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Influence of geographical location, crop type and crop residue cover on bacterial and fungal community structures

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ABSTRACT

The objective of this study was to evaluate the effects of geographical location, crop type, and residue coverage on soil microbial assemblages in Sherm soil from 30 geographically separate commercial fields in Ochiltree and Moore Counties of Texas. Crop residue coverage was derived from spectral data and used to classify sorghum and wheat fields into high and low crop residue categories. Denaturing gradient gel electrophoresis-polymerase chain reaction (DGGE-PCR) assays employing universal PCR primers that target prokaryotic and eukaryotic ribosomal genes were used to evaluate microbial community structure. An interaction between geographical location, crop type, and crop residue coverage was observed. A 50% similarity level was observed for overall bacterial community structure as determined using 16S data while a 59% similarity was observed for overall fungal community structure using 18S data. For the 16S composite dataset, high overall rates of correct classification (ORCC) were observed based on the user-defined groups of county by crop by residue coverage. A similar result was observed for fungal community structure using primer set FR1GC-FF390. Our data support the hypothesis that there are multiple provinces and multiple habitats that govern the assemblage of free-living taxa within the Moore–Ochiltree County agroecosystem. An ancient microbial assemblage based on historical features was identified and is still visible despite the presence of different crop types and cropping systems (Conventional vs. Conservation). For sorghum and wheat grown in Moore and Ochiltree Counties, a two-province state (Moore and Ochiltree Counties) was defined comprised of four habitats i.e. sorghum and wheat habitats influence by degree of residue coverage. Crop type and residue coverage can affect microbial assemblages within a geographical context.

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1. Introduction

Soils provide a complex microhabitat for harboring diverse groups of microorganisms (Borneman et al., 1996; Brodie et al., 2003) and a number of studies point to the inherently complex compositional structure of soil microbial communities (Gelsomino et al., 1999; Lord et al., 2002; Viaud et al., 2000). The interaction of soil type, crop type, agroecosystem, and land management practices may influence agricultural bacterial communities (Buckley and Schmidt, 2003; Girvan et al., 2003; Johnson et al., 2003; Steenwerth et al., 2002). Microorganisms in soil play key roles in the decomposition of plant organic matter, nutrient cycling, and soil structure. Fungi also are important in ecosystem functions such as organic matter decomposition processes (Lynch and Thorn, 2006; Thorn, 1997). Fungal community structure in relationship to a variety of grassland communities has been investigated (Jumpponen et al., 2005; de Souza et al., 2004; Oehl et al., 2003). Influences on fungal community structure and diversity by soil chemistry and structure (Gleeson et al., 2005); floristic composition; and agroecosystem manipulations such as crop rotation (Larkin, 2003), land management (Oehl et al., 2003), fertilizer usage (Jumpponen et al., 2005), and pesticide application (Girvan et al., 2004) have been demonstrated. The worldwide fungal community has been conservatively estimated to comprise approximately 1.5 million species (Hawksworth and Rossman, 1997).

Strong interest exists in understanding the environmental and geographical influences on the structure of terrestrial and aquatic microbial communities (Fierer and Jackson, 2006; Green et al., 2008). However, literature on the relative contributions of geographical distance and environmental habitat is limited and evolving. According to Martiny et al. (2006), habitat is defined as a suite of abiotic and biotic characteristics whereas province is defined as a region in which the biotic composition reflects the legacies of historical events. A number of hypotheses regarding the biogeographical distribution of microbial communities and a proposed framework for investigating microbial assemblages have recently been reviewed (Martiny et al., 2006; Fierer, 2008). Four hypotheses regarding the distribution of microorganisms over habitat and province were proposed. These include: a) null hypothesis — microorganisms are randomly distributed over space

Abbreviations: PCR, polymerase chain reaction; DGGE, Denaturing gradient gel electrophoresis; PCA–DA, principal components analysis–discriminant analysis; ORCC, overall rates of correct classification.

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(microorganisms experience only one habitat and one province); b) the biogeography of microorganisms reflects the influence of contemporary environmental variation (multiple habitats within a single province); c) all spatial variations are due to the lingering effects of historical events (multiple provinces but only one habitat); and d) the distributions of microbial taxa, like those of macroorganisms, reflect the influences of both past events and contemporary environmental conditions (multiple habitats and multiple provinces) (Martiny et al., 2006).

Evidence from a number of studies supports the idea that free-living microbial taxa exhibit biogeographic patterns. Non-random distributions of free-living microbial taxa have been identified in geographical scales ranging from 0.002 to 20,000 km. A null hypothesis that microorganisms are randomly distributed over space was clearly rejected in two studies (Cho and Tiedje, 2000; Oda et al., 2003). Non-random distributions of fluorescent pseudomonads were identified i.e., genetic distance correlated with geographical distance (Cho and Tiedje, 2000) at a scale of 20,000 km while genetic differences were identified in purple non-sulphur bacteria over a 10-meter transect (Oda et al., 2003). Non-random distributions of free-living microbial taxa have been identified in diverse ecosystems such as soil (Fulthorpe et al., 1998; Sliwinski and Goodman, 2004a,b), marine (Riemann and Middelboe, 2002; Pinhassi et al., 2003), agricultural soil (Franklin and Mills, 2003), river plume (Crump et al., 2004; Troussellier et al., 2002), salterns (Casamayor et al., 2002), groundwater (Franklin et al., 1999), and salt- and fresh-water marsh (Franklin et al., 2002; Oda et al., 2003). The distribution of free-living microbial taxa in these ecosystems has been correlated with geographical distance (Cho and Tiedje, 2000; Oda et al., 2003), latitude (Fulthorpe et al., 1998), depth (Garcia-Martinez and Rodriguez-Valera, 2000), salinity (Casamayor et al., 2002; Crump et al., 2004), oxygenation zone (Franklin et al., 1999) and forest vegetation (McArthur et al., 1988) for example. Elevation has been shown to influence patterns of bacterial diversity in the B soil horizon independent of plant diversity (Bryant et al., 2008). A variety of analytical methods were used to discern these influences. These include well accepted 16S analysis using denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (TRFLP), single strand conformation polymorphism (SSCP), restriction fragment length polymorphism (RFLP) and amplified rDNA restriction analysis (ARDRA), 16S/ITS (intergenic transcribe spacer) DNA sequence analysis, rep-PCR, random amplification of polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) assays, isolate allozymes and sole carbon source utilization studies.

All of the above-mentioned studies would reject the null hypothesis but lend little information regarding the relative contribution of spatial variability in microbial distribution and assemblages due to environmental conditions or historical contingencies, thus making it difficult to resolve between the remaining hypotheses. Resemblance matrices for biogeographical analyses were proposed as a potential method to assess the issues of relative contributions from environmental conditions or historical contingencies (Martiny et al., 2006). This requires carefully chosen sampling sites with adequate replicates to statistically assess contributions from province(s) and habitat(s) on microbial assemblages. At the time of the Martiny's review ten studies were identified that allowed definition of environmental segregation and biogeographical provincialism (Martiny et al., 2006). Some of these sites involve hot springs (Papke et al., 2003; Whitaker et al., 2003), lakes (Yannarell and Triplett, 2005; Reche et al., 2005) and soil (Green et al., 2004). Methods employed in these studies include 16S/ITS sequence, multi-locus sequencing (MLS) of isolates and DGGE of 16S rDNA. Recognition of divergence between isolated populations is also dependent upon specific DNA analysis methods employed to detect independent evolutionary events at the appropriate time scale. Bacterial life is estimated to have appeared between three and four billion years ago and thus provides this geological timescale via the 16S rRNA gene. The 16S rRNA evolves slowly over geological time and has been effective in revealing ancient biogeographical differentiation for a number of but not necessarily all microbial species (reviewed in Whitaker, 2006). The prokaryotic 16S rDNA gene has been proven to be a robust molecular chronometer that has greatly facilitated microbial taxonomy and allows for evaluation of microbial diversity and species richness in a variety of ecological systems (Hill et al., 2003). Thus ancient microbial assemblages are reflected in 16S rRNA composition.

The objective of this study was to evaluate geographical distance and climatic factors along with the effects of crop type and residue coverage on soil microbial populations. Both Moore and Ochiltree Counties are in the Panhandle region of Texas and soils in both counties are comprised of a silty clay Sherm soil series, thus potentially identifying a single habitat based on soil type. However Moore and Ochiltree Counties are geographically distinct with respect to elevation, rainfall, and temperature profiles and are separated by a distance of approximately 48 to 220 km from their closest to most distant points (Fig. 1) and may potentially represent more than one province. We defined groups such as county, crop type and residue coverage (potential habitats) and combinations of these groups (county by crop type, county by residue coverage and crop type by residue coverage) to create resemblance matrices to evaluate the influences of geographical factors, crop type and residue coverage on overall microbial community structure. Hierarchal cluster analysis, principal component analysis with discriminant and group separation analysis using two procedures were used to visualize and to assess the significance of interactions between crop type, crop residue coverage and geographical distance on overall microbial assemblages.

2. Methods

2.1. Field sampling

Field survey and soil samples were obtained from 14 field sites in Moore County and 16 field sites in Ochiltree County over the course of one day during the 2005 planting season. Field data including tillage type, soil moisture content using Time Domain Reflectometry, and soil samples for measuring organic and nitrogen content was conducted on 10 May 2005 in Ochiltree and on 17 May 2005 in Moore Counties as part of a regional tillage mapping study. More information on the data collection is provided in Gowda et al. (2008). Moore County is located in the north-central part of the Texas High Plains and has a total area of 236,826 ha and corn, sorghum, and wheat are the major crops in the county. The area of Ochiltree County is about 234,911 ha and sorghum, wheat and corn are the major crops in the county. Crop water needs are supplemented with groundwater from the underlying Ogallala Aquifer. Conventional tillage practice in the study area usually consists of offset disk in the fall. Common conservation tillage practice are no plowing in the fall and sweep or disk plowing at planting that leaves at least 30% of the surface covered with crop residue after planting. Moore County has a mean elevation range of 1006 to 1097 m while for Ochiltree the mean elevation is 914 m (Fig. 1). Annual average precipitation is about 481 mm and 562 mm for Moore and Ochiltree Counties, respectively. Typical planting dates for major crops in the study area vary from the second week of April to the third week of May. July average temperature for Ochiltree is 26.6 °C while for Moore it is 25.2 °C. For January, the average temperatures are 1.3 °C and 3.3 °C for Moore and Ochiltree Counties, respectively. Nearly all of the crop land in both Moore and Ochiltree Counties have nearly level plain to 0.5% with Sherm series (fine, mixed, superactive, mesic Torrertic Paleustolls) (USDA-SCS, 1975). Sherm association covers more than 50% of Moore and Ochiltree counties mainly croplands and Sherm soils make up 90% of the association. Small areas of Donley, Dalhart, Dumas, Harney, Ness, and Sunray soils cover the rest. Not surprisingly, there is not much variation in the organic matter and nitrogen content (Table 1).

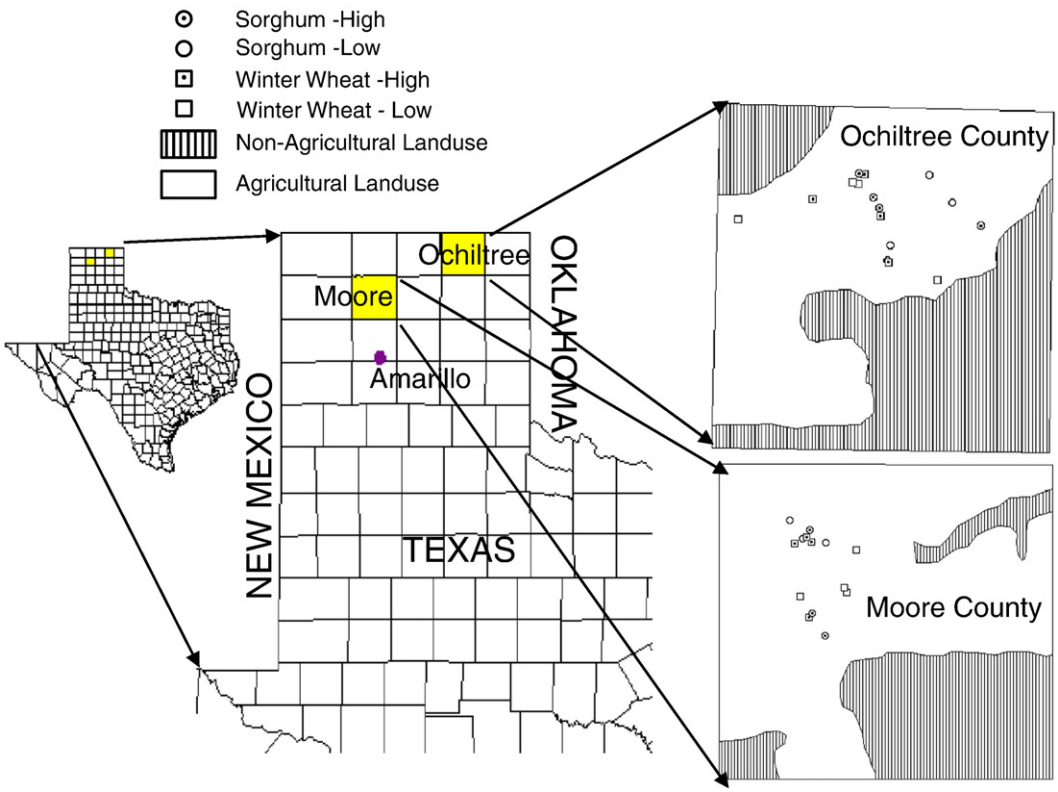

Fig. 1. Geospatial map indicating the location of field sampling sites in Moore and Ochiltree counties.

Five samples from the top 5 cm of soil were composited for each field to minimize sample variation within a field site. Soils were ground and oven dried at 60 °C prior to chemical analysis and DNA extraction. Aliquots used for subsequent DNA extraction were stored at −20 °C until they were extracted. Each field was photographed with a digital camera and a digital infrared camera to generate a composite image (Multispec™) to derive crop residue coverage. Multispec™[1] analyses created residue coverage values ranging from 5 to greater than 60% for both sorghum and wheat crops. Residue coverage less than 25% was assigned to low-residue classification while residue coverage greater than 37% was assigned to high-residue coverage for both sorghum and wheat crops (Table 1).

Table 1
Field characteristics.

County	Crop	Field no	Tillage*	Residue cover (%)	Soil water content (%)	N (%)	C (%)
Moore	Sorghum	33	0	10.8	0.16	0.13	1.36
Moore	Sorghum	34	0	16	0.13	0.17	1.52
Moore	Sorghum	38	0	20.4	0.28	0.16	1.59
Moore	Sorghum	29	1	34	0.23	0.13	1.48
Moore	Sorghum	4	1	38.1	0.25	0.11	1.17
Moore	Sorghum	7	1	45.7	0.19	0.1	0.93
Moore	Wheat	11	0	3.2	0.27	0.16	1.43
Moore	Wheat	14	0	14.2	0.11	0.13	1.27
Moore	Wheat	21	0	18.7	0.27	0.12	1.26
Moore	Wheat	10	0	23.7	0.17	0.12	1.12
Moore	Wheat	32	1	60.8	0.16	0.18	1.87
Moore	Wheat	35	1	67.1	0.18	0.23	2.28
Moore	Wheat	5	1	75.6	0.06	0.14	1.56
Moore	Wheat	37	1	82.1	0.11	0.13	1.37
Ochiltree	Sorghum	35	0	2	0.26	0.123	1.42
Ochiltree	Sorghum	42	0	9.1	0.16	0.124	1.32
Ochiltree	Sorghum	21	0	11.5	0.17	0.121	1.21
Ochiltree	Sorghum	19	0	13.4	0.21	0.11	1.13
Ochiltree	Sorghum	7	0	16.4	0.16	0.12	1.36
Ochiltree	Sorghum	29	1	42.4	0.25	0.112	1.19
Ochiltree	Sorghum	17	1	67.6	0.25	0.135	1.46
Ochiltree	Sorghum	2	1	68.5	0.21	0.12	1.27
Ochiltree	Wheat	26	0	6.1	0.02	0.149	1.48
Ochiltree	Wheat	5	0	23.2	0.2	0.12	1.37
Ochiltree	Wheat	4	0	26.9	0.12	0.13	1.36
Ochiltree	Wheat	3	1	40.9	0.19	0.13	1.43
Ochiltree	Wheat	16	1	45.8	0.26	0.157	1.53
Ochiltree	Wheat	22	1	54.6	0.21	0.161	1.49
Ochiltree	Wheat	6	1	62.3	0.19	0.12	1.27
Ochiltree	Wheat	1	1	69.5	0.25	0.11	1.04

1 — Conservation tillage, 0 — Conventional tillage.

2.2. DNA extraction

DNA was extracted using the UltraClean Soil DNA Isolation Kit according to the manufacturer's protocol (Mo-Bio Laboratories, Carlsbad, CA[1]). DNA was quantified using agarose gel electrophoresis.

2.3. Prokaryotic PCR

2.3.1. PCR primer set F341GC2 and R518

PCR amplifications of the V3 region of the 16S rRNA genes were carried out in a 50 µL reaction mixture containing 10× PCR Buffer, 2.0 mM $MgCl_2$ final concentration, 200 µM of each dNTP, 2.5 U JumpStart Taq DNA Polymerase (Sigma-Aldrich, St. Louis, MO), 20 pmol each of F341GC2 and R518 (Muyzer et al., 1993), and 25 ng of sample DNA. PCR was performed using the following parameters: initial denaturation at 95 °C for 5 min, 30 cycles of 95 °C for 1 min, 60 °C for 30 s, 72 °C for 1 min, final extension at 72 °C for 7.5 min. Three sets of PCR products were concentrated into 30 µL using the MinElute PCR purification kit (Qiagen, Valencia, CA).

[1] Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

2.3.2. Primer set 1092F and 1392RGC

PCR amplifications of the V7 region of the 16S rRNA genes were carried out in a 50 μL reaction mixture containing 10× PCR Buffer, 2.0 mM $MgCl_2$ final concentration, 200 μM of each dNTP, 2.5 U JumpStart Taq DNA Polymerase, 50 pmol each of 1092F and 1392R (Sun et al., 2004), and 25 ng of sample DNA. PCR was performed using the following parameters: initial denaturation at 95 °C for 2 min, 9 cycles of touchdown PCR at 95 °C for 30 s, 56 °C for 1 min, decreasing by 1 °C every cycle, and 72 °C for 40 s; 22 cycles of 95 °C for 30 s, 47 °C for 30 s, 72 °C for 1 min, final extension at 72 °C for 7.5 min. Two sets of PCR products were concentrated into 30 μL using the MinElute PCR purification kit.

2.4. Eukaryotic PCR

2.4.1. FF390 and FR1-GC

PCR amplifications of the 18S rRNA genes were carried out in a 50 μL reaction mixture containing of 10× PCR Buffer, 1.5 mM $MgCl_2$ final concentration, 200 μM of each dNTP, 2.5 U JumpStart Taq DNA Polymerase, 20 pmol each of FF390 (CGATAACGAACGAGACCT) and FR1-GC (AICCATTCAATCGGTAIT) (Kowalchuk and Smit, 2004), and 25 ng of sample DNA. PCR was performed using the following parameters: initial denaturation at 95 °C for 2 min, 8 cycles of touchdown PCR at 95 °C for 30 s, 55 °C for 30 s, decreasing by 2 °C every 2 cycles, and 72 °C for 1 min; 27 cycles of 95 °C for 30 s, 47 °C for 30 s, 72 °C for 1 min, final extension at 72 °C for 7.5 min. Two sets of PCR products were concentrated into 30 μL using the MinElute PCR purification kit.

2.5. DGGE analysis

DGGE analysis was performed using the DCode System (BioRad, Hercules, CA). Profiles of amplified 16S rRNA sequences were produced on 6.5% polyacrylamide gels with a 30–60% denaturing gradient (100% denaturant was 7 M urea and 40% [vol/vol] deionized formamide). Approximately 1.0–1.5 μg of DNA was loaded into the wells. The Low Molecular Weight DNA Ladder (Bioline, Randolph, MA) was used as a standard in combination with an amplicon generated from *E. coli* K12 DNA with the primer set F341GC2-R518. Gels were run for 16 h at 50 V at 60 °C. DNA fragments were stained for 30 min using SYBR Green I (Invitrogen, Eugene, OR).

2.6. Image and data analysis

Digital images of the gels were obtained using a Kodak Image Station 4000MM (Eastman Kodak Company, New Haven, CT). All digital images were processed using various algorithms within the Bionumerics v.5 software package (Applied Maths, Austin, TX). Processed images yielded normalized DNA fingerprints (based on reference DNAs) thus allowing for inter-gel comparison of various community DNA samples. For each fingerprint type, a whole-pattern (both the number of bands and the intensity of the bands) analysis was completed with the Pearson coefficient (creating a Pearson product-moment correlation). Cluster analysis on DNA fingerprint patterns was conducted using the unweighted paired-group method using mathematical averages (UPGMA) to generate a dendrogram (significant clusters were determined based on a cluster cutoff function — Point-bisectional correlation) using the Pearson coefficient. An ORCC analysis was performed on the assigned groups using bootstrap analysis using the Jackknife method with ties assigned to their own group. In this method, a sample is randomly deleted and the experiment is reanalyzed. This can be done by calculating the average similarity with each group or finding the maximum similarity with each group. The assessment of significance (internal stability) of an assigned group will be influenced if average and maximum similarity values of a group differ substantially. Both procedures were done for all entries to more clearly visualize the internal stability of each group assignment. This simulation then allows for a determination of the correct rate of classification of the individual groups and for an overall rate of correct classification (ORCC) of the various datasets based on user-defined groups. These results were reported in a group separation matrix and it must be noted that values in the matrix table were not reciprocal (see the following discussion). Band matching function and group assignment based on county, crop type and residue coverage, and group combinations (county by crop type, county by residue coverage and crop type by residue coverage) were conducted prior to principal components analysis–discriminant analysis (PCA–DA).

3. Results

3.1. Soil sample characteristics

Soil tillage, residue coverage, water, N and C content are presented in Table 1. The organic matter and nitrogen content in the study fields varied from 0.93 to 2.28% and 0.1 to 0.23%, respectively. The soil moisture content at the time of data collection varied from 0.02 to 0.27% with an average value of 0.19%.

3.2. 16S composite dataset analysis

3.2.1. Cluster analysis

A UPGMA derived dendrogram based on a 16S composite dataset indicated a bacterial community composition with an overall similarity of 50.4% (Fig. 2). Two main DNA similarity groups with one outlying cluster were determined (based on the number of clusters identified with greater than 60% similarity). Two significant subclusters (greater than 69% similarity) were present within one of the main clusters. Inspection of the dendrogram suggests an influence of county, crop type and residue coverage on overall microbial community composition. Groupings by county, crop type and residue coverage and county by crop type, county by residue coverage and crop type by residue coverage were contained within significant clusters at similarity levels approaching 70%.

3.2.2. County, crop type, and residue coverage

User-defined groups of county, crop type, and residue coverage were then assigned as group variables to more clearly define the influence of geographical location, crop type and tillage management on overall bacterial community composition. Two further methods of analysis were then applied to more clearly visualize the effect of the group variables on bacterial community structure. PCA–DA using county as the group variable revealed two groups with slight overlap of several members of each group (data not shown). The user-defined groups (county, crop type and residue coverage) yielded high ORCC (range, 83.3 to 100%) and a significant assessment using the maximum similarity method (Table 2). The average similarity resulted in lower ORCC (range, 56.7 to 86.7%) and evaluation of residue coverage using this method resulted in a non-significant assessment with 57% of the Moore samples assigned as Ochiltree samples. Sorghum was the crop type with the highest classification score (100% and 92.9% for the average- and maximum similarity methods) while residue coverage resulted in cross assignment using both methods (Table 2).

3.2.3. County by crop type

User-defined groups of county by crop type, county by residue coverage, and crop type by residue coverage were then assigned as group variables to more clearly define the influence of location, crop type and crop management on overall bacterial community composition. PCA–DA of the 16S composite dataset, using county by crop type (i.e., Moore-sorghum, Moore-wheat, Ochiltree-sorghum, and

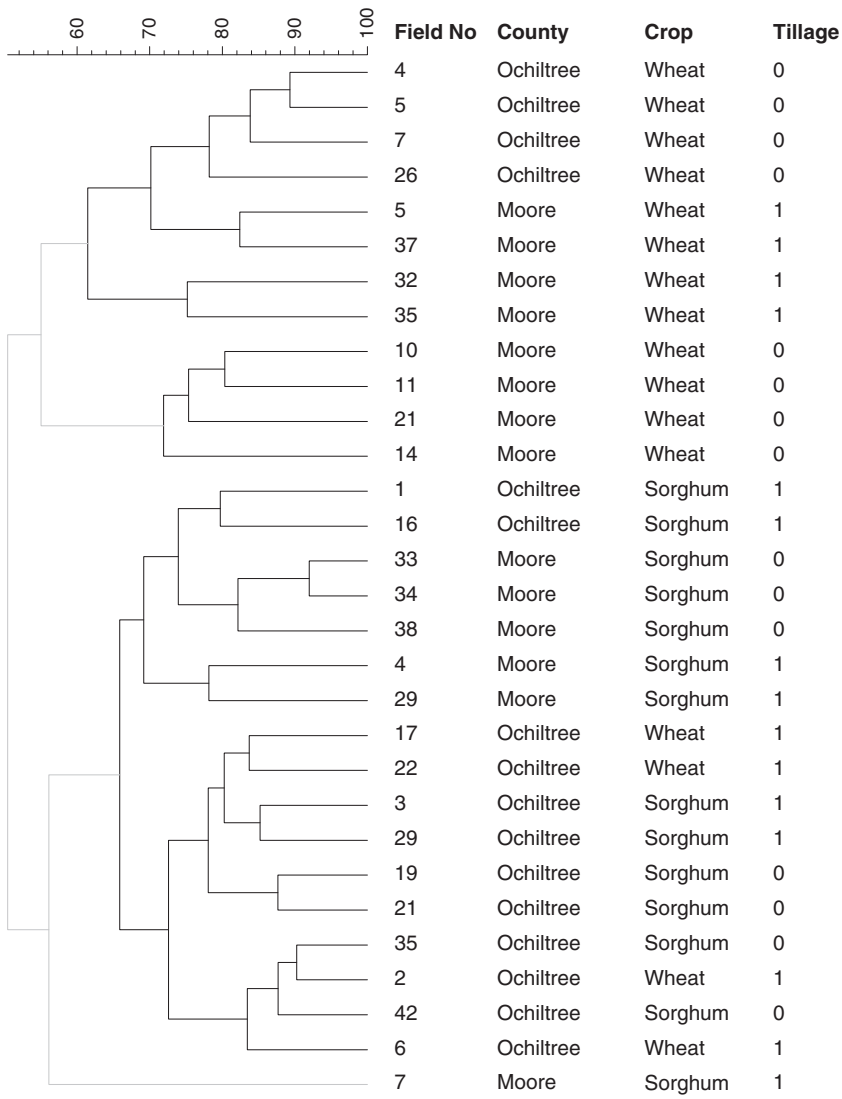

Fig. 2. A UPGMA derived dendrogram from cluster analysis of a similarity matrix calculated using the Pearson coefficient. The composite dataset used in this analysis was derived from DGGE-PCR experiments conducted on soil samples from 30 sites using 16S primer sets 341GC2-518R and 1092F-1392R. Dark solid lines indicate the location of significant clusters within the overall dendrogram.

Ochiltree-wheat) as the classification variable revealed four well-defined groups (Fig. 3a). Moore-sorghum was contained entirely within the lower right-hand quadrant while Moore-wheat was located mainly in the upper left-hand quadrant. Ochiltree-sorghum and Ochiltree-wheat were located in two well-defined although closely related groups. Moore-sorghum was the most tightly grouped variable and along with Moore-wheat had the highest correct rate of classification score of 100.0% with an ORCC of 86.7%, (Chi-square 68.00; $p<0.001$) analyzed using the maximum similarity procedure (Table 3). Ochiltree-wheat was the least correctly classified group (correct classification, 62.5%) while being assigned within the Ochiltree-sorghum group 37.5% of the time. Slightly lower ORCC values were observed using the average similarity procedure which gave an ORCC of 73.3% (Chi-square 47.14; $p<0.001$, data not shown).

Table 2
Composite 16S dataset group separation scores based on a) county, b) crop type and c) residue coverage.

Group	Ave-Sim		Group	Max-Sim	
County[a]	Moore	Ochiltree	County	Moore	Ochiltree
Moore	42.9	0.0	Moore	100.0	0.0
Ochiltree	57.1	100.0	Ochiltree	0.0	100.0
Crop type[b]	Sorghum	Wheat	Crop type	Sorghum	Wheat
Sorghum	100.0	25.0	Sorghum	92.9	18.8
Wheat	0.0	75.0	Wheat	7.1	81.3
Residue coverage[c]	Low	High	Residue coverage	Low	High
Low	53.3	40.0	Low	93.3	26.7
High	46.7	60.0	High	6.7	73.3

[a] OC% 73.3, X^2; 8.571, $p=0.003$, OC% 100.0, X^2; 30.000, $p<0.001$.
[b] OC% 86.7, X^2; 17.500, $p<0.001$, OC% 86.7, X^2; 16.476, $p<0.001$.
[c] OC% 56.7, X^2; 0.536, $p=0.464$, OC% 83.3, X^2; 13.889, $p<0.001$.

3.2.4. County by residue

PCA evaluation of the 16S composite dataset using county by residue as the grouping variable revealed two distinct groupings of Moore-low and -high residue field sites within the ordination plot whereas Ochiltree-low and -high residue field sites were contained within an overlapping group located primarily in the lower left-hand quadrant (Fig. 3b). The significance of this grouping variable yielded different results depending whether analyzed by using either the average or maximum similarity method. Moore-low residue was correctly identified 100% of the time followed by Moore-high and Ochiltree-low residue (85.7%) and Ochiltree-high residue (62.5%)

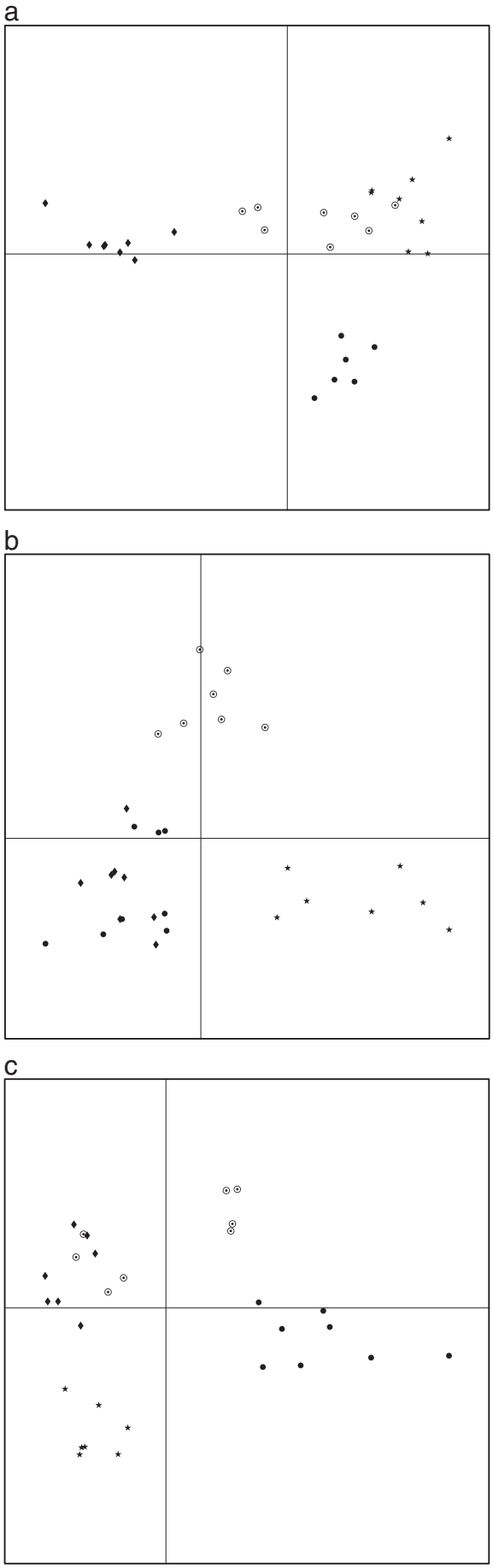

Table 3
Composite 16S group separation tables based on maximum similarity method for a) county by crop type, b) county by residue coverage and c) crop type by residue coverage.

County-crop type[a]	M-S	O-S	M-W	O-W
Moore-Sorghum (M-S)	100	0	0	0
Ochiltree-Sorghum (O-S)	0	87.5	0	37.5
Moore-Wheat (M-W)	0	0	100	0
Ochiltree-Wheat (O-W)	0	12.5	0	62.5
County-residue coverage[b]	**O-L**	**M-L**	**O-H**	**M-H**
Ochiltree-low (O-L)	87.5	0	37.5	0
Moore-low (M-L)	0	100	0	14.3
Ochiltree-high (O-H)	12.5	0	62.5	0
Moore-high (M-H)	0	0	0	85.7
Crop type-residue coverage[c]	**W-L**	**S-L**	**S-H**	**W-H**
Wheat-low (W-L)	100	0	0	0
Sorghum-low (S-L)	0	85.7	14.3	37.5
Sorghum-high (S-H)	0	0	85.7	0
Wheat-high (W-H)	0	14.3	0	62.5

[a] OC% 86.7, X^2: 68.00, $p<0.001$.
[b] OC% 83.3, X^2: 60.50, $p<0.001$.
[c] OC% 83.3, X^2: 61.29, $p<0.001$.

using the maximum similarity method (Table 3) that gave an ORCC of 83.3% (Chi-square; 60.5, $p<0.001$). The average similarity method indicates that Moore-high residue is incorrectly assigned (0.0%) to its own group. Since this zero value is present on the diagonal of the matrix, it is not possible to obtain a Chi-square score to assess the significance of this assessment using the average similarity method.

3.2.5. Crop type by residue

PCA ordination plots of crop type by residue analysis of the composite 16S dataset reveal that wheat- and sorghum-low residue field sites were contained within two well-resolved groups while there was an overlap of the wheat and sorghum-high residue field sites (Fig. 3c). A similar assessment of the internal stability of this group assignment was obtained using either the average similarity method which yielded an ORCC of 73.3% (Chi-square; 46.33, $p<0.001$) or the maximum similarity method leading to an ORCCORCC of 83.3% (Chi-square; 61.29, $p<0.001$). However there were some differences in the correct rates of classification of the various groups. Wheat-low residue was correctly assigned 100% of the time followed by sorghum-low and -high residue groups (85.7%) and wheat-low residue (62.5%) using the maximum similarity method (Table 3) whereas wheat-low residue was correctly assigned 87.5% of the time while sorghum-low residue was correctly assigned 100% of the time using the average similarity method. Both sorghum- and wheat-high residue had lower rates of correct classification in the average similarity method (71.4 and 37.5%, respectively) than the maximum similarity method (85.7% and 62.5%, respectively) with wheat-high residue being assigned 37.5% of the time to sorghum-high residue.

Fig. 3. a) PCA of the 16S composite dataset with the user-defined groups of county by crop type. Legend as follows: diamond — Moore-wheat, circle — Moore-sorghum, cylinder — Ochiltree-wheat and star — Ochiltree-sorghum. Explained variation; X = 52.2%, Y = 27.5%. b) PCA of the 16S composite dataset with the user-defined groups of county by residue coverage. Legend as follows: diamond — Ochiltree-Conservation tillage (>30%), circle — Ochiltree-Conventional tillage (<30%), cylinder — Moore-Conservation tillage (>30%), and star — Moore-Conventional tillage (<30%). Explained variation; X = 38.7%, Y = 33.5%. c) PCA of the 16S composite dataset with the user-defined groups of crop type by residue coverage. Legend as follows: diamond — sorghum-high, star — sorghum-low, cylinder — wheat-high and circle — wheat-low residue. Explained variation; X = 41.4%, Y = 30.8%.

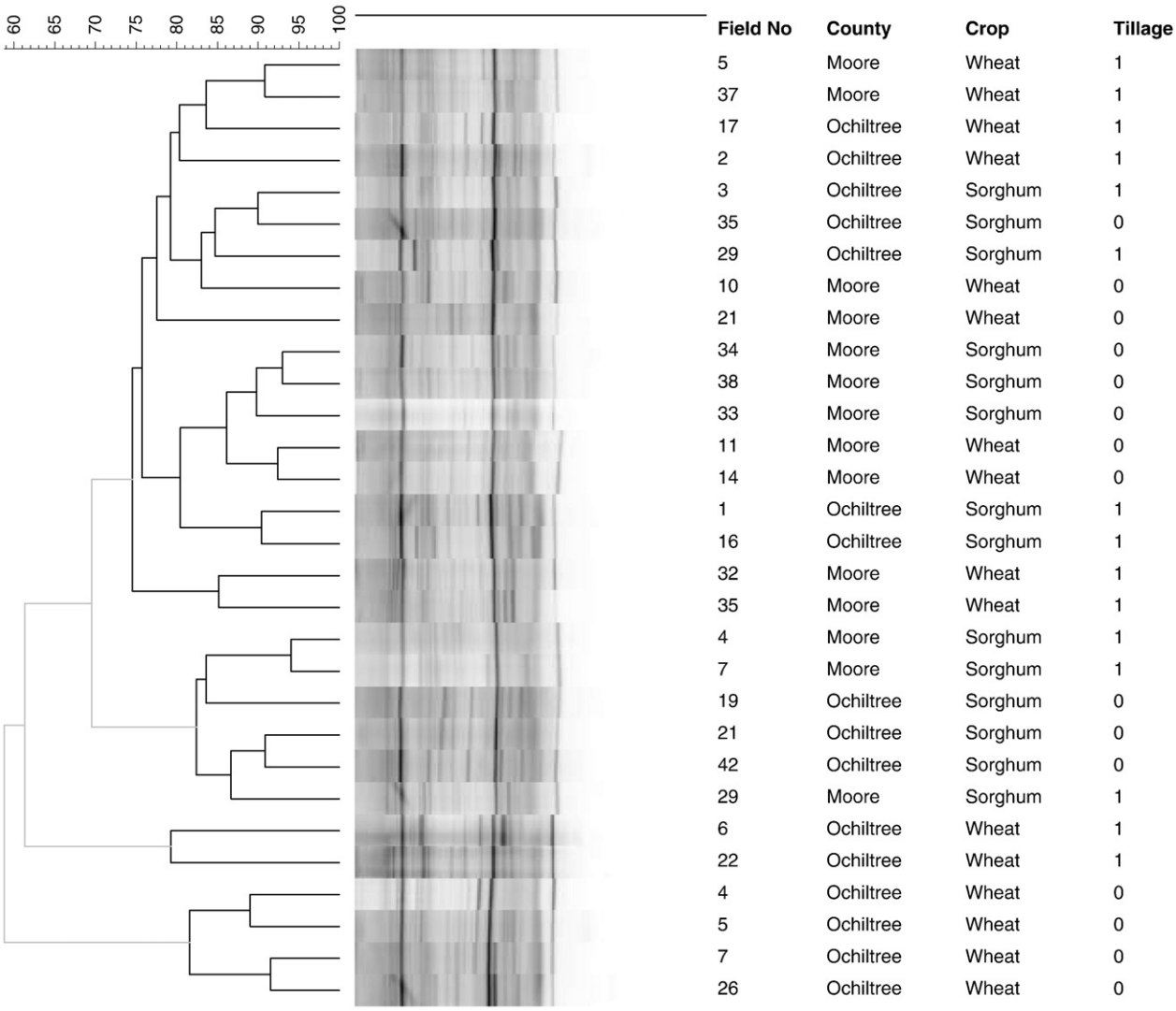

Fig. 4. A UPGMA derived dendrogram based on a similarity matrix determined from a cluster analysis using the Pearson coefficient. The dendrogram was determined from PCR reactions using fungal specific 18S primers FR1GC and FF390 in experiments from 30 sample sites. Dark solid lines indicate the location of significant clusters within the overall dendrogram.

3.3. Fungal community structure FR1GC-FF390 dataset

3.3.1. Cluster analysis

An overall fungal community structure with a similarity of 58.8% was observed based on analysis of fungal community composition using 18S fungal specific primers FR1GC and FF390 (Fig. 4). One large significant cluster comprised of several smaller clusters was observed with other insignificantly linked significant subclusters also observed. An interaction of county, crop type and residue coverage on overall fungal community structure was suggested, analogous to that seen with overall bacterial community structure based on ribosomal data.

3.3.2. County, crop type, and residue coverage

PCA–DA using county as the group variable revealed three groups with overlap of several members of each county present within two groups (data not shown). The user-defined groups of county, crop type and residue coverage gave an ORCC ranging from 83.3 to 90% and a significant assessment using the maximum similarity method (Table 4). Sorghum was the only correctly classified crop type based on the maximum similarity method whereas Moore County was correctly assigned 100% of the time based on the average similarity method. The average similarity method resulted in a lower ORCC (range, 66.7 to 76.7%) and a higher number of cross assignment of members from one group to the other group. Both high- and low-residue members were misclassified using both average and maximum similarity methods (Table 4).

3.3.3. County by crop type

An ordination plot based on PCA of 18S data using county by crop type as group variables revealed three well-resolved groups (Fig. 5a).

Table 4
18S dataset group separation scores based on a) county, b) crop type and c) residue coverage.

Group	Ave-Sim		Group	Max-Sim	
County[a]	Moore	Ochiltree	County	Moore	Ochiltree
Moore	100.0	62.5	Moore	78.6	12.5
Ochiltree	0.0	37.5	Ochiltree	21.4	87.5
Crop type[b]	Sorghum	Wheat	Crop type	Sorghum	Wheat
Sorghum	92.9	50.0	Sorghum	100.0	18.8
Wheat	7.1	50.0	Wheat	0.0	81.3
Residue coverage[c]	Low	High	Residue coverage	Low	High
Low	80.0	26.7	Low	86.7	20.0
High	20.0	73.3	High	13.3	80.0

[a] OC% 66.7, X^2; 6.563, $p = 0.010$, OC% 83.3, X^2; 13.274, $p < 0.001$.
[b] OC% 70.0, X^2; 6.531, $p = 0.011$, OC% 90.0, X^2; 20.074, $p < 0.001$.
[c] OC% 76.7, X^2; 8.571, $p = 0.003$, OC% 83.3, X^2; 13.393, $p < 0.001$.

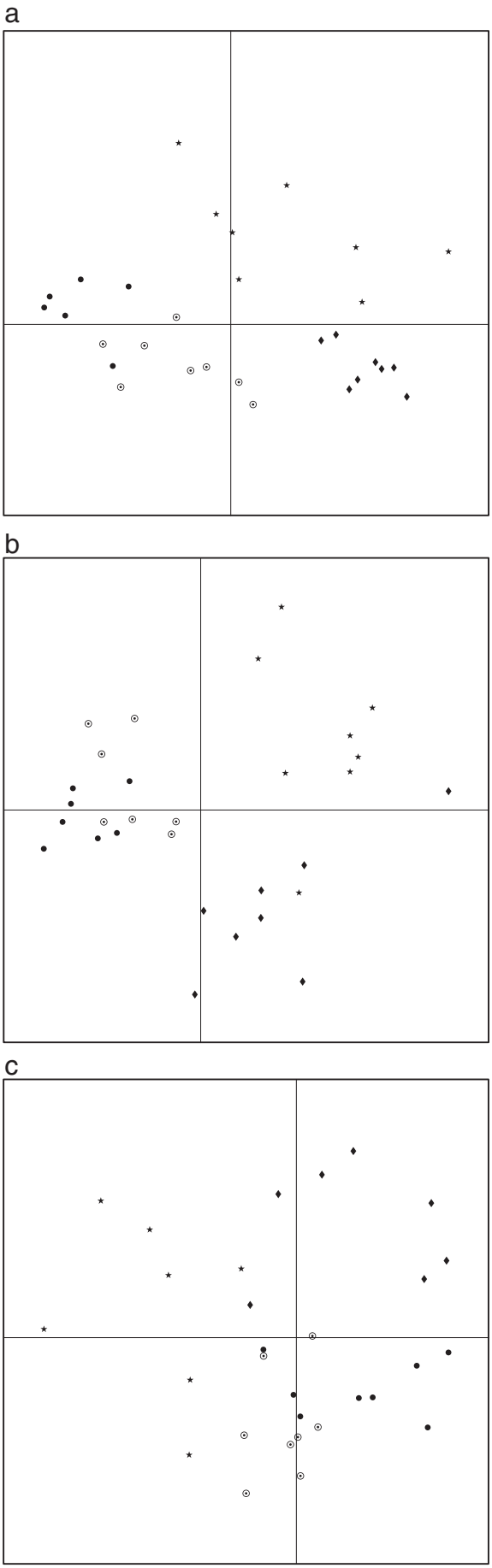

Table 5
Fungal 18S group separation tables based on maximum similarity method for a) county by crop type, b) county by residue coverage and c) crop type by residue coverage.

County-crop type[a]	M-S	O-S	M-W	O-W
Moore-Sorghum (M-S)	83.3	0	0	0
Ochiltree-Sorghum (O-S)	16.7	100	25	12.5
Moore-Wheat (M-W)	0	0	75	25
Ochiltree-Wheat (O-W)	0	0	0	62.5
County-residue coverage[b]	**O-L**	**M-L**	**O-H**	**M-H**
Ochiltree-low (O-L)	87.5	14.3	0	0
Moore-low (M-L)	0	71.4	25	14.3
Ochiltree-high (O-H)	12.5	14.3	50	0
Moore-high (M-H)	0	0	25	85.7
Crop type-residue coverage[c]	**W-L**	**S-L**	**S-H**	**W-H**
Wheat-low (W-L)	75	0	0	0
Sorghum-low (S-L)	12.5	85.7	28.6	12.5
Sorghum-high (S-H)	12.5	14.3	71.4	0
Wheat-high (W-H)	0	0	0	87.5

[a] OC% 80.0, X^2: 54.48, $p<0.001$.
[b] OC% 73.3, X^2: 42.78, $p<0.001$.
[c] OC% 80.0, X^2: 53.10, $p<0.001$.

Ochiltree-sorghum and -wheat were located in the upper and lower right quadrants, while Moore-sorghum and -wheat groups were located on the left-hand side. An ORCC of 80% (Chi-square; 54.48, $p<0.001$) was observed with the maximum similarity method (Table 5). The average similarity method failed to yield a significant assessment due to one group variable yielding a correct rate of classification of zero. Ochiltree-sorghum was correctly classified 100% of the time followed by Moore-sorghum (83.3%), Moore-wheat (75%) and Ochiltree-wheat (62.5%).

3.3.4. County by residue

Assignment of group variable of county by residue resulted in three well-defined although broadly dispersed groups visible in the ordination plot based on the fungal 18S dataset (Fig. 5b). Moore-low and -high residue occurred in one group while Ochiltree-low and -high residue sites were located in two large groups with one member of each classification present in the opposite group. This observation is supported by assessment of the internal stability of this group assignment by using both the average and maximum similarity methods (Table 5). An ORCC of 63.3% (Chi-square; 30.41, $p<0.001$) was observed for the average similarity procedure whereas for the maximum similarity procedure, an ORCC was 73.3% (Chi-square; 42.78, $p<0.001$). Moore-low residue was correctly classified 100% of the time using the average similarity method while Ochiltree-low residue was correctly classified 87.5% of the time with the maximum similarity method.

3.3.5. Crop type by residue

Assignment of group variable of crop type by residue resulted in discernable although well-dispersed groups visible in the ordination plot based on fungal 18S dataset (Fig. 5c). This observation is supported by assessment of the internal stability of this group assignment by using both the average and maximum similarity

Fig. 5. a) PCA of fungal DGGE-PCR analysis using primer sets FR1GC and FF390 based on user-defined groups of county by crop type. Legend as follows: circle — Moore-sorghum, cylinder — Moore-wheat, star — Ochiltree-sorghum, diamond — Ochiltree-wheat. Explained variation; X = 47.7%, Y = 26.0%. b) PCA of fungal DGGE-PCR analysis using primer sets FR1GC and FF390 based on user-defined groups of crop type by residue coverage. Legend as follows: circle — Moore-low, star — Ochiltree-low, diamond — Ochiltree-high and cylinder — Moore-high residue. Explained variation; X = 47.1%, Y = 31.4%. c) PCA of fungal DGGE-PCR analysis using primer sets FR1GC and FF390 based on user-defined groups for crop type by residue coverage. Legend as follows: circle — wheat-low, star — sorghum-low, diamond — sorghum-high and cylinder — wheat-high residue. Explained variation; X = 36.2%, Y = 34.3%.

methods. An ORCC of 60% (Chi-square; 30.98, $p<0.001$) was observed for the average similarity procedure whereas for the maximum similarity procedure (Table 5), an ORCC was 80% (Chi-square; 53.10, $p<0.001$). Wheat-high residue was correctly classified 87.5% by the maximum similarity method followed by sorghum-low residue (85.7%), wheat-low residue (75%), and sorghum-high residue (71.4%).

4. Discussion

4.1. Assessment of province

Combined analysis of 16S composite dataset and the 18S data using hierarchical clustering, PCA–DA and two group separation analysis methods to assess the internal stability (significance) of the assigned grouping variable(s) lends support to the idea that two distinct provinces are present. Both dendrograms (16S and 18S) suggested an interaction of geographical location (county), crop type and residue coverage on overall microbial assemblages. Assignment of the group variable county most strongly supports the idea that Ochiltree is one province and is distinct from the province defined by Moore County. For bacterial assemblages, Ochiltree County was consistently assigned within its own group based on both group separation procedures while Moore County was more frequently assigned as an Ochiltree group member based on the average similarity procedure (Table 2). Insight into province structure was also provided by data on fungal assemblages. Moore County was correctly classified 100% of the time based on the average similarity method while members of both Moore and Ochiltree Counties were cross assigned using the maximum similarity method (Table 4). Thus the effect of counties (province) is not as apparent or strong on fungal community composition as it is for bacterial composition. It may be that the effect of province on fungal community composition is somewhat confounded by the evolutionary timescale during which the 18S rRNA gene has had time to evolve. Fungi are thought to have originated between 760 million to 1.06 billion years ago (Lücking et al., 2009) thus they clearly represent a much shorter evolutionary timescale than that which can be revealed by the 16S rRNA bacterial gene.

Use of county by crop type and county by residue coverage as user-defined grouping variables was also consistent with the assessment of Moore and Ochiltree Counties as separate provinces. Low levels of county by group cross assignment were present thus suggesting the presence of a historical influence on microbial assemblages. No county field site was misclassified as another county (i.e. Moore as Ochiltree or vice versa) for the county by crop type and county by residue coverage group variables based on the maximum similarity procedure. However, Ochiltree-wheat was classified as Ochiltree-sorghum 37.5% of the time and Ochiltree-sorghum was classified as Ochiltree-wheat 12.5% of the time. For the average similarity method there were low instances (<17%) of county misclassification.

4.2. Assessment of habitat

Crop type and residue coverage potentially represent distinct habitats and their combination may represent four distinct habitats. Prokaryotic and eukaryotic rDNA datasets identify crop type as distinct habitats (Tables 2–5). Both 16S and 18S datasets support sorghum as providing the stronger effect as 'habitat' based on the analysis using both the average and maximum similarity methods. Sorghum was correctly classified from 93 to 100% of the time with either procedure. For the bacterial assemblage, wheat was correctly classified 75 to 81% of the time and for the fungal assemblage, wheat was correctly classified 50 to 81% of the time. Crop type by residue analysis is consistent with crop type as a major habitat. Crop type by residue coverage resulted in low instances of low- vs. high-residue misclassification and occasional instances of cross-classifications of sorghum-low residue to wheat-high residue, sorghum-high to sorghum-low residue and wheat-high to sorghum-low residue. Residue coverage was significantly assessed for both 16S and 18S datasets by the maximum similarity method (Tables 2 and 4) and tends to have the highest rate of cross group misclassification. Only the fungal 18S dataset gave a significant assessment using the average similarity method. However the bulk of the data still support the assignment of residue coverage as a habitat feature although this appears to be the weakest of the interactions that we have assessed herein.

The creation of resemblance matrices (user-defined groups) and application of hierarchical clustering, PCA–DA, and group separation analysis using two methods to assess the internal stability (significance) of the assigned grouping variables allows drawing conclusions regarding the biogeographical distribution of free-living taxa within this defined area. The slowly evolving 16S rRNA has effectively revealed ancient biogeographical evidence over geological time. An ancient microbial assemblage based on historical features was identified and was still visible despite the presence of different crop types and cropping systems (Conventional vs. Conservation) within the same soil series. Thus the combined effects of elevation, rainfall amount and distribution within the year, and temperature profile differences between Moore and Ochiltree Counties over geological time appears to and may have helped to established and may help to maintain this feature which is indicative of province. Imposed on this ancient assemblage are clear influences of crop type followed by tillage practice. Cropping practices over the last decade or so in both counties were fairly similar. Crop type along with agricultural management practices has been documented in a number of agricultural systems as influencing either or both the bacterial and fungal community composition. Our data overall clearly support this contention. For sorghum and wheat grown in Moore and Ochiltree Counties, we have defined a two-province state comprised of four habitats i.e. sorghum and wheat assemblages influence by residue state.

References

Borneman, J.P., Skroch, W., O'Sullivan, K.M., Palus, J.A., Rumjanek, N.G., Jansen, J.L., Nienhuis, J., Triplett, E.W., 1996. Molecular microbial diversity of an agricultural soil in Wisconsin. Appl. Environ. Microbiol. 62, 1935–1943.

Brodie, E., Edwards, S., Clipson, N., 2003. Soil fungal community structure in a temperate upland grassland soil. FEMS Microbiol. Ecol. 45, 105–114.

Bryant, J.A., Lamanna, C., Morlon, H., Kerkhoff, A.J., Enquist, B.J., Green, J.L., 2008. Microbes on mountainsides: contrasting elevational patterns of bacterial and plant diversity. Proc. Natl Acad. Sci. USA 105, 11505–11511.

Buckley, D.H., Schmidt, T.M., 2003. Diversity and dynamics of microbial communities in soils from agro-ecosystems. Environ. Microbiol. 5, 441–452.

Casamayor, E.O., Massana, R., Benlloch, S., Øvreas, L., Diez, B., Goddard, V.J., Gasol, J.M., Joint, I., Fodriguez-Valera, F., Pedros-Alio, C., 2002. Changes in archaeal, bacterial and eukaryal assemblages along a salinity gradient by comparison of genetic fingerprinting methods in a multipond solar saltern. Environ. Microbiol. 4, 338–348.

Cho, J.C., Tiedje, J.M., 2000. Biogeography and degree of endemicity of fluorescent *Pseudomonas* strains in soil. Appl. Environ. Microbiol. 66, 5448–5456.

Crump, B.C., Hopkinson, C.S., Sogin, M.L., Hobbie, J.E., 2004. Microbial biogeography along an estuarine salinity gradient: combined influences of bacterial growth and residence time. Appl. Environ. Microbiol. 70, 1494–1505.

de Souza, F.A., Kowalchuk, G.A., Leeflang, P., van Veen, J.A., Smit, E., 2004. PCR-denaturing gradient gel electrophoresis profiling of inter- and intraspecies 18S rRNA gene sequence heterogeneity is an accurate and sensitive method to assess species diversity of arbuscular mycorrhizal fungi of the genus *Gigaspora*. Appl. Environ. Microbiol. 1413–1424.

Fierer, N., 2008. Microbial biogeography: patterns in microbial diversity across space and time. In: Zengler, K. (Ed.), Accessing Uncultivated Microorganisms: from the Environment to Organisms and Genomes and Back. ASM Press, Washington DC, pp. 95–115.

Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proc. Natl Acad. Sci. USA 103, 626–631.

Franklin, R.B., Blum, L.K., McComb, A.C., Mills, A.L., 2002. A geostatistical analysis of small–scale spatial variability in bacterial abundance and community structure in salt marsh creek bank sediments. FEMS Microbiol. Ecol. 42, 71–80.

Franklin, R.B., Mills, A.L., 2003. Multi-scale variation in spatial heterogeneity for microbial community structure in an eastern Virginia agricultural field. FEMS Microbiol. Ecol. 44, 335–346.

Franklin, R.B., Taylor, D.R., Mills, A.L., 1999. The distribution of microbial communities in anaerobic and aerobic zones of a shallow coastal plain aquifer. Microb. Ecol. 38, 377–386.
Fulthorpe, R.R., Rhodes, A.N., Tiedje, J.M., 1998. High levels of endemicity of 3-chlorobenzoate-degrading soil bacteria. Appl. Environ. Microbiol. 64, 1620–1627.
Garcia-Martinez, J., Rodriguez-Valera, F., 2000. Microdiversity of uncultured marine prokaryotes: the SAR11 cluster and the marine Archaea of Group I. Mol. Ecol. 9, 935–948.
Gelsomino, A., Keijzer-Wolters, A.C., Cacco, G., van Elsas, J.D., 1999. Assessment of bacterial community structure in soil by polymerase chain reaction and denaturing gradient gel electrophoresis. J. Microbiol. Methods 38, 1–15.
Girvan, M.S., Bullimore, J., Pretty, N., Osborn, A.M., Ball, A.S., 2003. Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soil. Appl. Environ. Microbiol. 69, 1800–1809.
Girvan, M.S., Bullimore, J., Ball, A.S., Pretty, J.N., Osborn, A.M., 2004. Responses of active bacterial and fungal communities in soils under winter wheat to different fertilizer and pesticide regimens. Appl. Environ. Microbiol. 70, 2692–2701.
Gleeson, D.B., Clipson, N., Melville, K., Gadd, G.M., 2005. Characterization of fungal community structure on a weathered pegmatitic granite. Microb. Ecol. 50, 360–368.
Gowda, P.H., Howell, T.A., Evett, S.R., Chavez, J.L., New, L., 2008. Remote sensing of contrasting tillage practices in the Texas Panhandle. Int. J. Remote Sens. 29 (12), 3477–3487.
Green, J.L., Holmes, A.J., Westoby, M., Oliver, I., Briscoe, D., Dangerfield, M., Gillings, M., Beattie, A.J., 2004. Spatial scaling of microbial eukaryote diversity. Nature 432, 747–750.
Green, J.L., Bohannan, B.J.M., Whitaker, R.J., 2008. Microbial biogeography from taxonomy to traits. Science 320, 1039–1043.
Hawksworth, D.L., Rossman, A.Y., 1997. Where are all the undescribed fungi? Phytopath. 87, 888–891.
Hill, T.C.J., Walsh, K.A., Harris, J.A., Moffett, B.F., 2003. Using ecological diversity measures with bacterial communities. FEMS Microbiol. Ecol. 43, 1–11.
Johnson, M.J., Lee, K.Y., Scow, K.M., 2003. DNA fingerprinting reveals links among agricultural crops, soil properties, and composition of soil microbial communities. Geoderma 1954, 1–25.
Jumpponen, A., Trowbridge, J., Mandyam, K., Johnson, L., 2005. Nitrogen enrichment causes minimal changes in arbuscular mycorrhizal colonization but shifts community composition — evidence from rDNA data. Biol. Fertil. Soil. 41, 217–224.
Kowalchuk, G., Smit, E., 2004. Fungal community analysis using PCR-denaturing gradient gel electrophoresis (DGGE). In: Kowalchuk, G., de Bruijn, F.J., Head, I.M., Akkermans, A.D.L., van Elsas, J.D. (Eds.), Molecular Microbial Ecology Manual, 2nd Ed. Vol. 1. Kluwer Academic Publishers, Dordrecht, pp. 771–788.
Larkin, R.P., 2003. Characterization of soil microbial communities under different potato cropping systems by microbial population dynamics, substrate utilization, and fatty acid profiles. Soil Biol. Biochem. 35, 1451–1466.
Lord, N.S., Kaplan, C.W., Shank, P., Kitts, C.L., Elrod, S.L., 2002. Assessment of fungal diversity using terminal restriction fragment (TRF) pattern analysis: comparison of 18S and ITS ribosomal regions. FEMS Microbiol. Ecol. 42, 327–337.
Lücking, R., Huhndorf, S., Pfister, D.H., Plata, E.R., Lumbsch, H.T., 2009. Fungi evolved right on track. Mycologia 101, 810–822.
Lynch, M.D.J., Thorn, R.G., 2006. Diversity of Basidiomycetes in Michigan agricultural soils. Appl. Environ. Microbiol. 72, 7050–7056.
Martiny, J.B.H., Bohannan, B.J.M., Brown, J.H., Colwell, R.K., Fuhrman, J.A., Green, J.L., Horner-Devine, M.C., Kane, M., Krumins, J.A., Kuske, C.R., Morin, P.J., Naeem, S., Ovreas, L., Reysenbach, A.-L., Smith, V.H., Staley, J.T., 2006. Microbial biogeography: putting microorganisms on the map. Nat. Rev. Microbiol. 4, 102–112.
McArthur, J.V., Kovacic, D.A., Smith, M.H., 1988. Genetic diversity in natural populations of a soil bacterium across a landscape gradient. Proc. Natl Acad. Sci. USA 85, 9621–9624.
Muyzer, G., De Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59, 695–700.
Oda, Y., Star, B., Huisman, L.A., Gottschal, J.C., Forney, L.J., 2003. Biogeography of the purple nonsulfur bacterium *Rhodopseudomonas palustris*. Appl. Environ. Microbiol. 69, 5186–5191.
Oehl, F., Sieverding, E., Ineichen, K., Mader, P., Boller, T., Wiemken, A., 2003. Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. Appl. Environ. Microbiol. 69, 2816–2824.
Papke, R.T., Ramsing, N.B., Bateson, M.M., Ward, D.M., 2003. Geographical isolation in hot spring cyanobacteria. Environ. Microbiol. 5, 650–659.
Pinhassi, J., Winding, A., Binnerup, S.J., Ulla, L.Z., Riemann, B., Hagstrom, A., 2003. Spatial variability in bacterioplankton community composition at the Skagerrak–Kattegat Front. Mar. Ecol. Prog. Ser. 255, 1–13.
Reche, I., Pulido-Villena, E., Morales-Baquero, R., Casamayor, E.O., 2005. Does ecosystem size determine aquatic bacterial richness? Ecology 86, 1715–1722.
Riemann, L., Middelboe, M., 2002. Stability of bacterial and viral community compositions in Danish coastal waters as depicted by DNA fingerprinting techniques. Aquat. Microb. Ecol. 27, 219–232.
Sliwinski, M.K., Goodman, R.M., 2004a. Comparison of crenarchaeal consortia inhabiting the rhizosphere of diverse terrestrial plants with those in bulk soil in native environments. Appl. Environ. Microbiol. 70, 1821–1826.
Sliwinski, M.K., Goodman, R.M., 2004b. Spatial heterogeneity of crenarchaeal assemblages within mesophilic soil ecosystems as revealed by PCR-single stranded conformation polymorphism profiling. Appl. Environ. Microbiol. 70, 1811–1820.
Steenwerth, K.L., Jackson, L.E., Calderon, F.J., Stromberg, M.R., Scow, K.M., 2002. Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. Soil Biol. Biochem. 34, 1599–1611.
Sun, H.Y., Deng, S.P., Raun, W.R., 2004. Bacterial community structure and diversity in a century-old manure-treated agroecosystem. Appl. Environ. Microbiol. 70, 5868–5874.
Thorn, G., 1997. The fungi in soil. In: van Elsas, J.D., Wellington, E.M.H., Trevors, J.T. (Eds.), Modern Soil Microbiology. Dekker, New York, pp. 63–127.
Troussellier, M., Schafer, H., Batailler, N., Bernard, L., Courties, C., Lebaron, P., Muyzer, G., Servais, P., Vives-rego, J., 2002. Bacterial activity and genetic richness along an estuarine gradient (Rhone River plume, France). Aquat. Microb. Ecol. 28, 13–24.
USDA-SCS, 1975, Soil survey of Moore County, Texas. Soil Conservation Service, United States Department of Agriculture, Washington D.C. 20250, 57p.
Viaud, M., Pasquieur, A., Brygoo, Y., 2000. Diversity of soil fungi studied by PCR-RFLP of ITS. Mycol. Res. 104, 1027–1032.
Whitaker, R.J., 2006. Allopatric origins of microbial species. Philos. Trans. R. Soc. B 361, 1975–1984.
Whitaker, R.J., Grogan, D.W., Taylor, J.W., 2003. Geographic barriers isolate endemic populations of hyperthermophilic Archaea. Science 301, 976–978.
Yannarell, A.C., Triplett, E.W., 2005. Geographic and environmental sources of variation in lake bacterial community composition. Appl. Environ. Microbiol. 71, 227–239.