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Introduction

Functional diversity of the soil microbial community is commonly used in the assessment of soil health as it relates to the activity of soil microflora involved in carbon cycling. Soil microbes in different microenvironments will have varying responses to different substrates, thus catabolic fingerprint information, via substrate induced respiration, of each location-specific community can be obtained. These profiles are commonly used to assess the fertility potential and soil health under variable management or soil degradation scenarios. Arid land soils are exposed to extreme and highly variable abiotic stresses uncommon to most soils where on which Community Level Physiological Profiling (CLPP) by substrate induced respiration (SIR) methods are usually employed. This led to extremely specialized microbial consortia and associated metabolic activities with the heterotrophic activities in the surface cryptobiotic crust often dominating the system. Any activity that disturbs this crust is expected to have severe impacts on soil activity potential.

Objectives

The purpose of this study was to 1) evaluate total-soil enzymatic activity profiles and 2) their relationship to taxonomic changes in soil microbial consortia across three disturbance regimes in an arid desert grassland in the Southwestern United States.

General Methodology

Sampling sites:

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

Samples:

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

- Undisturbed grassland biological crust samples and surficial soil samples without the crust
- Adjacent grazed lands surficial soil and subsoil 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 CaCO₃ 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.

Five (5) random repeats were collected for each sample type.

Soil catabolic activity by MicroResp™ (CLPP-SIR)

Microbial activity of each sample was measured using the MicroResp™ system, which quantifies the CO₂ respired by microbes within whole soil samples supplemented with various carbon sources) using water as a control (REF). 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 CO₂ release. The hourly rates of CO₂ production per gram soil were used in these analyses (ug CO₂ g⁻¹ h⁻¹).

Bacterial abundance by Pyrosequencing (bTEFAP)

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 each (average read length >400bp).

Data analysis:

Metagenomic analyses, included sequence quality control, clustering and Blastn sequence identification. All were carried out using the pipeline available at (www.camera.calit2.net) and MEGAN (MetaGenome ANalyzer, <http://ab.inf.uni-tuebingen.de/software/megan/>). Similarity analyses for metagenomic data were carried out in SplitsTree 4 (www.splittree.org). All other statistics and visualization was carried on GenStat™11 and Minitab®. NOTE: Pyrosequencing reads obtained were assigned to the closest most similar taxonomic level. Thus OTU's have been assigned at all levels from species to kingdom.

Fig. 1. Discrimination of sampling sites by CLPP (PCA score and factor loading plots)

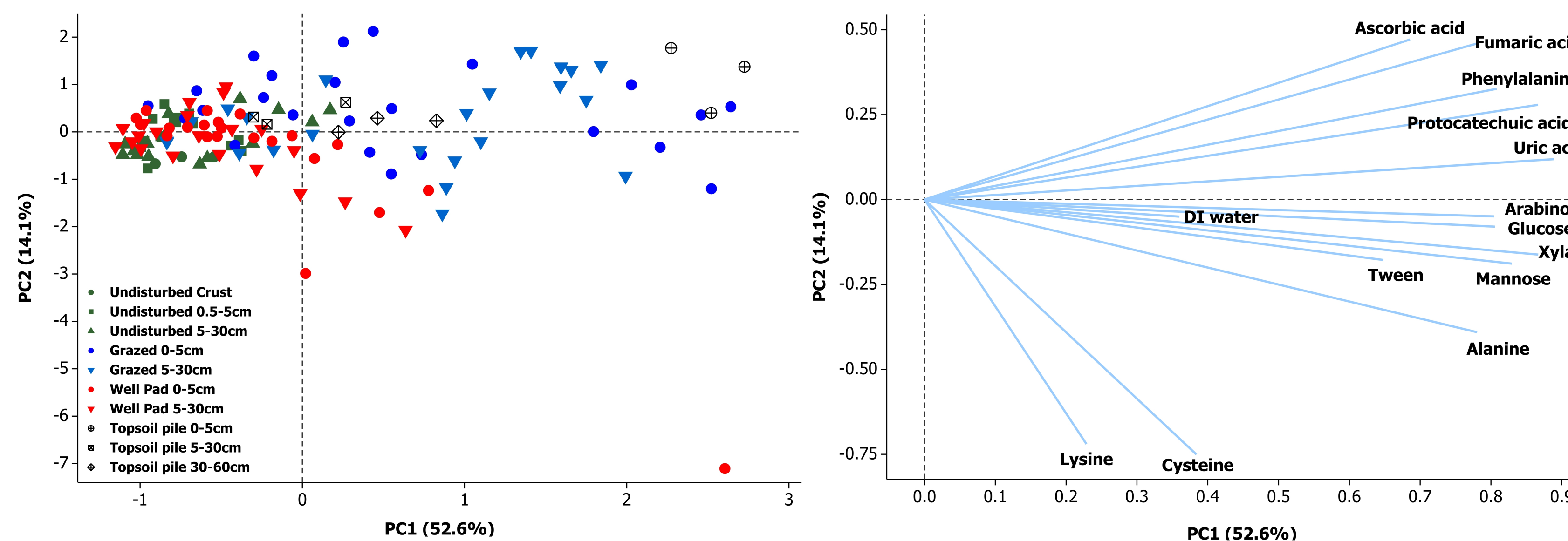


Fig 2. Rarefaction curves (all assigned bacterial taxa) Fig 3a. 16S rDNA similarity Fig 3b. CLPP-SIR similarity

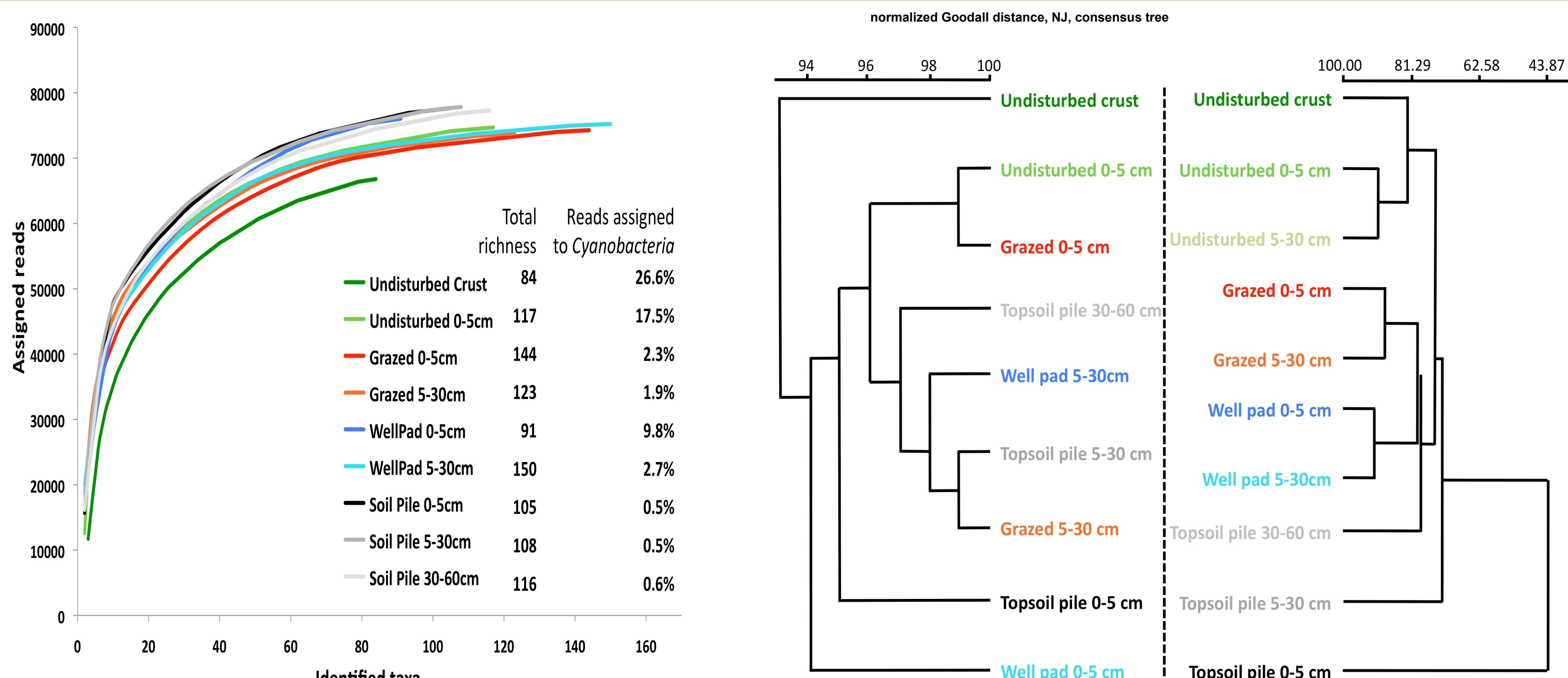
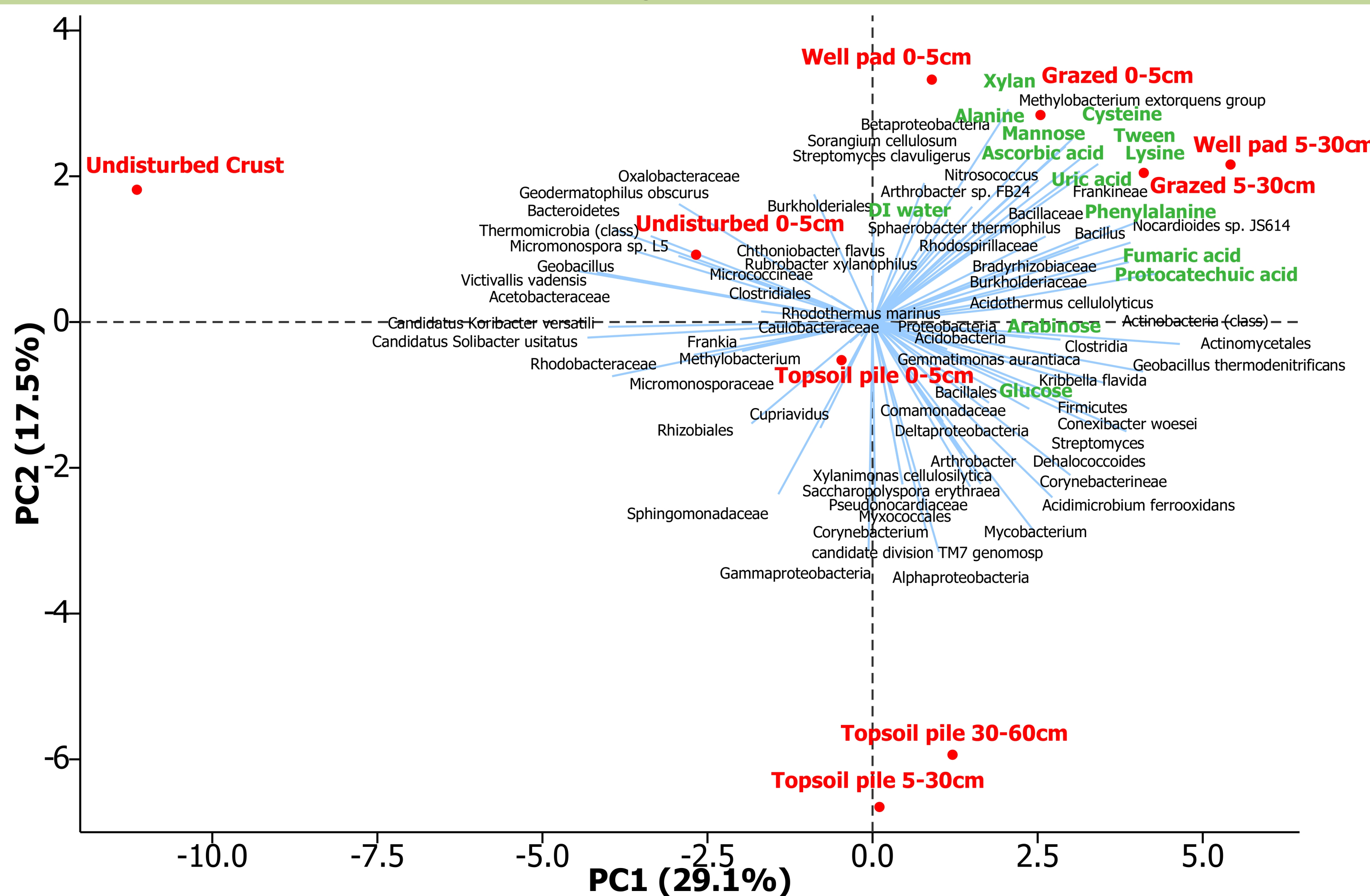
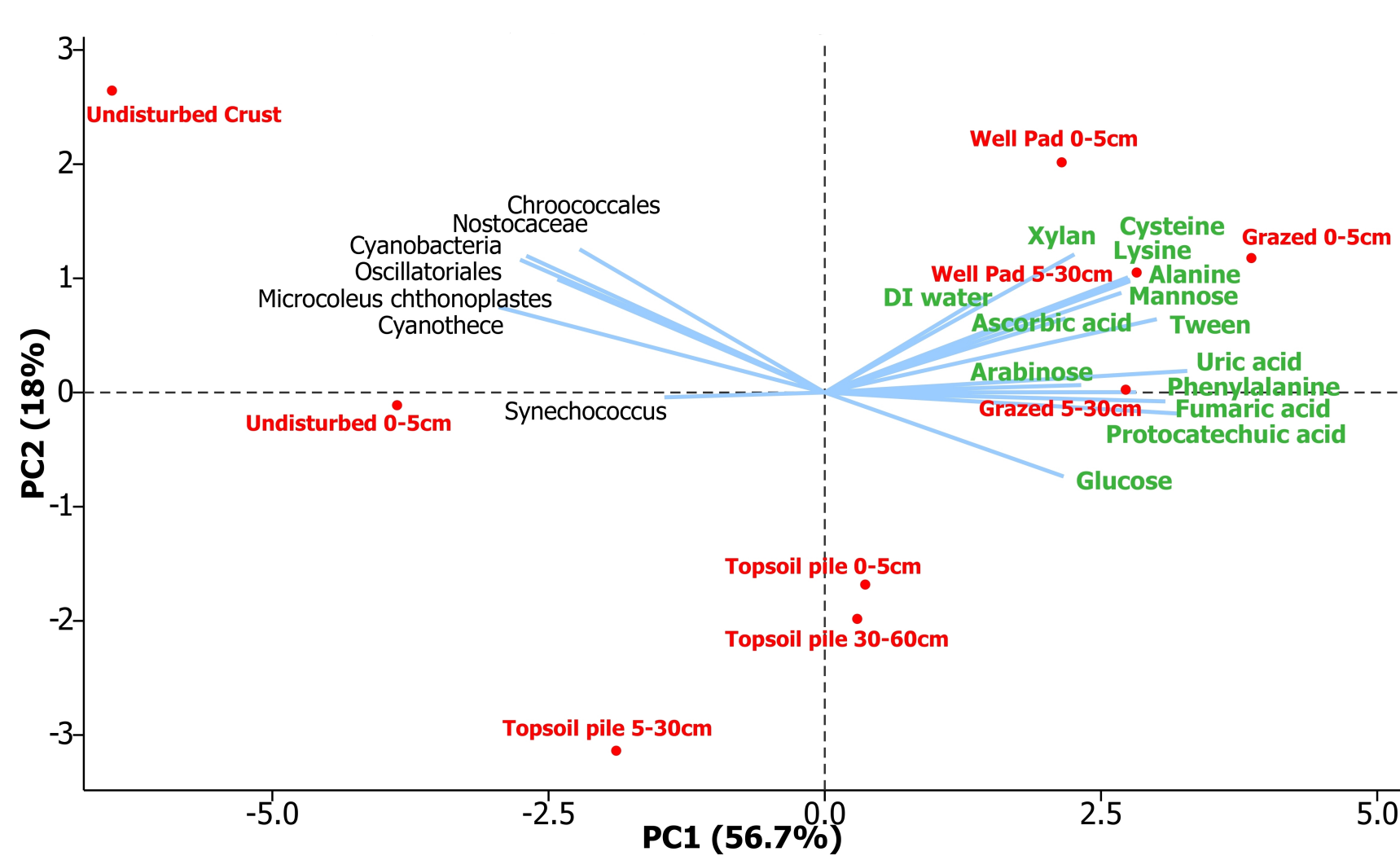


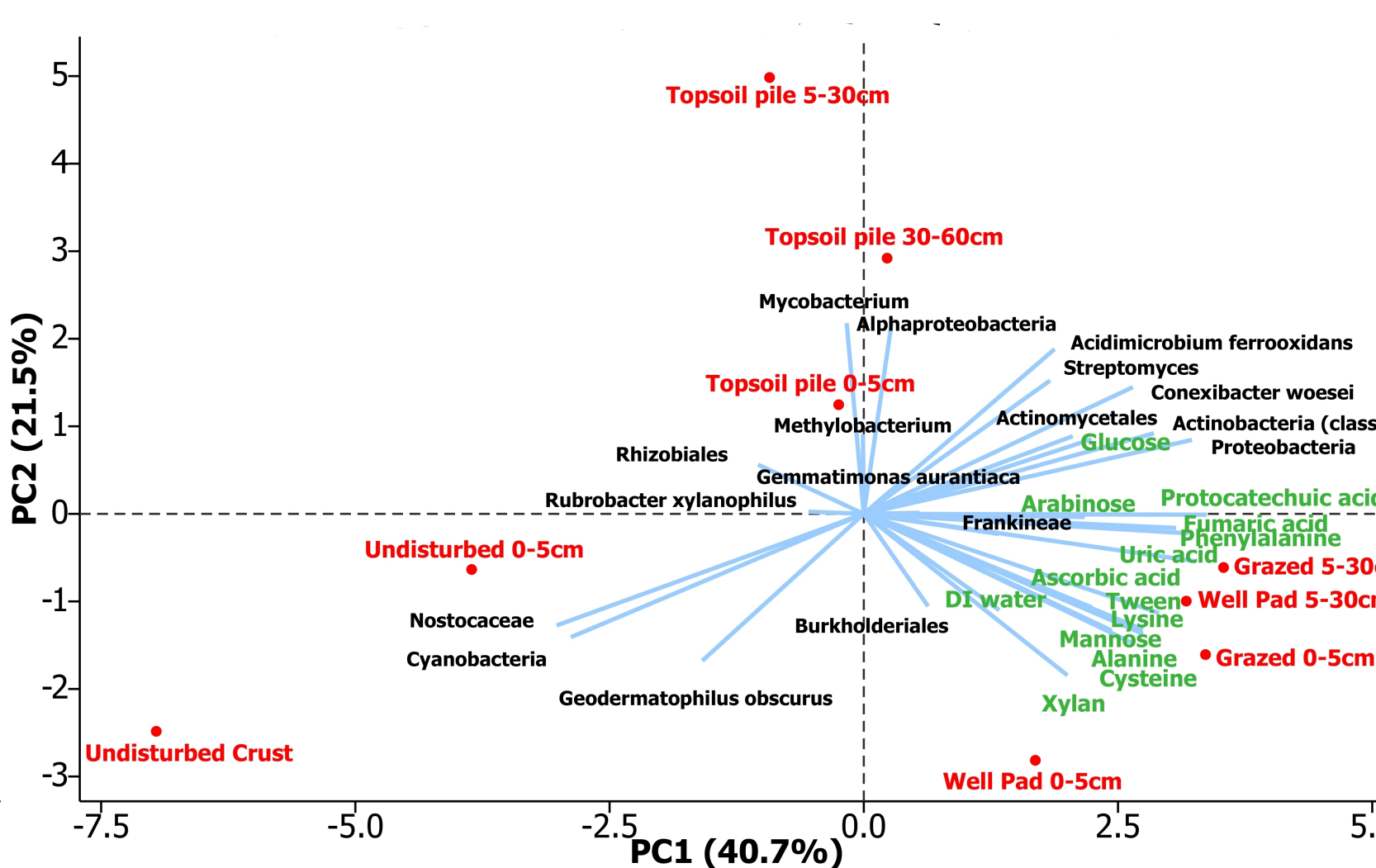
Fig 4. Sampling locations as described by bacterial diversity, and catabolic diversity a. CLPP taxa covering 90% cumulative abundance



b. SIR profiles and Cyanobacteria abundance



c. SIR profiles and dominant taxa (taxa with individual abundance of >1%)



Results

Community level physiological profiling

The first obvious observation is that the CLPP-SIR tests produced least respiration in the undisturbed soil samples including the crust and the subsoil samples.

All substrates induced more respiration in the disturbed systems (grazed and well pad soils).

Amino acid substrates especially lysine and cysteine) utilization was more variable in the well pad soils.

Grazed soils exhibited significant variability in the catabolic profiles most obvious for the organic acids and sugar utilization.

Metagenomics

The metagenomic survey also indicated that the undisturbed surface crust had the least diversity with Cyanobacteria as the dominant group. Several acidobacteria (*Candidatus Koribacter versatilis* and *Candidatus Solibacter usitatus*) were exclusively identified in the undisturbed cryptobiotic crust. Cyanobacteria dominance

Dominant taxa diversity (taxa with individual abundance of more than 1% of the total abundance) was rather low with Actinobacteria and Proteobacteria associated sequences dominating the disturbed systems and Cyanobacteria, Nostocaceae and Actinobacteria dominating the undisturbed crusts.

Conclusions

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 show that, for arid lands, any degradation-enhanced heterogeneity in soil's biotic and abiotic parameters may drive changes in soils towards higher functional diversity to adapt to the disturbance.

Re-spreading the soil, in an attempt to remediate degraded lands will possibly retain their diversity but will allow only for slow natural slow crust recovery.

Acknowledgements

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