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Diversity and dynamics of microbial communities in soils from agro-ecosystems

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Summary

Soil microbial communities are integrally involved in biogeochemical cycles and their activities are crucial to the productivity of terrestrial ecosystems. Despite the importance of soil microorganisms, little is known about the distribution of microorganisms in the soil or the manner in which microbial community structure responds to changes in land management. We investigated the structure of microbial communities in the soil over two years in a series of replicated plots, that included, cultivated fields, fields abandoned from cultivation and fields with no history of cultivation. Microbial community structure was examined by monitoring the relative abundance of ribosomal RNA (rRNA) from seven of the most common bacterial groups in soil (the Alpha and Beta Proteobacteria, Actinobacteria, Cytophagales, Planctomycetes, Verrucomicrobia and the Acidobacteria) and the Eukarya. These data reveal that soil microbial communities are dynamic, capable of significant change at temporal scales relative to seasonal events. However, despite temporal change in microbial community structure, the rRNA relative abundance of particular microbial groups is affected by the local environment such that recognizable patterns of community structure exist in relation to field management.

Introduction

Though of unquestionable importance in regards to the function of terrestrial ecosystems (Conrad, 1996; Whitman *et al*., 1998; Copley, 2000), we understand very little about the structure of soil microbial communities, how these communities respond to changes in their environment or the consequences that alterations in microbial community structure have on ecosystem function. The dearth of information about soil microbial communities is a consequence of their enormous complexity and genetic diversity (Torsvik *et al*., 1990) and the fact that the microorganisms that can be isolated from soil and studied in isolation represent only a small portion of the microbial groups present *in situ* (Hugenholtz *et al*., 1998). Soil microorganisms do not behave as a homogeneous trophic level (Mikola and Setala, 1998) and the species composition of a soil microbial community can influence microbial processes in the soil both qualitatively and quantitatively (Cavigelli and Robertson, 2000). As a result, it is important to examine the internal dynamics of soil microbial communities not simply for the sake of characterizing these fascinatingly complex biotic systems, but also to understand the impact that changes in microbial community composition may have on terrestrial ecosystems.

The recovery and analysis of rRNA genes has proven to be a useful tool in revealing the general taxonomic composition of soil microbial communities (Hugenholtz *et al*., 1998; Buckley and Schmidt, 2001a). Analyses of rRNA genes reveal a tremendous amount of species richness within soil microbial communities though a large fraction of the rRNA gene sequences recovered fall into one of several broad groups of organisms. Examination of the rRNA genes recovered from soil microbial communities at diverse sites reveal that eight bacterial groups are present in a majority of soil microbial communities (Buckley and Schmidt, 2001a). These groups are the Alpha, Beta, and Gamma groups of the Proteobacteria, the Actinobacteria (Gram-positive bacteria with high mol percentage G + C genome content) the Cytophagales, the Acidobacteria, the Planctomycetes and the Verrucomicrobia.

Whereas analysis of rRNA genes recovered from microbial communities provides a useful tool for estimating community richness, measurement of the relative proportions of the rRNA molecules themselves (the rRNA gene products) can be used to determine patterns of microbial distribution (Ward *et al*., 1992). It is important to note, however, that intracellular concentrations of rRNA increase with cellular growth rate and change in response to alterations in the nutrient status of a cell (Neidhardt and Magasanik, 1960, Jinks-Robertson and Nomura, 1987). Thus, high relative abundance of rRNA for a microbial

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Table 1. Summary of data from controls used in hybridization experiments.

Probe[a]	Target group	Match[b]	Controls[c]	Hybridization result[d] Rp	Rn
Alf1b	α Proteobacteria	83%	Kv (0), Pl (1), Ne (2), Ag (2), Pa (3)	0.212 ± 0.075	0.004 ± 0.001
Bet42a	β Proteobacteria	100%	Ne (0), Pa (1), Kv (–), Ag (–), Ac (–)	3.831 ± 0.392	0.049 ± 0.015
HGC69a	Actinobacteria	100%	Ag (0), Pa (2), Kv (–), Cj (–), Ac (–)	1.423 ± 0.277	0.028 ± 0.007
Pla46	Planctomycetes	78%	Pl (0), Vs (2), Kv (4), Ne (4), Cj (5)	2.384 ± 0.371	0.060 ± 0.010
Ver47	Verrucomicrobia	100%	Vs (0), Ne (3), Pl (3), Pa (3), Kv (4)	0.346 ± 0.045	0.004 ± 0.001
CF319a	Cytophaga-Flavo.	79%	Cj (0), Ag (2), Vs (2), Pa (2), Kv (3)	0.365 ± 0.059	0.013 ± 0.017
Acd31	Acidobacteria	65%	Ac (0), Ag (1), Pl (1), Ne (2), Kv (3)	0.952 ± 0.110	0.071 ± 0.009
Euk1195	Eukarya	82%	Sc (0), Kv (5), Ne (5), Ag (5), Pl (5)	0.417 ± 0.026	0.012 ± 0.001

a. References and sequences for each probe are provided in the *Experimental procedures* under the heading RNA hybridization.
b. Per cent of the target group that is recognized by the probe. Values are calculated for full length sequences in Release 8.1 of the Ribosomal Database Project (Maidak *et al.*, 2001). Sequences that were lacking information in the target region were excluded from calculations.
c. The number in parentheses indicates the number of mismatches between the probe and the 16S or 23S rRNA sequence of the organism that is specified (–) indicates that no sequence is available for the 23S rRNA of the organism that is specified.
d. The mean and standard error for probe hybridization to either positive control rRNA (Rp), or negative control rRNA (Rn), expressed as a ratio of universal probe (UNIV1390) hybridization. The values for each rRNA control were calculated from six pairs of membranes with the value for each pair of membranes calculated from five different spots representing a range of rRNA concentrations. The Rn values were further averaged over the four negative controls used for each probe.
Flavo, Flavobacteria; Kv, *Ketogulonogenium vulgare*; Ne, *Nitrosomonas europaea*; Cj, *Cytophaga johnsonae*; Ag, *Arthrobacter globiformis*; Vs, *Verrucomicrobium spinosum*; Pl, *Planctomyces limnophilus*; Ac, *Acidobacterium capsulatum*; Sc, *Saccharomyces cerevisiae*.

group in a community may indicate either that the group is numerically dominant or that it is growing rapidly within that community. We used radiolabelled oligonucleotide probes to determine the relative abundance of microbial group rRNA in relation to plant community composition and soil management history over a period of two years in a series of replicated fields that are part of the Long-term Experimental Research site at Michigan State University's Kellogg Biological Station (KBS-LTER). The microbial groups we choose to investigate were selected because they represent the bacteria most commonly found in soil microbial communities (Buckley and Schmidt, 2001a), though we have also examined the abundance of rRNA from Eurarya within the soil community. Each of the microbial groups we examined encompasses a considerable amount of taxonomic and functional diversity. Although this approach sacrifices information about individual microbial species, by focusing on the distribution and abundance of broad groups we have obtained an unprecedented view of soil microbial community structure. This research addresses basic questions about the structure of microbial communities, the distribution of microorganisms in the soil and the response of these microorganisms to environmental change.

Results

Overall microbial community structure

Microbial community structure was assessed by using rRNA-targeted oligonucleotide probes to determine the abundance of rRNA from eight microbial groups in relation to the total amount of rRNA present in the soil. A summary of the probes, and the results from experimental controls that were used in the calculation of rRNA relative abundance are provided in Table 1. Though there was considerable variation in the relative abundance of microbial groups in the 85 soil samples analysed, a general profile for the structure of the microbial communities was apparent in these soils (Fig. 1). The mean and standard deviation of rRNA relative abundance was determined for each microbial group across all of the samples analysed. The

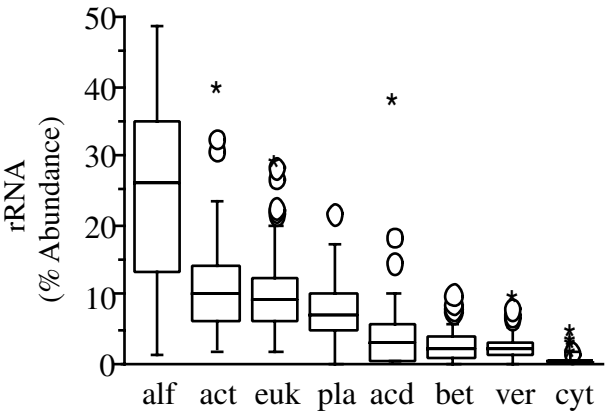

Fig. 1. Summary of values for rRNA abundance from Alpha Proteobacteria (alf), Actinobacteria (act), Eukarya (euk), Planctomycetes (pla), Acidobacteria (acd), Beta Proteobacteria (bet), Verrucomicrobia (ver) and Cytophaga-Flavobacteria (cyt) as measured in all samples analysed in this study ($n = 89$). For each set of observations the median is shown as a horizontal line whereas each box extends from the first to the third quartile of observations (IQR), whiskers represent data points within 1.5 IQR of each edge of the box, mild outliers are indicated by open circles whereas extreme outliers are indicated by asterisks.

microbial groups we examined accounted for 59% ± 23% of the total rRNA present in the soil microbial communities examined at the KBS-LTER site. The dominant microbial group observed was the Alpha Proteobacteria (24.7% ± 13.2%), followed in abundance by the Actinobacteria (11.1% ± 7.6%), the Eukarya (9.7% ± 5.7%), the Planctomycetes (7.2% ± 4.7%), the Acidobacteria (3.5% ± 5.7%), the Beta Proteobacteria (2.3% ± 1.9%), Verrucomicrobia (1.9% ± 1.9%), and the Cytophaga-Flavobacteria (0.4% ± 0.9%).

Effect of soil management practice

Microbial group rRNA abundance was measured for conventionally tilled (CT), historically cultivated successional (HCS), and never cultivated successional (NCS) fields at four different sampling times to determine whether characteristics of these fields such as plant community composition, current cultivation status, or cultivation history impact variability in the structure of soil microbial communities (Fig. 2). Characteristics of the fields are summarized in Table 2 and further described in *Experimental procedures*. Repeated multivariate analysis of variance (RMANOVA) of microbial group rRNA abundance revealed that the main effects of treatment and sampling time are significant (Table 3). There is also evidence for a significant interaction between the effects of treatment and sampling time, indicating that although there is temporal variation in microbial community structure this variation is influenced by treatment effects (Table 3). Subsequent

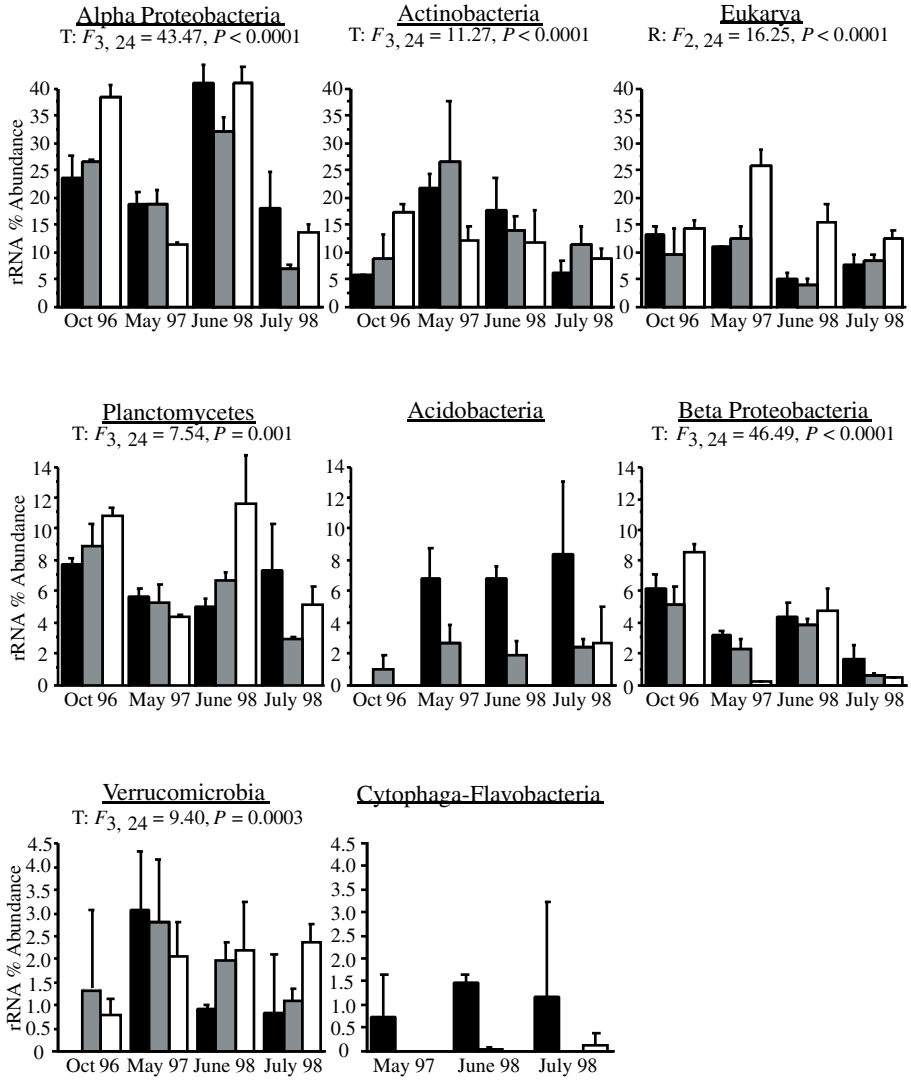

Fig. 2. Microbial group rRNA abundance in treatments CT (black bars), HCS (grey bars) and NCS (white bars) from October 1996 (October 96), May 1997, June 1998, and July 1998 (mean ± s.e., $n = 3$). F statistics are shown from ANOVA for the effects of treatment (R) and sampling time (T) where significant.

Table 2. Codes and descriptions of experimental treatments and reference communities on the KBS-LTER site.

Symbol	Description	Tillage	Management history	Plant community
CT	Cultivated field	Yes	Historically cultivated	Annual crop rotation, corn/soybean/wheat
PL	Poplar plantation	No	Historically cultivated *Populus* clones planted in 1989	Ground cover dominated by perennial grasses
HCS	Historically cultivated successional field	No	Historically cultivate dabandoned in 1989	Herbaceous perennials
HCST	Historically cultivated successional field, tilled subplot	Yes	Yearly tilled HCS subplots	Dominated by annual grasses
LS	Late successional field	No	Historically cultivated, abandoned in 1951	Herbaceous perennials
NCS	Never cultivated late successional field	No	Never cultivated	Herbaceous perennials

pairwise tests provided no evidence for differences in microbial community structure between CT and HCS while indicating that the microbial communities in both of these treatments differ significantly from the NCS fields (Table 3). Correspondance analysis was used to visually interpret the differences in overall microbial community structure that are shown to be significant in Table 3 (Fig. 3). Where Fig. 2 shows the mean relative abundance of each microbial group in each treatment, Fig. 3 reveals patterns in community structure where each point represents data from six microbial groups in a particular field at a particular time. Figure 3 reveals similarities in community structure between samples from October 1996 and June 1998, and between May 1997 and July 1998 (Fig. 3A). Closer observation reveals that the microbial communities in CT and HCS are most distinct from those in NCS in both June 1998 and May 1997, though such differences are not as apparent in October 1996 and July 1998 (Figs 2 and 3A). Data from the Acidobacteria and the Cytophaga-Flavobacteria were not used in multivariate analyses because a large proportion of zero abundance values rendered these data invalid for the tests performed.

By restricting the analysis to fields sampled in both June and July 1998 an additional tilled treatment, the tilled historically cultivated successional fields (HCST, Table 2) can be added to the analysis and the number of field replicates representing each treatment can be increased from three to five. MANOVA used to analyse these data from June and July 1998 continues to indicate that the main effects of treatment (Pillai's Trace: $F_{18,81} = 4.878$, $P < 0.0001$) and sampling time (Pillai's Trace: $F_{6,25} = 50.857$, $P < 0.0001$) are significant while also providing evidence for a significant interaction between the effects of treatment and sampling time (Pillai's Trace: $F_{18,81} = 3.526$, $P < 0.0001$). Additional pairwise tests confirm that the structures of the microbial communities in the NCS fields are significantly different from those shared in the historically cultivated fields (Hoetelling's T^2 test: $F_{6,29} = 11.88$, $P < 0.0001$).

Effects on microbial groups

Analysis of variance (ANOVA) was used to examine both treatment and sampling time effects for each microbial group individually. The main effect of sampling time was significant in five of the six microbial groups examined, whereas the treatment effect was only significant for the Eukarya (Fig. 2). This result is in contrast with the simultaneous analysis of all groups by MANOVA that showed treatment, time, and treatment–time interaction effects are all significant. Differences in eukaryal rRNA abundance

Table 3. Summary of parametric and non-parametric MANOVA examining the effects of treatment (CT, HCS, or NCS) and sampling time (October 1996, May 1997, June 1998, July 1998) on microbial community composition as measured by the rRNA abundance of the Alpha Proteobacteria, Beta Proteobacteria, Actinobacteria, Verrucomicrobia, Planctomycetes and the Eukarya.

	RMANOVA				RMANOVA by Ranks			
Effects	Pillai's Trace	d.f.	*F*-value	*P*-value	Pillai's Trace	d.f.	*L*-value	*P*-value
Treatment	1.307	12, 52	6.282	<0.0001	1.296	12	45.360	<0.001
Time	2.467	18, 81	16.202	<0.0001	2.461	18	86.135	<0.001
Treatment × Time	2.208	36, 180	2.328	0.0002	2.176	36	76.160	<0.001
Pairwise tests								
CT v HCS	0.216	6, 23	0.781	ns	0.544	6	12.512	ns
CT v NCS	0.613	6, 23	4.497	0.0066	0.827	6	19.021	<0.005
HCS v NCS	0.578	6, 23	3.880	0.0127	0.647	6	14.881	<0.025

Non-significant results ($P > 0.05$) are represented by the letters ns.

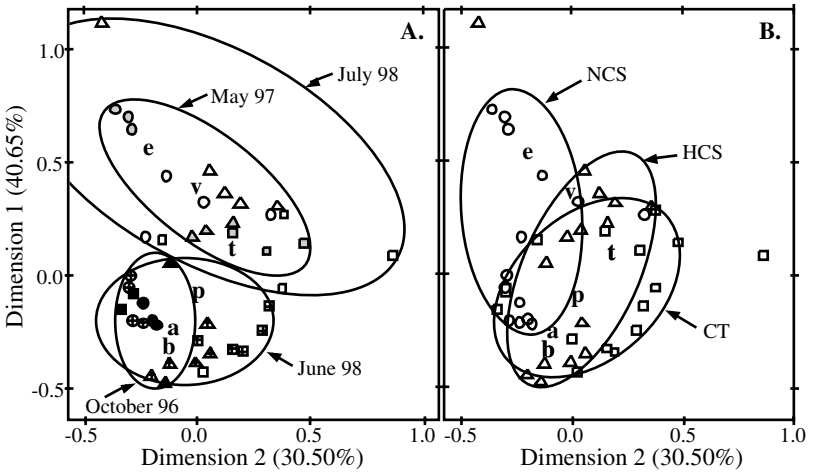

Fig. 3. Correspondence analysis of community structure in treatments CT (squares), HCS (triangles), and NCS (circles) at four sampling times. The same data is presented in both panels, ellipses and fills are used to help visualize differences in microbial community structure in relation to either sampling time (A) or treatment (B). In A symbols are filled to indicate sampling time for October 1996 (black fill), May 1997 (grey fill), June 1998 (crosses) and July 1998 (no fill). Community structure is represented by the rRNA abundance of alpha Proteobacteria (a), beta Proteobacteria (b), Actinobacteria (t), Verrucomicrobia (v), Planctomycetes (p) and the Eukarya (e).

were not solely responsible for the detection of significant treatment effects by MANOVA, as these effects were still significant when eukaryal data were excluded from analyses (Pillai's Trace: $F_{10,42} = 4.266$, $P = 0.0004$). The fact that a treatment effect was only observed for one microbial group, and that no interaction effects were observed, are likely a result of the decreased power of ANOVA relative to MANOVA. Correspondence analysis revealed that certain microbial groups have similar patterns of abundance at different sampling times (Fig. 3A). The rRNA abundance of the Eukarya, Verrucomicrobia and Actinobacteria were all maximal in May 1997 relative to the other sampling times while the Alpha Proteobacteria, the Beta Proteobacteria, and the Planctomycetes were maximal in abundance on October 1996 and June 1998 (Fig. 2, and Fig. 3).

Supplementary observations from July 1998

In July 1998 microbial community structure as defined by rRNA abundance was assessed for five field replicates from each of the following treatments: CT, HCS, HCST, NCS, poplar plantation (PL) and late successional (LS) (Fig. 4). The PL fields contain a ground cover that consists of perennial grasses, which are also abundant in the NCS fields (K.L. Gross, personal communication), and so if there were similarities in microbial community structure between PL and NCS it would point to the effects of plant communities on microbial community structure in the soil. The LS field, abandoned from cultivation prior to 1951, was sampled to investigate the length of time that is required for microbial communities in historically cultivated fields to resemble those in fields that have not been cultivated. The MANOVA for microbial group rRNA abundance in July 1998 provides strong evidence for a significant difference in community structure due to treatment (Table 4). Individual ANOVA reveal significant treatment effects for six of the seven microbial groups analysed (Fig. 4). Pairwise tests indicate that the microbial communities in the historically cultivated treatments (CT, PL, HCS, HCST) differed significantly from those in both the NCS and LS treatments (Fig. 2). Differences in community structure between the microbial communities in the NCS and LS treatments were not significant (Table 4) indicating that the structure of soil microbial communities can return to pre-agricultural conditions but that this process can take decades.

Depth distribution of microbial groups

We examined the effect of soil depth on microbial community composition by comparing the microbial communities in surface surface (0–5 cm) and deeper (0–10 cm) soil cores sampled from treatments CT, HCS, HCST and NCS in June 1998. We observed that microbial community structure differs significantly with soil depth (Pillai's Trace: $F_{7,23} = 8.647$, $P < 0.0001$). There was no evidence for any interaction between field treatment and depth on microbial community structure, indicating that neither tillage nor the historical effects of cultivation influenced differences seen in community structure between 5 cm and 10 cm cores. Calculating the difference in rRNA abundance between pairs of soil cores from the two different depths reveals the effect of soil depth on the relative abundance of individual microbial groups (Fig. 5). The rRNA abundance of three microbial groups differed significantly with depth: rRNA from the Alpha Proteobacteria ($F_{1,36} = 5.197$, $P = 0.0288$) and Beta Proteobacteria ($F_{1,36} = 16.765$, $P = 0.0002$) was, respectively, 18% and 61% less abundant in surface cores than in deeper cores, whereas rRNA from Verrucomicrobia was 35% more abundant in surface cores than in deeper cores ($F_{1,36} = 16.897$, $P = 0.0002$).

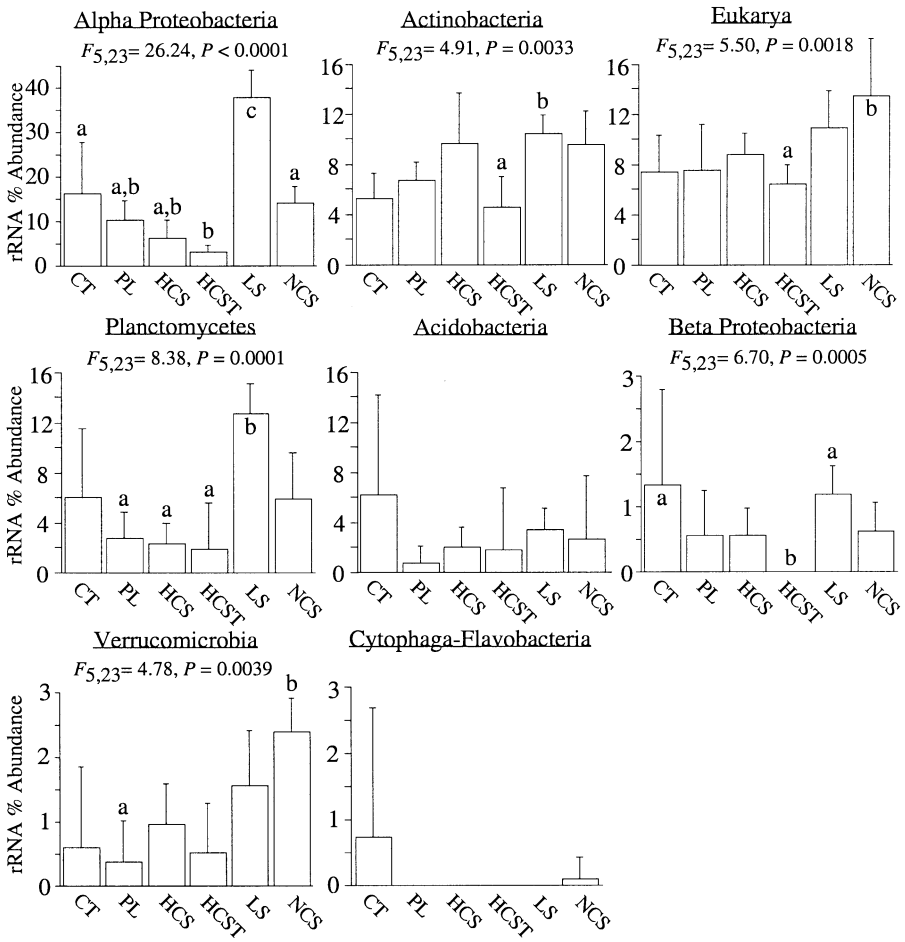

Fig. 4. Microbial rRNA abundance (mean ± s.e., $n = 5$) in all treatments sampled in July 1998. F statistics are shown, where significant after Bonferroni correction, for treatment differences in microbial rRNA abundance. Values that were found to be significantly different ($P < 0.05$) by the Scheffe test are indicated by different letters.

Microbial community variability

Variability in microbial community structure as a result of treatment and sampling time was examined by determining the coefficient of variation (CV) for the rRNA abundance of each microbial group surveyed across the field replicates for each treatment. Microbial community variability was influenced by the main effects of treatment ($F_{2,54} = 6.91$, $P = 0.0059$), sampling time ($F_{3,54} = 3.08$, $P = 0.0348$), and by the interaction of these effects ($F_{6,54} = 4.33$, $P = 0.0012$). Interestingly, variability in community structure was highest among HCS fields (20.34 ± 2.48) and lower in the CT (14.44 ± 2.03) and NCS fields (10.87 ± 1.78). Microbial community variability

Table 4. Results of both parametric and non-parametric one-way MANOVA used to examine treatment effects on microbial community composition in July 1998 samples (CT, PL, HCS, HCST, LS, NCS).

	MANOVA				MANOVA by Ranks			
Effects	Pillai's Trace	d.f.	*F*-value	*P*-value	Pillai's Trace	d.f.	*L*-value	*P*-value
All treatments	2.538	35, 140	3.091	<0.0001	2.286	35	64.008	<0.001
Pairwise tests								
HC v NCS	0.805	7, 23	9.432	<0.0001	0.716	7	16.468	<0.025
HC v LS	0.873	7, 24	16.743	<0.0001	0.707	7	16.968	<0.020
NCS v LS	0.985	7, 8	9.249	ns	0.993	7	8.937	ns

HC includes all of the historically cultivated fields on the main experimental site (CT, PL, HCS, HCST). Non-significant results ($P > 0.05$) are represented by the letters ns.

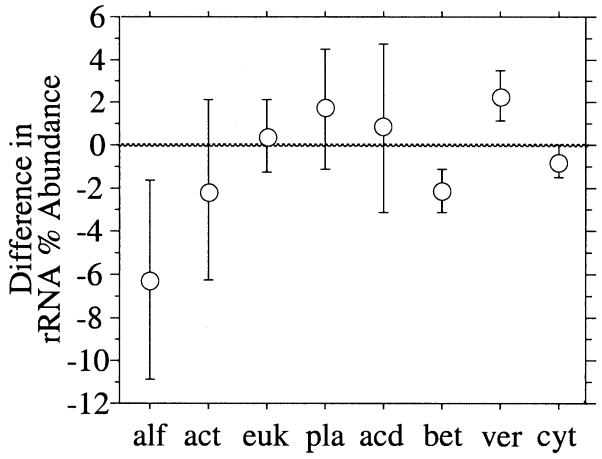

Fig. 5. Mean differences in rRNA abundance between surface (0–5 cm) and deeper (0–10 cm) soil cores are shown with 95% confidence intervals. Values are estimated from all fields sampled in June 1998 ($n = 19$). Microbial groups depicted are the Alpha Proteobacteria (alf), Actinobacteria (act), Eukarya (euk), Planctomycetes (pla), Acidobacteria (acd), Beta Proteobacteria (bet), Verrucomicrobia (ver) and Cytophaga-Flavobacteria (cyt). Positive values indicate that rRNA relative abundance values in surface cores exceeds those found in deeper cores.

due to sampling time was highest in July 1998 (20.44 ± 2.52) relative to October 1996 (14.28 ± 3.63), May 1997 ($11.58\% \pm 1.72\%$), and June 1998 (14.58 ± 1.62). The high variability for July 1998 samples relative to other sampling times is evident in Fig. 3A by the wide distribution of points representing these samples compared to the smaller distributions seen for the other sampling times.

Discussion

Our analyses show that microbial community composition did not differ significantly between conventionally managed agricultural fields (CT) and fields that had been abandoned from cultivation for nine years (HCS) (Table 3). In contrast, the microbial communities in both of these treatments differed significantly from those in nearby fields that had never been cultivated (NCS) (Table 3). Only successional fields that had been abandoned from cultivation for > 45 years (LS) had microbial communities that were similar in structure to those found in the NCS fields (Table 4). These results provide further support for previous observations indicating that the long-term effects of cultivation influence community structure in the soil at the KBS-LTER site (Klug and Tiedje, 1993; Buckley and Schmidt, 2001b). Cavigelli and Robertson (2000) have shown that there are differences between CT and NCS fields in denitrifier community composition, whereas Bruns *et al.* (1999) have shown that the composition of autotrophic ammonia oxidizer communities also differ between these fields. Furthermore, rates of methane consumption by microbes in the NCS fields are much greater than methane consumption in either CT or HCS fields (Robertson *et al.*, 2000). These observations are consistent with the idea that soil microorganisms are influenced by historical soil characteristics that are retained long after changes in land management.

Although microbial community composition did not differ between the CT and HCS fields, there is some evidence that after nearly 10 years community-level differences may be emerging among the historically cultivated fields. For example, microbial community variability as assessed by the mean CV of microbial rRNA abundance is significantly higher in the HCS fields than in either the CT or NCS fields. Such variability could result from successional processes occurring as HCS recovers from the effects of agriculture and may be related to changes in plant community composition (Gross *et al.*, 1995). There is growing evidence that plants influence microbial community structure in soil immediately adjacent to plant roots (Duineveld *et al.*, 1998; Ibekwe and Kennedy, 1998; Ohtonen *et al.*, 1999; Yang and Crowley, 2000), but there is still conflicting evidence as to whether plant communities influence microbial distribution across individual fields (Cavigelli *et al.*, 1995; Felske and Akkermans, 1998; Pennanen *et al.*, 1999; Broughton and Gross, 2000).

This study does not provide convincing evidence that plant community composition is influencing soil microbial community structure at the KBS-LTER site. The observation that there is not any significant variability in microbial community structure between the historically cultivated fields (CT, HCS, HCST, PL) is consistent with previous reports that microbial community composition is largely homogeneous across continuous landscapes (Felske and Akkermans, 1998). There are several possible explanations for why a relationship between plant community composition and microbial community composition may not have been observed even if such a relationship actually exists. First, it is possible that the effect of plant community composition on microbial communities in these fields is masked by the overwhelming influence of past agricultural practices that cause changes in soil structure and depletion of soil carbon and nitrogen levels (Drinkwater *et al.*, 1998). Second, microbial community structure in the historically cultivated treatments could differ as a result of plant community composition at spatial scales below the resolution of the analyses presented in this study. A final possibility is that differences in microbial community structure that occur as a result of plant community composition occur at taxonomic levels that cannot be resolved by determining the abundance of entire microbial groups. For example, genetic variability within specific bacterial populations has been observed to coincide with differences in plant community composition (Fulthorpe

et al., 1998; Minamisawa *et al*., 1999; Achouak *et al*., 2000), these differences in population structure are probably due to non-specific changes in local soil characteristics caused by the long-term deposition of plant materials (Gross *et al*., 1995; Wardle *et al*., 1999; Degens *et al*., 2000).

Temporal variability in the composition of soil microbial communities may complicate the interpretation of spatial patterns of microbial abundance in relation to the field characteristics. Studies of whole-community phospholipid fatty acid profiles have previously shown that physiological changes can occur in soil microbial communities in response to seasonal cues (Bossio *et al*., 1998; Bardgett *et al*., 1999). Our results show that soil microbial community structure exhibits significant temporal variability in the treatments examined (Fig. 2 and Table 3). Indeed, microbial community structure changed considerably even during the seven weeks separating the sampling times in June and July 1998 (Figs 2 and 3A). Changes in rRNA abundance are a function of population density and growth rate and so the observed temporal variability in community rRNA abundance reflects alterations in the activity of different microbial populations in the soil. These changes in activity could be associated with environmental variables such as soil moisture and temperature that vary on similar time scales. It is apparent from our data that microbial community composition in the soil can change dramatically at temporal scales relevant to seasonal or perhaps even meteorological events.

Correspondence analysis revealed that differences in community structure due to sampling time are largely driven by changes in the abundance of two sets of microbial groups (Fig. 3). The Eukarya, Verrucomicrobia and Actinobacteria each achieve their highest rRNA relative abundance in May 1997 with lower abundance values seen in the other sampling times (Figs 2 and 3A). In contrast, the Alpha Proteobacteria, Beta Proteobacteria and the Planctomycetes all have peaks of abundance in both October 1996 and June 1998 with lower abundance values seen in May 1997 and July 1998 (Figs 2 and 3A). It is possible that these microbial groups may be responding in concert to environmental characteristics that are temporally variable, though we could not identify specific environmental parameters that influence the abundance of individual microbial groups.

To determine whether microbial groups are homogeneously distributed with depth in the soil, we examined community structure in both 0–5 cm and 0–10 cm deep soil cores. We observed significant differences in microbial community structure due to depth. Both the Alpha and Beta Proteobacteria were significantly more abundant in deeper soil cores, while the Verrucomicrobia were significantly more abundant in surface soil (Fig. 5). Soil depth has previously been shown to influence the community composition of nitrogen fixing bacteria in the soil (Nalin *et al*., 1997; Shaffer *et al*., 2000). It is interesting that microbial community structure varied with depth even in soils exposed to the homogenizing effects of tillage. Soil parameters such as total organic carbon, total nitrogen, and soil moisture have been observed to decrease with depth in agricultural fields with no significant change due to increases in tillage intensity (van Gestel *et al*., 1992). It is likely that variation in microbial group abundance with depth results as the organisms respond to soil characteristics that also vary with depth.

A possible explanation of the observation that microbial communities in fields abandoned from cultivation for 10 years continue to resemble those in currently cultivated fields is that soil microbial communities respond to soil characteristics that require long periods of time to recover from disturbance. Long-term continuous agricultural management can cause soil carbon and nitrogen pools to be depleted by as much as 89% and 75% respectively (Knops and Tilman, 2000). The distribution of soil nutrients in post-agricultural fields can require decades to recover from the homogenizing effects of tillage (Robertson *et al*., 1988; 1993), and the recovery of soil carbon and nitrogen pools to pre-agricultural levels may require decades or even centuries (Drinkwater *et al*., 1998; Knops and Tilman, 2000). Consistent with these observations the soil organic carbon or total nitrogen content did not differ significantly among the historically cultivated fields (CT, PL, HCS, HCST) at the KBS-LTER site as of 1994 (Paul *et al*., 1999). In contrast, both soil organic carbon and total nitrogen content were significantly lower in the HCS fields than in the NCS fields (Paul *et al*., 1999; Cavigelli and Robertson, 2000). Relationships have been observed between microbial respiration and nitrogen content in the soil (Abril and Bucher, 1999), and between carbon availability and microbial biomass in the soil (Zak *et al*., 1994). We hypothesize that changes in the composition of microbial communities are strongly influenced by soil characteristics such as soil carbon and nitrogen content that are slow to recover from the influence of cultivation. This hypothesis is supported by recent observations that soils with similar carbon and nitrogen contents have similar microbial communities as determined by both PLFA profiles (Wardle *et al*., 1999; Zak *et al*., 2000) and catabolic diversity (Degens *et al*., 2000). These data suggest that long-term management practices are more likely to influence microbial community composition than are contemporary land-use or plant community composition.

This research shows that soil microbial communities are heterogeneous entities with distinct components that are each capable of responding differently to environmental characteristics. Microbial community composition was shown to change with depth in the soil and with sampling time. Temporal changes in microbial community composi-

tion were observed to occur at scales that are relevant to seasonal events. In addition, it was demonstrated that cultivation has a significant impact on the composition of soil microbial communities and that the effects of cultivation on these communities are long lasting. As processes mediated by microorganisms in the soil are affected by the taxonomic composition of soil microbial communities (Schimel, 1995; Cavigelli and Robertson, 2000; Holtan-Hartwig *et al*., 2000), determining the impact that microbial community dynamics have on terrestrial ecosystems will require further examination of microbial community composition in relation to soil characteristics and soil processes. By observing the distribution of microbial groups in relation to environmental stimuli we can begin to generate and test hypotheses regarding the rules which govern the organization and distribution of microorganisms in terrestrial ecosystems.

Experimental procedures

Site description and soil sampling

Soil samples were taken from the Long-term Ecological Research (LTER) site located at the Michigan State University W. K. Kellogg Biological Station (KBS) in Hickory Corners, Michigan. The KBS-LTER site, established in 1989 to study ecological processes in agro-ecosystems, includes a large-scale replicated field experiment with seven treatments representing different cropping systems and types of management, as well as several nearby successional sites (for detailed agronomic protocols see http://lter.kbs.msu.edu). The main field experiment is located on 48 hectares of land that had been uniformly farmed for over 50 years prior to establishment of the LTER site (Robertson *et al*., 1997). Soils are typic hapludalfs, sandy to silty clay loam and are of moderate fertility (Robertson *et al*., 1997). The site has a mean annual air temperature of 9.4°C and a mean annual rainfall of 860 mm (Cavigelli and Robertson, 2000). Three to five fields were sampled to represent each treatment. Fields were sampled by taking a single soil core (2.5 cm diameter, 10 cm depth) from each of five permanent sampling locations distributed across each field replicate. The soil cores from each field were pooled, sieved (4 mm mesh), frozen in liquid nitrogen (generally within 10 min of sampling) and stored at −80°C.

Soil samples were obtained at four times over a period of two years. On 3 October 1996 and 23 May 1997 soil was sampled from three field replicates of two of the historically cultivated treatments [the Cultivated Tilled (CT) and Historically Cultivated Sucessional (HCS) fields] and from a site that had never been cultivated, that was adjacent to the LTER experimental site [the Never Cultivated Successional (NCS) fields] (Table 2). At the time of sampling, in October 1996, corn was present on CT fields. On 6 June 1998 soil was sampled from five field replicates of the CT, HCS and NCS fields and from tilled subplots of the HCS fields (HCST). In addition, June 1998 sampling included both 0–5 cm deep soil cores and 0–10 cm deep soil cores to assess potential differences in microbial community structure due to soil depth. Wheat had been planted on CT fields at the end of 1997 and was present on CT fields in June and July. On 28 July 1998, five field replicates were sampled from all of the treatments mentioned above (CT, HCS, HCST and NCS) from a treatment consisting of poplar plantations grown on historically cultivated fields (PL) and from a treatment consisting of late successional fields (LS) that had been historically cultivated prior to abandonment in 1951 (Table 2). The successional fields sampled (HCS, NCS, LS) were all dominated by herbaceous forbs (Burbank *et al*., 1992; Gross *et al*., 1995; Huberty *et al*., 1998). Following abandonment from cultivation in 1989 the plant communities in the historically cultivated successional fields (HCS) had progressed from initial dominance by annual species to be dominated by herbaceous perennial forbs (Huberty *et al*., 1998). Further information about the chemical and physical characteristics of the soils sampled can be found in the online data catalogue of the KBS-LTER site (http://lter.kbs.msu.edu).

Sample processing

RNA extraction. The RNA for use in hybridization experiments was extracted as previously described (Buckley *et al*., 1998; Buckley and Schmidt, 2001b). In brief, a 10 g portion of each frozen soil sample was suspended in a buffer suitable for sample homogenization and RNA stabilization. Microbial cells were lysed using beadmill homogenization with 10 g of 0.1 mm silica/zirconia beads in a 32 ml chamber for a duration of two minutes (Beadbeater, Biospec Products). The RNA from homogenized samples was concentrated by precipitation with polyethelene glycol and then purified using both hydroxyapatite and Sephadex G-75 columns. The RNA samples were finally precipitated, resuspended in 200 μl of Rnase-free ddH_2O, and stored at −20°C.

RNA hybridization. Quantitative filter hybridization was performed as previously described (Stahl *et al*., 1988; Buckley and Schmidt, 2001b). Nucleic acids from soil samples and standards were denatured with 0.5% glutaraldehyde-50 mM Na_2HPO_4, serially diluted to provide a range of sample concentrations, blotted onto nylon membranes using a 96-well dot blot manifold and immobilized by UV crosslinking. The RNA isolated from pure cultures [Ketogulonogenium vulgare DSM 4025, Nitrosomonas europaea ATCC 25978, Cytophaga johnsonae ATCC 17061, Arthrobacter globiformis ATCC 8010, Verrucomicrobium spinosum ATCC 43997, Planctomyces limnophilus ATCC 43296, Acidobacterium capsulatum ATCC 51196 and Saccharomyces cerevisiae American Ale Yeast 1056 (Wyeast Laboratories, INCS)] were included on all membranes as standards to control for differences in the specific activity of labelled probes and to account for the possibility of non-specific probe binding. Hybridization protocols for [^{32}P]-5′-labelled oligonucleotide probes were previously described in detail (Stahl *et al*., 1988). Radiolabelled oligonucleotide probes that bind to the rRNA molecules from specific microbial groups were then used to determine the relative abundance of microbial group rRNA. Oligonucleotide

probes specific for bacteria from the alpha subclass of the Proteobacteria (Alf1b, 5′-CGTTCGYTCTGAGCCAG-3′), the beta subclass of the Proteobacteria (Bet42a, 5′-GCCT TCCCACTTCGTTT-3′), the Actinobacteria (HGC69a, 5′-TATAGTTACCACCGCCGT-3′), the Planctomycetes (Pla46R, 5′-GACTTGCATGCCTAATCC-3′), the Verrucomicrobia (Ver47, 5′-GACTTGCATGTCTTAWC-3′), and the Cytophaga-Flavobacterium cluster of the Cytophagales (CF319a, 5′-TGGTCCGTGTCTCAGTAC-3′) have all been previously described (Amann *et al*., 1995; Neef *et al*., 1998; Buckley and Schmidt, 2001c). The probe specific for the Acidobacteria (Acd31, 5′-GATTCTGAGCCAGGATC-3′) was designed (modified from Barns *et al*., 1999) and verified empirically as specifically recognizing acidobacterial 16S rRNA under the hybridization conditions indicated below. In addition, the probe specific for all of the Eukarya (Euk1195, 5′-GGGCAT CACAGACCTG-3′), and the probe universal to all 16S rRNA (Univ1390, 5′-GACGGGCGGTGTGTACAA-3′) have also been described previously (Amann *et al*., 1995). Hybridization between labelled probes and RNA immobilized on filters proceeded at 45°C for at least 12 h. Following probe hybridization, filters were washed for 30 min at 45°C and then washed for an additional 30 min at a higher temperature to provide stringency (45°C for Univ1390, Euk1195, and Ver47; 50°C for HGC69a; 53°C for Acd31; 55°C for Alf1b and CF319a; and 62°C for Bet42a). The specifically bound probe that remained on the membrane was visualized using a phosphorimaging system (Storm 860, Molecular Dynamics), signal intensity was quantified using Image Quant software v 5.0 (Molecular Dynamics).

Determination of rRNA abundance

Within a soil sample, the relative abundance of rRNA derived from a specific group was measured as the ratio of the signal derived from a group-specific probe to the signal derived from the universal probe. This approach for determining microbial rRNA abundance has been used previously to describe aspects of microbial community structure (Stahl *et al*., 1988). Relating specific probe binding to universal probe binding controls for variability in the total amount of RNA recovered from each soil sample, and also controls for the presence of hybridization inhibitors that may co-purify with RNA from soil. Positive controls were included on each membrane to correct for variations in the labelling efficiency of different oligonucleotide probes while negative controls were used to correct for the possibility of non-specific probe binding. Every RNA sample was represented by five aliquots in a dilution series to examine potential differences in signal intensity due to inhibition or membrane saturation. The ratio of signal intensities obtained for specific and universal probe binding to an RNA sample was defined as $R = \Sigma^{n}_{i=1}[G_i(U_i)^{-1}]n^{-1}$, where G_i and U_i represent, respectively, the corresponding signal intensities obtained for group specific and universal probe binding to each aliquot representing the sample, and n equals the total number of aliquots representing the RNA sample. The value R was calculated for each soil RNA sample (R_S), and a mean value of R was determined for all positive (R_P) and negative (R_N) controls present on each membrane. The relative abundance of rRNA from a specific microbial group was then defined as $(R_S - R_N)(R_P - R_N)^{-1} \times 100$.

Data analysis

Per cent rRNA abundance data was arcsine transformed before statistical analyses to compensate for relationships observed between the mean and variance of samples. After transformation certain data suffered from both departures from normalty and heteroscedasticity, as a result all parametric tests were verified through comparable non-parametric tests. Except where explicitly stated to the contrary, all conclusions drawn from parametric statistical tests were supported by non-parametric analyses as well. In addition, abundance values for the Cytophaga-Flavobacteria group and the Acidobacteria frequently fell below detection limits (approximately 0.5% rRNA abundance). A large number of zero values may bias statistical tests, so where noted these groups have been omitted from community-level analyses. Statistical tests were performed using STATVIEW v 5.0 (SAS Institute), and SAS v 7.0 (SAS Institute).

Repeated measure MANOVA (RMANOVA) was used to examine the main effects of treatment and sampling time, and their interaction for all microbial groups simultaneously in treatments CT, HCS, and NCS over all four sampling dates (with three field replicates for each treatment at each time). Significant MANOVA results were investigated by using Hoetelling's T^2 test to perform pairwise comparisons, whereas ANOVA was used to examine the effects within each individual microbial group. Non-parametric ANOVA and MANOVA were calculated using a generalized rank-order method (Thomas *et al*., 1999). Correspondence analysis was used to graphically represent relationships between the fields sampled at different times and the microbial groups from those fields. In addition, one way MANOVA and ANOVA were used to examine treatment effects for all fields sampled in July 1998 (with five field replicates representing each treatment from July 1998).

To determine if soil depth has an effect on microbial community structure in samples from June 1998, community structure in 0–5 cm cores was compared to community structure in 0–10 cm cores using Hoetelling's T^2 test. The effect of the treatments CT, HCS, HCST and NCS on changes in community structure with depth were investigated by subtracting microbial group abundance in 5 cm cores from that in 10 cm cores from the same field replicate. Depth differences due to treatment were examined using one way MANOVA and ANOVA.

In addition to multivariate analyses of community structure, analyses were made of community variability in the treatments sampled. Variability in community structure was measured by first determining the coefficient of variation (CV) of rRNA relative abundance for each microbial group in each treatment. Variability in overall community structure in each treatment was then assessed as the mean of the CVs of relative abundance for all of the microbial groups examined in each treatment. ANOVA was used to examine treatment effects on microbial community variability.

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References

Abril, A., and Bucher, E.H. (1999) The effects of overgrazing on soil microbial community and fertility in the Chaco dry savannas of Argentina. *Appl Soil Ecol* **12:** 159–167.

Achouak, W., Thiery, J.M., Roubaud, P., and Heulin, T. (2000) Impact of crop management on intraspecific diversity of *Pseudomonas corrugata* in bulk soil. *FEMS Microbial Ecol* **31:** 11–19.

Amann, R.I., Ludwig, W., and Schleifer, K.H. (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol Rev* **59:** 143–169.

Bardgett, R.D., Lovell, R.D., Hobbs, P.J., and Jarvis, S.C. (1999) Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. *Soil Biol Biochem* **31:** 1021–1030.

Barns, S.M., Takala, S.L., and Kuske, C.R. (1999) Wide distribution of the bacterial Kingdom Acidobacteria in the environment. *Appl Environ Microbiol* **65:** 1731–1737.

Bossio, D.A., Scow, K.M., Gunapala, N., and Graham, K.J. (1998) Determinants of soil microbial communities: Effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. *Microbial Ecol* **36:** 1–12.

Broughton, L.C., and Gross, K.L. (2000) Patterns of diversity in plant and soil microbial communities along a productivity gradient in a Michigan old-field. *Oecologia* **125:** 420–427.

Bruns, M.A., Stephen, J.R., Kowalchuck, G.A., Prosser, J.I., and Paul, E.A. (1999) Comparitive diversity of ammonia oxidizer 16S rRNA gene sequences in native, tilled, and successional soils. *Appl Environ Microbiol* **65:** 2994–3000.

Buckley, D.H., and Schmidt, T.M. (2001a) Exploring the diversity of soil – a microbial rainforest. In *Biodiversity of Microbial Life: Foundation of Earth's Biosphere*. Staley, J.T., and Reysenbach, A.L. (eds). New York: Wiley-Liss, pp. 183–208.

Buckley, D.H., and Schmidt, T.M. (2001b) The structure of microbial communities in soil and the lasting impact of cultivation. *Microbial Ecol* **42:** 11–21.

Buckley, D.H., and Schmidt, T.M. (2001c) Environmental factors influencing the distribution of rRNA from Verrucomicrobia in soil. *FEMS Microbiol Ecol* **35:** 105–112.

Buckley, D.H., Graber, J.R., and Schmidt, T.M. (1998) Phylogenetic analysis of nonthermophilic members of the kingdom *Crenarchaeota* and their diversity and abundance in soils. *Appl Environ Microbiol* **64:** 4333–4339.

Burbank, D.H., Pregitzer, K.S., and Gross, K.L. (1992) Vegetation of the W. K. Kellogg Biological Station, Kalamazoo County, Michigan. Research Report 510. Agricultural Experiment Station, East Lansing: Michigan State University.

Cavigelli, M.A., and Robertson, G.P. (2000) The functional significance of denitrifier community composition in a terrestrial ecosystem. *Ecology* **81:** 1402–1414.

Cavigelli, M.A., Robertson, G.P., and Klug, M.J. (1995) Fatty Acid Methyl Ester (F.A.M.E.) profiles as measures of soil microbial community structure. InThe significance and regulation of soil biodiversity. Collins, H.P., Robertson, G.P., and Klug, M.J. (eds). Boston: Kluwer Academic Publishers, pp. 99–113.

Conrad, R. (1996) Soil microorganisms as controllers of atmospheric trace gasses. *Microbiol Rev* **60:** 609–640.

Copley, J. (2000) Ecology goes underground. *Nature* **406:** 452–454.

Degens, B.P., Schipper, L.A., Sparling, G.P., and Vojvodic-Vukovic, M. (2000) Decreases in organic C reserves in soils can reduce the catabolic diversity of soil microbial communities. *Soil Biol Biochem* **32:** 189–196.

Drinkwater, L.E., Wagoner, P., and Sarrantonio, M. (1998) Legume-based cropping systems have reduced carbon and nitrogen losses. *Nature* **396:** 262–265.

Duineveld, B.M., Rosado, A.S., van Elsas, J.D., and van Veen, J.A. (1998) Analysis of the dynamics of bacterial communities in the rhizosphere of the Chrysanthemum via denaturing gradient gel electrophoresis and substrate utilization patterns. *Appl Environ Microbiol* **64:** 4950–4957.

Felske, A., and Akkermans, A.D.L. (1998) Spatial homogeneity of abundant bacterial 16S rRNA molecules in grassland soils. *Microbial Ecol* **36:** 31–36.

Fulthorpe, R.R., Rhodes, A.N., and Tiedje, J.M. (1998) High levels of endemicity of 3-chlorobenzoate-degrading soil bacteria. *Appl Environ Microbiol* **64:** 1620–1627.

van Gestel, M., Ladd, J.N., and Amato, M. (1992) Microbial biomass responses to seasonal change and imposed drying regimes at increasing depths of undisturbed topsoil profiles. *Soil Biol Biochem* **24:** 103–111.

Gross, K.L., Pregitzer, K.S., and Burton, A.J. (1995) Spatial variation in nitrogen availability in three successional plant communities. *J Ecol* **83:** 357–367.

Holtan-Hartwig, L., Dorsch, P., and Bakken, L.R. (2000) Comparison of denitrifying communities in organic soils: kinetics of NO_3^- and N_2O reduction. *Soil Biol Biochem* **32:** 833–843.

Huberty, L.E., Gross, K.L., and Miller, C.J. (1998) Effects of nitrogen addition on successional dynamics and species diversity in Michigan old-fields. *J Ecol* **86:** 794–803.

Hugenholtz, P., Goebel, B.M., and Pace, N.R. (1998) Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *J Bacteriol* **180:** 4765–4774.

Ibekwe, A.M., and Kennedy, A.C. (1998) Fatty acid methyl ester (FAME) profiles as a tool to investigate community structure of two agricultural soils. *Plant Soil* **206:** 151–161.

Jinks-Robertson, S., and Nomura, M. (1987) Ribosomes and tRNA. In *Escherichia Coli and Salmonella Typhimurium*. Neidhardt, F.C. (ed.). Washington, D.C: American Society for Microbiology, pp. 1358–1385.

Klug, M.J., and Tiedje, J.M. (1993) Response of microbial communities to changing environmental conditions: chemical and physiological approaches. In *Trends in Microbial Ecology*. Guerrero, R., and Pedros-Alio, C. (eds). Barcelona: Spanish Society for Microbiology, pp. 371–374.

Knops, J.M.H., and Tilman, D. (2000) Dynamics of soil nitro-

gen and carbon accumulation for 61 years after agricultural abandonment. *Ecology* **81:** 88–98.

Maidak, B.L., Cole, J.R., Lilburn, T.G., Parker, C.T., Saxman, P.R., Farris, R.J., *et al.* (2001) The RDP-II (Ribosomal Database Project). *Nucleic Acids Res* **29:** 173–174.

Mikola, J., and Setala, H. (1998) No evidence of trophic cascades in an experimental microbial-based soil food web. *Ecology* **79:** 153–164.

Minamisawa, K., Nakatsuka, Y., and Isawa, T. (1999) Diversity and field site variation of indigenous populations of soybean bradyrhizobia in Japan by fingerprints with repeated sequences RSα and RSβ. *FEMS Microbiol Ecol* **29:** 171–178.

Nalin, R., Normand, P., and Domenach, A. (1997) Distribution and N_2-fixing activity of *Frankia* strains in relation to soil depth. *Physiologia Plantarum* **99:** 732–738.

Neef, A., Amann, R., Schlesner, H., and Schleifer, K.H. (1998) Monitoring a widespread bacterial group: *in situ* detection of Planctomycetes with 16S rRNA-targeted probes. *Microbiology-UK* **144:** 3257–3266.

Neidhardt, F.C., and Magasanik, B. (1960) Studies on the role of ribonucleic acid in the growth of bacteria. *Biochimica Biophysica Acta* **42:** 99–116.

Ohtonen, R., Fritze, H., Pennanen, T., Jumpponen, A., and Trapp, J.E. (1999) Ecosystem properties and microbial community changes in primary succession on a glacier forefront. *Oecologia* **119:** 239–246.

Paul, E.A., Harris, D., Collins, H.P., Schulthess, U., and Robertson, G.P. (1999) Evolution of CO_2 and soil carbon dynamics in biologically managed, row-crop agroecosystems. *Appl Soil Ecol* **11:** 53–65.

Pennanen, T., Liski, J., Baath, E., Kitunen, V., Uotila, J., Westman, C.J., and Fritze, H. (1999) Structure of the microbial communities in coniferous forest soils in relation to the site fertility and strand development stage. *Microbial Ecol* **38:** 168–179.

Robertson, G.P., Huston, M.A., Evans, F.C., and Tiedje, J.M. (1988) Spatial variability in a successional plant community: patterns of nitrogen availability. *Ecology* **69:** 1517–1524.

Robertson, G.P., Crum, J.R., and Ellis, B.G. (1993) The spatial variability of soil resources following long-term disturbance. *Oecologia* **96:** 451–456.

Robertson, G.P., Klingensmith, K.M., Klug, M.J., Paul, E.A., Crum, J.R., and Ellis, B.G. (1997) Soil resources, microbial activity, and primary production across an agricultural ecosystem. *Ecol Applications* **7:** 158–170.

Robertson, G.P., Paul, E.A., and Harwood, R.R. (2000) Greenhouse gases in intensive agriculture: contributions of individual gases to the radiative forcing of the atmosphere. *Science* **289:** 1922–1925.

Schimel, J. (1995) Ecosystem consequences of microbial diversity and community structure. In *Artic and Alpine Diversity: Patterns, Causes, and Ecosystem Consequences.* Chapin, F. S., and Korner, C. (eds). New York: Springer, pp. 241–254.

Shaffer, B.T., Widmer, F., Porteous, L.A., and Seidler, R.J. (2000) Temporal and spatial distribution of the *nifH* gene of N_2 fixing bacteria in forests and clearcuts in western Oregon. *Microbial Ecol* **39:** 12–21.

Stahl, D.A., Flesher, B.H., Mansfield, R., and Montgomery, L. (1988) Use of phylogenetically based probes for studies of ruminal microbial ecology. *Appl Environ Microbiol* **54:** 1079–1084.

Thomas, J.R., Nelson, J.K., and Thomas, K.T. (1999) A generalized rank-order method for nonparametric analysis of data from exercise science: a tutorial. *Res Q for Exercise Sport* **70:** 11–23.

Torsvik, V., Goksoyr, J., and Daae, F.L. (1990) High diversity in DNA of soil bacteria. *Appl Environ Microbiol* **56:** 782–787.

Ward, D.M., Bateson, M.M., Weller, R., and Ruff-Roberts, A.L. (1992) Ribosomal RNA analysis of microorganisms as they occur in nature. *Adv Microbial Ecol* **12:** 219–268.

Wardle, D.A., Bonner, K.I., Barker, G.M., Yeates, G.W., Nicholson, K.S., Bardgett, R.D., *et al.* (1999) Plant removals in perennial grassland: vegetation dynamics, decomposer, soil biodiversity, and ecosystem properties. *Ecol Monographs* **69:** 535–568.

Whitman, W.B., Coleman, D.C., and Wiebe, W.J. (1998) Prokaryotes: The unseen majority. *Proc Natl Acad Sci USA* **95:** 6578–6583.

Yang, C., and Crowley, D.E. (2000) Rhizosphere microbial community structure in relation to root location and plant iron nutritional status. *Appl Environ Microbiol* **66:** 345–351.

Zak, D.R., Tilman, D., Parmenter, R.P., Rice, C.W., Fisher, F.M., Vose, J., *et al.* (1994) Plant production and soil microorganisms in late-successional ecosystems: a continental-scale study. *Ecology* **75:** 2333–2347.

Zak, D.R., Pregitzer, K.S., Curtis, P.S., and Holmes, W.E. (2000) Atmospheric CO_2 and the composition and function of soil microbial communities. *Ecol Applications* **10:** 47–59.