decades*. Cambridge University Press, Cambridge, UK.
- Roesch, L.F.W., Fulthorpe, R.R., Riva, A., Casella, G., Hadwin, A.K.M., Kent, A.D., Daroub, S.H., Camargo, F.A.O., Farmerie, W.G., Triplett, E.W., 2007. Pyrosequencing enumerates and contrasts soil microbial diversity. *The ISME Journal* 1, 283–290.
- Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M., Wall, D.H., 2000. Biodiversity – global biodiversity scenarios for the year 2100. *Science* 287, 1770–1774.
- Singh, B.K., Munro, S., Reid, E., Ord, B., Potts, J.M., Paterson, E., Millard, P., 2006. Investigating microbial community structure in soils by physiological, biochemical and molecular fingerprinting methods. *European Journal of Soil Science* 57, 72–82.
- Singh, B.K., Nunan, N., Ridgway, K.P., McNicol, J., Young, J.P.W., Daniell, T.J., Prosser, J. I., Millard, P., 2008. Relationship between assemblages of mycorrhizal fungi and bacteria on grass roots. *Environmental Microbiology* 10, 534–541.
- Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Science Society of America Journal* 70, 555–569.
- Smit, E., Leeflang, P., Glandorf, B., van Elsas, J.D., Wernars, K., 1999. Analysis of fungal diversity in the wheat rhizosphere by sequencing of cloned PCR-amplified genes encoding 18S rRNA and temperature gradient gel electrophoresis. *Applied and Environmental Microbiology* 65, 2614–2621.
- Steenwerth, K.L., Jackson, L.E., Calderon, F.J., Stromberg, M.R., Scow, K.M., 2002. Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. *Soil Biology and Biochemistry* 34, 1599–1611.
- Swift, M.J., 1982. Basidiomycetes as components of forest ecosystems. In: Frankland, J.C., Hedger, J.N., Swift, M.J. (Eds.), *Decomposer Basidiomycetes: their Biology and Ecology*. British Mycological Symposium 4. Cambridge University Press, Cambridge, UK, pp. 307–337.
- Thomas, G.W., 1996. Soil pH and Soil Acidity. In: Sparks, D.L. (Ed.), *Methods of Soil Analysis, Part 3 – Chemical Methods*. Soil Science Society of America, Madison, Wisconsin, USA, pp. 475–490.
- Treseder, K.K., 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO<sub>2</sub> in field studies. *New Phytologist* 164, 347–355.
- van der Wal, A., van Veen, J.A., Smant, W., Boschker, H.T.S., Bloem, J., Kardol, P., van der Putten, W.H., de Boer, W., 2006. Fungal biomass development in a chronosequence of land abandonment. *Soil Biology and Biochemistry* 38, 51–60.
- Yu, Y.N., Breitbart, M., McNairnie, P., Rohwer, F., 2006. FastGroupII: a web-based bioinformatics platform for analyses of large 16S rDNA libraries. *BMC Bioinformatics* 7.
- Zelles, L., Bai, Q.Y., Beck, T., Beese, F., 1992. Signature fatty-acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. *Soil Biology and Biochemistry* 24, 317–323.