Soil bacterial community composition and diversity as affected by animal manure application in pasture and cropping systems of the Southern Piedmont USA

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Abstract

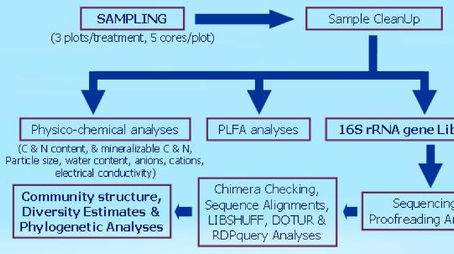
While land management practices are known to have a tremendous impact on agro-ecosystems and their microbial activities, their effects on prokaryotic diversity are not well described. Seven management systems at the J. Phil Campbell, Sr., Natural Resource Conservation Center (JPCSNRCC) near Watkinsville, Georgia were investigated: cropping with inorganic fertilizer (A1), cropping with poultry litter fertilizer (A2), bermudagrass hay with inorganic fertilizer (B1), bermudagrass hay with poultry litter fertilizer (B2), bermudagrass grazed by cattle receiving inorganic fertilizer (C1), bermudagrass grazed by cattle receiving poultry litter fertilizer (C2), and control forest without agriculture since the Civil War (D0). Mixed community DNA was extracted from soil, and the bacterial 16S rRNA genes were amplified in 15-cycle PCR, cloned, and then sequenced. The resulting 3706 sequences were used to analyze the community composition and diversity by RDPquery, LIBSHUFF, and other methods. LIBSHUFF analyses indicated that the bacterial communities from soils under all seven treatments were significantly different. While the forest soils (D0) contained the highest numbers of *Acidobacteria*, the poultry litter treated soils (A2, B2 and C2) contained the least. Similarly, *γ-Proteobacteria* were unusually abundant in soils from cropland with inorganic fertilizer (A1). Interestingly, *Nitrospira* were specifically present only in the poultry litter treated soils (A2, B2 and C2). Seasonal differences were also observed for communities in the cropland (A1 and A2) and grazed pasture receiving inorganic fertilizer (C1), but not in the other treatments. *Gemmatimonadetes* occurred more frequently in the inorganic fertilizer treated summer from both cropland (A1) and grazed pasture (C1) than in the winter. The reasons for differences could be readily explained in only a few cases at this stage. Further analyses will be conducted to better understand the effects of land management on soil prokaryotic communities.

Introduction

Although soils represent one of the most complex and difficult environments to study, it is amongst the largest and extremely diverse reservoirs of prokaryotes in the biosphere. Estimates show that it contains on the order of 2.6 x 10^{29} cells worldwide or ~5% of all prokaryotic cells on earth (Whitman et al., 1998). Agriculture is one of the most important human activities that depends upon soil. While agriculture is well known to affect the activity of prokaryotic community of soil, the effects on prokaryotic diversity are not well described (Kennedy, 1999). Previously published reports that studied the prokaryotic diversity of soil used different methodologies, lacked correlation and were based on insufficient number of 16S rRNA gene clones. Hence, there is a need for a more systematic study that could be used as a standard for future studies.

Experimental Strategy

Mixed community DNA was extracted from soil, and the bacterial 16S rRNA genes were amplified in 15-cycle PCR, cloned in pCR2.1® vector (Invitrogen), and then sequenced.



Site Description

The J. Phil Campbell, Sr., Natural Resource Conservation Center (JPCSNRCC), Watkinsville, Georgia

- 10 by 30 m plots with Cecil sandy loam soil conventionally cropped with corn/rye since 1991.
- Control neighboring forest in an upland field with Cecil sandy loam soil with loblolly pine plantation protected from cultivation since the 1860's.
- Plots conventionally cultivated with various rowcrops prior to grassland establishment in 1991.

Treatments Studied to test affects of manure on soil bacteria

Sample Code	Type of Field and Fertilizer	Type of Manure	Seasons Sampled*
A	Conventionally Cropped		
A1	Inorganic	None	S/W
A2	Broiler litter	Poultry	S/W
B	Unharvested Pasture Grass		
B1	Inorganic	None	S/W
B2	Broiler litter	Poultry	S/W
C	Cattle grazed Pasture Grass		
C1	Inorganic	Cattle	S/W
C2	Broiler litter	Cattle + Poultry	S/W
D	Undisturbed forest with no inputs	None	S/W

*S= Summer, W= Winter

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 16S rRNA gene clones were sequenced at the Pratt Lab, Department of Plant Biology, University of Georgia

Results

Diversity indices for the soil 16S rRNA gene libraries[a]

Diversity Index	Cropland				Forest (D0)	
	Inorganic (A1)		Poultry (A2)			
	A1S	A1W	A2S	A2W	S	W
S[b]	107	148	195	167	142	135
N[c]	259	260	263	263	277	258
Eveness[d]	2.15	2.17	2.20	2.21	2.12	2.13
H/Hmax	0.79	0.85	0.91	0.88	0.81	0.82
Chao 1[e]	125	448	752	318	382	386
95% lci[f]	115	305	517	256	269	265
95% hci[g]	145	653	1068	408	546	561

Diversity Index	Ungrazed Pasture				Grazed Pasture			
	Inorganic (B1)		Poultry (B2)		Inorganic (C1)		Poultry (C2)	
	B1S	B1W	B2S	B2W	C1S	C1W	C2S	C2W
S[b]	195	178	197	188	184	151	200	204
N[c]	272	262	275	264	274	252	260	267
Eveness[d]	2.23	2.22	2.24	2.23	2.20	2.18	2.23	2.24
H/Hmax	0.91	0.90	0.91	0.91	0.89	0.86	0.93	0.93
Chao 1[e]	911	455	529	743	534	429	1040	638
95% lci[f]	585	341	397	499	389	301	662	467
95% hci[g]	1351	613	708	1075	733	610	1547	867

[a] Calculations were based on OTUs formed using DOTUR (Schloss & Handelsman, 2005) at an evolutionary distance of <0.03. S= Summer, W= Winter
[b] Number of OTUs.
[c] Number of clones in the library.
[d] Minimum and maximum eveness values were 0 & 2.3, respectively.
[e] Chao1= S + $n_1^2/2n_2$, where n_2 is the number of clones that occur twice.
[f] 95% lower confidence interval for Chao1 estimator.
[g] 95% higher confidence interval for Chao1 estimator.

LIBSHUFF comparisons of the soil 16S rRNA gene libraries[a]

Type of Treatment	Differences*	
	Within Replicates	Between Seasons
Conventionally Cropped		
A1	S	Y
A2	S, W	Y
Unharvested Pasture Grass		
B1	–	–
B2	S	–
Cattle grazed Pasture Grass		
C1	S, W	Y
C2	W	–
Undisturbed forest (D0)	–	–

[a] Comparisons were made using LIBSHUFF (Singleton et al., 2001). The experimentwise p-value calculated from the Bonferroni correction was 0.002 for all the treatments.
* S=Summer, W=Winter, Y=Significant differences

JPCSNRCC, Forest site

Phylogenetic Assignments of 16S rRNA gene Clones

Taxa	Cropland (#of clones)[a]			
	A1		A2	
	S*	W*	S	W
Acidobacteria	58	71	39	36
Actinobacteria	4	4	5	9
Bacteroidetes	14	24	18	22
Flavobacteria	4	7	13	10
Sphingobacteria	9	16	3	10
Unclassified[b]	1	1	2	2
Chloroflexi				
Cyanobacteria		11	3	6
Fibrobacteres	2		2	
Firmicutes	30	30	56	53
Bacilli	5	1	15	5
Clostridia	1		3	1
Unclassified[b]	24	29	38	47
Gemmatimonadetes	7	1	7	9
Nitrospira			3	2
Planctomycetes	15	10	8	12
Proteobacteria				
α-proteobacteria	13	13	22	18
β-proteobacteria	23	36	27	49
δ-proteobacteria	13	6	30	13
γ-proteobacteria	63	45	18	15
Unclassified[b]	7	5	12	9
Spirochaetes	2			
Thermomicrobia				
Verrucomicrobia	2	2	4	3
Genera incertae sedis		1	2	
Unclassified Bacteria[c]	6	1	7	7
Unclassified				
Total (all taxa)	259	260	263	263

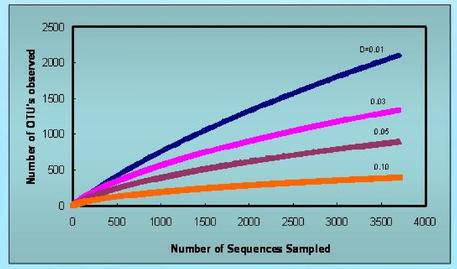
Taxa	Forest (# of clones)[a]	
	S*	W*
Acidobacteria	136	119
Actinobacteria	9	6
Bacteroidetes	5	7
Flavobacteria	1	
Sphingobacteria	1	1
Unclassified[b]	3	6
Chloroflexi		
Cyanobacteria		
Fibrobacteres		
Firmicutes	39	32
Bacilli	4	
Clostridia		
Unclassified[b]	35	32
Gemmatimonadetes		1
Nitrospira		4
Planctomycetes	25	13
Proteobacteria		
α-proteobacteria	27	30
β-proteobacteria	12	20
δ-proteobacteria	1	2
γ-proteobacteria	5	4
Unclassified[b]	4	7
Spirochaetes		
Thermomicrobia		
Verrucomicrobia	13	11
Genera incertae sedis		1
Unclassified Bacteria[c]	1	1
Unclassified		
Total (all taxa)	277	258

Taxa	Pasture (# of clones)[a]							
	B1		B2		C1		C2	
	S*	W*	S	W	S	W	S	W
Acidobacteria	63	54	37	54	76	72	55	47
Actinobacteria	9	3	6	6	7	12	7	10
Bacteroidetes	14	13	9	22	14	8	24	16
Flavobacteria	10	11	6	16	3	6	12	11
Sphingobacteria	3		3	4	9	2	6	4
Unclassified[b]	1	2		2	2		6	1
Chloroflexi			1					
Cyanobacteria		1					1	2
Fibrobacteres				2	2			1
Firmicutes	61	58	60	49	55	64	59	66
Bacilli	11	21	14	5	28	25	19	16
Clostridia							1	
Unclassified[b]	50	37	46	44	27	39	39	50
Gemmatimonadetes	5	3	3	4	12	2	8	7
Nitrospira	2		5	6			6	3
Planctomycetes	12	25	19	15	12	9	13	16
Proteobacteria								
α-proteobacteria	27	29	26	28	24	24	23	21
β-proteobacteria	32	42	47	42	30	17	25	35
δ-proteobacteria	10	7	14	7	8	8	11	9
γ-proteobacteria	17	8	14	9	18	19	7	7
Unclassified[b]	10	9	24	13	4	9	10	19
Spirochaetes								
Thermomicrobia					1			
Verrucomicrobia	4	6	2	2	5	2	3	2
Genera incertae sedis	2	1	1	2	3	1	1	2
Unclassified Bacteria[c]	4	3	7	3	3	4	6	4
Unclassified						1	1	
Total (all taxa)	272	262	275	264	274	252	260	267

[a] Phylogenetic assignments were based upon comparisons to sequences in the RDP database performed by RDPquery (http://simo.marsci.uga.edu/public_db/rdp_query.htm). Clones with ≥75% or ≥85% sequence similarity to a type strain in the RDP were assigned to the same phylum or class, respectively.
[b] Clones with ≥75% but <85% sequence similarity to a type species.
[c] Clones with <75% sequence similarity to a type species in the RDP.
* S=Summer, W=Winter

Rarefaction of clone libraries

Analysis of the clones from all libraries with OTUs defined at different evolutionary distances (D)



[a] Calculations were based on OTUs formed using DOTUR (Schloss & Handelsman, 2005)

Conclusions

LIBSHUFF analyses indicated that the bacterial communities from soils under all seven treatments were significantly different. Forest soils (D0) contained the highest numbers of *Acidobacteria*, poultry litter treated soils (A2, B2 and C2) contained the least. *γ-proteobacteria* were unusually abundant in soils from cropland with inorganic fertilizer (A1). *Nitrospira* were specifically present only in the poultry litter treated soils (A2, B2 and C2). Seasonal differences observed for communities in the cropland (A1 and A2) and grazed pasture receiving inorganic fertilizer (C1), but not in the other treatments. Further analyses will be conducted to better understand the effects of land management on soil prokaryotic communities.

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