

BUFFELGRASS EFFECTS ABOVE AND BELOWGROUND IN THE SONORAN DESERT

by

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***Abstract***

The Sonoran Desert has highly diverse environmental characteristics that produce an extraordinary variety of endemic plant species as well as shaping interactions between them. One crucial interaction in this ecosystem is the dynamic of nurse species, often observed between dominant trees and other native species. Buffelgrass (*Cenchrus ciliaris*), introduced for cattle production, has become one of the most detrimental invaders in the Sonoran Desert, threatening the dynamic of nurse species since it is known to begin its invasion process in desertscrub under the canopy of trees. This field study investigates the impact of buffelgrass invasion on native vegetation and soil microbial communities under dominant tree species canopies in the Sonoran Desert. Vegetation and soil were sampled from two adjacent sites situated in the Plains of Sonora subdivision of the Sonoran Desert in the core region of buffelgrass invasion in the state of Sonora, Mexico. One of the sites underwent significant transformation, forming a buffelgrass pasture, while the other site retained its native desertscrub vegetation with buffelgrass patches resulting from the invasion process. The results of the vegetation analysis show that site is the primary factor that determines the vegetation richness. In contrast to the vegetation results, the results of the soil analyses indicate that cover type is the primary factor determining the richness and composition of soil microbial communities. Overall, our results provide insights of the above and belowground effects of buffelgrass invasion which can inform management and restoration strategies in uninvaded and invaded desertscrub in the Sonoran Desert.

## ***Chapter 1: Buffelgrass Invasion and Tree Species Identity Affect Plant Composition of Desert Diversity Islands***

### **1.1 Abstract**

The Sonoran Desert, characterized by extreme environmental conditions, hosts a diverse array of endemic plant species and interactions between them. However, anthropogenic disturbances such as invasion by introduced species, threaten this biodiversity and their interactions. One crucial interaction in this ecosystem is the nurse – protégé/seedling interaction, often observed between dominant trees species such as *Parkinsonia spp.* (palo verde), *Prosopis spp.* (mesquite), and *Olneya tesota* (ironwood) and other native plant species. *Cenchrus ciliaris* (buffelgrass), one of the most detrimental invasive species in the Sonoran Desert, tends to invade desertscrub starting from under the canopy of nurse species. This field study investigates the impact of buffelgrass invasion on native plant diversity, richness, and composition under nurse species canopies in the Sonoran Desert. Vegetation sampling was conducted at a site that had undergone significant transformation, forming a buffelgrass pasture and a nearby site that has retained its native desertscrub vegetation with buffelgrass patches. The results show that the transformed site had higher species richness potentially due to the higher proportion of invasives and ruderals. Buffelgrass likely promotes the establishment of other invasives in the transformed site, and buffelgrass presence could be making the conditions under nurse species no longer favorable for annual native grasses like six-weeks grama and for cacti in the transformed site.

### **1.2 Introduction**

The Sonoran Desert, shared by Mexico and the United States, has highly diverse environmental characteristics including high temperatures, low precipitation, and high variance in both. These produce an extraordinary variety of endemic plant species as well as interactions between them (Búrquez & Martínez-Yrizar, 1997). Logging, land clearing, and invasion of introduced species

are some of the potential threats that put in danger this diversity and their interactions (Búrquez & Martínez-Yrizar, 1997). A common interaction between plants in the Sonoran Desert is the nurse – protégé/seedling interaction (Flores & Jurado, 2003; K. A. Franklin et al., 2016). The dynamic of nurse species is common in limited resources systems (Filazzola & Lortie, 2014). A classic example in the Sonoran Desert is the interaction of dominant trees and *Carnegiea gigantea* (saguaro cactus) (Turner et al., 1966). Saguaro seedlings are frequently found growing under the canopy of *Parkinsonia spp.* (palo verde), *Prosopis spp.* (mesquite), and *Olneya tesota* (ironwood) trees (Turner et al., 1966). Most of the important nurse species in the Sonoran Desert are trees, specifically legume trees (K. A. Franklin et al., 2016). Trees and shrubs create conditions under their canopies that ameliorate stressful conditions such as excessive sun exposure, cold stress, trampling, and herbivory that seedlings and plants otherwise would experience outside their canopies (Flores & Jurado, 2003; Franco & Nobel, 1989; Shreve, 1931). Trees in the Fabaceae plant family are also known to have mutualistic associations with bacteria and fungi that enrich in nitrogen the soils under their canopy. These conditions are beneficial for the establishment of both native and invasive species (K. A. Franklin et al., 2016). Sometimes, they even more strongly facilitate the establishment of invasive species (Lucero et al., 2019). Not only columnar cacti find adequate conditions for recruitment and prosper under trees, but many species of shrubs and herbs concentrate forming “islands of diversity” (Búrquez & Quintana, 1994; Tewksbury & Lloyd, 2001).

*Cenchrus ciliaris* (buffelgrass), an invasive C<sub>4</sub> perennial grass considered to be one of the most detrimental to the Sonoran Desert, has been observed to begin its invasion in undisturbed desertscrub by establishing underneath the canopy of trees (Brenner & Kanda, 2013; Farrell & Gornish, 2019; Lyons et al., 2013). This African graminoid was introduced in the early

nineteenth century to Arizona and later in the century to Sonora to be used as forage for cattle (Búrquez-Montijo et al., 2002; Marshall et al., 2012). Today, this species is listed as a noxious weed by the Arizona Department of Agriculture, while in Sonora, there have been efforts to spread awareness, but it is still being harvested, sold, and widely used by ranchers to increase the grass cover of the range. At the turn of the century, approximately 1.6 million hectares of desertscrub in Sonora were reportedly been converted into pastures for buffelgrass (Búrquez-Montijo et al., 2002). This acreage has steadily increased in the last 20 years. There is limited research on how the presence of buffelgrass underneath dominant legume trees' canopies can modify native plant species diversity and composition and alter the community dynamics of natural and transformed desertscrub in the Sonoran Desert. Understanding plant composition under nurse species canopies invaded with buffelgrass can help further our understanding of the impacts of buffelgrass invasion and inform more effective management and restoration strategies in uninvaded and invaded desertscrub.

In this study, we sampled vegetation from two adjacent sites situated within the Plains of Sonora subdivision of the Sonoran Desert. One of the sites had undergone significant transformation, forming a buffelgrass pasture, while the other site retained its native desertscrub vegetation with naturally occurring buffelgrass patches resulting from the invasive process. Our research questions were: (1) Does the plant richness and diversity differ between sites and plant cover type (i.e., under the canopy of mesquite, palo verde, and ironwood trees, and in open areas with buffelgrass and open areas with native species)? and (2) How is the plant composition different between sites and plant cover types?

### **1.3 Materials & Methods**

#### **1.3.1 Study Site**

The two sites selected for the study are located within Patronato del Centro de Investigaciones Pecuarias del Estado de Sonora (PATROCIPES) land near the town of Carbó, approximately 55 km north of Hermosillo, the state capital of Sonora, Mexico. One of the sites, referred to as the transformed site (29°34'36.90" N, 111° 3'37.97" W; elevation 494 m), underwent clearing to remove native species and trees and extensive seeding with buffelgrass in the early 1970s to be used as a pasture for cattle grazing, and, since then, has been managed as a grassland according to records of PATROCIPES (Búrquez-Montijo et al., 2002). The other site, referred to as the natural site (29°33'51.43" N, 111° 5'18.84"W; elevation 498 m), did not experience such transformation, however buffelgrass is naturally spreading predominantly under the canopy of trees and along desert drainages. According to the Instituto Nacional de Estadística y Geografía (INEGI), the prevailing soils are Regosols and Leptosols in a geological setting of Cretaceous-Tertiary granites, extrusive volcanic rocks, and Quaternary alluvium. According to data from the Servicio Meteorológico Nacional, the average annual temperature is 22.3°C, ranging from -4°C to 47°C, with an annual precipitation of 373 mm. Most of the rainfall occurs during the monsoon season from July to September, with additional rainfall in the winter-spring period. The vegetation is an arbosuffrutescent community typical of the Plains of Sonora subdivision of the Sonoran Desert. Trees and shrubs like *Prosopis velutina* (mesquite), *Olneya tesota* (ironwood), *Parkinsonia praecox* (palo verde), *Encelia farinosa* (brittlebush), and *Mimosa distachya* (Mexican mimosa) are dominant, along with cacti like *Lophocereus schottii* (senita) and *Stenocereus thurberi* (organ pipe). Besides *Cenchrus ciliaris* (buffelgrass), the dominant native grasses are *Bouteloua barbata* (six-weeks grama), *Bouteloua aristidoides* (needle grama), and *Setaria macrostachya* (plains bristle grass).

### 1.3.2 Plant Sampling

Plant sampling was conducted at the end of the monsoon season in late September of 2021. At both sites, we recorded plant species abundance from eight plant cover types: 1) under the canopy of mesquite trees (*Prosopis velutina*) with and 2) without buffelgrass, 3) under ironwood trees (*Olneya tesota*) with and 4) without buffelgrass, 5) under palo verde trees (*Parkinsonia praecox*) with and 6) without buffelgrass, 7) in open areas with only buffelgrass, and 8) in open areas with native annual grasses. Within each site, we selected adult, fully grown trees of each of the studied species as well as open areas of equivalent mean area. Tree height was measured using a measuring stick to the nearest 10 cm. Two diameters perpendicular to each other were measured using an open reel metric tape measure. The canopy cover was estimated by using the circle area formula taking the average of the two diameter measurements. The transformed site, a buffelgrass dominated savannah, has no trees without buffelgrass under their canopy so we could not assess these cover types. Thus, a total of 20 samples (four cover types and five replicates) in the transformed site, and 35 in the natural site (seven cover types and five replicates) were used. Species abundance was measured in the field as the proportion of the area covered by each species, ranging from 1 (less than 5%) to 5 (covering more than 75% of the area).

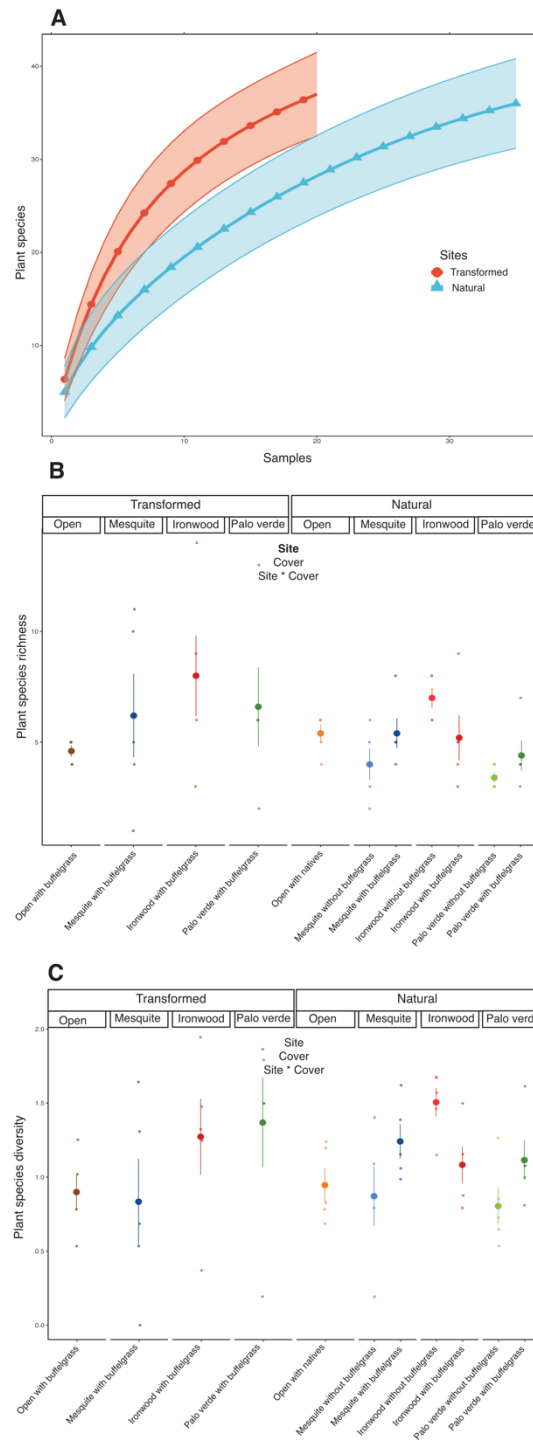
### 1.3.3 Statistical Analyses

Species accumulation curves were generated to compare the number of species per sample on each site. To estimate the effect of site and cover type on plant species richness and diversity (Shannon index) on each area sampled, we used linear models including the interaction between site and cover. Similarly, linear models including the interaction between site and cover were used for the proportion of invasive and ruderal species. The compositional changes were evaluated by Bray-Curtis dissimilarities and visualized using non-metric multidimensional scaling (NMDS) ordination plot. To assess the effect of site and cover type (and their interaction)

on compositional changes, we used permutational multivariate analysis of variance (PERMANOVA). We used the function `envfit` to assess the species driving the compositional patterns and function `betadisper` to assess the compositional variation between sites. Multivariate statistics were performed using the `vegan` package version 2.6-4 in R v. 4.2.2.

#### 1.4 Results

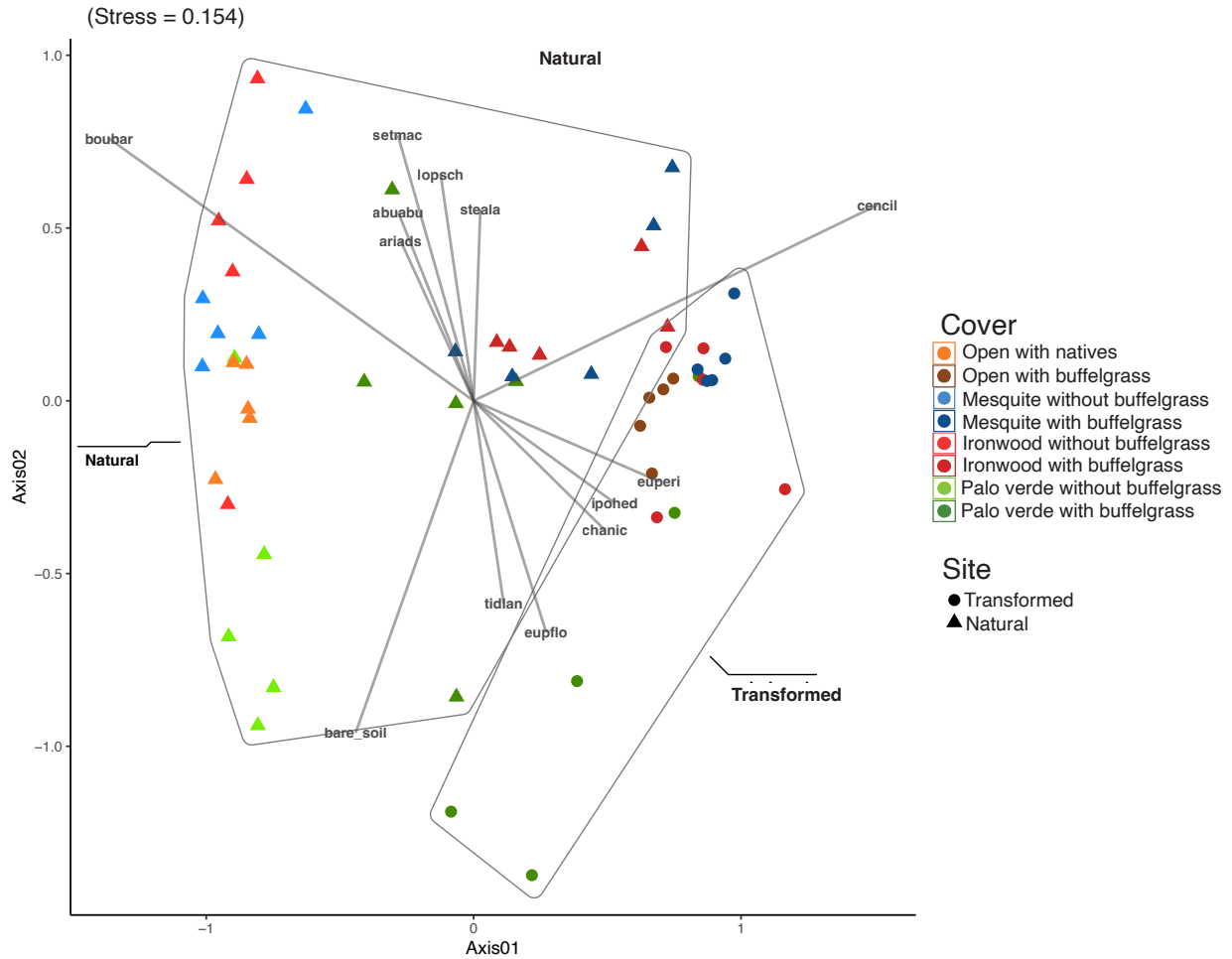
We observed a total of 52 plant species in the 55 samples. Samples collected in the transformed site had more plant species than the ones collected in the natural site (ANOVA,  $F = 4.11$ ,  $P = 0.049$ ; **Figure 1A, B**). Plant richness (i.e., number of different species) was not significantly different among cover types (ANOVA,  $F = 1.59$ ,  $P = 0.16$ ) nor in the interaction between cover and site (ANOVA,  $F = 0.45$ ,  $P = 0.64$ ) (**Figure 1B**). Plant diversity measured using the Shannon index showed a similar pattern to plant richness although it did not result in significant differences between sites, type covers, or the interaction of the two (ANOVA, Site:  $F = 0.01$ ,  $P = 0.91$ ; Cover:  $F = 1.76$ ,  $P = 0.12$ ; Site\*Cover:  $F = 1.92$ ,  $P = 0.16$ ) (**Figure 1C**).



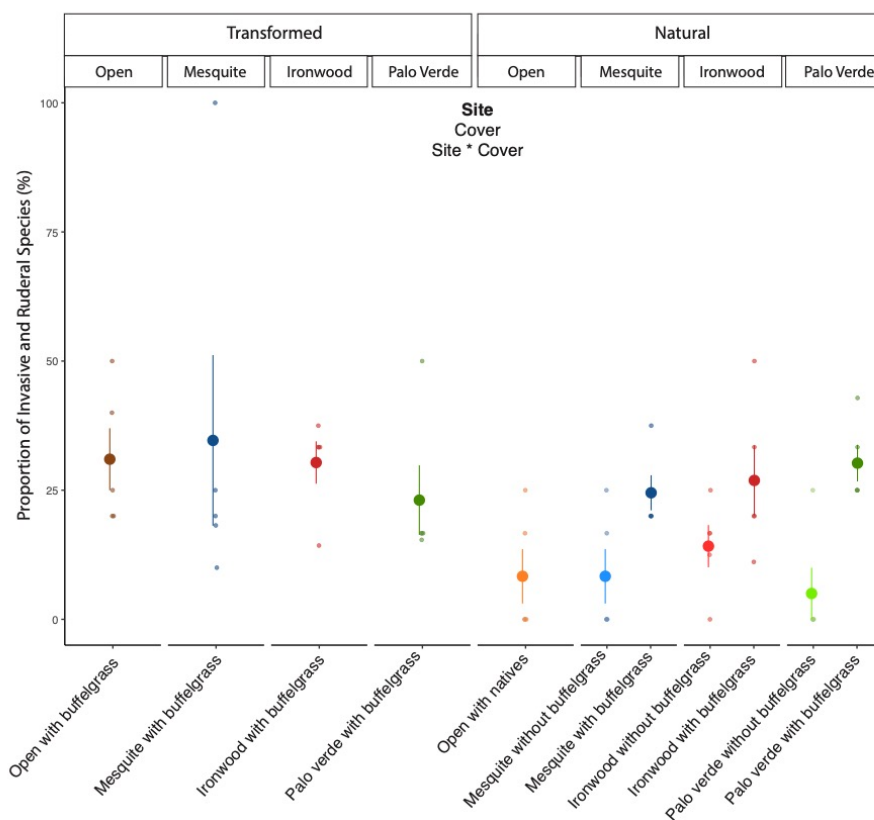
**Figure 1.** A) Species accumulation curves in the transformed and natural sites. Plant species richness (B) and diversity (C) across sites and cover types. Bold variables indicate significant results ( $P < 0.05$ ).

Plant composition (i.e., species present and their relative abundance in each area sampled) was significantly different across sites (PERMANOVA,  $F = 39.23$ ,  $P < 0.001$ ,  $R^2 = 0.299$ ) and covers (PERMANOVA,  $F = 6.35$ ,  $P < 0.001$ ,  $R^2 = 0.339$ ), but not for the interaction between the two variables (PERMANOVA,  $F = 1.69$ ,  $P = 0.12$ ,  $R^2 = 0.026$ ) (**Figure 2A**). Plant composition had a significant difference in variation between sites (Betadisper,  $P < 0.01$ ; **Figure 2**). The proportion of invasive and ruderal species per sample was significantly different between sites (ANOVA,  $F = 8.77$ ,  $P = 0.005$ ), with the transformed site having the highest proportion of invasive and ruderal species per sample (**Figure 3**). However, it was not significantly different between types of cover (ANOVA,  $F = 1.82$ ,  $P = 0.106$ ) nor in the interaction between site and cover (ANOVA,  $F = 0.78$ ,  $P = 0.466$ ).

The height, diameter, and cover of the trees that were sampled were not significantly different across sites (ANOVA, Height:  $F = 3.44$ ,  $P = 0.07$ ; Diameter:  $F = 0.60$ ,  $P = 0.44$ ; Cover:  $F = 0.42$ ,  $P = 0.52$ ), types of cover (ANOVA, Height:  $F = 1.39$ ,  $P = 0.25$ ; Diameter:  $F = 0.76$ ,  $P = 0.58$ ; Cover:  $F = 0.81$ ,  $P = 0.55$ ), nor in the interaction of the two (ANOVA, Height:  $F = 2.57$ ,  $P = 0.09$ ; Diameter:  $F = 0.40$ ,  $P = 0.67$ ; Cover:  $F = 0.31$ ,  $P = 0.73$ ) (**Table 1**).



**Figure 2.** Non-metric multidimensional scaling (NMDS) ordination plot of plant community dissimilarity with vectors representing observed plant species. Only the species that had significant correlation ( $P < 0.05$ ) were included as vectors. The plant species that had a significant correlation with the data visualized in the NMDS plot were abuabu - *Abutilon abutiloides*, ariads - *Aristida adscensionis*, boubar - *Bouteloua barbata*, cencil - *Cenchrus ciliaris*, chanic - *Chamaecrista nictitans*, euperi - *Euphorbia eriantha*, eupflo - *Euphorbia florida*, ipohed - *Ipomoea hederaceae*, lopsch - *Lophocereus schottii*, setmac - *Setaria macrostachya*, steala - *Stenocereus alamosensis*, and tidlan - *Tidestromia lanuginosa*.



**Figure 3.** Proportion of invasive and ruderal species across sites and cover types. Bold variables indicate significant results ( $P < 0.05$ ).

**Table 1.** Height, diameter, and cover of each tree sampled.

Sample Number	Site	Cover	Height (m)	Diameter (m)	Cover ( $\pi * r^2$ )
1	Transformed	Palo verde with buffelgrass	3.50	4.80	18.10
2	Transformed	Palo verde with buffelgrass	3.60	5.60	24.63
3	Transformed	Palo verde with buffelgrass	2.50	4.30	14.52
4	Transformed	Palo verde with	3.50	5.40	22.90

		buffelgrass			
5	Transformed	Palo verde with buffelgrass	6.00	9.80	75.43
1	Transformed	Mesquite with buffelgrass	7.00	5.50	23.76
2	Transformed	Mesquite with buffelgrass	4.50	7.50	44.18
3	Transformed	Mesquite with buffelgrass	4.50	5.30	22.06
4	Transformed	Mesquite with buffelgrass	4.50	5.50	23.76
5	Transformed	Mesquite with buffelgrass	5.50	6.80	36.32
1	Transformed	Ironwood with buffelgrass	4.50	8.00	50.27
2	Transformed	Ironwood with buffelgrass	4.00	5.50	23.76
3	Transformed	Ironwood with buffelgrass	4.50	6.50	33.18
4	Transformed	Ironwood with buffelgrass	4.50	6.70	35.26
5	Transformed	Ironwood with buffelgrass	4.50	6.30	31.17
1	Natural	Palo verde with buffelgrass	3.50	4.60	16.62
2	Natural	Palo verde with buffelgrass	3.00	4.50	15.90
3	Natural	Palo verde with buffelgrass	3.00	6.50	33.18
4	Natural	Palo verde with buffelgrass	5.00	7.10	39.59
5	Natural	Palo verde with buffelgrass	4.00	5.30	22.06
1	Natural	Mesquite with buffelgrass	3.50	6.00	28.27
2	Natural	Mesquite with buffelgrass	4.00	5.50	23.76
3	Natural	Mesquite with buffelgrass	4.00	6.90	37.39
4	Natural	Mesquite with	3.00	6.00	28.27

		buffelgrass			
5	Natural	Mesquite with buffelgrass	4.50	7.40	43.01
1	Natural	Ironwood with buffelgrass	4.50	5.40	22.90
2	Natural	Ironwood with buffelgrass	4.50	5.60	24.63
3	Natural	Ironwood with buffelgrass	5.00	6.00	28.27
4	Natural	Ironwood with buffelgrass	4.50	5.20	21.24
5	Natural	Ironwood with buffelgrass	4.50	6.30	31.17
1	Natural	Palo verde without buffelgrass	2.70	5.00	19.63
2	Natural	Palo verde without buffelgrass	3.00	5.00	19.63
3	Natural	Palo verde without buffelgrass	3.00	5.40	22.90
4	Natural	Palo verde without buffelgrass	4.50	7.40	43.01
5	Natural	Palo verde without buffelgrass	5.00	11.30	100.29
1	Natural	Mesquite without buffelgrass	3.20	4.50	15.90
2	Natural	Mesquite without buffelgrass	4.50	6.50	33.18
3	Natural	Mesquite without buffelgrass	4.50	4.70	17.35
4	Natural	Mesquite without buffelgrass	3.60	4.50	15.90
5	Natural	Mesquite without buffelgrass	3.50	6.80	36.32
1	Natural	Ironwood without buffelgrass	4.00	5.10	20.43

2	Natural	Ironwood without buffelgrass	3.50	4.30	14.52
3	Natural	Ironwood without buffelgrass	5.00	5.70	25.52
4	Natural	Ironwood without buffelgrass	3.50	5.30	22.06
5	Natural	Ironwood without buffelgrass	5.50	6.80	36.32

### 1.5 Discussion

Facilitation by nurse species is an interaction between plants that is frequently observed in systems with extreme climates, such as the Sonoran Desert (Bertness & Callaway, 1994). Nurse species are able to provide beneficial or/and ameliorate stressful conditions for other plant species (Flores & Jurado, 2003). This important interaction is being threatened by anthropogenic disturbances that affect the native vegetation of the Sonoran Desert (Búrquez & Martínez-Yrizar, 1997). In our field study, we observed that land transformation to an artificial grassland and the natural invasion of a non-native species do affect the plant community structure of desert scrub.

In contrast to a previous study that found that buffelgrass pastures had lower richness than native plant communities, our results show that species richness increased in the transformed site (K. Franklin & Molina-Freaner, 2010). The increase in richness in the transformed appears to be due to the presence of other invasive and ruderal species observed in the transformed site but not in the natural site. The presence of more invasive and ruderal species could be explained by the hypothesis of “invasional meltdown” (Cavieres, 2021), which states that non-native species are able to directly facilitate the establishment of other non-native species (Cavieres, 2021). Among the mechanisms that have been shown to support the facilitation

between non-native species is the modification of soil microbiota (Cavieres, 2021). It has been reported that samples from soil surrounding buffelgrass roots have a different microbial composition than samples collected from uninvaded soil (Gornish et al., 2020). Another reason that could explain why invasives and ruderal species were more abundant in the transformed site is the clearing of native species the site went through in order to transform it into a buffelgrass pasture (Búrquez-Montijo et al., 2002). It is known that disturbance reduces the competition of native species, which increases resource availability and favors the establishment of opportunistic species like invasives and ruderals (Pearson et al., 2023).

Our results showed that the composition of plant species observed in each of the sampled areas differed between sites and covers. The presence of buffelgrass seemed to reduce the presence of six-weeks grama as six-weeks grama was very abundant in the natural site. This is interesting since a previous greenhouse study showed that buffelgrass developed the greatest shoot biomass when growing with grasses with drought-escaping traits such as six-weeks grama (Farrell et al., 2022), suggesting that buffelgrass tends to outcompete six-weeks grama. Additionally, the two cacti species, *Lophocereus schottii* and *Stenocereus alamosensis*, were more abundant in the natural site. This could be due to cattle trampling or a possible higher occurrence of fire in the transformed site compared to the natural site because buffelgrass forms a continuous fine fuel cover that supports fire, and cacti are highly susceptible to fire (Búrquez-Montijo et al., 2002; Hultine et al., 2023). This suggests that buffelgrass presence could be the reason driving the low abundance of cacti in the transformed site, as a previous field study reported that buffelgrass invasion reduced the abundance of another cactus species compared to uninvaded areas (Bracamonte et al., 2017).

The findings of our study showed that the transformed site had higher species richness, buffelgrass likely promotes the establishment of other invasives in the transformed site, and buffelgrass presence could be making the conditions under nurse species no longer favorable for annual native grasses like six-weeks grama and for cacti in the transformed site. Larger scale research is needed to confirm if these are patterns frequently observed in other areas of the Sonoran Desert to have substantial data that can inform policies and management across this whole system. Additionally, research on the potential belowground effects of buffelgrass could help to understand the patterns observed aboveground. Nevertheless, this study supports that buffelgrass has a detrimental effect on the structure of native plant communities in the Sonoran Desert.

## ***Chapter 2: Buffelgrass Invasion Effects on Soil Microbial Communities Under Dominant Trees in the Sonoran Desert***

### **2.1 Abstract**

Invasive species pose a significant threat to biodiversity, particularly in arid ecosystems like the Sonoran Desert, where extreme environmental conditions hinder rapid vegetation recovery. Buffelgrass (*Cenchrus ciliaris*), introduced for cattle production, has become one of the most detrimental invaders in the Sonoran Desert. This species is known to begin its invasion process in desert scrub under the canopy of trees. This study investigates how buffelgrass invasion affects soil microbial communities under native tree canopies in an artificial buffelgrass pasture and in a neighboring desert scrub naturally invaded by buffelgrass in Sonora, Mexico. Soil samples were collected from under the canopy of mesquite, ironwood, and palo verde trees, as well as open areas with different vegetation cover types. Physiochemical properties, micronutrients and microbial communities of the soil were analyzed. Results indicate that cover type is the primary factor that determines the richness and composition of soil microbial communities. Interestingly, our research suggests that desert trees could potentially mitigate the impact of buffelgrass on soil microbial communities, and palo verde trees might even have the potential to be used for the restoration of semi-arid ecosystems affected by buffelgrass.

### **2.2 Introduction**

Invasive species are considered one of the main threats to biodiversity worldwide (Bellard et al., 2022). Biodiversity in arid ecosystems is particularly vulnerable to the effects of invasive species due to the extreme environmental conditions which do not support rapid recovery of vegetation (Y. Zhang et al., 2023). In the Sonoran Desert, biological diversity is threatened by numerous non-native grass species (e.g., Lehmann's lovegrass, Johnsongrass, fountain grass, cheatgrass, red brome) capable of filling open gaps between native vegetation (Wilder et al., 2021).

Buffelgrass (*Cenchrus ciliaris*) is one of the most noxious and detrimental invasive species to the integrity of the Sonoran Desert (Farrell & Gornish, 2019; Olsson, Betancourt, Crimmins, et al., 2012; Yetman & Búrquez, 1994). This African perennial C<sub>4</sub> bunchgrass was introduced with the objective of optimizing cattle production (K. A. Franklin et al., 2006). However, buffelgrass has rapidly expanded outside of its planted range (Olsson, Betancourt, McClaran, et al., 2012), and has been a cause of concern due to its negative effects on the desert ecosystem (K. A. Franklin et al., 2006). This invasive species has significantly reduced native species' cover and richness (Farrell & Gornish, 2019; Lyons et al., 2013; Marshall et al., 2012; Tinoco-Ojanguren et al., 2013) by promoting the accumulation of a continuous fine-fuel loads between patches of vegetation that imposes a grass-fire cycle unsuited for native species, mainly fire-sensitive cacti and tree species (Búrquez-Montijo et al., 2002; Fusco et al., 2019; McDonald & McPherson, 2013; Olsson, Betancourt, Crimmins, et al., 2012).

Trees play a significant role in the ecosystem stability of the Sonoran Desert (K. A. Franklin et al., 2016; Virginia, 1990). By creating resource islands, trees support the recruitment and success of other species below their canopies (Darrouzet-Nardi et al., 2023; K. A. Franklin et al., 2016; Gornish et al., 2021). Certain tree species can provide more complete resource islands than others (Bashan & de-Bashan, 2010; K. A. Franklin et al., 2016). For example, leguminous trees like mesquite (*Prosopis velutina*) and ironwood (*Olneya tesota*), form symbiotic relationships with nitrogen-fixing bacteria (Búrquez & Quintana, 1994; K. A. Franklin et al., 2016; Rodríguez-Echeverría et al., 2016), while other legume trees like some palo verde trees (*Parkinsonia spp*), neither produce nodules (Rodríguez-Zaragoza et al., 2008; Waldon, 1987) nor show enhanced growth when inoculated with nitrogen-fixing, symbiotic bacteria (Álvarez & Pérez, 2018). Biologically fixed nitrogen is one of the few sources of nitrogen for other non-

nitrogen fixing species in the Sonoran Desert (K. A. Franklin et al., 2016; Lopez-Lozano et al., 2016). Additionally, the conditions below the canopy of leguminous trees create microhabitats that support the activity of soil microbial communities (Carrillo-Garcia et al., 1999). Among the active soil microorganisms, there are groups, such as the arbuscular-mycorrhizal fungi, which can alleviate plant drought stress and thus, are recognized as essential microorganisms for these arid ecosystems (Porter et al., 2020). However, the extensive resources of the fertile islands that leguminous trees provide can benefit both native and invasive species (Allen et al., 2011; Novoa et al., 2021).

Buffelgrass establishment in uninvaded desert scrub in the Sonoran Desert tends to begin under a canopy of trees and shrubs (Lyons et al., 2013). It is known that buffelgrass can impact mature trees and shrubs through resource competition (Eilts & Huxman, 2013). The findings of a field study suggested that soil invaded with buffelgrass has a distinct soil microbiome characterized by a higher amount of nitrifiers, fungal symbionts, and methanotrophs compared to uninvaded soil (Gornish et al., 2020). However, there is a lack of knowledge about if and how buffelgrass could modify soil microbial communities under the canopy of native leguminous trees, and if that directly or indirectly favors its invasion success in the Sonoran Desert. Invasive species are capable of modifying the soil microbial communities in a variety of ways that impact surrounding vegetation and enhance their invasion (Wolfe & Klironomos, 2005). For example, there are invasive species that reduce the occurrence of soil microorganisms that are beneficial to native species by altering the soil physiochemical properties through allelopathic exudates (Qu et al., 2021). Alternatively, there are invasive species that can promote the presence of local pathogens detrimental to native species (Eppinga et al., 2006). The ecosystem-level impacts of buffelgrass invasion could be better understood by exploring the interactions of buffelgrass with

native tree species and soil microbial communities since these interactions regulate and support multiple ecosystem services in the Sonoran Desert.

In this field study, we sampled soils from two neighboring sites located in the core region of buffelgrass invasion in the state of Sonora, Mexico (Búrquez-Montijo et al., 2002). One of the sites was extensively transformed creating a buffelgrass-dominated savannah, and the other site is a native desert scrub with natural (i.e., not purposely seeded) buffelgrass patches. Our research questions were: (1) Does soil microbial richness and composition differ between the open areas in transformed and natural sites? (2) Does soil microbial richness and composition differ among desert trees with and without buffelgrass under their canopy in each site? and (3) Does the abundance of particular microbial functional groups differ between sites and the type of plant cover?

## 2.3 Materials & Methods

### 2.3.1 Study Site

We selected two sites (29° 34' 12'' N, 111° 04' 38'' W; elevation 497 m) on the land of Patronato del Centro de Investigaciones Pecuarias del Estado de Sonora (PATROCIPES) located near the town of Carbó (29°41'05'' N, 110°57'16'' W; elevation 462 m), approximately 55 km north of Hermosillo, the state capital of Sonora, Mexico. The dominant soils are Regosols and Leptosols in a geological setting of Cretaceous-Tertiary granites, extrusive volcanic rocks, and Quaternary alluvium (INEGI, [https://www.inegi.org.mx/contenidos/productos/prod\\_serv/contenidos/espanol/bvinegi/productos/geografia/tematicas/Geologia\\_hist/1\\_250\\_000/702825675516.pdf](https://www.inegi.org.mx/contenidos/productos/prod_serv/contenidos/espanol/bvinegi/productos/geografia/tematicas/Geologia_hist/1_250_000/702825675516.pdf)), According to the Servicio Meteorológico Nacional, ([https://smn.conagua.gob.mx/tools/RECURSOS/Normales\\_Climatologicas/Normales9120/son/](https://smn.conagua.gob.mx/tools/RECURSOS/Normales_Climatologicas/Normales9120/son/)

[nor9120\\_26016.TXT](#)), the mean annual temperature is 22.3°C (-4°C - 47°C), and the annual precipitation 373 mm primarily occurring from July to September during the monsoon season, and secondarily during the winter-spring season. On PATROCIPES records, one of the sites was cleared and extensively seeded with buffelgrass in the early 1970s to be used as a pasture for cattle grazing (Búrquez-Montijo et al., 2002; Lyons et al., 2013). The site, hereafter referred to as the transformed site, has been managed as a grassland since then. The other site, hereafter referred to as the natural site, has not suffered transformation, but buffelgrass is naturally spreading, mainly under the canopy of trees and along desert drainages. Disturbance favors buffelgrass establishment (Búrquez-Montijo et al., 2002), and natural colonization tends to start along roads, and streams (Búrquez-Montijo et al., 2002). Eventually, it moves into the desertscrub via seeds that accumulate in surface litter and under trees (Búrquez-Montijo et al., 2002). The dominant trees and shrubs are *Prosopis velutina* (mesquite), *Olneya tesota* (ironwood), *Parkinsonia praecox* (palo verde), *Encelia farinosa* (brittlebush), and *Mimosa distachya* (Mexican mimosa). The dominant cacti include *Lophocereus schottii* (senita) and *Stenocereus thurberi* (organ pipe). Besides *Cenchrus ciliaris* (buffelgrass), the dominant grasses are *Bouteloua barbata* (six-weeks grama), *Bouteloua aristidoides* (needle grama), and *Setaria macrostachya* (plains bristle grass).

### 2.3.2 Soil Sampling

Soils were collected at the end of the monsoon season in late September of 2021. In both sites, we aimed to collect soil samples from nine environmental conditions representing specific types of cover: 1) under the canopy of mesquite (*Prosopis velutina*) with and 2) without buffelgrass, 3) under ironwood (*Olneya tesota*) with and 4) without buffelgrass, 5) under palo verde trees (*Parkinsonia praecox*) with and 6) without buffelgrass, 7) in open areas more than 5 m from any

tree or shrub, 8) in open areas with only buffelgrass, and 9) in open areas with native annual grasses. Five replicate samples were taken from each condition. As the transformed site, a buffelgrass dominated savannah, has no trees without buffelgrass under their canopy, we could not assess these cover types. Thus, a total of 30 soil samples (six cover types and five replicates) in the transformed site, and 45 in the natural site (nine cover types and five replicates) were processed for molecular analyses. Soil samples for these analyses were collected near the plant roots with a shovel and were placed in a sterile Whirl-Pack plastic bag. Soil bags were kept on ice and immediately stored in a -80 °C freezer.

### 2.3.3 Soil Physicochemical Properties and Micronutrients Analyses

To ensure the differences between sites were due to the type of buffelgrass invasion (cover types), soil physicochemical properties and micronutrient analyses were performed on soil subsamples from the samples collected for molecular analyses. These were first passed through a 2 mm sieve and left to air dry overnight. Soil moisture content was determined by subtracting the weight of the soil after it was oven-dried from its initial weight. Soil pH was measured by mixing soil with deionized water at a 1:1 weight/volume ratio and using a FiveEasy Plus pH meter (Mettler Toledo, Columbus, OH, USA). Soil electroconductivity (EC) was measured by combining soil with deionized water at a 1:5 weight/volume ratio and utilizing a FiveEasy Plus pH meter equipped with a Cond probe LE703 (Mettler Toledo, Columbus, OH, USA). To assess total carbon (C) and nitrogen (N), samples were dried in an oven at 105 °C overnight, manually ground with a mortar, and weighed at 1 g for thermal combustion analysis using a Vario MAX Cube instrument (Elementar, Langenselbold, DE). Micronutrient concentrations including iron (Fe), copper (Cu), potassium (K), magnesium (Mg), calcium (Ca), phosphorous (P), manganese (Mn), zinc (Zn), and sulfur (S) were determined using a 3 g soil sample mixed with 30 mL of

Menlich III solution in a 50 mL polyethylene bottle. The mixture was shaken for 10 minutes on a reciprocating shaker at 180 cycles per minute, then filtered through quantitative filter paper. The filtrate was analyzed for the target elements using an iCAP 7200 inductively coupled plasma-optical emission spectroscopy (ICP-OES) Duo instrument (Thermo Scientific, Waltham, MA).

#### 2.2.4 Microbial Molecular Analyses

We employed the DNeasy PowerLyzer PowerSoil kit (Qiagen, Hilden, Germany) to extract total genomic DNA following the guidelines provided by the manufacturer. To assess bacterial/archaeal communities, we performed PCR amplification of the V4 hypervariable region of 16S rRNA gene using the primer sets 515-F (GTGCCAGCMGCCGCGGTAA) and 806-R (GGACTACHVGGGTWTCTAAT) (Walters et al., 2015). For fungal communities, PCR amplification targeted the first internal transcriber region (ITS1) using the primers ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGCGTTCTTCATCGATGC) (Walters et al., 2015). The primers included Illumina adapters, and the reverse primer pairs included unique error-correcting 12-base-pair barcode specific to each sample for demultiplexing. Each sample underwent triplicate PCR reactions in a 40  $\mu$ l volume, consisting of 3  $\mu$ l of extracted DNA, 3  $\mu$ l of each primer, 20  $\mu$ l of MyFi PCR Mix (Bioline, Taunton, MA, USA), and 11  $\mu$ l of water. PCR conditions involved an initial denaturation step at 95 °C for 1 minute, followed by 35 cycles of amplification (95 °C for 15 seconds, 60 °C for 15 seconds, and 72 °C for 15 seconds), and a final elongation step at 72 °C for 3 minutes. Negative controls were included to identify potential contamination. The PCR products were cleaned using the Ultra-Clean PCR Clean-Up kit (MoBio Laboratories, Carlsbad, CA, USA) and quantified with the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Waltham, MA, USA). The purified PCR products were then pooled in equimolar concentrations and sequenced on an Illumina MiSeq platform with 2x150 base pair reads. The

sequencing was performed at the PANDA Core for Genomics and Microbiome Research, University of Arizona.

### 2.3.5 Microbial Sequence Processing

Demultiplexing of the raw sequenced reads was performed using idemp, and subsequent processing was carried out using DADA2 (Callahan et al., 2016). To ensure uniformity, the length of the 16S amplicon sequences were trimmed. As the ITS region usually exhibits high variation in length, specific base pairs were removed from the reverse reads and primers were eliminated using cutadapt (Martin, 2011). The resulting filtered reads were employed to train the error model and infer amplicon sequence variants (ASVs). Paired-end reads were merged, and any chimera sequences were eliminated. Taxonomic identities were assigned to each ASV using the Ribosomal Database Project (RDP) classifier (Wang et al., 2007). The 16S rRNA sequences were annotated using the SILVA database (Quast et al., 2013), while the ITS sequences were annotated using the UNITE database (Nilsson et al., 2019). Any 16S ASVs that were not classified within the bacterial or archaeal domain or were classified as mitochondria or chloroplasts were removed. Similarly, ITS ASVs without a fungal domain assignment were discarded. The number of bacterial/archaeal sequences per sample ranged from 40,286 to 250,394, while fungal sequences per sample ranged from 27,640 to 122,154. Functional groups of Bacteria/Archaea were determined using FAPROTAX (Louca et al., 2016), and fungal guilds with a "highly probable" or "probable" confidence ranking were inferred using FUNGuild (Nguyen et al., 2016). Sequencing data have been deposited in NCBI Sequencing Read Archive under BioProject accession XXXXXX.

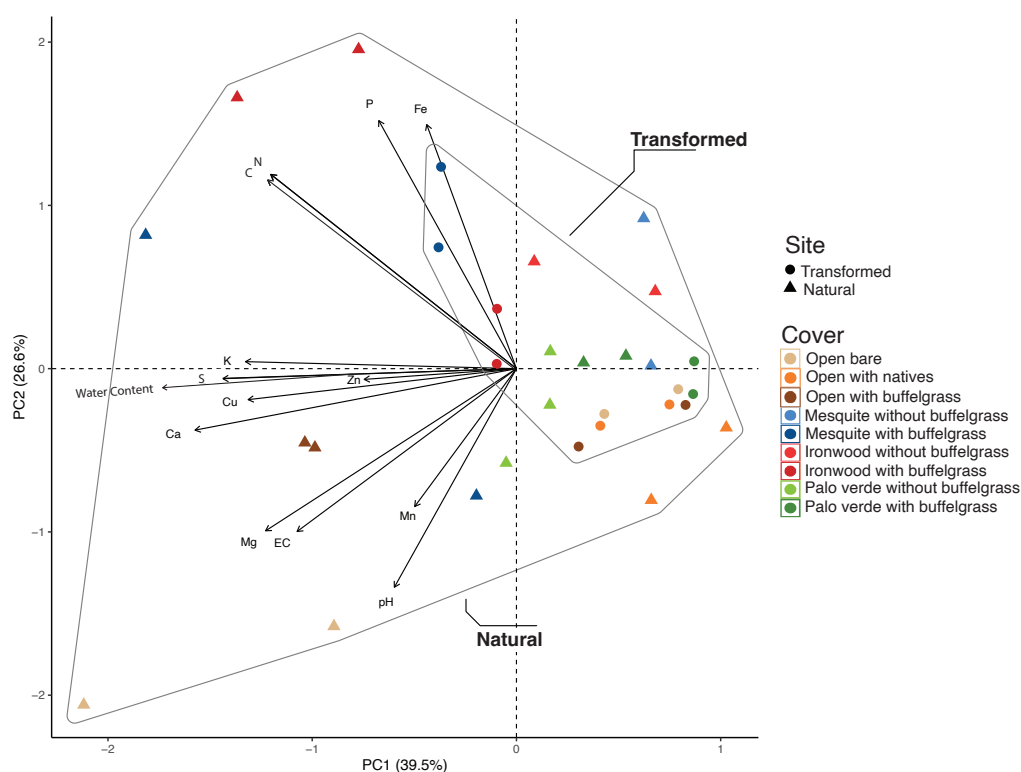
### 2.3.6 Statistical Analyses

The soil properties and micronutrients (pH, EC, water content, C, N, P, K, Fe, Cu, Mg, Ca, Mn, Zn, S) association to site (i.e. transformed and natural) and cover type (i.e., mesquite, ironwood, palo verde, bare ground, native grasses, and buffelgrass) were tested individually using linear models including the interaction between site and cover type (i.e., 2-way ANOVA), and also collectively using permutational multivariate analysis of variance (PERMANOVA) after calculating an Euclidean distance matrix. In addition, we assessed the relationships among soil properties and micronutrients using principal component analysis (PCA). To estimate the effect of site and cover type on microbial richness (number of different phylotypes) and diversity (measured using the Shannon index), we first rarefied the sequence counts for bacteria/archaea to 40,000 and for fungi to 20,000 to control for differences in sequencing depth. Then, we used linear models including the interaction between site and cover. The compositional changes of soil microbial communities were evaluated by Bray-Curtis dissimilarities and visualized using non-metric multidimensional scaling (NMDS). To assess the effect of site and cover type (and their interaction) on compositional changes, we used PERMANOVA. Multivariate statistics were performed using the vegan package version 2.6-4. Microbial functional group differences among sites and cover type were tested using generalized linear models with negative binomial error structures due to overdispersion and log link functions including the total number of sequences as offset in the MASS package version 7.3-58.4. R version 4.2.2 and RStudio version 2022.12.0+353 were used to perform all data analyses and visualizations.

## 2.4 Results

Overall, soil variables were different among sites, cover type, and their interaction, but the highest explanatory variable was cover type (**Fig. 1, Table 1**). In particular, all soil properties (water content, pH, EC, N and C) and most micronutrients, except for Cu and Zn, showed

significant differences among cover types (**Table 2**). Although soil variables were mostly different between the two sites (i.e., transformed and natural), there were some similarities in their physiochemical and micronutrient composition (**Fig. 1**). For instance, C and N concentration were not significantly different between sites (**Table 2**). Remarkably, soil samples from the transformed site clustered within the soils from the natural site in ordination space (**Fig. 1**).



**Figure 1.** Principal component analysis (PCA) ordination biplot of soil properties and micronutrients across sites and cover types. Arrows correspond to the loadings of each soil variable.

**Table 1.** Effects of site, cover type, and their interaction (Site \* Cover) on soil variables and soil microbial communities. F corresponds to F-statistic in the case of microbial richness, and pseudo-F in the case of soil variables (Euclidean distance) and microbial composition (Bray-

Curtis distance). Bold values indicate significant results ( $P < 0.05$ ). Partial  $R^2$  (i.e., sum of squares for the variable as a proportion of the total sum of squares) are shown for PERMANOVA results.

Response	Explanation	F	P	R <sup>2</sup>
Soil composition	Site	8.16	< <b>0.001</b>	0.097
	Cover	5.14	< <b>0.001</b>	0.48
	Site * Cover	3.74	< <b>0.001</b>	0.22
Bacterial/Archaeal richness	Site	1.90	0.173	0.35
	Cover	3.56	<b>0.002</b>	
	Site * Cover	0.15	0.978	
Bacterial/Archaeal composition	Site	7.10	< <b>0.001</b>	0.07
	Cover	3.19	< <b>0.001</b>	0.25
	Site * Cover	2.24	< <b>0.001</b>	0.11
Fungal richness	Site	0.02	0.890	0.66
	Cover	14.28	< <b>0.001</b>	
	Site * Cover	0.26	0.934	
Fungal composition	Site	3.97	< <b>0.001</b>	0.04
	Cover	3.20	< <b>0.001</b>	0.26
	Site * Cover	2.06	< <b>0.001</b>	0.10

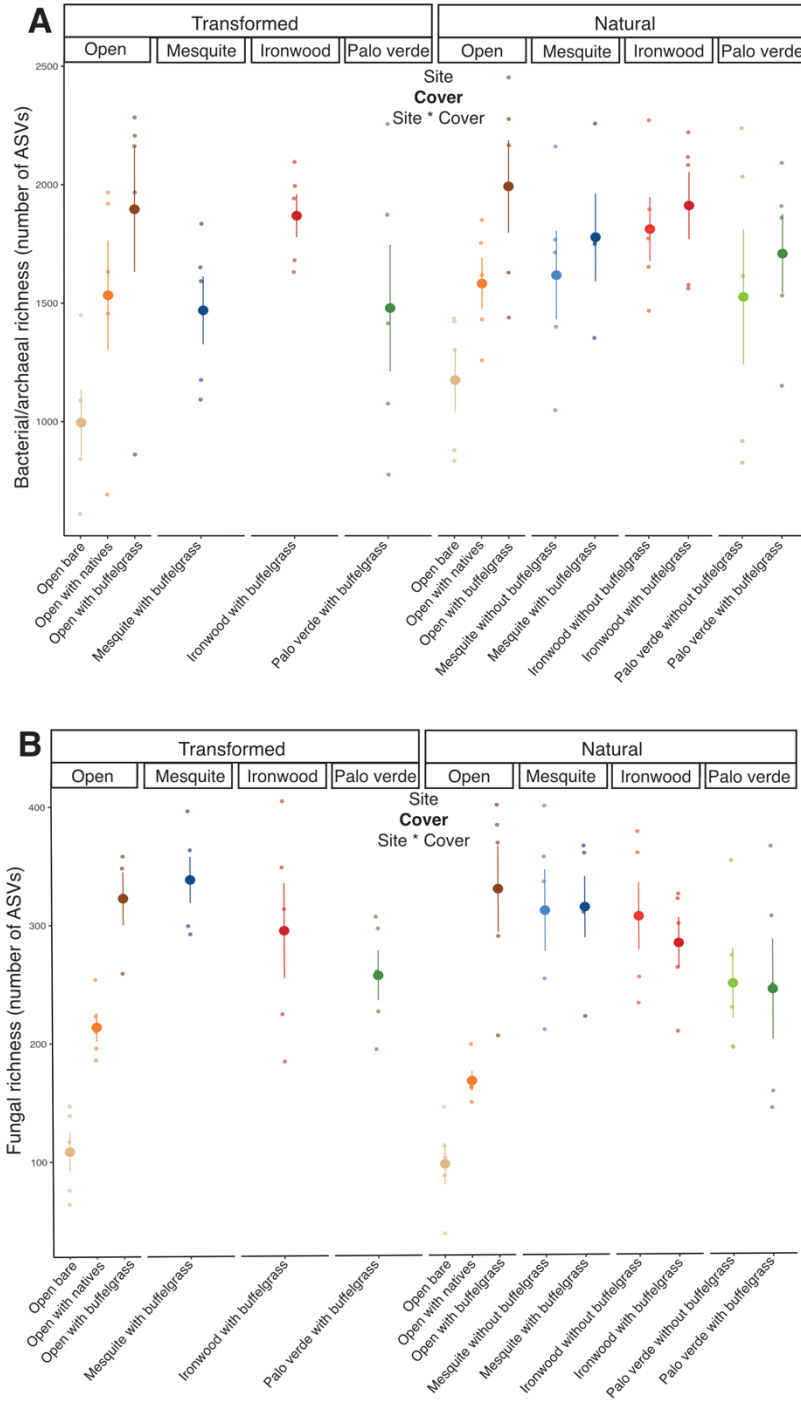
**Table 2.** Effects of site, cover, and their interaction (Site \* Cover) on soil properties and micronutrients. Bold values indicate significant results ( $P < 0.05$ ).

Response	Explanatory	F	P
Water Content (g)	Site	7.80	<b>0.013</b>
	Cover	3.91	<b>0.010</b>
	Site * Cover	2.41	0.082
pH	Site	12.22	<b>0.003</b>
	Cover	5.37	<b>0.002</b>
	Site * Cover	5.16	<b>0.005</b>
EC	Site	7.91	<b>0.013</b>
	Cover	9.86	< <b>0.001</b>
	Site * Cover	12.36	< <b>0.001</b>
N %	Site	2.99	0.103
	Cover	5.28	<b>0.002</b>
	Site * Cover	1.33	0.301

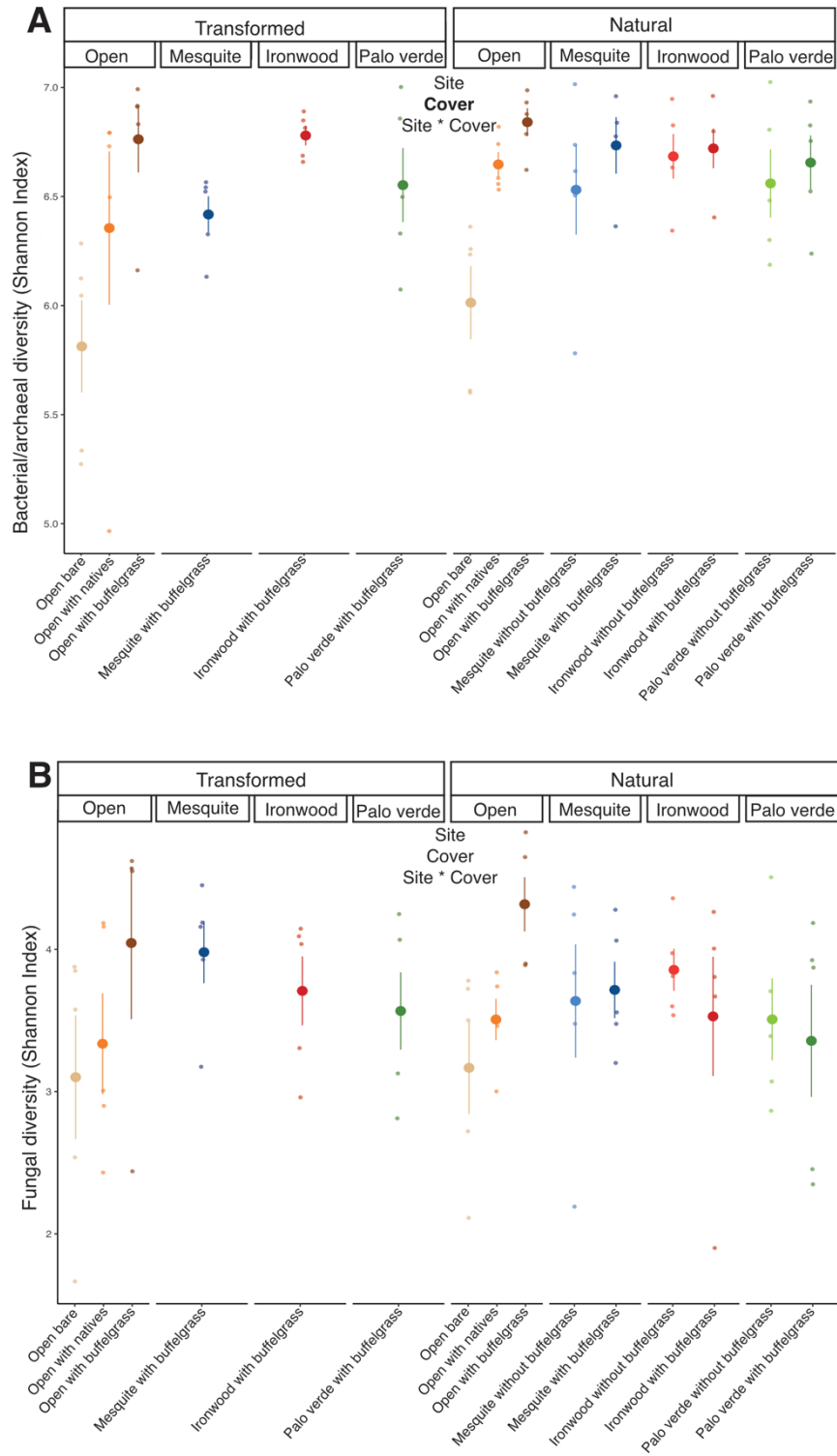
C %	Site	3.85	0.068
	Cover	5.99	<b>0.001</b>
	Site * Cover	1.84	0.162
Fe (ppm)	Site	0.43	0.519
	Cover	4.93	<b>0.003</b>
	Site * Cover	9.44	<b>&lt; 0.001</b>
Cu (ppm)	Site	1.44	0.247
	Cover	2.12	0.096
	Site * Cover	1.92	0.147
Zn (ppm)	Site	4.65	<b>0.047</b>
	Cover	1.49	0.237
	Site * Cover	1.92	0.147
K (ppm)	Site	0.01	0.943
	Cover	14.56	<b>&lt; 0.001</b>
	Site * Cover	11.95	<b>&lt; 0.001</b>
Mg (ppm)	Site	30.28	<b>&lt; 0.001</b>
	Cover	5.38	<b>0.002</b>
	Site * Cover	3.39	<b>0.028</b>
Ca (ppm)	Site	65.97	<b>&lt; 0.001</b>
	Cover	10.91	<b>&lt; 0.001</b>
	Site * Cover	6.36	<b>0.002</b>
P (ppm)	Site	5.28	<b>0.035</b>
	Cover	9.47	<b>&lt; 0.001</b>
	Site * Cover	4.12	<b>0.013</b>
Mn (ppm)	Site	5.98	<b>0.026</b>
	Cover	4.35	<b>0.006</b>
	Site * Cover	2.52	0.072
S (ppm)	Site	9.31	<b>0.008</b>
	Cover	8.11	<b>&lt; 0.001</b>
	Site * Cover	4.21	<b>0.012</b>

The total number of different bacterial/archaeal ASVs after rarefaction was 119,899. Bacterial/archaeal richness per sample ranged from 608 to 2,450. The total number of different fungal ASVs was 18,818. Fungal richness per sample ranged from 38 to 404. Bacterial/archaeal and fungal richness showed significant differences among cover types, but not sites or the

interaction between site and cover (**Table 1**). Among the soil samples collected from below the canopy of the different tree species (i.e., mesquite, ironwood, palo verde), soil from under ironwood had the highest bacterial richness (**Fig. 2A**) and soil from under mesquite the highest fungal richness (**Fig. 2B**). Meanwhile, soil from under palo verde trees showed the lowest bacterial and fungal richness (**Fig. 2**). Additionally, there were no significant differences in microbial richness between trees with buffelgrass and trees without buffelgrass in the natural site (**Fig. 2**). However, consistently trees with buffelgrass had a slightly higher bacterial richness than those without buffelgrass (**Fig. 2A**). There was a clear pattern among the soils collected from the open areas with different vegetation cover types that could be observed on both sites (**Fig. 2**). That is, both bacterial and fungal richness were highest in soils from open areas with buffelgrass, followed by open areas with native grasses, and then open areas with no vegetation cover (bare ground) (**Fig. 2**). Similar patterns were observed for Shannon diversity index (**Fig. 3**).



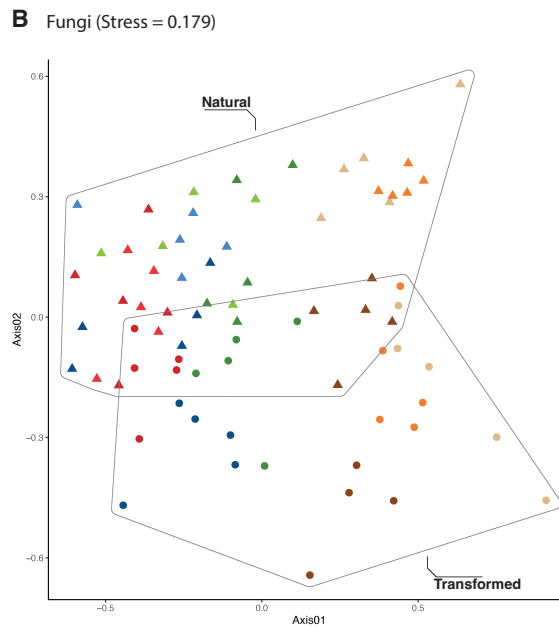
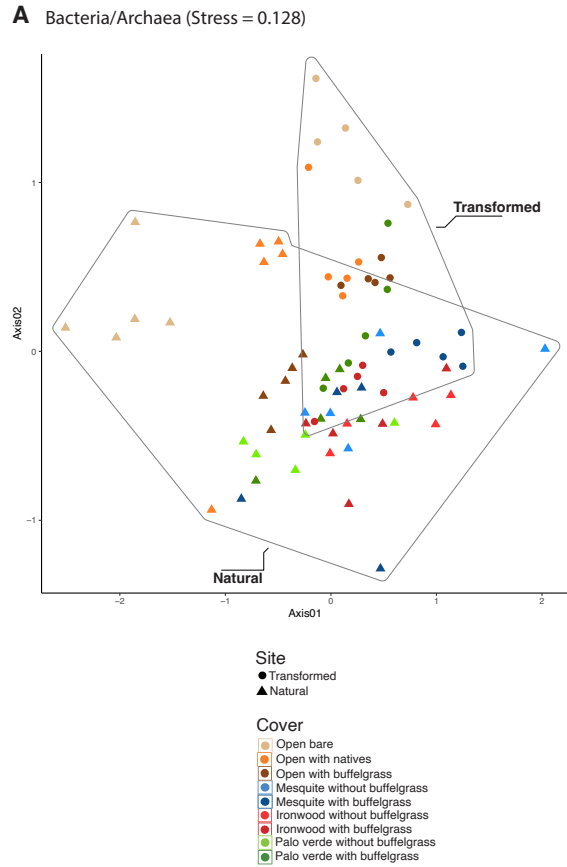
**Figure 2.** Soil bacterial/archaeal (A) and fungal (B) richness across sites and different cover types. Point ranges show means and standard errors. Bold variables indicate significant results ( $P < 0.05$ ) according to statistics in Table 1.



**Figure 3.** Soil bacterial/archaeal (A) and fungal (B) Shannon diversity across sites and different covers. Point ranges show means and standard errors. For bacterial/archaeal diversity ( $R^2 =$

0.43): Site:  $F = 3.12$ ,  $P = 0.083$ ; Cover:  $F = 5.01$ ,  $P = <0.001$ ; Site\*Cover:  $F = 0.39$ ,  $P = 0.853$ . For fungal diversity ( $R^2 = 0.21$ ): Site:  $F = 0.01$ ,  $P = 0.938$ ; Cover:  $F = 1.72$ ,  $P = 0.112$ ; Site\*Cover:  $F = 0.22$ ,  $P = 0.952$ . Bold values in figure indicate significant results ( $P < 0.05$ ).

Soil bacterial/archaeal communities were dominated by Actinobacteria (35.97%), Proteobacteria (23.94%), Acidobacteria (9.22%), Chloroflexi (8.28%), Firmicutes (6.83%), and Gemmatimonadetes (3.20%). Soil fungal communities were dominated by Ascomycota (79.57%), followed by Basidiomycota (12.40%), Mortierellomycota (6.61%), Glomeromycota (0.51%), Chytridiomycota (0.44%), and Rozellomycota (0.37%). Overall, soil bacterial/archaeal and fungal compositions were significantly different across sites and cover types, with significant interaction among variables. Even though, cover type was the variable with the highest explanatory power (**Table 1**). There was a clear microbial compositional difference between soils collected under the canopy of trees and soil collected from open cover type (**Fig. 4**). Additionally, there were large compositional differences between the soils collected from open bare areas in the transformed site and in the natural site (**Fig. 4**). As to the overall microbial composition, the abundance of soil microbial functional groups varied significantly across different cover types (**Table 3**). For example, soil samples from under palo verde trees had a higher relative abundance of nitrifiers (mainly genera *Nitrososphaera* and *Nitrospira*), especially those without buffelgrass invasion (**Fig. 5A; Table 3**). There were few functional groups in which abundance differed among sites. For example, in the transformed site the abundance of putative nitrogen fixing bacteria (mainly the genera *Azospirillum* and *Bradyrhizobium*) was generally higher than the natural site except for the samples under palo verde trees, which had more nitrogen fixing bacteria in the natural site (**Fig. 5B; Table 3**).

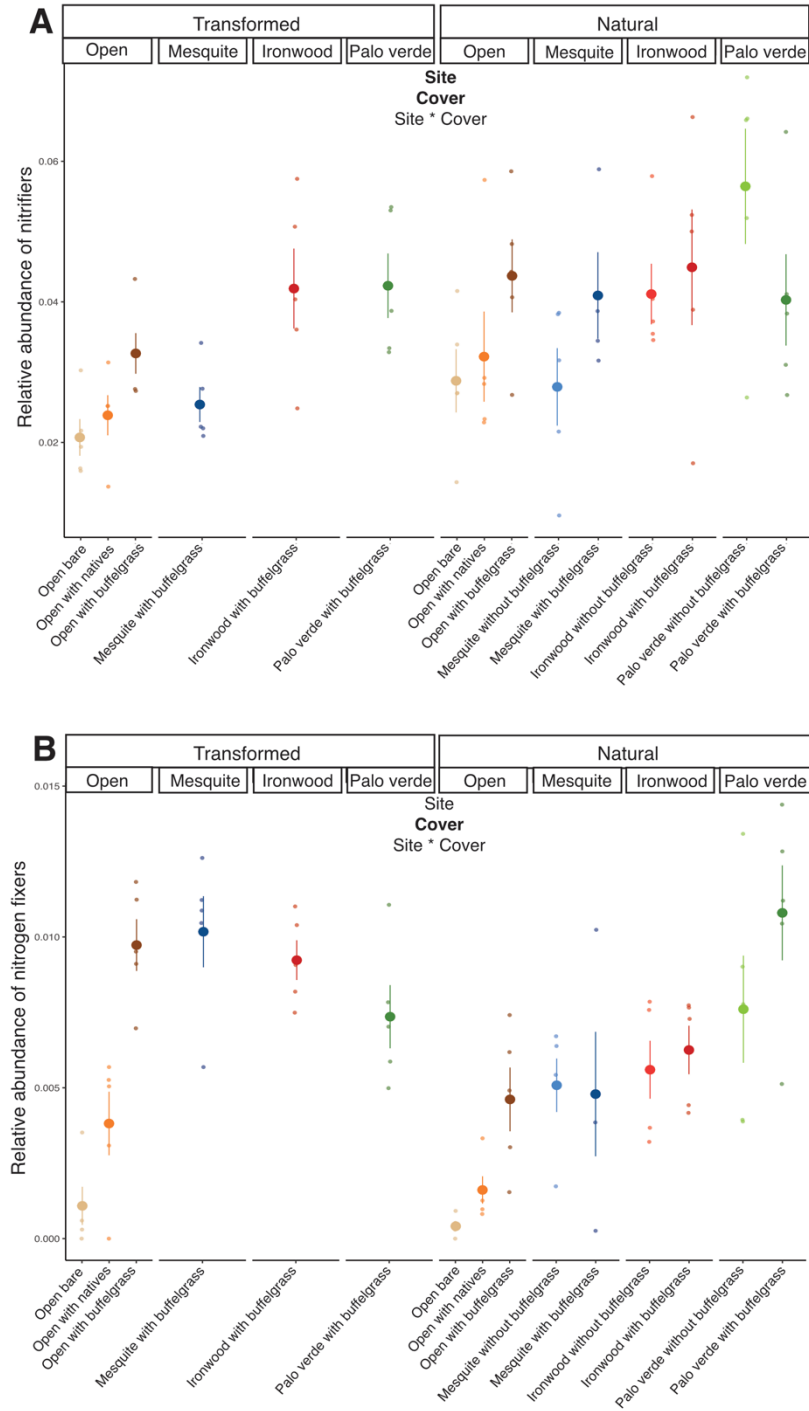


**Figure 4.** Non-metric multidimensional scaling (NMDS) ordination plot of soil bacterial/archaeal (A) and fungal (B) community dissimilarity.

**Table 3.** Effect of site, cover type, and their interaction (Site \* Cover) on the abundance of microbial functional groups. Bold values indicate significant results ( $P < 0.05$ ).

Response	Explanatory	X <sup>2</sup>	P	R <sup>2</sup>
Methylotrophs	Site	6.54	<b>0.011</b>	0.666
	Cover	39.65	<b>&lt; 0.001</b>	
	Site * Cover	15.33	<b>0.009</b>	
Nitrifiers	Site	11.33	<b>&lt; 0.001</b>	0.663
	Cover	43.91	<b>&lt; 0.001</b>	
	Site * Cover	4.97	0.419	
Denitrifiers	Site	3.45	0.063	0.623
	Cover	51.49	<b>&lt; 0.001</b>	
	Site * Cover	2.75	0.739	
Chitinolytic bacteria	Site	5.13	<b>0.024</b>	0.554
	Cover	28.29	<b>&lt; 0.001</b>	
	Site * Cover	11.59	<b>0.041</b>	
Nitrogen fixers	Site	3.21	0.073	0.773
	Cover	80.13	<b>&lt; 0.001</b>	
	Site * Cover	6.77	0.239	
Cellulolytic bacteria	Site	2.90	0.089	0.697
	Cover	44.92	<b>&lt; 0.001</b>	
	Site * Cover	23.17	<b>&lt; 0.001</b>	
Fermenters	Site	0.00	0.952	0.78
	Cover	67.14	<b>&lt; 0.001</b>	
	Site * Cover	22.98	<b>&lt; 0.001</b>	
Aerobic chemoheterotrophic bacteria	Site	2.40	0.122	0.761
	Cover	65.23	<b>&lt; 0.001</b>	
	Site * Cover	14.11	<b>0.015</b>	
Oxygenic photoautotrophic bacteria	Site	0.59	0.441	0.913
	Cover	127.36	<b>&lt; 0.001</b>	
	Site * Cover	27.83	<b>&lt; 0.001</b>	
Ureolytic bacteria	Site	0.46	0.499	0.783
	Cover	79.86	<b>&lt; 0.001</b>	
	Site * Cover	8.62	0.125	
Arbuscular mycorrhizal fungi	Site	8.68	<b>0.003</b>	0.625
	Cover	32.50	<b>&lt; 0.001</b>	
	Site * Cover	15.14	<b>0.010</b>	

Ectomycorrhizal fungi	Site	0	0.998	0.596
	Cover	25.12	<b>0.001</b>	
	Site * Cover	15.96	<b>0.007</b>	
Fungal plant pathogen	Site	2.17	0.141	0.475
	Cover	21.20	<b>0.007</b>	
	Site * Cover	13.01	<b>0.023</b>	
Fungal saprotrophs	Site	3.56	0.059	0.552
	Cover	23.01	<b>0.003</b>	
	Site * Cover	18.92	<b>0.002</b>	



**Figure 5.** Relative abundance of nitrifiers (A) and nitrogen fixers (B) across sites and different cover types. Bold values indicate significant results ( $P < 0.05$ ) according to statistics in Table 3.

## 2.5 Discussion

Grasses are one of the most aggressive invasive plant species threatening the biodiversity of arid and semi-arid ecosystems of North America (D'Antonio & Vitousek, 1992). In the Sonoran Desert, buffelgrass has been recognized as one of the most noxious invasives due to its ability to establish a fire regime not present before in the native vegetation (Búrquez-Montijo et al., 2002; Fusco et al., 2019; Olsson, Betancourt, Crimmins, et al., 2012). This novel grass-fire regime is especially detrimental since fire-sensitive trees and large succulent species play a vital role as keystone species in this ecosystem (Darrouzet-Nardi et al., 2023; K. A. Franklin et al., 2016; Gornish et al., 2021). In particular, legume trees can incorporate nitrogen for non-nitrogen fixing species in addition to providing a microhabitat where favorable soil conditions exist and beneficial soil microorganisms can thrive (Carrillo-Garcia et al., 1999; K. A. Franklin et al., 2016; Lopez-Lozano et al., 2016). Buffelgrass uses these resource islands to spread and invade nearby desert scrub (Lyons et al., 2013). Since buffelgrass has been suggested to have a characteristic soil microbial community (Gornish et al., 2020), its spread and successful establishment could affect soil microbial communities under mesquite, palo verde, and ironwood trees in a way that favors its invasion. In this study, we found that soil microbial communities are not affected by how buffelgrass was introduced to a site; instead, we found that the cover type matters.

During the 1970s, buffelgrass was introduced to the state of Sonora, Mexico, and after three decades it is estimated that about 1.6 million hectares of desert scrub have been transformed into buffelgrass pastures in Sonora (Búrquez-Montijo et al., 2002). To transform the area into buffelgrass pastures, the process of clearing (“desmonte” in Spanish) was carried out stripping the land of native vegetation and seeding with buffelgrass (Búrquez-Montijo et al., 2002). In

agreement with other studies, we found, land clearing and buffelgrass establishment do not have a significant impact on soil total C and N but have a substantial impact on some soil micronutrients such as Mg (e.g., Ibarra-Flores et al., 1999). Additionally, we observed that soil samples from the transformed site were a subset of the larger variety of soil profiles represented in the natural site, suggesting a relative soil homogenization and loss of biodiversity compared to the natural desert scrub (Dickens et al., 2013; Trammell et al., 2022).

Our results showed differences between the soil microbial composition of open bare areas in the transformed and the natural sites. The open bare areas in the transformed site were small and sparse, while these in the natural site were larger and more connected. These differences between the open bare areas could potentially explain our results. However, we found no differences in microbial richness between the transformed and natural sites but differences in microbial composition. Therefore, the changes in microbial composition could potentially be explained by legacy effects caused by the clearing process or by the presence of buffelgrass near the bare areas sampled in the transformed site. Land use changes are known to influence soil properties and nutrient content (Lauber et al., 2008), and, as our results show, pH, EC, and some micronutrients analyzed differed significantly between sites despite their closeness and shared common landscape dynamics. Soil properties, especially pH, have been proven to be the primary influence on soil microbial communities (Fierer & Jackson, 2006), which could explain some of our microbial findings. Another explanation could be that buffelgrass can produce about three times more biomass than native species (Martin-R et al., 1995). Therefore, there is a greater litter input to the soil in the transformed site that can be potentially modifying soil microbial communities indirectly by altering soil properties (P. Zhang et al., 2019). Moreover, the transformation of the arborescent desert into an open grassland adds up to 20 Mg of dead

biomass (Búrquez et al., 2010) that slowly incorporate into soils. Additionally, buffelgrass releases secondary metabolites that are usually detrimental to surrounding plant species (Hussain et al., 2011; Jara-Servin et al., 2023). A recent study has demonstrated the impact of buffelgrass allelopathic exudates on the soil microbial communities of its rhizosphere (Jara-Servin et al., 2023). Remarkably, in our comparative study the microbial richness of the open areas in both sites had the same pattern, showing the highest bacterial and fungal richness in soils from open areas with buffelgrass, followed by open areas with native grasses, and then open areas with no vegetation cover. The higher microbial richness in open areas with buffelgrass could be explained by the allelochemicals exudated by buffelgrass since these chemicals have been shown to increase the microbial diversity of the rhizosphere of buffelgrass and other plant species (Jara-Servin et al., 2023; Torres et al., 2021).

The conditions created by buffelgrass, either through allelopathy or litter input, could potentially lead to the establishment of a microbial community resembling that of trees. For example, the samples collected from open areas with buffelgrass as cover had a more similar microbial composition to those collected from under the canopy of trees than those collected from other open areas. Additionally, buffelgrass-invaded soils had a similar microbial richness to soils below tree canopies, even higher in some cases. This was a consistent pattern observed at both sites. For instance, the bacterial richness was highest in open areas with buffelgrass in both sites. In the natural site, trees with buffelgrass underneath their canopy had a slightly higher bacterial and fungal richness than trees without buffelgrass. Based on our results, mesquite, ironwood, and palo verde trees could be moderating the effect of buffelgrass on soil microbial richness as plant communities have been shown to mediate the effect of invasive species on soil microbial communities through litter input, root exudation, and microclimate effects (Fahey et

al., 2020), as well as by providing shade altering photon flux density and soil surface temperature (Shreve, 1931).

There were noteworthy results from palo verde tree interactions. Our study species, *Parkinsonia praecox*, is claimed not bearing root nodules (Perroni-Ventura et al., 2010; Rodríguez-Zaragoza et al., 2008). However, our results showed that the soil collected under palo verde trees had a higher relative abundance of nitrogen fixers with and without buffelgrass below their canopies in the natural site than nodule-forming ironwood and mesquite (Felker & Clark, 1980, 1981). This finding merits further research to elucidate the source of *P. praecox* higher abundance of nitrogen fixers, including the possibility that environmental variables and soil characteristics are factors determining the facultative formation of nitrogen fixing root nodules in this species (Mortier et al., 2012). In the field, specifically in the natural site, we observed palo verde trees with a complete absence of buffelgrass underneath their canopies while being right beside mesquite or ironwood trees with buffelgrass invading below their canopies. Instead, palo verde trees had native vegetation like brittlebush (*Encelia farinosa*) and annual grama grasses (genus *Bouteloua*). In addition, we found that for both fungal and bacterial soil communities, palo verde had the lowest soil microbial richness among the trees. This could suggest that there are specific beneficial microorganisms for buffelgrass not present under the canopy of palo verde trees, potentially explaining the preference of buffelgrass for mesquite and ironwood over palo verde trees. Therefore, the palo verde species we looked at in this study shows potential to be utilized for restoration purposes since it can provide a “safe site” under its canopy for native species to grow without buffelgrass competition (Gornish et al., 2021).

In this study, we analyzed the effect of buffelgrass on soil microbial communities at two sites in the core region of its invasion in the state of Sonora, Mexico. One site was cleared and

seeded transforming the desert into a grassland for cattle grazing, while the other site has been invaded naturally but retains the original structure of arborescent desert communities (Búrquez et al., 1999). Our findings show that the cover type is the primary factor that determines the richness and composition of soil microbial communities. Interestingly, our research suggests that desert trees could potentially mitigate the impact of buffelgrass on soil microbial communities, and palo verde trees might even have the potential to be used for the restoration of semi-arid ecosystems affected by buffelgrass. Therefore, our findings further highlight the importance of tree species in the Sonoran Desert.

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