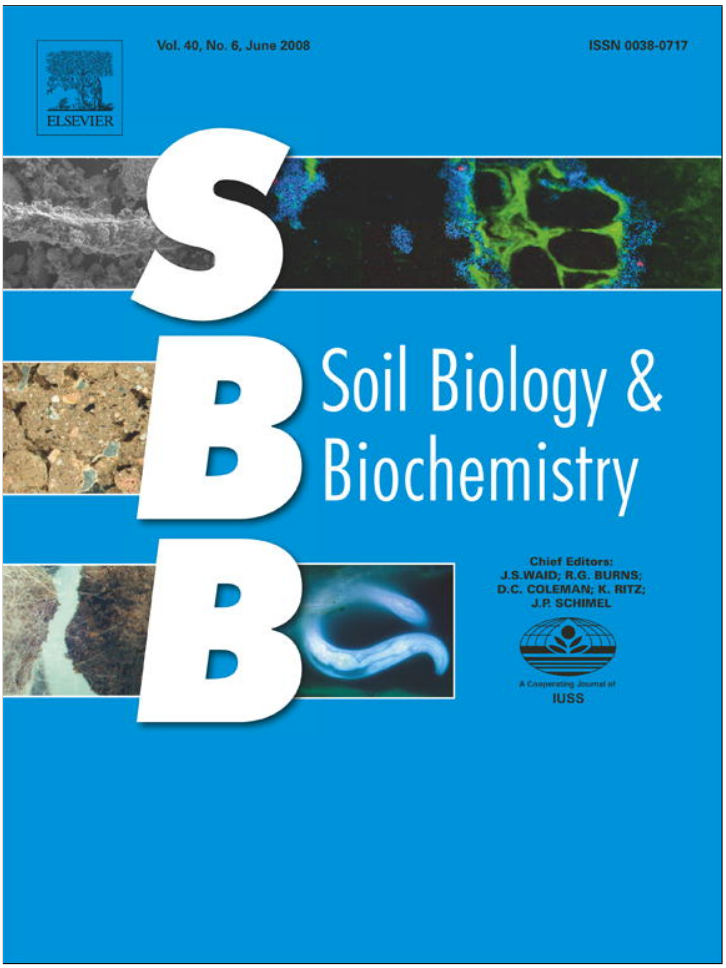

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Differences in the composition and diversity of bacterial communities from agricultural and forest soils

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Abstract

Differences in the bacterial communities of soils caused by disturbances and land management were identified in rRNA gene libraries prepared from conventional tilled (CT) and no tilled (NT) cropland, a successional forest after 30 y of regrowth (NF) and an old forest of >65 y (OF) at Horseshoe Bend, in the southern Piedmont of Georgia (USA). Libraries were also prepared from forests after 80 y of regrowth at the Coweeta Long Term Ecological Research site (CWT) in the Southern Appalachians of western North Carolina (USA). The composition of the bacterial communities in cropland soils differed from those of the Horseshoe Bend OF and CWT forest soils, and many of the most abundant OTUs were different. Likewise, the diversity of bacterial communities from forest was less than that from cropland. The lower diversity in forest soils was attributed to the presence of a few, very abundant taxa in forest soils that were of reduced abundance or absent in cropland soils. After 30 y of regrowth, the composition of the bacterial soil community of the NF was similar to that of the OF, but the diversity was greater. These results suggested that the bacterial community of soil changes slowly within the time scale of these studies. In contrast, the composition and diversity of the bacterial communities in the Horseshoe Bend OF and Coweeta soils were very similar. Thus, this forest soil bacterial community was widely distributed in spite of the differences in soil properties, vegetation, and climate as well as resilient to disturbances of the above ground vegetation.

Keywords: Bacterial community structure; 16S rRNA genes; No tillage; Forest

1. Introduction

The effects of disturbances on soil bacterial diversity have not been well documented (Kennedy, 1999). One of the most important human activities that depends on and has the potential to exert a large impact on soil is agriculture. Increases in agriculturally utilized land, up to $\frac{1}{3}$ of the total land area on earth, have caused increases in erosion, losses of soil organic matter (SOM), and changes within bacterial communities within soil (Matson et al., 1997; Tilman et al., 2001). Common disturbances in forest soils include timber harvesting and damage caused by wind (Wright and Coleman, 2002).

Soil disturbances caused by natural or human activities have direct impacts on ecosystem properties and function, such as nutrient cycling and physical and chemical complexity (Sohlenius, 1982; Greenberg and McNab, 1998; Ulanova, 2000; Wright and Coleman, 2002). Soil, one of the largest reservoirs for bacteria on earth, and its processes are greatly influenced by bacterial community structure, activity, and stability (Whitman et al., 1998; Dunbar et al., 1999, 2002; McCaig et al., 2001; Coleman and Whitman, 2005).

Analysis of bacterial communities and diversity in soil has traditionally begun with cultivating microorganisms from the environment. This technique is limited because of the inability of a wide range of bacteria to be efficiently cultured in the laboratory (Torsvik and Øvreås, 2002; Torsvik et al., 2002). The use of molecular, culture-independent

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based techniques has led to a new understanding of bacterial diversity (for reviews see: von Wintzingerode et al., 1997; Hugenholtz et al., 1998; Zhou et al., 2004; Janssen, 2006). A commonly used molecular method to study communities utilizes 16S rRNA gene clone libraries constructed from DNA extracted from whole soil communities. Libraries have the potential to determine the composition and diversity within soil microbial environments, which is essential to understanding the role of these communities and their effects on ecosystem processes. To identify differences in the composition and diversity of the bacterial communities resulting from differences in land management and human and natural disturbances, two research sites, Horseshoe Bend (HSB) in northern Georgia and Coweeta Hydrologic Laboratory in western North Carolina, were studied. At these sites, different tillage regimes, namely conventional deep moldboard plowing and no-tillage (NT) agriculture, were compared to the effects of disturbance by previous agricultural practices, hurricane wind damage, and removal of understory vegetation on forest soils.

2. Materials and methods

2.1. Sample site description

2.1.1. HSB site

The HSB research site, located in Athens, Georgia on the floodplain of the Oconee River, consists of a sandy loam (typic Kanhapludult) soil (Furlong et al., 2002). Winter cover crops are rye (*Secale cereale* L.) and crimson clover (*Trifolium pratense* L.), followed by summer crops of maize (*Zea mays* L.) and, since 1998, cotton (*Gossypium hirsutum* L.; Hendrix et al., 2001). Annual precipitation was 1010 mm (30 y average).

HSB samples were taken from four locations. Two locations were within eight 0.1 ha field plots that have been agriculturally managed using deep moldboard plowing followed by disking or conventional tillage (CT) and plots managed by direct seed drilling or NT agriculture. A third location, called new forest or NF, denoted a transect 10 m from the edge of the CT and NT plots in an area of successional forest. This forest developed from pasture that was abandoned in 1973 (Odum et al., 1974). Old forest, or OF, referred to a strip of land adjacent to the river and within 100 m of the NF. This site had been forested since the earliest record of the site in 1938. Both new and old forests were dominated by water oak (Quercus nigra L.) and sweetgum (Liquidambar styraciflua L.).

Three cores with a diameter of 5 cm were collected at each HSB location on March 28, 2002. Libraries were constructed using the 5–10 cm layer of each soil core. These libraries were labeled NT5, CT5, NF5 and OF5 depending on the location. At the locations NT and OF, libraries were also constructed from the 2–5 cm layer and labeled NT2 and OF2, respectively. Other properties of these soils are summarized in Table 1.

2.1.2. Coweeta LTER site

The Coweeta Hydrologic Laboratory (CWT) is a Long Term Ecological Research (LTER) site located in Otto, North Carolina in the Southern Appalachians. It possesses Humic Hapludults near the transects at lower elevations (760 m) with silty loam texture (Maxwell and Coleman, 1995). At higher elevations, Typic Haplumbrepts are found (Knoepp et al., 2000). Samples were taken from three plots. Cut (CU) and Storm (ST) were in watershed 55 where chestnut oak (*Q. prinus* L.), tulip poplar (*Liriodendron tulipifera* L.), and an understory of rosebay rhododendron (*Rhododendron maximum* L.) dominated the riparian forest. In the CU plot, rhododendron stems were cut in August 1995 (Wright and Coleman, 2002). The ST plot was damaged by Hurricane Opal in October, 1995, which knocked down most of the over story (Wright and Coleman, 2002). Summit (SM) was at 1340 m elevation and dominated by northern hardwoods (Betula alleghaniensis and *Q. rubra*; Knoepp et al., 2000). Three cores of 5 cm in diameter were collected at each location on April 20, 2002. For these samples soil from the 0–10 cm depths

Table 1
Physical and chemical characteristics of Horseshoe Bend (HSB) and Coweeta (CWT) soils[a]

Soil name	Description	Depth (cm)	Typic	Description	pH	Total C[b] ($g\,kg^{-1}$)	Total N[b] ($g\,kg^{-1}$)
NT2	No till agriculture	2–5	Rhodic Kanhapludult	Sandy loam	6.4	14.9	0.94
NT5	No till agriculture	5–10	Rhodic Kanhapludult	Sandy loam	5.5	10.5	1.02
CT5	Conventional tillage	5–10	Rhodic Kanhapludult	Sandy loam	6.0	11.5	1.08
NF5	New forest	5–10	Rhodic Kanhapludult	Sandy loam	ND	9.9	0.89
OF2	Old forest	2-5	Rhodic Kanhapludult	Sandy loam	ND	28.6	1.28
OF5	Old forest	5–10	Rhodic Kanhapludult	Sandy loam	ND	20.2	1.25
CU	Old forest, cut	0–10	Humic Hapludult	Chandler gravelly loam	5.3	34.8	1.93
ST	Old forest, storm	0–10	Humic Hapludult	Chandler gravelly loam	5.5	31.4	1.80
SM	Old forest, summit	0–10	Haplumbrept	Evard-Cowee gravelly loam	4.0	99.0	7.00

[a]The Horseshoe Bend sites (NT2, NT5, CT5, NF5, OF2, and OF5) are described in greater detail in Coleman et al. (2006). The pH values reported are from Groffman et al. (1986). The Coweeta sites (CU, ST and SM) are described in greater detail in Maxwell and Coleman (1995), Wright and Coleman (2002) and Knoepp et al. (2000), which are the sources of the physical and chemical characteristics reported here.
[b]Mean of three determinations.

were utilized. Other properties of these soils are summarized in Table 1.

2.2. DNA extraction and preparation for 16S rRNA gene libraries

Soils from sections of each core were mixed, and large roots, rocks, and animals were removed. Total environmental DNA was extracted from 1 g of soil using an UltraClean Soil DNA Extraction kit (MoBio Industries). The 16S rRNA genes were amplified using low-cycle PCR. Each reaction consisted of 1 Ready-To-Go bead (Amersham Biosciences), 1 μl of DNA (about 10–100 ng), 2 μl of 27F primer (10 μmol) [5′-AGA GTT TGA TCM TGG CTC AG-3′], 2 μl of 1492R primer (10 μmol) [5′-ACG GYT ACC TTG TTA CGA CTT-3′], and enough dH_2O to bring the total volume to 25 μl. Each PCR included 1 cycle of 95° C for 5 min followed by 15 cycles of 95° C for 1 min, 62° C for 1 min, and 72° C for 2 min with a final cycle of 72° C for 4 min on a Mastercycler Gradient (Eppendorf).

After PCR the products were immediately cloned using a TOPO TA cloning kit (Invitrogen) using the pCR2.1 vector. Plates were incubated overnight at 37° C and then 24 h at 4° C. Well-isolated white colonies were picked into 96-well blocks with Luria-Bertani broth plus kanamycin and grown overnight. Sterile glycerol was added to a final concentration of 10%, and an aliquot of this was transferred to a 96-well sequencing block. Both the sequencing and the original culture blocks with LB broth and glycerol were stored at −80° C.

2.3. Sequence and alignment viewing and editing

Sequencing plates were sent to Iowa State Plant Sciences sequencing facility or Seqwright, Inc., in Houston, Texas. Robotics were used to prepare plasmids from the bacterial cultures, and the 16S rRNA genes were partially sequenced from the plasmids using the primer 27F. The 16S rRNA gene for selected clones was further sequenced using the primers 533F [5′-GTG CCA GCM GCC GCG GTA A-3′] and 1492R at the University of Michigan's sequencing facility. Chromatographs of sequences were viewed and manually edited for quality using Sequencer version 2.0 (Gene Codes Corporation) and ChromasPro (Technelysium Pty Ltd). Sequences that were less than 410 bp were resequenced or not included in further analyses.

Sequence alignments were constructed using PILEUP in the GCG Wisconsin package or Clustal W. Alignments were viewed and manually edited using version 2.6.02 of the program GeneDoc using the guidelines of Furlong et al. (2002). All sequences with low similarity to an RDP sequence were tested for chimeras. In this test, the top BLAST hits for the GenBank database were compared for the full sequence and the 5′- and 3′-halves. When the top BLAST hits were from different phylogenetic groups, the sequence was considered chimeric, and it was removed from the data set. The edited sequences were submitted to GenBank, accession numbers DQ128321-DQ128395 and DQ128397-DQ129236.

2.4. Library comparisons

Edited alignments were used to calculate distance matrices using the Jukes–Cantor algorithm in the program DNADIST from the Phylip package (Felsenstein, 2004). These matrices were then used as the input file for the program PRELIBSHUFF. This program in the Perl scripting language formats a distance matrix into a sample file for use by the program LIBSHUFF version 1.2 (Singleton et al., 2001). The Bonferroni correction was used to correct for experiment wise error when doing multiple LIBSHUFF comparisons. Two diversity measurements were used to compare clone libraries independent of their phylogenetic composition. The Shannon diversity index was used as a measure of general diversity, including richness and evenness (Shannon and Weaver, 1963). The Chao1 estimator was calculated as an alternative to Shannon diversity (Chao, 1984). Both of these calculations were determined at evolutionary distances of 0.03 or a sequence similarity value of about 97%. To analyze the distribution of abundant taxa within libraries, groups were constructed using DOTUR at a distance of less than or equal to 0.03 (Schloss and Handelsman, 2005). These groups were then analyzed using the binomial test (Siegel, 1956).

2.5. Taxonomic assessment

Taxonomic identifications were made using RDPquery written by Wade Shelton and Glen Dyszynski at the University of Georgia (http://simo.marsci.uga.edu/public_db/rdp_query.htm). This program compared clone sequences to type species in the RDP, calculated the percent similarity between individual sequences and the closest-related type species, and used these similarities to make taxonomic assessments. The taxonomic ranks were assigned using similarity values for species, genus, family, order, class and phylum as 100%, 95%, 92%, 91%, 85% and 80%, respectively.

2.6. Tree construction and analysis

Phylogenetic trees were constructed from the edited alignments of the soil libraries. Distance matrices were calculated using DNADIST. These matrices were used to construct neighbor-joining or Fitch-Margoliash trees with bootstrap values in the Phylip package. Trees shown were constructed using a representative clone sequence for groups with multiple sequences.

3. Results

3.1. Construction of bacterial gene libraries

Between 30 and 50 clones of 16S rRNA genes were sequenced from each sample taken at HSB and CWT (Table 2). Because each site was represented by three replicate samples, the total number of clones per site was approximately 100. LIBSHUFF comparisons of these replicates indicated that they were not significantly different from one another with one exception. The first and third replicates of OF5 were different, $p = 0.002$. Thus, the methods for extracting DNA and cloning appeared to be reproducible, and the samples generally appeared to be representative of each site. Sequences from replica cores were then combined for further analyses. Because all libraries contained sequences from bacterial groups that were difficult to lyse (e.g., Actinobacteria), cell lysis during the extraction was considered complete (More et al., 1994). Moreover, LIBSHUFF comparisons of libraries from the 2–5 cm and 5–10 cm depths were not significantly different. Therefore, these libraries were combined for some analyses.

3.2. Phylogenic groups represented in clone libraries

Close to one-half of the sequences within each library were only distantly related to cultured organisms. These clones were placed by RDPquery into the ''unclassified'' group (Table 2), indicating that they possessed less than 80% sequence similarity to a sequence from a type stain in the RDP database. In spite of their low similarity to genes from cultured organisms, many of these clones were closely related to other environmental clones obtained from soil. For instance, about 50% of the clones possessed $\geqslant$98% sequence similarity to a previously discovered environmental clone (Fig. 1). In contrast, only 50% of the clones possessed $\geqslant$81% sequence similarly to a type strain. Thus, the composition of these libraries was similar to that found in other soil communities, and these communities are well represented in the databases. The large number of unclassified clones was due to poor representation of soil bacteria among the type strains in culture collections. This conclusion was confirmed by sequencing a longer region of some of the ''unclassified'' clones. The longer sequences ($>$1300 bp) were necessary to examine their relatedness at the phylum level. Many of these clones represented deep branches of phyla with only a few cultured representatives, such as *Acidobacteria, Nitrospira* and *Chloroflexi* (Fig. 2). Others represented lineages for which no cultured representatives existed, such as TM7 and OP3.

For the clones that were classified by RDPquery, the most abundant phylum was Proteobacteria, which consisted of 32% of the total number of clones (Table 2). Moreover, the α-Proteobacteria was the largest proteobacterial group within all the libraries and included clones similar to many common soil bacteria, such as nitrifying bacteria and Rhizobiaceae (Fig. 3). The second most abundant phylogenetic group was *Actinobacteria*, with

Table 2
Phylogenetic assignments of HSB and CWT clones

Phylogenetic group	Number of clones in each library[a]								
	Horseshoe Bend						Coweeta		
	CT5	NT2	NT5	NF5	OF2	OF5	CU	SM	ST
Actinobacteria	4	11	16	10	1	7	4	7	3
Bacteroidetes									
Flavobacteria	1	0	0	0	0	0	0	0	0
Sphingobacteria	3	2	2	2	1	0	0	1	0
Other	0	0	0	1	1	0	2	0	0
Chloroflexi	1	0	0	0	0	0	0	0	0
Firmicutes	1	2	1	4	0	1	2	0	1
Gemmatimonadetes	5	2	8	2	1	1	1	0	0
Nitrospira	1	0	0	1	0	1	0	1	0
Planctomycetes	0	8	1	2	1	3	3	7	3
Proteobacteria									
α-Proteobacteria	20	20	28	17	16	12	25	21	37
β-Proteobacteria	8	8	5	4	2	3	4	1	0
γ-Proteobacteria	2	0	3	3	3	1	2	1	1
δ-Proteobacteria	1	1	1	0	1	1	1	0	0
Unclassified[b]	0	1	2	4	6	8	10	5	5
Verrucomicrobia	4	7	6	4	12	3	10	2	8
Unclassified bacteria[c]	42	47	43	45	46	46	48	54	48
Total (all taxa)	93	109	116	99	91	87	114	100	106

[a]Phylogenetic assignments were based upon comparisons to sequences in the RDP database performed by RDPquery. Clones with $\geqslant$80% of $\geqslant$85% sequence similarity to a type strain in the RDP were assigned to the same phylum or class, respectively.
[b]Clones with $\geqslant$80% but $<$85% sequence similarity to a type species of Proteobacteria.
[c]Clones with $<$80% sequence similarity to a type species in the RDP.

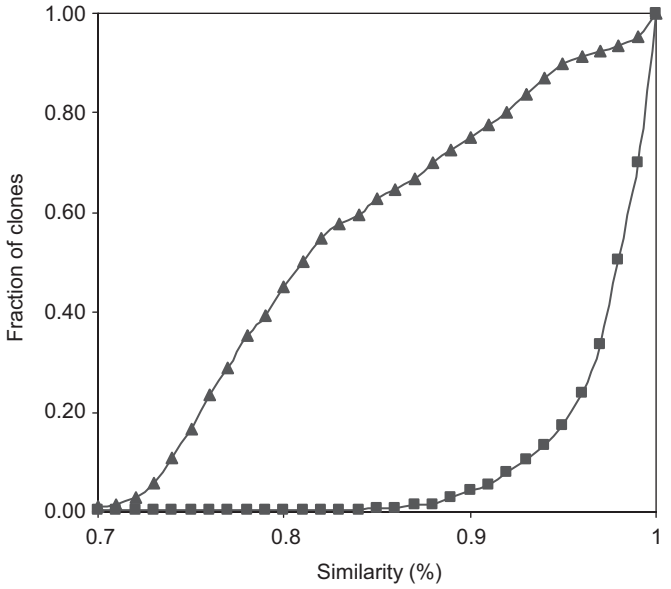

Fig. 1. Similarity of the soil clones to sequences in the Ribosomal Database Project (RDP). Cumulative curve of the fraction of clones with less than the indicated similarity to an RDP sequence. Comparison of the clone sequences to those of type strains in the RDP of $\geqslant$1200 bp (▲). Comparison of clone sequences to the entire RDP including both environmental and organismal genes with lengths both $<$or $\geqslant$1200 bp (■).

about 7% of clones. An important decomposer of plant matter in the soil, this phylum has previously been shown to be abundant in some of these soils by culture-dependent and independent techniques (Furlong et al., 2002). Even though this group is fairly well represented in culture collections, only a few of the clones were closely related to cultured organisms (data not shown). This observation suggests that a large amount of the actinobacterial diversity remains to be characterized by culturing. The third largest phylum, about 6% of clones, represented another well known soil group, *Verrucomicrobia*. Only a few representatives of this group have been cultivated, and their properties are largely unexplored. The remaining phyla present within the libraries included *Planctomycetes, Bacteroidetes*, and *Gemmatimonadetes*. Each consisted of less than 3% of the clones.

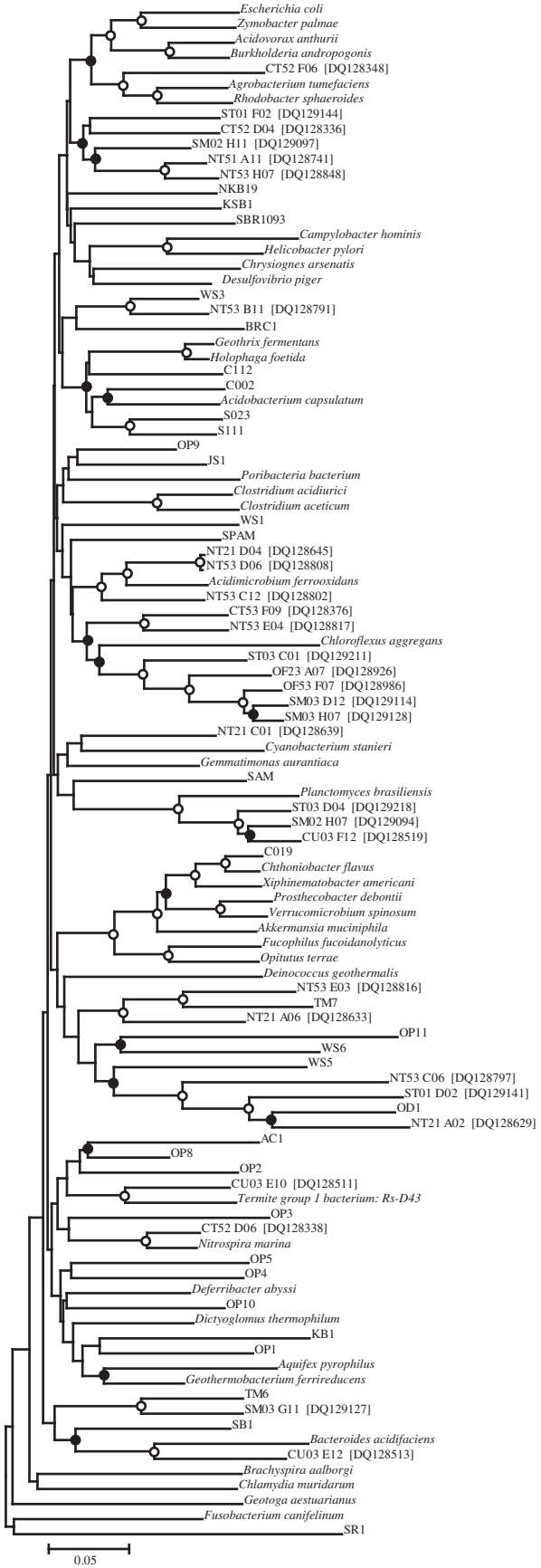

Fig. 2. Phylogeny of representative "unclassified" clones. This neighbor-joining tree was constructed from nearly complete sequences of representative clones. The scale bar represents the Jukes–Cantor evolutionary distance. Open circles (○) denote bootstrap values of $\geqslant$95% and closed circles (●) denote bootstrap values of $\geqslant$50% based upon 100 replicates. Clones are named according to the following conventions: the first two letters indicate the source of the sample (CT, NT, NF, OF, CU, SM, or ST). The first number indicates depth: 0 for 0–10 cm for the CWT cores, 2 for the 2–5 cm depths for some HSB cores, and 5 for the 5–10 cm depths for other HSB cores. The second number indicates the sample replicate. The last letter and number combination indicates the position in the sequencing block and are used to uniquely identify each clone. The GenBank accession number is given in brackets immediately after each clone name.

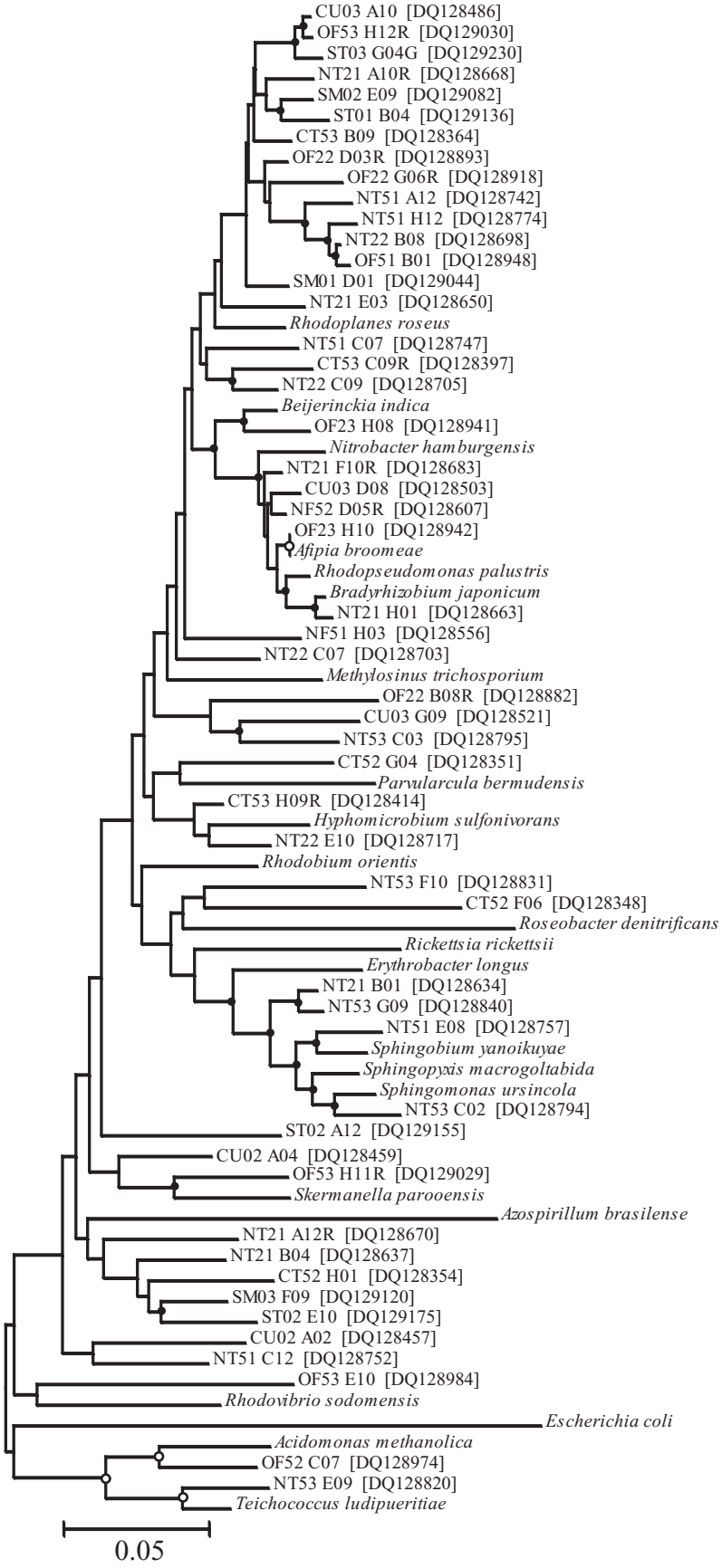

Fig. 3. Phylogeny of representative α-*Proteobacteria* clones from Horseshoe Bend and Coweeta. Otherwise, as Fig. 2.

3.3. Diversity indices

The soil bacterial community was very diverse. For calculation of diversity indices, OTUs were formed at an evolutionary distance of $\leqslant 0.03$ (about 97% sequence similarity). This level is conservative because many OTUs would be expected to include multiple species (Keswani and Whitman, 2001). Because PCR amplification and sequencing can introduce sequencing errors in the range of $\leqslant 1\%$ sequence similarity, a conservative OTU definition was chosen to minimize the effect of these artifacts. Even with this conservative definition of OTUs, the Shannon indices for each library were 0.8–0.9 of their maximum values, and the Chao1 estimators were much higher than the number of sequences examined (Table 3). Similarly, the rarefaction curves failed to plateau, even when similar libraries were combined (Fig. 4). For these reasons, the libraries only sampled a small portion of the bacterial diversity present in the samples.

The bacterial communities in the agricultural soils were more diverse than those of the forest soils. For the three libraries from agricultural soil, the Shannon index was 0.90–0.92 of its maximum value, and the Chao1 estimator was always greater than 200 (Table 3). Except for the NF, the Shannon indices for the forest libraries were less, 0.78–0.86. Likewise, the Chao1 estimator was always below 200. In terms of bacterial diversity, the NF soil more closely resembled the agricultural than the forest soils. This conclusion was also supported by the rarefaction curves, which were nearly identical for the NT, CT and NF libraries (Fig. 4). Likewise, the rarefaction curves for the Coweeta and OF libraries were nearly identical and less steep than those of the agricultural libraries.

Examination of the sizes of abundant OTUs provided a rationale for the higher diversity of the agricultural soil community (Table 4). For the NT soil, one-half of the clones were found in OTUs that were composed of only one member. In the OF soil, only one-third of the clones were in single-member OTUs. Instead, the OF soil contained a few very large OTUs, and about 21% of the clones were found in the two most abundant OTUs. These

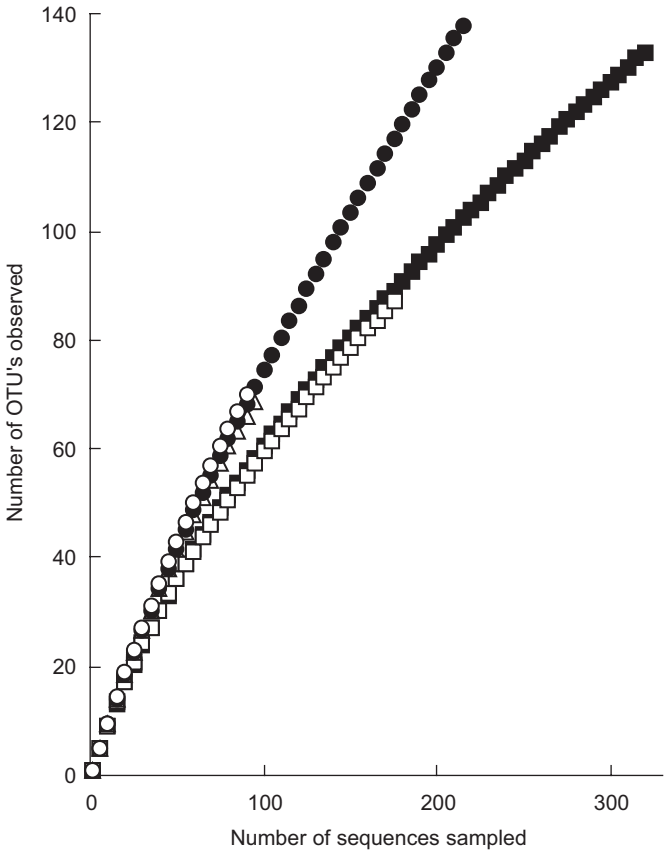

Fig. 4. Rarefaction curves for Horseshoe Bend and Coweeta libraries. OTUs were formed at an evolutionary distance of 0.03 or about 97% sequence similarity. CWT, pooled CU, ST and SM libraries (■); OF, pooled OF2 and OF5 libraries (□); NF, NF5 library (Δ); NT, pooled NT2 and NT5 libraries (●); and CT, CT5 library (○).

Table 3
Diversity indices for the bacterial communities as represented in the 16S rRNA gene libraries[a]

Index	Horseshoe Bend						Coweeta		
	CT5	NT2	NT5	NF5	OF2	OF5	CU	ST	SM
S[b]	72	80	86	71	56	55	68	53	62
N[c]	93	109	116	99	91	87	114	106	100
Evenness[d]	0.98[e]	0.97	0.96	0.97	0.94	0.95	0.94	0.91	0.94
Richness[f]	0.77	0.73	0.74	0.71	0.61	0.63	0.59	0.49	0.62
Shannon	0.92	0.91	0.90	0.90	0.84	0.86	0.84	0.78	0.84
Chao1	211 (393,146)[g]	276 (352,183)	452 (933, 262)	201 (354,140)	127 (243,92)	170 (477,96)	158 (304,116)	89 (206,71)	152 (263,109)

[a]Calculations were based on OTUs formed at an evolutionary distance of ≤ 0.03 (or about 97% sequence similarity).
[b]S defined as the number of OTUs.
[c]N defined as the number of sequences.
[d]Evenness defined as the Shannon index or $H/\log S$, where the maximum value is 2.3.
[e]Evenness, richness, and Shannon diversity (H) are reported as a ratio of actual value to maximum value such that closer to 1 denotes values approaching maximum.
[f]Richness = (number of singleton OTUs-1)/log N. The maximum value is (N-1)/log N
[g]Confidence intervals for the Chao1 estimator are shown in parenthesis.

Table 4
Abundances of OTUs in NT and OF 16S rRNA gene libraries[a]

Size of OTUs (n)	Number of ribotypes of this size in:	
	NT[b]	OF[c]
1	118	62
2	17	13
3	2	3
4	1	2
5	3	4
6	1	0
7	3	0
8	1	1
13	1	0
16	0	1
22	0	1

[a]The number of OTUs with the indicated size (N = number of individuals) found in each of the libraries. OTUs were formed at an evolutionary distance of <0.03 (or about 97% similarity)
[b]Includes both NT2 and NT5, $N = 225$.
[c]Includes both OF2 and OF5, $N = 178$.

Table 5
LIBSHUFF comparisons of Horseshoe Bend samples, including agricultural and forest soils[a]

X library	Y library CT	NT	NF	OF
CT	–[b]	0.643	0.427	0.003
NT	0.013	–	0.159	0.001
NF	0.002	0.012	–	0.106
OF	0.001	0.001	0.307	–

[a]Experimentwise p-value calculated from the Bonferroni correction for all HSB comparisons was 0.012.
[b]No comparison.

OTUs were found in all of the replicate samples, so they could not be attributed to a single, very large concentration of one organism. In contrast, only 9% of the NT clones were in the two most abundant OTUs (Table 4). Thus, the lower diversity of the forest soil resulted from the presence of a few very abundant OTUs.

3.4. Composition of soil bacterial communities

The composition of the bacterial communities in the two agricultural soils differed from each other and from the forest soil communities. First, LIBSHUFF analyses indicated that the CT and NT communities differed (Table 5). The heterologous coverage of the CT by the NT library was not significantly different ($p = 0.643$) from the homologous coverage of the CT library by itself. This implies that the sequences within CT were well represented by the NT library (Singleton et al., 2001). However, the reciprocal comparison was significantly different ($p = 0.013$). Thus, the NT library contained sequences that were absent from CT. In contrast, the comparisons of either NT or CT to OF were significantly different in both directions, indicating that portions of the NT and CT communities were not found in the OF community and that a portion of the OF community was absent from the agricultural communities (Table 5).

In contrast, the composition of the NF community was intermediate between the agricultural and OF communities. By LIBSHUFF analyses, NF was not significantly different from the OF community (Table 5). Likewise, the heterologous coverage of NT and CT by NF was not significantly different from the homologous coverage curves ($p = 0.159$ and 0.427, respectively), indicating that the sequences in NT and CT were also present in NF. However, the reciprocal comparisons were significantly different ($p = 0.012$ and 0.002, respectively), indicating that portions of the NF community were absent from both NT and CT. Hence, while the community in NF was different from that in the agricultural soils, it was not as different as the OF community. These relationships are shown diagrammatically in Fig. 5. This conclusion was further supported by comparison to the bacterial communities in the Coweeta soils. LIBSHUFF did not find significant differences within the Coweeta libraries (experimentwise p-value after Bonferroni correction = 0.34). Likewise, differences were not observed between the OF and Coweeta libraries (experimentwise p-value = 0.23). In contrast, the composition of the NF library was significantly different from all three Coweeta communities, with experimentwise p-values of 0.006 (data not shown). Therefore, while the NF library was more similar to the forest libraries than were the libraries from agricultural soil, it was still somewhat different.

As predicted from the LIBSHUFF analyses, many of the abundant OTUs were differentially enriched in either the agricultural or forest libraries. Nearly one-third of all the clones were in abundant OTUs with a size $\geqslant 8$ (Table 6). These clones were representative of a variety of phyla, including the Proteobacteria, Verrucomicrobia, and many unclassified groups. Because most of these OTUs were not closely related to previously cultured organisms, they are identified by the name of a representative clone.

Four OTUs were significantly enriched in agricultural soils. One of the two α-proteobacterial OTUs, NT21_B01, was related to *Sphingomonas* spp. (Table 6). The other α-proteobacterial OTU, NT51_H12, was distantly related

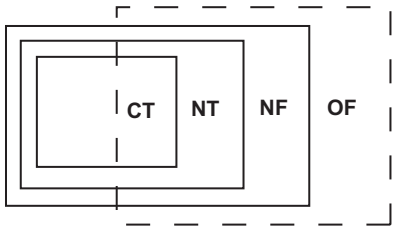

Fig. 5. Schematic representation of the overlap between the compositions of the agricultural and forest libraries suggested by LIBSHUFF analyses. CT is a subset of NT and NF. NT is a subset of NF. NF is a subset of OF, while CT and NT are different from OF.

Table 6
Distribution of the largest OTUs in agricultural and forest soils[a]

Group (N[b])	Clone name[c]	Accession number[d]	AG[e]	FOR[f]	Group (*N*)	Clone name	Accession number	AG	FOR
α-Proteobacteria					Unclassified				
43	CT53_B09*	DQ128364	6	37	23	ST01_A04*	DQ129133	1	22
41	NT21_H01	DQ128663	10	31	19	NF52_F08*	DQ128578	0	19
20	NT51_H12*	DQ128774	13	7	18	CU02_F03		2	16
13	ST02_E10*	DQ129175	0	13	17	OF53_D12	DQ128981	4	13
9	NT21_B01*	DQ128634	9	0	14	OF22_H02RU*	DQ128920	0	14
Verrucomicrobia					11	CT52_D03*	DQ128335	11	0
22	NT53_D02	DQ128804	8	14	10	CT52_B01	DQ128324	3	7
10	CU03_C09	DQ128497	1	9	10	NF52_A05RU*	DQ128594	7	3
10	OF22_E04RU	DQ128909	3	7	9	NT21_B10RU	DQ128671	4	5
Other[g]					9	CU03_F10	DQ128517	2	7
10	CU01_G01	DQ128444	1	9					
8	NT51_B07*	DQ128743	8	0					

[a]OTUs formed at an evolutionary distance $\leqslant 0.03$ (or about $>97\%$ similarity). OTUs whose distribution was not random, $p<0.05$ by the binomial test, are marked by an asterisk. The total number of AG and FOR clones were 318 and 597, respectively.
[b]Number of clones in OTU
[c]Representative clone for each OTU
[d]NCBI Genbank accession number for the representative clone
[e]Number of clones from the libraries of agricultural soils CT5, NT2 and NT5.
[f]Number of clones from the libraries of forest soils NF5, OF2, OF5, CU, SM and ST.
[g]Other includes γ-Proteobacteria, CU01_G01, and β-Proteobacteria, NT51_B07.

to *Rhodoplanes* spp. The remaining OTUs were unclassified and not closely related to any cultured organisms (Table 6). Similarly, five OTUs were significantly enriched in the forest soils. One of the α-proteobacterial OTUs, CT53_B09, was also related to *Rhodoplanes* spp. The different distributions of the two abundant *Rhodoplanes* OTUs in agricultural and forest soils provide evidence for phenotypic or other functional differences between the groups. However, it is also possible that the differences may reflect the individual histories of the soils. The remaining abundant OTUs were distributed in both soil types. Interestingly, the OTU NT21_H01 was closely related to *Bradyrhizobium*, possibly indicating the potential for symbiotic N_2 fixation in both soils. Importantly, because these OTUs represented groups of closely related species, it is possible that some of the individual species within these OTUs may be differentially enriched. For instance, a species group that is evenly distributed between agricultural and forest soils might be composed of multiple species, some of which are specifically enriched in one soil type or the other. However, these species enrichments cannot be detected when only the group is observed.

4. Discussion

4.1. Distinctive bacterial population of agricultural soils

Agriculture causes major changes in the composition and structure of the bacterial communities in soil (Borneman et al., 1997; Kennedy, 1999; McCaig et al., 1999; Nüsslein and Tiedje, 1999; Buckley and Schmidt, 2003). These effects are long-lasting and may persist for decades (Buckley and Schmidt, 2003). The studies at HSB generally confirmed these observations. At this site, the original vegetation was presumably similar to that of the old forest or OF. Prior to 1937, the locations that eventually became the agricultural and successional forest plots were pasture. In 1968, a portion of the pasture was converted to the conventional and no tillage agricultural plots CT and NT. The remainder of the pasture was left unmanaged after 1973 to become the successional forest NF. Evidence that the composition of the bacterial communities within the agricultural plots differed dramatically from those of the original forest at HSB are three fold. First, significant differences in composition were detected with LIBSHUFF analyses. Second, about one-half of the most abundant bacterial groups or OTUs identified at HSB were distributed differently between agricultural and forest soils. Lastly, the diversity indices were different (see below). Even after more than 30 y, the diversity of the successional forest still more closely resembled that of the agricultural plots than the original forest. This level of diversity may represent that of the original pasture from which both were derived. Alternatively, it could also be a coincidence that the agricultural and successional forest communities possess similar levels of diversity that are different from the original soil community. In any case, allowing invasion of the pasture by native vegetation did not fully restore the bacterial community of the soil to its original structure within the time scale of these observations.

While the functional significance of the differences between agricultural and forest bacterial communities is not well understood, they are likely to play important roles in fertility and other soil properties. In this regard, the

identification of a large number of previously uncultured OTUs whose distributions differ between the agricultural and forest communities is particularly important because it provides an experimental approach to linking changes in soil properties to specific bacterial groups. Although outside the scope of the current study, such investigations have the potential to identify specific functional roles for some of these abundant groups.

4.2. Increased diversity in agricultural soil

Diversity indices in the agricultural soil were higher than the indices of old forest soil at HSB as well as Coweeta. While both communities possessed large numbers of rare OTUs, the forest was distinguished by the presence of a few, very abundant OTUs. Since the soils were not exhaustively sampled, the total number of OTUs present was not determined. Thus, the differences in the diversity indices reflect the distributions of abundant OTUs rather than the total number of OTUs. Bearing these points in mind, some possible explanations for the observed increase in diversity in cropland are as follows. One, seasonal variation in the plant community of cropland may create a more dynamic and hence more diverse bacterial community. Two, the cropland is more exposed than forest. Thus, immigration of wind or animal borne bacteria may be a more common occurrence. Three, tillage may disrupt the existing community and increase the likelihood of successful immigration. In contrast, in forest soils disturbances may be relatively rare, which may allow large populations of particularly well-suited bacteria to develop. Four, weeds were common in the HSB fields because of the low application of herbicides. Possibly, the presence of weeds may have resulted in a higher plant diversity and, hence, higher bacterial diversity.

In contrast to this work, previous studies found that tillage reduced the diversity of the bacterial communities of soil (Øvreås and Torsvik, 1998). However, these studies relied upon fingerprinting methods, which were very sensitive to intra-species differences. The methods used here measured diversity at the interspecies level, which may be less sensitive to the effects of tillage. While previous studies found that bacterial diversity was correlated with soil pH (Fierer and Jackson, 2006), a similar correlation was not observed here. Given that the effect of pH on diversity was modest, the correlation coefficients were only 0.6–0.7, the sample sizes in this study may have been too small to observe it.

No tillage or conventional moldboard plowing have profound affects on the physical and chemical properties of soils (Haynes, 2000; Dominy and Haynes, 2002; Balota et al., 2003). At HSB, the composition of the bacterial communities differed in conventional and no tillage plots. Changes in composition of the community are expected given the differences in SOM and microbial biomass and respiration between conventional and no tillage soils. Moreover, the abundance of other soil biota, including nematodes, microarthropods, and protozoans, is much higher under NT than CT management (Garrett et al., 2001; Adl et al., 2006). In microcosm studies, protozoan predation has significant effects on the composition of the bacterial community (Rønn et al., 2002), and differences in the bacterial community at HSB could have resulted from effects of tillage on faunal predation. In contrast, the effects of tillage on the bacterial diversity at HSB were small. Thus, the diversity of the bacterial soil community did not reflect the increased diversity expected for the soil fauna. Given the much higher biomass of the bacterial community in these soils, the fauna-associated bacteria may be a relatively small fraction of the total community, and changes in the fauna may have small affects on the bacterial diversity. Lastly, it is also possible that the type of tillage has a more dramatic affect on diversity at the species or subspecies level than at the species-group level, which was observed here.

4.3. Stability of the bacterial community in forest soils

The similarities of the HSB old forest and Coweeta forest bacterial soil communities were also striking. The libraries were indistinguishable by LIBSHUFF, possessed the same abundant OTUs, and had similar levels of diversities. Few biogeographic studies have been conducted to compare rRNA gene libraries prepared from soil bacterial communities at different locations. Because rRNA gene libraries are very sensitive to the preparation methods, almost by necessity they must be performed within the same laboratory using standardized procedures. The similarities of the communities in forests from these disparate soils suggest that communities of bacteria exist that are widely distributed and presumably highly specialized for these soils.

The implications of these observations are profound. They imply the existence of a large and well adapted soil bacterial community that is largely independent of the specific vegetation and small changes in climate in forest soils. This community is evenly distributed with depth, at least within the first 10 cm.Moreover, it is stable to changes in the surface vegetation such as hurricane wind damage and removal of the understory.

Even after more than 30 y, the diversity of the successional forest still more closely resembled that of the agricultural plots than the original forest. This level of diversity may represent that of the original pasture from which both were derived. Alternatively, it could also be a coincidence that the agricultural and successional forest communities possess similar levels of diversity that are different from the original soil community. In any case, allowing invasion of the pasture by native vegetation did not fully restore the bacterial community of the soil to its original structure within the time scale of these observations. Possibly, the bacterial forest soil communities may take decades to develop their mature forms.

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