Effect of Land Management on Soil Bacterial Community Composition and Diversity in the Southern Peidmont USA

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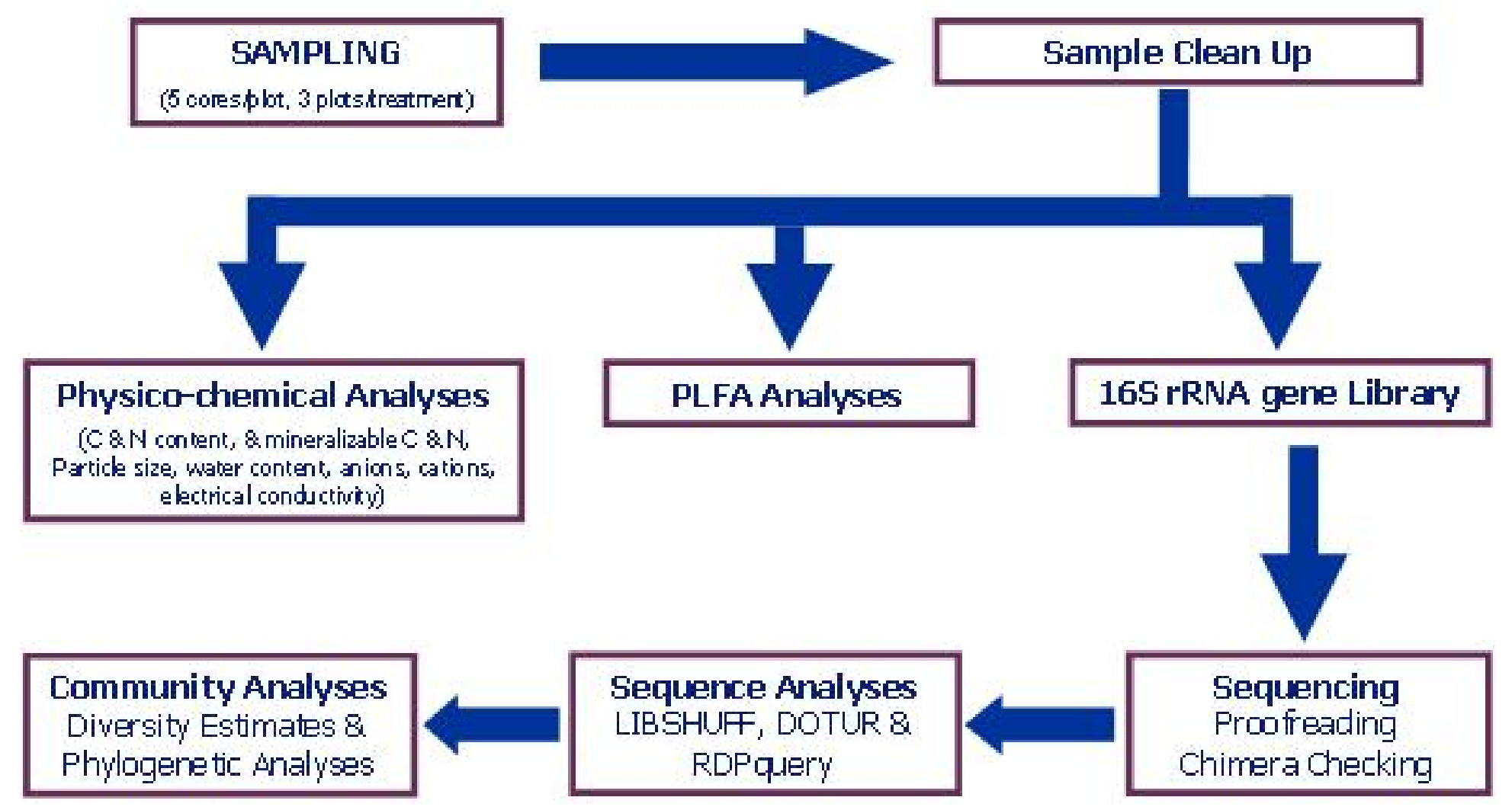
Introduction

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.



SAMPLING
(5 cores/plot, 3 plots/treatment)

Sample Clean Up

Physico-chemical Analyses
(C & N content, & mineralizable C & N, Particle size, water content, anions, cations, electrical conductivity)

PLFA Analyses

16S rRNA gene Library

Sequencing
Proofreading
Chimera Checking

Sequence Analyses
LIBSHUFF, DOTUR & RDPquery

Community Analyses
Diversity Estimates & Phylogenetic Analyses

Study Site

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[a]	Type of Field and Fertilizer	Type of Manure	Seasons Sampled[b]
A	Conventionally Cropped		
A1	Inorganic	None	S/W
A2	Poultry litter	Poultry	S/W
B	Un-grazed Pasture		
B1	Inorganic	None	S/W
B2	Poultry litter	Poultry	S/W
C	Cattle-grazed Pasture		
C1	Inorganic	Cattle	S/W
C2	Poultry litter	Cattle + Poultry	S/W
D	Undisturbed forest with no inputs	None	S/W

[a] For a sample code of WA1S2, the first letter "W" stands for the sampling site Watkinsville, the second letter "A" stands for the type of field (in this case, Cropland), the number "1" indicates the fertilizer added (in this case, Inorganic), the third letter "S" stands for the Summer season and the fourth number "2" stands for the replicate number.
[b] S= Summer, W= Winter

Abstract

While land management practices are known to have a tremendous impact on agro-ecosystems and their microbial activities, its affects on prokaryotic diversity are not well described. Seven management systems at the J. Phil Campbell, Sr., Natural Resource Conservation Center near Watkinsville, Georgia were investigated: (1) control forest without agriculture since the Civil War, (2) cropping with inorganic fertilizer, (3) cropping with poultry litter fertilizer, (4) bermudagrass hay with inorganic fertilizer, (5) bermudagrass hay with poultry litter fertilizer, (6) bermudagrass grazed by cattle receiving inorganic fertilizer, and (7) bermudagrass grazed by cattle receiving poultry litter fertilizer. Mixed community DNA was extracted from soil, and the bacterial 16S rRNA genes were amplified in 15-cycle PCR, cloned, and then sequenced. The community composition and diversity were analyzed by RDPquery, LIBSHUFF and DOTUR. The resulting 3706 sequences formed 1335 operational taxonomic units (OTUs) with Chao1 estimated total richness of 3104 OTUs at 97 % sequence similarity. The RDPquery analyses indicated that the *Acidobacteria* and the *Firmicutes* are the two most abundant taxa in all soil libraries. While the forest soils (1) contained the highest numbers of *Acidobacteria*, the poultry litter treated soils (3, 5 and 7) contained the least. Similarly, *γ-Proteobacteria* were unusually abundant in soils from cropland with inorganic fertilizer (2). Interestingly, *Nitrospira* were present predominantly in the poultry litter treated soils (3, 5 and 7). Seasonal differences were observed for the communities in cropland (2 and 3) and grazed pasture receiving inorganic fertilizer (6), but not in the other treatments. *Gemmatimonadetes* occurred more frequently in the inorganic fertilizer treated summer soils from both cropland (2) and grazed pasture (6). The LIBSHUFF analyses indicated that the bacterial communities from soils under all seven treatments were significantly different. While the *Acidobacteria* and *Firmicutes* caused the differences in the pasture systems (4 through 7), all major taxa except the *Planctomycetes*, were different in the cropping systems (1 and 2). Seasonal differences within a treatment were observed for the cropping systems (1 and 2) and the cattle-grazed pasture soils receiving inorganic fertilizer (6). Most of these differences could be specifically attributed to the seasonal differences in *Acidobacteria*, *Firmicutes* and *Proteobacteria* associated with these soil libraries. The DOTUR analyses for the most abundant OTUs indicated treatment specific OTUs. The addition of poultry litter specifically altered the composition of the *Firmicutes*. A huge prokaryotic diversity still remains unexplored.

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 Molecular Genomics Instrumentation Facility, University of Georgia

Phylogenetic Assignments of Clones

Taxa	Cropland		Ungrazed Pasture		Grazed Pasture		Forest
	Inorganic	Poultry	Inorganic	Poultry	Inorganic	Poultry	
Acidobacteria	129	75	117	91	148	102	255
Actinobacteria	8	14	12	12	19	17	15
Bacteroidetes	38	40	27	31	22	40	12
Firmicutes	60	109	119	109	119	125	71
Planctomycetes	25	20	37	34	21	29	38
Proteobacteria	**224**	**213**	**191**	**224**	**161**	**167**	**112**
Alpha	26	40	56	54	48	44	57
Beta	59	76	74	89	47	60	32
Delta	19	43	17	21	16	20	3
Gamma	108	33	25	23	37	14	9
Unclassified	12	21	19	37	13	29	11
Others	35	55	31	38	36	47	32
Total - All Taxa	**519**	**526**	**534**	**539**	**526**	**527**	**535**

[a] Number of clones is reported. 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% sequence similarity to a type species were assigned as unclassified bacteria.
[b] Clones with ≥75% or ≥85% sequence similarity to a type strain in the RDP were assigned to the same phylum or class, respectively.
[c] Clones with ≥75% but <85% sequence similarity to a type species were assigned to the same phylum as unclassified.
[d] Includes taxa with <10 total clones in the whole library were grouped together as others.
* S=Summer, W=Winter

Diversity Indices

Diversity Index[a]	Cropland		Ungrazed Pasture		Grazed Pasture		Forest	All Sites
	Inorganic	Poultry	Inorganic	Poultry	Inorganic	Poultry		
S[b]	219	316	314	335	277	346	225	1333
N[c]	519	526	534	539	526	527	535	3706
Eveness[d]	2.12	2.18	2.18	2.21	2.15	2.21	2.06	2.08
H/Hmax	0.80	0.87	0.87	0.89	0.84	0.90	0.77	0.79
Chao 1[e]	383	748	910	966	752	972	699	3103
95 % lci[f]	322	613	715	763	586	781	511	2776
95 % hci[g]	481	945	1190	1255	998	1241	987	3481

[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 Analyses of the Clone Libraries

Type of treatment	Differences*		
	Within Replicates	Between Seasons	Season Specific Groups
Cropland			
Inorganic	S	Y	*Firmicutes*, *Proteobacteria* (α & γ)
Poultry litter	S, W	Y	*Acidobacteria*, *Proteobacteria* (unclass)
Un-grazed Pasture			
Inorganic	--	--	--
Poultry litter	S	--	*Proteobacteria* (unclass)
Grazed Pasture			
Inorganic	S, W	Y	*Acidobacteria*, *Firmicutes*, *Proteobacteria*
Poultry litter	W	--	--
Undisturbed forest No inputs	--	--	*Planctomycetes*

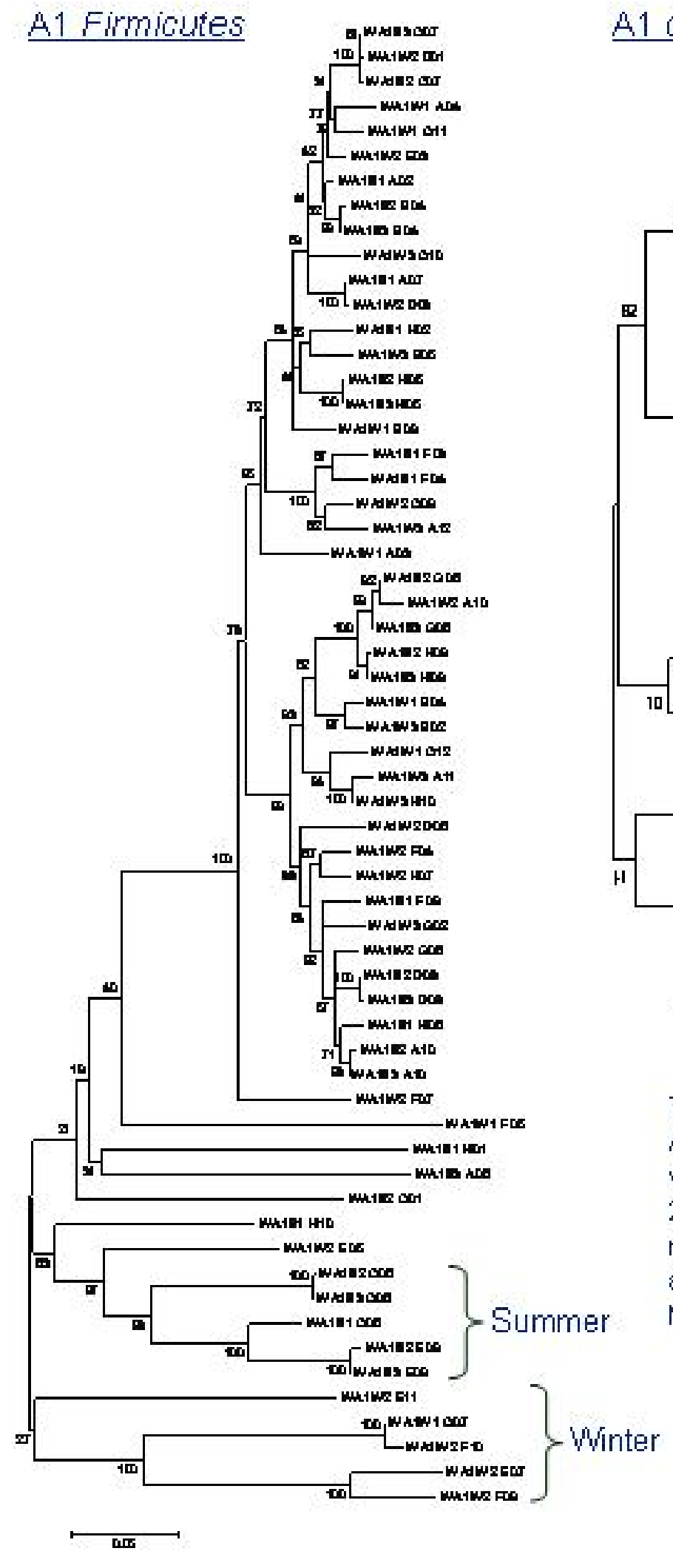
Comparisons between Un-grazed and Grazed pasture

Type of treatment	Differences* (Specific Groups)
Inorganic Fertilizer (Un-grazed vs Grazed)	*Acidobacteria*, *Bacteroidetes*, *Firmicutes*, *Planctomycetes*, *Proteobacteria*
Poultry Litter (Un-grazed vs Grazed)	*Firmicutes*
Inorganic vs Poultry (Both Un-grazed & Grazed)	*Acidobacteria*, *Firmicutes*, *Proteobacteria*

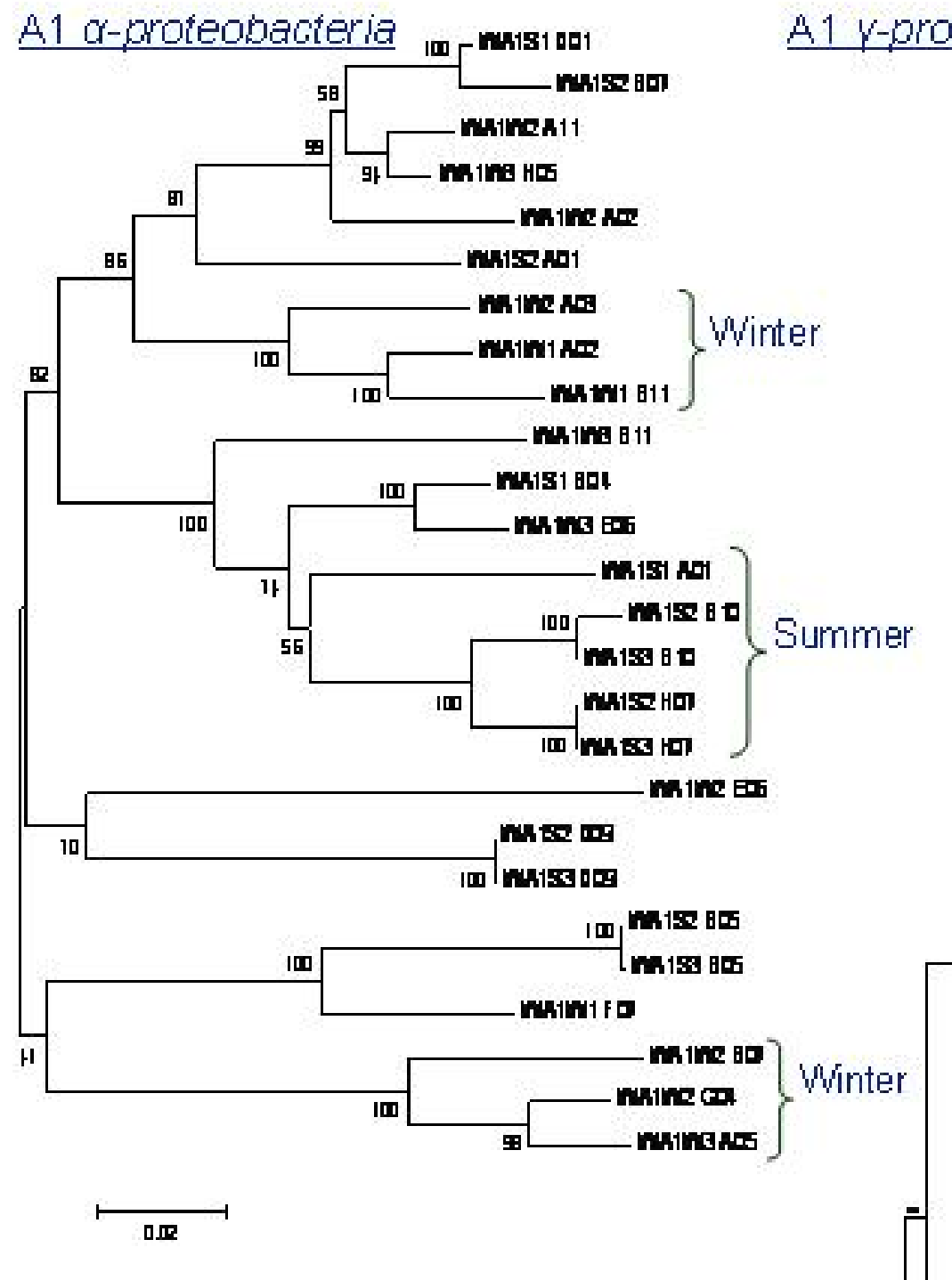
[a] Comparisons were made using LIBSHUFF (Singleton et al., 2001).
* The experimentwise p-value calculated from the Bonferroni correction was 0.002 for all the treatments. For our analysis we considered 0.01 as siginifcant.

Season Specific Clades

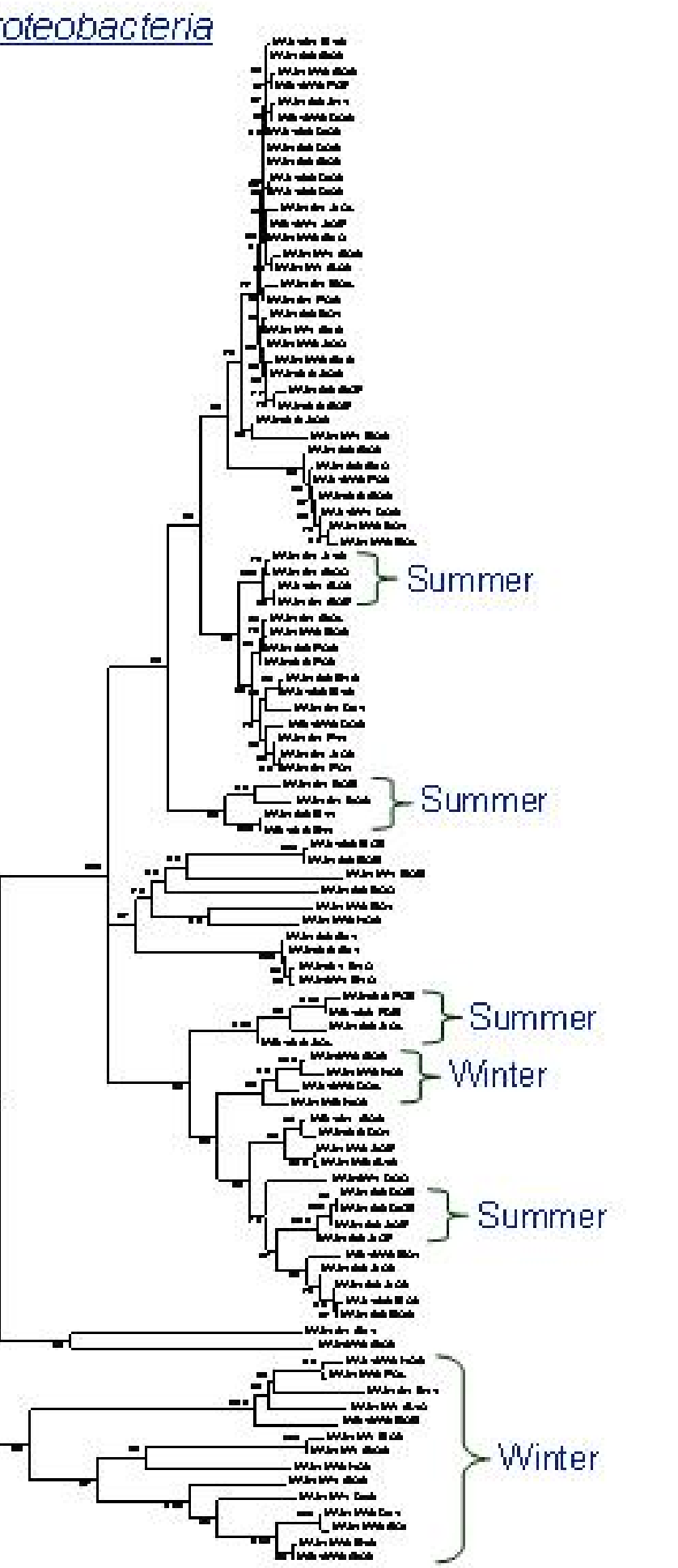
A1 *Firmicutes*



A1 *α-proteobacteria*

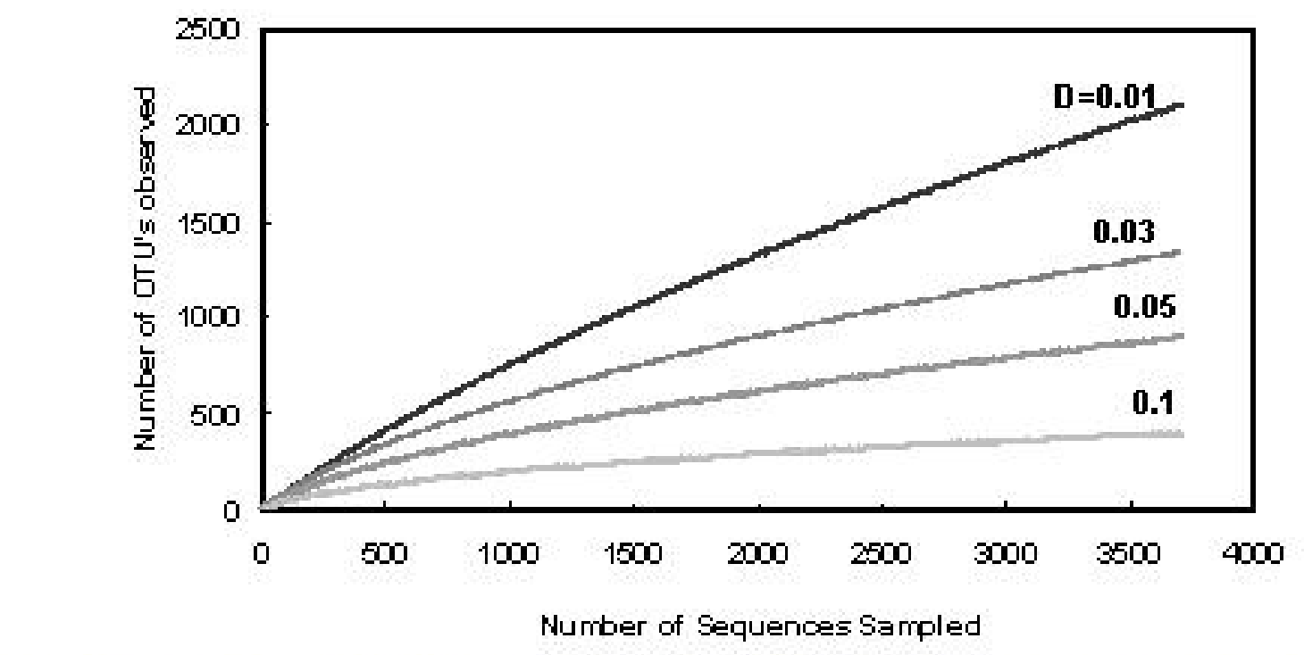


A1 *γ-proteobacteria*



The data presented is representative for the A1 treatment only. Group specific sequences were aligned with NAST (DeSantis et al., 2006). Trees were constructed using 1000 replicates in the Neighbor-joining algorithm and K2P distances with pairwise deletions in MEGA v3.1 (Kumar et al., 2004).

Rarefaction of Clone Libraries



Calculations were based on OTUs formed using DOTUR (Schloss & Handelsman, 2005).

Distribution of Representative Abundant OTUs[a]

Taxa	Clone Name[b]	Cropland		Un-grazed Pature		Grazed Pasture		Forest	N[c]
		A1	A2	B1	B2	C1	C2	D0	
Acido	WA1S1_A08	**16**	**3**	1	1	**16**	**1**	3	41
Acido	WA1W1_B03	4	3	**8**	**1**	4	5	13	38
Bacillus	WA2S1_D12		2	**7**	**1**	2	2	7	21
Acido	WA1S1_G02	1	1		**5**	1	4	4	16
Acido	WA1S1_B09	8	2	**11**		7	4	1	33
Acido	WA1S2_A05	**7**	**1**	1	2	2	2		15
Bacillus	WA1S1_H03	**11**	**1**	2	3	5	2		24
Unclassi.	WA2S1_A10		**34**	7	7	17	16		81
Acido	WA1S1_H05	**8**	**1**	1	3	4			17
Flavobac	WB1S3_B01			**9**	**2**	3	2		16
Acido	WA1S2_B06	**5**		2		1		13	21
Acido	WA1S1_B01	**7**		1		4		5	17
Firmi.	WA2S1_H07		**5**		2		**5**		12
Beta	WA2S2_C07		**7**	3	1				11
Acido	WA1S1_D02	**16**			1			5	22
Gamma	WA1S1_A03	**15**				3	1		19
Sphingo	WA2S2_A05		**9**	1					10
Nitrospira	WB2S1_A03				**7**		3		10
Firmi.	WA1S1_A04	**27**				**6**			33
Acido	WDOS1_D09							**30**	30
Acido	WDOS1_A05							**20**	20

[a] The data in the table is representative. It neither contains all OTUs nor mentions the seasonal distribution of the most abundant OTUs for each treatment. OTUs were formed at an evolutionary distance <0.03. Distributions where p≤0.05 by the binomial test are in bold.
[b] Representative clone for each OTU.
[c] Number of clones in OTU.
[d] OTUs in bold were specifically absent from poultry litter treated soil libraries.

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). The differences between various treatments were specifically attributed to the differences between the *Acidobacteria*, the *Firmicutes* and the *Proteobacteria* present in those soils. The application of poultry litter specifically altered the composition of these three taxonomic groups. Some OTUs were specifically associated with some treatments. Treatment and season specific trends were also observed for some taxonomic groups. The land management in cropping system decreased the prokaryotic diversity. A huge amount of unexplored diversity exists.

References

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