B-1086 Analysis of Diazotrophic Community Structure within an Urbanized Desert Ecosystem

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Abstract

Nitrogen-fixation plays a crucial role in the ecology of soils, particularly in arid environments where complex biological soil crusts form. These mature crusts function as a reserve for fixed nitrogen. However, many of these soils have been anthropogenically disturbed, resulting in significant decreases in nitrogenase activity. This change is likely indicative of the variation in bacterial community structure observed at these sites. The goal of this molecular-based study was to use the *nifH* gene to analyze diazotrophic communities within the Central Arizona Phoenix Long Term Ecological Research (CAP LTER) site, a section of the Sonoran desert that is composed mainly of rugose crusts outside of the city and disturbed crusts within urbanized areas.

Soil samples were collected from fifteen CAP LTER sites. The sites were categorized as either urban, open desert, or desert remnant. PCR amplification of *nifH* gene fragments was performed using a modified protocol and primer set initially used by Poly et al. (2000). Clone libraries were generated using a TOPO TA kit. Clones were sequenced and identified using the BLAST search tool, and aligned using the MEGA3 sequence alignment editor.

Overall, the majority (~80%) of *nifH* sequences were *Nostoc*-like, which is in agreement with past arid soil studies that find that nitrogen fixation is primarily conducted by heterocystous cyanobacteria. However, *nifH* sequence pools obtained from open desert soils appear to differ from urbanized counterparts in terms of overall proportions of *nifH* sequence clusters. The urban soils also contained slightly higher proportions of non-cyanobacterial species, revealing a more diverse assemblage of nitrogen-fixing bacteria in disturbed soils.

The results indicate that anthropogenic factors associated with urbanization of desert soils not only effect overall bacterial community structure, but also those populations involved in nitrogen fixation. These differences are likely related to the increased water retention and flow in urbanized soils. This permits a wider range of nitrogen-fixing bacteria to reside in urban soils. However, this increase in *nifH* diversity may also reflect the degradation of natural desert crust flora, thereby limiting nitrogenase activity and overall health of the soil.

Introduction

Knowledge of the diversity of complex bacterial assemblages in soil has expanded tremendously with the increased efficiency and robustness of ribosomal RNA gene-based molecular techniques and statistical analyses. However, many of these studies are deficient in assaying the functional diversity within soil environments that can lead to a more resolved understanding of microbial ecology. One fundamental component of ecosystem viability is nitrogen fixation; a process fueled by microbes via the nitrogenase protein complex. Nitrogenase gene diversity, typically represented by *nifH* sequence data, has been shown to change drastically across various terrestrial and aquatic environments (6). Furthermore, the degree of variation in diazotrophic communities within soils is often significant (4-6). One such example of this phenomenon is in arid soils, which are typically encased by a biological crust that is composed mostly of cyanobacteria. However, recent disturbances in these crusts can lead to dramatic long-term transformations in diazotrophic community composition (4). Changes in the diversity of nitrogen-fixing bacteria in these soils can lead to a substantial decrease in the rates of nitrogen-fixation activity and the overall nitrogen content (1). Land use change, particularly those that arise from a wide range of intense urban and agricultural activities, is one parameter that may qualify as disturbances that may affect nitrogen-fixation in soil. Potentially, land use change could disrupt critical roles that nitrogen-fixing bacteria fill in terms of soil stability, water retention, and overall soil quality.

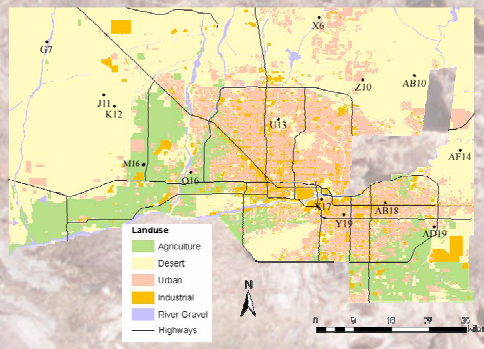

Figure 1. MAG land use map of CAP LTER site and sample plot collection locations used for *nifH* gene clone library construction

The Central Arizona-Phoenix Long Term Ecological Research (CAP LTER) site is located in the heart of the Phoenix metropolitan area, as well as surrounding rural areas (Figure 1). The site provides for an arid landscape (<50mm annual rainfall) that contains a diverse array of land use types, including 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 health of other rapidly urbanized areas. This work involves a database of over 500 *nifH* gene clones extracted from fifteen CAP LTER samples (Figure 1). The sites were all categorized based upon land use type, including open desert, agricultural, urban, and desert remnant groups. Analysis of community *nifH* gene sequence data was used to examine the relationship between diazotrophic bacterial diversity and the rapid urbanization of the Phoenix metropolitan area and the open desert soils that encircle it.

Methods

Sample Collection and DNA Extraction. The samples used in the study were part of the 204 soil samples collected aseptically from various recorded sites of the CAP LTER using a dual-density, randomized, tessellation-stratified design to obtain an unbiased, spatially-dispersed sample that allowed for maximum postdesign stratification. This sampling approach ensures representative and unbiased characterization of ecological resources. The sampling unit was a 30 X 30m plot. One hundred gram soil samples were dug from the upper 5 cm of soil crust using a sterile scoop. The sampling area within the plot was chosen based on the absence of vegetation and leaf litter. Classification of land use type at each of the surveyed sites was according to the Maricopa Association of Governments land-use classification scheme (1997). Community DNA was extracted from soil samples using a modified method of Herrick et al. (1993).

PCR, cloning, and sequencing. Community *nifH* DNAs were amplified by PCR from 20 to 200 ng of DNA in reaction mixtures totaling 50-ml using *nifH*-specific primers *PolF* [5'TGCGAYCCSAARGCBFACTC3'] and *PolR* [5'ATSGCCATCATYTCRCCGGA3'] (3). PCR conditions consisted of an initial denaturation step of 94°C (6 min), a hot start step at 90°C (30 s), 30 cycles at 94°C (1 min), 1 min for annealing at 55°C (30 s), and 72°C (2 min), with a 5 min extension at 72°C for the final cycle. PCR products were cloned with a TOPO TA cloning kit in accordance with the manufacturer's instructions (Invitrogen Corp.).

Analysis of *nifH* gene sequence data. Based on the BLAST search results, *nifH* clones were assigned into their respective phylogenetic groups. Alignment of DNA sequences was performed using ClustalW (Higgins et al. 1994) and visually inspected using BioEdit sequence alignment editor (Hall 1999). Translations, percent dissimilarity distances, phylogenetic analysis of Amino acid sequence data was performed using the MEGA software package version 3 (Kumar et al. 2004). NifH dendrograms were also constructed in MEGA using the minimum evolution function to analyze initial neighbor joining (NJ) trees.

Results

***nifH* sequence analysis.** Using *nifH*-specific primers, PCR products from selected CAP LTER soils of varying land use type were cloned and sequenced (Table 1). Sequences that shared 95% similarity or greater were categorized into *nifH* cluster types (Table 2). Overall, fourteen clusters were comprised of cyanobacteria commonly found in arid and seimiarid soil crusts. The majority (72%) of *nifH* sequences obtained from the sites were closely associated with members of the cyanobacterial genus *Nostoc*. All *Nostoc*-like sequences were at least 87% similar to each other and placed into 8 different *nifH* clusters (A-H). Three additional clusters (I-K) were slightly diverged from *Nostoc*, and more closely related to the cyanobacterial genus *Anabaena*. Two other clusters detected were related to an Utah soil crust isolate M and *Microcoleus chthonoplastes*, respectively. Overall, cyanobacterial abundance and diversity within the clone libraries was higher in open desert and desert remnant soils than urban and agricultural soils.

Five percent of the *nifH* sequences were associated with members of the alpha-subclass of the phylum *Proteobacteria*. Clusters P and R were closely related to the Nickel mine clone SERD-40A obtained from New Caledonia and clone YC06 collected from a acidic German forest soil. Cluster Q, containing a single urban soil clone, was nearly identical to *Rhodovaculum strictum*, a purple non-sulfur bacterium isolated from a Japanese tidal pool that is classified as a halophilic, facultatively aerobic photoheterotroph. Cluster S clones were associated with *Azospirillum brasilense*, a common free-living, surface-colonizing rhizobacterium that has been shown to promote plant-growth in many soils. The majority of these sequences were collected from urban and agricultural soils.

Table 1 - Description of CAP LTER soils used in this study

Soil Type	Soil ID	Land Description	No. of clones
Urban	AB18	Urban;residential;low density;xeric	82
Open	AB10	Open; Desert	49
Remnant	X6	Open; Desert	73
Open	AF14	Open; Desert	43
Urban	O16	Urban; transportation	11
Open	J11	Open; Desert	35
Open	K12	Open; Desert	35
Open	G7	Open; Desert	20
Urban	AD19	Urban;residential;low density;mixed	39
Remnant	U13	Undisturbed desert	24
Urban	X17	Urban;residential;high density;xeric	14
Urban	Y19	Urban;residential;low density;xeric	30
Agricultural	M16	Agricultural;cropland;fallow	14
Remnant	Z10	Open; Desert	11

Five additional *nifH* clusters (31 total sequences) were found to be unique to the urban CAP LTER sites. All of these clusters appear to be phylogenetically related to members of the low G+C Gram positive *Paenibacillus*, a genus that have been found to frequently associate with the rhizosphere of various plants species across different environments. These bacteria also have the ability for high rates of nitrogen fixation, even in limiting environmental conditions. The remaining three clusters (V,W, and Y) contain sequences that are nearly identical to clones obtained from a post-fire mixed conifer forest located in northern New Mexico.

Table 2 - Sequence analysis of *nifH* gene clones from CAP LTER samples

nifH cluster(s)[a]	Closest relative[b]	% Similarity		% of each cluster type: Urban	Open	Remnant	Agricultural
A-H	*Nostoc* spp.	98-99	Cyanobacteria	60	78	86	58
I-K	*Anabaena* spp.	96-99		6	9	9	0
L-M	Cluster M clone	95-99		2	5	5	0
N	*Microcoleus* spp.	91-95		2	3	<1	7
O	*Sinorhizobium* spp.	91-94		2	<1	0	14
P	clone SERD-40A	95-96	Alpha-proteobacteria	<1	2	0	0
Q	*Rhodovaculum strictum*	91		<1	0	0	0
R	clone YC06	92-96		4	<1	0	7
S	*Azospirillum brasilense*	91-95		1	2	0	14
T	*Paenibacillus azotofixans*	92-96	Low G+C Gram-positive	8	0	0	0
U	clone M1a-88	95-96		5	0	0	0
V	*Paenibacillus wynnii*	88		2	0	0	0
W	clone BIc-9	92-93		2	0	0	0
Y	clone M1a-82	95-96		2	0	0	0

[a] Sequences that shared 95% similarity or greater were categorized into nifH cluster types

[b] Based upon BLAST search results

Phylogenetic analysis. Amino acid sequences translated from representative *nifH* clones revealed phylogenetic divergence between nitrogen-fixing communities of varying land use type (Figure 2). NifH protein sequences were associated with subclusters IJ, IB, and IE as defined by Zehr et al (6). Members of the 1B subcluster include all cyanobacteria and over 350 unique sequences from a wide range of environments. Only 3 out of 14 (21%) representative sequence groups within subcluster 1B were detected in all four land use types. The majority of NifH sequence types were found in open desert, desert remnant, and urban soils (64%, 50%, and 65% respectively). In contrast, agricultural sequence types in the 1B subcluster were detected only once. Representative NifH sequences related to subcluster 1J were also detected. This group is composed of members of the family *Rhizobiaceae* within the alpha proteobacteria. Within the database, only open desert and urban samples contained sequences from this group. Furthermore, four of the six sequence types within this subcluster were detected in a single land use type. The final subcluster found in the CAP LTER soils was 1J, a group complied of members of the genus *Paenibacillus*. Representative sequences associated with this subcluster were only detected in the urban soils.

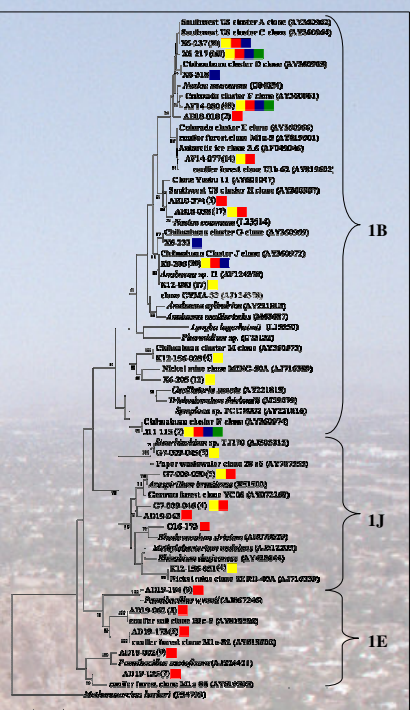

Figure 2. Dendrogram of NifH sequences (103 derived amino acid positions) translated from clones collected from CAP LTER samples (shown by CAP LTER soil type and followed by clone number; e.g., AD19-135). Numbers in parentheses represent the number of additional *nifH* clones with 95% shared DNA similarity with the corresponding representative sequence. Bootstrap values from 100 resamplings are shown outside of nodes if larger than 50. Accession numbers are listed next to previously described sequences. Colored blocks adjacent to representative sequences denote presence in an agricultural (yellow), urban (red), remnant (blue), and/or agricultural (green).

Discussion

This culture-independent study provides evidence for shifts in diazotrophic community diversity across land use types at the CAP LTER site. Cyanobacteria appear to dominate the open desert soils where undisturbed, mature crusts facilitate the substantial growth of heterocystous, *Nostoc*-like species. *nifH* clone libraries constructed from the nearby Colorado plateau and Chihuahuan desert regions yielded similar community patterns (4), suggesting that the open desert outside of Phoenix is retaining the microflora typical of later stages of succession that was present before the urbanization event occurred. The desert remnant sites also appear to remain nearly identical to the open desert in terms of being dominated by members of the 1J subcluster. However, there remains multiple instances in which open desert sequence types were not detected in desert remnant samples, despite no visible differences in land cover. The data suggests only a slight deviation in nitrogen-fixing communities within these areas despite its close proximity to heavily urbanized settings, where multiple changes in the physical environment have been observed, including a reduction in C/N ratios (2). In terms of the community structure, the results indicate that in desert remnant areas, sparsely populated cyanobacterial species are becoming rare, while the more dominant species maintain a strong presence and are generally unaffected or aided by limited amounts of anthropogenic disturbances.

The *nifH* sequences of urban and agricultural CAP LTER sites revealed a more diverse assemblage of nitrogen-fixing bacteria from three highly diverged phyla. This is likely an indication of the increased vegetation in urbanized and agricultural settings that reverts the crust to a poorly developed state, thereby selecting for rhizobacterial nitrogen-fixing species which reside near the plant-soil interface. Unlike the cyanobacteria, the rhizobacteria maintain symbiotic interactions with the urban or agricultural vegetation and posses the ability to produce and degrade antimicrobial toxins from other competitors. The increased presence of spore-forming *Paenibacillus* spp. suggests major disturbances in these soils that inhibits or kills normal desert flora and allows spore formers to quickly colonize the area in the early stages of ecological succession. These urban and agricultural communities appear to mimic less arid environments and diverge from typical desert soils. The overall ecological impacts of these shifts in nitrogen-fixing bacterial community structure in terms of overall soil health has yet to be determined.

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exploring changes in microbial diversity across landuse gradients

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