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The effects of urbanization and agriculture on soil bacterial diversity at the phylum level

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Abstract

The limiting factor involved in past assessments of soil bacterial diversity has been the lack of sampling and replication when employing culture-independent techniques. In this study, 23 16S rDNA gene clone libraries consisting in total of over 11,000 clones sampled from soils at the Central Arizona Phoenix Long Term Ecological Research (CAP LTER) site were constructed, allowing for a more robust statistical analysis of potential factors, including land use type, that may significantly alter bacterial species composition. Overall bacterial diversity based on Simpson's reciprocal index is found to increase when comparing urbanized and agricultural samples located outside the city to open desert samples (P=0.0537). The reasons for this apparent gradient is unclear, but may involve an increased presence of carbon and an overall increase in disturbance within urbanized soils as well as the lack of high energy-yielding metabolic substrates in open desert samples, attributing to fewer numbers of species according to the energy limitation hypothesis. When resolving the analysis to the phylum level, changes in abundance amongst land use types are evident in *Proteobacteria* (P=0.0054), in *Acidobacteria* (P=0.0584) and *Firmicutes* (P=0.0091). Diversity estimates based upon ARDRA fingerprints reveal no significant difference in the five most abundant phyla associated with land use. Inter-phylum LIBSHUFF analysis of the clones also shows the highest degree of phylogenetic partitioning between land use categories within the *Acidobacteria* and *Actinobacteria* phyla. This provides further evidence that the members of this relatively unknown phylum may be one of the most metabolically diverse groups. An increase in the numbers of shared taxa in agricultural soils was also observed, indicating that agricultural environments provide an environment that is more conducive to bacterial competition and survival. Overall, this study suggests that urbanization of soil is changing the overall bacterial species regime, particularly in some specific phylogenetic groups.

Introduction

Knowledge of the diversity of complex bacterial assemblages in soil has increased tremendously with the increasing efficiency of molecular-based techniques and new statistical analyses. These methods increase understanding of possible factors that influence species composition in soil habitats. Land use change, particularly those that arise from urban and agricultural activities, is one parameter that may explain this observed heterogeneity in species composition. However, due to the excessive amount of bacterial species existing in soils, a more practical approach to diversity studies is focusing on more resolved phylogenetic groups. Increased awareness of the diversity and function of these groups could ultimately lead to changes in soil management policy in and around urban and agricultural environments to improve overall quality.

The Central Arizona-Phoenix Long Term Ecological Research (CAP LTER) site is located in the heart of the Phoenix metropolitan area, an arid landscape (<50mm annual rainfall) that contains a diverse array of land use types, from urban industrial and residential areas, agricultural regions, and pristine open deserts outside the city. Furthermore, patches of desert surrounded by the urban landscape, known as "desert remnants", provide an estimate of change in natural bacterial communities that reside in close proximity to the urban sector. The confinement of several diverse land use types within a compact geographical region (6400 km^2) makes the CAP LTER a suitable model for testing the potential fluxes in bacterial diversity across land use gradients and may serve as a microcosm in predicting changes in soil of other rapidly urbanized areas.

This work involves a database of over 11,000 16S rRNA gene clones extracted from 23 CAP LTER sites. The sample sites were all categorized based upon land use type, including open desert, agricultural, urbanized, and desert remnant groups. Using DNA fingerprinting counts and partial 16S rRNA gene sequence data, dominant phyla were classified by land use type and compared using various statistical methodologies to discover if shifts in any species composition amongst phyla changes from natural to urbanized/agricultural settings is observed and if so, what specific phyla appear to be most affected.

Objectives

1. **Do relative abundances of Bacterial phyla change significantly across land use types?**
   - The phylogeny of members of large 16S rRNA gene clone libraries (330-509 clones) were identified and designated into respective phyla based upon Amplified Ribosomal DNA Restriction Analysis (ARDRA) fingerprints and extensive sequencing. Relative abundances were categorized by land use type and ANOVA was performed to search for significant changes in mean abundance.
2. **Are there cases of phyla exhibiting higher diversity in a certain land use type?**
   - Unique ARDRA fingerprints were defined as Operational Taxonomic Units (OTUs) and used to calculate diversity measurements based upon Simpson's reciprocal index for the five most abundant phyla. Indices measured were averaged according to land use type and ANOVA was performed to search for any significant changes in diversity
3. **Do intra-phyla sequence libraries differ in respect to land use?**
   - Partial DNA sequences were assigned to all 16S rRNA gene clones based upon ARDRA fingerprint data. All sequences associated with the most abundant phyla corresponding to a single land use type were compiled and subjected to LIBSHUFF analysis to determine shifts in sequence diversity across land use types.
4. **Are desert remnant samples being influenced by surrounding urban areas?**
   - Based upon experimental and statistical data, comparisons of desert remnants with open desert and urbanized samples will provide evidence as to whether these soils are retaining natural bacterial populations or are more representative of urban communities.

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Methods

Sample Collection and DNA Extraction. Soil samples were collected from the upper 2 in. of 200 sites within the CAP LTER area. DNA was extracted from samples using the technique of Smalla et al.(2) Nucleic acids were purified using a Prep-A-Gene kit (BioRad Industries).

PCR and cloning . Community 16S rDNAs were amplified by PCR from 20 to 200 ng of DNA in reaction mixtures totaling 50-µl using universal bacterial primers 27f and 1492r. PCR products were cloned with a TOPO XL cloning kit in accordance with the manufacturer's instructions (Invitrogen Corp.).

ARDRA and Sequencing. A 10µl aliquot of each cloned PCR product mixture was digested with *Taq*I restriction endonuclease for 1 hr at 65^{0}C and separately with *Rsa*I restriction endonuclease for 1 hr at 37^{0}C. The reaction products were analyzed by Nuseive agarose (BioWhittaker Molecular Applications) (3% wt/vol) gel electrophoresis in 1X TBE buffer. The gel was then stained with 10ml of ethidium bromide and visualized by UV illumination. Restriction fragment fingerprints were converted to densitometric curves and subjected to neighbor-joining cluster analysis to identify groups of unique banding patterns using Bionumerics software (Applied Maths). Clones with unique patterns were considered operational taxonomic units (OTUs) with >97% genetic similarity. Banding patterns of interest were sequenced and imported into the BLAST interface of GENBANK for the most closely related sequence in the database.

Analysis of 16S rRNA gene sequence data. Based on the BLAST search results, clones were designated into their respective phyla. Sequence data corresponding to bases 101 to 600 to *Escherichia coli* were used to generate phylogenetic trees and calculate evolutionary distances using the Jukes-Cantor algorithm in the PHYLIP software package.

Chimera Checking. All clones exhibiting less than 90% sequence similarity to an existing GenBank sequence were subsequently sequenced from the 3' end, placed into phylogenetic trees, and compared to the phylogenetic tree generated from the sequencing of the 5' end to check for discrepancies that would suggest for chimeric sequences. The proportions of overall chimeras each sample was below 7%.

Diversity Indices and Statistical Analysis. Percent abundance values were arcsine transformed before statistical analyses were performed. The reciprocal of Simpson's index (1/D) was chosen as a diversity estimate due to its frequent use in ecological studies. The statistical significance of differences in the compositions of pairs of libraries was tested by using the LIBSHUFF software using distances matrices generated as described above (1). Experimental pairwise error rate altered using the Bonferroni correction for n=6. Statistical analyses of diversity estimates and taxonomic group abundance were performed using Least Significant Difference (LSD) adjustment and Correspondence analysis in the SAS statistical package. Phyla with very low abundances (<3%)were omitted from the statistical datasets due to the biases inherent in using large numbers of zero values.

Results

Phylum Abundance. Representatives of 15 phyla were identified from the clone library data. Two phyla displayed abrupt stratification across the four land use types (Fig. 1, P<0.05). The entire *Proteobacteria* phylum was more dominant in open desert soils than urban and desert remnant soils. Most of this land use effect is explained by the abundance of alpha *Proteobacteria*, while the Beta *Proteobacteria* and *Acidobacteria* are slightly more prevalent in urban and agricultural soils. Moreover, correspondence analysis revealed that inter-phyla abundance patterns are different between urban, agricultural and desert soils, while desert remnants soils show some similarity to all other land use types (Fig 2.)

TABLE 2. LIBSHUFF comparisons of specific phyla contained in CAP LTER soil clone libraries[a]

Comparison no.	Homologous (X) data	Heterologous (Y) data	*Acidobacteria*			*Actinobacteria*			*Firmicutes*			*Gemmatimonas*		
	Library	Library	n_X	n_Y	P	n_X	n_Y	P	n_X	n_Y	P	n_X	n_X	P
1 A	Agricultural	Open	179	343	0.001	90	302	0.001	261	172	0.573	103	224	0.001
B	Open	Agricultural			0.001			0.001			0.443			0.001
2 A	Agricultural	Remnant	179	299	0.260	90	89	0.033	261	110	0.595	103	205	0.881
B	Remnant	Agricultural			0.001			0.038			0.001			0.001
3 A	Agricultural	Urban	179	285	0.001	90	245	0.001	261	209	0.001	103	206	0.134
B	Urban	Agricultural			0.001			0.001			0.001			0.001
4 A	Open	Remnant	343	299	0.001	302	89	0.001	172	110	0.054	224	205	0.001
B	Remnant	Open			0.001			0.001			0.185			0.001
5 A	Open	Urban	343	285	0.002	302	245	0.001	172	209	0.001	224	206	0.001
B	Urban	Open			0.813			0.001			0.001			0.001
6 A	Remnant	Urban	299	285	0.015	89	245	0.853	110	209	0.068	206	205	0.549
B	Urban	Remnant			0.001			0.121			0.003			0.671

[a] P-values in red indicate a non-rejection of the null hypothesis that the two libraries are equal using the Bonferroni correction.

Inter-phyla diversity estimates. Statistical comparisons of the diversity within the five most abundant phyla were performed and compared with overall bacterial diversity (Table 1). Despite a definitive transition of overall diversity, inter-phyla diversity estimates were highly variable within land use categories and therefore means were strongly insignificant (P>0.1).

LIBSHUFF Analysis. Significant phylogenetic heterogeneity in the 16S rRNA genes of bacterial phyla across the land use types was observed using LIBSHUFF analysis (Table 2). However, symmetric similarity was discovered in five comparisons, indicating the sequences contained within these land use types are similar. Nearly all agricultural sequences were similar to remnants (Comparison 2A), while nearly all remnant sequences were found in urbanized samples (Comparison 6A).

TABLE 1. Effects of land use on bacterial diversity*.

Phylogenetic group	1/D values				
	Agricultural	Open	Urban	Remnant	F and P values
Proteobacteria	6.79	9.61	12.65	8.49	0.56 (0.6482)
Acidobacteria	38.20	29.73	39.07	21.23	1.00 (0.4167)
Actinobacteria	32.50	35.78	41.42	50.08	0.34 (0.7932)
Firmicutes	5.67	18.01	5.67	11.78	1.77 (0.1881)
Gemmatimonas	38.10	38.91	39.10	22.64	0.62 (0.6133)
Bacteria	61.97	27.41	56.90	41.57	2.86 (0.0537)

*Effects of land use on domain and phylum-level diversity. All F and P values are derived from analysis of variance (ANOVA), with P values determined on the basis of F(3,18) for the experimental study.

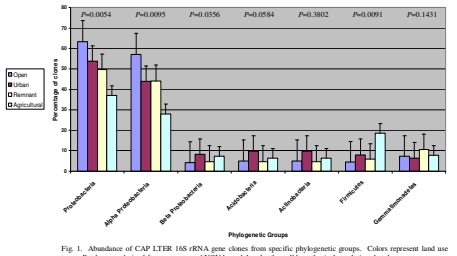

Fig. 1. Abundance of CAP LTER 16S rRNA gene clones from specific phylogenetic groups. Colors represent land use type. P values are derived from a one-way ANOVA model under the null hypothesis that relative abundance means across land use types are equal.

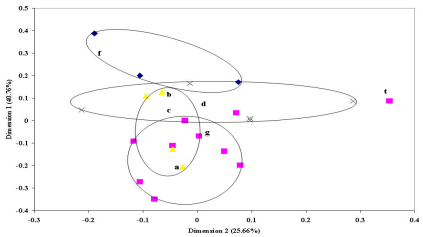

Fig. 2. Correspondence analysis of community structure in land use types open desert (squares), desert remnants (triangles), urban (crosses), and agricultural (diamonds) soils. Ellipses are used to visually aid differences in bacterial diversity structure. Community is represented by alpha *Proteobacteria* (a), beta *Proteobacteria* (b), *Cytophagales* (c), *Acidobacteria* (d), *Gemmatimonadetes* (g), and *Actinobacteria* (t).

Discussion

Changes in abundances of bacterial phyla across land use types suggest selective advantages occurring in CAP LTER soils, resulting in unequal allocation of specific populations. Increases in *Proteobacteria*, and more specifically members of the alpha subdivision, may be the result of *r*-selected reproduction strategies associated with many of its members. Increased urban and agricultural presence of the beta *Proteobacteria* and *Acidobacteria* may be indicative of the importance of these groups in rhizosphere ecology. The increased numbers of *Firmicutes* in agricultural soils is unclear, but is most likely influenced by a weakened presence of *Actinobacteria* and the antimicrobial agents they produce, which are most effective against these bacteria.

Inter-phyla diversity estimates vary widely with land use type samples and therefore no significant stratification in richness and evenness is observed, which is contrary to the changes present in overall bacterial diversity values. This suggests that in a given land type there are similar proportions of species within the more abundant phyla, while the limited presence or absence of other more rare phyla are strongly contributing to overall diversity of the soil.

While similar distributions of species within phyla exist, the total phylogenetic composition of the 16S rRNA gene sequences are vastly different in many instances. Since the bacterial species concept is based primarily upon 16S rRNA gene divergence, these overall differences suggest species partitioning across land use types. Sequence libraries of *Actinobacteria* and *Firmicutes* in two land use comparisons each do not significantly differ, implying that closely related phylogenetic groups are present, presumably filling similar ecological roles in these environments. Furthermore, in all four phyla studied, nearly all desert remnant sequences were also found in their urban counterparts (Comparison 6a, Table 3). In contrast, these remnant sequences are not related to those found in open desert soils in three of the four phyla (Comparison 4b, Table 3). Coupled with the assumption that these remnant soils contained natural open desert bacterial populations previous to the rapid population growth of the area, the LIBSHUFF data provides evidence of urbanization not only affecting communities within the urban sector, but those perceived nearby natural settings as well.

Cumulatively, these results indicate some significant changes in the composition of bacterial groups across land use types. Thus, an important factor in these observed shifts may relate to the disturbance of natural desert communities via urbanization and agricultural practices. Properties most likely associated with this disruption include artificial irrigation systems and increasing diversity in metabolic substrates. Increased moisture content within urban and agricultural soils allows for frequent immigration of species and materials, disturbing local desert populations and introducing new species based upon amended ecological constraints on the system which could weaken overall soil health. Further analysis of those phyla more strongly influenced by urbanization may lead to amended management policies to improve soil quality of urbanized environments.

Conclusions

Significant changes found in *Proteobacteria* and *Firmicutes* abundance across land use types.

Land use categorization does not predict significant change in intra-phyla diversity, although fluxes in overall bacterial species diversity is observed.

Desert remnant soil 16S rRNA gene sequence libraries are more closely related to urbanized soils than open desert soils.

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