Arid Ecosystem Vegetation Canopy-Gap Dichotomy: Influence on Soil Microbial Composition and Nutrient Cycling Functional Potential

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ABSTRACT

Increasing temperatures and drought in desert ecosystems are predicted to cause decreased vegetation density combined with barren ground expansion. It remains unclear how nutrient availability, microbial diversity, and the associated functional capacity vary between vegetated-canopy and gap soils. The specific aim of this study was to characterize canopy vs gap microsite effect on soil microbial diversity, the capacity of gap soils to serve as a canopy soil microbial reservoir, nitrogen (N)-mineralization genetic potential (*ureC* gene abundance) and urease enzyme activity, and microbial-nutrient pool associations in four arid-hyperarid geolocations of the western Sonoran Desert, Arizona (USA). Microsite combined with geolocation explained 57% and 45.8% of the observed variation in bacterial/archaeal and fungal community composition, respectively. A core microbiome of amplicon sequence variants was shared between the canopy and gap soil communities; however, canopy soils included abundant taxa that were not present in associated gap communities, thereby suggesting that these taxa cannot be sourced from the associated gap soils. Linear mixed-effects models showed that canopy-soils have significantly higher microbial richness, nutrient content, and organic N-mineralization genetic and functional capacity. Furthermore, *ureC* gene abundance was detected in all samples suggesting that *ureC* is a relevant indicator of N-mineralization in deserts. Additionally, novel phylogenetic associations were observed for *ureC* with the majority belonging to *Actinobacteria* and uncharacterized bacteria. Thus, key N-mineralization functional capacity is associated with a dominant desert phylum. Overall, these results suggest that lower microbial diversity and functional capacity in gap soils may impact ecosystem sustainability as aridity drives open-space expansion in deserts.

IMPORTANCE

Increasing aridity will drive a shift in desert vegetation and interspace gap (microsite) structure toward gap expansion. To evaluate the impact of gap expansion, we assess microsite effects on soil nutrients, microbiome community composition and functional capacity, and the potential of gap soils to serve as microbial reservoirs for plant-root associated microbiomes in an arid ecosystem. Results indicate that gap soils have significantly lower bioavailable nutrients, microbial richness, and N-mineralization functional capacity. Further, abundance of the bacterial urease gene (*ureC*) correlates strongly with N-availability and its major phylogenetic association is with *Actinobacteria*, the dominant phylum found in deserts. This finding is relevant because it identifies an important N-mineralization capacity indicator in the arid soil microbiome. Such indicators are needed to understand the relationships between interplant gap expansion and microbial diversity and functional potential associated with plant sustainability. This will be a critical step in recovery of land degraded by aridity stress.

1. INTRODUCTION

Desert ecosystems are characterized by plant spatial heterogeneity, where vegetation cover is present in patches separated by gaps (1). Vegetation patches capture dust, water, and nutrients from wind and water erosion, forming fertility or resource islands in soils (2, 3). According to climate model predictions, a rise in global temperatures with associated extreme weather events and drought conditions will lead to an increase in aridity in these regions (4). This in turn will affect essential ecosystem services (5) through transformation of vegetation patterns as well as gap expansion in drylands (6), and might result in irreversible shifts from healthy to degraded soils (7).

Presently, there is limited knowledge of how loss of vegetative cover accompanied by expanded gaps will affect arid ecosystem services.

Previous work has shown that desert soil microbial communities are taxonomically distinct from other biomes and have lower functional diversity with respect to nutrient cycling (8). In addition, studies conducted in drylands have demonstrated that an increase in aridity results in a decrease in microbial abundance and diversity (9, 10). It is also known that microbial abundance and diversity in deserts are controlled by microsites (vegetation and gap microsites includesoils under plant canopies versus interspace gaps devoid of any vegetation) (11, 12), size of the fertility island (13, 14), and precipitation (15). However, the link between microbial diversity and the associated genetic potential and functional capacity remains largely unclear in deserts. Predicted global climatic changes that shift desert microsite structure may in turn influence the microsite-specific microbial community composition and its functional capacity. Therefore, it is critical to understand microbial functions not only under the vegetation canopy soils but also in the gaps.

A critical nutrient cycling service in desert ecosystems is soil organic matter mineralization, a process carried out by heterotrophic bacteria and fungi. Microbial mineralization of organic matter is a primary source of important inorganic nutrients including nitrogen (N) (16). In fact, N-availability is the main limiting factor for net primary productivity and ecosystem function in desert ecosystems. However, knowledge concerning the consortia of microorganisms that drive mineralization of organic N pools to inorganic forms in deserts is limited. N-mineralization is an important function impacting plant available nitrogen supplies. N-mineralization begins with proteolysis, the breakdown of proteins into peptides, amines, and amides (17). These proteolysis products are converted into urea and urea is further hydrolyzed into ammonium by the enzyme urease (18). Whereas proteases are ubiquitous and produced by all forms of life (19), the processes of ureolysis are driven by specialized microbial guilds (18). Thus, the genetic potential and functional capacity of these ureolytic microorganisms should be evaluated to assess N-availability between canopy and gap soil microsites in deserts.

The goal of this study is to evaluate the effects of soil microsites (canopy vs gap) in different geolocations on the coupled association of microbial community composition, functional nutrient cycling capacity, and nutrient pools in the Sonoran Desert of the southwestern US. To do so, we compare microbial community composition and mineralization potential of gap and vegetated fertility islands at four arid to hyperarid locations (geolocations) in the *Larrea tridentata-Ambrosia dumosa* (creosote bush-white bursage) ecosystem of the western Sonoran Desert, Arizona (Fig. 1). The creosote bush-white bursage is the dominant vegetation ecosystem of the western Sonoran Desert and vegetation density is controlled by aridity (20). The study was designed to evaluate the impact of desert microsite and geolocation on: 1) microbial diversity and community composition, 2) capacity of the gap soils to serve as a reservoir for the plant root-zone microbiome, and 3) associations between N-mineralization gene abundance (bacterial *ureC*, ureolysis), N enzyme activity (urease), and soil nutrient pools in the four Sonoran Desert geolocations. Abundance of the *ureC* gene was targeted to compare microbial populations critical to the cycling of organic N and to address whether urease functional capacities shift to alternate taxa in desert ecosystems.

2. RESULTS

2.1 Soil Physicochemical Properties

Physicochemical analysis was performed on soil samples collected from the vegetation canopy and gap soil microsites. Vegetation canopy was defined as the soil samples collected under the canopy of the creosote bush-white bursage vegetation, and gap was defined as the interplant barren spaces devoid of vegetation or biocrust (Fig. 1). Physicochemical analysis indicated that nutrient values, particularly dissolved organic carbon (DOC), total organic carbon (TOC), total nitrogen (TN), dissolved nitrogen (DN), ammonium (NH_4-N), nitrate (NO_3-N), and bioavailable phosphorus (BAP) were significantly higher in canopy soils compared to gap soils (Table 1; Table S1). The soil chemical parameters that most strongly and significantly associated with canopy soils as opposed to gap soils included DN, NH_4-N, TN, BAP, and DOC (Linear mixed-effect models; marginal R^2>0.30; Table 1). Specifically, the content of DN was on average 1.5-to 4.6 -fold higher in canopy soils than in gap soils. Total carbon (TC) and DOC also showed significant geolocation effects, thereby indicating that certain nutrient pools varied with different geographic sites. Additionally, the major fraction of TN and DN was primarily organic as inorganic N values (NH_4-N and NO_3-N) were relatively low (Table S1).

2.2 Microbial Community Composition

The bacterial/archaeal communities across the four arid to hyperarid geolocations of the western Sonoran Desert were dominated by: *Actinobacteria* (40.9%), *Proteobacteria* (24.2%), *Firmicutes* (7.4%), *Gemmatimonadetes* (6%), *Chloroflexi* (5.2%), *Acidobacteria* (4.9%), *Planctomycetes* (2.1%), *Bacteroidetes* (1.5%), and *Verrucomicrobia* (0.9%) (Fig. S1). A comparison of this community composition with other desert and non-desert biomes revealed a profile distinct from non-desert biomes (Fig. S1; tropical/temperate forests and prairie grasslands). Specifically, the relative abundance of *Actinobacteria* (15.3-45.3%) and *Gemmatimonadetes* (1.3-6.7%) was consistently greater in desert biomes (Fig. S1), whereas *Acidobacteria* (17.3-28.1%), and *Verrucomicrobia* (22.8-28.1%) were more abundant in forests and grasslands (Fig. S1; Table S2).

For the sites sampled for this study, compositional differences between microbial communities in canopy and gap soils were assessed using *DESeq2* (Fig. S2 & S3). For bacteria/archaea, the relative abundances of *Actinobacteria*, *Firmicutes*, *Chloroflexi*, and

Armatimonadetes were significantly higher in gap soils (*p<0.05*), whereas *Proteobacteria*, *Gemmatimonadetes*, *Entotheonellaeota*, *Deinococcus-Thermus*, *Dependentiae*, and *Nanoarchaeota* had higher relative abundances in canopy soils (Fig. S2A). At the class level, *Alphaproteobacteria* and *Deltaproteobacteria* had greater relative abundances in the canopy soils, whereas *Bacilli*, *Rubrobacteria*, and *Thermoleophilia* classes had higher relative abundances in gap soils (Fig. S2B). There were 58 and 31 genera that were significantly more abundant in canopy and gap soils, respectively (Fig. S2C). Of these, genera belonging to *Actinobacteria* were split in relative abundance; eleven and nine genera had greater relative abundance in canopy and gap soils, respectively (Fig. S2C). Twenty *Alphaproteobacteria* and eight *Gammaproteobacteria* genera were more abundant in canopy soils and seven *Bacilli* genera were more abundant in gap soils (Fig. S2C).

Likewise, fungal community composition was significantly different between canopy and gap soils (Fig. S3). Ascomycota had greater relative abundance in canopy soils (*p<0.05*), whereas the relative abundances of Basidiomycota and Glomeromycota were significantly greater in gap soils (Fig. S3A). At the class level, Leotiomycetes was the most abundant class in the canopy soils, whereas Geminibasidiomycetes, Glomeromycetes, and Pezizomycetes were more abundant in the gap soils (Fig. S3B). In all, 37 fungal genera were significantly different between canopy and gap soil microsites, and of these, 32 genera had greater relative abundance in canopy soils and only five were more abundant in gap soils (Fig. S3C). The majority of the genera that were abundant in canopy soils belonged to Ascomycota, whereas the five genera with greater relative abundance in gap soils belonged to Ascomycota, Basidiomycota, Calcarisporiellomycota, and Glomeromycota (Fig. S3C).

2.3 Microbial Community Diversity

Linear mixed-effects models showed that the effect of microsite was greater in canopy soils than in gap soils and this effect was stronger for fungi (marginal R^2=0.58) than for bacteria and archaea (marginal R^2=0.20; Table 1). For bacteria/archaea, there was no difference between canopy and gap soil richness at sites 1 and 2; however, richness was greater in canopy soils than gap soils for the more arid sites 3 and 4 (Fig. 2A). In contrast, fungal richness was consistently greater under plant canopies across all the four geolocations (Fig. 2C). Non-metric multidimensional scaling (NMDS) revealed that soil microbial community composition was different between canopy and gap soils and among geolocations (Fig. 2B & D). In addition, geolocation 4 clustered separately from the other sites. Permutational multivariate analysis of variance (PERMANOVA) showed that geolocation explained 20% of the variation in bacterial/archaeal community composition ($p=0.001$); and microsite within each location explained an additional 37% variation ($p=0.001$; Fig. 2B). For fungal communities, 16.3% of the variation in community composition ($p=0.001$) was explained by geolocation, and microsite within geolocation explained an additional 29.5% variation ($p=0.001$; Fig. 2D). Additionally, multivariate dispersion analysis revealed that the bacterial/archaeal ($p=0.28$) and fungal ($p=0.19$) community dispersions were not significantly different between microsites (Fig. S4), suggesting that the PERMANOVA results were not biased by the variation within microsites.

2.4 Core Microbiome and Microsite Specific ASVs

The number of unique and shared amplicon sequence variants (ASVs) in canopy and gap soil microbial communities was examined to investigate whether the gap microbial community has the capacity to serve as a reservoir for the plant root-zone microbiome community recruitment. Unique ASVs are identified as those present in either the canopy or the gap soils, but absent from the other, whereas shared ASVs refer to those present in both microsites. Here we define the core microbiome of this system as the shared ASVs that are present in both canopy and gap soils. In this analysis, ASV number represents richness and ASV relative abundance (at the class level) represents community composition (Fig. 3). A total of 16,484 ASVs were identified for bacteria/archaea across all the sites. The richness of unique bacterial/archaeal ASVs was higher in canopy soils (7,573) than in gap soils (5,254) and the core microbiome was comprised of 3,657 ASVs (Fig. 3A). At the site level, unique canopy soil ASVs represented 34-51% of the richness across the four geolocations (Fig. 3B) and 18-49% of the canopy community composition (Fig. 3C; Table S3). In contrast, the core microbiome represented 51-82% (Fig. 3C; Table S4) and 71-81% (Table S4) of the community composition in the canopy and gap soil communities, respectively.

There was a total of 2,234 ASVs identified for fungi across all sites. Similar to the bacteria/archaea, fungal richness was also higher in canopy soils (1,295 ASVs) than gap (500 ASVs) soils (Fig. 3D). However, the difference was much more pronounced for fungi; the ratio of unique canopy soil to gap soil fungal ASVs was 1.8 times greater than for bacterial/archaeal ASVs. Further, the core microbiome was comprised of just 439 ASVs (Fig. 3D). At the site level, unique fungal ASVs in canopy soils represented 48-73% of the richness while the core microbiome represented only 10-21% of the richness (Fig. 3E). For community composition, unique fungal canopy soils ASVs comprised 26-70% of the community (Fig. 3F; Table S5), in contrast to the core microbiome which represented 30-75% of the relative abundance of fungal taxa in canopy soils (Fig. 3F; Table S6).

2.5 Fungal Guild Predictions

Fungal functional guilds were determined from fungal ASVs using the FUNGuild tool. Linear mixed-effects models showed that the functional guild variability was influenced more significantly by microsite than geolocation (Table 1). Arbuscular mycorrhizal fungi represented the only guild that associated more significantly with gap soils (marginal R^2=0.38; Fig. 4A). In contrast, plant pathogens (marginal R^2=0.68) and wood saprotrophs (marginal R^2=0.57) were more abundant in canopy soils (Fig. 4B & 4C; Table 1). In addition, epiphytes and endophytes also had higher abundance in canopy soils, which are functionally associated with plants (Table 1).

2.6 qPCR Bacterial Gene Abundance

The 16S rRNA and *ureC* bacterial genes were used to quantify bacteria and evaluate specific N-mineralization genetic potential of the bacterial community, respectively (Fig. 5). Bacterial abundance ranged from 7.83 to 8.65 log 16S rRNA gene copies g^{-1} soil (Fig. 5A). The 16S rRNA gene abundance indicated that bacteria were significantly more abundant in canopy soils than gap soils; however, the variability in bacterial abundance was explained more by geolocation than microsite (Table 1, compare the marginal and conditional R^2 values). In contrast, the bacterial *ureC* gene abundance showed a strong and significant microsite effect and a weaker geolocation effect (Table 1). The *ureC* gene was detected in all but one sample and the abundance ranged from 6.5-7.5 log gene copies g^{-1} soil (Fig. 5B). Similar to the *ureC* gene abundance pattern, urease enzyme activity was detected in all the samples (n=24; Table S1). Both urease enzyme activity (marginal R^2=0.31) and *ureC* gene abundance (marginal R^2=0.38) were greater in canopy soils (Table 1). A limited clone library was prepared from Sonoran Desert soil samples and a phylogenetic analysis of associated *ureC* sequences revealed that 51% of the clones did not have a phylogenetic affiliation in the database, 42% belonged to *Actinobacteria*, and 4% of the clones were associated with *Proteobacteria* (Table S7).

2.7 Correlations between Soil Nutrients and Gene Abundance

The organic matter pool is an important source of limiting nutrients such as nitrogen. Correlations were evaluated between TOC, different N forms and N-mineralization functional potential to characterize the associations between N-pools and microbial N-mineralization capacity. Correlations between TOC and TN ($r=0.72$, $p<0.001$) and TOC and DN ($r=0.59$, $p<0.01$) were stronger and more significant than between TOC and ammonium or nitrate (Table S8) indicating that the major N pool in the deserts is organic. Further analysis of N forms and gene abundance showed significant positive correlations between 16S rRNA gene abundance and both TN and DN (Table S9). In addition, bacterial *ureC* gene abundance strongly correlated with all N forms, but the strongest correlations were observed between *ureC* and TN and DN ($r>0.70$; Table S9).

3. DISCUSSION

Climate change-induced acceleration of dryland expansion is predicted to stress arid ecosystems and transform vegetation patterns leading to expansion of interplant gaps (6, 7). Thus, there is a need to understand the significance of the canopy-gap microsite dichotomy to the soil nutrient pools and microbial nutrient cycling capacity of desert ecosystems to predict potential impacts of factors such as gap expansion. This study describes the effect of microsite (canopy versus gap soils) on microbial community composition, functional potential, and microbial associations with soil nutrient pools across four geolocations in the western Sonoran Desert, AZ. Specifically, we demonstrate that (1) there is a stronger microsite than geolocation effect on the microbial community composition; 2) there is significantly greater phylogenetic, genetic, and functional N-mineralization capacity associated with canopy soils relative to the gap soils; and 3) gap soil microbial community lacks sufficient microbial diversity to serve as a complete reservoir for the plant root-zone microbiome.

3.1 Microsite Differences in Microbial Diversity

The Sonoran Desert samples showed that canopy soils have a higher microbial diversity than the gap soils. These results are in contrast to a study of desert palms conducted in semi-arid to arid regions of Tunisian Saharan Desert that found bacterial diversity to be greater in bulk soils compared to rhizosphere in five out of the seven sampling sites (21). Interestingly, a study in the Namib Desert demonstrated different patterns for bacterial and fungal diversity in bulk and speargrass rhizosphere soils. Bulk soils had lower fungal diversity than speargrass rhizosphere soils, whereas bacterial diversity was comparable in bulk and rhizosphere soils (22). It is important to note that the aridity conditions of our Sonoran Desert sites are more similar to the Namib Desert (arid-hyperarid) whereas the Tunisian Saharan Desert sites range from semi-arid to arid. Taken together, our results and the results from these previous studies suggest that the ratio of microbial richness of bulk to rhizosphere soils decreases as aridity increases.

This study also revealed interesting microsite impacts on microbial community composition. For the bacteria/archaea, *Actinobacteria*, *Firmicutes*, and *Chloroflexi* had significantly higher relative abundance in gap soils as compared to under plant canopy soils. This is likely a result of gap areas being more prone to desiccation due to the absence of the protective (shading) vegetation canopy (23). Previous research has shown that the relative abundance of *Actinobacteria* increases with decreased water availability and that desiccation stimulates ribosome synthesis for a quick head start during favorable conditions in members of this phylum (24). For example, Actinobacteria classes of *Rubrobacteria* and *Thermoleophilia*, known to be radio-tolerant and thermophilic, respectively, were abundant in gap soils (25, 26). Similarly, *Firmicutes* are known for a resistance response strategy that maintains a stable ribosome content in both drought and wet conditions (24) and *Chloroflexi* exhibit adaptation strategies including resistance to desiccation and ultraviolet radiation (27). Taken together, adaptation to desiccation and dormancy are common strategies that facilitate microbial survival under the oligotrophic arid conditions of gap soils (28). In contrast, examples of populations found predominantly in canopy soils are the Alphaproteobacteria genera *Rhizobiales* and *Sphingomonadales*. These microbes are known for their ability to fix nitrogen and carry out phototrophy in deserts, respectively (29).

A strong significant microsite impact was also observed for fungal communities. Functionally, desiccation stress of gaps does not affect fungi as they are considered more resistant than bacteria and have hyphae to aid in accessing nutrients and water (30, 31). The higher fungal richness in canopy soils is likely driven by plant litter inputs in canopy soils (32). FUNGuild correctly predicted that plant pathogen, epiphyte, endophyte, and saprotrophs would be more associated with canopy soils. Ascomycetes were more abundant under plant canopies and these fungi play an important role in carbon and nitrogen cycling as they break down complex molecules, such as cellulose and lignin (33). Of the *Ascomycota*, the fungal classes enriched in canopy soils were Dothideomycetes, Eurotiomycetes, and Sordariomycetes. The characterized members of Sordariomycetes are involved in decomposition and nutrient cycling and act as plant and animal pathogens, endophytes, and saprobes (34, 35). Further, members of the order Sordariales (Sordariomycetes) and Agaricales (Agaricomycetes) are potent lignin degraders (36, 37).

Based on FUNGuild results, arbuscular mycorrhizae are more abundant in gap soils than under canopy soils. This is surprising, as arbuscular mycorrhizae are typically found in association with plant roots. It is noted that arbuscular mycorrhizae belonging to *Glomeromycetes* were also found to have higher abundance in gap rather canopy soils. Some species of this taxa are found associated both with plants and free-living (38). Alternatively, an explanation for this observed pattern is the fungal loop hypothesis where fungi act as biological networks for transforming and translocating nutrient resources across barren spaces between plants (39).

In summary, microsite was a strong driver of microbial community composition for both bacteria/archaea and fungi. Possible explanations for this difference include: 1) the plant fertility island effect under oligotrophic conditions provides enhanced nutrient availability that supports a plant rhizosphere microbial community that is quite different from gaps (40–42), and 2) the plant-protective effect provides a safe haven for canopy soil microbial communities by buffering extreme temperatures, providing UV protection, and preventing desiccation by maintaining soil moisture (43).

3.2 Gap Soils are not a Complete Reservoir for the Plant Canopy Soil Microbiome

Studies of global drylands have demonstrated that increased aridity reduces microbial diversity (9, 10). Thus, it is logical to hypothesize that without the protection of plants, gap soil microbial diversity may be more susceptible to aridity stress. Berdugo and colleagues described the response of drylands to increasing aridity as a three-step sequential process, initiated by a "vegetation decline" followed by a phase of "soil disruption", and finally resulting in a "systemic breakdown" along with a reduction in relative abundance of saprotrophic and ectomycorrhizal fungi (5). This scenario raises two related questions: 1) does gap microbial diversity function as an ecosystem reservoir from which plants recruit microbes for plant-rhizosphere soils, ? (44) and 2) if the gap does not provide a plant microbiome reservoir, will aridity-induced gap expansion leave vegetation patches as the only reservoirs of critical microbial capacity?.

Our results demonstrate that the core microbiome of the plant root-zone was comprised of numerous taxa that were sourced from surrounding bulk (gap) soils; however, there was a recognizable number of unique plant canopy soil microbial taxa that were not shared with the associated gap microbial communities. Importantly, the relative abundance of unique taxa in canopy soils increased with aridity; sites 3 & 4 had higher relative abundance of unique canopy soil microbial taxa than sites 1 & 2. These patterns suggest that gap soils do not provide a complete microbial reservoir for the plant root-zone microbiome and the source of the unique taxa in the canopy soil microbiome is unknown. For this reason, we suggest a potential loss of this plant-associated haven of microbial diversity as a result of decreasing plant density or death (45) in the scenario of increasing aridity (4). In this case, the ecosystem would lose a reservoir of microbial diversity that may be critical to plant survival in arid ecosystems (45). Therefore, the canopy-gap dichotomy documented in our study suggests that plant death could lead to ecosystem loss of critical microbial genetic potential, possibly causing the “soil disruption” and “systemic breakdown” described by Bergudo et al. (5).

3.3 Organic Nitrogen is the Major Nitrogen Pool in the Sonoran Desert

The carbon, nitrogen, and phosphorus levels found in this study are similar to that observed in many desert ecosystems that exhibit fertility island effects (3, 32, 46). TOC was 3.1 to 13.7 times higher in canopy soils than in gap soils across the geolocations studied. In desert ecosystems, organic carbon is an important nutrient source and a good indicator of the plant-associated fertility. Organic carbon supports heterotrophic microbes that carry out depolymerization and mineralization processes needed to increase N availability (47, 48). Multi-model analyses performed by Delgado-Baquerizo et al. (48) to characterize N availability found that aridity is the primary driver for N availability in gaps, but organic carbon is as significant as aridity for N availability under plant canopies. In another study by Delgado-Baquerizo et al., their model explained that increasing aridity will decrease N and C concentrations and increase phosphorus

(P) concentrations, hence decoupling C, N, and P cycles (49). Such reduced availability of C and N is predicted to disrupt the stoichiometric balance between C, N, and P, resulting in reduced plant productivity and microbial diversity and activity. Results from our study showed that inorganic N content in desert soils is relatively low and TOC strongly correlates with TN/DN. Therefore, we contend that organic N is the major N pool in the Sonoran Desert soils. Given that organic N is relevant source of N in this ecosystem, predicted changes in C and N availability due to climatic changes could disrupt the stoichiometric nutrient balance and subsequently lead to loss of ecosystem services.

3.4 Nitrogen Availability is Indicated by *ureC* in Sonoran Desert Soils

The bacterial *ureC* gene abundance was significantly greater in canopy soils than gap soils; however, little is known about *ureC* gene abundance in natural ecosystems. Most studies on *ureC* gene abundance have been conducted in agricultural settings. The *ureC* abundance values detected in two unfertilized control agricultural soils, an organic farm soil, a Black soil, and vegetated sandstone and siltstone surface mine rock fragments were 4.5-9.5 x 10^7, 1-1.8 x 10^8, 4.8-8.4 x 10^7, and 0.68- 3.4 x 10^5 gene copies g^{-1} soil, respectively (50–54). Given that the *ureC* gene abundance in the Sonoran Desert ecosystem is just slightly lower (0.35-2.87 x 10^7 gene copies g^{-1} soil) than unamended agricultural and Black soils, it suggests that canopy and gap soils have substantial N-mineralization genetic potential among ureloytic bacteria. Additionally, in a recently published organic-inorganic co-composting study, *ureC* gene was validated as a marker for ammonification (i.e. conversion of urea to ammonium) (55). Thus, our study supports the use of *ureC* as a marker for N-ammonification/mineralization in the desert ecosystems. Urease enzyme activity measurements, like *ureC* gene abundance, were significantly more abundant in canopy soils relative to gap locations indicating a strong link between genetic potential and functional capacity.

Additionally, *ureC* gene abundance correlated strongly with TN and DN. These correlations support the contention that mineralization of organic matter is an abundant and relevant source of N in this arid ecosystem.

3.5 Phylogenetic Associations of *ureC* in the Sonoran Desert Soils

Like *ureC* gene abundance, the diversity of ureolytic bacteria have been primarily studied in agricultural soils. *Proteobacteria* are the most abundant ureolytic bacteria in urea-amended or non-amended agricultural soils followed by *Actinobacteria*, *Verrucomicrobia*, and *Nitrospirae* (50, 53). Other low-abundance phyla containing *ureC* taxa include *Acidobacteria*, *Bacteroidetes*, *Cloroflexi*, *Firmicutes*, and *Planctomycetes* (50, 53). In contrast, the majority of the identifiable *ureC* sequences in our limited clone library phylogenetically associated with *Actinobacteria* (42%; Table S7), whereas only 4% of the clones were associated with *Proteobacteria*. As discussed previously, *Actinobacteria* are more abundant in desert soils than other biomes. Therefore, this novel association of critical ureolysis functional capacity with the dominant desert phylum suggests that the limited and distinct microbial diversity observed in desert soils may not imply a loss of functional capacity, but rather an important shift in the association of that functional capacity to phylogenetic groups dominant in desert ecosystems. Moreover, it is noteworthy that the *ureC* primers used in this study were designed using conserved regions within *ureC* gene from sequences of *Proteobacteria* (56). Clearly, these primers are compatible with *Actinobacteria*, indicating that the *ureC* gene sequence is conserved across taxa. Further, 51% of the *ureC* gene clones from this study represented taxa that did not have a phylogenetic affiliation in the database (Table S7). This suggests that the *ureC* phylogenetic distribution in desert soils includes many unidentified bacteria. These results confirm the limitations of current databases and contend that important functional nutrient cycling capacities in desert ecosystems are associated with novel organisms that are currently poorly characterized.

In summary, canopy soils of the Sonoran Desert were found to have significantly higher nutrient content, greater bacterial and fungal diversity, as well as greater N-mineralization genetic potential and urease functional capacity when compared to the associated gap soils. The *ureC* gene marker for N-mineralization was not only identified as a significant indicator of functional capacity in this desert ecosystem, but the shift in association of that functional capacity to phylogenetic groups dominant in desert ecosystems demonstrated an important pattern for desert ecosystem services. Additionally, we showed that Sonoran Desert gap soil microbial community lacks sufficient microbial diversity to serve as a complete reservoir for the plant root-zone microbiome. Therefore, it is critical that future research further characterizes the low microbial diversity and functional capacity of arid-ecosystem gap soils and determines whether the diversity of these soils is necessary as a reservoir for plant recruitment to the rhizosphere community. We propose that future studies investigate the microbial genetic capacity of gap soils along a wide aridity gradient, continue to identify novel associations between phylogenetic and functional diversity, and extensively evaluate the significance of the gap soil microbiome to the sustainability of plant vegetation density in desert ecosystems.

4. MATERIAL AND METHODS

4.1 Site Description and Sample Collection

This study focused on arid ($0.05 \leq$ Aridity Index < 0.2) and hyper arid (Aridity Index < 0.05) regions of the Western Sonoran Desert, AZ. Samples were collected from four distinct geographic locations along a 77 km north-south transect (Fig. 1) in October 2017. Site 1 was located in the Kofa National Wildlife Refuge; site 2 was on route 60 in Pioneer, AZ; site 3 on route 95 north of

Quartzsite, AZ; and site 4 south of Parker, AZ (Table S1). The mean annual temperature (MAT) and mean annual precipitation (MAP) of the geolocations were compiled from the WorldClim-Global database (57) (Table S1). The aridity index (AI) was compiled from the CGIAR-CSI's Global Potential Evapotranspiration and Global Aridity Index (Table S1; https://cgiarcsi.community/2019/01/24/global-aridity-index-and-potential-evapotranspiration -climate-database-v2/).

At each geolocation, a 30 m x 20 m sampling area was delineated, and three 30 m transects were laid out in a N/S direction within the sampling area beginning with transect 1 at the NW corner (Fig. 1D). Transects 2 and 3 were started 10 m east of the first transect. Vegetation metrics including number of vegetation patch and gaps, inter-distance between each vegetation patch, and number of grasses/shrubs/trees in each patch along the three transects were recorded (Table S1). The shrub species *Larrea tridentata* (creosote bush) and *Ambrosia dumosa* (white bursage) comprised the dominant vegetation (~70%) and the remaining plants were mixed species. For soil and microbial analyses, soil samples were collected from vegetation canopy and gap soil microsites, where 1) canopy is defined as under the canopy soil of the creosote bush-white bursage vegetation and 2) gap is defined as the interplant barren spaces devoid of any vegetation or biocrusts. Six grab samples were collected along each transect, three under the shrub canopy (canopy) and three in interplant barren spaces (gap; Fig. S5). At each transect, soil was removed from a 20 cm diameter soil pit excavated to a depth of 20 cm and combined with soils from the other two canopy or gap soil-pits along the same transect. The three composite soil samples were homogenized, sieved (2 mm pore size), and subsampled for soil chemical analyses in the field. This was done separately for under shrub canopy and gap soils along each transect. Soils were sampled to a depth of 20 cm to include soils relevant to root development and to minimize the impact of UV radiation on surface soil microbial communities (58). Composite samples for each microsite were collected to capture the heterogeneity of the microsites along the transect. For microbial analysis, samples were collected from each sampling pit sidewall to a depth of 20 cm using sterile instruments. Microbial samples from three pits along the transect were homogenized and combined. Thus, a single microbial composite sample was generated for canopy and gap soil microsites from each transect. These samples were transported on ice to the lab and frozen at -80ºC until ready for DNA extractions. Selected soil chemistry measurements (TOC, DOC, DN, and BAP) and 16S rRNA gene amplicon analyses have been reported by Chen et al. (2020) (59). In our present study, a detailed analyses of soil physicochemical and enzymatic properties, microbial diversity and composition, and gene abundance have been described.

4.2 Soil Physicochemical and Enzymatic Analyses

Soil pH and electric conductivity (EC) were determined from a 1:2 soil-to-distilled water (dH_2O) slurry after 30 min of shaking (60). Dry soils were milled (SPEX 8000D Mixer/Mill, Metuchen, NJ) prior to analysis for TC, TN, DOC, dissolved inorganic carbon (DIC), and DN. The TC and TN were analyzed using ECS 4010 C and N analyzer (Costech Analytical Technologies, Valencia, CA). The DOC, DIC, and DN (1:15 soil: dH_2O; shaken at 200 rpm for 24 h at room temperature) were quantified by TOC-L analyzer (Shimadzu Scientific Instruments, Columbia, MD). For measurement of TOC, soil samples were digested with HCl to remove dissolved carbonates followed by washing with water and drying at 70°C. The dried samples were then combusted in the presence of CuO in vacuum and the released carbon dioxide was cryogenically distilled from the mixture of combustion gases, manometrically quantified, and converted to carbon mass (University of Arizona Accelerator Mass Spectrometry Lab). The NO_3-N (1:5 w/v in KCl solution) was measured using the Cd-reduction method (61). The NH_4-N (1:2 w/v in KCl solution) was quantified using the HACH Ammonia Salicylate Reagent and Ammonia Cyanurate Reagent (HACH, Loveland, CO; Sinsabaugh Lab protocol 2005). BAP was measured using the Olsen method (62) and soil texture was determined using the hydrometer method (63). Urease enzyme activity was measured using the Kandeler and Gerber (64) method with modifications as per Fioretto et al. (65).

4.3 Molecular Analyses of Microbial Communities

DNA extraction from 0.5 g soil samples was carried out using Fast DNA SPIN for Soil Kit™ (MP Biomedicals, Solon OH, USA) with modifications to enhance DNA recovery from oligotrophic arid soil samples. All consumables and reagents lacking biomolecules were sterilized with UV light for 30 minutes. Further, modifications include: (1) 0.8 mL of binding matrix was used to bind DNA, and after mixing and settling the binding matrix, all supernatant was removed and discarded without filtering through the spin filter; (2) binding matrix was washed 1-2x times with 0.5 mL of 6 M Guanidine thiocyanate (Sigma-Aldrich, St. Louis, MO) to remove organic matter; (3) the wash with SEWS-M reagent was repeated twice to remove co-extracted salts; (4) spin filters were dried at 37°C for 10 min under a laminar flow hood prior to DNA elution; (5) a two-fold DNA elution was performed using two sequential aliquots of 50 µL of UV-sterilized DEPC treated, nuclease-free water (Growcells, Irvine, CA) preheated to 60°C in which each 50 µL aliquot was followed by incubation at 60°C for 10 min and centrifugation for 1 min. The extracted DNA was quantified using the Qubit® dsDNA High Sensitivity Assay Kit (Life Technologies, NY, USA). All the samples had quantifiable DNA ranging from 3.17 to 41.5 ng μL^{-1}. Paired-end amplicon sequencing of DNA extracts was performed using 16S rRNA gene primers 515F/806R (bacteria/ archaea) and ITS primers ITS1f-ITS2 (fungi) as described in Walters et al. (66). The purified PCR products from all samples were pooled together in equimolar concentrations and sequenced on a 2×150

bp Illumina MiSeq platform. All sequencing runs were conducted at the Microbiome Core, Steele Children's Research Center, University of Arizona.

4.4 Sequence Processing

Raw reads were demultiplexed using idemp tool (https://github.com/yhwu/idemp). Further bioinformatics analyses were carried out using DADA2 pipeline (67). The demultiplexed reads were trimmed to retain 140 bases of the forward and reverse reads. The paired-end reads were joined using the default overlap of at least 12 bases and then grouped into ASVs. The ASVs were subjected to chimera removal. Post quality filtering, a total of 4,193,573 sequence reads remained for 16S rRNA gene and 2,901,758 for ITS, with an average of 149,770 ± 33,143 and 120,907 ± 37,410 sequence reads per sample for 16S rRNA gene and ITS region, respectively. Taxonomy assignments were determined with the RDP Classifier (68) using SILVA (69) and UNITE ITS (70) databases for b18acterial/archaeal and fungal communities, respectively. Contaminants were removed from the ASV tables through comparisons of samples and the blanks, leaving 16,4844 and 2,234 ASVs for bacterial/archaeal and fungal communities, respectively. The taxonomy tables obtained were then normalized using a cumulative-sum scaling approach (71). The taxonomic distribution of bacterial/archaeal communities were compared to other desert and non-desert biomes using published datasets (8, 10). Additionally, the FUNGuild tool was utilized to predict fungal guilds from ITS taxonomic assignments (72).

4.5 Bacterial Gene Abundance Using qPCR

Bacterial 16S rRNA and *ureC* genes were quantified to assess the bacterial copy number and N-mineralization capacity, respectively. The V3-V4 variable region of the 16S rRNA gene was amplified using primers 338F/518R (73). The bacterial *ureC* gene, encoding a subunit of the urease enzyme, was amplified using the primers ureC1F/ureC2R (56). Conserved regions within *ureC* gene from *Ralstonia eutropha* and other ureolytic proteobacteria including species from the genera *Nitrosospira* and *Nitrosococcus* were used to design degenerate primers for the *ureC* gene (56).

qPCR standard for 16S rRNA gene quantification was described previously (74). For the bacterial *ureC* gene, a limited clone library was constructed from the collected soil samples as described in (75) and the resulting *ureC* sequences were matched to NCBI protein database using BLASTX. The clone for the standard was selected based on 100% sequence match to the query in the NCBI database (Table S7). Plasmids were quantified using Qubit® dsDNA High Sensitivity Assay Kit. The qPCR reactions were performed in triplicate using a 96-well plate on a CFX96 Real-Time Detection System (Bio-Rad Laboratories, Hercules, CA). In a 10 µL reaction, 5 µL SsoFast™ EvaGreen® Supermix (Bio-Rad Laboratories, Hercules, CA), either 0.2 µM (*ureC*) or 0.3 µM (16S rRNA gene) primers, and 1 µL of sample DNA extract or plasmid standards were used.

All qPCR standards and samples were run in triplicate. Amplification conditions for 16S rRNA gene were as described previously (74) with an amplification efficiency of 97-100%, R^2 of 0.99, and a calibration curve with 10^3 to 10^8 copies of 16S rRNA gene. The amplification conditions for *ureC* gene were 98ºC for 3 min, 50 cycles of 98ºC for 20s, 60ºC for 25s with an amplification efficiency greater than 91%, an R^2 of 0.98-0.99 and a calibration curve of 10^2 to 10^6 gene copies. Melt curves were generated at the end of each run with a temperature range of 65 to 95ºC in 0.5ºC increments. Amplicon specificity was confirmed by melt curve analysis and gel electrophoresis. Inter-plate calibration was performed to normalize for inter-plate variations in amplification efficiencies among plates being assayed for the same gene (CFX Manager™ software).

4.6 Statistical Analyses

Statistical analyses were implemented in R (R Core Team, 2019). Significant differences of soil physicochemical properties, microbial richness, and gene abundance between canopy and gap soils were determined using linear mixed-effects models using the *lme4* and *lmerTest* packages. Microsite and geolocation were used as fixed and random effects, respectively. The Satterthwaite's (76) approximations were used to estimate the statistical significance of the fixed effect. R^2-value of the fixed effect was assessed as described in Nakagawa & Schielzeth (77). Community dissimilarity was calculated using the Bray-Curtis distance and NMDS ordination was used for visualization. Microbial community differences among geolocations and microsites were examined using PERMANOVA (78). Further, community dispersion within microsite was (multivariate dispersion) was calculated. FUNGuild differences were also evaluated using negative binomial generalized linear mixed-effects models. Additional statistical tests are described in Supplemental methods. The R scripts used for the statistical analyses are available on GitHub: https://github.com/kush-priyanka/Arid_Soil_Microbiome.

4.7 Data Availability

Raw sequence data for 16S rRNA gene and ITS were submitted to NCBI's Sequence Read Archive under the accession number PRJNA610058.

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6. REFERENCES

1. Aguiar MR, Sala OE. 1999. Patch structure, dynamics and implications for the functioning of arid ecosystems. Trends Ecol Evol 14:273–277.
2. Schlesinger WH, Raikks JA, Hartley AE, Cross AF. 1996. On the spatial pattern of soil nutrients in desert ecosystems. Ecology 77:364–374.
3. de Graaff M-A, Throop HL, Verburg PSJ, Iii JAA, Campos X. 2014. A synthesis of climate and vegetation cover effects on biogeochemical cycling in shrub-dominated drylands. Ecosystems 17:931–945.
4. Seager R, Ting M, Held I, Kushnir Y, Lu J, Vecchi G, Huang HP, Harnik N, Leetmaa A, Lau NC, Li C, Velez J, Naik N. 2007. Model projections of an imminent transition to a more arid climate in southwestern North America. Science (80-) 316:1181–1184.
5. Berdugo M, Delgado-Baquerizo M, Soliveres S, Hernández-Clemente R, Zhao Y, Gaitán JJ, Gross N, Saiz H, Maire V, Lehman A, Rillig MC, Solé R V., Maestre FT. 2020. Global ecosystem thresholds driven by aridity. Science (80-) 367:787–790.
6. Sun GQ, Wang CH, Chang LL, Wu YP, Li L, Jin Z. 2018. Effects of feedback regulation on vegetation patterns in semi-arid environments. Appl Math Model 61:200–215.
7. Kéfi S, Rietkerk M, Alados CL, Pueyo Y, Papanastasis VP, ElAich A, De Ruiter PC. 2007. Spatial vegetation patterns and imminent desertification in Mediterranean arid ecosystems. Nature 449:213–217.
8. Fierer N, Leff JW, Adams BJ, Nielsen UN, Bates ST, Lauber CL, Owens S, Gilbert JA, Wall DH, Caporaso JG. 2012. Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. PNAS 109:21390–21395.
9. Maestre FT, Delgado-Baquerizo M, Jeffries TC, Eldridge DJ, Ochoa V, Gozalo B, Quero

JL, García-Gómez M, Gallardo A, Ulrich W, Bowker MA, Arredondo T, Barraza-Zepeda C, Bran D, Florentino A, Gaitán J, Gutiérrez JR, Huber-Sannwald E, Jankju M, Mau RL, Miriti M, Naseri K, Ospina A, Stavi I, Wang D, Woods NN, Yuan X, Zaady E, Singh BK. 2015. Increasing aridity reduces soil microbial diversity and abundance in global drylands. PNAS 112:15684–15689.

10. Neilson JW, Califf K, Cardona C, Copeland A, van Treuren W, Josephson KL, Knight R, Gilbert JA, Quade J, Caporaso JG, Maier RM. 2017. Significant impacts of increasing aridity on the arid soil microbiome. mSystems 2:e00195-16.

11. Bachar A, Ines M, Soares M, Gillor O. 2012. The effect of resource islands on abundance and diversity of bacteria in arid soils. Microb Ecol 63:694–700.

12. Ben-David EA, Zaady E, Sher Y, Nejidat A. 2011. Assessment of the spatial distribution of soil microbial communities in patchy arid and semi-arid landscapes of the Negev Desert using combined PLFA and DGGE analyses. FEMS Microbiol Ecol 76:492–503.

13. Hortal S, Bastida F, Armas C, Lozano YM, Moreno JL, García C, Pugnaire FI. 2013. Soil microbial community under a nurse-plant species changes in composition, biomass and activity as the nurse grows. Soil Biol Biochem 64:139–146.

14. Garcia DE, Lopez BR, de-Bashan LE, Hirsch AM, Maymon M, Bashan Y. 2018. Functional metabolic diversity of the bacterial community in undisturbed resource island soils in the southern Sonoran Desert. L Degrad Dev 29:1467–1477.

15. Nielsen UN, Ball BA. 2015. Impacts of altered precipitation regimes on soil communities and biogeochemistry in arid and semi-arid ecosystems. Glob Chang Biol 21:1407–1421.

16. De Neve S. 2017. Organic matter mineralization as a source of nitrogen, p. 65–83. *In* Advances in research on fertilization management of vegetable crops. Springer.

17. Jan MT, Roberts P, Tonheim SK, Jones DL. 2009. Protein breakdown represents a major bottleneck in nitrogen cycling in grassland soils. Soil Biol Biochem 41:2272–2282.

18. Gresham TLT, Sheridan PP, Watwood ME, Fujita Y, Colwell FS. 2007. Validation of ureC-based primers for groundwater detection of urea-hydrolyzing bacteria. Geomicrobiol J 24:353–364.

19. Baraniya D, Puglisi E, Ceccherini MT, Pietramellara G, Giagnoni L, Arenella M, Nannipieri P, Renella G. 2016. Protease encoding microbial communities and protease activity of the rhizosphere and bulk soils of two maize lines with different N uptake efficiency. Soil Biol Biochem 96:176–179.

20. Butterfield BJ, Betancourt JL, Turner RM, Briggs JM. 2010. Facilitation drives 65 years of vegetation change in the Sonoran Desert. Ecology 91:1132–1139.

21. Mosqueira MJ, Marasco R, Fusi M, Michoud G, Merlino G, Cherif A, Daffonchio D. 2019. Consistent bacterial selection by date palm root system across heterogeneous desert oasis agroecosystems. Sci Rep 9:1–12.

22. Marasco R, Mosqueira MJ, Fusi M, Ramond J-B, Merlino G, Booth JM, Maggs-Kölling G, Cowan DA, Daffonchio D. 2018. Rhizosheath microbial community assembly of sympatric desert speargrasses is independent of the plant host. Microbiome 6:215.

23. Butterfield BJ, Bradford JB, Armas C, Prieto I, Pugnaire FI. 2016. Does the stress-gradient hypothesis hold water? Disentangling spatial and temporal variation in plant effects on soil moisture in dryland systems. Funct Ecol 30:10–19.

24. Barnard RL, Osborne CA, Firestone MK. 2013. Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. ISME J 7:2229–2241.

25. Chanal A, Chapon V, Benzerara K, Barakat M, Christen R, Achouak W, Barras F, Heulin

T. 2006. The desert of Tataouine: An extreme environment that hosts a wide diversity of microorganisms and radiotolerant bacteria. Environ Microbiol 8:514–525.

26. Hu D, Zang Y, Mao Y, Gao B. 2019. Identification of molecular markers that are specific to the class thermoleophilia. Front Microbiol 10:1–13.

27. Battistuzzi FU, Hedges SB. 2009. A major clade of prokaryotes with ancient adaptations to life on land. Mol Biol Evol 26:335–343.

28. Leung PM, Bay SK, Meier D V., Chiri E, Cowan DA, Gillor O, Woebken D, Greening C. 2020. Energetic basis of microbial growth and persistence in desert ecosystems. mSystems 5:e00495-19.

29. Van Goethem MW, Makhalanyane TP, Cowan DA, Valverde A. 2017. Cyanobacteria and Alphaproteobacteria may facilitate cooperative interactions in niche communities. Front Microbiol 8:1–11.

30. Gordon H, Haygarth PM, Bardgett RD. 2008. Drying and rewetting effects on soil microbial community composition and nutrient leaching. Soil Biol Biochem 40:302–311.

31. de Vries FT, Liiri ME, Bjørnlund L, Bowker MA, Christensen S, Setälä HM, Bardgett RD. 2012. Land use alters the resistance and resilience of soil food webs to drought. Nat Clim Chang 2:276–280.

32. Ochoa-Hueso R, Eldridge DJ, Delgado-Baquerizo M, Soliveres S, Bowker MA, Gross N, Le Bagousse-Pinguet Y, Quero JL, García-Gómez M, Valencia E, Arredondo T, Beinticinco L, Bran D, Cea A, Coaguila D, Dougill AJ, Espinosa CI, Gaitán J, Guuroh RT, Guzman E, Gutiérrez JR, Hernández RM, Huber-Sannwald E, Jeffries T, Linstädter A, Mau RL, Monerris J, Prina A, Pucheta E, Stavi I, Thomas AD, Zaady E, Singh BK, Maestre FT. 2018. Soil fungal abundance and plant functional traits drive fertile island

formation in global drylands. J Ecol 106:242–253.

33. Zeng Q, Liu Y, Xiao L, An S. 2020. Climate and soil properties regulate soil fungal communities on the Loess Plateau. Pedobiologia (Jena) 81–82:150668.

34. Suleiman MK, Dixon K, Commander L, Nevill P, Quoreshi AM, Bhat NR, Manuvel AJ, Sivadasan MT. 2019. Assessment of the diversity of fungal community composition associated with Vachellia pachyceras and its rhizosphere soil from Kuwait Desert. Front Microbiol 10:63.

35. Qin H, Wang H, Strong PJ, Li Y, Xu Q, Wu Q. 2014. Rapid soil fungal community response to intensive management in a bamboo forest developed from rice paddies. Soil Biol Biochem 68:177–184.

36. Pöggeler S. 2011. Evolution of multicopper oxidase genes in Coprophilous and Non-Coprophilous members of the order Sordariales. Curr Genomics 12:95–103.

37. Entwistle EM, Zak DR, Edwards IP. 2013. Long-term experimental nitrogen deposition alters the composition of the active fungal community in the forest floor. Soil Sci Soc Am J 77:1648–1658.

38. Hempel S, Renker C, Buscot F. 2007. Differences in the species composition of arbuscular mycorrhizal fungi in spore, root and soil communities in a grassland ecosystem. Environ Microbiol 9:1930–1938.

39. Collins SL, Sinsabaugh RL, Crenshaw C, Green L, Porras-Alfaro A, Stursova M, Zeglin LH. 2008. Pulse dynamics and microbial processes in aridland ecosystems. J Ecol 96:413–420.

40. Pointing SB, Belnap J. 2012. Microbial colonization and controls in dryland systems. Nat Rev Microbiol 10:551–562.

41. Mueller RC, Belnap J, Kuske CR. 2015. Soil bacterial and fungal community responses to nitrogen addition across soil depth and microhabitat in an arid shrubland. Front Microbiol 6:891.

42. Steven B, Gallegos-Graves LV, Yeager C, Belnap J, Kuske CR. 2014. Common and distinguishing features of the bacterial and fungal communities in biological soil crusts and shrub root zone soils 69:302–312.

43. Cortina J, Maestre FT. 2005. Plant effects on soils in drylands: implications for community dynamics and ecosystem restoration. Kluwer Academic Publishers, Dordrecht, the Netherlands.

44. Yeoh YK, Dennis PG, Paungfoo-Lonhienne C, Weber L, Brackin R, Ragan MA, Schmidt S, Hugenholtz P. 2017. Evolutionary conservation of a core root microbiome across plant phyla along a tropical soil chronosequence. Nat Commun 8:1–9.

45. Honeker LK, Gullo CF, Neilson JW, Chorover J, Maier RM. 2019. Effect of re-acidification on buffalo grass rhizosphere and bulk microbial communities during phytostabilization of metalliferous mine tailings. Front Microbiol 10:1209.

46. Butterfield BJ, Briggs JM. 2009. Patch dynamics of soil biotic feedbacks in the Sonoran Desert. J Arid Env 73:96–102.

47. Cookson WR, Müller C, O'Brien PA, Murphy D V., Grierson PF. 2006. Nitrogen dynamics in an Australian semiarid grassland soil. Ecology 87:2047–2057.

48. Delgado-Baquerizo M, Maestre FT, Gallardo A, Quero JL, Ochoa V. 2013. Aridity modulates N availability in arid and semiarid mediterranean grasslands. PLoS One 8:59807.

49. Delgado-Baquerizo M, Maestre FT, Gallardo A, Bowker MA, Wallenstein MD, Luis

Quero J, Ochoa V, Gozalo B, García-Gómez M, Soliveres S, García-Palacios P, Berdugo M, Valencia E, Escolar C, Arredondo T, Barraza-Zepeda C, Bran D, Carreira JA, Chaieb M, Conceição AA, Derak M, Eldridge DJ, Escudero A, Espinosa CI, Gaitán J, Gatica MG, Gómez-González S, Guzman E, Gutiérrez JR, Florentino A, Hepper E, Hernández RM, Huber-Sannwald E, Jankju M, Liu J, Mau RL, Miriti M, Monerris J, Naseri K, Noumi Z, Polo V, Prina A, Pucheta E, Ramírez E, Ramírez-Collantes DA, Romão R, Tighe M, Torres D, Torres-Díaz C, Ungar ED, Val J, Wamiti W, Wang D, Zaady E. 2013. Decoupling of soil nutrient cycles as a function of aridity in global drylands. Nature 502:672–676.

50. Sun R, Li W, Hu C, Liu B. 2019. Long-term urea fertilization alters the composition and increases the abundance of soil ureolytic bacterial communities in an upland soil. FEMS Microbiol Ecol 95:44.

51. Ouyang Y, Reeve JR, Norton JM. 2018. Soil enzyme activities and abundance of microbial functional genes involved in nitrogen transformations in an organic farming system. Biol Fertil Soils 54:437–450.

52. Ouyang Y, Norton JM. 2020. Short-term nitrogen fertilization affects microbial community composition and nitrogen mineralization functions in an agricultural soil. Appl Environ Microbiol 86.

53. Wang L, Luo X, Liao H, Chen W, Wei D, Cai P, Huang Q. 2018. Ureolytic microbial community is modulated by fertilization regimes and particle-size fractions in a Black soil of Northeastern China. Soil Biol Biochem 116:171–178.

54. Yarwood S, Wick A, Williams M, Daniels WL. 2015. Parent material and vegetation influence soil microbial community structure following 30-Years of rock weathering and

pedogenesis. Microb Ecol 69:383–394.

55. Yu H, Xie B, Khan R, Yan H, Shen G. 2020. The changes in functional marker genes associated with nitrogen biological transformation during organic-inorganic co-composting. Bioresour Technol 295.

56. Koper TE, El-Sheikh AF, Norton JM, Klotz MG. 2004. Urease-encoding genes in ammonia-oxidizing bacteria. Appl Env Microbiol 70:2342–2348.

57. Fick SE, Hijmans RJ. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. Int J Clim 37:4302–4315.

58. Maier RM, Drees KP, Neilson JW, Henderson DA, Quade J, Betancourt JL, Navarro-Gonzalez R, Rainey FA, McKay CP. 2004. Microbial life in the Atacama Desert. Science (80-) 306:1289–1291.

59. Chen Y, Neilson JW, Kushwaha P, Maier RM, Barberán A. 2020. Life-history strategies of soil microbial communities in an arid ecosystem. ISME J 1–9.

60. Honeker LK, Neilson JW, Root RA, Gil-Loaiza J, Chorover J, Maier RM. 2017. Bacterial rhizoplane colonization patterns of Buchloe dactyloides growing in metalliferous mine tailings reflect plant status and biogeochemical conditions HHS Public Access. Microb Ecol 74:853–867.

61. Dorich RA, Nelson DW. 1984. Evaluation of manual cadmium reduction methods for determination of nitrate in potassium chloride extracts of soils. Soil Sci Soc Am J 48:72–75.

62. Olsen S, Cole C, Watanabe E, Dean L. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonateU.S. Gov. Print Office. Washington, DC.

63. Gee GW, Bauder JW. 1979. Particle size analysis by hydrometer: A simplified method for

routine textural analysis and a sensitivity test of measurement parameters. Soil Sci Soc Am J 43:1004–1007.

64. Kandeler E, Gerber H. 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. Biol Fert Soils 6:68–72.

65. Fioretto A, Papa S, Pellegrino A, Ferrigno A. 2009. Microbial activities in soils of a Mediterranean ecosystem in different successional stages. Soil Biol Biochem 41:2061–2068.

66. Walters W, Hyde ER, Berg-Lyons D, Ackermann G, Humphrey G, Parada A, Gilbert JA, Jansson JK, Gregory Caporaso J, Fuhrman JA, Apprill A, Knight R, Walters CW. 2016. Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. mSystems 1:e00009-15.

67. Callahan BJ, Mcmurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: high-resolution sample inference from illumina amplicon data. Nat Methods 13:581–583.

68. Wang Q, Garrity GM, Tiedje JM, Cole JR. 2007. Naive bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Env Microbiol 73:5261–5267.

69. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Rg Peplies J, Glö Ckner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596.

70. Nilsson RH, Larsson K-H, Taylor AFS, Bengtsson-Palme J, Jeppesen TS, Schigel D, Kennedy P, Picard K, Gï Ockner 10 FO, Tedersoo L, Saar I, Abarenkov K. 2018. The UNITE database for molecular identification of fungi: handling dark taxa and parallel

taxonomic classifications. Nucleic Acid Res 47:D259–D264.

71. Paulson JN, Pop M, Bravo HC. 2013. metagenomeSeq: Statistical analysis for sparse high-throughput sequencing. Bioconductor Packag 1:191.

72. Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG. 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol 20:241–248.

73. Einen J, Thorseth IH, Øvreås L, Øvreås Ø. 2008. Enumeration of Archaea and Bacteria in seafoor basalt using real-time quantitative PCR and fuorescence microscopy. FEMS Microbiol Lett 282:182–187.

74. Ortiz M, Legatzki A, Neilson JW, Fryslie B, Nelson WM, Wing RA, Soderlund CA, Pryor BM, Maier RM. 2013. Making a living while starving in the dark: metagenomic insights into the energy dynamics of a carbonate cave. ISME J 8:478–491.

75. Delgado-Baquerizo M, Maestre FT, Eldridge DJ, Singh BK. 2016. Microsite differentiation drives the abundance of soil ammonia oxidizing bacteria along aridity gradients. Front Microbiol 7:505.

76. Satterthwaite FE. 1941. Synthesis of variance. Psychometrika 6:309–316.

77. Nakagawa S, Schielzeth H. 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods Ecol Evol 4:133–142.

78. Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46.

TABLE 1: Abiotic and biotic differences between canopy and gap soil microsites. Results of linear mixed-effects models are shown. Microsite and geolocation were used as fixed and random effects, respectively. A positive number in the canopy and gap differences column (difference in average canopy and gap values) represents a higher value in canopy soils and a negative number denotes higher value in gap soils. Significant *p-values* are represented as * $p<0.05$; ** $p<0.01$; *** $p<0.001$, and ns, not significant. The marginal R^2 represents only the variance of the microsite. The conditional R^2 takes both the microsite and geolocation into account. The variables are ranked based on their decreasing marginal R^2 values.

Abiotic variables[a]	**Canopy and Gap differences**	**Significance**	**Marginal R^2**	**Conditional R^2**
DN (μg g^{-1})	20.80	***	0.51	0.65
DIC (μg g^{-1})	24	***	0.50	0.73
Ammonium (μg g^{-1})	1.7	***	0.46	0.60
TN (μg g^{-1})	154.3	***	0.43	0.74
BAP (μg g^{-1})	3.5	***	0.42	0.43
DOC (μg g^{-1})	94.2	***	0.31	0.84
Nitrate (μg g^{-1})	10.8	**	0.30	0.41
EC (μs cm^{-1})	153	**	0.26	0.58
TOC (%)	0.24	*	0.22	0.22
TC (μg g^{-1})	1372.5	**	0.14	0.75
pH	-0.15	ns	0.07	0.49
Sand (%)	-1.4	ns	0.01	0.82
Silt (%)	1.6	ns	0.01	0.78
Clay (%)	-0.13	ns	0.00	0.18
Biotic variables	**Canopy and Gap differences**	**Significance**	**Marginal R^2**	**Conditional R^2**
ITS Richness	131.7	***	0.58	0.63
16S Richness	261.3	*	0.20	0.21
***ureC* gene abundance (copy # g^{-1} soil)**	0.23	***	0.38	0.49
Urease enzyme activity (μg NH_4-N g^{-1} h^{-1})	5.4	**	0.31	0.49
16S rRNA gene abundance (copy # g^{-1} soil)	0.18	**	0.18	0.56
Fungal Functional Guild				
Epiphyte	0.0001	***	0.89	0.98
Endophyte	0.02	***	0.71	0.71

Plant Pathogen	0.09	***	0.68	0.73
Wood Saprotroph	0.01	**	0.57	0.57
Ericoid Mycorrhizal fungi	0.002	*	0.44	0.44
Fungal Parasite	0.001	**	0.44	0.44
Ectomycorrhizal fungi	0.003	*	0.40	0.40
Arbuscular Mycorrhizal fungi	-0.02	*	0.38	0.40
Soil Saprotroph	0.001	-	0.05	0.05

[a]DN=dissolved nitrogen, DIC=dissolved inorganic carbon, TN=total nitrogen, BAP=bioavailable phosphorus, DOC=dissolved organic carbon, EC=electrical conductivity, TOC=total organic carbon, TC=total carbon

FIG 1: Sampling design in this study. A) map of the Sonoran, Mojave, and Chihuahuan Deserts in North America; B) map of the Sonoran Desert sampling area in the state of Arizona; C) aridity index map showing the soil sampling sites; and D) field sampling layout. Along each transect, a composite sample of canopy (C) and gap (G) soils was collected by combining three canopy and three gap samples.

FIG 2: Richness and non-metric dimensional scaling (NMDS) ordination plots for the microbial community. A) observed number of bacterial/archaeal amplicon sequence variants (ASV) or phylotypes; B) ordination plot for bacterial/archaeal community composition; C) observed number of fungal ASVs (or phylotypes); and D) ordination plot for fungal community composition. Richness is depicted as the mean value and standard deviation.

FIG 3: Unique and shared ASVs across the canopy and gap soil microsites. A) venn-diagram represents unique and shared bacterial/archaeal amplicon sequence variants (ASVs) in the microsites; B) number of unique and shared bacterial/archaeal ASVs in the microsites at site level; C) relative abundance of canopy soil bacterial/archaeal ASVs; D) venn-diagram represents unique and shared fungal ASVs in the microsites; E) number of unique and shared fungal ASVs in the microsites at site level; and F) relative abundance of canopy soil fungal ASVs. The relative abundance values of unique and shared bacterial/archaeal and fungal ASVs (at class level) across the microsites are represented in Tables S3-6.

FIG 4: Predicted fungal guilds in Sonoran Desert soil samples: A) arbuscular mycorrhizal fungi, B) plant pathogen, and C) wood saprotroph. Proportions are depicted as the mean value and standard deviation.

FIG 5: **Gene abundance in Sonoran Desert soil samples.** A) 16S rRNA gene and B) *ureC*. Copy numbers were calculated per gram of dry soil and are depicted as the mean value and standard deviation.