Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems

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ABSTRACT

Soil microbial communities under three agricultural management systems (conventionally tilled cropland, hayed pasture, and grazed pasture) and two fertilizer systems (inorganic fertilizer and poultry litter) were compared to that of a 150-y-old forest near Watkinsville, Georgia. Both 16S rRNA gene clone libraries and phospholipid fatty acid (PLFA) analyses indicated that the structure and composition of bacterial communities in the forest soil were significantly different than in the agricultural soils. Within the agricultural soils, the effect of fertilizer amendment on bacterial communities was more dramatic than either land use or season. Fertilizer amendment altered the abundance of more bacterial groups throughout the agricultural soils. In addition, the changes in the composition of bacterial groups were more pronounced in cropland than in pastures. There was much less seasonal variation between the soil libraries. Community-level differences were associated with differences in soil pH, mineralizable carbon and nitrogen, and extractable nutrients. Bacterial community diversity exhibited a complex relationship with the land use intensity in these agro-ecosystems. The pastures had the highest bacterial diversity and could be characterized as having an intermediate degree of intervention compared to low intervention in forest and high intervention in cropland. Changes in bacterial diversity could be attributed to the abundance of a few operational taxonomic units (OTUs). The microdiversity of abundant OTUs in both forest and cropland was consistent with an increase in abundance of many phenotypically similar species rather than a single species for each OTU. Soil microbial communities were significantly altered by long-term agricultural management systems, especially fertilizer amendment, and these results provide a basis for promoting conservation agricultural systems.

1. Introduction

Soil bacteria are abundant, diverse, and play important roles in biogeochemical cycles that drive terrestrial ecosystems (Gans et al., 2005). The activity and diversity of soil microorganisms are directly influenced by changes in the soil environment. Changes in soil water content (Bossio and Scow, 1998), pH (Fierer and Jackson, 2005), soil type and field properties (Wu et al., 2008), plant diversity and composition (Carney and Matson, 2006) have all been shown to influence the composition of soil microbial communities. Similarly, agricultural land management is one of the most significant anthropogenic activities that greatly alter soil characteristics, including physical, chemical, and biological properties and processes. Thus, while agriculture is expected to affect the diversity and structure of soil microbial communities, the specific responses of various bacterial groups to the changing environment in agricultural soils are not well understood (Buckley and Schmidt, 2001).

The microbial communities in forest and managed soils differ dramatically, but the responsible factors are not well documented. Recently, Upchurch et al. (2008) proposed that the higher bacterial diversity in managed agricultural soils than in neighbouring, undisturbed forest soils in Georgia could have resulted from greater seasonal and plant variability and increased opportunities for immigration by animal or air borne bacteria. If these results are generalizable to other sites, the response of microbial communities to agricultural land management practices, such as tillage and fertilizer amendments, which have different degrees of habitat disturbance, would be expected to depend on the intensity of...
human intervention. In contrast to the results of Upchurch et al. (2008), the bacterial diversity decreased with the intensity of tillage in sandy Norwegian soils (Øvreås and Torsvik, 1998). These results suggest that microbial diversity and habitat disturbance are not linearly correlated and that the relationship is more complex. To test this hypothesis, the current research explored further the response of soil microbial communities to the intensity of land use.

Historically, farmyard manures, including poultry litter, swine, and cattle manures, have been applied to agricultural land to improve soil fertility and crop yield in Georgia (Troels-Smith, 1984). Currently, inorganic fertilizer has become more widely used. Although, much of the poultry litter is applied to pastures, a small percentage is also applied to cropland (Endale et al., 2002). While organic fertilizers such as swine and cattle manure and sewage sludge promote the activities of soil microbial communities (Emwall et al., 2007; Ruppel et al., 2007), the impact of these amendments on soil microbial community composition, structure and diversity remains unclear. Repeated application of manures may pose environmental hazards, because they introduce fecal microbial flora into the soil and have the potential to alter the endogenous microbial community structure (Soupir et al., 2006). The accumulation in soil and runoff from soil of trace elements, nitrate, and phosphate are an additional environmental concern (Edwards et al., 2000).

Soil microbial communities respond to changes in carbon and other substrates in soil (Kilham, 1994). Whether nutrients are supplied to soil via inorganic fertilizer or poultry litter, fertilizer amendment would impact soil microbial communities. Previous studies on poultry litter amendment have focused on changes in soil chemical characteristics (Franzleubbers et al., 2004), crop production (Endale et al., 2002), water quality impacts (Sharpley, 2003), and shifts in total microbial communities using enzymatic and broad community fatty acid markers (Acosta-Martínez and Harmel, 2006). The current research examined the responses of soil microbial communities to the intensity of land use. In addition, the specific changes in microbial communities that occurred with fertilizer amendment were also elucidated. We investigated the effects of three major agricultural land management systems in the southeastern USA (i.e., cropland, hayed pasture, and grazed pasture) and the specific impact of inorganic fertilizer and poultry litter amendments within these agricultural systems on the composition of soil microbial communities using 16S rRNA gene libraries and phospholipid fatty acid (PLFA) analyses.

2. Materials and methods

2.1. Sampling site

Field sites were located at the USDA – Agricultural Research Service, J. Phil Campbell Sr. Natural Resource Conservation Center in Watkinsville, Georgia, USA (33°54′N, 83°24′W). Soils were mostly Cecil sandy loam (fine, kaolinitic, thermic Typic Kanhapludoll) and have been described in detail (Bruce et al., 1983). The area has a warm temperate climate with an annual average temperature of 16.5 ℃, long-growing season (280 frost-free days), and ample annual rainfall (1250 mm). Six land-use treatments with > 10 y of continuous application were examined: conventionally tilled cropland with inorganic fertilizer (CTIF), conventionally tilled cropland with poultry litter (CTPL), hayed pasture with inorganic fertilizer (HPIF), hayed pasture with poultry litter (HPPL), grazed pasture with inorganic fertilizer (GPIF), and grazed pasture with poultry litter (GPPL). In addition, a control plot consisted of a neighbouring forest that had not been cultivated since the 1860s. Croplands CTIF and CTPL were conventionally tilled with chisel and disk plowing. Fertilizer treatments were applied each year to the same plots, i.e., inorganic fertilizer (CTIF) with an equivalent of nitrogen (58–60 kg ha⁻¹), phosphorus (56 kg ha⁻¹), and potassium (56 kg ha⁻¹) (Endale et al., 2002) and poultry litter at 4.5 Mg ha⁻¹ y⁻¹ (CTPL) with 3.6% N (116 kg ha⁻¹ equivalent to 58 kg ha⁻¹ as available) (Franzleubbers et al., 2004). Poultry litter was from a local poultry house that generates three flocks per cleaning from concrete floors covered with saw-dust and shavings. Fresh litter was transported to the research site and kept under cover for not more than 2 weeks before being applied on the plots with a specially designed spreader. From 1991 to 1995, corn was grown with a rye cover crop. From 1996 to 2000, cotton was grown with a rye cover crop (Endale et al., 2002). From 2001 to 2005, corn was grown with a rye cover crop. The experimental design was a randomized complete block with three replicate plots of 300 m² for each treatment.

Pastures were located in a 15 ha upland field (33°22′N, 83°24′W) near Farmington, Georgia that had previously been conventionally cultivated with row crops of soybean [Glycine max (L.) Merr.], sorghum [Sorghum bicolor (L.) Moench], and cotton [Gossypium hirsutum L.] for several decades. Pastures were established by sprigging of ‘Coastal’ bermudagrass (Cynodon dactylon (L.) Pers.) in 1991 and received varied concentrations of either inorganic fertilizer (NH₄NO₃) or poultry litter since 1994 (Franzleubbers et al., 2001). While the fertilizer application was targeted to supply 200 kg N ha⁻¹ y⁻¹ during the first 5 y, it was targeted to supply 270 kg N ha⁻¹ y⁻¹ during the next 7 y following overseeding with ‘Georgia 5’ tall fescue [Lolium arundinaceum (Schreb.) S.J. Darbyshire]. From 1999 to the end of summer 2005, fertilizers were applied in Apr/May, Jul, and Sep/Nov. For each type of fertilization, the pastures were either left unharvested (HPIF and HPPL) or grazed by cattle (GPIF and GPPL). The experimental design utilized 300 m² plots and was a randomized complete block with three replicates of each treatment.

The control forest was a 1 ha single plot in an upland field protected from cultivation. The forest was established in the 1860s and planted with loblolly pine (Pinus taeda L.). Timber was harvested in the mid 1960s, and hardwoods were allowed to regrow (Franzleubbers et al., 2000). At the time of sampling, overstory in the forest was ~ 90% hickory (Carya spp.) and oak (Quercus spp.) and ~ 10% loblolly pine (age 20–50 y old trees). The understory was sparse, with < 3% cover and composed predominantly of Smilax, Vitis, and Ligustrum spp. Three 10 × 30 m areas were selected in the forest as pseudo-replicates.

2.2. Sample collection

Soil samples were collected in summer (7 July 2005) and in winter (7 December 2005). The two sampling dates allowed a limited seasonal comparison. For each plot, five cores with a diameter of 4 cm were collected from a depth of 1–10 cm with a metallic corer and combined in 1.61 Whirlpak™ bags. The top of the mineral layer was considered the soil surface, and the dust or litter layer was not included. During sampling within a plot, the corer was wiped clean of obvious soil particles between cores with a paper towel. Between plots, the corer was rinsed with 70% ethanol and then wiped dry. Soil cores were screened to remove roots, small animals and rocks upon arrival in the laboratory. Soil cores from the same plot were thoroughly mixed and stored at ~80 ℃.

2.3. Physico-chemical analysis of soils

A portion of soil was air dried overnight and passed through a 2 mm sieve. pH measurements were carried out after 30 min equilibrations on a 1:2.5 soil:water dilution. Electrical conductivity was determined on a 1:2 soil:water mixture following equilibration for 24 h and filtering through Whatman 42 filter paper. Soil textural
2.4. Phospholipid fatty acid analysis of soil communities

The PLFAs were analyzed to infer relatedness of microbial communities, microbial biomass and the relative ratio of fungal to bacterial biomass. Microbial lipids were extracted from 15-g wet weight of soil by the modified one-phase Bligh and Dyer method (White et al., 1996). Phospholipids were purified and methylated to fatty acid methyl esters for analysis as described previously (Butler et al., 2003). Mol% of dominant fatty acids was used for non-metric multidimensional scaling (NMS) analysis, a non-parametric method for graphical ordination of experimental data (Williams et al., 2006).

2.5. DNA extraction and preparation of 16S rRNA gene libraries

For each soil sample, DNA was extracted from 5 g of soil using the PowerMax™ Soil DNA isolation kit (MoBio Laboratories, Inc.). The extracted DNA was dissolved in 400 µl of nuclease-free water, and its concentration was determined electrophoretically. The bacterial 16S rRNA genes were amplified in a 15-cycle PCR. Each 25-µl PCR reaction contained 20–50 ng of DNA, 10 pmol each of 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-GTT ACC TGT TGA AGC ACT T3') primers, and a single PuReTaq™ Ready-To-Go™ PCR bead (Amersham Biosciences). Reaction conditions included an initial denaturation at 95 °C for 3 min; 15 cycles of 94 °C for 1 min, 58 °C for 45 s, and 72 °C for 2 min; and a final elongation at 72 °C for 4 min on a Mastercycler® Gradient (Eppendorf). The PCR products were immediately cloned in plasmid T-vector and transformed into chemically competent Escherichia coli TOP10™ cells (Invitrogen). Preliminary studies on bacterial transformation indicated that the viability of the heat-shocked recombinant bacteria increased rapidly for 20 min without an increase in cell number (data not shown). By about 40 min, the cells began to divide. Thus, a 30-min recovery period was chosen before plating to avoid the appearance of duplicate clones in the libraries. Transformed cells were plated on Luria-Bertani (LB) agar plates with ampicillin (100 mg l-1) and kanamycin (10 mg l-1). Plates were incubated overnight at 37 °C and then stored at 4 °C for 24 h. White colonies were inoculated into 96-well culture blocks using the autoanalyzer technique (Bundy and Meisinger, 1994). Particulate phospholipid fatty acid analysis was performed by the hydromethid method (Gee and Bauder, 1979) at 19.5 °C. Extractable nutrients were determined using the Mehlich-3 extraction protocol (Elrashidi et al., 1996). Potential N mineralization was calculated from the difference in inorganic N concentration (NH4-N + NO2-N + NO3-N) between 0 and 24 d of incubation (25 °C) determined using the autoanalyzer technique (Bundy and Meisinger, 1994). Partially organic C (POC) and N (PON) were determined from a 33-g subsample that was shaken with 100 ml of 0.01 M Na2P2O7 for 16 h on a reciprocating shaker and passed through a 0.053-mm screen. Soil and sand organic material not passing the screen were dried at 55 °C for 72 h, ground in a ball mill for 5 min and analyzed for total C and N using dry combustion.

2.6. Sequencing and editing

Sequencing was performed at the Molecular Genomics Instrumentation Facility at the University of Georgia, where cultures were regrown, plasmids were isolated, and the 16S rRNA genes were partially sequenced using the primer 27F. The complete 16S rRNA gene for selected clones was determined by further sequencing using the primer 1492R. Sequence chromatogram files were viewed for quality in Sequence Scanner v1 (Applied Biosystems, Inc.) and manually edited using CodonCode Aligner v1.6.3 (CodonCode Corporation). Sequences that were less than 400 base pairs were not included in further analyses. All sequences were checked for artificial chimeras using Mallard v1.02 (Ashelford et al., 2006) and Pintail v1.1 (Ashelford et al., 2005). Sequence alignments were carried out using NAST aligner (DeSantis et al., 2006a), while alignment format conversions were carried out using GenDoc v2.7 (http://www.nrbsc.org/downloads/). The edited sequences were submitted to GenBank with accession numbers EF071996–EF073040 and EF073270–EF075930 (Supplementary Table S1).

2.7. Taxonomic assignment and phylogenetic analysis

Taxonomic assignment of sequences was made using a combination of RDPquery written by Wade Sheldon and Glen Dyszyanski at the University of Georgia (http://simogmarsi.uga.edu/public_db/rdp_query.htm) and the Greengenes Classifier (DeSantis et al., 2006b). The former program calculated percent similarity of the query sequence with its closest type-species sequence in the RDP database (http://rdp.cme.msu.edu/). Based on this percentage similarity, taxonomic assignments were then made. The similarity cutoff values were 75%, 85%, 91%, 92%, 95%, and 100% for phylum, class, order, family, genus, and species designations, respectively. Following RDPquery, the sequences assigned to Acidobacteria and Firmicutes were reassigned using the Greengenes Classifier program. This was necessary because of low representation of acidobacterial type strains in the RDP.

2.8. Library comparisons, diversity estimates and statistical analyses

Sequence alignments were used to calculate distance matrices using the Jukes–Cantor algorithm in the program DNADIST of the Phylip package (Felsenstein, 2004). These distance matrices were then formatted and used to compare libraries in the program LIBSHUFF v1.2 (Singleton et al., 2001). Bonferroni correction was used to correct for experiment-wise error when performing multiple LIBSHUFF comparisons. For group-specific LIBSHUFF analyses, sequences were extracted from the larger data set containing all libraries using the data extraction tools available at http://www.arches.uga.edu/~whitman/detoools.html. These tools generated preformatted output files that were directly imported into LIBSHUFF.

Diversity measurements were used to compare the clone libraries independent of their phylogenetic composition. The Shannon diversity index (H) was used as a measure of general diversity, including richness and evenness (Shannon and Weaver, 1963). Chao1 estimator was calculated as an alternative to H (Chao, 1984). Because H is more sensitive to changes in the abundance of rare groups, the reciprocal of Simpson’s index was also calculated. The number of operational taxonomic units (OTUs) was determined at an evolutionary distance (D) of 0.03 using the average neighbor algorithm in DOTUR (Schloss and Handelsman, 2005). At this D value, closely related species were grouped together. These OTUs would, therefore, contain taxa that share a range of common physiological properties.
A hierarchy of log-linear models was used to examine differences in the taxonomic composition among rRNA gene libraries for the agricultural soils. The association between each taxonomic group and a particular soil treatment was modeled separately with a hierarchy of 16 log-linear models aimed to explain the occurrence as a function of land use, fertilizer, and season using the GENMOD procedure in SAS® software. This procedure was used to fit generalized linear models to the data using maximum likelihood estimation. The response variable, i.e., separate counts for each taxon from replicate libraries, was assumed to approximate a Poisson distribution. The null model for each taxon assumed that the distribution of all clones across all experimental conditions was the same, subject to statistical variability. A general model for the analysis was:

$$\ln(\lambda_{ijk}) = \mu + a_i + b_j + c_k + d_{ij} + e_{ik} + f_{jk} + \eta_{ijk}$$

where $a_i$, $b_j$, and $c_k$ represent the $i$th, $j$th, and $k$th level of field, fertilizer and season variables, respectively, $d_{ij}$, $e_{ik}$, and $f_{jk}$ indicate double-interaction terms and $\eta_{ijk}$ indicates the triple interaction term. The $\lambda$ term on the left-hand side of the model represents the true mean number of counts of a taxon under conditions $(i, j, k)$ on the natural log scale. Parameter estimates for the intercept ($\mu$), two levels of field, one level of fertilizer, and one level of season were calculated since the winter sampling of hayed pasture with inorganic fertilizer amendment was set as the baseline. Model selection was evaluated using a variant of the Bayesian information criterion, BIC (Schwarz, 1978). To correct for overdispersion, a modification of BIC, QBIC was used:

$$QBIC = -2 \log (\tilde{\theta}) / \tilde{c} + K \log (n)$$

where $\tilde{\theta}$ is the likelihood of the model, $K$ is the number of parameters in the model, $n$ is the effective sample size, and $\tilde{c}$ is the measure of overdispersion evaluated for each taxon. The measure of overdispersion was calculated as follows:

$$\tilde{c} = \chi^2 / df$$

where $\chi^2$ is the Pearson chi-square statistic and $df$ is the associated degrees of freedom. The minimum QBIC model was considered to be the most likely model in a candidate model set.

### 3. Results

Libraries of 16S rRNA genes were prepared from DNA extracted from triplicate soil samples collected in winter and summer. Of the 96 clones sequenced for each library, between 73 and 95 clones yielded good quality sequences for each library. From a total of 3719 clone sequences, only 12 sequences were confirmed chimeras. Another sequence was from a vector. Hence, a total of 3706 sequences (∼88 per library) were used for further analyses. For most of the replicates, no significant differences were detected in LIBSHUFF analysis, indicating that the experimental strategy used for sampling, extracting DNA and preparing clone libraries was reproducible. However, Replicate 2 from the GPPL plots differed from the other replicates in both the summer and winter. Similarly, summer Replicate 2 for both the CTIF and HPPL plots differed from Replicate 3. These results suggested that small differences existed between the bacterial communities in some of the plots. Because the effect of season was relatively small, libraries were pooled in some analyses to investigate the broader impact of land management regimes.

#### 3.1. Comparison between agricultural and forest soil

In general, the characteristics of agricultural and forest soils were comparable to those reported previously from the same sites (Franzluebbers et al., 1999, 2000). The forest soil showed higher sand content (Table 1) and was significantly enriched in Fe (Supplementary Table S2). In contrast, the agricultural soils were more enriched in Mg, P, Ca, and Cu, inorganic N, microbial biomass C, and soil organic C and N fractions (Supplementary Table S3).

Significant differences in microbial communities between agricultural and forest soils were detected. Multivariate NMS plots and multi-response permutation tests indicated that the total microbial PLFA distribution under forest was significantly different than under agricultural management (Fig. 1). Similar results were obtained when only bacterial PFLAs were examined (data not shown). The Gram-positive markers (10methyl 16:0 and a15:0) and the fungal marker (18:2ω6,9) were positively and negatively correlated with the Axis 1 ($r > 0.75$), respectively, suggesting that fungi were relatively more abundant in the forest and Gram-positive bacteria were more abundant in the pasture and cropped

<table>
<thead>
<tr>
<th>Property¹</th>
<th>Cropland</th>
<th>Hayed Pasture</th>
<th>Grazed Pasture</th>
<th>Forest</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (pH)</td>
<td>4.7 (0.2)</td>
<td>5.7 (0.1)</td>
<td>5.3 (0.1)</td>
<td>5.3 (0.3)</td>
</tr>
<tr>
<td>% Sand</td>
<td>66 (4)</td>
<td>62 (3)</td>
<td>66 (6)</td>
<td>61 (13)</td>
</tr>
<tr>
<td>% Silt</td>
<td>13 (3)</td>
<td>18 (3)</td>
<td>17 (4)</td>
<td>18 (6)</td>
</tr>
<tr>
<td>% Clay</td>
<td>21 (4)</td>
<td>20 (0)</td>
<td>17 (4)</td>
<td>21 (8)</td>
</tr>
<tr>
<td>EC (μS cm⁻¹)</td>
<td>84 (6)</td>
<td>118 (2)</td>
<td>65 (14)</td>
<td>135 (17)</td>
</tr>
<tr>
<td>Total organic C (mg kg⁻¹ soil)</td>
<td>12 (2)</td>
<td>14 (3)</td>
<td>17 (1)</td>
<td>19 (2)</td>
</tr>
<tr>
<td>Total N (mg kg⁻¹ soil)</td>
<td>0.9 (1)</td>
<td>1.3 (2)</td>
<td>1.4 (0.2)</td>
<td>1.4 (0.2)</td>
</tr>
<tr>
<td>Inorganic N (mg kg⁻¹ soil)</td>
<td>29 (3)</td>
<td>30 (4)</td>
<td>28 (2)</td>
<td>35 (3)</td>
</tr>
<tr>
<td>POC (g kg⁻¹ soil)</td>
<td>2.3 (0.3)</td>
<td>2.9 (0.3)</td>
<td>3.6 (0.9)</td>
<td>4.5 (0.7)</td>
</tr>
<tr>
<td>PLFAWIN (nmol g⁻¹ soil)</td>
<td>101 (6)</td>
<td>201 (24)</td>
<td>150 (4)</td>
<td>224 (34)</td>
</tr>
<tr>
<td>Cmin (mg CO₂-C kg soil 24 d⁻¹)</td>
<td>351 (22)</td>
<td>551 (40)</td>
<td>447 (21)</td>
<td>597 (86)</td>
</tr>
<tr>
<td>Nmin (mg kg⁻¹ soil 24 d⁻¹)</td>
<td>23 (14)</td>
<td>43 (3)</td>
<td>49 (9)</td>
<td>88 (34)</td>
</tr>
<tr>
<td>SMBC (mg kg⁻¹ soil)</td>
<td>480 (33)</td>
<td>689 (24)</td>
<td>580 (51)</td>
<td>617 (31)</td>
</tr>
<tr>
<td>PLFA SUM (nmol g⁻¹ soil)²</td>
<td>60 (10)</td>
<td>89 (11)</td>
<td>72 (5)</td>
<td>94 (4)</td>
</tr>
<tr>
<td>PLFA N (nmol g⁻¹ soil)³</td>
<td>90 (4)</td>
<td>123 (6)</td>
<td>117 (8)</td>
<td>138 (9)</td>
</tr>
<tr>
<td>Fungal:bacterial ratio</td>
<td>0.28 (0.02)</td>
<td>0.37 (0.01)</td>
<td>0.25 (0.01)</td>
<td>0.25 (0.01)</td>
</tr>
</tbody>
</table>

¹ Values are mean (standard deviation) of three soil samples collected in summer. CTIF, HPPL and GPPL are inorganic fertilizer-amended treatments and CTPL, HPPL and GPPL are poultry litter-amended treatments.

² EC = electrical conductivity; POC = particulate organic C; PON = particulate organic N; Cmin = flush of CO₂-C during the first 3 d following rewetting of dried soil; Cmin – mineralizable C; Nmin – mineralizable N; and SMBC = soil microbial biomass C.

³ Values for PLFA and fungal/bacterial ratio are from summer (SUM) and winter (WIN).
soils. Likewise, the fungal:bacterial PLFA ratio was higher in the forest soil than most of the agricultural soils (Table 1).

The bacterial diversity was lower in the forest soil than most of the agricultural soils studied (Table 2). An exception was the bacterial diversity in CTIF soil, which was lower than in other agricultural soils and approached the value observed under forest. The low diversity and evenness in CTIF was due to the dominance of six highly abundant OTUs (Supplementary Table S4) represented by the GASP (Georgia Survey of Prokaryotes) clones WA151_A03, WA151_A04, WA151_A08, WA151_A09, WA151_D02, and WA151_H03. Most members of these six OTUs were found in the CTIF summer soil, which was considerably less diverse than in winter. Hence, the lower diversity index of the pooled CTIF soils could be attributed in part to the lower diversity in summer. Conclusions based upon the diversity indices were supported by rarefaction curves (Fig. 2a). These curves failed to plateau for any of the treatments, indicating that the diversity was not fully sampled. Nevertheless, except for CTIF, the agricultural soils possessed steeper slopes, consistent with overall higher diversity.

The bacterial communities in forest soil were readily distinguishable from those in agricultural soils (Table 3). While the Acidobacteria were among the most abundant phyllogenetic groups in all soils, their abundance in forest soil was close to 50%, or about twice that in other soils. Similarly, the Verrucomicrobia comprised 4.5% of the bacterial community in forest soil, but only 0.7–1.9% in agricultural soils. In contrast, the Bacteroidetes, Firmicutes, β-, Δ-, γ-Proteobacteria and Gemmatimonadetes were more abundant in agricultural soils.

LIBSHUFF analysis of rRNA gene libraries also indicated that the composition of the bacterial communities differed significantly between agricultural and forest soils (P = 0.002). Group-specific LIBSHUFF comparisons confirmed that the composition of all abundant phyllogenetic groups, such as Acidobacteria, Bacteroidetes, Planctomycetes, Proteobacteria, and the three proteobacterial classes (α-, β-, and γ-Proteobacteria) differed significantly between the forest and agricultural soils (data not shown). Therefore, not only did the abundance of many groups differ between forest and agricultural soils, but the composition of groups also differed.

3.2. Comparison of cropland vs. pasture

The pasture soils were richer in organic C and N than cropped soils, especially in potential N mineralization (Table 1 and Supplementary Table S3). The Mg, Ca and Mn concentrations were also greater in the pasture than in the cropland.

Differences in the microbial PLFAs were also detected. Lower fungal:bacterial PLFA ratios in pasture soils indicated greater bacterial biomass (Table 1). The greater bacterial biomass was positively correlated to the increased abundance of Gram-positive bacteria in the pasture soils and Axis 1 in the NMS analysis of the molPLFAs (Fig. 1). However, the difference in community structure between cropland and pasture soils was not as dramatic as the difference between agricultural and forest soils.

Bacterial diversity was greater in the pasture soils than cropped soils. The greater diversity was due to increases in both species richness and evenness in the pasture (Table 2). Within each fertilizer amendment, the diversity of the pastures was always higher than that of the cropland. Similarly, when the libraries from each treatment were pooled or when all the pasture libraries were pooled, the diversity indices of the pastures always exceeded that of the cropland (data not shown). Greater diversity was also reflected in the steeper rarefaction curves for pasture soils (Fig. 2a).

The abundance and composition of bacterial taxa also differed between cropland and pasture. The most likely model within the hierarchy of potential log-linear models revealed a significant increase in the abundance of Δ- and γ-Proteobacteria and a decrease in the abundance of Firmicutes and α-Proteobacteria under cropland (Table 4). Moreover, the composition of the entire bacterial communities was significantly different between cropland and pasture by LIBSHUFF analyses. Group-specific LIBSHUFF comparisons confirmed that the composition of Acidobacteria, Bacteroidetes, Firmicutes, Planctomycetes, and the three proteobacterial classes (β-, Δ- and γ-Proteobacteria) differed significantly between cropland and pasture soils. The composition of all these

![Fig. 1. Non-metric multidimensional scaling analysis of total microbial community phospholipid fatty acids in soils from Watkinsville GA. Groupings used to separate communities were based on mo% of 22 fatty acids. Percentages denote the amount of variability associated with each axis.](Author's personal copy)

<table>
<thead>
<tr>
<th>Diversity index</th>
<th>Cropland</th>
<th>Hayed pasture</th>
<th>Grazed pasture</th>
<th>Forest</th>
<th>All sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of clones, N</td>
<td>519 526</td>
<td>534 539</td>
<td>526 527</td>
<td>535 3706</td>
<td></td>
</tr>
<tr>
<td>Number of OTUs, S</td>
<td>219 316</td>
<td>314 335</td>
<td>277 346</td>
<td>225 1333</td>
<td></td>
</tr>
<tr>
<td>Shannon index, H = -Σ(n/N)[ln(n/N)]</td>
<td>4.97 5.44</td>
<td>5.45 5.57</td>
<td>5.25 5.60</td>
<td>4.56 6.49</td>
<td></td>
</tr>
<tr>
<td>Reciprocal of Simpson’s index, 1/D = N(N-1)/Σ(n(n-1))</td>
<td>105 152</td>
<td>227 270</td>
<td>143 178</td>
<td>79 333</td>
<td></td>
</tr>
<tr>
<td>Evenness – H/log(S)</td>
<td>2.12 2.18</td>
<td>2.18 2.21</td>
<td>2.15 2.21</td>
<td>2.06 2.08</td>
<td></td>
</tr>
<tr>
<td>H/Hmax at Hmax n = N; see above</td>
<td>0.80 0.87</td>
<td>0.87 0.89</td>
<td>0.84 0.90</td>
<td>0.77 0.79</td>
<td></td>
</tr>
<tr>
<td>95% Lower confidence interval for Chaol</td>
<td>383 748</td>
<td>910 966</td>
<td>752 972</td>
<td>699 3103</td>
<td></td>
</tr>
</tbody>
</table>

* Calculations based on OTUs formed at D = 0.03 using DOTUR (Schloss and Handelsman, 2005). CTIF, HPIF and GIPF are inorganic fertilizer-amended treatments and CTPL, HPPL and GPPL are poultry litter-amended treatments. Summer and winter libraries were combined for these analyses.

* n2 = number of clones that occur once, n3 = number of clones that occur twice.
groups, except Δ-Proteobacteria, differed significantly within the inorganic fertilizer-amended soils, whereas only Bacteroidetes, and the β- and Δ-Proteobacteria differed significantly between poultry litter-amended cropland and pasture soils.

3.3. Impact of grazing on pasture soils

Grazing on pastures did not significantly alter the soil characteristics. Except for Cu, the elemental nutrient concentrations, soil microbial biomass C, mol% PLFA or fungal:bacterial PLFA ratio of the two pasture soils were similar (Supplementary Table S2).

Bacterial diversity of grazed pastures was lower than the hayed pastures, especially in the inorganic fertilizer-amended soils (Table 2). The lower diversity index with grazing was due to a decrease in both species richness and evenness. In contrast, grazing had little effect on the bacterial diversity in poultry litter-amended soil. The rarefaction curves confirmed these conclusions (Fig. 2a).

The most likely model indicated a significant decrease in the abundance of β-Proteobacteria and an increase in the abundance of Firmicutes with grazing (Table 4). The bacterial community composition also differed significantly between grazing and haying. In addition to differences found with LIBSHUFF for the entire community, group-specific comparisons indicated that the composition of Acidobacteria, and α- and γ-Proteobacteria differed significantly between hayed and grazed pastures. No difference was detected in the composition of Bacteroidetes, Firmicutes, Planctomycetes, and β- and Δ-Proteobacteria. In addition, the composition of Acidobacteria, Bacteroidetes and Firmicutes differed significantly within the inorganic fertilizer-amended hayed and grazed pastures. In contrast, none of these phylogenetic groups differed in composition between hayed and grazed pastures amended with poultry litter.

3.4. Effect of fertilizer amendments

Amendment with poultry litter changed multiple soil characteristics compared with inorganic fertilizer. Poultry litter-amended soils were less acidic and had greater electrical conductivity, potential C and N mineralization, biomass C and higher concentrations of extractable nutrients than inorganic fertilizer-amended soils (Table 1, Supplementary Tables S2 and S3). The total C and N concentrations in CTIF soil were lower than in the other five agricultural soils, reaching a level similar to that in forest soil. A clear correlation between biomass C and mol% PLFA was noted, with higher values under poultry litter amendment than with inorganic fertilizer amendment. In terms of PLFA composition, the three poultry litter-amended soils (CTPL, HPPL and GPPL) and the inorganic fertilizer-amended hayed pasture (HPIF) grouped together and were well differentiated from inorganic fertilizer-amended cropland (CTIF) and grazed pasture (GPIF) (Fig. 1).

Bacterial diversity was always higher in poultry litter-amended soils than inorganic fertilizer soils. The greater diversity was due to an increase in both species richness and evenness (Table 2). The rarefaction curves confirmed these conclusions (Fig. 2a). Slopes of the curves for poultry litter-amended soils exceeded those of inorganic fertilizer-amended soils.

Significant differences in the bacterial communities between fertilizer amendments were also noted. The phylogenetic groups whose composition differed between fertilizer treatments were identified using group-specific LIBSHUFF analysis. The composition of Acidobacteria, Bacteroidetes, and α-β-Δ- and γ-Proteobacteria differed significantly between fertilizer amendments in cropland (CTIF and CTPL). No difference was detected in the composition of Firmicutes and Planctomycetes. While grazed pastures (HPIF vs HPPL) differed only in the composition of Acidobacteria, grazed pastures (GPFL vs GPIF) differed in the composition of both Acidobacteria and Firmicutes. Thus, fertilizer amendments had a larger effect on bacterial communities in cropland than in pastures.

Fertilizer amendment affected bacterial abundance only for some of the bacterial groups whose composition also differed. The most likely model included fertilizer amendment as a factor for abundance of six of the 14 groups examined (Table 4). While the abundance of Acidobacteria was lower with poultry litter amendment, the abundances of β-, Δ-, and unclassified-Proteobacteria, unclassified bacteria and the “others” group were higher. In addition, abundance of γ-Proteobacteria was strongly affected by an
interaction between fertilizer amendment and land use, lower with poultry litter-amendment under both cropping and grazing.

3.5. Effect of season

Microbial communities were influenced by season as observed in the PLFA analyses. Without exception, the total microbial biomass was greater in winter (average 1.3 and 2 times the quantity found in summer (average 1.39 nmol PLFA g\(^{-1}\) soil). In addition, the positive (18:1w7) and negative (10methyl 17:0, i16:0, i17:0, a17:0) correlation of specific PLFAs to Axis 2 (r > 0.75) explained much of the seasonal variation (Fig. 1), indicating that Gram-positive bacteria were favored in winter and Gram-negative bacteria were favored in summer. However, the functional and ecological significance of this observation is not well understood. Although there was a difference in total microbial biomass between seasons, the fungal:bacterial ratio remained similar.

Both diversity and composition of some bacterial communities differed significantly between seasons. However, these differences were only observed for cropland (CTIF and CTL) and inorganic fertilizer-amended grazed pasture (GPFL) soils. For the CTIF soils, the composition of bacterial communities differed significantly between seasons. Group specific LIBSHUFF analysis revealed that the composition of Acidobacteria, Bacteroidetes, Planctomycetes, and three proteobacterial classes (∂-, β-, and γ-Proteobacteria) differed significantly between seasons. Similarly, the diversity was lower in summer than in winter. For instance, the reciprocal of Simpson’s index was 71 in summer and 119 in winter. Only the compositions of the Acidobacteria and Proteobacteria differed significantly between seasons for both CTPL and GPFL. Similarly, season affected the abundance of only a few bacterial taxa. The most likely model suggested greater abundance of ∂- and γ-Proteobacteria in summer (Table 4). Seasonal differences in relative abundance of these bacterial taxa between fertilizer amendments were also observed (data not shown).

3.6. Specific associations of most abundant OTUs

For some abundant OTUs, it was possible to detect specific associations with land use. The 72 most abundant OTUs (N ≥ 10) represented ~38% of the total clones. The distribution of some of these OTUs correlated with specific land use and/or fertilizer amendments (Table 5, Supplementary Table S4). For instance, seven OTUs represented by the GASP clones WB1S2_C06, WB1W2_A03, WD0S1_A01, WD0S1_A05, WD0S1_B12, WD0S1_D04, and WD0S1_D09 were found in high numbers under forest, but were either less abundant or completely absent under agricultural management. Six of these OTUs were closely related to Acidobacteria. Similarly, 41 other OTUs were specifically absent in forest (data not shown).

While some OTUs were evenly distributed in agricultural soils, others were specifically associated with either cropland or pasture soils. For instance, the OTU represented by GASP clone WA1S1_D06 was significantly enriched in one land use or the other.
Acidobacteria

4.1. Response of bacterial communities to habitat intervention

Microbial community composition and diversity of crop and pasture soils were significantly different from forest soils, an observation that was consistent with earlier studies (Buckley and Schmidt, 2001; Upchurch et al., 2008). In addition, bacterial communities in agricultural and forest soils differed significantly in both composition and structure.

Response of soil biota to land use intensity in agro-ecosystems is organism and treatment dependent. For macrobiota, there is either a direct decline in richness and diversity or a unimodal response to the degree of habitat disturbance (Grime, 1973; Anderson, 1994).

Table 5

<table>
<thead>
<tr>
<th>GASP Clone namea</th>
<th>Taxonomic affiliation (Genbank accession number)</th>
<th>Clone library</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>WA1S1_A08</td>
<td>Acidobacteria (D26171)</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>WA1W1_B03</td>
<td>Acidobacteria (D26171)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>WA1S1_B06</td>
<td>Firmicutes (X09238)</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>WA1S2_A07</td>
<td>Acidobacteria (D26171)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>WA1S1_F01</td>
<td>Firmicutes (X09238)</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>WA1S1_A04</td>
<td>Acidobacteria (D26171)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>WA1S1_B09</td>
<td>Acidobacteria (D26171)</td>
<td>9</td>
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</tr>
<tr>
<td>WA1S1_G02</td>
<td>Acidobacteria (D26171)</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>WA2S3_C09</td>
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<td>34</td>
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</tr>
<tr>
<td>WA1S1_H03</td>
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<td></td>
</tr>
<tr>
<td>WA2W1_B03</td>
<td>Acidobacteria (D26171)</td>
<td>10</td>
<td></td>
</tr>
<tr>
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<td>WA1S1_H06</td>
<td>Acidobacteria (D26171)</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>WA1S1_F03</td>
<td>Acidobacteria (D26171)</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

For instance, a significant increase in species diversity of nematodes, microarthropods, Gymnamoebae, testate amoebae, flagellates, ciliates, and protozoans occurred with increase in years of no-tillage management (Adl et al., 2006). In contrast, the response of both plant species and functional group diversity to the intensity of habitat disturbance in pastures was unimodal, exhibiting maximum plant species diversity in semi-intensive treatments and lower diversity at both low and high interventions (Mills and Adl, 2006). Bacterial functional diversity determined on the basis of sole-carbon-source utilization also showed a unimodal response. In this study, a unimodal response of bacterial diversity to a disturbance (or management intervention) gradient was also observed. Although poultry litter amendments increased the diversity, it was generally higher in pastures, which was an intermediate intervention relative to cropland (greatest intervention) and forest (least intervention).

It has been suggested that plants determine the composition and activity of a soil microbial community (Wardle et al., 1997). Sowing plant seed mixtures on abandoned agricultural land promoted the growth of bacterial community as compared to plots that were left to be naturally colonized by plants (Hedlund, 2002). In contrast, a recent survey conducted on 98 soil samples collected from a wide array of ecosystem types in North and South America indicates that the diversity of microbial communities responds to differences in pH but not in above ground flora and other factors tested (Fierer and Jackson, 2006). Although the range of pH was small (4.7–5.9), our results also suggest a relationship between soil pH and bacterial diversity, measured by either $H/H_{max}$ ($r^2 = 0.8$) or Chao1 ($r^2 = 0.7$). However, it is worth noting that other factors, such as mineralizable C, not studied by Fierer and Jackson, 2006) also showed a strong relationship with these same measures of diversity. Even though C mineralization and pH may associate with diversity, they do not necessarily provide a mechanism that controls diversity.

Changes in bacterial diversity reflected to a large degree the abundance of a few OTUs. The seven abundant OTUs that were preferentially enriched in forest soils represented 23% of the forest soil clones. This was a much higher percentage than the most abundant OTUs under pasture. With one exception, the most abundant clones were Acidobacteria. These oligotrophic bacteria grow slowly and are likely to outcompete copiotrophic in environments where microorganisms are exposed to sustained environmental...
stress, such as resource limitation, suggesting that these OTUs may have increased in abundance very slowly with the maturation of the forest (Fierer et al., 2007). Similarly, the five abundant OTUs that were preferentially enriched in inorganic fertilizer-amended cropland (CTIF) represented 16% of the clones. They were affiliated to Acidobacteria, γ-Proteobacteria, and Firmicutes. The most abundant OTU was a γ-Proteobacteria related to an environmental clone from vegetable fields (GenBank accession number DQ011842). The closest cultured relative was also found in soil, fixes N₂, and tolerates acidic conditions (Swings et al., 1980). Because cropland soil was regularly disturbed with tillage, these abundant OTUs may represent populations that have adapted to both growth and survival under persistent tillage conditions.

Two models could explain the development of these highly abundant OTUs (Fuhrman and Campbell, 1998). Both models assume that soils initially contain large numbers of species, each one of which is represented by only a few individuals. In a clonal model, abundant OTUs arise from a few well-adapted species that expand slowly to occupy large portions of the soil habitat. In this case, microdiversity of the OTUs would be low and sequences would be nearly identical. Microdiversity encompasses the small differences in sequences at the species or subspecies level, in this case within an OTU (Moore et al., 1998). In an environmental model, large portions of the habitat are uniform, allowing for simultaneous increases in abundance for many phenotypically similar species. In this case, the microdiversity would be high and sequences that compose the OTUs, while related, would not be identical. The microdiversity of the abundant OTUs in this study was high, consistent with the environmental model. This conclusion follows from the presence of relatively larger differences in the 16S rRNA sequences within the six most abundant acidobacterial OTUs in forest soil, one γ-proteobacterial OTU in CTIF soil, and one Firmicutes OTU in pasture soil than would be expected from a microevolution in a clonal population.

4.2. Impact of fertilizer amendments on soil microbial communities

Within the agricultural soils, the type of fertilizer amendment had a stronger effect on microbial communities than land use or season. This conclusion was derived consistently from analyses of PLFA, microbial biomass, and 16S rRNA gene libraries. For instance, from 16S rRNA gene libraries, the bacterial diversity was always higher in poultry litter-amended soils regardless of land use or season. Similarly, abundance of more bacterial groups changed as a response to fertilizer amendment than due to land use or season. While LIBSHUFF analysis indicated that the bacterial communities were different between fertilizer amendments, group-specific LIBSHUFF confirmed that the changes to the community composition were more profound in cropland than in pastures. Similar observations have been made in other studies. For instance, amendment of soils with poultry litter in a Florida tomato field increased bacterial diversity (Wu et al., 2008). Long-term fertilization of agricultural soils in Northern China with organic manure yielded distinct community structures with higher richness and diversity (Ge et al., 2008).

The lower bacterial diversity of inorganic fertilizer-amended soils was associated with an increase in abundant inorganic fertilizer-specific OTUs affiliated to Acidobacteria. The Acidobacteria are usually oligotrophic and more abundant in acidic soils (Sait et al., 2006). Therefore, the lower pH and lower organic matter content of the inorganic fertilizer-amended soils may partly explain the higher abundance of members of this phylogenetic group. Because of their abundance, these OTUs may play major roles in soil properties and fertility. In order to validate this hypothesis, the distribution of these OTUs should be examined further, and the organisms should be characterized in pure culture.

Soil microbial responses to poultry litter amendment could be due to either alteration in soil physicochemical characteristics or introduction of microorganisms in the poultry litter. Application of poultry litter caused increases in labile pools of C, microbial biomass, mineralizable and inorganic N, and elemental nutrients with time (Franzluebbers et al., 2004). Although poultry litter amendment includes large populations of exogenous bacteria, common poultry litter-associated bacteria were not abundant in these soils. In addition to enteric bacteria, such as Enterococcus spp. and coliforms, the microbial communities in poultry litter are often comprised of multiple families in the Firmicutes, Actinobacteria and Proteobacteria (Lu et al., 2003). However, the abundance of Firmicutes and Actinobacteria did not change with poultry litter amendment. Even though some proteobacterial groups increased, only two clones showed >95% sequence similarity to environmental clones previously identified in poultry litter (Lu et al., 2003). Similarly, none of the poultry litter-specific OTUs was closely related to species or environmental clones previously identified in poultry litter. However, some were similar to bacteria identified in swine manure (Cotta et al., 2003) and aerobic digestors (Manaa et al., 2003). These results indicate that poultry litter-associated bacteria were not abundant in these poultry litter-amended soils, an observation consistent with other studies (Sinton et al., 2007).

4.3. Seasonal effects on soil microbial communities

Environmental variables govern the structure of microbial communities in soil (Bossio et al., 1998). Microbial biomass and activity are higher during the summer in Scottish grassland systems and correlated with increases in carbon source availability due to root growth (Griffiths et al., 2003). For this reason, the microbial communities in summer were expected to be greatly impacted by short-term effects of the management practices. Thus, summer samples were expected to provide the most sensitive measure of differences between treatments. It was also expected that the microbial populations would be dormant in winter with the lower temperatures causing a decrease in total microbial activity. However, these expectations were not realized in these soils, and seasonal variation in the abundance and composition of specific soil bacterial groups were small relative to the differences associated with fertilizer amendment and land use. The absence of seasonal differences in bacterial communities in most of the pasture and forest soils indicated that these communities were uniform and stable. However, with PLFA, the biomass of the bacterial community actually increased 1.5 ± 0.1 fold in winter. A remarkably similar change in the fungal community (1.4 ± 0.2 fold) was also detected. It is worth noting that the change in microbial biomass (PLFA) with season was not associated with a larger change in the microbial community. This suggests that there was a rather even response to environmental change among the various soil microbial populations with little change in the dominant groups and that the changes in PLFA may reflect physiological adaptation to season rather than changes in cellular abundance. Continuous cultures of yeasts grown at relatively low rates (dilution = 0.03 h⁻¹) and with limited amounts of available nitrogen favor the accumulation of phospholipids at the expense of neutral and glycolipids (Gill et al., 1977). Hence, as available C and N pools are continuously depleted at the end of the growing season, microorganisms may continue to slowly accumulate phospholipids but without a change in cellular abundance.

4.4. Conclusions

Similar to many macrobiota, the biodiversity of soil bacteria in agro-ecosystems has a complex relationship with the intensity of human intervention. A considerable difference in structure and composition existed between microbial communities in forest and
agricultural soils. Similarly, the effect of fertilizer amendment on soil microbial communities was much stronger than either land use or season. The lower bacterial diversity in the inorganic fertilizer-amended soils was due to decreased evenness. The lower evenness was associated with a concomitant increase in abundance of land-use- and/or fertilizer-specific OTUs affiliated to Acidobacteria and γ-Proteobacteria, suggesting a possible physiological adaptation and ecological selection of these groups of bacteria due to the altered soil characteristics. These findings contribute significantly toward an understanding of the specific changes in soil microbial communities in response to long-term agricultural management practices.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.soilbio.2008.07.030

References


