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Importance of spatially structured environmental heterogeneity in controlling microbial community composition at small spatial scales in an agricultural field

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ABSTRACT

Spatial heterogeneity is an inherent feature of soils that has significant functional implications, particularly when the activities of soil microbial communities are considered. The main goal of this study was to determine the physical–chemical properties best correlated with changes in microbial community composition in an agricultural ecosystem, as part of an effort to better understand what environmental factors control the distribution and organization of soil microbes. In addition, we sought to determine: (i) whether these factors vary depending on the spatial extent considered, and (ii) whether different subsets of the microbial community were linked with different environmental variables. This analysis was conducted in a spatially explicit manner via a series of Mantel and partial Mantel tests to examine the relationship between the microbial community and the soil microenvironment while controlling for any shared spatial structure. Two-hundred soil samples were collected with separation distances ranging from 2.5 cm to 11 m, and the multi-scale spatial distributions of soil carbon (C), nitrogen (N), organic matter (OM), texture, and bacterial abundance were compared with previously published analyses of microbial community structure. The results of the spatial analysis of soil properties were similar to those obtained for the microbial communities, and considerable spatial structure was detected, even at very small scales (i.e., $\leq$40 cm). A strong link between the microbial community and the soil physical–chemical properties was established, and different subsets of the microbial community responded differently to the various environmental properties. C and N affected the widest portion of the microbial community, while patterns in OM distribution and soil texture were selectively correlated with specific groups of microbes. Collectively, these results demonstrate the value of considering multiple spatial scales when studying community–environment interactions, and that one's interpretation of these relationships is critically dependent on the scale of the investigation and the aspect of the community considered. Understanding how microbial communities develop and organize will help scientists interpret the interplay of dispersal, disturbance, and local dynamics in spatial mosaics, and may have important implications for land management following natural disturbances or human alterations.

1. Introduction

There are a variety of abiotic and biotic factors that shape the structure and dynamics of microbial communities. However, the relative importance of these factors, and the specific mechanisms by which each can affect the diversity and composition of microbial assemblages are poorly understood. In soils, this is partially due to methodological constraints that limit ecologists' ability to study microbial diversity, and further complicated by the fact that any investigation of soil is faced with the problem that the substrate is highly variable on small scales, both horizontally and vertically. This variability may result from a complex set of geological, pedological, and biological processes, as well as different types of land use. Even within a single field, the distribution of soil physical and chemical properties is the result of many superimposed processes acting at different spatial and temporal scales. These properties may cause differences in both the structure and function of soil microbial communities, which may further impact ecosystem-level processes by altering things like soil chemistry, organic matter content, and vegetation growth.

Understanding microbial community organization across various scales in an important first step in evaluating microbial community structure–function relationships, and knowledge of the spatial patchiness of bacteria is important for determining the appropriate

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sampling scales and for addressing basic ecological questions. However, despite the assumed importance of spatial variability in soil microbiology, studies that specifically consider spatial scale when examining the distribution patterns of microorganisms, and the possible causes of these patterns, are rare. Moreover, most of the studies that have investigated the spatial variability of soil physical, chemical, and microbiological properties only focus on a single spatial scale (Morris, 1999; Mottonen et al., 1999; Robertson et al., 1988; Saetre, 1999; Savin et al., 2001), even though spatial heterogeneity in community composition, and the analysis of its relationship to habitat heterogeneity, is scale-dependent (Pinel-Alloul et al., 1995).

Recently, scientists have begun to focus on multi-scale comparisons, and have found evidence for nested scales of spatial structure for both soil physical–chemical properties and the associated microbial communities (Bach et al., 2008; Becker et al., 2006; Ettema and Wardle, 2002; Garten et al., 2007; Morris and Boerner, 1998, 1999; Nunan et al., 2002; Robertson and Gross, 1994; Ritz et al., 2004; Robertson et al., 1997; Saetre and Bååth, 2000; Stenger et al., 2002). In an earlier published work, we explored the multi-scale spatial distribution of microbial community structure in an agricultural wheat field, and demonstrated that several scales of spatial autocorrelation may exist within the cm- to 10-m scale (Franklin and Mills, 2003). In the present manuscript, we present results concerning the multi-scale distribution of soil environmental properties, and compare this to the previously reported data concerning microbial community structure, as part of an effort to better understand what environmental factors help control the distribution and organization of soil microbial communities. A strong link between the structure of the microbial community and the soil physical–chemical properties was established, and different subsets of the microbial community responded differently to the various environmental properties. These results suggest that the environmental factors regulating the development of the microbial communities in this soil may operate at different scales, and a simultaneous analysis of the multi-scale spatial variability of microbial community structure and soil microenvironment is often necessary to identify these factors and determine their relative importance.

2. Materials and methods

2.1. Site description and sampling design

Soil samples were collected from an agricultural field on the eastern shore of Virginia (USA). The field was located along the lagoonal shoreline of the Delmarva Peninsula, approximately 700 m from the edge of the open water (37° 17.62′N, 75° 55.53′W). The soil at this site was very sandy, with low organic matter content, and had been plowed as a single crop for many years. At the time of sampling, the field was planted with durum wheat (*Triticum turgidum*), which was approximately 75 days old.

The basic sampling design was a square with 7.1-m edges and 10-m diagonals (Fig. 1). Samples were collected at regular intervals around the perimeter of the block (1.8-m separation distance), and at 1-m intervals along the diagonals. At each node (A, B, C, D, and X), more concentrated sampling efforts were employed. Nested within the original sampling grid, a second set of samples was collected at 10-cm increments in a cross shape surrounding each node. Five samples were collected in each direction (north, south, east, and west) away from the center node. Nested within this area, a third set of samples was collected at 2.5-cm increments around each node, following the same pattern (2.5, 5.0, 7.5, and 10 cm in each direction). A total of 193 soil samples were collected, 33 at each node and 28 at larger separation distances.

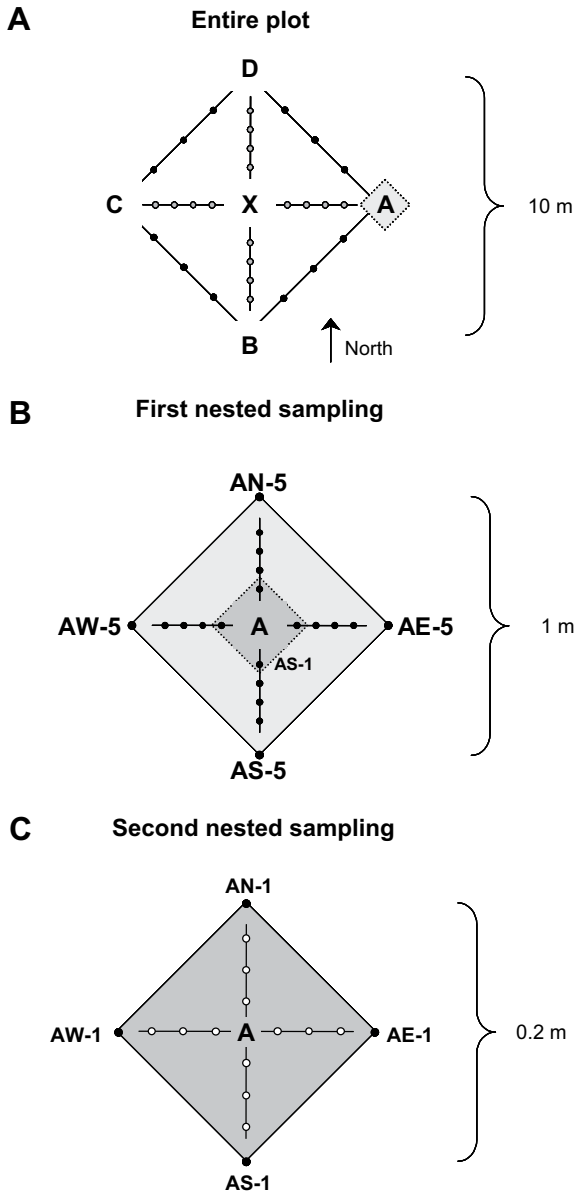

Fig. 1. Map of the sampling scheme. (A) The sampling area was a 50 m^2 square (diamond) with 10 m diagonals. Around the perimeter of the square, samples were collected at 1.8 m increments, and at 1 m increments along the diagonals. At each node (A–X), more concentrated sampling efforts were employed. A nested sampling pattern was applied at each location, and node A is presented in the figure as an example. Additional samples were collected at 10 cm increments (B) and 2.5 cm increments (C) in a cross shape surrounding each node.

At each sampling location, the loose layer of surface material was removed, and a small hole (1.5 cm diameter) was dug to collect 5–10 g of soil. The samples were placed on ice for transport to the lab, where they were sifted (approximately 750-μm mesh size) to remove gravel, plant, and root material, and stored at −80 °C.

2.2. Analysis of soil properties

Originally, these samples were collected with the goal of examining the spatial distribution of the soil microbes via whole-community DNA fingerprinting (Franklin and Mills, 2003). The results of that study prompted us to further analyze the initial samples, though this data collection was limited in two ways. First, the soil samples we originally collected were necessarily small

(5–10 g), to permit an analysis of small-scale (cm) spatial variability; this small sample size restricted the number and type of soil physical–chemical properties that could be analyzed. Second, because these analyses were performed on stored, frozen samples, certain parameters could not be evaluated (e.g., moisture content). Within these constraints, we selected environmental properties that were likely to directly influence soil microbial communities, and measured total soil carbon (C) and nitrogen (N) content, organic matter (OM) content , and soil texture (sand, silt, and clay fractions).

The C and N content of the soil was determined using an elemental analyzer (CE Elantech, Lakewood, NJ). A modified version of the pipette method was used for particle-size determination, based on the technique described by Kettler et al. (2001). Percent sand, silt, and clay were calculated, and a small subsample of soil was also analyzed for OM content by determining the amount of material lost upon ignition (450 °C, 24 h).

2.3. Analysis of microbial community structure and abundance

Microbial community structure was analyzed using Amplified Fragment Length Polymorphism (AFLP) DNA fingerprinting (Franklin and Mills, 2003). Once analysis of community structure and soil properties was complete, microscopic counts of total bacterial abundance were carried out on any samples with material remaining (59 samples, from locations throughout the plot). Approximately 0.5 g of sample was combined with 50 ml filter sterilized water, and blended for 1 min. The samples were allowed to settle for 2 min, and a 1 ml aliquot was then removed. Acridine orange direct counts (AODC) was performed (Bottomley, 1994), and the number of cells g^{-1} dry weight of soil was calculated.

2.4. Data analysis

2.4.1. Approach

The relationship between microbial community structure, soil microenvironment, and spatial separation was examined using Mantel and partial Mantel tests and a multi-scale approach. For the first set of analyses, the relationships among all pairs of sampling locations were considered, to obtain an average portrait of the overall spatial variability in this plot. Subsets of these data, varying in maximum separation distance, were then analyzed to quantify autocorrelation at different spatial scales. These scales were named based on relative size, and the following designations were used: (i) *plot scale* (all sampling locations), (ii) *large scale* ($\leq$5 m), (iii) *small scale* ($\leq$1 m), and (iv) *fine scale* ($\leq$0.4 m). Each analysis was performed at each analytical scale to study how the perception/detection of the community–environment relationship changed when different spatial extents were considered.

In order to account for the shared correlation between different soil properties, the environmental variables were separated into three groups: (i) overall soil texture – combining sand, silt, and clay measurements, (ii) soil carbon and nitrogen (CN), and (iii) organic matter (OM) content. Organic matter content was maintained as a separate variable based on the fact that it did not correlate significantly with any of the other soil properties considered (Pearson correlation analysis, $p > 0.1$). The relationship of each of these macrovariables (texture, CN, or OM) to either bacterial abundance (AODC) or similarity in microbial community structure (as determined by AFLP DNA fingerprinting) was examined.

2.4.2. Preparation of data matrices

Since Mantel tests are used to compare distance/dissimilarity matrices, Gower's coefficient was used to quantify the resemblance among samples (Gower, 1971; Legendre and Legendre, 1998); separate similarity matrices were developed for each set of environmental variables (OM, CN, or texture) and for bacterial abundance. Geographic distance was computed for all pairs of sampling locations to assemble a spatial distance matrix. For the community structure data, relative similarity values were calculated using the Jaccard coefficient (Franklin and Mills, 2003). When necessary, similarity matrices were transformed to dissimilarity matrices as: Dissimilarity = 1 – Similarity.

In addition to studying the relationship of the environmental variables with *overall* community structure, we also wanted to examine whether different subsets of the microbial community responded to different environmental variables. A principal components analysis (PCA) was used to reduce the multivariate community data (the AFLP patterns) into a set of derived variables describing different aspects of community structure (Legendre, 1993). Each of the resulting principal components (PCs) correlates with a distinct pattern of variability, manifest with different groups of AFLP bands. Together, these components explained 27% of the total variance in community structure (PC1 = 12.3, PC2 = 8.7, PC3 = 5.6). Conceptually, these PCs may be considered to represent different "subsets" of the communities' overall genetic composition, and are related to the distribution and relative abundance of different populations or groups of organisms. Separate similarity matrices were calculated for each community subset/PC using Gower's coefficient.

2.4.3. Mantel and partial Mantel tests

A Mantel test is a regression in which the variables are actually similarity or distance matrices summarizing pairwise comparisons among samples; the Mantel statistic (r_M) is computed by determining the sum of the cross-products of the corresponding values in each of these matrices (Rossi, 1996). The partial Mantel test, as developed by Smouse et al. (1986), allows testing for the correlation between two matrices while controlling for the effect of a third matrix, and is analogous to a partial correlation. We used these techniques in two ways: (i) to establish whether significant spatial structure was present in the environmental dataset (Mantel test with a matrix of geographic distances and each matrix of environmental or community similarity); and (ii) to address the question "is there a relationship between community similarity and environmental similarity, after removing the shared correlation of these variables with spatial separation?" (partial Mantel test using a matrix of community similarity and a matrix of environmental similarity, controlling for the effect of a third matrix of geographic spatial separation distances).

Normalized Mantel and partial Mantel statistics were calculated using the R statistical package (Legendre and Vaudor, 1991). The results (r_M values) range from -1 to $+1$, and the statistical significance of each test was evaluated through permutation (a Monte Carlo approach, with 1000 permutations). The significance level was corrected for multiple comparisons using a Bonferroni approach. For each overall community model that was developed, 40 simultaneous tests were performed, and a corrected significance level of 0.001 was used (0.05/40 comparisons).

3. Results

3.1. Characterization of spatial structure

Before any spatial statistics were computed, an exploratory data analysis was performed for each of the environmental variables and the bacterial abundance data (Table 1); a Kolmogorov–Smirnov test demonstrated that each variable was normally distributed (results not presented). Next, a simple Mantel test was used to test each variable for the presence of spatial structure at each scale (Table 2). At both the plot- and large-scale, significant spatial structure was

Table 1
Basic statistics for the environmental variables and bacterial abundance.

Parameter (%)	Min	Max	Mean	SD	CV (%)
Carbon (C)	0.35	0.89	0.60	0.104	17.3
Nitrogen (N)	0.03	0.09	0.06	0.008	12.9
Organic matter (OM)	0.61	1.58	1.05	0.21	20.0
Sand	61.6	78.1	70.1	3.35	4.6
Silt	19.6	34.4	26.1	2.63	10.1
Clay	0.36	5.46	2.69	1.12	41.6
Bacterial abundance[a]	1.0×10^9	3.1×10^9	1.9×10^9	5.4×10^8	28.4

[a] Cells g^{-1} dry weight of soil.

detected for all of the environmental variables, bacterial abundance, overall community structure, and for community subsets 2 and 3. At smaller spatial scales, fewer significant relationships were detected, particularly for the fine scale, where only two nearly significant patterns were revealed.

3.2. Relationship of community properties and environmental variables

Partial Mantel tests, removing the effect of spatial separation, were used to examine the relationship between each microbial community variable and each environmental variable (Table 3). When overall community structure was considered, no significant results were obtained. However, when the individual community subsets were examined, strong correlations were observed. Changes in community subset PC 1 were significantly correlated with changes in soil CN at separations distances larger than 1 m (plot-, large-, and small-scale analyses), but not at the finest scale. Changes in community subset PC 2 were associated with changes in both CN and OM at all of the scales considered. A significant relationship was detected between community subset PC 3 and both CN and texture for all four scales.

Because of the relatively small number of samples analyzed for bacterial abundance, the correlation analysis was only performed at the plot and large scales (Table 3). At both scales, OM was correlated with bacterial abundance; at the large scale (>5 m), a significant correlation with texture was also detected.

4. Discussion

4.1. Spatial structure for microbial and physical–chemical parameters

Spatial heterogeneity is an inherent feature of soils and has significant functional implications, particularly when the activities and distribution of microorganisms are considered. In this study, we sought to correlate differences in the distribution of a soil microbial community with differences in the soil physical–chemical parameters, as part of an effort to understand which aspects of the environment are most strongly associated with differences in microbial community composition. The agricultural field described in this study was originally selected to represent a *relatively* homogeneous system, given that it had been plowed and planted as a single crop for several years. Despite this history, and the small area sampled (50 m^2), a great deal of spatial variability was observed in the physical–chemical properties of the soil (Table 2). In addition to the Mantel tests, a series of geostatistical semi-variogram analyses were conducted to examine the type of spatial pattern displayed in each variable using the approach outlined in Franklin and Mills (2003). Geostatistics demonstrated the presence of spatial autocorrelation for all of the microbial and environmental variables (Supplemental Fig. 1 and Supplemental Table 1), and kriging was used to generate maps of the spatial distribution of each parameter (Supplemental Fig. 2). The maps of soil texture displayed a large-scale gradient pattern across the sampling plot, while those for C and N showed a more patchy structure, with multiple levels of autocorrelation, nested within this larger-scale gradient. These patterns were quite similar to those previously observed for community structure (Franklin and Mills, 2003).

While significant spatial structure was detected for all of the variables considered at the larger analytical scales, only texture and community structure displayed spatial autocorrelation at the fine scale (≤0.4 m). Previously, we found that the spatial distribution of community structure at the finer scales was different at the different nodes in the plot, so it is possible that a clear pattern was simply not detected because the data from all of these locations was pooled in the present analysis. If the fine scale spatial structure of a variable changes across the sampling plot, it may not be possible to observe an overall "average" pattern. The decreased number of samples at the fine scale ($N = 1670$ pairwise comparisons), compared to the large ($N = 8288$) and plot ($N = 18\,528$) scale analyses, could also limit our ability to detect fine scale relationships.

Overall, the results demonstrate that physical–chemical factors can vary substantially at small spatial scales in soils and within an area that would be classified as a single habitat. These results are particularly striking because the effect of most agricultural practices is to homogenize soils and thus remove variation from nearby

Table 2
Mantel test results to assess for spatial structure in each soil physical–chemical property and each aspect of microbial community structure.

Soil property	Spatial scale			
	Plot	Large	Small	Fine
CN	−0.31[a]	−0.39[a]	−0.15[a]	−0.04
OM	−0.05[b]	−0.11[a]	−0.09[a]	−0.02
Texture	−0.42[a]	−0.34[a]	−0.12[a]	−0.05[b]
Overall Community Structure	−0.26[a]	−0.22[a]	−0.12[a]	−0.06[b]
Community PC 1	−0.02	−0.03	−0.05[b]	−0.04
Community PC 2	−0.10[a]	−0.22[a]	−0.03	−0.01
Community PC 3	−0.38[a]	−0.23[a]	−0.01	−0.04
Bacterial abundance	0.02	−0.22[a]	ND	ND

ND, not determined due to small sample size.
[a] $p \leq 0.0001$.
[b] $0.001 < p \leq 0.01$.

Table 3
Partial Mantel test results, removing the effect of spatial structure, to study the relationship between each environmental variable and each microbial property.

Microbial property	Spatial scale	Environmental variables		
		CN	OM	Texture
Bacteria abundance	Plot	−0.03	0.19[a]	−0.07
	Large	−0.03	0.11[a]	−0.13[a]
Overall community structure	Plot	−0.04	0.00	−0.06
	Large	0.00	0.02	−0.02
	Small	−0.02	0.02	−0.04
	Fine	0.04	0.06	−0.01
Community subset PC 1	Plot	0.14[a]	−0.05	−0.01
	Large	0.12[a]	−0.05	−0.02
	Small	0.06[a]	0.02	0.00
	Fine	0.03	0.05	−0.01
Community subset PC 2	Plot	0.07[a]	0.09[a]	0.02
	Large	0.11[a]	0.14[a]	−0.02
	Small	0.14[a]	0.05[a]	0.04
	Fine	0.21[a]	0.08[b]	0.04
Community subset PC 3	Plot	0.11[a]	−0.05	0.07[a]
	Large	0.05[a]	−0.05	0.09[a]
	Small	0.13[a]	0.00	0.08[a]
	Fine	0.16[a]	0.04	0.08[a]

[a] $p \leq 0.0001$.
[b] $0.001 < p \leq 0.01$.

sites (Buckley and Schmidt, 2001; Eo and Nakamoto, 2008; Robertson et al., 1993). Nonetheless, they are consistent with results from several earlier studies (Beckett and Webster, 1971; Robertson et al., 1988, 1993; Webster and Butler, 1976). For example, Robertson et al. (1997) found that more than 50% of the variability in soil properties in a cultivated field resulted from spatial structures between 5 and 60 m. In abandoned fields, Tilman (1982) has shown that levels of important soil nutrients can vary at a scale of meters. Though agricultural practices can result in reductions in variability at certain scales, much of the variability in soil properties (particularly those relevant to microorganisms) exists at finer spatial scales – well below even the scales of this study – and so remains relatively unaffected by human actions. For instance, inner and outer portions of soil microaggregates have been found to contain different bacterial communities (Mummey and Stahl, 2004), as have different soil microenvironments (Ranjard et al., 2000).

4.2. Relationship between environmental variables and microbial community structure

Significant spatial structure was detected for the abiotic and biotic variables, and the spatial patterns observed for the environmental variables were quite similar to those found in the earlier analysis of community structure (Franklin and Mills, 2003). These similarities could be the result of an active response of the microbial populations to changes in soil microenvironment, or the result of a spurious correlation between the two sets of variables induced by a common spatial gradient. These two possibilities were explicitly examined using a series of partial Mantel tests (Table 3) to determine whether changes in the microbiological properties were correlated with changes in the environmental variables while considering the spatial separation of sampling locations. These analyses were conducted at each analytical scale (plot, large, small, and fine) to determine whether different environmental factors are associated with different microbial communities at each spatial scale.

In the first set of analyses, overall community structure was considered using a similarity matrix derived from the AFLP fingerprinting data. The variability in the AFLP patterns was strongly spatially structured at all of the analytical scales considered (Table 2), but did not correlate with environmental variability for any of the physical–chemical properties we measured once the shared autocorrelation with space was removed (Table 3). One explanation for this result is that the dominant environmental factor controlling the microbial community was not among those we analyzed. Alternately, the fact that different members of the microbial community respond in different and possibly divergent ways to environmental variability means that a single coherent signal is not present in this dataset.

Despite the fact that no significant pattern was observed between the "overall" structure of the microbial community and the pooled environmental variables, analysis of the different subsets of the community (each PC) revealed a strong relationship to both spatial separation and environmental variability. Overall, CN seemed to be the most important environmental factor, as it was correlated to each of the community subsets. At each analytical scale, community subset PC 2 was also influenced by changes in OM, and changes in community subset PC 3 were correlated with changes in soil texture.

It is not necessarily surprising to discover that patterns in soil C, N, and OM were strong controls on microbial community structure. It is generally accepted that microbial community composition is partially controlled by the amount and type of substrate available (Rodriguez-Zaraqoza et al., 2008), and C is often a limiting factor for microbial growth in soil (Aldén et al., 2001). Previous work has also demonstrated the existence of "hot spots" (zones of intense microbial activity and large microbial populations) in relation to available organic matter (Gonod et al., 2003); in our study, a strong relationship was observed between bacterial abundance and soil organic matter (Table 3).

The response of the microbial community to the distribution patterns for C, N, and OM could indirectly suggest a relationship between the microorganisms and the distribution of vegetation in the plot. The structure and functional diversity of microbial communities in soils has been shown to be tightly related to plant species composition and distribution (Bach et al., 2008; Grayston et al., 2001; Kourtev et al., 2003), and there is evidence that certain components of microbial community structure can vary at spatial scales consistent with the distribution of individual plants (Cavigelli et al., 1995; Robertson et al., 1997). It is likely that differences in the age or health of the individual plants can also alter microbial community structure. These changes may, in turn, influence plant growth. For example, changes in the efficiency with which microbial communities decompose organic matter and/or changes in the size or composition of the microbial community have been demonstrated to cause changes in nutrient cycling (Boerner and Rebbeck, 1995; Cotrufo et al., 1994) and in the structure of the plant community (Lambers, 1993).

Compared to C, N and OM, much less is known about the way the physical soil matrix affects microbial community structure and function (Feeney et al., 2006), making it more challenging to interpret the correlations presented here between texture, community structure, and bacterial abundance. Soil texture has the potential to affect the accessibility of substrate to soil organisms as well as influence many aspects of the soil microenvironment (e.g., the exchange of water, nutrients, and oxygen). In addition, texture is also thought to influence microbial community structure by affecting biological interactions between organisms such as competition and predation (e.g., by providing physical protection to prey species (Wright et al., 1995)). Recent work by Feeney et al. (2006) demonstrated that soil microbes may even microengineer their habitats by changing the porosity and aggregation, and further hypothesized that the soil–plant–microbe complex is self-organized. In the present study, soil texture was correlated with bacterial abundance and with some aspects of microbial community structure (community subset PC 3), and, though the mechanism by which this correlation occurs was not tested, it likely relates to water availability and oxygenation. For example, since this is a relatively sandy soil, wetter, possibly anaerobic, microsites associated with clay particles could reasonably harbour a unique set of organisms.

There are a number of soil factors that may influence microbial community properties besides those we measured. For example, evidence shows a close relationship of soil microbial communities and pH, soil moisture, and soil temperature (Bach et al., 2008; Emmerling et al., 2001; Liu et al., 2008). There are also a number of biotic factors that may be important, including small-scale relationships among the microorganisms (e.g., competition or predation). Furthermore, cm- to meter-scale patterns of spatial variability have also been observed for soil fungi and nematodes, and have been linked with microbial patterns (Ettema and Yeates, 2003; Gorres et al., 1998;; Kuperman et al., 1998; Mottonen et al., 1999; Robertson and Freckman, 1995).

Table 3 shows how differences in each community were correlated with differences in each environmental variable, but does not provide any information about the overall environmental regime associated with each community. To investigate this, a PCA was performed on the environmental data collected from each of the nodes; a correlation matrix was used to prepare the PCA as the variables were each measured on different scales. Combined, the first 2 PCs explained ~ 75% of the variance associated with the environmental properties (Fig. 2A). The variables important for

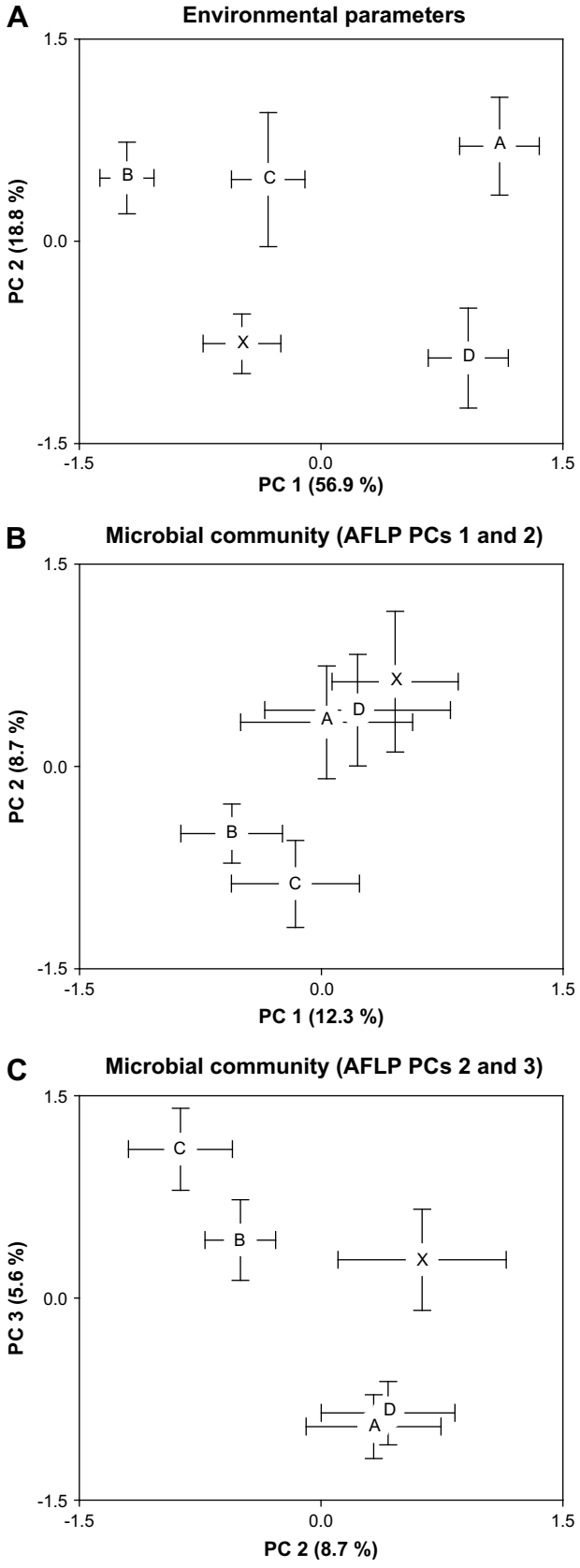

Fig. 2. Principal components plots for environmental data (A) and community structure data (B & C). Error bars represent 99% confidence intervals around each centroid.

PC 1 were: sand (factor loading = 0.96), silt (- 0.91), C (0.86), and clay (- 0.80), and the variables important for PC 2 were: OM (0.86). Soils from nodes A and D have high carbon and sand content, and low amounts of silt and clay, and the most similar microbial communities (Fig. 2B and C). In contrast, soils from nodes B and C have lower carbon and sand content, and relatively high amounts of silt and clay, and more similar microbial communities. The microbial community from node X was intermediate, as was its overall soil environment.

4.3. Organization of microbial communities

It has been suggested that conclusions about the organization of microbial communities, the effects of disturbance, or the roles of various limiting factors are likely to differ at different spatial scales. Paradoxes may arise when different investigators, studying similar communities but at different scales, arrive at different conclusions about the factors that structure these communities; these disagreements may reflect viewpoints of different scales, and not necessarily differences in the way communities are organized (Rahel, 1990). The analytical approach employed in this paper was motivated partially by this consideration, and a desire to demonstrate how important the "scale" used in an analysis is – even if that difference in scale is small as perceived by the researcher (e.g., changing from 0.5 m to 1 m). To accomplish this, we subdivided the data to represent scales commonly employed by researchers, and then compared the results. While each scale was "logical" and, further, reflects a person's intuitive visual assessment of an environment – they are not necessarily scales that are particularly meaningful to microorganisms.

The results of this study indicate that one's conclusions regarding the factors that are important for controlling community structure in this system can indeed change depending on the analytical scale used (even within the range of cm to 10 m), but even *greater* differences were found to depend on the portion of the community studied. In particular, the different 'community subsets' were found to correlate with different environmental properties. This finding has important implications considering that the different techniques available to characterize microbial community structure all have limitations, and are generally biased and focus on particular portions of the microbial community. For example, traditional microbiological methods are selective for microorganisms that are capable of growing on culture media, community level physiological profiling (CLPP) may be biased toward faster growing organisms (Konopka et al., 1998), and the numerous molecular genetic methods may provide very different results depending on differences in nucleic acid extraction procedures, PCR conditions and primers, or the resolution associated with a particular technique. The results of our study suggest that researchers must be especially careful about comparing separate community-environment studies that use different assays to evaluate community structure; the findings may change considerably depending on the portion of the community actually evaluated.

Spatial variability, such as we observed in this study, is likely to exist in most ecosystems, and needs to be considered when making inferences about ecological relationships and when developing strategies to sample the environment. In particular, understanding the scale at which a parameter must be measured is essential to creating a sampling design that will result in a sound ecological evaluation of that parameter, and in determining sample sizes and appropriate statistical techniques for data analysis. In addition to considering horizontal patterns, as was done with this study, depth must also be taken in account. Much less is known about the nature of the microbial communities found throughout the soil profile, and deeper layers of soil may contain microbial communities that are specialized for their environment and fundamentally different from

the surface communities (Blume et al., 2002; Fierer et al., 2003; Griffiths et al., 2003; Rodriguez-Zaraqoza et al., 2008; Snajdr et al., 2008; Zyagintse, 1994).

These results provide a snapshot of the relationships in this field at a single time, and do not consider the role of temporal variability, or its interaction with spatial heterogeneity, in determining community patterns. Because different environmental variables are important not only at different spatial scales, but also at different temporal scales, studies that examine both simultaneously are needed. Some of the soil properties we measured are expected to be relatively static (e.g., sand or silt content), while other, such as C, N, or OM are expected to be more seasonally dynamic. Still others, which we did not measure, such as temperature or water content, may cause variations over even shorter time scales. For example, it has been reported that microbial biomass can fluctuate sharply over days following agricultural management or natural influences like drying and rewetting of a soil (Fierer and Schimel, 2002; Ocio et al., 1991a,b; Peixotoa et al., 2006; Wyland et al., 1995).

While increased knowledge of these many factors influencing microbial community structure, and the role of space and time in this relationship, is important, there is particular interest in understanding how these factors may affect the activity of microorganisms in an ecosystem. Soil microbes play a crucial role in keeping the main nutrients cycling in soils (C, N, P, S), and are fundamental for the long-term functioning of ecosystems. The results from the present study demonstrate that changes in community structure may occur is association with different environmental conditions; however, it remains to be determined how/if this change in structure will manifest as a change in microbial community function. Little is known of the importance of microbial community structure and diversity in determining the functioning of soils. It is often hypothesized that diversity is important for the maintenance of soil processes, and that reductions in soil microbial diversity will disrupt the functional capability of soils (Giller et al., 1997); however, the few studies available that specifically address this question in soils present conflicting results (Atlas et al., 1991; Klein et al., 1986; Noguez et al., 2008; Salonius, 1981; Zak et al., 2003). Increased research into the relationship between community structure and function is necessary before scientists can anticipate how habitat disruption and changes in community structure may impact community activity and ecosystem performance, and a better understanding of the role of spatial heterogeneity in microbial communities will help ecologists to determine the relevance of small-scale observations and experiments for large-scale patterns and processes.

Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.soilbio.2009.06.003.

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