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Common and distinguishing features of the bacterial and fungal communities in biological soil crusts and shrub root zone soils

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ABSTRACT

Soil microbial communities in dryland ecosystems play important roles as root associates of the widely spaced plants and as the dominant members of biological soil crusts (biocrusts) colonizing the plant interspaces. We employed rRNA gene sequencing (bacterial 16S/fungal large subunit) and shotgun metagenomic sequencing to compare the microbial communities inhabiting the root zones of the dominant shrub, *Larrea tridentata* (creosote bush), and the interspace biocrusts in a Mojave desert shrubland within the Nevada Free Air CO_2 Enrichment (FACE) experiment. Most of the numerically abundant bacteria and fungi were present in both the biocrusts and root zones, although the proportional abundance of those members differed significantly between habitats. Biocrust bacteria were predominantly Cyanobacteria while root zones harbored significantly more Actinobacteria and Proteobacteria. Pezizomycetes fungi dominated the biocrusts while Dothideomycetes were highest in root zones. Functional gene abundances in metagenome sequence datasets reflected the taxonomic differences noted in the 16S rRNA datasets. For example, functional categories related to photosynthesis, circadian clock proteins, and heterocyst-associated genes were enriched in the biocrusts, where populations of Cyanobacteria were larger. Genes related to potassium metabolism were also more abundant in the biocrusts, suggesting differences in nutrient cycling between biocrusts and root zones. Finally, ten years of elevated atmospheric CO_2 did not result in large shifts in taxonomic composition of the bacterial or fungal communities or the functional gene inventories in the shotgun metagenomes.

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1. Introduction

Soil microbial communities in dryland ecosystems play critical roles in nutrient cycling, both as associates of the widely spaced shrubs and grasses and as components of biological soil crusts (hereafter referred to as biocrusts) that colonize plant interspaces (Belnap, 2003; Belnap et al., 2003a). Plants in arid ecosystems exert strong influences on soil nutrient and moisture content through root exudates, increased water infiltration along roots, and to some extent litter production (Schade and Hobbie, 2005). Dryland plants can increase soil fertility and microbial biomass in the root zones, and create "islands of fertility" (e.g. Gallardo and Schlesinger, 1992; Herman et al., 1995; Housman et al., 2007; Schade and Hobbie, 2005; Tongway and Ludwig, 2005). Organic carbon, as well as nutrients, may concentrate under plant canopies although in some instances soil nitrogen and phosphorus concentrations may not increase in the presence of plants (Charley and West, 1975; Schade and Hobbie, 2005; Titus et al., 2002). In patchy landscapes, plant nutrient capture is complex and varies with plant species, edaphic factors, and environmental gradients (Butterfield and Briggs, 2009; Housman et al., 2007; Smith et al., 2002). Biocrusts, consisting of cyanobacteria, algae, mosses and lichens along with their associated heterotrophic bacteria and fungi, colonize the soil between the patchily distributed plants and lie primarily outside the plant canopies and influence of plant roots (Belnap, 2003; Belnap et al., 2003a). Biocrusts can cover up to 70% of the dryland surface soil and they provide many important ecosystem functions, such as moisture trapping, nitrogen fixation, and soil stabilization (Evans and Lange, 2001; Lange, 2001; Langhans et al., 2009). Biocrust Cyanobacteria fix the majority of carbon in dryland surface soils, which likely subsidizes underlying soil food webs (Darby et al., 2010; Evans and Lange, 2001; Lange, 2001). Biocrusts also stabilize soils from erosion, influence soil water content, and can fix atmospheric nitrogen.

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Prior studies have demonstrated taxonomic differences in either the bacterial or fungal communities between root zone (soil containing live roots under a plant canopy) and biocrust soils and the relative abundance of bacterial and fungal taxa that inhabit the different patches. Yet, how those bacterial and fungal communities might vary in concert and the functional potential of the collective soil microbiota have not been defined. General trends have been noted; biocrust bacterial communities are enriched with Cyanobacteria and anoxygenic phototrophs, whereas un-crusted soils under plant canopies harbor larger populations of heterotrophic bacteria (Kuske et al., 2002; Steven et al., 2012a). Prior surveys of soil fungi, through microscopic counts, culturing, denaturing gradient gel electrophoresis pattern comparisons, and limited DNA sequencing suggest that fungi are more abundant and diverse in association with dryland shrub root zones than in the interspace biocrusts (Bates and Garcia-Pichel, 2009; Bates et al., 2012, 2010; Porras-Alfaro et al., 2010; Steven et al., 2012a; Titus et al., 2002; Weber et al., 2011). In this regard, dryland soil microbial communities worldwide are united by similar taxonomic structures and ecological roles so that observations from synonymous ecosystems may be generalized to arid ecosystems at a more global scale.

Both the plant and biocrust patches contribute to landscape-level ecosystem productivity, but the extent to which nutrient cycling between the plant and biocrust patches is connected is unknown. The collective activities of soil fungi and bacteria could provide links between the patches of this mosaic landscape. For example, bacterial activity in biocrusts can positively influence plant growth through increased nitrogen inputs and soil stability (Belnap et al., 2003b) and filamentous fungi may colonize both root zones and biocrusts, potentially forming nutritional bridges between the patches (Green et al., 2008; Porras-Alfaro et al., 2010). In aquatic systems algae may 'prime' decomposition of recalcitrant carbon by exuding labile carbon compounds (Danger et al., 2013; Guenet et al., 2010). Whether biocrust cyanobacteria are capable of a similar priming role is unknown. Although prior studies suggest patch interactions may be mediated by soil bacteria and fungi, potential microbial links and their relative importance between plants and biocrusts remain speculative. One limitation is that the structure of the collective soil community has not been characterized in a uniform way. We used a combination of targeted bacterial 16S rRNA gene (hereafter termed bacterial rRNA) and fungal large subunit rRNA gene (hereafter termed fungal rRNA) and shotgun metagenomic sequencing to characterize the taxonomic and functional profiles of both the bacterial and fungal communities inhabiting the interspace biocrusts and root zones of creosote bush (*Larrea tridentate*), the dominant species of the Mojave desert shrubland.

Our study was conducted at the Nevada Free Air CO_2 Enrichment (FACE) experiment located in the Mojave Desert. This experiment was initiated to determine the effects of long-term elevated atmospheric CO_2 on a natural dryland ecosystem (Jordan et al., 1999). At this FACE site, elevated CO_2 conditions have resulted in increases of above-ground shrub biomass and productivity (Housman et al., 2006; Nowak et al., 2004). However, elevated CO_2 may have negatively impacted biocrust cyanobacteria (Steven et al., 2012b). We hypothesized that the changes noted in plants and biocrust cyanobacteria in response to elevated CO_2 would translate into shifts in the taxonomic composition and functional characteristics of the soil bacterial and fungal communities that would differ between the biocrusts and root zones.

2. Materials and methods

2.1. Site description and sample collection

Soil samples were collected in July 2007 from the Nevada desert FACE experiment located in the Mojave Desert, USA (36°49′N, 115°55W), previously described by Jordan et al. (1999). The dominant shrub is creosote bush (*Larrea tridentata*) and cyanobacterial biocrusts colonize 35–65% of the soil surface of the inter-plant spaces (Jordan et al., 1999). Soils at the Nevada FACE site are loamy sand Aridosols (Jordan et al., 1999). Soil characteristics associated with root zones and biocrusts have been documented previously by Dunbar et al. (2012) and a comparison to other studies looking at similar habitats is presented in Table 1. Across the different studies, organic matter, nitrate, total nitrogen, and phosphorus were all higher in the root zones than the biocrusts (Table 1). At the time of sampling soil moisture was similar between the root zone and biocrusts and was extremely low.

This site contains three replicate FACE rings that received elevated atmospheric CO_2 (550 ppm) and three replicate face rings that received ambient CO_2 (360 ppm), arranged as blocked pairs (Jordan et al., 1999). Within each of the six FACE rings, soil samples were collected from four compass points along the canopy drip-line of a creosote bush (*c.a.* 1 m diameter bush size) using a 5 cm wide by 5 cm deep coring device. The four samples were then pooled into a single sample, resulting in a single soil sample per FACE ring ($n = 3$ per $\pm$ CO_2 treatment). Similarly, four 5 cm $\times$ 5 cm core samples were collected from an interspace biocrust patch from random points within an approximately 1 m square area and pooled into one sample representing each FACE ring. The pooled soil samples were homogenized and sieved through a 2 mm sieve to remove any

Table 1
Characteristics of biocrust and root zone soils from three studies.

Soil	pH	OM (%)	NO_3^- (ppm)	P (ppm)	K^+ (ppm)	Mg^{2+} (ppm)	Ca^{2+} (ppm)	Na^+ (ppm)	TOC[4] ($g\ m^{-2}$)	TON[5] ($g\ m^{-2}$)
Biocrusts										
1	7.5	0.8	4.3	3.9	36	22.9	155	6.3	.	.
2	8.1	.[6]	.	.	.	.	.	.	361	29.0
3	8.1	.	.	.	.	.	.	.	142	19.1
Root zones										
1	7.9	1.4 (1.8×)[7]	21.0 (4.9×)	11.0 (2.8×)	156 (4.3×)	49.2 (2.1×)	315 (2×)	11.5 (1.8×)	.	.
2	8.2	.	.	.	.	.	.	.	999 (2.8×)	89.5 (3.1×)
3	8.2	.	.	.	.	.	.	.	379 (2.7×)	40.4 (2.1×)

[1]This study. Raw data presented in the Supplemental Material in Dunbar et al. (2012).
[2]From Schaeffer et al. (2003).
[3]From Schaeffer et al. (2007).
[4]Total organic carbon.
[5]Total organic nitrogen.
[6]Dot indicates that parameter was not measured in that study.
[7]Numbers in parentheses indicate the fold-increase in root zones compared to biocrusts for that variable.