Inorganic Fertilizer and Poultry-Litter Manure Amendments Alter the Soil Microbial Communities in Agricultural Systems

N-076

Kamlesh Jangid[1], Mark A. Williams[2], Alan J. Franzluebbers[3], Jamie S Sanderlin[1], Jaxk H Reeves[1], Michael B. Jenkins[3], Dinku Endale[3], David C. Coleman[1], William B. Whitman[1]

jangidk@uga.edu

whitman@uga.edu

[1]University of Georgia, Athens, GA, [2]Mississippi State University, Mississippi, MS,[3]J. Phil Campbell, Sr., Natural Resource Conservation Ctr., USDA-ARS, Watkinsville, GA

ABSTRACT

Effects of agricultural land management practices on soil prokaryotic diversity have not been well described. Soil microbial communities under three agricultural management systems (conventionally tilled cropland, hayed pasture, and grazed pasture) and two fertilizer systems (inorganic fertilizer or IF and poultry litter or PL) were compared to that of a ~150-year-old forest at the J. Phil Campbell, Sr., Natural resource Conservation Center, 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 pasture. 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 were 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 the basis for promoting conservation agricultural systems.

INTRODUCTION

Bacteria are the most abundant and diverse group of organisms in soil and play a critical role in terrestrial ecosystems and global biogeochemical cycles. However, the activity and diversity of soil microorganisms are directly influenced by changes in the soil environment. Agricultural land management tremendously impacts agro-ecosystems and affects the structure and diversity of soil microbial communities. The results presented here are a part of a larger study investigating the effect of several land management practices on microbial communities. This poster presents work that investigated the composition and structure of soil microbial communities in conventionally tilled cropland, pasture, and forest soils in Georgia using the 16S rRNA gene library approach.

METHODS

Study Site

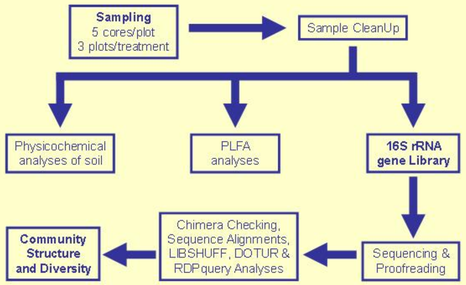
The J. Phil Campbell, Sr., Natural Resource Conservation Center

- 10 X 30 m plots with Cecil sandy loam soil, conventionally cropped with corn/rye since 1991.
- Pastures established in 1991, previously cultivated with rowcrops.
- Control forest with Cecil sandy loam soil, Loblolly pine plantation, no cultivation since 1860's.
- Two seasons Summer (S) and Winter (W) were sampled for each treatment.

Treatments Studied

Sample Code	Type of Field and Fertilizer	Color Code
A	Cropland	
A1	Inorganic	▲
A2	Poultry litter	△
B	Hayed Pasture	
B1	Inorganic	■
B2	Poultry litter	□
C	Grazed Pasture	
C1	Inorganic	●
C2	Poultry litter	○
D0	Undisturbed forest	+

Experimental Strategy



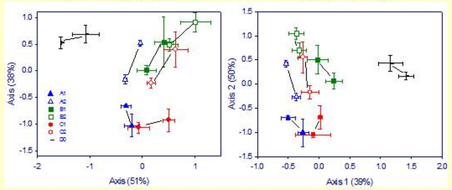
Sequence Data Summary

Clones prepared	: 4032
Sequences obtained	: 3719
Chimeric sequences	: 12
Non-16S rRNA sequences	: 1
Sequences used for analyses	**: 3706**
Mean Read length	: 842 bp

The sequencing was performed at the Molecular Genomics Instrumentation Facility, University of Georgia.

RESULTS

Distinct Microbial Communities in Forests



Non-metric multidimensional scaling plot of the mol% distribution of PLFAs with treatment and season. Standard errors are presented for each treatment in each season (n=3). The summer and winter sampling times are connected by a straight line.

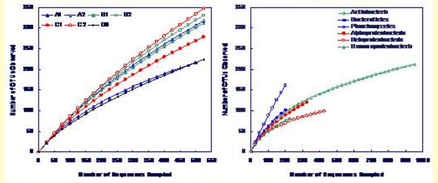
Diversity Indices

Complex Relationship Between Bacterial Diversity & Intensity of Human Intervention

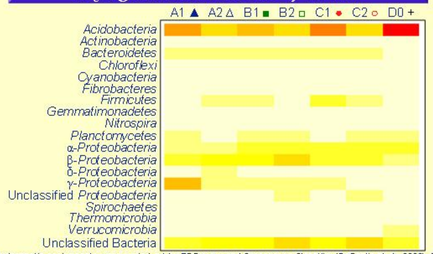
Diversity Index[a]	Treatments A1 ▲	A2 △	B1 ■	B2 □	C1 ●	C2 ○	D0 +	All Sites
N[b]	519	526	534	539	526	527	535	3706
S[c]	219	316	314	335	277	346	225	1333
H[d]	4.97	5.44	5.45	5.57	5.25	5.60	4.56	6.49
1/D[e]	105	152	227	270	143	278	79	333
Eveness[f]	2.12	2.18	2.18	2.21	2.15	2.21	2.06	2.08
H/H_{max}	0.80	0.87	0.87	0.89	0.84	0.90	0.77	0.79
Chao 1[g]	383	748	910	966	752	972	699	3103

[a] Calculations were based on OTUs formed at D= 0.03 using DOTUR (Schloss and Handelsman, 2005), summer and winter libraries were pooled for this analysis; [b] Total number of clones in the library; [c]Total number of OTUs; [d] Shannon diversity index, $H = \Sigma[(ni)(ln(ni/n))]$; at H_{max} n= N, [e] Simpson's index, $D = \Sigma n_i(n_i-1)/N(N-1)$; [f] Minimum and maximum eveness values were 0 & 2.3, respectively; [g] Chao1 = $S + n_1^2/2n_2$, where n_1 is the number of clones that occur twice.

Rarefaction of Clone Libraries



Phylogenetic Distribution of Clones[a]



[a] Phylogenetic assignments were carried out by RDPquery and Greengenes Classifier (DeSantis et al., 2006). The cutoff's used were 75% and 85% for the phylum and class designations, respectively. Color gradation from RED to WHITE indicates decreasing abundance from 50% to 0%.

Log-Linear Modeling[a]

Fertilizer Amendment Strongly Affected Taxa Abundance

Taxa	Intercept	A ▲△	C ●○	2 △□○	Sum
Acidobacteria	26.4			-1.6**	
Firmicutes	4.9	-1.9*	2.0*		
Gemmatimonadetes	1.6				1.8
Planctomycetes	5.3				
Alphaproteobacteria	10.4	-1.9**	-1.4		
Betaproteobacteria	13.7	-1.4	-1.7*	1.4*	
Deltaproteobacteria	2.0	1.8*	-1.2	1.8*	1.9*
Gammaproteobacteria[b]	4.1	4.9**	1.7	-1.2	1.5*
Unclass.*Proteobacteria*	2.8			2.3**	
Unclassified Bacteria	13.1			1.5*	
Others	1.5			2.1*	

[a]Winter sampling of hayed pasture with inorganic fertilizer amendment was set as the baseline. Values were calculated as the natural logarithm of the number of clones in each library and then converted to arithmetic values for an average library size of 100 for clarity. ** and * denote estimates where $p \leq 0.001$ and $p \leq 0.05$ for the interaction, respectively; [b] Parameter estimates for the interaction of cropland*poultry litter amendment and grazed pasture*poultry litter amendment for γ-*Proteobacteria* were -3.42 ($p \leq 0.01$) and -2.76 ($p \leq 0.05$), respectively. No effect on *Actinobacteria*, *Bacteroidetes* and *Verrucomicrobia* was detected.

LIBSHUFF Analyses[a]

Type of treatment	Differences Observed[b] Within Replicates	Between Seasons	Season Specific Groups
Cropland			
Inorganic	S	Y	*Proteobacteria* (α & γ)
Poultry litter	S, W	Y	*Acidobacteria*, Unclass. *Proteo.*
Hayed Pasture			
Inorganic	--	--	--
Poultry litter	S	--	Unclass. *Proteo.*
Grazed Pasture			
Inorganic	S, W	Y	*Acidobacteria*, *Proteobacteria*
Poultry litter	W	--	--
Forest	--	--	*Planctomycetes*

[a]Comparisons were made using LIBSHUFF (Singleton et al., 2001); [b] The experimentwise p-value calculated from the Bonferroni correction was 0.002 for all the treatments. For our analysis we considered 0.01 as significant.

References

- DeSantis, *et al.* 2006. *Applied & Environmental Microbiology* **72:** 5069-5072.
- Schloss, P.D., Handelsman, J. 2005. *Applied & Environmental Microbiology* **71:** 1501-1506.
- Singleton, *et al.* 2001. *Applied & Environmental Microbiology* **67:** 4374-4376.

Treatment Specific OTUs[a]

Clone Name[b]	Taxonomic affiliation	Clone Library A1 ▲	A2 △	B1 ■	B2 □	C1 ●	C2 ○	D0 +
A1S1_A08	*Acidobacteria*	**16**	**3**	1	1	**16**	**1**	**3**
A1W1_B03	*Acidobacteria*	4	3	**8**	**1**	4	5	13
A2S1_D12	α-*Proteobacteria*		2	7	1	2	2	7
A1S1_B09	*Acidobacteria*	8	2	**11**		7	4	**1**
A1S1_A02	*Firmicutes*	9	3	7	3	4		2
A1S1_D06	β-*Proteobacteria*	5	6	7	14	6	9	
A2W3_C01	*Acidobacteria*		1	2	6	8	3	
A2S1_A10	Unclassified		**34**	7	7	17	16	
A1S2_B06	*Acidobacteria*	5		2		1		**13**
A1S1_D02	*Acidobacteria*	**16**			1			5
A1S1_A04	γ-*Proteobacteria*	**27**				6		
DOS1_D09	*Acidobacteria*							**30**
DOS1_A05	*Acidobacteria*							**20**

[a] Only the most abundant OTUs with a N ≥20 are presented. OTUs were formed at D = 0.03. Distributions where $p \leq 0.05$ by the binomial test are represented in bold; [b] Representative clone name for each OTU. GREY shading indicates absence of clones.

CONCLUSIONS

This work suggests that 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 exists between microbial communities in forest and agricultural soils. Similarly, the effect of fertilizer amendment on soil microbial communities is much stronger than either land use or season. The lower bacterial diversity in the inorganic fertilizer-amended soils is due to decreased evenness. This is associated with an increase in abundance of land use- and/or fertilizer-specific OTUs affiliated to the *Acidobacteria* and γ-*Proteobacteria*. This suggests a possible physiological adaptation of these groups of bacteria to more favorable soil environment. These findings contribute significantly toward an understanding of the specific changes in soil microbial communities in response to long-term agricultural management practices.

Acknowledgement

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