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## Introduction

Cryptobiotic soil crusts in arid regions contribute to ecosystem stability through increased water infiltration, soil aggregate stability, and nutrient cycling between the soil community and vascular plants. Loss of this crust may drastically affect the potential of these soils to sustain microbial activity and limits the likelihood of success for re-vegetation efforts. Managing the fertility of these soils requires understanding of the soil microbial ecological networks. Unfortunately desert soil fertility is an often-overlooked area of investigation. We employed and cross-validated three distinct methods to estimate residual soil fertility potential of soils following grazing and establishment of natural gas extraction pads in the northern Chihuahua Desert. Actual activity is described through the catabolic profiles obtained by estimation of total soil respiration (MicroResp™) induced by a range of amino acids, carboxylic acids, sugars and polymeric lipids. Nitrogen cycling potential was estimated by quantification of genetic markers relevant to the N cycle. Total bacterial and fungal diversity was evaluated by targeted metagenomic pyrosequencing.

## Objectives

- Determine effect of land disturbance on genetic and functional diversity of soil microbial community
- Evaluate the discriminant power of genetic diversity and physiological profiling tools for assessing arid land degradation and remediation success

## General Methodology

### Sampling sites:

Sampling was carried out in the northern Chihuahua desert in New Mexico, USA. The site has an average elevation of 1557m, and receives an average annual precipitation of 25cm.

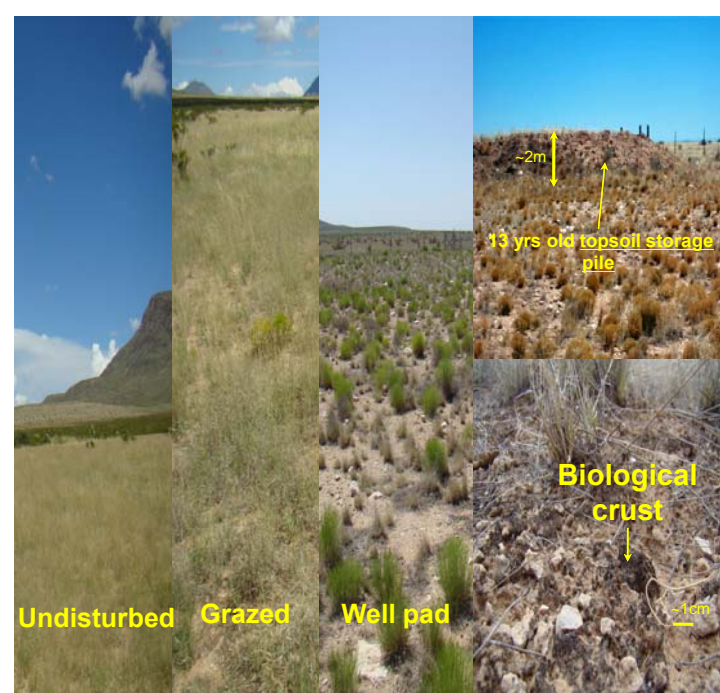
Undisturbed arid land grassland, grazed areas and oil well pad disturbed soils were collected (sandy and sandy loam soils):

- Undisturbed grassland biological crust samples and surficial soil samples without the crust were collected.

- Adjacent grazed lands surficial soil and subsurface samples.

- During the preparation of an oil extraction pad the surface soil was scraped and pushed into a pile with an average height of about 2m. The soil pile thus is a mix of plant material, soil crust and subsurface CaCO<sub>3</sub> fragments. At the time of sampling the soil had been stored in such a pile for 13 years (since 1997). Both topsoil storage pile and the scraped well pad surface were sampled.

- 5 random repeats were collected for each sample type.



### Tests:

Soils were sieved through a 2mm sieve. Total soil catabolic activity (MicroResp™ measurements) and the identification and quantification of total bacteria and associated nitrogen cycling genes (qPCR) were carried out for each individual sample in triplicate. Total bacterial diversity was estimated by Pyrosequencing (bTEFAP).

### Data analysis:

Metagenomic analyses were carried out using the pipeline available at ([www.camera.calit2.net](http://www.camera.calit2.net)) and MEGAN (MEtaGenome ANalyzer, <http://ab.inf.uni-tuebingen.de/software/megan/>). All other statistics and visualization was carried on GenStat™11 and Minitab®.

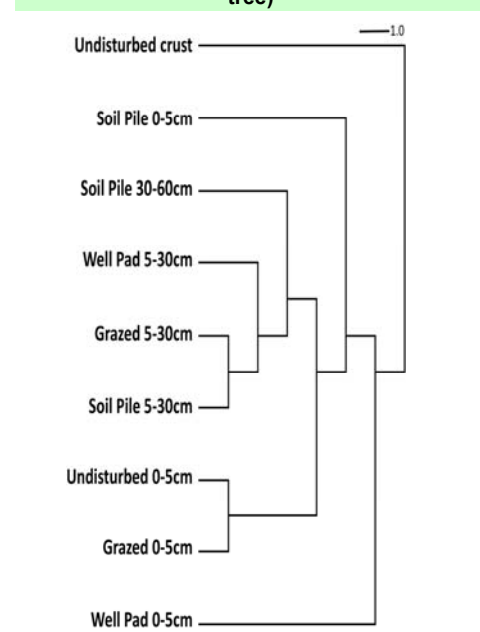
### Bacterial abundance by Pyrosequencing (bTEFAP)

#### Methodology

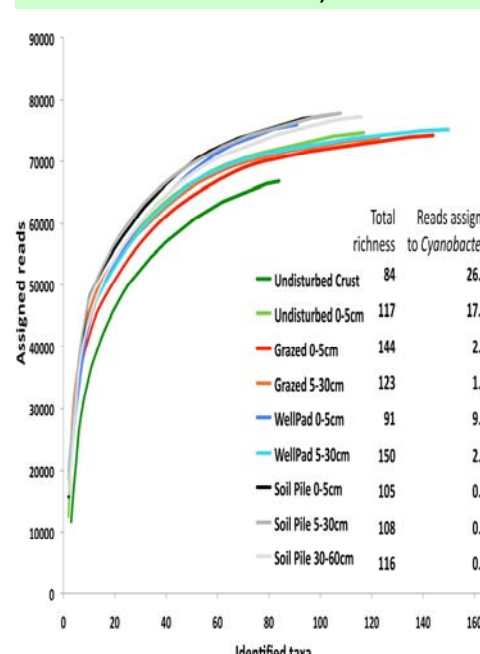
16S rDNA based bacterial tag-encoded amplicon Pyrosequencing (bTEFAP) using titanium plates were used to generate an average of 5000 reads per sample of over 300bp (average read length >400bp). The reads were clustered in a number of consensus sequences ([www.camera.calit2.net](http://www.camera.calit2.net)) and analyzed in MEGAN and Genstat.

### Site similarity according to the total estimated bacterial diversity (16S rDNA)

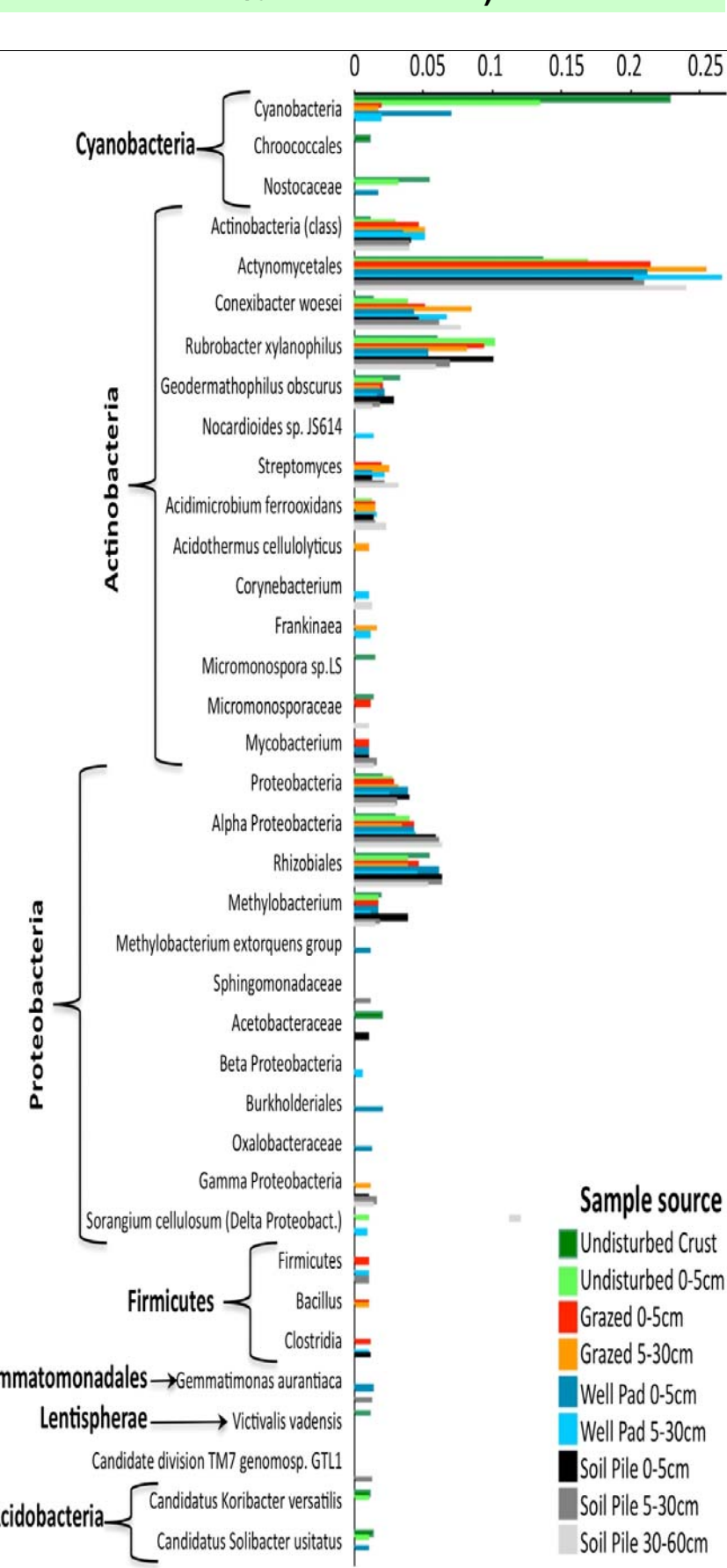
(normalized Goodall distance, NJ, consensus tree)



### Rarefaction curves (all assigned bacterial taxa)

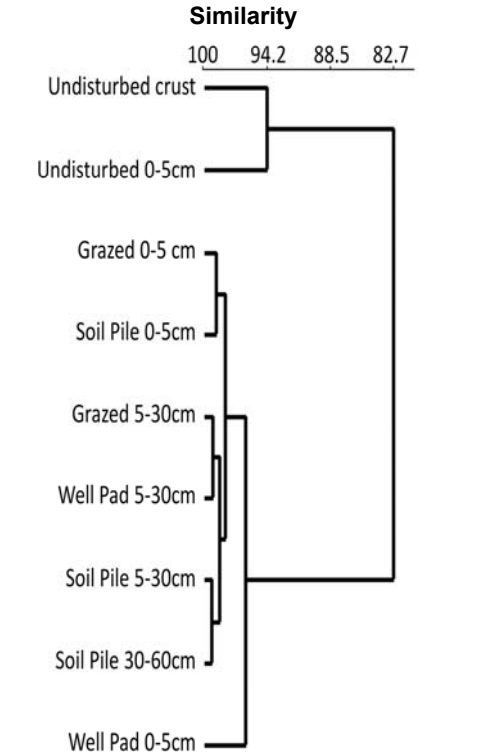


### bTEFAP abundance of dominant taxa (each >1% of total reads)



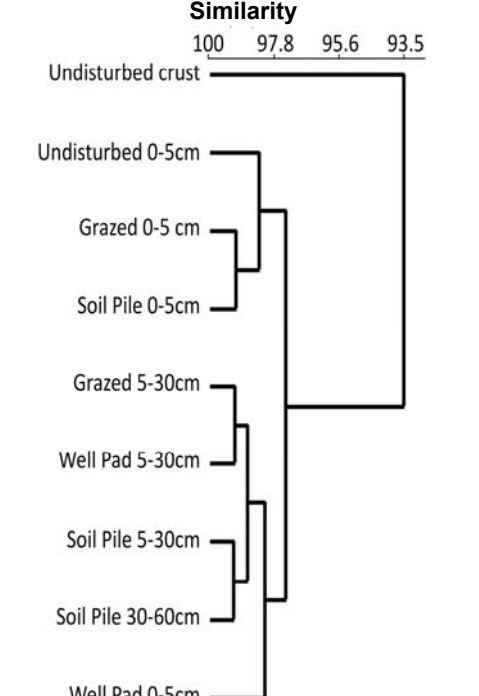
### Sites similarity according to the dominant bacterial populations including Cyanobacteria

(correlation coefficient distance, average linkage)

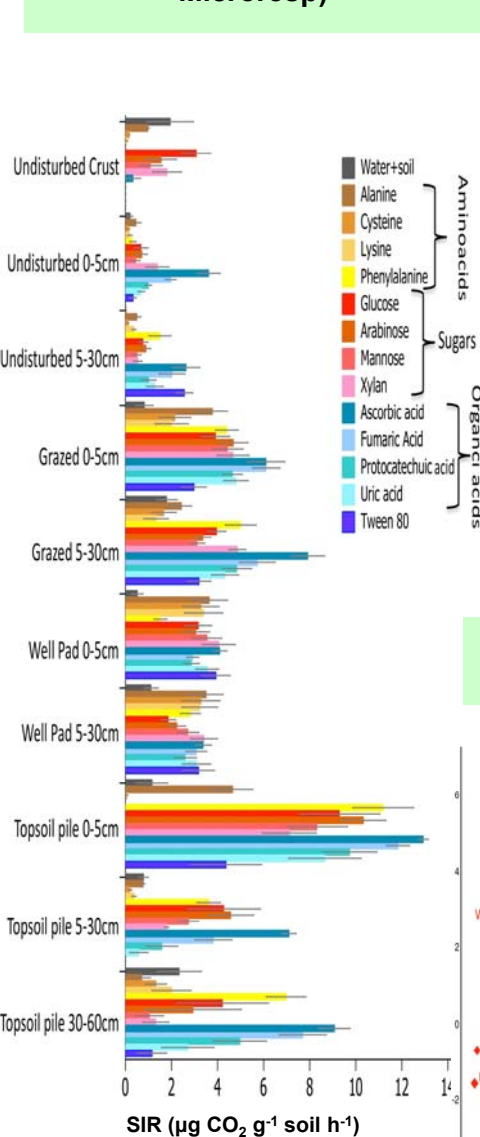


### Sites similarity according to the dominant bacterial populations excluding Cyanobacteria

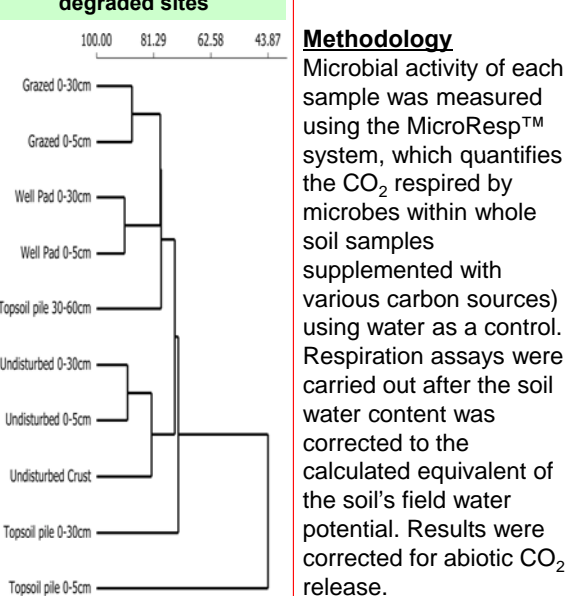
(correlation coefficient distance, average linkage)



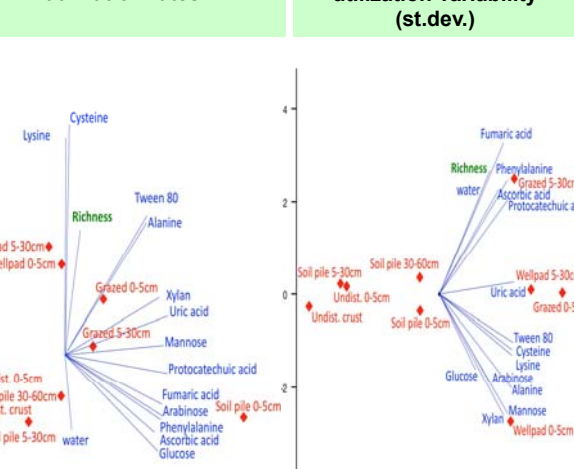
### Soil catabolic profiles (SIR via MicroResp)



### Correlation of catabolic profiles of undisturbed and degraded sites



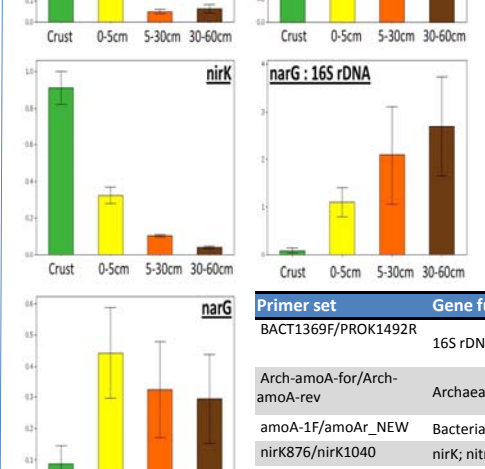
### Biplot: Site vs. substrate utilization rates



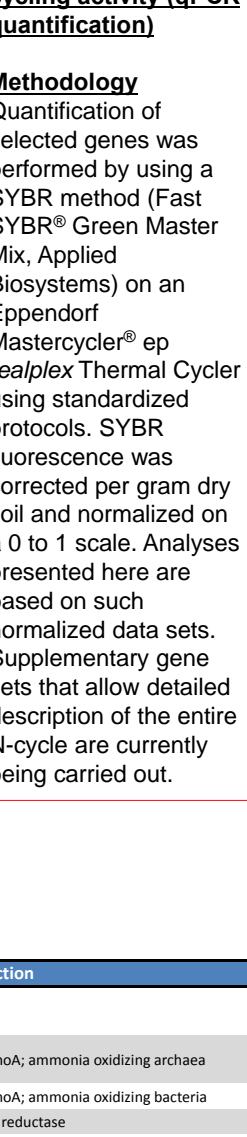
### Soil catabolic activity by MicroResp™

**Methodology**  
Microbial activity of each sample was measured using the MicroResp™ system, which quantifies the CO<sub>2</sub> respired by microbes within whole soil samples supplemented with various carbon sources) using water as a control. Respiration assays were carried out after the soil water content was corrected to the calculated equivalent of the soil's field water potential. Results were corrected for abiotic CO<sub>2</sub> release.

### Biplot: Site vs. substrate utilization variability (st.dev.)



### 16S rDNA and N-cycling genes in native crust and stored soil: qPCR evaluation



## Results

### Bacterial Diversity

Cyanobacteria were confirmed to be dominant, diagnostic indicator bacterial group for the biological surface crust, specific to arid lands, and the one group to induce clear dissimilarities in microbial diversities between the crust and no-crust soils, or between surface and deep soil strata. Common soil microbial groups *Actinobacteria*, *Proteobacteria* (alpha, beta, gamma and delta) and *Firmicutes* were found to dominate all degraded and undisturbed surface and deep soils. *Acidobacteria* candidates were found to associate mainly with surface soil biological crusts where the role of the arid land ubiquitous calcium carbonate is diminished. Thus the distinctiveness of the bacterial diversity profile of soil crust, even when cyanobacteria were eliminated from analysis, was still easily discernible. Removal of the few inches of topsoil, and thus crust, from the oil well pad created a severely degraded habitat however after 13 years it is regaining some of the lost cyanobacterial populations, while still closely resembling deeper soil strata.

Taxonomic richness increased with depth and after removal of crusts. Soil piles, that originally received the topsoil and crust removed from oil pad, have clearly lost the autotrophic cyanobacteria and even 13 years later they do hardly re-establish on the pile's surface. Diversity of heterotrophic bacteria in the buried soils is very similar to the original, non-cyanobacterial, diversity in the undisturbed top soils indicating origin and persistence. Grazed areas have shown an increase in putative pathogens.

### Catabolic potential diversity

Biological crusts and non-crust surface undisturbed soils have a low catabolic activity potential. Carbohydrates increased the respiration of soil crust while organic acids did induce some activity in the non-crust undisturbed surficial soils. The stored topsoil has shown the most complete catabolic profile potential. The catabolic potential for the grazed or oil well pad soils was very similar and for both greater than that of undisturbed soils. These observations suggest that decoupling the C cycle from the C-fixing cyanobacteria may induce more diverse C uptake pathways associated with a more diverse microbial population. The stored topsoil has shown the greatest catabolic activity and diversity profile suggesting an enhanced C utilization metabolic profile. Taxonomic richness was directly correlated with utilization of amino acids. Greater taxonomic richness in grazed and well pad soils correlated with greater variability in the SIR results (i.e. patchy catabolic activity).

Similarities in taxonomic diversity and C substrate utilization patterns suggest that relatively low energy impact activities such as low-density grazing may have impacts similar to destructive removal of the topsoil. Degradation-introduced heterogeneity in soil's biotic and abiotic parameters may induce increases in soil microbial diversity.

### N-cycle genes distribution

Targeted quantification of N-cycling genes and 16S rDNA was carried out for the topsoil pile and crust samples only. Bacterial concentration decreased drastically with depth. Different groups of organisms are likely governing nitrification (amoA genes) and denitrification (nirK and narG) in the soil crust versus the buried soils. Nitrification in soil crust is clearly dominated by archaea while bacteria dominates nitrification within soil. Denitrification, while carried out by proteobacteria, is likely carried out by different groups of organisms in the crust and buried soils environments.

In the topsoil storage pile while the capacity for degradation of sugars and complex sugars decreases with depth, bacteria persisting at greater depths may have a greater count of N cycling genes per unit 16S rDNA (e.g. the bacterial amoA and narG genes); possibly the most efficient cyclers may dominate the diversity at species level.

## Conclusions

Degradation of arid lands topsoil drastically affects cyanobacteria but maintains the diversity and activity of heterotrophic bacteria also common in soil crusts. Kinetic nutrient availability trials are required to estimate the absolute potential for these organisms to facilitate revegetation of degraded arid lands.

## Acknowledgements

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