EFFECTS OF BUFFELGRASS REMOVAL AND NITROGEN ADDITION ON SOIL MICROBIAL COMMUNITIES DURING AN EXTREME DROUGHT IN THE SONORAN DESERT

by

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As members of the Master's Committee, we certify that we have read the thesis prepared by Jared Williams, titled "Effects of buffelgrass removal and nitrogen addition on soil microbial communities during an extreme drought in the Sonoran Desert" and recommend that it be accepted as fulfilling the dissertation requirement for the Master's Degree.

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Final approval and acceptance of this thesis is contingent upon the candidate's submission of the final copies of the thesis to the Graduate College.

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ABSTRACT

Understanding the aboveground-belowground links between buffelgrass (*Cenchrus ciliaris*) invasion and soil microbial communities will be critical for developing a comprehensive understanding of arid ecosystems and for deploying successful control strategies. Buffelgrass, an invasive grass in the arid areas of the US, has drastically modified natural ecosystems. Buffelgrass control efforts have been generally unsuccessful, partly due to the insufficient understanding of how this species might alter belowground conditions in a way that promotes its own spread. In a randomized-block field experiment located at Tumamoc Hill, Arizona, we investigated the effects of buffelgrass removal via hand pulling and nitrogen addition (and their interaction) on soil microbial communities during an extreme drought. We found that these treatments did not significantly impact bacterial and archaeal community diversity and composition, while plant removal weakly affected fungal community diversity and composition. In addition, the removal treatment increased the proportion of putative chitinolytic bacteria (genus *Lysobacter*) and decreased the proportion of putative fungal endophytes (genus *Darksidea*). Buffelgrass manual removal may favor fungal endophyte death around and inside of leftover intact roots of buffelgrass, which may result in an increment of chitinolytic bacteria thriving on the degradation of fungal cell walls. Overall, my results suggest that buffelgrass removal can alter soil fungal communities and the proportion of certain microbial functional groups, and low levels of nitrogen addition during an extreme drought may not influence the effects of buffelgrass on soil microbial communities.

1. INTRODUCTION

Buffelgrass (*Cenchrus ciliaris*) was transported by the US Department of Agriculture (USDA) to the southwestern United States for erosion control and cattle forage due to its drought tolerance and ability to produce large numbers of seeds[1]. Since its arrival to arid areas of the US in the 1930s, buffelgrass has immensely altered natural systems by decreasing native plant community cover and richness (Jackson 2005), as well as by enhancing fire cycle frequency and intensity (Marshall et al. 2012). Plant invasion can enhance fire cycle frequency and nitrogen volatilization during fire, which leads to nitrogen loss (Evans et al. 2001). Even in the absence of fire, invasive plants can affect soil microorganisms. For example, buffelgrass invasion continuously decreases plant diversity and richness over time without fire (Olsson et al. 2012b), which may impact soil microbial community composition (Wolfe & Klironomos 2005). These impacts may persist even after the removal of the invader, and these 'invasive plant legacies' may be key for effective restoration of invaded areas (Corbin & D'Antonio 2012). In a study located in southern Arizona, the authors found that buffelgrass has spread at a fairly consistent rate since 1988 (Olsson et al. 2012a) (**Figure 1**).

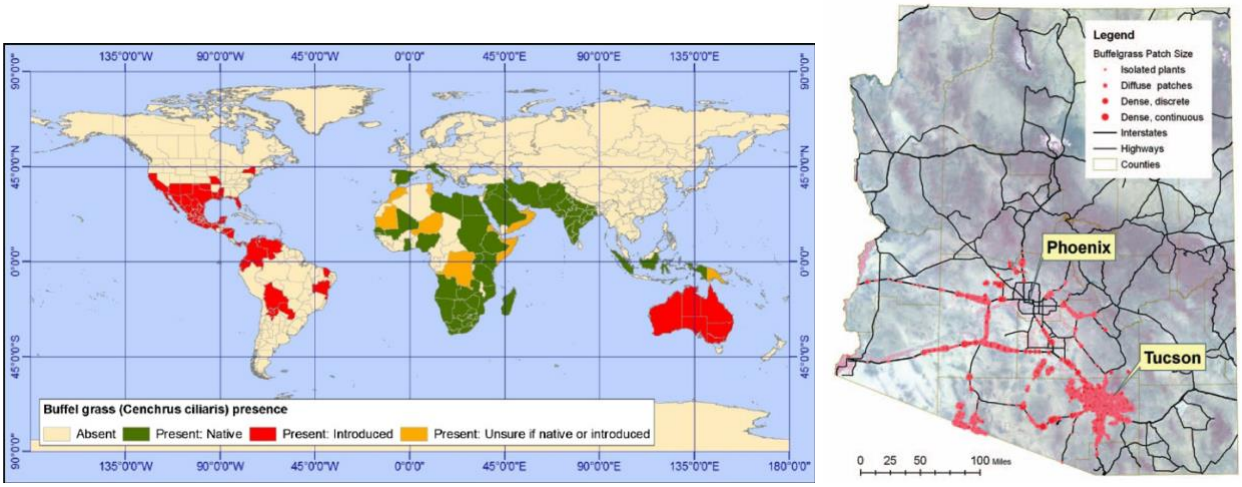

Figure 1. The native and invaded global distribution of buffelgrass (Marshall et al. 2012) (left). Buffelgrass distribution in Arizona (Stevens & Falk 2009) (right).

[1] US National Park Service (www.nps.gov/sagu/learn/nature/buffelgrass.htm)

One of the reasons why buffelgrass appears to be so resilient in the face of management efforts is that researchers and restoration managers still have an incomplete understanding of how this species might modify invaded habitat in a way that facilitates its own spread. For instance, many invasive plants can alter the soil microbiome in a way that directly or indirectly affects its success and dominance (Hawkes et al. 2005; Inderjit & van der Putten 2010). In general, plant invasion can directly change soil microbial community function and composition via litter addition, root exudation, nutrient acquisition strategies that can modify biogeochemical processes, production of chemicals that have antimicrobial properties, and rhizosphere alterations caused by root function or structure (Wolfe & Klironomos 2005). Invasive plants can also modify the interactions between native plants and soil microbial communities (Wolfe & Klironomos 2005). For example, the rate of nitrogen fixation in native symbiotic N-fixing plants can be impacted by nearby non-N-fixing invasive plants (Ehrenfeld 2003). Recent work suggests that buffelgrass is associated with increases in the relative abundance of nitrogen-cycling microorganisms and mycorrhizal fungi in invaded habitat (Gornish et al. 2020).

One aspect of global change that might be particularly relevant to buffelgrass invasion in the Southwest US is increased nitrogen deposition due to anthropogenic activities such as fossil fuel combustion and fertilizer application. At a rate of 50 kg N ha^{-1} yr^{-1}, nitrogen input can reduce native plant cover more than buffelgrass cover (Lyons et al. 2013). However, it commonly takes multiple years of nitrogen addition to alter plant species composition (Maron & Jefferies 1999). Nitrogen input may increase the biomass of native and invasive plants in the first year of nitrogen fertilization, and it may result in the dominance of invasive plants within two years (Huenneke et al. 1990).

Nitrogen addition can also impact soil microorganisms directly. According to a meta-analysis of nitrogen addition experiments, nitrogen input typically reduces the microbial C:N ratio, decreases the soil fungal:bacterial ratio, and increases the Gram positive:Gram negative bacteria ratio (Zhou et al. 2017). Nitrogen input may affect soil nitrification rates, as ammonia-oxidizing bacteria in soils may be positively affected by enhanced ammonium content, and ammonia-oxidizing archaea may be negatively impacted by increased nitrate content (Ying et al. 2017). Additionally, a recent meta-analysis showed how long-term nitrogen fertilization increases the relative abundance of

Actinobacteria and decreases the relative abundance of Acidobacteria in agro-ecosystems (Dai et al. 2018). Furthermore, soil pH is altered by nitrogen addition (Zhou et al. 2017) and can be altered by certain invasive plants (Ehrenfeld et al. 2001; Kourtev et al. 2003; Teixeira et al. 2020).

Some invasive plants increase (Ehrenfeld et al. 2001; Kourtev et al. 2003) or decrease (Teixeira et al. 2020) soil pH, which may affect the phylogenetic diversity of soil bacterial communities, as well as bacterial community composition (Lauber et al. 2009). For example, the relative abundances of Bacteroidetes and Actinobacteria are positively correlated with soil pH, and the relative abundance of Acidobacteria is negatively correlated with soil pH (Lauber et al. 2009). Moreover, acidification reduces ammonia-oxidizing bacteria abundance and mostly enhances ammonia-oxidizing archaea abundance, except in very low pH conditions (Ying et al. 2017). This soil property shapes bacterial community composition more than fungal community composition (Rousk et al. 2010), and thus the soil fungal:bacterial ratio (Bååth & Anderson 2003). Soil pH may strongly modulate bacterial community composition, and soil nutrient availability may be a larger influence on fungal community composition across land-use types (Lauber et al. 2008).

Plant invasion can also affect nutrient fluxes and stocks, as well as the composition and function of soil microorganisms associated with nutrient cycling, by changing light penetration, soil chemistry, geomorphology, hydrology, and other ecosystem processes (Ehrenfeld 2004). Through processes controlled by soil microbial communities, invasive plants usually increase soil nitrogen stocks, fluxes, and pools (Rout & Callaway 2009; Castro-Díez & Alonso 2017). For example, invasive plants can enhance nitrogen mineralization rates (Ehrenfeld et al. 2001); thus, invasive plants may increase nitrate and ammonium content (Niu et al. 2007). Also, invasive grasses can alter the composition and increase the abundance of ammonia-oxidizing bacteria, which partly explains their higher gross nitrification rates (Hawkes et al. 2005). On the other hand, Carey et al. (2017) found that two invasive grasses can decrease denitrification and nitrification potentials, as well as soil nitrate availability. Functional differences mostly determine the effect of invasive plants on N pools, and N pools are modified more during plant invasion when invasives and natives have different plant height, plant/leaf habit (evergreen or perennial plant compared to deciduous or annual plant), and nitrogen-fixation ability (Castro-Díez et al. 2014). Even after the removal of

invasive plants, their legacy on total soil N, net N mineralization rates, and inorganic N pool sizes may persist for several years (Maron & Jefferies 2001).

The largest nitrogen flux increase during invasions is plant nitrogen uptake (Castro-Díez et al. 2014). Thus, invasive plants usually grow faster (Pyšek & Richardson 2007), have higher plant and litter nitrogen concentrations, and have lower litter lignin:N and C:N ratios compared to native plants (Liao et al. 2007). The rate of net N mineralization and nitrification during plant invasion may be increased by higher aboveground net primary productivity, litter and plant biomass, higher litter and plant nitrogen concentrations, and lower litter C:N ratio (Liao et al. 2007). During decomposition, nitrogen may be released faster and in larger amounts from invasive plant litter compared to native plant litter (Allison & Vitousek 2004). Overall, plant invasion may impact belowground nitrogen-cycling microorganisms (Gornish et al. 2020).

During water pulses, soil microbial activity (decomposition, mineralization, and release of inorganic C and N) escalates due to a resource pulse (Schimel 2018). Soil microbial communities increase the availability of soil nutrients following rainfall pulses, which enhances plant survival and ecosystem functionality (Collins et al. 2014). There may be enhanced gross nitrification, gross N mineralization, and microbial N pools for up to 10 days following a 1 cm precipitation event (Dijkstra et al. 2012). Also, pulses of rainfall lead to net nitrogen mineralization (Austin et al. 2004), but also increase nitrogen losses (Austin et al. 2004), including nitrous oxide and nitric oxide effluxes (Davidson et al. 1993), ammonia volatilization (McCalley & Sparks 2008), denitrification (Peterjohn & Schlesinger 1991; Yahdjian & Sala 2010), and nitrate leaching (Yahdjian & Sala 2010). The magnitude of a precipitation event can also influence the effect of rainfall on soil microorganisms (Dijkstra et al. 2012). Following extreme rainfall events, soil fungal abundance is not impacted; however, soil bacterial abundance increases, which may affect soil ammonium and nitrate contents (Zhang et al. 2019). Additionally, large rainfall events in arid ecosystems promote a decrease in soil diversity, higher abundance of anaerobic bacteria, more Firmicutes probably linked to dormancy exit, and lower abundance of dry-tolerant Actinobacteria (McHugh et al. 2014; Štovíček et al. 2017; Cregger et al. 2012).

In this study, we investigated the effect of buffelgrass on soil microbial communities using a randomized blocks field experiment (buffelgrass removal and N addition as treatments). My hypotheses were: 1) Buffelgrass removal and N addition (and their interaction) would have a large effect on soil microbial community diversity and composition; and 2) N addition would have a larger effect than buffelgrass removal particularly due to changes in nitrifiers' relative abundance.

2. MATERIALS AND METHODS

2.1. Study site

The field experiment was deployed at Tumamoc Hill (32°13'N, 111°00'W) (**Figure 2**) in Tucson, Arizona (USA). Tumamoc Hill is an ecological reserve and study site of the University of Arizona that is covered with a variety of native and invasive plants (Ge et al. 2019). Tucson experiences a semi-arid climate of hot, dry summers and warm, dry winters. The average annual air temperature in Tucson is 20.6°C (69.0°F), and the average annual precipitation is 301 mm (11.9 inches)[2]. The wet monsoon season mainly occurs in July and August, which is when plant biomass peaks. During the summer of 2020, the Tucson area experienced an extreme drought with 62.7 mm of rain[3] compared to 117 mm average of rain[4].

There are 68 soil series in the eastern part of Pima County (Cochran & Richardson 2003). The site has a Lehmans-Delthorny-Lajitas complex soil (40% is Lehmans and similar soils, 25% is Delthorny and similar soils, 15% is Lajitas and similar soils, and 20% is minor components)[5]. The taxonomic class for Lehmans series is Clayey, smectitic, thermic Lithic Haplargids[6], Delthorny series is Loamy-skeletal, mixed, superactive, thermic Calcic Lithic Petrocalcids[7], and Lajitas series is Loamy-skeletal, mixed, superactive, nonacid, thermic Lithic Torriorthents[8]. The soil texture for Lehmans series in the B horizon is clay loam or clay (35-60% clay)[6], Delthorny series in the Bk horizon is fine sandy loam, sandy loam, or loam[7], and Lajitas series in the A horizons is fine sandy loam, loam, or sandy loam[8]. Also, the maximum calcium carbonate content for Lehmans series is 5%, Delthorny series is 20%, and Lajitas series is 2%[5]. All three of these soil series are Hydrologic Soil Group D[5]. Lastly, soil pH in this area ranges between 7.5 and 8.0.

[2] en.climate-data.org/north-america/united-states-of-america/arizona/tucson-1467
[3] https://cals.arizona.edu/AZMET/data/0120em.txt
[4] https://www.rssweather.com/climate/Arizona/Tucson/
[5] websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx
[6] https://soilseries.sc.egov.usda.gov/OSD_Docs/L/LEHMANS.html
[7] https://soilseries.sc.egov.usda.gov/OSD_Docs/D/DELTHORNY.html
[8] https://soilseries.sc.egov.usda.gov/OSD_Docs/L/LAJITAS.html

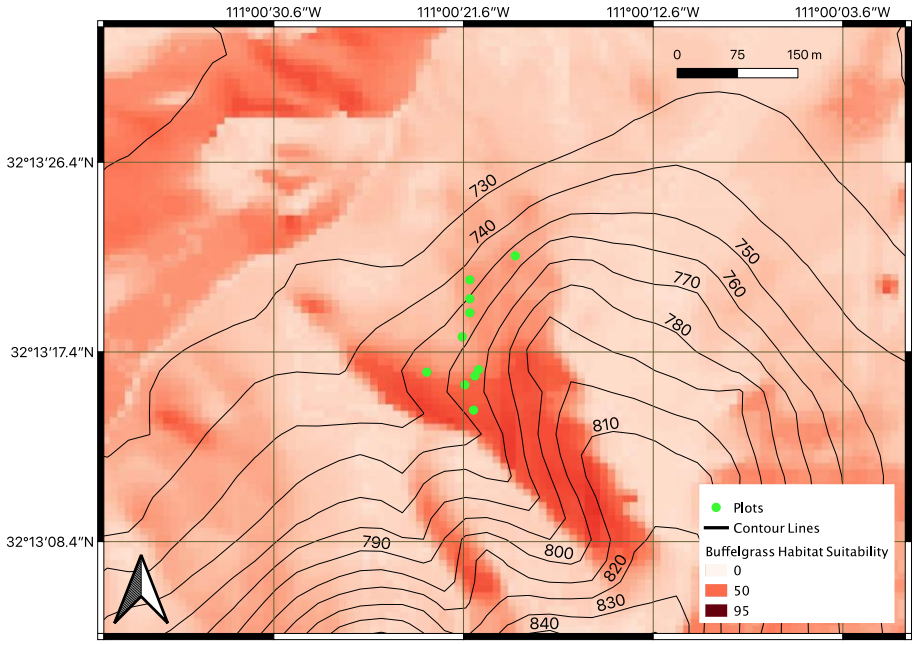

Figure 2. Location of Tumamoc Hill sampling area. This map was generated with QGIS (https://www.qgis.org/) overlaying buffelgrass habitat suitability from Jarnevich and Young (2018).

2.2. Experimental design

The treatments consisted of crossed manipulations of buffelgrass manual removal and N addition, plus controls within 10 randomized blocks (**Figure 3**). In each block, we identified 8 individual buffelgrass plants and randomly assigned each one to undergo one of the following treatments: (1) none (control); (2) buffelgrass removal via hand pulling; (3) N addition; and (4) buffelgrass removal combined with N addition. All treatments were applied in April 2020 and prior to treatment application we assessed cover of all buffelgrass and native plant species in a 1m-squared quadrat centered on the target buffelgrass. Each treatment was assigned to two buffelgrass plants in each block. The N addition treatments consisted of the application of six grams of granular N fertilizer in the form of NH_4NO_3 sprinkled evenly across the quadrat. For buffelgrass plants that were assigned the buffelgrass removal combined with N addition treatment, the buffelgrass plants were removed first, and then the fertilizer was applied. Hand pulled buffelgrass plants were removed from the site. 400 ml of water was added to each quadrat regardless of the treatment. In early August 2020, the entire study site was homogeneously exposed to an herbicide application of 4% glyphosate (N-(phosphonomethy)glycine) solution (Ranger Pro) at a rate of 1 gallon per

acre which is typical for annual buffelgrass control. Glyphosate is the most widely applied herbicide in the US (Weidenhamer & Callway 2010), and in a recent field study, this herbicide did not significantly alter the soil microbiome of buffelgrass in the Sonoran Desert (Gornish et al. 2020).

Figure 3. Assessing focal and total buffelgrass cover (left). Removing buffelgrass via hand pulling (center). N addition and watering (right).

2.3. Soil sampling

During peak plant biomass (September 2020), soil samples from every quadrat were collected to a depth of 10 cm. Soil was collected near the plant roots using a spatula, and soil from each quadrat was placed in a sterile Whirl/Pack plastic bag after being sieved to 2 mm. These soil samples were immediately transported to the University of Arizona on ice and stored in a -80°C freezer until molecular analyses.

2.4. Molecular analyses

Soil genomic DNA was extracted from 0.3 g of each sample using QIAGEN DNeasy PowerLyzer PowerSoil kits. Microbial diversity was evaluated utilizing high-throughput amplicon sequencing methods to assess the diversity and composition of taxonomic marker gene sequences. Extracted DNA undergone amplicon paired-end Illumina MiSeq sequencing using the 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) primer pair for bacterial and archaeal V4 hypervariable region of the 16S rRNA gene (Caporaso et al. 2012), and

the ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGCGTTCTTCATCGATGC) primer pair for fungal first internal transcribed spacer (ITS) region of the rRNA operon (Schoch et al. 2012). Primers included Illumina adapters and unique error-correcting 12-bp barcodes. PCR products were quantified fluorescently with the Quant-It PicoGreen dsDNA Assay Kit and pooled in equimolar concentrations for sequencing on an Illumina MiSeq instrument at the Microbiome Core, Steele Children Research Center, University of Arizona.

2.5. Sequence processing

After demultiplexing, reads were subject to quality filtering, dereplication, chimera detection, and merging of paired-end reads using DADA2 (Callahan et al. 2016). The ITS region is highly variable in length and thus, the first step prior to sequence processing was the removal of primers using cutadapt (Martin 2011). Unique phylotypes were assigned taxonomy using the Ribosomal Database Project (RDP) classifier (Wang et al. 2007) trained on the 16S rRNA SILVA database (Quast et al. 2013) or the ITS UNITE database (Abarenkov et al. 2010), for bacteria and archaea and fungi, respectively. Sequences unclassified at the domain level or classified as mitochondria, chloroplasts or Eukaryota were removed. We inferred putative bacterial and archaeal metabolisms using FAPROTAX (Louca et al. 2016) and putative fungal functional guilds using FUNGuild (Nguyen et al. 2016).

2.6. Statistical analyses

Linear mixed-effects models fit by restricted maximum likelihood were used to determine richness (number of different phylotypes) and Shannon diversity (phylotype evenness) differences among treatments (Harrison et al. 2018). Buffelgrass removal and N addition were set as fixed factors and block as random effects. Non-metric multidimensional scaling (NMDS) based on Bray-Curtis distance was utilized to visualize patterns in the composition of bacterial and fungal communities across treatments. Permutational multivariate analysis of variance (PERMANOVA) was used to assess the effect of treatments (permutations constrained within block) on community similarity patterns (Anderson 2001). Taxonomic or inferred functional differences among treatments were assessed using a negative binomial generalized linear mixed-effects model (response variables are overdispersed count data) with treatments as fixed effects and block as random effects. All

statistical analyses were executed in the R environment[9] using the lme4[10], lmerTest[11], and vegan[12] packages.

[9] www.r-project.org
[10] https://cran.r-project.org/web/packages/lme4/index.html
[11] https://cran.r-project.org/web/packages/lmerTest/index.html
[12] https://cran.r-project.org/web/packages/vegan/index.html

3. RESULTS

3.1. General description

There were 21,761 unique phylotypes for bacteria and archaea, as well as 6,128 unique phylotypes for fungi across the 75 soil samples. The average number of different phylotypes in each soil sample was 1,651 (sd = 186) for bacteria and archaea and 326 (sd = 67) for fungi. The taxonomic classes Alphaproteobacteria (11.2%), Thermoleophilia (9.4%), Deltaproteobacteria (8.0%), Actinobacteria (7.7%), Planctomycetacia (7.0%), and Bacteroidia (6.1%) dominated prokaryotic community composition. The taxonomic classes Dothideomycetes (26.9%), Sordariomycetes (23.1%), Agaricomycetes (15.1%), Eurotiomycetes (7.7%), Pezizomycetes (5.4%), and Tremellomycetes (5.2%) dominated fungal community composition.

3.2. Bacterial and archaeal community diversity

Bacterial and archaeal richness showed no significant differences with the removal treatment, nitrogen treatment, or interaction between nitrogen and removal within blocks (linear mixed-effects: F = 1.16, P = 0.286; F = 0.412, P = 0.523; F = 2.26, P = 0.138, respectively) (**Figure 4A**). Fixed effects only explained 4.6% of the variability in bacterial and archaeal richness, while block random effects explained an additional 13.4%. Similar patterns were observed for Shannon diversity. Bacterial and archaeal diversity showed no significant differences with the removal treatment, nitrogen treatment, or interaction between nitrogen and removal within blocks (linear mixed-effects: F = 0.00, P = 0.995; F = 1.55, P = 0.217; F = 1.73, P = 0.193, respectively) (**Figure 4B**). Overall, the treatments only explained 3.6% of the variability in bacterial and archaeal Shannon diversity, while block effects explained an additional 21.8%.

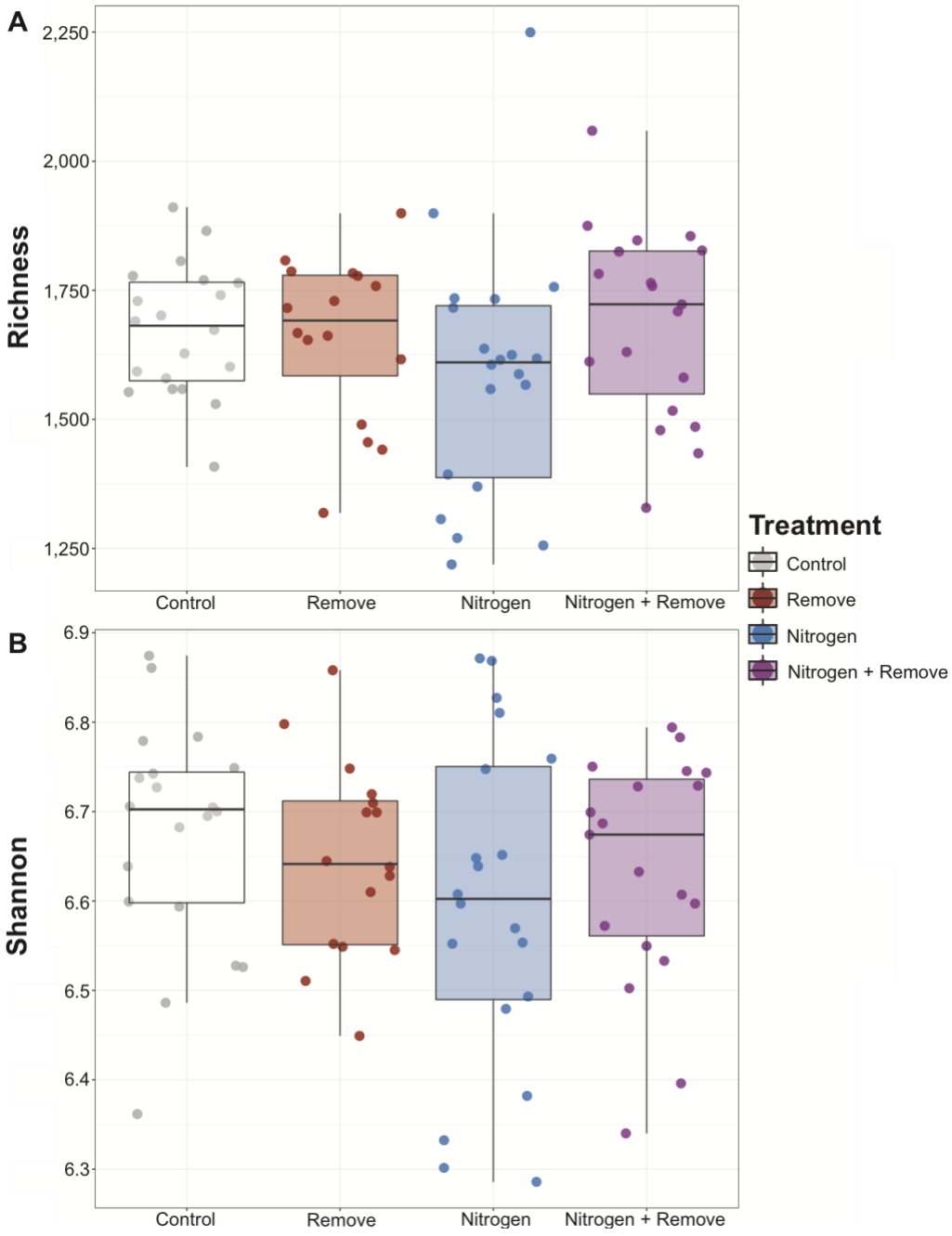

Figure 4. Bacterial and archaeal richness results (A). Bacterial and archaeal Shannon diversity (B).

3.3. Bacterial and archaeal community similarity patterns

Bacterial and archaeal community similarity patterns also exhibited no significant differences with the removal treatment, nitrogen treatment, or interaction between nitrogen and removal within

blocks (PERMANOVA, R^2 = 0.017, P = 0.066; R^2 = 0.010, P = 0.696; R^2 = 0.011, P = 0.583, respectively). Bacterial and archaeal community composition of removal and nitrogen treatments overlapped, and these treatments were not distinct from the control (**Figure 5**).

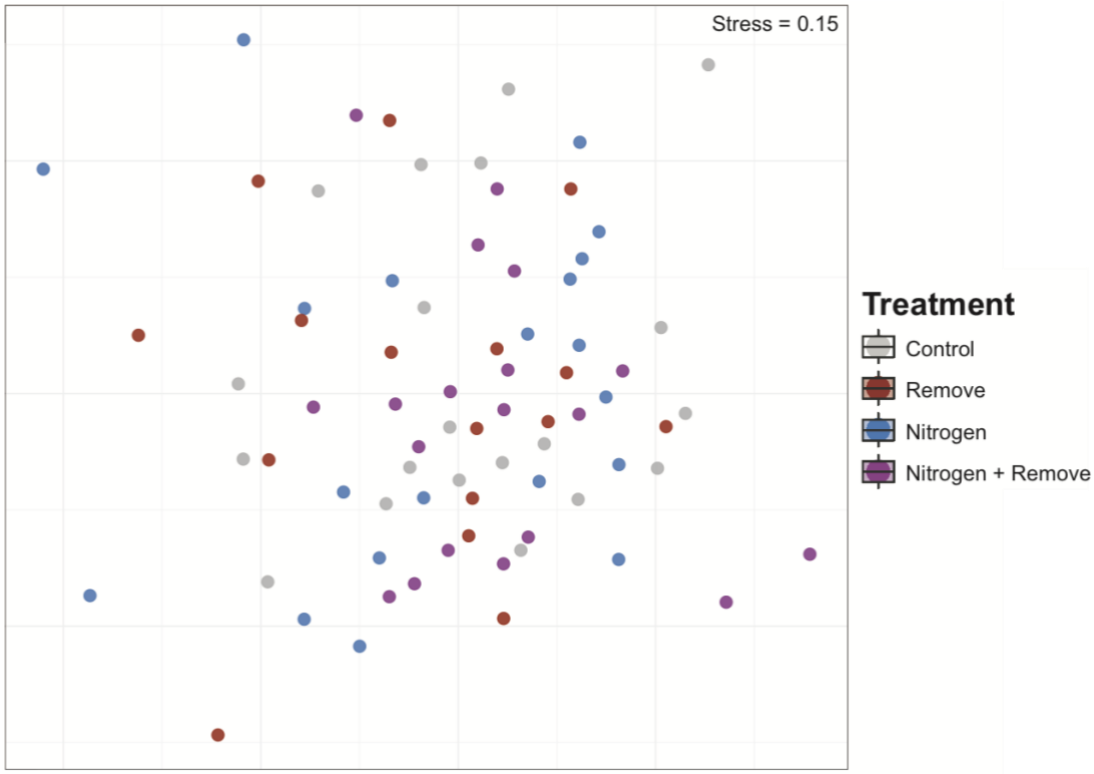

Figure 5. Bacterial and archaeal non-metric multidimensional scaling (NMDS) ordination plot.

3.4. Fungal community diversity

Fungal richness showed a significant decrease with the removal treatment within blocks (linear mixed-effects: F = 12.39, P = 0.001) (**Figure 6A**). The nitrogen treatment or the interaction between nitrogen and removal showed no significant differences (linear mixed-effects: F = 1.64, P = 0.205; F = 1.37, P = 0.245, respectively) (**Figure 6A**). Overall, fixed effects explained 17.5% of the variability in fungal richness, and block random effects explained an additional 1.8%. Similar patterns were observed for Shannon diversity. Fungal diversity exhibited a significant decrease with the removal treatment within blocks (linear mixed-effects: F = 8.17, P = 0.006) (**Figure 6B**). The nitrogen treatment or the interaction between nitrogen and removal showed no significant differences (linear mixed-effects: F = 2.02, P = 0.160; F = 1.48, P = 0.229, respectively)

(**Figure 6B**). Overall, fixed effects explained 12.2% of the variability in fungal Shannon diversity, and block random effects explained an additional 14.3%.

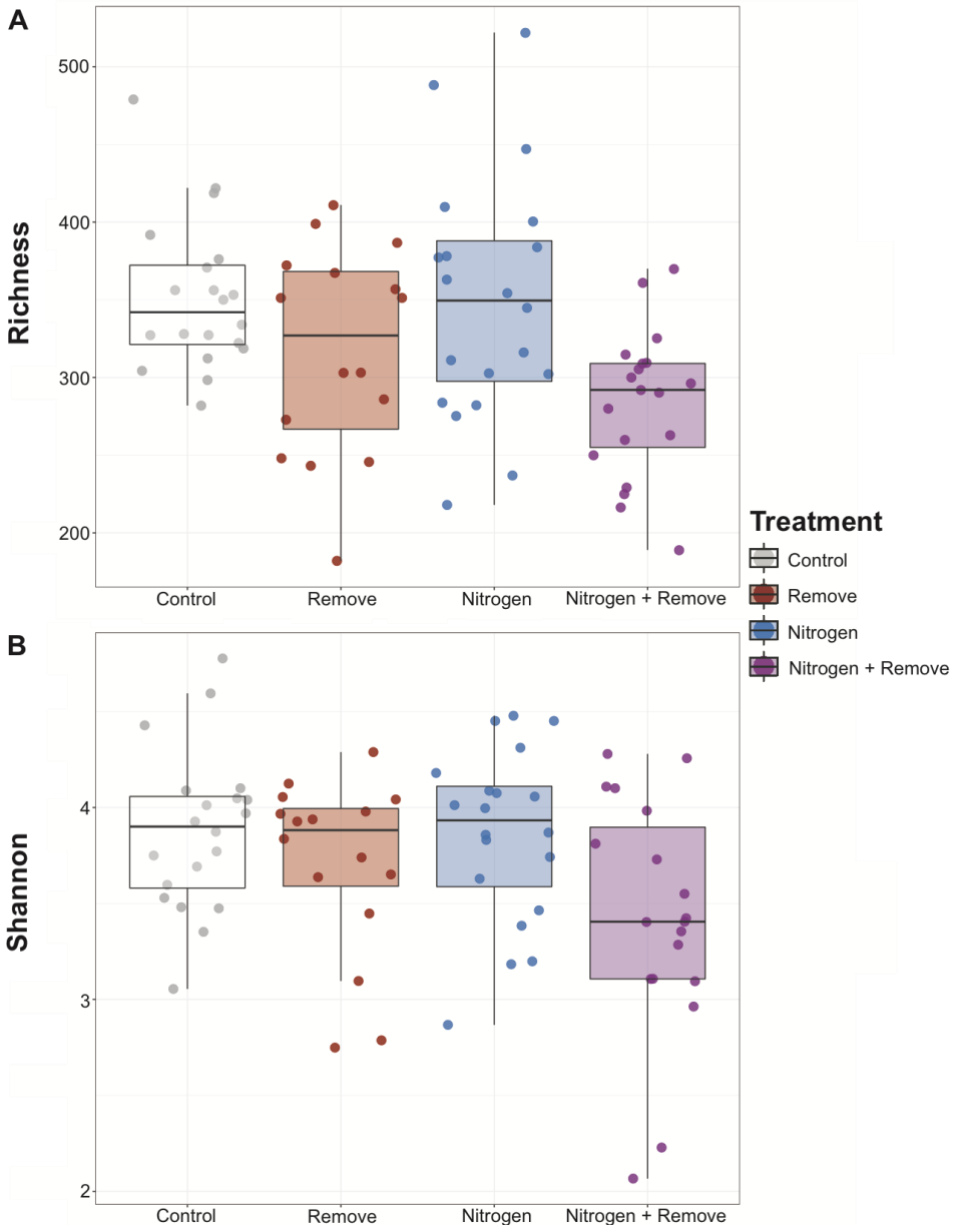

Figure 6. Fungal richness results (A). Fungal Shannon diversity (B).

3.5. Fungal community similarity patterns

Fungal community similarity patterns slightly varied with the removal treatment within blocks (PERMANOVA, $R^2 = 0.040$, $P < 0.001$). The nitrogen treatment or the interaction between nitrogen and removal showed no significant differences (PERMANOVA: $R^2 = 0.012$, $P = 0.357$;

R^2 = 0.011, P = 0.750, respectively). Fungal community composition of removal and nitrogen treatments overlapped, and the removal treatment was to a small degree distinct from the control (**Figure 7**).

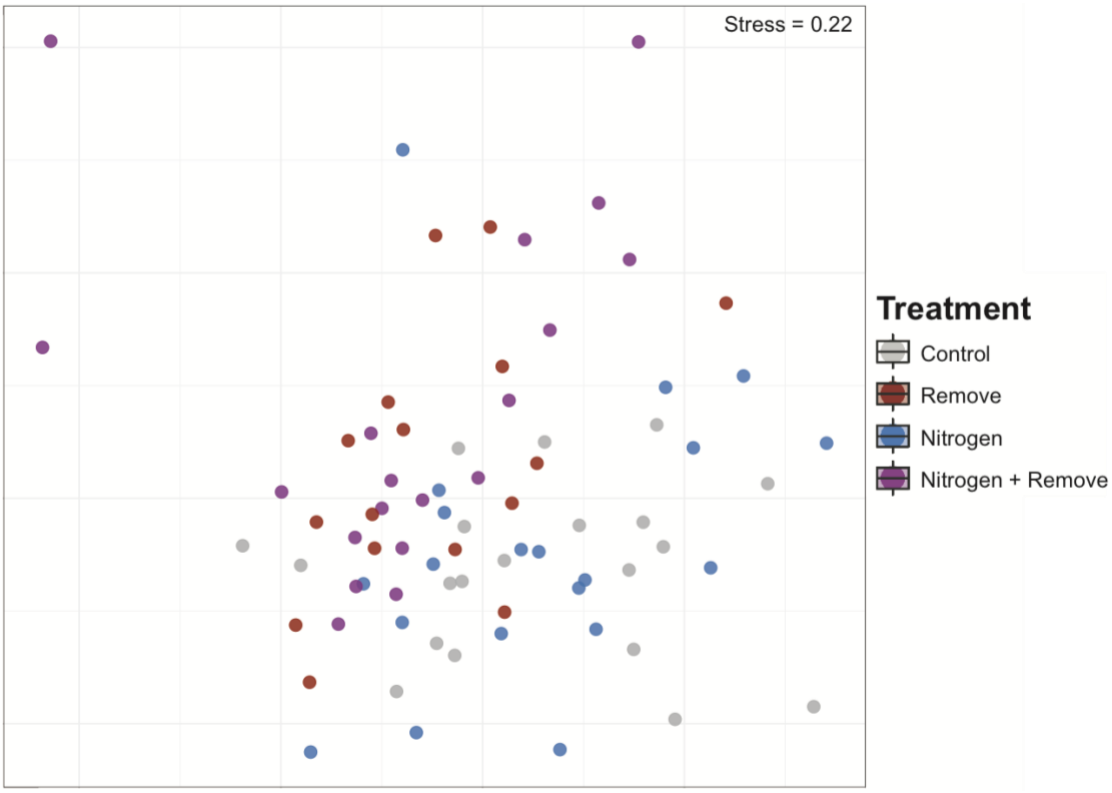

Figure 7. Fungal non-metric multidimensional scaling (NMDS) ordination plot.

3.6. Inferred microbial functional groups

After assessing the effects of my experimental treatments on overall bacterial and archaeal and fungal soil communities, we further explored the effect of the removal and nitrogen treatments on the proportion of inferred functional microbial groups. Putative chitinolytic taxa (mainly Xanthomonadales from the genus *Lysobacter*) showed a significant increase with the removal treatment within blocks (negative binomial generalized linear mixed-effects: R^2 = 0.308, P < 0.001) (**Figure 8A**). Conversely, buffelgrass removal reduced the proportion of fungal endophytes (mainly Pleosporales from the genus *Darksidea*) (negative binomial generalized linear mixed-effects: R^2 = 0.116, P = 0.038) (**Figure 8B**).

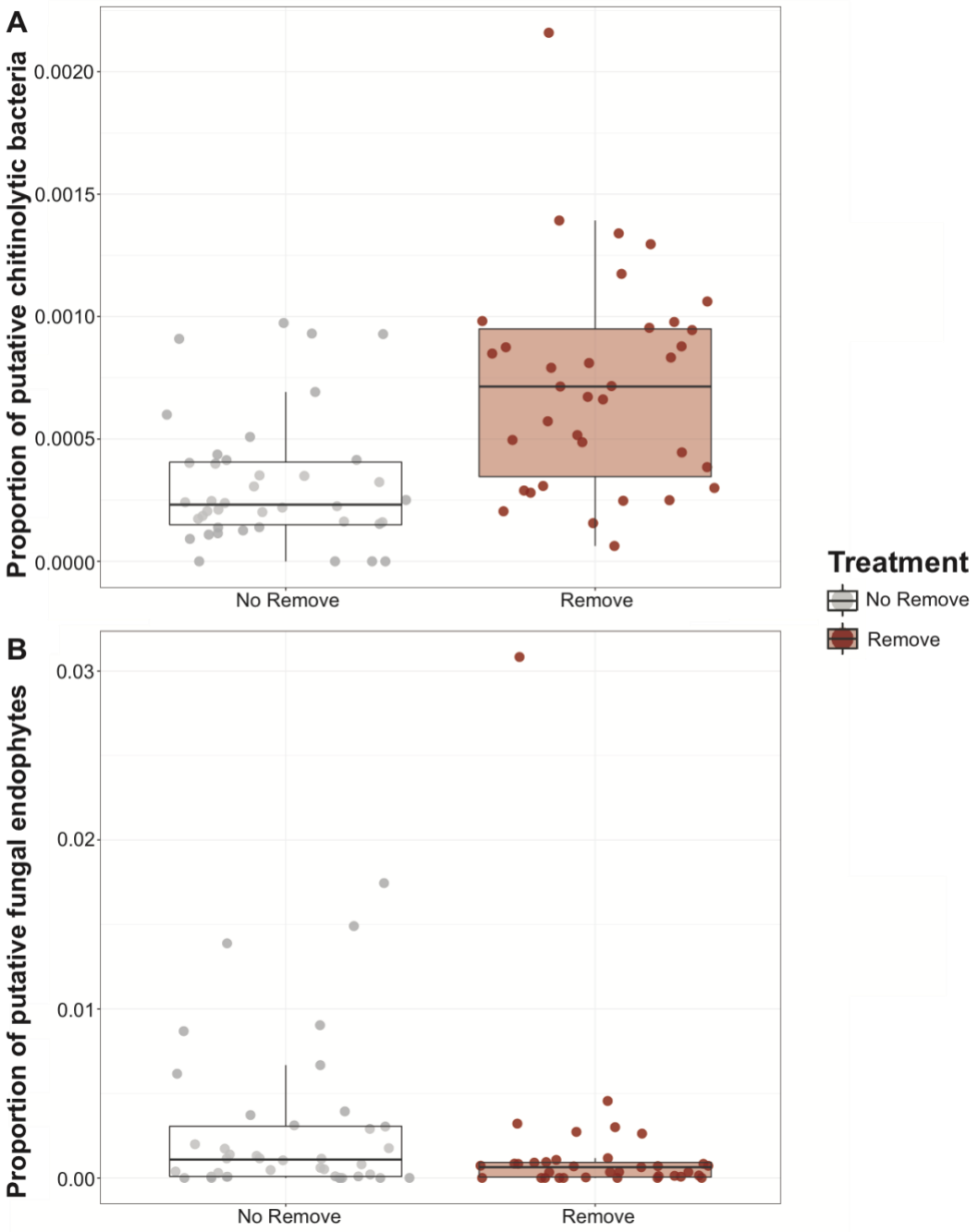

Figure 8. Proportion of putative chitinolytic bacteria (A). Proportion of putative fungal endophytes (B).

4. DISCUSSION

Nonnative plants from different continents, such as buffelgrass in the US, are more likely to be invasive compared to nonnative plants from the same continent (Li & Keping 2010). These invasive plants can impact ecosystems in various ways, such as changing soil microbial community structure and function (Kourtev et al. 2002). Soil bacterial, archaeal, and fungal communities are key components in terrestrial ecosystems, as soil microbial communities modulate numerous ecosystem processes (e.g., nutrient cycling and plant productivity) (Chu et al. 2020). Thus, understanding how buffelgrass alters soil microbial communities would aid our comprehension of the effects of plant invasion. To the best of my knowledge, this is the first field manipulative experiment that examines the influence of buffelgrass on soil microbial communities.

4.1. Hypothesis 1: Buffelgrass removal and N addition (and their interaction) would have a large effect on soil microbial community diversity and composition

We hypothesized that buffelgrass removal and N addition (and their interaction) would have a large effect on soil microbial community diversity and composition. We did not observe large treatment effects on bacterial and archaeal communities, with only weak but statistically significant effects of plant removal on fungal communities. The effects of the treatments on bacterial richness, diversity, and community composition might have been small in comparison to the impacts of the extreme drought during the summer of 2020. In arid ecosystems, nutrients and water are mainly accessible in pulses of precipitation (Collins et al. 2008). The activity of soil microorganisms is thus indirectly controlled by drying-rewetting cycles, which affects nitrogen cycling (Austin et al. 2004). During drought, soil microorganisms experience reduced diffusion and resource supply (Schimel 2018). Drought actually increases soil inorganic nitrogen content; however, it is not available to soil microorganisms during drought due to limited diffusion (Evans & Burke 2013). Diffusion limitation may explain why nitrogen addition did not have a significant effect on soil bacteria and fungi in my experiment. Furthermore, invasive plants can uptake more nitrogen compared to native plants during drought (Barros et al. 2020). The impact of extreme drought on invasive plant density may be reduced by nitrogen and phosphorous input (Kelso et al. 2020), and nitrogen addition may promote plant invasion during an extreme drought (Valliere et al. 2017).

The removal treatment may have influenced fungal communities more than bacterial communities because drought tends to impact fungi less than bacteria (Preece et al. 2019). In general, potentially active soil bacterial communities are more sensitive to desiccation, as well as precipitation pulses, than potentially active soil fungal communities (Barnard et al. 2013). In summary, the extreme drought might have mitigated the effects of the treatments on soil microbial communities, especially bacterial and archaeal communities.

4.2. Hypothesis 2: N addition would have a larger effect than buffelgrass removal particularly due to changes in nitrifiers' relative abundance

We also hypothesized that N addition would have a larger effect due to changes in the relative abundance of nitrifying microorganisms. This second hypothesis can also be rejected, because the nitrogen treatment had no effect on the diversity, composition, or proportion of inferred functional microbial groups. Only the plant removal treatment affected fungal richness, diversity, and community composition. These results are in agreement with Anthony et al. (2020). In this study, the authors reported that the interaction between plant invasion and nitrogen input did not significantly impact fungal community composition. Overall, nitrogen addition may not alter the relative abundance of nitrifiers near buffelgrass roots during an extreme drought in the Sonoran Desert.

As discussed in regards with my first hypothesis, the dramatic impacts of the extreme drought may have prevented the treatments from significantly affecting the relative abundance of nitrifiers. There is increased activity of autotrophic nitrifier populations during frequent drying-rewetting events (Fierer & Schimel 2002). However, there may be decreased nitrification rates during drought; thus, nitrifying bacteria may have lower activity during drought (Stark & Firestone 1995). In contrast, Barrett et al. (2002) found that soil water content has little effect on in situ net nitrogen mineralization, as well as nitrification. Although nitrifiers were not affected by the treatments, buffelgrass removal significantly impacted the proportion of putative chitinolytic bacteria (mainly *Lysobacter*) and fungal endophytes (mainly *Darksidea*).

In my field experiment, buffelgrass removal increased the proportion of putative chitinolytic bacteria, in particular members of the genus *Lysobacter*. Chitin, an unbranched polymer (Duo-

Chuan 2006), is a cell wall component in fungi (Peberdy 1990). Chitin-degrading enzymes, chitinases, occur in bacteria, fungi, and other organisms (Duo-Chuan 2006). For example, several strains of *Lysobacter* can produce chitinases and have antagonistic activity against various pathogens (Gómez Expósito et al. 2015). Plant removal may favor fungal death around and inside of leftover intact roots (Dickie et al. 2016); thus, more chitinolytic bacteria (e.g., *Lysobacter*) may be breaking down chitin (**Figure 9**).

Buffelgrass removal decreased the proportion of putative fungal endophytes in surrounding soils, in particular members of the genus *Darksidea*. Fungal endophytes reside within plant tissues, such as leaves, stems, and/or roots (Carroll 1988; Stone et al. 2004). This genus of dark septate endophytes includes endophytic fungi that can reside within the roots of grasses in arid and semiarid areas (Knapp et al. 2015). Overall, buffelgrass removal might have removed the endophytes (e.g., *Darksidea*) living inside and in close association with the buffelgrass roots (**Figure 9**).

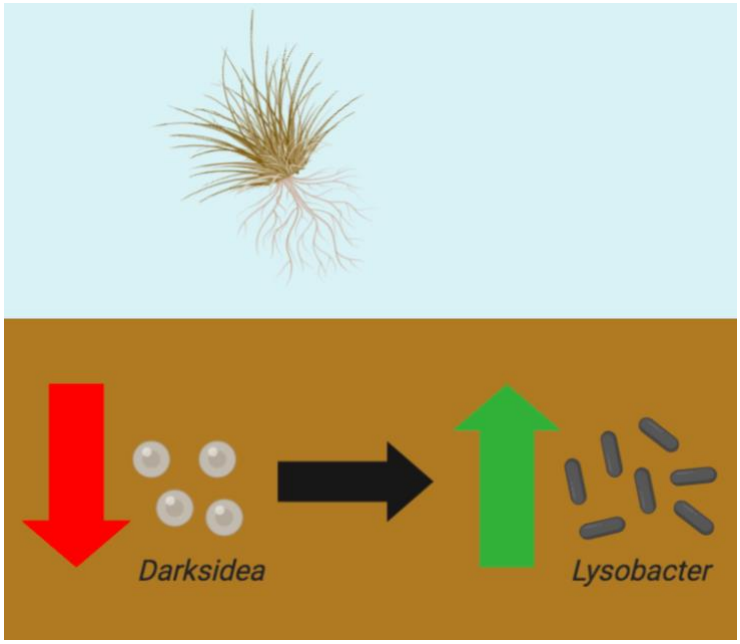

Figure 9. Conceptual diagram showing the effects of buffelgrass removal on the proportion of putative chitinolytic bacteria (e.g., Lysobacter) and fungal endophytes (e.g., Darksidea). Created with Biorender.com.

5. CONCLUSION

Understanding the fundamental relationships between buffelgrass invasion and soil microbial communities is critical for developing a more comprehensive understanding of arid land ecology as well as establishing a platform on which to create and deploy effective control strategies. My results suggest that nitrogen fertilizer application does not influence the effects of established buffelgrass plants on soil microbial communities during an extreme drought in the Sonoran Desert. However, buffelgrass removal via hand pulling affected soil fungal communities, the proportion of putative chitinolytic bacteria (mainly *Lysobacter*), and the proportion of putative fungal endophytes (mainly *Darksidea*). These findings could be a result of fungal endophyte death around and inside of leftover intact roots of buffelgrass, and the subsequent proportional enrichment of chitinolytic bacteria thriving on the degradation of fungal cell walls. In summary, buffelgrass removal can impact soil fungal communities and the proportion of specific microbial functional groups, and low levels of nitrogen input during an extreme drought may not alter the effects of buffelgrass on soil microbial communities.

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