Metagenomics of Karst Soil Microbial Systems and their Role(s) in Carbon Cycling and Native Plant Restoration

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Abstract

Soil metagenomic analyses were conducted at Wild Basin Wilderness Preserve (WBWP) within the black-capped vireo habitat restoration treatment site in May 2012. Microbial (archeae, bacterial and fungal) rDNA was sequenced using Illumina technology from each of 5 treatment areas: 1) The control treatment is mulch socks only; 2) Onsite mulch containing local juniper and hardwoods recently processed; 3) Onsite composted mulch containing local juniper and hardwoods that have been fermenting on the ground for 10-15 years; 4) Mulch and biochar combined; 5) Composted mulch and biochar combined. A preliminary analysis of the total data obtained (not parsed based on treatment) has identified: 1302, 1356, 1693 operational taxonomic units (OTUs) for archeae, bacteria and fungi, respectively. The prevalence of archeae present (1302 OTUs) in these karst soils is significant, and may reflect microbial adaptations to this unique environment. Further analyses will identify how this diversity is distributed among each of the five treatment areas.

Introduction

Wild Basin Wilderness Preserve (WBWP) is part of the Balcones Canyonlands Preserve (BCP) since its creation in1996, protecting eight endangered species, as well as 27 species of concern. As part of an adaptive management experiment designed to improve habitat restoration techniques for black-capped vireos, this project aims to characterize the soil microbial communities present in the karst soils, and their response to soil treatments applied by employing metagenomic sequencing approaches. Central Texas has a region of uplifted limestone, the Edwards Plateau, providing an island of karst soils that fostered the speciation of many organisms and forms one of North America's areas of endemism. With an extreme and unpredictably variable climate, the survival of regional flora is increasing recognized to be dependent on tight relationships with soil fauna, none of which have been described. The soils are extremely alkaline, requiring unexplored adaptations and novel biochemistry. The region was deforested in the late 1800's to provide the railroad ties that still underlie the nation's train network. Post-deforestation, the region was heavily grazed, principally with goats. Reforestation efforts have been largely unsuccessful because of insufficient soils and a lack of understanding of plant-soil microbial relationships that underlie biotic yields. The climate of central Texas has historically been extremely unpredictable. However as demonstrated by the historic drought and heat wave of 2011 and recent climatic trends, we can expect increasing variability in the near future. Soil microorganisms constitute a significant fraction of the Earth's biomass, with surface soils estimated to contain 10^9- 10^{10} microbial cells per gram including bacterial, archaeal, fungal species, plus viruses and protists (Torsvik et al., 2002, Tringe et al., 2005, Gans et al., 2005). Despite this abundance and the importance of soil microorganisms for key ecosystem functions, the diversity and structure of soil

microbial communities remain poorly studied. With the development of metagenomic techniques, efforts to characterize the full extent of microbial diversity, their role in a variety of global ecological functions including carbon balance, cycling of nutrients and promoting plant growth have been initiated. Recent large-scale surveys have revealed that different ecosystems support unique microbial populations (Angel et al., 2010, Fierer et al., 2008). The goals of this project are to: (1) characterize the genomic complexity of the bacterial, archaea and fungal communities characteristic of karst soils, (2) Determine the effect(s) on plant-microbial communities/interactions following treatment as an indication of the capacity of the microbial community to sequester carbon. The long-term goal is to better manage BCP lands for black-capped vireo habitat and ecological health.

Materials and Methods

Soil Samples

Soil samples were collected from the black-capped vireo adaptive management treatment area. The treatment area is comprised of 3 rows, with each row consisting of five separate treatments: 1) The control treatment is mulch socks only; 2) Onsite mulch containing local juniper and hardwoods recently processed; 3) Onsite composted mulch containing local juniper and hardwoods that have been fermenting on the ground for 10-15 years; 4) Mulch and biochar combined; 5) Composted mulch and biochar combined. Samples were obtained from each treatment within rows 1 and 2 (Fig. 1). Soil samples were stored -80 prior to DAN isolation.

DNA Isolation

Total DNA was prepared using PowerSoil DNA Isolation Kit (Mo Bio Labs). DNA was prepared in triplicate from each treatment area and pooled to generate a composite sample for sequence analysis.

Metagenomic Sequencing

rDNA regions from the total DNA were amplified using PCR primer sets designed to amplify: archaea, bacterial, and fungal, and PCR products sequenced using Illumina platform (MrDNA).

Sequence Analyses

Sequences were depleted of barcodes and primers and short sequences (< 200bp) were removed. Sequences with ambiguous base calls, and homopolymer runs exceeding 6bp were also removed. Operational taxonomic units were defined after removal of singleton sequences, clustering at 3% divergence (97% similarity). (Dowd, Callaway et al. 2008; Dowd, Sun et al. 2008; Edgar 2010; Capone, Dowd et al. 2011; Dowd, Delton Hanson et al. 2011; Eren, Zozaya et al. 2011; Swanson, Dowd et al. 2011). OTUs were then taxonomically classified using BLASTn against a curated GreenGenes database (DeSantis, Hugenholtz et al. 2006) and compiled into each taxonomic level.

Principal component analysis and k-means clustering was applied to the OTU and species analysis. Principal component analysis ranks the data based on variance. The first two principal components are graphed as "pca1" and "pca2". pca1 contains the principal component analysis run considering each sample as a different feature to each OTU or species.

Results

The first phase of the black-capped vireo adaptive management experiment spans three plots across the City of Austin's Vireo Preserve. Two plots occupy the south-facing slopes and the third is NE of the central knoll. The plots have already been prepared with mulch socks laid perpendicular to the angle of the slope to reduce soil erosion and

capture soil runoff during rain events. The south facing plots each contain three tiers. Each treatment is arranged vertically along the slope across the three tiers so there is no contamination of lower tiers by upper tier treatments. The data obtained in this study were obtained from the westernmost south-facing plot (Figure 1).

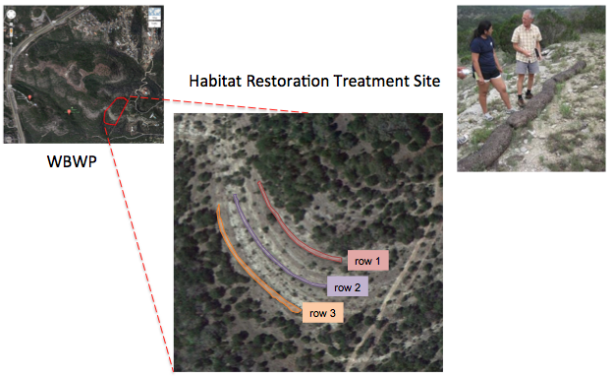

Fig. 1 Soil Metagenomic Research Site. Wild Basin Wilderness Preserve Overview (left), Soil sample sites (middle), mulch sock (right).

Students obtained soil samples from each of the five treatment areas from rows 1 and 2 in May 2011 (Fig. 1): 1) The control treatment is mulch socks only; 2) Onsite mulch containing local juniper and hardwoods recently processed; 3) Onsite composted mulch containing local juniper and hardwoods that have been fermenting on the ground for 10-15 years; 4) Mulch and biochar combined; 5) Composted mulch and biochar combined. DNA was isolated in in triplicate from each soil sample obtained from row 1 samples and pooled. The pooled samples from each treatment was amplified using rDNA primer sets for archeae, fungi and bacteria, and sequenced using Illumina technology (Fig. 2).



Fig. 2. Sample Collection and DNA Isolation. Student collecting soil sample (lt), and isolating DNA (rt).

A preliminary analysis of the microbial diversity contained within the fiev treatment areas identified: 1302, 1356, 1693 operational taxonomic units (OTUs) for archeae, bacteria and fungi, respectively. These data represent total diversity for a domain, and the distribution of OTUs among the five treatment areas has not yet been determined.

To estimate the degree of diversity within each domain, a preliminary principal component analysis (PCA) of the sequence data was performed (Fig. 3). Based on these

preliminary analyses, bacterial and fungal diversity exceeds diversity present within the archaea.

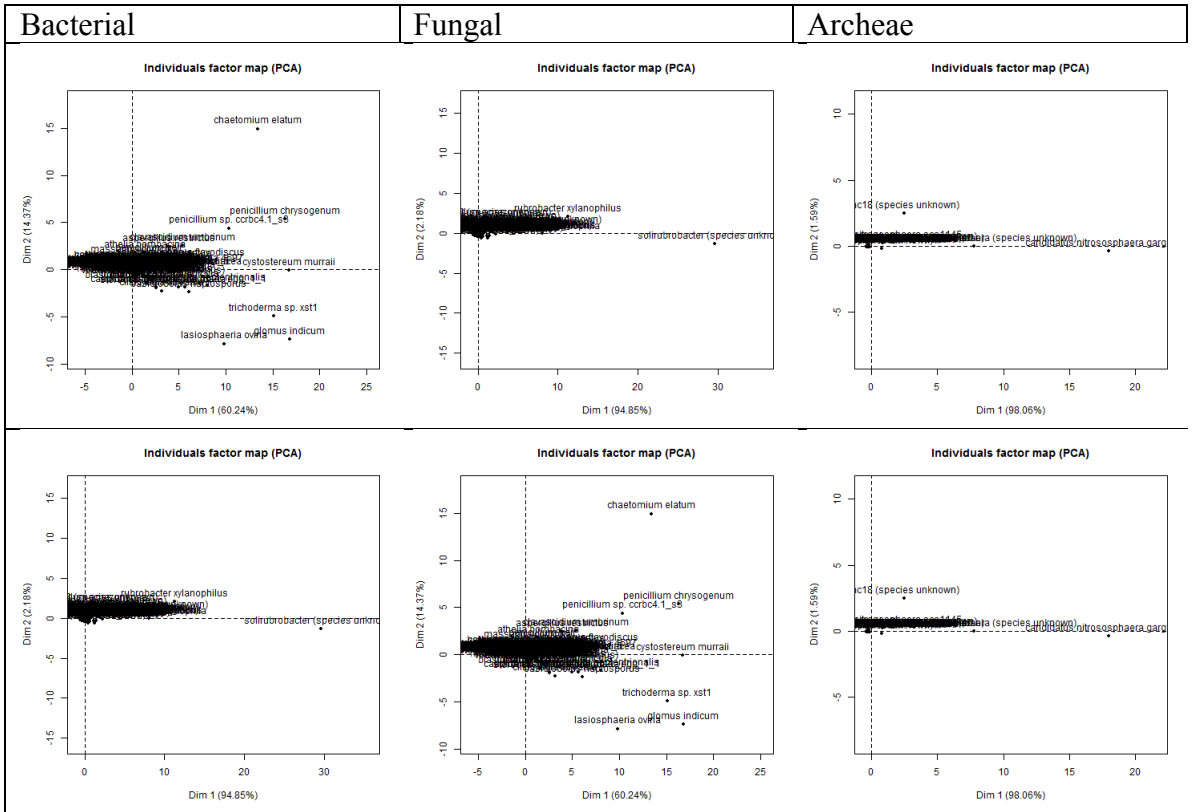

Fig. 3 Principal Component Analysis. PCA analyses of sequences obtained from bacterial, fungal and archeae domains. pca2 (top) pca1(bottom).

Discussion and Conclusions

To our knowledge this study represents the first metagenomic analyses of the karst soils microbial biome at Wild Basin. The prevalence of archeae present (1302 OTUs) in these karst soils is significant, and may reflect required microbial adaptations to this unique environment.

Further analyses are required to identify the microbial composition within each treatment area. The primary benefits of a longitudinal metagenomic analyses will be to provide data regarding: the efficacy of each the five treatments to restore black capped vireo habitat; the effects of soil treatments on microbial populations, and potentially identify unique plant-microbe interactions unique to the karst soils present at WBWP.

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