Soil Biology & Biochemistry 42 (2010) 1138–1147

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Spatial analysis reveals differences in soil microbial community interactions between adjacent coniferous forest and clearcut ecosystems

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ARTICLE INFO

Article history:
Received 16 July 2009
Received in revised form
21 March 2010
Accepted 22 March 2010
Available online 8 April 2010

Keywords:
Spatial analysis
PLFA
PCNM
Coniferous forest
Clearcut

ABSTRACT

Knowledge of how forest management influences soil microbial community interactions is necessary for complete understanding of forest ecology. In this study, soil microbial communities, vegetation characteristics and soil physical and chemical properties were examined across a rectangular 4.57 × 36.58 m sample grid spanning adjacent coniferous forest and clearcut areas. Based on analysis of soil extracted phospholipid fatty acids, total microbial biomass, fungi and Gram-negative bacteria were found to be significantly reduced in soil of the clearcut area relative to the forest. Concurrent with changes in microbial communities, soil macroaggregate stability was reduced in the clearcut area, while no significant differences in soil pH and organic matter content were found. Variography indicated that the range at which spatial autocorrelation between samples was evident (patch size) was greater for all microbial groups analyzed in the clearcut area. Overall, less spatial structure could be resolved in the forest. Variance decomposition using principal coordinates of neighbor matrices spatial variables indicated that soil aggregate stability and vegetation characteristics accounted for significant microbial community spatial variation in analyses that included the entire plot. When clearcut and forest areas were analyzed separately, different environmental variables (pH in the forest area and soil organic matter in the clearcut) were found to account for variation in soil microbial communities, but little of this variation could be ascribed to spatial interactions. Most microbial variation explained by different components of microbial communities occurred at spatial scales other than those analyzed. Fungi accounted for over 50% of the variation in bacteria of the forest area but less than 11% in the clearcut. Conversely, AMF accounted for significant variation in clearcut area, but not forest, bacteria. These results indicate broadly disparate controls on soil microbial community composition in the two systems. We present multiple lines of evidence pointing toward shifts in fungi functional groups as a salient mechanism responsible for qualitative, quantitative and spatial distribution differences in soil microbial communities.

1. Introduction

Ecosystems show characteristic biotic and abiotic variability on a range of spatial and temporal scales. Microorganisms are integral to soil function, playing central roles in many ecosystem processes, including organic matter decomposition, nutrient mineralization and immobilization, and the development and maintenance of soil structure. The structure of soil microbial communities is determined by a range of superimposed biotic and abiotic factors acting on a multitude of different spatial and temporal scales. This results in aggregation of soil microbial communities at many, typically nested, spatial scales (e.g. Ettema and Wardle, 2002; Franklin and Mills, 2003, 2009; Bach et al., 2008). Elucidation of relationships between microbial communities and environmental factors that influence their composition requires measurement at the scales which these factors operate (Legendre and Legendre, 1998).

In coniferous forests, spatial heterogeneity of microbial communities and the processes they mediate can be pronounced at scales relevant to individual plants and plant communities (Pennanen et al., 1999; Saetre et al., 1999; Saetre and Bååth, 2000). Spatial distributions of soil microbial communities in these ecosystems, while being influenced by plant community composition (e.g. Leckie et al., 2004), may also be one of the primary determinants of plant community structure via a number of positive and negative feedback mechanisms (e.g. van der Heijden et al., 2008).

It is well established that the age and species composition of plant communities can influence soil nutrient cycling dynamics

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doi:10.1016/j.soilbio.2010.03.020

and microbial communities, in part due to differences in the quality and quantity of root exudates, litter and root materials (Myers et al., 2001). Changes in soil physical properties can also influence microbial communities and processes. For example, heavy equipment traffic and ground-based skidding during timber harvest are known to decrease soil structure (e.g. Chatterjee et al., 2008). Soil compaction resulting from these activities has been implicated in causing decreases in microbial biomass (Tan et al., 2005) and amounts of fungi relative to bacteria (Schnurr-Pütz et al., 2006), presumably due to alteration of soil aeration status and pore volumes accessible to different soil organisms and roots. However, the degree to which soil structure is impacted by these practices, and the time required for soil to recover to the pre-harvest condition, are highly variable due to differences in soil biotic and abiotic properties (e.g. Busse et al., 2006).

Temperature and moisture regime is an important determinant of soil microbial community composition and processes. Removal of the forest canopy is known to alter soil temperature amplitudes (Heithecker and Halpern, 2006), in part due to changes in the radiation balance at the soil surface, as well as wind, shade and snow accumulation and seasonal cover (Ballard, 2000). Removal of this insulation layer, combined with canopy removal, can result in alteration of temperature and moisture regimes, potentially influencing the frequency, intensity and duration of wet-dry and freeze–thaw cycles. In addition to influences on microbial and plant communities, alteration of temperature and moisture regime can impact soil aggregate stability both directly and indirectly via physical and biological influences (Denef et al., 2001; Kvaernø and Øygarden, 2006).

Although numerous mechanisms that determine forest microbial community composition have been identified, how spatial interactions of forest soil microbial communities and processes change after tree canopy removal is not well understood (Morris and Dress, 2007). Since spatial differences in ecosystem characteristics are indicative of disparate controls on communities, populations and processes (Levin, 1992), knowledge of microbial community spatial distributions may provide insights into factors that determine how forest ecosystems are organized.

The aim of this study was to examine how soil physical, chemical and biotic properties vary and covary spatially in adjacent forest and clearcut areas of a coniferous forest ecosystem. We chose analysis scales expected to capture the influence of the dominant plant species and based on reports of microbial distributions in other forest systems (reviewed by Morris and Dress, 2007). A goal of this study was to examine controls on microbial community composition and how these differ between forest and clearcut systems.

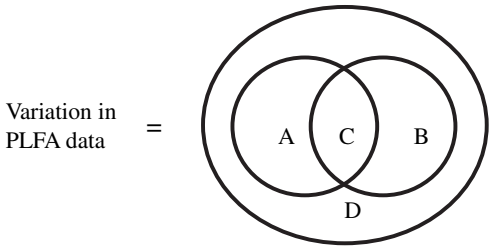
2. Materials and methods

2.1. Site description

The study site is located within the Swan Valley of Montana (47° 19′N, 114° 5′W; elevation 883 m). At the nearby town of Condon annual temperature and precipitation average 7.57 °C and 40.4 cm, respectively. Soils of the site (Typic Dystrocryepts), formed from thick quaternary alluvial, colluvial, and glacial till deposits, are loamy in texture, well-drained, and skeletal (USDA Soil Survey Staff, 1999).

2.2. Sampling design

Our sample strategy was designed to facilitate analysis of spatial characteristics at scales greater than 1.2 m. Samples were collected from 140 locations on a 4.57 × 36.58 m rectangular grid (Fig. 1A). The northern half of the grid is positioned in an area clearcut harvested 9 years prior to sampling (1999; Plum Creek Timber Company, personal communication). Vegetation on the clearcut



Outer circle represents 100% of the variance.
A) Variation that can accounted for by space (PCNM model).
B) Variation that can be accounted for by environment.
C) Variation shared by space and environment.
D) Variation that cannot be accounted for by space or environment.

Fig. 1. Diagram depicting variance decomposition.

portion of the plot consists of native grasses and forbs, although exotic plant species [*Centaurea maculosa* (spotted knapweed), *Chrysanthemum leucanthemum* (oxeye daisy), and *Cirsium arvense* (Canada thistle)] are present in low abundance. The southern half of the grid is positioned in an area where timber was most recently clearcut harvested in 1966, 45 years prior to this study. Although the area was not planted after timber harvest, three different tree species [*Abies grandis* (grand fir), *Pinus contorta* (lodgepole pine) and *Pseudotsuga menziesii* (douglas fir)] and an understory of native grasses, forbs and shrubs, including *Vaccinium caespitosum* (huckleberry), *Arctostaphylos uva-ursi* (L.) Spreng. (kinnikinnick), have established on the site.

In September 2008, two mineral soil samples (0–10 cm depth) were collected from each sample location after removal of surface litter. One sample was placed on ice in the field and stored at −20 °C prior to analysis of microbial characteristics (see below). The second sample was placed in a paper bag, air dried at ambient room temperature and analyzed for soil physical and chemical properties as described below. Samples collected under grasses or forbs (or their litter), in bare areas or under woody debris were noted for each sample location. We also documented areas within the drip line of all trees on the site.

2.3. Soil analysis

Soil macroaggregate (>250 μm diameter) water stability was measured for the <2000 μm size class. Soil samples (4 g) were placed on a 250 μm mesh sieve and submerged in deionized water for 5 min. Water-stable macroaggregates were separated from whole soil by moving the sieve up and down 1.4 cm for 5 min using a wet-sieving machine (Five Star Scientific, Twin Falls, ID). Material that didn't pass through the sieve was dried (105 °C, 24 h) and subsequently weighed. We corrected for coarse matter by dispersing these materials in 0.5% hexametaphosphate, sieving (250 μm mesh) and determining the dry weight of coarse materials (Denef et al., 2001).

Soil organic matter (SOM) contents were estimated as loss on ignition. Soil (8 g) was dried at 105 °C, weighed and combusted in ceramic crucibles at 360 °C for 2 h. Pre- and post combustion weights were used to calculate percent SOM.

Soil pH was determined in a soil slurry (1:1 soil to diH_2O) using an electrode.

Soil Ca, Mg, K and available P was analyzed for a subset of 28 samples representive of forest and clearcut areas. Soil Ca, Mg and K were extracted in 1 N NH_4OAc and amounts determined using atomic absorption spectroscopy. Available P was estimated using the Bray-Kurtz method.

2.4. Hyphal lengths

Fungal hyphae were extracted from 4 g soil and total length measured using an aqueous membrane filtration method with subsequent microscopic examination at 200× (Rillig et al., 1999). Hyphal length was measured using the line intersect method as described in Jakobsen et al. (1992). We analyzed both arbuscular mycorrhizae (AM) and non-AM fungal hyphae, which were distinguished based on morphological criteria described in Rillig et al. (1999).

2.5. PLFA analysis

Microbial community structure was examined in each soil sample using phospholipid fatty acid analysis (PLFA). Phospholipid fatty acids were extracted from two replicate 4 g soil samples and analyzed according to White and Ringelberg (1998). Briefly, lipids were removed from samples into chloroform using a modified Bligh and Dyer (1956) extraction procedure. Phospholipids were separated from other lipids by silica acid chromatography and derivatized to their fatty acid methyl esters (FAMEs) for analysis by gas chromatography. FAMEs were identified by relative retention times, co-elution with purchased standards, and comparison of samples between capillary columns of differing polarity (HP-5 (crosslinked 5% phenal methyl silicon) 50 m × 0.32 mm × 0.52 um film, HP-225 (50% CNPrPh Me Siloxane) 30 m × 0.32 mm × 0.25 um film). Final verification of peak identity was made by gas-chromatography/mass spectroscopy on representative samples. FAMEs were quantified on an HP-225 column using an HP 6890 series GC system and protocol according to Frostegård et al. (1993). The sum of total extractable PLFAs have been shown previously to correlate well with other microbial biomass measures (e.g. Bailey et al., 2002; Fierer et al., 2003) and was used as a proxy for total microbial biomass in each sample. For all analyses directed at elucidation of microbial community compositional differences, including all spatial analyses, we converted total amount of each PLFA to mol% of all PLFAs within the sample to reduce the effect of biomass differences on the analysis.

We used the sum of PLFAs 14:0, a15:0, 16:1ω7c and i17:0 as bacterial biomass markers (Zak et al., 1996; Frostegård et al., 1993; Zelles, 1999), 10Mel6:0, 10Mel7:0 and 10Mel8:0 as actinobacteria biomarkers (Zelles, 1999), i15:0 and i16:0 for Gram-positive bacteria (Frostegård et al., 1993; Zelles, 1999), cy17:0 and 18:1ω7 for Gram-negative bacteria (Frostegård et al., 1993; Zelles, 1999), 16:1ω5 for arbuscular mycorrhizal fungi (AMF) (Olsson et al., 1995; Hedlund, 2002) and 18:2ω6 and 18:1ω9 as biomarkers for total fungi (Frostegård et al., 1993; Zelles, 1999). Since the use of different PLFAs as biomarkers can be problematic due to overlap in the PLFA composition of organisms, our lack of knowledge regarding the PLFA composition for soil organisms, and conformational shifts in membrane PLFAs in response to stress and nutritional status, the proposed microbial groupings should be considered approximate (Guckert et al., 1986; Zelles et al., 1994; Zelles, 1999).

2.6. Data analysis

A significant north-south spatial trend was evident for a number of variables (data not shown) indicating spatial structure at a scale larger than the sampling extent. To achieve stationary (Isaaks and Srivastava, 1989; Webster and Oliver, 1990), trends were removed from the data using linear regressions of sample coordinates (Borcard and Legendre, 2002) prior to geostatistical or multivariate spatial analyses. For analyses specifically focused on clearcut or forest areas, all sample points occurring 18.3 m from the south end of the plot were considered to be in the forest area and sample points 18.3 m from the north end in the clearcut area. No significant spatial trends were detected in these areas and further de-trending wasn't performed prior to analysis of sub-plots.

Data distributions were examined for all variables and, when necessary, transformed to meet analysis normality assumptions. SOM, non-AMF hyphae, Ca, Mg and K were ln-transformed. AMF hyphal lengths were indicator-transformed with quartiles labeled from 1 to 4. Categorical variables (grass, wood, forbs, bare areas, and areas under tree drip-lines) were encoded using binary "dummy" variables indicating presence or absence.

Differences in PLFA biomarkers (both total abundance and calculated as mol%), SOM, soil aggregate stability, AMF and non-AMF hyphae, pH and soil chemical variables between forest and harvested areas were examined using one-way ANOVA (SPSS ver. 15.0).

Correlation matrices of all variables analyzed were constructed for the entire plot and for forest and clearcut areas separately (SPSS ver. 15.0).

2.7. Geostatistical approaches

Geostatistical methods are commonly used to describe how variance depends on the distance between observations. For our analyses spatial structure was modeled using variography (Isaaks and Srivastava, 1989). Omnidirectional semivariograms were fit to the data using the program Variowin 2.21. Outliers were identified by examining variogram clouds and h-scattergrams and removed from the dataset. Depending on the spatial characteristics described by the data, spherical, linear and exponential models were fit to different variables. When exponential models were used the range was calculated as the distance where the semivariance equals 95% of the sill (Isaaks and Srivastava, 1989).

Plotted variogram models have three important informative features 1) the sill (the distance where semivariance is maximal), 2) the range (the distance at which the sill is reached), and 3) the nugget variance (the semivariance at zero distance). Nugget variance indicates the variation that cannot be explained due to measurement errors or spatial variance at distances smaller than the smallest sampling interval (Webster and Oliver, 1990). We calculated spatial structure as the proportion of the modeled variance between the model intercept and the sill (e.g. Saetre and Bååth, 2000).

Ordinary kriging was used for interpolation of values between sampling points. Estimates were interpolated at 1 m intervals and used to construct filled contour plots (Sigma Plot ver. 10), enabling graphical presentation of spatial distributions.

2.8. Ordination-based methods

Principal coordinates of neighbor matrixes (PCNM) allows for examination of spatial structure at all scales possible within the sampling scheme (Borcard and Legendre, 2002; Borcard et al., 2004; Dray et al., 2006). Via spectral decomposition of spatial relationships among sampled sites, PCNM generates a finite set of explanatory spatial variables (PCNM variables), each of which corresponds to a specific spatial structure, that can be directly linked to spatial patterns of environmental variables using canonical analysis (Borcard et al., 2004).

Three separate matrices of PCNM variables were constructed (entire plot, forest only and harvested only) by computing principle coordinates of a truncated matrix of Euclidean distances between sample sites using the computer program SpaceMaker2 (Borcard and Legendre, 2004). Canonical variance partitioning was subsequently used to examine spatial and environmental influences on PLFA biomarkers. PCNM and environmental variables which significantly explained patterns of variation in the microbial data were determined via forward selection (499 Monte Carlo permutations) and used as explanatory or co-variables in redundancy

analysis (RDA) analyses to partition the variance in microbial data into pure spatial effects, pure environmental variable effects, variance shared between spatial and environmental components, and variation not accounted for by spatial or environmental variables (Borcard et al., 1992; Peres-Neto et al., 2006) (Fig. 1). For analyses examining relationships between all PLFAs, including those not used as biomarkers for specific microbial groups, Euclidian distance matrices were constructed and analyzed using distance-based RDA (db-RDA) (Legendre and Anderson, 1999).

3. Results

3.1. Univariate analyses

3.1.1. Soil properties

Soil aggregate stability averaged 26.9% (Std. err. = 0.56) across the plot but differed significantly with time since timber harvest ($F = 61.95$, $P < 0.001$), averaging 30.50% and 23.20% for forest and clearcut areas, respectively. Variography indicated that spatial structure, and the range for which spatial dependence was evident, was greater for the forest area than for the clearcut area (Table 1). A contour map of kriged estimates for soil aggregate stability shows clear differences between forest and clearcut areas of the plot, with high and low values corresponding with forest and clearcut areas, respectively (Fig. 2).

Soil pH averaged 4.89 across all samples. While differences in pH between clearcut and forest areas were not significant ($F = 1.98$, $P = 0.16$), both spatial structure and spatial dependence range were found to be greater for the forest area (Table 1). A contour plot of kriged pH estimates further indicates spatial stratification over the entire plot, as well as in clearcut and forest areas (Fig. 2).

Soil organic matter in all samples averaged 3.0% (std. err. = 0.126). Differences between forest and clearcut areas were not significant ($F = 2.03$, $P = 0.157$), averaging 3.1% and 2.9% SOM for forest and clearcut areas, respectively. Spatial dependence range was similar for analyses of whole plot, forest and clearcut areas (Table 1). Spatial stratification of SOM and the patchy nature of this variable are evident from the contour map of kriged estimates (Fig. 2).

Analyses of 28 samples distributed across the plot indicated that soil Ca, Mg, K and P averaged 621, 104, 96 and 22 μg g soil^{-1}, respectively. No significant differences for any of these variables were found between samples collected from clearcut and forest areas (data not shown).

3.1.2. Hyphae

AMF extraradical hyphae averaged 0.62 m g soil^{-1} overall, and 0.75 and 0.50 m g soil^{-1} for samples of clearcut and forest areas, respectively (data not shown). Differences between forest and clearcut areas were not significant ($F = 2.8$, $P = 0.09$). AMF extraradical hyphae exhibited spatial structure in the clearcut area but not in forest (pure nugget effect; Table 1).

Non-AMF hyphae averaged 4.70 m g soil^{-1} overall, and 4.90 and 4.40 m g soil^{-1} for samples obtained from clearcut and forest areas, respectively. Differences between areas were not significant ($F = 0.84$, $P = 0.36$). Similar to what was found for AMF hyphae, non-AMF hyphae exhibited spatial structure in the clearcut area but not in forest (Table 1).

3.1.3. PLFA

Total microbial biomass (the sum of extracted PLFAs) differed significantly between clearcut and forest areas ($F = 367.9$; $P < 0.001$), averaging 2.83 μg g soil^{-1} (std. err. = 0.06) in the forest area and 1.12 μg g soil^{-1} (std. err. = 0.06) in the clearcut area. Total amounts of PLFA biomarkers for all microbial groups analyzed were significantly greater in the forest area (Fig. 3b).

The proportion of PLFAs for total bacteria and Gram-positive bacteria (expressed as mol%) were significantly greater in samples from the clearcut, whereas PLFA biomarkers for fungi, Gram-negative bacteria and microbial biomass were greater for forest samples (Fig. 3a). The ratio of fungi to bacteria biomass was significantly greater ($F = 11.1$; $P < 0.005$) in samples collected from the forest area (mean = 1.10; std. err. = 0.045) than in samples collected from the clearcut area (mean = 0.89; std. err. = 0.046).

Variography of the whole plot indicated that spatial structure differed greatly for different PLFA biomarkers (Table 1), with spatial structure being greatest for the fungi biomarkers and least for the AMF-specific biomarker. The range at which spatial structure was evident was greatest for Gram-negative bacteria, Gram-positive bacteria and total bacteria biomarkers.

Spatial structure and spatial dependence range for PLFA biomarkers typically differed greatly for analyses based on the entire plot and clearcut or forest areas (Table 1). Spatial structure wasn't apparent for Gram-negative, Gram-positive and biomass biomarkers in forest area. The range for which spatial autocorrelation was evident was greater for biomarkers in the clearcut (Table 1). Contour maps of kriged estimates for bacteria, fungi, the fungi:bacteria ratio and microbial biomass are depicted in Fig. 2.

3.2. Multivariate analyses

For entire plot analyses PCNM spatial models accounted for greater than 60% of the variation in all microbial groups with the exception of actinobacteria (Table S1). The scale of PCNM variables inversely corresponds to spatial scale and the amount of variance

Table 1
Spatial structure and the range for which spatial autocorrelation was evident for microbial community and soil variables in analyses of the entire plot, forest and clearcut areas.

	Calculated using PLFA mol%						Calculated using PLFA μg g soil^{-1}			
	Entire plot		Forest		Clearcut		Forest		Clearcut	
	Spat. struct.	Range	Spat. struct.	Range	Spat. struct.	Range	Spat. struct.	Range	Spat. struct.	Range
Bacteria	0.43	8.2	0.65	3.6	0.75	6.1	0.33	4.3	0.42	1.8
Actinobacteria	0.74	4.6	0.81	4.6	0.39	6.1	0.57	1.9	0.64	2.0
Fungi	0.85	7.6	0.42	3.3	0.25	5.4	0.22	5.3	0.73	3.8
Gram-negative	0.15	9.1	–	–	0.31	4.9	–	–	0.62	3.3
Gram-positive	0.16	9.1	–	–	0.24	6.1	0.24	4.5	0.60	1.8
Biomass	0.17	7.6	–	–	0.10	6.1				
Aggregate stab.	0.22	7.6	0.21	4.6	0.35	3.0				
SOM	0.40	4.6	0.53	4.6	0.67	4.6				
pH	0.16	5.5	0.39	4.6	0.44	5.5				
AMF hyphae	0.19	10.7	–	–	0.43	9.1				
Non-AMF hyphae	0.22	6.1	–	–	0.38	9.1				

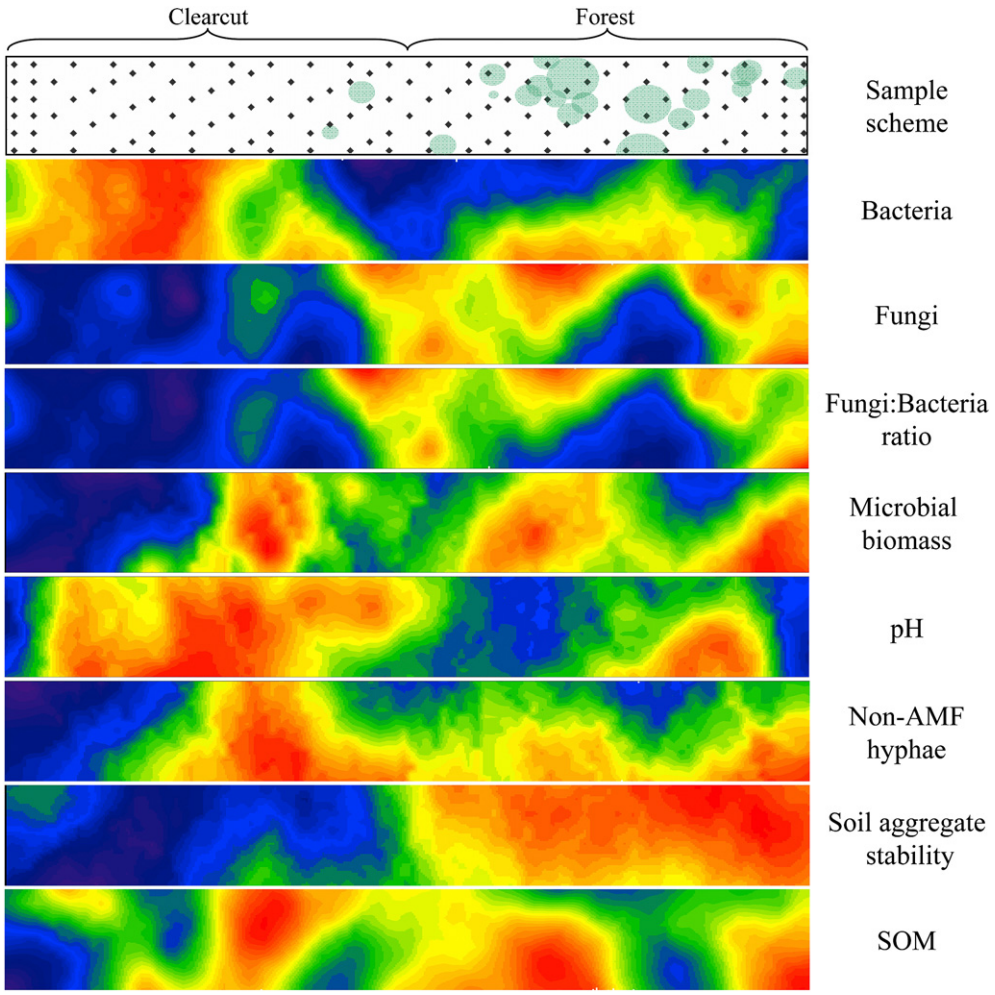


Fig. 2. Sample scheme (top panel) indicating sample locations, forest vs harvested areas and tree drip-lines (green transparent circles). Contour maps (lower panels) represent kriged estimates for select soil and microbial variables. Red indicates areas having high values for the variable and dark blue areas having low values. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

accounted for by the PCNM models was generally greatest for lower numbered PCNM scales, especially PCNM scale 1. When analyses were limited to forest (Table S2) or clearcut (Table S3) areas, PCNM spatial models accounted for relatively little of the variation in the PLFA biomarkers; however, relatively more variation could be accounted for at finer scales.

PCNM spatial models accounted for larger amounts of variation in PLFA biomarkers for bacteria, fungi, Gram-positive bacteria, Gram-negative bacteria, actinobacteria and the fungi:bacteria ratio for the clearcut area than for forest (Tables S2 and S3). For the forest area, spatial models accounted for more of the variation in all PLFAs (Table S3). PCNM spatial models accounted for similar microbial biomass variance in forest and clearcut areas (Tables S2 and S3).

Variance decomposition of data for the entire plot indicated that much of the variation in the microbial data could be accounted for by spatial interactions with soil macroaggregate stability and, to a lesser extent, soil macroaggregate stability pure effects (Table 2). Non-AMF hyphae spatial interactions and pure effects explained small, but significant, amounts of variation in all microbial groups but not in total microbial biomass. Although significant amounts of variation in each microbial group could be explained by spatial and non-spatial interactions with at least one vegetation variable, in all cases spatial interactions accounted for greater variation than non-spatial interactions (Table 2).

In contrast to analyses based on the entire plot, soil macroaggregate stability did not account for significant amounts of variation in PLFA biomarkers for either clearcut or forest areas (Table 3). In the forest area, pH accounted for a significant amount of the variation in bacteria, fungi and microbial biomass, as well as all PLFAs. In the clearcut area, SOM predicted a significant amount of the variation in bacteria, Gram-negative bacteria, Gram-positive bacteria and all PLFAs. In all cases environmental variable pure effects predicted greater amounts of variance than spatial interactions (Table 3).

Non-AMF hyphae, as was found for analyses of the entire plot, predicted significant amounts of microbial variation in both forest and clearcut areas (Table 3). In the forest area, non-AMF hyphae accounted for a significant amount of the variance in bacteria, fungi, the fungi:bacteria ratio, biomass and all PLFAs. In the clearcut area, non-AMF hyphae predicted a significant amount of the variation in Gram-negative and Gram-positive bacteria, the fungi:bacteria ratio and all PLFAs.

Unlike analysis of the entire plot (Table 2), vegetation didn't account for a significant amount of microbial variation in separate analyses of clearcut and forest areas, with the exception of bacteria in the clearcut area where forbs predicted a significant amount of the variance (Table 3). Environmental variables failed to predict significant amounts of the variation in Gram-negative or Gram-positive

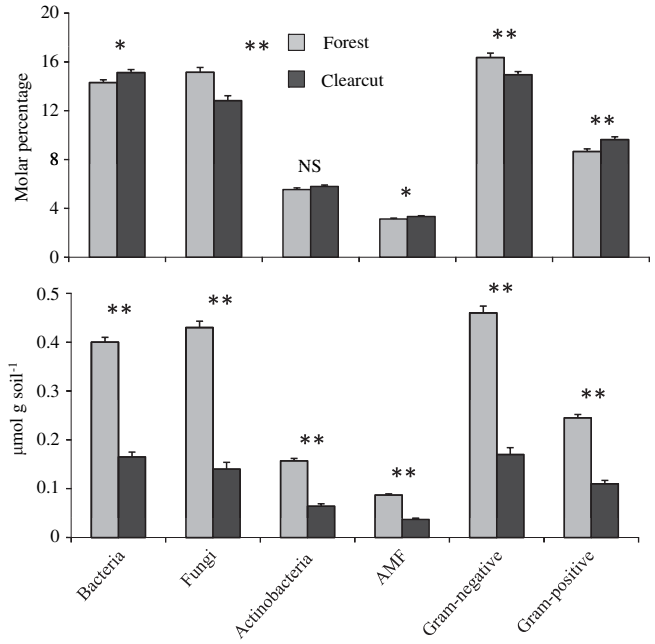


Fig. 3. PLFA biomarker amounts for microbial groups in forest and clearcut harvested areas. *, ** and *** indicate significant differences at 0.05, 0.01 and 0.001 levels of significance, respectively.

bacteria biomarkers in the forest area or actinobacteria biomarkers in any analysis.

Analyses examining interactions between microbial groups indicated that significant amounts of bacteria variation could be accounted for by fungi pure effects and spatial interactions in both forest and clearcut areas. Fungi pure effects and, to a lesser extent, spatial interactions accounted for much more bacteria variation in the forest area than in the clearcut (Table 4). Gram-negative bacteria explained significant amounts bacteria spatial variation in forest, and Gram-positive bacteria, actinobacteria and biomass in the clearcut area. Significant predictors of fungi variation included bacteria and actinobacteria in forest and bacteria, AMF, Gram-negative bacteria, actinobacteria and biomass in clearcut (Table 4).

4. Discussion

Considering the large temporal soil microbial biomass changes documented in other studies (e.g. Gundale et al., 2005; Moore-Kucera and Dick, 2008), our forest soil microbial biomass estimates are within the range found in other studies of coniferous forest ecosystems, which typically vary between 1 and 4 μg g soil^{-1} (Bååth et al., 1995; Saetre and Bååth, 2000; Gundale et al., 2005; Moore-Kucera and Dick, 2008). Likely reflecting differences in carbon inputs, microbial biomass decreased from an average of 2.83 μg g soil^{-1} in the forest area to 1.12 μg g soil^{-1} in the clearcut. Many studies have found reductions in microbial biomass following clearcut timber harvest, although changes this large are unusual. For example, Bååth et al. (1995) found that total PLFAs of a scots pine system decreased from 2.65 to 1.9 μmol g soil^{-1} three years after clearcut. A study examining soil microbial structure in Douglas fir ecosystems (Moore-Kucera and Dick, 2008) found that microbial biomass of old-growth forest soils averaged 2.1 μmol g soil^{-1}, while soils of sites clearcut 8 years prior to analysis averaged only 1.4 μmol g soil^{-1}. The reasons why microbial biomass differences were greater in our study system are not clear, but could be due to climate differences and the relative importance of tree canopies to modulation of temperature and moisture.

Table 2
Variance partitioning of microbial community data from the entire plot. Only environmental variables accounting for significant variation of each microbial group are presented.

		Spatial-not shared[1]	Env. + spatial[2]	Environ. not shared[3]
Bacteria	Ag. Stab.	15.1	53.7	9.7
	Non-AMF	62.9	3.1	2.6
	Grass	51.0	17.9	1.0
	Tree	58.3	10.6	3.2
Fungi	Ag. Stab.	14.6	54.0	10.7
	Non-AMF	66.2	2.5	2.0
	Tree	59.3	9.4	2.4
	Bare	64.8	3.9	1.0
AMF	Ag. Stab.	16.7	53.7	8.0
	tree	59.4	11.0	3.5
G-bact.	Ag. Stab.	14.5	52.3	9.0
	Non-AMF	64.5	2.4	1.3
	Grass	47.5	19.4	2.1
	Tree	58.5	8.4	1.6
G+ bact.	Ag. Stab.	15.1	56.8	7.3
	Non-AMF	68.6	3.3	2.7
	Grass	54.6	17.3	1.3
	Tree	61.1	10.9	1.8
Fungi/Bacteria	Ag. Stab.	15.7	53.5	9.4
	non-AMF	66.2	3.0	2.8
	Tree	59.2	9.9	2.8
Biomass	Ag. Stab.	14.7	59.2	8.6
	Grass	53.3	20.6	2.4
	Tree	63.4	10.5	1.8
All PLFA	Ag. Stab.	15.3	59.3	8.3
	Non-AMF	72.6	1.7	1.0
	Grass	56.0	18.6	1.5
	Tree	63.8	10.8	1.8

[1] Spatial structure in the microbial data that is not shared by the environmental variable.
[2] Spatial structure in the microbial data that is shared with the environmental variable.
[3] The fraction of the microbial data that can be explained independently of spatial structure.

Microbial biomass qualitative differences were detected between forest and clearcut areas (Fig. 3). In agreement with our results, other studies have documented decreased fungi to bacteria ratios after timber harvest and multiple biological and physical mechanisms have been invoked to explain these changes, including alteration of substrate quality (Bååth et al., 1995; Pietikäinen and Fritze, 1995; Siira-Pietikäinen et al., 2001; Busse et al., 2006; Chatterjee et al., 2008). Our results indicating changes in microbial biomass, but not total SOM, suggest differences in SOM quality (Bååth et al., 1995; Pietikäinen and Fritze, 1995). Numerous studies indicate that the ratio of fungal to bacterial biomass typically increases with succession, time since ecosystem disturbance or tighter nutrient cycles (Bardgett and McAlister, 1999; Högberg et al., 2007; Maharning et al., 2008; Mummey et al., 2002). Fungi, which are typically favored in soils low in available nutrients (e.g. Högberg et al., 2007), generally use substrates more efficiently than bacteria (Six et al., 2006) and are important to wood and recalcitrant OM decomposition. Bacteria, on the other hand, are typically favored in nutrient-rich soils that support a more rapid turnover of carbon and faster nutrient cycling (Ingwersen et al., 2008).

All-scale analyses based on PCNM spatial variables typically accounted for >60% of the variation in PLFA biomarkers across the entire plot (Table S1). In almost all cases most of the variation was explained at the largest spatial scales possible (PCNM scale 1). Since larger spatial scales predominantly capture differences between clearcut and forest areas, this result highlights differences

Table 3
Variance partitioning of environmental factor influence on microbial communities in separate analyses of forest and clearcut areas.

		Spatial-not shared	Env. + spatial	Environ. not shared
Forest				
Bacteria	pH	6.3	<1.0	9.0
	Non-AMF	4.0	2.5	1.6
Fungi	pH	11.8	1.6	9.3
	Non-AMF	11.3	2.1	5.4
Fungi/Bacteria	pH	8.0	<1.0	11.1
	Non-AMF	5.9	2.2	9.6
Biomass	SOM	31.5	2.2	7.8
	Non-AMF	26.8	6.9	7.9
All PLFAs	pH	9.8	<1.0	4.1
	Non-AMF	8.8	1.4	5.3
Clearcut				
Bacteria	SOM	10.2	1.0	5.5
	Forbs	20.2	1.0	5.6
G-bact.	SOM	17.8	3.4	4.9
	AMF	19.6	1.6	4.7
	Non-AMF	21.5	<1.0	6.5
G+ bact.	SOM	15.6	1.0	8.9
	Non-AMF	15.9	1.0	9.1
Fungi/Bacteria	AMF	22.8	1.6	7.3
	Non-AMF	25.2	<1.0	11.9
Biomass	SOM	32.2	<1.0	4.4
All PLFA	SOM	3.8	1.2	6.6
	Non-AMF	3.9	1.1	5.2

in microbial properties between these areas found using other analyses (Table 1; Fig. 3). Compared to the entire plot, PCNM spatial models for forest (Table S2) and clearcut (Table S3) areas accounted for relatively small amounts of microbial community variation, further indicating that much of the variation in entire plot analyses was due to differences between forest and clearcut areas.

Different environmental factors accounted for significant variation in microbial communities depending on the area of the plot analyzed. Analysis of the entire plot indicated soil macroaggregate water-stability accounted for over 50% of the variability in each microbial group and for all PLFAs combined (Table 2). Similar to the structuring of soil microbial communities, many physical, chemical and biological factors (and their interactions) contribute to, and are influenced by, soil structure (Six et al., 2004, 2006). Changes in soil structure are thus indicative of broad changes in ecological processes and soil function.

Most studies comparing the relative contributions of bacteria and fungi to soil aggregate formation and stability indicate that the later are more important (Chotte, 2005). Our results, similar to what was found by Bååth et al. (1995), indicate a three-fold reduction in fungal biomass after clearcut harvest. Fungal hyphae can enmesh and entangle soil particles, facilitating aggregate formation and stability. Mycorrhizae, which serve as channels for photosynthates between plants and the soil, where these exudates or decomposition products can act as soil binding agents (Miller and Jastrow, 2000), are recognized as being of especial importance to soil aggregation (Rillig and Mummey, 2006). Ectomycorrhizal fungi may be especially important in this regard to coniferous forest ecosystems (Högberg and Högberg, 2002). Plant species able to host these fungi and total fungi biomass were greatly reduced in the clearcut area. Large reductions in fungal and total microbial biomass C have been demonstrated to occur with decreased supplementation of ectomycorrhizal networks following tree girdling (Högberg and Högberg, 2002). Although it is not possible to disentangle use of heavy equipment at the time of timber harvest from other factors known to influence soil structure, disruption of this important photosynthate channel (Högberg et al., 2001), if not the primary cause of soil structure loss, could slow soil structure development.

Vegetation characteristics predicted significant amounts of the variation in microbial communities for entire plot analyses (Table 2). Relatively more variation could be accounted for by vegetation spatial interactions than by pure effects (Table 2). Tree location accounted for significant amounts of the variance in all microbial groups and grass accounted for significant amounts of variation in all microbial groups except fungi. Since trees were nearly absent, and grasses more abundant, in the clearcut area, these results may reflect broad differences in vegetation and other factors and not necessarily individual plant influences. This is supported by the results of separate analyses of clearcut and forest areas where vegetation characteristics, with the exception of significant bacteria variance accounted for by for pure effects in the clearcut area, failed to predict significant microbial community variation (Table 3).

Our results indicating that tree position predicted only insignificant variation in forest microbial community composition contrasts with other studies suggesting that coniferous trees can strongly influence microbial community composition (e.g. Pennanen et al., 1999; Saetre and Bååth, 2000; Leckie et al., 2004; but see Mottonen et al., 1999). However, areas directly influenced by plants are difficult to define and it could be argued that all of the forest area was influenced to different degrees by coniferous trees. Moreover, our study wasn't designed to examine the influence of individual plants and the scales examined may not have been optimal for detection of localized plant influences.

Unlike analyses of the entire plot, specific analysis of the forest area indicated that pH accounted for significant variation in bacteria, fungi and all PLFAs, while SOM accounted for significant biomass variation (Table 3). Most of the variation in microbial communities accounted for by pH was due to pH pure effects, suggesting that pH influenced microbes at different spatial scales than were analyzed. pH ranged from 4.1 to 5.6 on the forest site. Even small changes in pH can influence a multitude of soil properties which directly or indirectly influence the composition and activity of soil microbial communities (Lauber et al., 2008). Numerous studies have demonstrated the importance of pH to soil microbial community structure in forest ecosystems (i.e. Bååth and Arnebrant, 1994; Bååth and Anderson, 2003; Högberg et al., 2007).

Analysis of the clearcut area indicated that pH, although having a mean value and range similar to the forest site, did not account for

Table 4
Variance partitioning of microbial community indicators in separate analyses of forest and clearcut areas.

		Spatial-not shared	Env. + spatial	Environ. not shared
Forest				
Bacteria	Fungi	<1.0	6.0	45.7
	Gr-bact	9.5	3.1	20.3
Fungi	Bacteria	4.0	<1.0	42.3
	Actino.	5.9	<1.0	18.6
Clearcut				
Bacteria	Fungi	24.7	3.8	7.0
	AMF	13.2	7.7	17.4
	Gr+ bact	1.8	<1.0	48.5
	Biomass	9.6	<1.0	7.2
	Actino.	7.5	<1.0	21.2
Fungi	Bacteria	24.0	3.8	7.0
	AMF	21.2	<1.0	14.4
	Gr-bact	12.6	<1.0	26.9
	Actino.	24.6	<1.0	5.0
	Biomass	18.7	<1.0	23.9

significant amounts of microbial community variation. Instead, SOM content predicted significant variation in bacteria biomarkers (total bacteria, Gram-positive and Gram-negative bacteria), biomass and all PLFAs (Table 3). Unlike the forest, no environmental variable predicted significant fungal biomarker variation. Similar to what was found for pH in the forest area, most of the microbial variation accounted for by SOM was due to SOM pure effects, again suggesting that influences on microbial communities occur predominantly at spatial scales other than those analyzed. Covariation between bacteria and SOM, and decreased fungi to bacteria ratio, in the clearcut suggests a shift towards bacterial decomposition pathways.

While non-AMF extraradical hyphae accounted for significant variation in microbial community composition in both forest and clearcut areas, AMF extraradical hyphae accounted for significant microbial community variation only in the clearcut. This again suggests the importance of mycorrhizal energy channel differences to the structuring of microbial communities of forest and clearcut areas.

Variance decomposition indicated that over 50% of bacteria variation in the forest could be accounted for by fungi pure effects and, to a lesser extent, spatial interactions with fungi (Table 4). Similarly, bacteria pure effects accounted for over 40% of the variation in fungi. These results indicate that the abundance of broad microbial groups are closely linked, although primarily at local scales (Borcard et al., 1992). Fungi pure effects and spatial interactions, although significant, accounted for much less bacteria variation in the clearcut area, suggesting that fungi and bacteria relative abundance are not as tightly coupled at any scale. These relationships are visually apparent from examination of contour plots of proportional bacteria and fungi abundance (Fig. 2); both fungi and bacteria are distributed relatively evenly in the clearcut area, whereas fungi and bacteria in the forest exhibit a relatively strong negative relationship.

Potential explanations for the observed decreased coupling of fungi and bacteria abundance in the clearcut area include differences in fungi functional group influences on microbial community composition. Unlike the forest, AMF pure effects and spatial interactions accounted for significant amounts of bacteria variation in the clearcut area (Table 4). Positive relationships between AMF and bacteria may reflect distributions of AMF host plant roots and their relative importance in the two systems to provision of bacteria growth substrates. Both AMF and roots may preferentially forage for nutrients in areas more favorable for growth of bacteria. However, there are reasons to question the validity of the AMF biomarker (16:1ω5) for determination of AMF biomass in this study system. Despite the relatively low abundance of AMF host plants in the forest, amounts of 16:1ω5 in forest soils were close to twice what was found in the clearcut (Fig. 3). Unlike total fungi biomarkers, which exhibited a significant, positive correlation with non-AMF extraradical hyphal lengths ($R = 0.31$, $P < 0.001$), significant correlation between 16:1ω5 and AMF hyphae were not found ($R = 0.04$, $P = 0.68$). Although Gram-negative bacteria are known to produce 16:1ω5, no relationships between Gram-negative biomarkers and 16:1ω5 were found.

Ectomycorrhizae, which would be expected to be much more abundant in the forest, play a greater direct role in organic matter decomposition than arbuscular mycorrhizae by freeing nutrients from organic materials that are subsequently used by themselves and their hosts. This can result in increased competition for nutrients (Lindahl et al., 2001), which may have decreased bacterial biomass relative to fungi in the forest. There is evidence that ectomycorrhizae may have access to their own litter (Langley and Hungate, 2003; Bending, 2003) resulting in internal nutrient cycling which may further limit nutrients available to bacteria.

A number of microbial variable pure effects, but not their spatial interactions, accounted for significant variation in bacteria and fungi in the clearcut area and fungi in the forest (Table 4), suggesting the importance of local effects. Gram-negative bacteria pure effects and spatial interactions accounted for significant bacteria variation in the forest but not in the clearcut. Conversely, Gram-positive bacteria and actinobacteria pure effects accounted for significant bacteria variation in the clearcut but not in the forest. The importance of these different bacteria groups to prediction of total bacteria is reflected by differences in their relative abundance and contribution to the bacteria biomass pools in forest and clearcut areas (Fig. 3). Differences in organic substrates for microbial growth and presumed alteration of temperature and moisture regimes due to canopy and surface organic layer removal may have selected for Gram-positive bacteria, which sometimes increase under drought conditions (Nazih et al., 2001), in the clearcut relative to the forest.

Differences in microbial community spatial structure between clearcut and forest areas were indicated by variography. Spatial autocorrelation range was greater for all microbial biomarkers in the clearcut area relative to the forest area, indicating increased patch size and decreased spatial heterogeneity (Table 1). While our analyses indicate spatial structure for most, but not all, PLFA biomarkers and soil variables at the scales measured, much of the variation could not be accounted for at the spatial scales examined. This was especially true for the forest area where spatial structure was not evident for Gram-negative bacteria, Gram-positive bacteria or total microbial biomass (Table 1). Analyses based on PCNM spatial models also suggested that soil microbial communities are structured at different scales in forest and clearcut areas. Compared with analyses of the entire plot (Table S1) or clearcut area (Table S3), PCNM spatial models predicted less variation for most microbial biomarkers in the forest (Table S2).

Although greater microbial community spatial structure was generally evident in the clearcut area (Table S1 and S3), variography indicated that less than 25% of the variation in fungi, Gram-positive bacteria and biomass could be accounted for by spatial structure (Table 1). This contrasts with the results of a spatial analysis conducted in a mixed spruce-birch stand that found relatively pronounced spatial structure for all PLFAs analyzed (Saetre and Bååth, 2000), although in some cases at scales smaller than our experimental design allowed. However, the range at which these authors found spatial structure to occur (1–11 m) brackets ranges found in the current study. Other studies have detected spatial structure in soil microbial properties of forest ecosystems at and below the scales we analyzed (reviewed by Morris and Dress, 2007).

In support of the results of PCNM analyses, the high nugget variance of variogram models suggests that sampling on a finer scale, possibly at the scale of individual plants or smaller, would be required to fully capture their spatial variation (Webster and Oliver, 1990). Taken as a whole, our results indicate that analysis at smaller spatial scales than analyzed here will be required to fully elucidate spatial interactions between different components of microbial communities.

Acknowledgements

This work was supported by Paul and Jessica Gurinas and arose from conversations with Tom Parker, Mark Vander Meer, and Paul Gurinas. Dr. Cory Cleveland is acknowledged for equipment use.

Appendix. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.soilbio.2010.03.020.

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