OBSERVING PATTERNS OF PROKARYOTIC DIVERSITY ALONG LAND USE GRADIENTS OF THE CENTRAL ARIZONA-PHOENIX LONG-TERM ECOLOGICAL RESEARCH SITE (CAP-LTER).

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Abstract

The CAP-LTER project is located in the Phoenix metropolitan area, an urban ecosystem situated within an arid landscape. The study area comprises the city of Phoenix and surrounding areas including suburbs, agricultural regions and undeveloped natural environments. The rapid expansion of the city into former pristine settings provides a unique opportunity to monitor human induced ecological transformations resulting from rapid land-use change. A total of 200 soil samples representing a variety of land use sites have been used in this study. Both culturing and non-culturing methods have been employed to study microbial populations in these soils. This presentation will focus on metabolic diversity that has been studied using BIOLOG Eco plates. BIOLOG Eco plates are specifically designed for characterization of microbial communities in environmental samples. Direct comparison of patterns generated in the BIOLOG plates has allowed grouping of the samples according to the similarity of their community profiles. The contribution of various members of the microbial community to these patterns is established by direct analysis of BIOLOG wells. DGGE analysis provides data on phylogenetic diversity of the organisms metabolizing each type of substrate. The DGGE patterns obtained from BIOLOG wells can be compared with the DGGE profiles of the original sample.

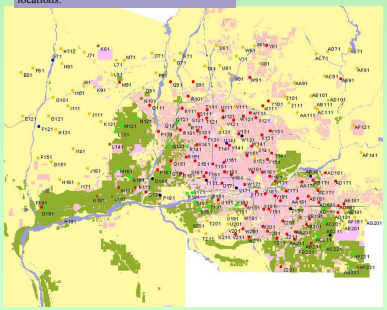
Introductio n

The Central Arizona Phoenix Long-Term Ecological Research (CAP-LTER) is located in the Phoenix metropolitan area. With less than 7 inches of annual rainfall, a high evaporation rate and a continued threat of desertification this is a truly arid environment. Phoenix is one of the largest and most rapidly growing cities of the American west. The spectacular growth of the city (population has doubled twice in the last 35 years) has lead to natural desert habitats being converted for different land-use such as agriculture and residential. The objectives of the CAP-LTER center on understanding the structure and function of the urban ecosystem, especially how ecological patterns and processes are altered as land-use changes during urban growth. In this project patterns of community composition and function will be observed in relationship to land use, which exhibits both temporal and spatial variation in this urban environment, using both culturing and non-culturing techniques.

200 samples were chosen at random within the site and the locations of these are shown in Figure 1. Total viable counts of organisms have been determined for each sample by dilution plating on both nutrient rich and dilute culture media. The functional diversity or microbial metabolic diversity of the soils has been demonstrated and compared on the basis of the communities ability to oxidize a range of carbon sources using the BIOLOG Microplate system. BIOLOG Eco plates are specifically designed for characterization of microbial communities in environmental samples. The plates contain three sets of 31 carbon sources along with other nutrients and a tetrazolium dye that indicates oxidation of the substrate. Previous studies have shown that the method can be used to detect temporal and spatial differences from various different environments including soils (Zak *et al*., 1994; Bossio and Scow, 1995; Wünsche *et al*., 1995). The contribution of various members of the microbial community to the BIOLOG patterns can be determined by direct analysis of the BIOLOG wells. Denaturing gradient gel electrophoresis (DGGE) is being used to analyze 16S rDNA PCR products amplified from the positive BIOLOG well samples. PCR products of the same length can be separated by DGGE on the basis of nucleotide composition. The number, intensity and migrated distance of the individual bands reflect both the number and relative abundance of major 16S rDNA types within the sample.

This presentation focuses on the metabolic diversity from each sample as determined from BIOLOG analysis and comparisons of patterns from the different land use sites.

Figure 1. CAP-LTER site and sample locations.



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Results

Total viable counts have been recorded from all of the sample sites. Higher counts are seen on dilute media and counts from urban residential and agricultural samples are generally higher than those from open desert sample sites. Isolates from these plate counts have been collected and stored in a culture collection for future analysis.

BIOLOG analysis was carried out on all samples and plates were incubated for 10 days at 26°C. Color changes in the wells were measured at OD 595nm on an Ascent Plate reader (Labsystems, Helsinki, Finland) after 24 hours, 72 hours, 7 days and 10 days. Images of the plates were also recorded at 72 hours and 10 days. Examples of patterns generated from two different open desert sample sites after 10 days are shown in Figure 2.

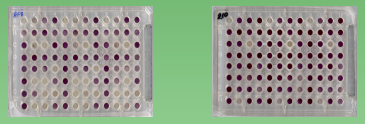


Figure 2. Example of BIOLOG patterns after 10 days incubation. Both are Open desert sites.

Substrate utilization patterns from each plate can be compared using the Bionumerics program (Applied Math, Kortrijk, Belgium). The program compares color intensities from each plate and generates a dendogram based on similarity of the patterns. Figure 3 shows a dendogram generated from all the sample sites after 10 days incubation.The major sample sites Urban residential, Agricultural and Open desert are shown in colors to illustrate grouping.

The functional diversity of each sample is established by the substrate utilization patterns of the BIOLOG plates. The next step is to determine what specific organisms are present in each sample and creating these patterns.

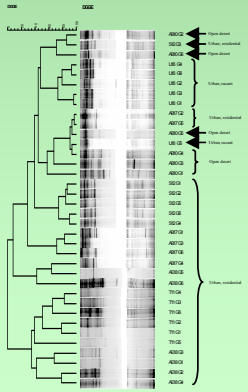
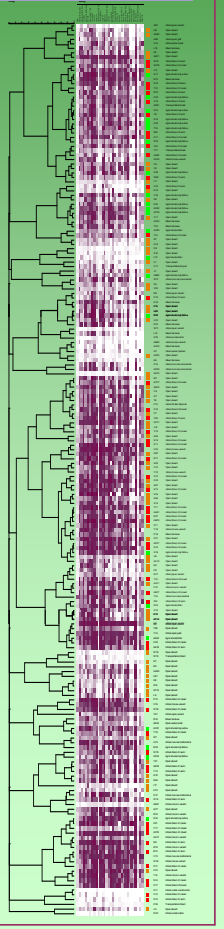


Figure 4. Comparison of patterns generated by DGGE from six sample sites which showed growth on all six guilds of the BIOLOG plate.

BIOLOG plates contain 31 different carbon sources, this means that there could be a maximum 31 different mixed cultures growing for each sample. Samples were collected and DNA extracted from each positive well from each BIOLOG plate. A total of 2711 DNA samples were taken for further analysis. To cut down on the amount of samples that are to undergo DGGE analysis positive samples from each plate have been grouped into Guilds by substrate type according to Zak *et al*., 1994. These are 1) Carbohydrates, 2) Carboxylic Acids, 3) Polymers, 4) Amino Acids, 5) Amines/Amides and 6) Miscellaneous. Grouping into guilds means that if growth was seen in all the wells of the BIOLOG plate the maximum number of samples to be analyzed per plate is 6 instead of 31. DGGE analysis is performed on these samples using primers GC-GM5f and 907r (Teske *et al*.,1996). Patterns generated by DGGE can also be compared using the Bionumerics program and Figure 4 shows a comparison of six different sample sites all of which exhibited growth in all 6 guilds. From this it can be seen that banding patterns tend to group by site rather than by substrate type.

Figure 3. Dendogram showing comparison of BIOLOG patterns for all sample sites.



Summary

Total counts from natural undisturbed desert and open desert samples were generally lower compared to those measured from urban residential, agricultural, and vacant lot samples. These lower total counts do not necessarily mean a lower functional diversity as open desert soils utilized a high number of different carbon sources in the BIOLOG analysis. Also, higher total counts but utilized only a few different carbon sources. This may indicate that the microbial populations from the natural desert soils is made up of a lesser number of organisms adapted to desert soils is made up of a lesser number of organisms adapted to using a wide range of substrates, whereas microbial populations from agricultural and residential samples have a higher number of organisms adapted to a narrower range of substrates.

Comparisons of the utilization patterns generated by the BIOLOG analysis has shown that there does seem to be some grouping of land use sites. When a narrower comparison was made between natural desert sites and agricultural sites (results not shown) more distinct differences were noticed. Open desert has received no external inputs whereas agricultural soils will be highly treated by tillage, addition of fertilizers and irrigation. It is expected that these two land-use types would provide very different environments for the microbial populations so differences in functional diversity between the sites would be expected. It should be considered that the actual location within the CAP-LTER site will have a significant influence on the microbial population of the sample. Soil types and conditions can vary greatly within the land-use types described and that is why it is important to know location of the sites in relation to each other. Recently it has been noticed that certain bacterial divisions have been found in diverse habitats (Hugenholtz *et al*, 1998) and suggests that they exhibit a broad range of metabolic capabilities. The presence of ubiquitous taxonomic groups throughout the CAP-LTER site irrespective of land use may link functional diversity of different sites.

DGGE analysis of the sample from the BIOLOG plates is work in progress. Initial results suggest that patterns generated from different substrates group with other patterns from the same site rather than with patterns from the same substrate of different sites. More gels need to be analyzed before any conclusions can really be drawn from the DGGE study. Dominant bands from DGGE gels are to be excised and sequenced to identify the organisms present in the sample this will identify any ubiquitous taxonomic groups and any that are specific to any particular land use type.

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