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The Structure of Microbial Communities in Soil and the Lasting Impact of Cultivation

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ABSTRACT

The structure of microbial communities was examined as a function of community composition and the relative abundance of specific microbial groups to examine the effects that plant community composition and land-use history have on microbial communities in the soil. The sites sampled were part of the Long Term Ecological Research (LTER) project in agricultural ecology at the W.K. Kellogg Biological Station of Michigan State University (Hickory Corners, MI) and included both active and abandoned agricultural fields as well as nearby fields that had never been cultivated. Microbial community structure was assessed by extracting total RNA from soil samples and using 16S rRNA-targeted oligonucleotide probes to quantify the abundance of rRNA from the alpha, beta, and gamma Proteobacteria, the Actinobacteria (Gram positive bacteria with a high mol % G+C genome), the Bacteria, and the Eukarya. In addition, soil microbial communities were characterized by examining fluorescently tagged terminal restriction fragment length polymorphisms (T-RFLP) in PCR amplified 16S rDNA. Microbial community structure was observed to be remarkably similar among plots that shared a long-term history of agricultural management despite differences in plant community composition and land management that have been maintained on the plots in recent years. In contrast, microbial community structure differed significantly between fields that had never been cultivated and those having a long-term history of cultivation.

Introduction

Soil microbial communities regulate nutrient cycles in terrestrial ecosystems, yet there remains a scarcity of basic knowledge about the structure of soil microbial communities and the factors that influence it in soils. This lack of knowledge arises, in part, from the extraordinary complexity of soil microbial communities, estimated to contain more than 4,000 different genomic equivalents in a single gram of soil [47]. Further complicating matters is the observation that the organisms isolated from soil represent only a portion of the microbial groups present *in situ*, while the vast majority of soil microorganisms have yet to be cultivated [26]. Recently, cultivation-independent approaches utilizing 16S rRNA genes have been used to explore the taxonomic

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diversity of soil microbial communities [5, 14, 19, 21, 30, 31, 35, 38, 45, 48, 53]. These 16S rRNA-based techniques can also be exploited to examine the distribution of specific microbial groups in relation to environmental characteristics.

There is little doubt that microbial communities are sensitive to changes in the surrounding soil. Comparative studies have documented that microbial communities can change in response to soil disturbance [2, 15, 28, 35, 38], and differences have been observed between microbial communities in fields with different histories of soil amendment, irrigation, tillage, and plant community structure [3, 4, 7, 8, 23, 24, 49]. Additionally, there is evidence that certain components of community structure can vary at spatial scales consistent with the distribution of individual plants [12, 44]. Analyses of microbial community structure are commonly restricted to a determination of whether microbial communities are similar or different. Such analyses do not permit any examination of variation in the abundance of specific microbial groups in the environment or the scale at which variation in microbial abundance is significant. By examining how specific microbial groups respond to environmental manipulation, it should be possible to identify environmental factors that influence the structure of microbial communities and the scale at which these environmental factors influence the distribution of individual microbial groups in the soil.

Microbial communities in plots at the W.K. Kellogg Biological Station Long Term Ecological Research (KBS-LTER) site were analyzed using both 16S ribosomal RNA and DNA extracted from soils. The KBS-LTER site includes a large-scale experiment with replicated plots under distinct management regimes ranging from conventionally tilled, annual cropping systems to abandoned fields. The site provided an opportunity to evaluate the effects of tillage, fertilization, and plant community composition on the structure of microbial communities. In addition, since at the KBS-LTER site both cultivated fields and fields abandoned from cultivation are present on a contiguous parcel of land that had been uniformly cultivated for greater than 50 years prior to 1989, it is possible to evaluate the lasting impact of agricultural management on soil microorganisms. The relative abundance of microbial groups was determined by extracting total RNA from soils and challenging the extracted RNA with oligonucleotide probes specific for rRNA from the alpha, beta, and gamma Proteobacteria, the Actinobacteria (Gram positive bacteria with high mol % G+C content), the Bacteria, and the Eukarya. In addition, microbial communities were compared on the basis of patterns generated from 16S rDNA terminal restriction fragment length polymorphisms (T-RFLP) [33]. The objective of this research was to examine the distribution of microbial groups across a managed landscape, to determine whether or not patterns of microbial community structure are apparent at the scale of individual fields, and to identify environmental factors that affect the distribution of specific microbial groups in soil.

Materials and Methods

Site Description and Soil Sampling

Soil samples were taken in October 1996 from the KBS-LTER site located at the Michigan State University W.K. Kellogg Biological Station (Hickory Corners, MI). The KBS-LTER site, established in 1989 to study ecological processes in agroecosystems, includes a large-scale replicated field experiment with seven treatments representing different cropping systems and types of management (for a more detailed site description see http://lter.kbs.msu.edu). The main site is located on 48 hectares of land that had been uniformly farmed for more than 50 years prior to establishment [44]. Soil was sampled from five of the main site treatments and from a field area that had never been cultivated but was adjacent to the LTER experimental site (Table 1). The conventional till (CT), no till (NT), and no input (NI) treatments received a corn/soybean/wheat crop rotation that was in corn at the time of sampling. These treatments were maintained with or without chemical inputs, tillage, and the presence of cover crops (Table 1). The alfalfa treatment (AF) received fertilization but no tillage and differed from the previous three treatments because the plant community was dominated by perennial instead of annual crops. 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 dominance by biennials and herbaceous forbs, which dominated for 3 years prior to sampling [25]. The plant communities in the never cultivated successional field (NCS) were also dominated by herbaceous forbs and closely resembled the plant communities in the HCS fields.

Soil was sampled from three of the six replicate plots (KBS-LTER field replicates 2, 3, and 4) from each main site treatment and from three replicate plots within the HCS field area. Soils at the site were Typic Hapludalfs, sandy to silty clay loam and were of moderate fertility [44]. Plots were sampled by taking a soil core (2.5 cm diameter, 10 cm depth) from each of the five permanent sampling locations in each replicate plot. The soil cores from each replicate plot were pooled, sieved (4 mm mesh), frozen in liquid nitrogen, and stored at −80°C.

Nucleic Acid Extraction

DNA suitable for use in PCR amplification was purified from 1 g of soil using the method of Purdy et al. [41]. RNA for use in hybridization experiments was extracted as previously indicated [11]. Briefly, 10 g of soil was suspended in a homogenization buffer

Table 1. Codes and descriptions of experimental treatments and reference communities on the KBS-LTER site

Name	Chemical inputs[a]	Current tillage	Management history	NPP (g/m^2)[b]	Plant community
Conventional till (CT)	Yes	Yes	Cultivated >50 years	929 ± 104	Annual crop, corn/soybean/wheat rotation
No till (NT)	Yes	No	Cultivated >50 years	1082 ± 184	Annual crop, corn/soybean/wheat rotation
No input (NI)	No	Yes	Cultivated >50 years	1017 ± 75	Annual crop, corn/soybean/wheat rotation with legume cover crop
Alfalfa (AF)	Yes	No	Cultivated >50 years	959 ± 39	Perennial crop
Historically cultivated successional (HCS)	No	No	Cultivated >50 years, abandoned in 1989	634 ± 38	Herbaceous perennials dominated by dicots
Never cultivated successional (NCS)	No	No	Never cultivated	460 ± 47	Herbaceous perennials dominated by dicots

[a] Chemical additions to CT and NT consisted of standard agronomic inputs of fertilizer and herbicide, while AF received fertilizer and insecticide. More specific information may be found at http://lter.kbs.msu.edu.
[b] Values for aboveground Net Primary Productivity (NPP) in 1996 were obtained with permission from http://lter.kbs.msu.edu.

containing guanidium isothiocyanate to prevent RNA degradation and bead milling was used to disrupt cells (Beadbeater, Biospec Products, Inc.). After solids were removed by centrifugation, purification of RNA was achieved by precipitation with polyethylene glycol followed by an organic extraction and passage through both hydroxyapatite and Sephadex G-75 spin columns. The total RNA concentrations of samples were estimated by using an orcinol reaction to determine ribose concentration [17].

Quantitative Filter Hybridization

Quantitative filter hybridizations were performed as previously described with minor modifications [46]. Nucleic acids from soil samples and cultures 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. RNA isolated from pure cultures (*Ketogulonogenium vulgarum* DSM 4025, *Nitrosomonas europaea* ATCC 25978, *Pseudomonas aeruginosa* ATCC 10145, *Cytophaga johnsonae* ATCC 17061, *Arthrobacter globiformis* ATCC 8010, *Bacillus subtilis* ATCC 6051, and *Saccharomyces cerevisiae* American Ale Yeast 1056 (Wyeast Labs, Inc.)) were included on all filters for use as positive and negative controls. Hybridization protocols were used for ^{32}P-5′-labeled oligonucleotide probes as previously described [46]. Replicate filters were prepared and used for hybridization with the following probes: Univ1390, Eub338, Euk1195, Alf1b, Bet42a, Gam42a, and HGC69a [1]. All filters were hybridized for >12 hr at 45°C, washed for 30 min at 45°C, and then washed for an additional 30 min to provide stringency (45°C for Univ1390, Eub338, Euk1195; 50°C for HGC69a; 55°C for Alf1b; and 62°C for Bet42a and Gam42a). Specifically bound probe was quantified using a radioanalytic imaging system (AMBIS, Inc).

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 [46]. 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 copurify with RNA from soil. Positive controls were included on each membrane to correct for variations in the labeling efficiency of different oligonucleotide probes while negative controls were used to correct for the possibility of nonspecific 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 $R = \Sigma_{i=1}^{n} [G_i(U_i)^{-1}]n^{-1}$, 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 $(R_s - R_n)(R_p - R_n)^{-1} \times 100$. To calculate the amount of 16S rRNA g^{-1} of soil, the relative abundance determined for samples was multiplied by the total amount of 16S rRNA present in soil samples as estimated from measurements of soil RNA content.

16S rDNA T-RFLP Analysis

Bacterial community composition was investigated in fields from the treatments CT, HCS, and NCS on the basis of T-RFLP analysis of 16S rDNA amplified from soil DNA extracts. Bacterial 16S rDNA from soil extracts was PCR amplified using the oligonucleotide primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′), labeled at the 5′ end with the phosphoramidite dye 5-hexachlorofluorescein, and

Table 2. Relative abundance of rRNA from specific groups of microorganisms in the KBS LTER plots

Treatment	μg RNA[a]	Mean % rRNA abundance (±s.e.)[b]					
		Alf1b	Bet42a	Gam42a	HGC69a	Euk1195	Eub338
CT	3.53 ± 0.76	28.8 ± 5.1	6.2 ± 1.6	3.8 ± 0.2	5.8 ± 2.7	13.1 ± 2.6	59.0 ± 5.8
NT	4.39 ± 0.33	30.8 ± 2.0	5.1 ± 1.3	3.3 ± 0.5	10.9 ± 5.2	9.7 ± 4.9	51.8 ± 4.2
NI	3.99 ± 0.78	26.1 ± 2.1	4.3 ± 0.6	3.3 ± 0.2	6.8 ± 1.4	12.3 ± 3.9	51.0 ± 2.9
AF	4.29 ± 0.60	26.3 ± 1.7	3.7 ± 0.5	3.0 ± 0.2	7.0 ± 1.3	7.7 ± 3.0	52.3 ± 2.5
HCS	4.50 ± 0.76	26.6 ± 1.7	5.2 ± 1.3	3.3 ± 0.3	9.0 ± 2.9	9.5 ± 5.1	53.4 ± 1.8
NCS	7.41 ± 1.20	38.6 ± 2.4	8.5 ± 0.9	3.6 ± 0.4	17.1 ± 1.3	14.3 ± 1.8	62.8 ± 2.4

[a] $\mu g g^{-1}$ soil dry weight
[b] Values for each treatment determined from three replicate plots

1492R (5′-GGTTACCTTGTTACGACTT-3′) [33]. PCR was carried out in a volume of 50 μl with 50 ng template DNA, 0.05% Nonidet P-40, 0.05% bovine serum albumin, 1.5 mM $MgCl_2$, 200 nM of each dNTP, 0.5 μM primer 8F, 0.3 μM primer 1492R, and 1.25 U Taq polymerase with 1× concentration of the supplied buffer (Gibco BRL). Reactions were performed in a Gene Amp 9600 thermocycler (Perkin-Elmer) for 30 cycles (1 min at 92°C, 1 min at 37°C, and 1 min at 72°C). Amplified 16S rDNA was purified using Ultrafree-MC (30,000 NMWL) filtration units (Millipore) according to the manufacturer's specifications. After purification, amplified 16S rDNA was separately digested with the restriction endonucleases *Msp*I (Boehringer Mannheim), *Rsa*I (Gibco BRL), or *Hae*III (Gibco BRL) according to the manufacturer's instructions. As a result each field replicate was represented by three distinct T-RFLP profiles. The exact lengths of fluorescently labeled terminal restriction fragments from each restriction digestion were determined by electrophoresis of 50 ng sample through a 36 mm 6% polyacrylamide gel on a model 373A automated sequencer (Applied Biosystem Instruments, Inc.).

Community T-RFLP profiles were compared solely on the basis of fragment size, and without respect to band intensity. The number of bands shared between any two T-RFLP profiles was calculated for all pairwise comparisons of samples using the Sorenson index of similarity: $S = 2ab/(a + b)$, where a and b are the number of bands in any two samples and ab is the number of bands shared between those samples [34]. Bands were considered identical provided that their calculated fragment sizes differed by less than one base pair.

Data Analysis

Measurements of 16S rRNA relative abundance were analyzed using nonparametric statistical tests to compensate for heteroscedasticity (inequality of variance among samples) observed in these data. The effects of the five main site treatments (CT, NT, NI, AF, and HCS) on microbial group abundance were analyzed using MANOVA by ranks for all groups simultaneously and by using the Kruskal–Wallis test independently on each group of organisms. Mann–Whitney U tests were used to examine relationships in microbial group abundance between specific pairs of treatments and between the historically cultivated fields of the main site and the NCS fields. In addition, MANOVA by ranks was used to examine differences in microbial community structure between the historically cultivated fields and NCS fields.

The average similarity in microbial community structure between treatments CT, HCS, and NCS was also estimated based on the Sorenson index calculated from T-RFLP patterns. These similarity values were compared using ANOVA following arcsine data transformation. Between-treatment similarities in T-RFLP patterns were compared using ANOVA where each comparison was represented by nine measurements made with each of the three restriction enzymes. Comparisons of within-treatment variability and numbers of discrete T-RFLP bands were analyzed using ANOVA where each treatment was represented by three measurements made with each restriction enzyme. Post hoc analyses were performed using the Scheffe test to identify differences between specific treatments. All statistical analyses were performed using StatView v5.0 (SAS Institute, Inc.).

Results

Relative Abundance of Microbial Groups in Soil Samples

Ribosomal RNA was readily detected from all of the microbial groups surveyed in the soils examined (Table 2). The ratios of HGC69a/Univ1390 probe binding are displayed for CT, NCS, HCS samples as well as for relevant controls in order to represent the manner in which 16S rRNA relative abundance was calculated for all other samples and probes (Fig. 1). Negative controls are used to adjust for nonspecific binding, and positive controls are used to adjust for differences in probe specific activities as discussed in the methods. ANCOVA revealed that differences between the slopes of the probe binding ratios for samples and controls are not significant. The homogeneity of slopes for the probe binding ratios indicates that differences in sample RNA concentra-

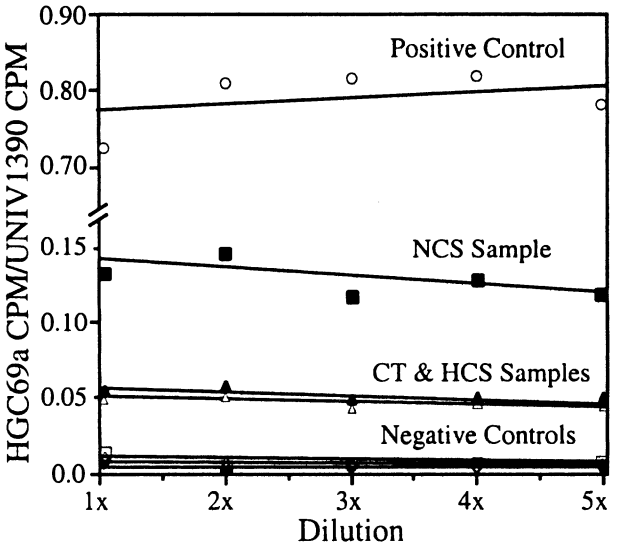

Fig. 1. Data generated from hybridization experiments with the ^{32}P-labeled oligonucleotide probes HGC69a and Univ1390 reveal the characteristic linear response of the ratio of specific to universal probe binding over a range of sample concentrations. The symbols represent values obtained for RNA from *A. globiformis* (○), *B. subtilis* (□), *K. vulgarum* (◆), *N. europaea* (●), *P. aeruginosa* (◇), and RNA from CT (▲), HCS (△), and NCS (■) soil samples.

tion and the possible presence of hybridization inhibitors in soil RNA extracts will not affect calculations of rRNA relative abundance. Of the bacterial groups surveyed, the alpha Proteobacteria composed the largest fraction of community rRNA in all plots (29.8% ± 1.6%; mean ± standard error). The Actinobacteria (9.7% ± 1.5%) were the second most abundant group surveyed, followed by the beta Proteobacteria (5.5% ± 0.6%), and the gamma Proteobacteria (3.3% ± 0.2%) (Table 2). The bacterial groups examined in this study represent 88.0% ± 9.7% of the total bacterial signal as measured by the probe Eub338.

Effects of Environmental Characteristics on Microbial Community Structure

Microbial community structure was remarkably similar among the fields of the five historically cultivated treatments at the main experimental site (CT, NT, NI, AF, HCS), despite the wide variation in plant community composition that existed between the fields in these treatments at the time of sampling (Tables 1, 2). In addition, analysis of RNA yields indicated that the total amount of RNA present in the soil did not vary appreciably among the historically cultivated treatments (Table 2). In contrast, differences in microbial group rRNA abundance were readily observed when the historically cultivated fields were compared to the NCS fields (Fig. 2). The rRNA relative abundance of the alpha Proteobacteria, the beta Proteobacteria, and the Actinobacteria were significantly higher in the NCS fields than in the fields that shared a history of cultivation (Fig. 2A). In addition, the total amount of 16S rRNA for all of the microbial groups examined was significantly higher in the NCS fields than in the historically cultivated fields (Table 2 and Fig. 2B). MANOVA revealed that the differences in microbial community structure between the historically cultivated fields and the NCS fields were significant (Pillai's trace, $P < 0.0001$). Also, RNA yields in the fields that had never been cultivated differed significantly (t-test, $P < 0.05$) from the RNA yields obtained from the historically cultivated main site treatments (Table 2).

Analysis of T-RFLP Profiles

Relationships between bacterial communities in the never cultivated reference fields (NCS), the historically cultivated successional fields (HCS), and the conventionally managed agricultural fields (CT) were explored using T-RFLP analysis of amplified 16S rDNA (Fig. 3). By comparing the microbial community composition in the HCS fields relative to those in the CT and NCS fields, it is possible to assess the lasting impact of cultivation on the microbial communities in fields that have been abandoned from cultivation for 7 years. T-RFLP profiles reveal a different level of community structure than rRNA probing, providing a broader view of the phylogenetic diversity within microbial communities while sacrificing the ability to quantify individual microbial groups. Analysis of Sorenson similarity values calculated from 16S rDNA T-RFLP profiles revealed that there are significant differences in the composition of bacterial communities between the treatments CT, HCS, and NCS (ANOVA; $F(2,24) = 35.43$, $P < 0.01$; Fig. 4). Post-hoc tests indicate that the similarity between bacterial communities in the CT and HCS fields (0.53 ± 0.15) was significantly higher than the similarities between bacterial communities in either the CT and NCS (0.34 ± 0.13) or the HCS and NCS fields (0.34 ± 0.12) (Scheffe test, $P < 0.01$). An analysis of variance for within-treatment community similarity in CT (0.61 ± 0.16), HCS (0.54 ± 0.17), and NCS (0.38 ± 0.19) also revealed a significant treatment effect ($F(3, 6) = 6.015$, $P < 0.05$), and additional tests revealed that the T-RFLP patterns from the CT fields were significantly less variable than that those in the NCS fields (Scheffe test, $P < 0.05$).

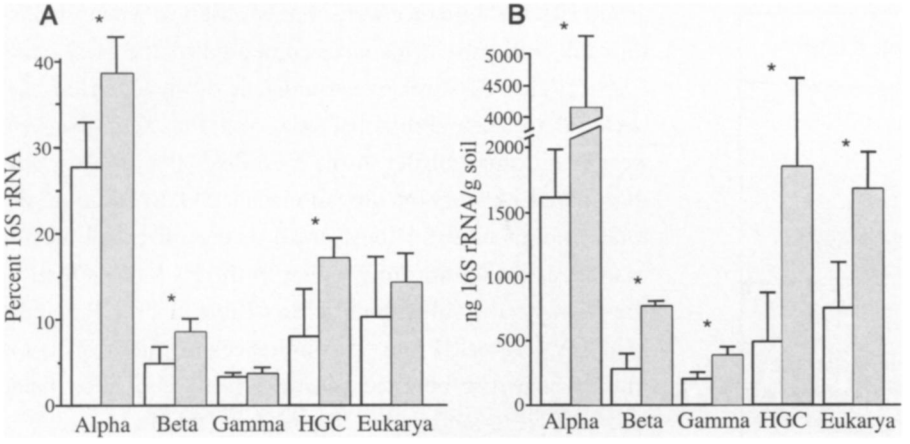

Fig. 2. Relative abundance of 16S rRNA (A) and quantity of 16S rRNA (g^{-1} dry weight of soil) (B) for microbial groups in fields sharing a history of agricultural disturbance (CT,NT,NI,AF,HCS; open bars) and in fields that have never been cultivated (NCS; shaded bars). Groups shown in the figure are the alpha, beta, and gamma Proteobacteria, Actinobacteria (HGC), and Eukarya. The differences between historically cultivated and never cultivated fields that are indicated by an asterisk were found to be significant by Mann–Whitney U-tests ($p < 0.05$). Error bars indicate one standard deviation from the mean.

Discussion

Hybridization of extracted RNA with 16S rRNA-targeted oligonucleotide probes provides a quantitative measurement of the protein synthetic capacity of microorganisms in the environment, which, since cellular rRNA concentrations increase with growth rate, is influenced by both the number and metabolic activity of cells in the environment [50]. The relative abundance of rRNA for a microorganism in a microbial community may differ from the relative abundance of rDNA for that organism if there are large differences in the growth rates (in the case of rRNA) or in the rRNA gene copy number (as in the case of rDNA) of the microorganisms in that community. It is interesting to note that the relative abundance of rRNA determined for the microbial groups at the KBS-LTER site (Table 2) roughly corresponds to the relative abundance of these same microbial groups in 16S rDNA clone libraries that have been generated from soil samples. Analysis of 733 16S rDNA clones originating from diverse soil samples taken from sites on three continents reveals the average abundance of clones from the alpha, beta, and gamma Proteobacteria to be 16%, 4%, and 3% respectively, whereas Actinobacteria compose 9% of clones [5, 6, 30–32, 35, 48, 53]. That the relative abundance of microbial groups as reflected by representation in clone libraries and rRNA probing is roughly similar in many diverse soil samples may reveal that there are certain characteristics of

	CT-R2	CT-R3	CT-R4	HCS-R2	HCS-R3	HCS-R4	NCS-R2	NCS-R3	NCS-R4
CT-R2	-	-	-	-	-	-	-	-	-
CT-R3	0.69 ± 0.04	-	-	-	-	-	-	-	-
CT-R4	0.64 ± 0.17	0.49 ± 0.21	-	-	-	-	-	-	-
HCS-R2	0.57 ± 0.14	0.48 ± 0.28	0.50 ± 0.16	-	-	-	-	-	-
HCS-R3	0.53 ± 0.10	0.35 ± 0.14	0.62 ± 0.19	0.48 ± 0.11	-	-	-	-	-
HCS-R4	0.59 ± 0.05	0.55 ± 0.03	0.49 ± 0.11	0.54 ± 0.22	0.59 ± 0.22	-	-	-	-
NCS-R2	0.37 ± 0.19	0.36 ± 0.18	0.34 ± 0.21	0.29 ± 0.13	0.31 ± 0.15	0.35 ± 0.06	-	-	-
NCS-R3	0.32 ± 0.14	0.34 ± 0.15	0.37 ± 0.16	0.30 ± 0.04	0.33 ± 0.15	0.38 ± 0.17	0.38 ± 0.25	-	-
NCS-R4	0.31 ± 0.03	0.27 ± 0.10	0.38 ± 0.02	0.36 ± 0.19	0.37 ± 0.14	0.41 ± 0.14	0.31 ± 0.17	0.46 ± 0.21	-

Fig. 3. Pairwise comparisons of T-RFLP patterns representing soil communities from three field replicates of the treatments CT, HCS, and NCS. The values were calculated using the Sorenson index and represent the mean and standard deviation of comparisons made with three different T-RFLP patterns generated from digesting the 16S rDNA from each soil sample with enzymes *Msp*I, *Hae*III, and *Rsa*I. The shaded regions along the diagonal of the matrix contain the data for the within-treatment comparisons in Fig. 4B, whereas the open and shaded blocks in the body of the matrix contain the data for the between-treatment comparisons in Fig. 4A.

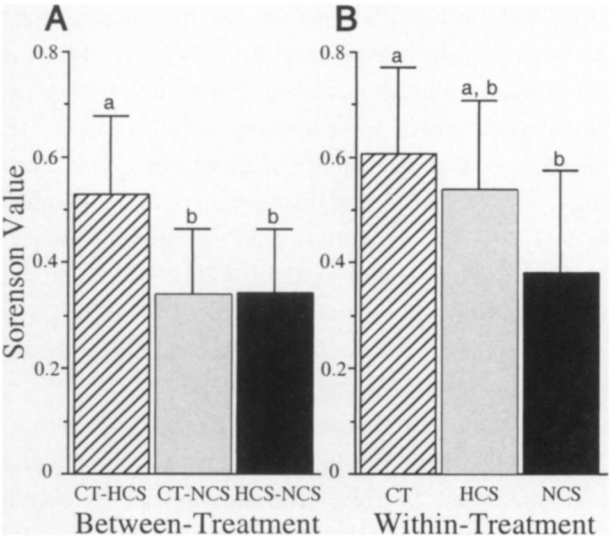

Fig. 4. Sorenson similarity indices generated from T-RFLP patterns reveal the relative similarity in community structure between and within treatments CT, HCS, and NCS. Bars with different letters were revealed to be significantly different (Scheffe test, $p < 0.05$); error bars indicate one standard deviation from the mean.

soil environments that lead to overall similarities in microbial community structure.

Although there may be similarities among microbial communities from different soils, we have shown that community structure can change significantly in a contiguous landscape as the result of changes in the soil environment brought about by the long-term impacts of cultivation (Fig. 2). Fields that had been cultivated prior to 1989 had significantly lower proportions of rRNA from the alpha Proteobacteria, beta Proteobacteria, and Actinobacteria, and had 16S rDNA T-RFLP profiles that were significantly different from those in the NCS fields (Fig. 2, Fig. 4). In addition, the total amount of 16S rRNA from each of the microbial groups and the total amount of RNA g^{-1} soil was significantly lower in fields that had been cultivated relative to NCS fields (Table 2, Fig. 2). These differences in the total amount of RNA g^{-1} soil between the historically cultivated and NCS fields are most likely a reflection of similar differences observed in the size of the total microbial biomass between these fields [39]. Differences observed between the CT and NCS fields in the composition of both the denitrifying and the autotrophic ammonia oxidizing microbial communities are also consistent with the conclusion that microbial community structure differs significantly as a result of the lasting impact of cultivation [10, 13].

In this study the Eub338 and Euk1195 probes together accounted for only 66.0% ± 13.9% of the community rRNA detected with the universal probe (Univ1390). Archaeal rRNA in these soils was previously measured to be 1.5% ± 0.6% [11]. Although the Eub338 probe is generally specific for the majority of the Bacteria and the Euk1195 probe is specific for the majority of the Eukarya, both probes will miss a portion of the sequence diversity within their respective groups. For example, the Eub338 probe does not recognize two groups of Bacteria known to occur in soil, the Planctomycetes and the Verrucomicrobia [16]. These two microbial groups accounted for 9.6% ± 3.5% of the Univ1390 signal in KBS-LTER plots (Buckley and Schmidt, unpublished data). Even if the results are adjusted to include the Planctomycetes and Verrucomicrobia, approximately 23% of the Univ1390 signal remains unidentified. Although experimental error could account for a portion of the discrepancy from 100% coverage, a more likely explanation is that the unaccounted portion of the microbial community is composed of rRNA from the Bacteria and Eukarya that bind the Univ1390 probe but are not recognized by the Eub338 and Euk1195 probes. As a result, there are certainly microorganisms in the soil that have not been targeted by the probes used in this study, but it is clear that the microorganisms that have been detected by these probes are influenced by the lasting impact of cultivation on the soil.

The bacterial probe Eub338 accounted for 55% of the rRNA molecules extracted from soil microbial communities. As mentioned above, this estimate is likely an underestimate of the actual bacterial contribution to community rRNA [16]. It is interesting to note that fluorescent *in situ* hybridization (FISH) studies in soil have found that cell counts made using the fluorescently labeled Eub338 probe detect only 40% to 45% of the total DAPI stained cells [14, 52]. The low ratio of Eub338 FISH stained cells to DAPI stained cells could be interpreted as evidence for low permeability of cells to flourescently labeled oligonucleotide probes. However, in light of the fact that Eub338 identified on average only 55% of community rRNA in this hybridization analysis, it is possible that discrepancies between counts of FISH stained cells and DAPI stained cells may be due to limitations in the specificity of the Eub338 probe.

Microbial community structure, as assessed by rRNA probing, did not vary significantly across the historically cultivated fields at the KBS-LTER site (CT, NT, NI, AF, HCS) despite differences in chemical inputs, tillage, plant community composition, and productivity that existed in

these fields at the time of sampling. It is possible that slight differences exist in community structure between these fields, and that these differences were not detected because the number of samples analyzed was low relative to the natural variability in the microbial communities. However, differences between microbial community structure in the historically cultivated fields and the NCS fields were readily detected. These observations indicate that any differences that exist among the historically cultivated fields are small in comparison to differences between these fields and the fields that had never been cultivated.

Probing of rRNA provides a quantitative view of a very broad level of microbial community structure. A great deal of biological diversity can exist within each of the microbial groups examined in this study. Analyses of 16S rDNA T-RFLPs were performed to assess changes in community composition that were not detected by quantitative probing of rRNA. T-RFLP analysis of amplified 16S rDNA can be used to provide a general comparison of the overall phylogenetic similarity between microbial communities at a finer level of resolution than is provided through quantitative analysis of microbial group rRNA abundance. T-RFLP analyses supported the results obtained from probing rRNA as similarities in bacterial community T-RFLP profiles among the historically cultivated plots (CT and HCS) were significantly higher than those between the historically cultivated plots and the NCS plots (Fig. 4). As measured by T-RFLP, variability in bacterial community structure was lowest among the CT replicates, whereas such variability was highest among the NCS plots. The low number of replicates and high variability made it difficult to determine whether the T-RFLP variability among the HCS replicates was either significantly different from the other treatments or truly occupies an intermediate level of variability between the two.

These data allowed us to assess the influence of plant community composition, fertilization, tillage, and the effect of historical cultivation on microbial community structure across different treatments at the KBS-LTER site. At the time of sampling, despite maintenance for 7 years under several different management practices, the microbial community structure was not appreciably different in fields sharing a common long-term history of cultivation. In addition, whereas the plant community composition and productivity in the HCS fields closely resembled those of fields that had never been cultivated, the microbial communities in the HCS fields were still indistinguishable from the microbial communities found in active agricultural fields. Previous studies have also identified patterns of microbial community structure that are consistent across sites that vary in plant composition and agricultural treatment [9, 22, 28, 49]. It is clear that plants influence microbial community structure in soil immediately adjacent to plant roots [20, 27, 36, 37, 51], but there is conflicting evidence as to whether plant communities influence microbial distribution across individual fields [9, 12, 22, 40]. This study does not provide any evidence that plant community composition is influencing soil microbial community structure at the KBS-LTER site, though any plant effects may be masked by the overwhelming influence of past agricultural practices.

It is important to note that the two methods of community analysis that were employed both have a fairly coarse level of resolution. Microbial communities whose overall structure appears similar by rRNA probing and T-RFLP analyses may still possess ecologically significant differences in community composition, as these methods are insensitive to changes in community composition that may occur at the level of individual strains or even species. Such strain- or species-level changes in community composition could be responsible for differences in the physiological capacity of microbial communities whose overall structure is very similar. Although these data are unable to account for absolute differences in community composition between the fields examined, it is clear that there are surprising similarities in community structure between the CT and HCS fields. At some level there are probably differences between the composition of the microbial communities in the CT and HCS fields; however, the fact that the communities in these fields are still more similar to each other than to the communities in NCS fields suggests strongly that after 7 years of abandonment the microbial communities in the HCS fields have still not recovered from the effects of cultivation.

Microbial communities can respond rapidly to changes in their local environment, so it may seem odd that the microbial communities in abandoned fields remain similar to those in agricultural fields. A possible explanation of this observation is that soil microbial communities respond to soil characteristics that require long periods of time to recover from disturbance. The soil organic carbon and total soil nitrogen pools are examples of soil characteristics that can be depleted by long-term agricultural practices and can require decades or even centuries to recover to preagricultural levels [18, 29, 39]. In addition, studies of spatial variability in soil resources indicate that the distribution of soil nutrients in postagricultural fields can require decades to recover from the homogenizing effects of tillage [42, 43]. Consistent with these observations, total carbon and nitro-

gen content of soil were significantly lower in the historically cultivated fields than in NCS fields at the KBS-LTER site [13, 39]. Further study, leading to the identification of specific soil characteristics that influence the dynamics and spatial variability of microbial community structure, should aid in understanding the long term effects of disturbance on microbial communities and on ecosystem function.

In this study rRNA-based phylogenetic probes were used to characterize the abundance of specific microbial groups in the soil and to determine the relative importance of certain environmental variables in influencing patterns of community structure across a replicated field site. Patterns of microbial community structure, as assessed both by quantitative rRNA probing and by analysis of 16S rDNA T-RFLP profiles, revealed similarities in microbial community structure among fields sharing a history of cultivation, despite differences in chemical inputs, tillage, plant community composition, and productivity. Microbial communities in fields abandoned from agriculture for 7 years retained the characteristics of contemporary agricultural fields. Meanwhile, community structure in those fields sharing a history of cultivation was shown to differ significantly from that in fields that had never been cultivated. Additional studies are currently underway to assess specific factors that may influence soil microbial community structure, and to determine if the patterns observed continue to hold true during different times of the year and with increasing time since abandonment.

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