

PLANT-PLANT AND PLANT-SOIL INTERACTIONS UNDER DROUGHT  
AND THE PRESENCE OF INVASIVE BUFFELGRASS

by

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## **Abstract**

Drylands are predicted to become more arid with future climate change scenarios and have the potential for expansion. Invasive grasses with drought-tolerating strategies proliferate in these areas although limited resources characterize these ecosystems. Buffelgrass is a particularly invasive grass species with a high dominance in the southwestern United States. Plant-plant and plant-soil interactions could play a major role in mechanisms of invasion and could prove to be an essential link in understanding potential ecosystem alteration. However, we currently have a poor understanding of how soil microbial communities potentially enhance or obstruct invasive dominance. We used a greenhouse experiment in Tucson, Arizona to assess changes in native plant traits and soil bacterial and fungal communities in response to drought, buffelgrass presence, and their interaction. We found that buffelgrass increases its growth from growing in the presence of a native and that this effect may be mediated by increases in bacterial richness and a higher abundance of nitrogen-fixing soil microorganisms. A simulated drought had no obvious effects on plants or soil microbial communities in this experiment. Overall, plant-plant and plant-soil interactions should be integrated into the science of invasion and restoration ecology, with significant potential for mediating the effects of interacting abiotic and biotic anthropogenic stressors.

## 1. Introduction

Global climate models forecast precipitation changes, with more intense droughts that will promote the expansion of drylands (Huang et al. 2016). Specifically, the southwest USA is predicted to become more arid with more intense, sporadic precipitation events delivering less water overall (Seager et al. 2007). Altered precipitation regimes can substantially influence plant root-shoot ratios, litter quantity and quality, and changes in root vertical distribution and exudate composition (Preece & Peñuelas 2016; Bingwei Zhang et al. 2019). For example, in arid ecosystems of the southwest USA native plants have developed smaller leaf areas and long, lateral roots (Phillips & Comus 2000). Water availability also regulates soil microorganisms by direct physiological stress and indirectly by limiting diffusion and thus, nutrient supply (Schimel 2018). As a consequence, drought can modify both plant competitive ability and plant-soil interactions (Suttle et al. 2007; Pugnaire et al. 2019). For instance, a trade-off between growth rate and water-use efficiency can influence interspecific interactions in desert annual plants (Huxman et al. 2008), and positive interactions with mycorrhizal fungi can alleviate plant drought stress (Porter et al. 2020). These drought-driven plant-plant and plant-soil interactions have in turn cascading effects such as biodiversity loss, reduced soil carbon sequestration, increased soil erosion, and altered plant invasiveness (Meisner et al. 2013; Berdugo et al. 2020).

Invasive plant species are a threat to biodiversity and ecosystem services globally (Pyšek et al. 2012) and consequently, billions of dollars are spent to control and manage them (Diagne et al. 2021). Although it is expected that native plants have a competitive advantage over invasives in low-resource environments (Funk 2013), and plant-soil responses to drought are expected to be generally positive when plant and soil microorganisms are co-adapted, that is, they share a history of coexistence (de Vries et al. 2023), invasive grasses in the arid southwest USA have rapidly

become dominant (A. D. Olsson, J. L. Betancourt, et al. 2012). Buffelgrass (*Cenchrus ciliaris*), an African perennial C<sub>4</sub> bunchgrass, was introduced in the Sonoran Desert for cattle forage and erosion control (Marshall et al. 2012) due to its capability of withstanding stressful climactic conditions (Marshall et al. 2012). The success of buffelgrass outside its native range is due to its low water requirements (Ward et al. 2006), high germination rates (Tinoco-Ojanguren et al. 2016), rapid nitrogen acquisition (Lyons et al. 2013), and its ability to modify native neighboring vegetation through allelopathy (Espinoza et al. 2020). In addition, the presence of buffelgrass has been associated with an idiosyncratic soil microbiome (Gornish et al. 2020). Buffelgrass fills barren gaps between native grasses, shrubs and succulents, and these continuous grass stands promote fire hazards, creating a new fire regime unsuitable for native species (McDonald & McPherson 2013). The altered fire regimes also create favorable conditions for buffelgrass, which recover and spread postfire, resulting in a grass-fire cycle (Fusco et al. 2019). As a consequence, buffelgrass invasion can reduce the number of native plant species by more than 50% (Aaryn D. Olsson, Julio Betancourt, et al. 2012; Tinoco-Ojanguren et al. 2013), but correlations to individual native species abundance vary across studies. Effective control by herbicides occurs (Dixon et al. 2003; Tjelmeland et al. 2008), though manual extraction remains a common treatment method. Although a buffelgrass environmental niche model suggested changes in the present distribution and the invasion of areas previously unaffected (de Albuquerque et al. 2019), this model lacked biotic information related to plant competition and interactions with the surrounding soil microbiome. Such changes to components of ecosystems could have legacy effects that influence management before and after treatment (Marshall et al. 2012; Aaryn D. Olsson, Julio Betancourt, et al. 2012). Thus, understanding how drought and native-invasive competition interactively affect

plant growth, biomass allocation, and its concomitant influence on soil microbial communities is critical to the management and restoration of invaded ecosystems.

In a greenhouse study, we examined competition between buffelgrass and two native Sonoran Desert plants (i.e., *Aristida purpurea* and *Plantago patagonica*) under two water treatments (well-watered vs. drought) by measuring plant traits, and soil microbial diversity and composition. Our hypotheses were (1) buffelgrass would grow at a high rate even under the drought treatment (Valliere 2019); (2) native plants' biomass allocation to roots would increase with competition and drought (Poorter et al. 2012); (3) competition treatments (i.e., two different species planted together) would provide a larger diversity of plant resources and thus, would support more diverse soil microbial communities and a higher abundance of fungal saprotrophs (Eisenhauer et al. 2010), and (4) presence of buffelgrass would modify the abundance of nitrogen-cycling soil microorganisms (Gornish et al. 2020). Overall, we aimed to generate an improved mechanistic understanding of plant trait and soil microbial responses to the interactive effects of abiotic and biotic co-occurring stressors.

## **2. METHODS**

### **Greenhouse experiment**

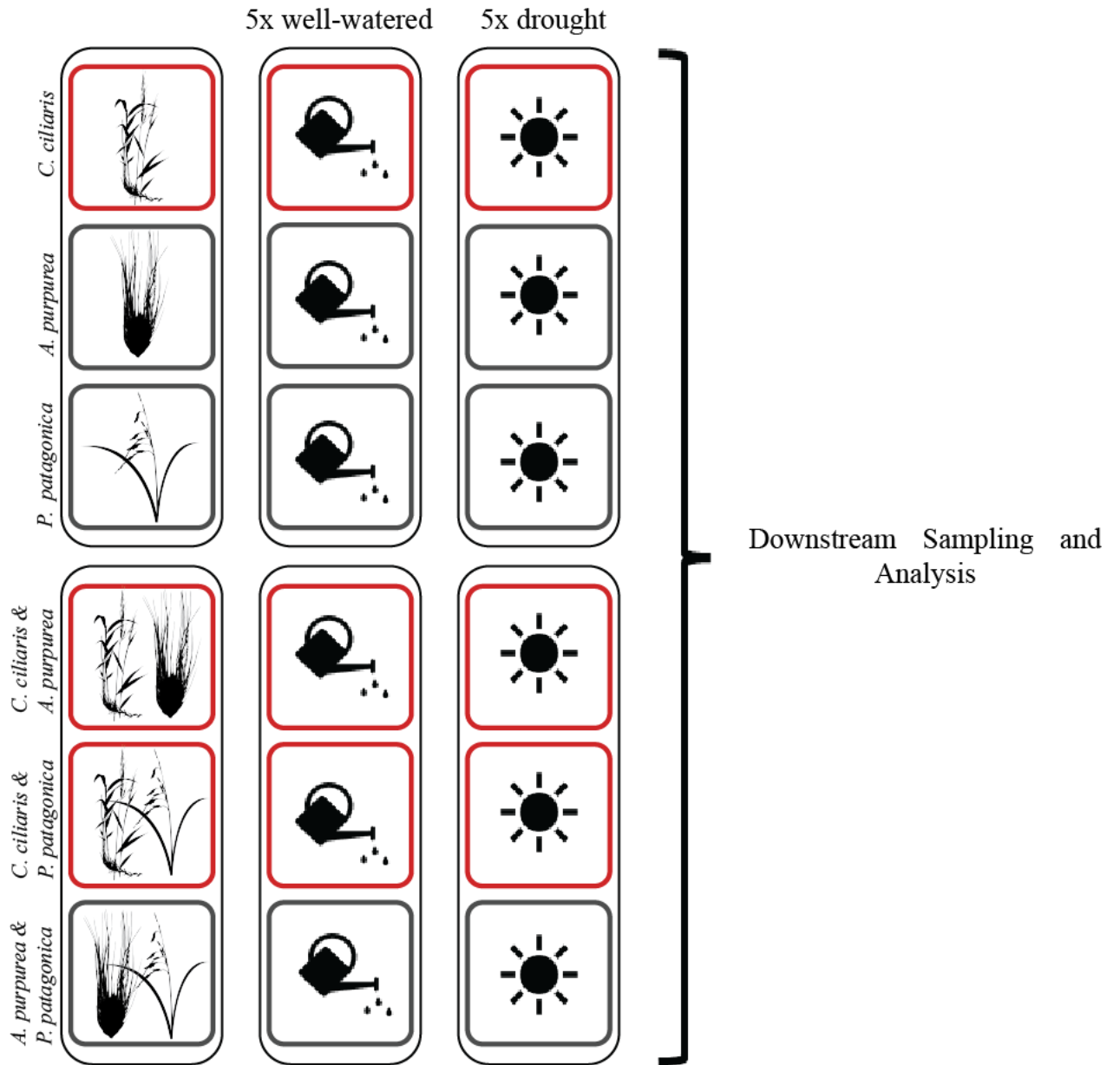
The experiment was conducted in a greenhouse facility at the University of Arizona Campus Agricultural Center, Tucson, Arizona (USA). We selected warm-season plant species that grow in the same habitat as buffelgrass in southern Arizona: *Aristida purpurea* (purple three-awn, perennial C<sub>4</sub> grass), and *Plantago patagonica* (woolly plantain, annual forb). Native seeds were purchased from local vendors (Borderland Restoration Network, Patagonia, and Arizona Revegetation and Monitoring Co, Tucson), and buffelgrass seed was collected along the roadside



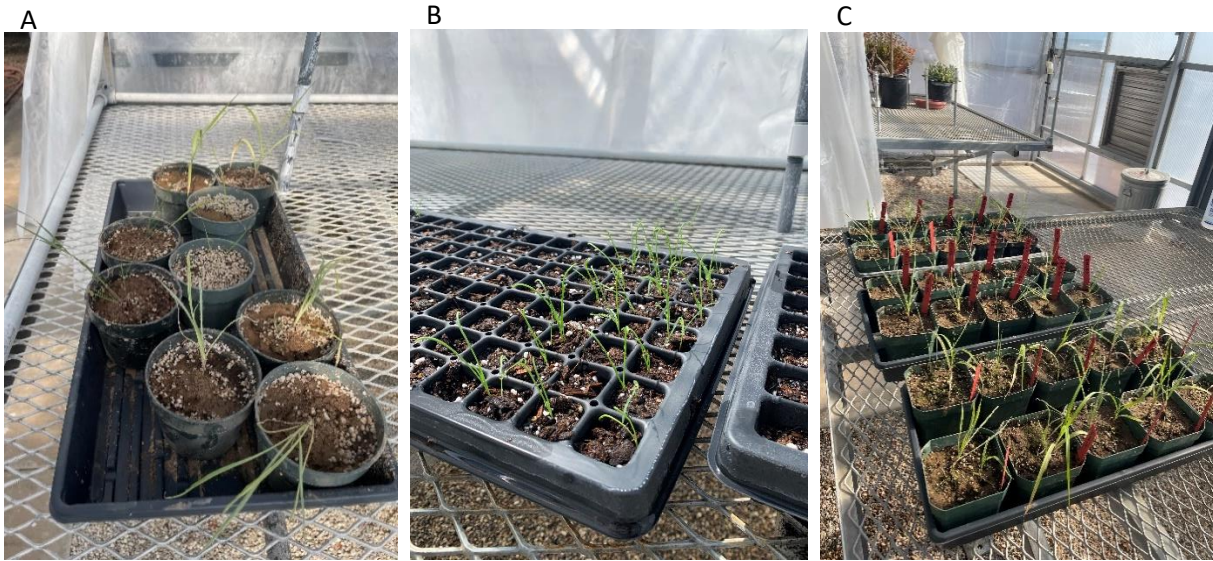
near the intersection of S La Cholla Blvd and W Ajo Way in Tucson, AZ. A preliminary experiment was conducted to uncover the seed germination rates of each plant species. Ten individual seeds of each plant were placed in a brightly lit and damp space and left to germinate for 2 weeks. The seed germination rates were as follows: buffelgrass – 0.5; *A. purpurea* – 0.5; and *P. patagonica* – 0.33. Plants were germinated in an 8x16 germination tray with approximately 1” wide pools of 2” depth filled with potting soil (Espoma Organic Potting Mix, The Espoma Company, Millville, New Jersey, USA). The tray was initially well-watered carefully by hand to avoid destruction of the sowed seeds, then the automatic mister was set for 15 seconds every 15 minutes for 5 weeks (Farrell et al. 2022) (Fig 2B). Upon adequate germination wherein the plants entered the fast-growing vegetative stage, the plants were thinned into individual specimens. We filled 60 1-pint pots with a 2:1 mix of sandy, gravely soil gathered from a local soil pit (Marana, Arizona, USA) and perlite. This mix resulted in a standardized soil medium with good drainage emulating desert soil. All pots were filled 2.5 cm below the rim of the pot. Each species was planted alone in a pot (1 plant/pot) and planted in a crossed design with the two other species (Fig 1, Fig 2C).

We examined the impact of drought using two watering conditions (well-watered vs. drought). Watering treatments were chosen based on summer monsoon season patterns in the region. That is, the well-watered treatment represented a similar average monsoon year (4 days between watering events), while the drought treatment (8 days between watering events) represented predicted changes in precipitation frequency in the region (Farrell et al. 2022). A preliminary watering experiment for the drought treatment concluded that 8 days between watering events was adequate to alter native plant traits but did not result in extreme senescence (Fig 2A). All watering events were done to soil field capacity. Greenhouse daytime temperature averaged

26 °C with 75% relative humidity and nighttime temperature averaged 20 °C with 40% relative humidity. Each treatment combination had five replicates (6 plant treatments, 2 watering treatments, 5 replicates; n = 60) (Fig 1).



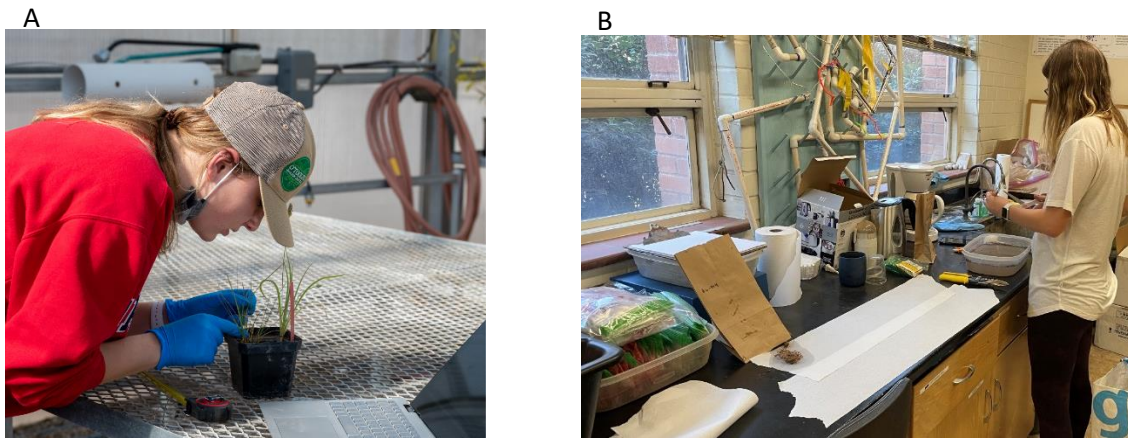
**Figure 1.** Experimental Design



**Figure 2.** A) Preliminary watering rate experiment, B) Germination tray, C) One-week post-transplanting of germinated plants to treatment pots.

### **Plant and soil collection**

Plant and soil samples were collected on January 30<sup>th</sup>, 2022, 10 weeks after the experiment was initiated (Fig 3). Each individual plant was carefully removed to avoid damaging roots, and the number of flowering culms and aboveground height were measured. Plants were washed of excess soil and debris using brushes, dried on paper towels, and separated at the crown or the location where the roots meet the culms. Shoot and root samples were dried in an oven at 70 °C until constant weight (i.e., approximately 72 hours), and weighed for total dry root and shoot biomass measurements. Upon plant collection, soil from within the pots and around the roots was collected and placed into Whirl-Pak bags (Sigma-Aldrich, St. Louis, MO, U.S.A). Soil bags were then kept on ice and immediately stored in a -80 °C freezer.



**Figure 3.** A) Collecting plant trait data at the University of Arizona Campus Agricultural Center Greenhouses, B) Washing and drying individual plant samples at the University of Arizona

### **Molecular analyses and sequence processing**

We used the DNeasy PowerLyzer PowerSoil kit (Qiagen, Hilden, Germany) to extract total genomic DNA following the manufacturer's protocols. To characterize bacterial/archaeal communities, we PCR amplified the V4 hypervariable region of the 16S RNA gene using the primers 515-F (GTGCCAGCMGCCGCGGTAA) and the 806-R (GGACTACHVGGGTWTCTAAT) (Walters et al. 2016). For fungal communities, the first internal transcriber region (ITS1) was PCR amplified using the primers ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGCGTTCTTCATCGATGC) (Walters et al. 2016). PCR was conducted in 40  $\mu$ l triplicate reactions per sample, using 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 consisted of an initial denaturing step at 95 °C for 1 min, 35 cycles of amplification (95 °C for 15 s, 60 °C for 15 s, and 72 °C for 15 s), and a final elongation step of 72 °C for 3 min. Negative controls were included to detect potential contamination. PCR products were quantified

with the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Waltham, MA USA), pooled in equimolar concentrations, and sequenced on a 2x150 bp Illumina MiSeq platform. Sequencing was conducted at the PANDA Core for Genomics and Microbiome Research, University of Arizona.

Sequence reads were demultiplexed using `idemp` and processed using the DADA2 pipeline (Callahan et al. 2016). 16S rRNA reads were trimmed, and due to length variation in the ITS region, fungal primers were removed with `cutadapt` (Martin 2011). Reads that exceeded a maximum error of 2 or more bp were removed. Filtered reads were used to train the error model and to infer amplicon sequence variants (ASVs). Paired-end reads were merged, and chimera sequences were removed. We assigned taxonomy using the Ribosomal Database Project (RDP) classifier (Wang et al. 2007) with the SILVA database (Quast et al. 2012) for 16S ASVs and the UNITE database (Nilsson et al. 2019) for ITS ASVs. Any 16S ASVs without a bacterial or archaeal domain classification or classified as mitochondria or chloroplasts were removed. ITS ASVs without a fungal domain assignment were removed. The number of bacterial/archaeal sequences per sample ranged from 37,623 to 305,825. Fungal sequences per sample ranged from 4,418 to 82,435. Functional groups of Bacteria/Archaea were inferred 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 PRJNA984306.

## Statistical analyses

All data analyses and visualizations were performed in R version 4.3.0. We explored the associations among plant functional traits (i.e., height, number of flowering culms, total dry biomass, root-shoot ratio, and root mass fraction) using principal component analysis (PCA). We assessed plant and drought treatment differences in plant functional traits using linear models. We calculated a competitive response index ( $C_r$ ) by dividing the mean total dry biomass in the presence of neighboring individuals of a different species to the mean total dry biomass in monoculture. A higher  $C_r$  value indicates a stronger ability to resist competitive suppression (Goldberg & Fleetwood 1987).

To study soil microbial diversity, we rarefied sequence counts to 35,000 for Bacteria/Archaea and 4,400 for Fungi to control for differences in sequencing depth. We assessed the effect of plant and drought treatments on soil microbial richness (number of different ASVs) and diversity (measured using the Shannon index) using linear models. To evaluate soil microbial compositional changes, we calculated Bray-Curtis dissimilarities. Non-metric multidimensional scaling (NMDS) was used to visualize differences in microbial composition, and permutational multivariate analysis of variance (PERMANOVA) was used to evaluate the effect of the plant and drought treatments (Anderson 2001). Multivariate statistics were performed using the vegan package version 2.6-4. Microbial functional group differences among treatments 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.

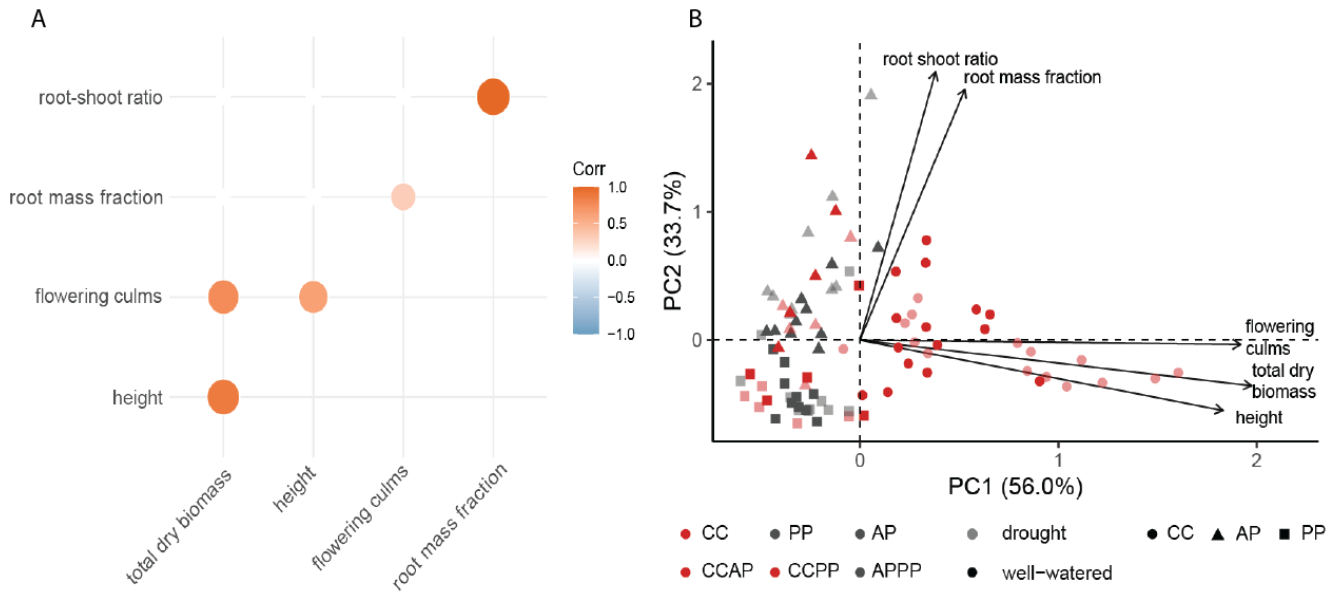
### 3. RESULTS

Buffelgrass grew bigger, taller and had more flowering culms in monoculture or in competition than native species, particularly under the drought treatment (Table 1; Fig. 4B; Appendix). Overall, buffelgrass showed a stronger competitive response to *A. purpurea* ( $C_r = 2.60$ ) than to *P. patagonica* ( $C_r = 2.04$ ). Overall, biomass allocation to roots was not significantly different between the watering treatments, but plant treatments within watering treatments (i.e., interaction) were significantly different (Table 1; Fig. 4B; Appendix). For instance, when planted alone, *A. purpurea* tended to increase its biomass root allocation under drought conditions, but in competition under drought root allocation was reduced compared to solo planted (Table 1; Fig. 4B; Appendix).

**Table 1.** Competitive response indices for buffelgrass and *A. purpurea* according to relative plant ID's. Linear model results of the effect of treatment and competition on plant functional traits. Bold values indicate significant effects ( $P < 0.05$ ).

|   | Plant of Interest  | $C_r$  |                |       |
|---|--------------------|--------|----------------|-------|
| Buffelgrass + <i>A. purpurea</i>          | Buffelgrass        | 2.597  |                |       |
| Buffelgrass + <i>P. patagonica</i>        | Buffelgrass        | 2.036  |                |       |
| <i>A. purpurea</i> + <i>P. patagonica</i> | <i>A. purpurea</i> | 0.835  |                |       |
|   |                    | F      | P              | $R^2$ |
| Root-Shoot Ratio                          | Plant              | 0.636  | 0.673          | 0.43  |
|   | Drought            | 0.003  | 0.959          |       |
|   | Interaction        | 4.124  | <b>0.002</b>   |       |
| Root Mass Fraction                        | Plant              | 0.534  | 0.750          | 0.53  |
|   | Drought            | 0.075  | 0.786          |       |
|   | Interaction        | 3.307  | <b>0.009</b>   |       |
| Height                                    | Plant              | 9.131  | < <b>0.001</b> | 0.84  |
|   | Drought            | 0.091  | 0.764          |       |
|   | Interaction        | 3.494  | <b>0.007</b>   |       |
| Total Dry Biomass                         | Plant              | 15.719 | < <b>0.001</b> | 0.82  |
|   | Drought            | 12.601 | < <b>0.001</b> |       |
|   | Interaction        | 1.834  | 0.117          |       |
| Number of Flowering Culms                 | Plant              | 7.106  | < <b>0.001</b> | 0.75  |
|   | Drought            | 21.465 | < <b>0.001</b> |       |
|   | Interaction        | 3.713  | <b>0.004</b>   |       |





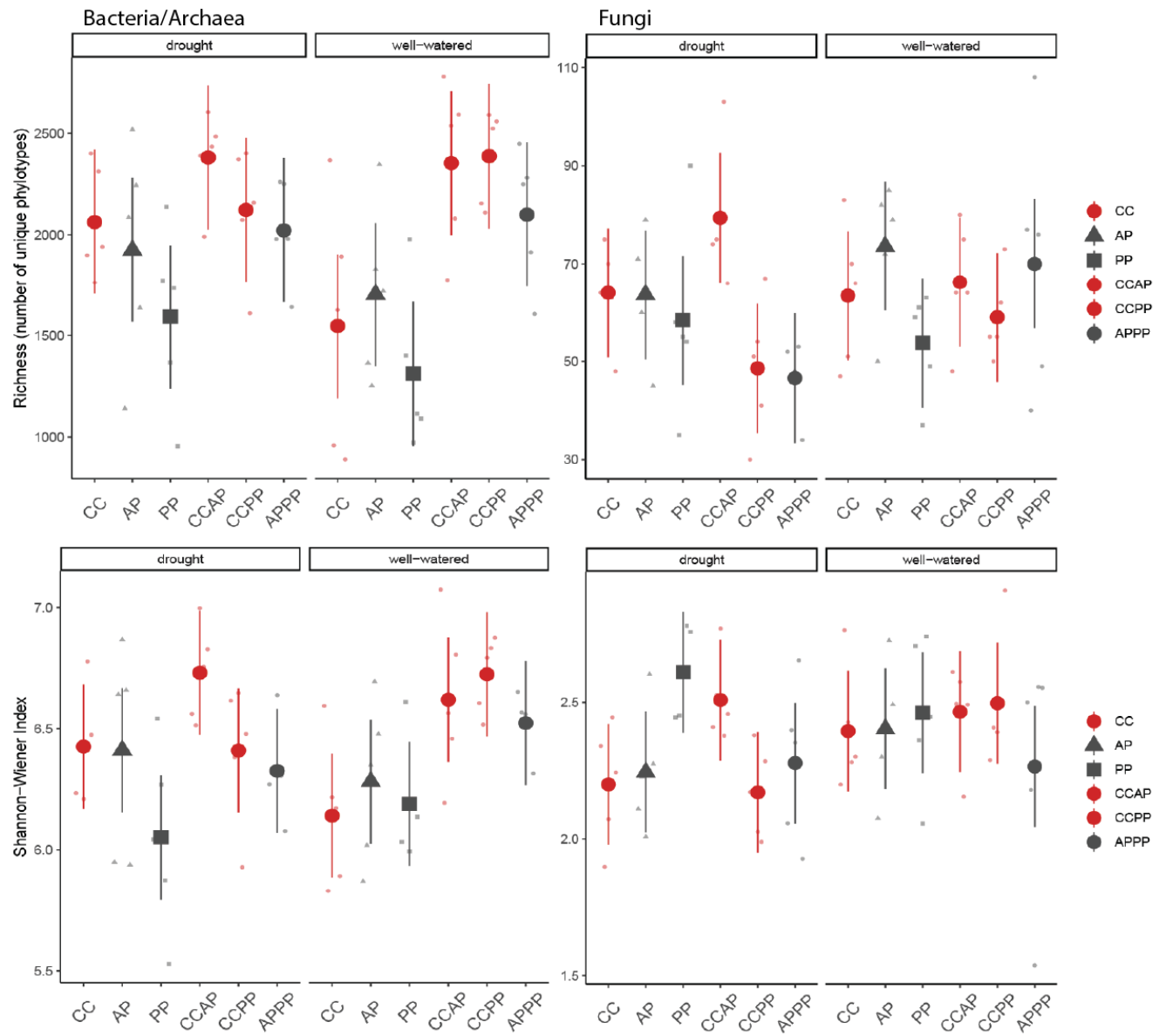
**Figure 4.** A) Significant Spearman's correlations ( $P < 0.05$ ) among plant functional traits. Larger circles and higher color intensity indicate stronger correlations, B) Principal Component Analysis (PCA) of plant functional traits.

The total number of different bacterial/archaeal ASVs after rarefaction was 20,597. Bacterial/archaeal richness per sample ranged from 890 to 2,766. The total number of different fungal ASVs was 1,001. Fungal richness per sample ranged from 30 to 108. Bacterial/archaeal richness was significantly different among plant treatments (Table 2; Fig. 5). In particular, plant treatments with any combination of two species had higher bacterial/archaeal richness than monocultures, and pots with buffelgrass (solo or in combination) tended to present higher richness (Fig. 5). On the contrary, fungal richness did not vary across any treatments (Table 2; Fig. 5). For both bacterial/archaeal and fungal richness, we did not observe a significant effect of the drought treatment (Table 2; Fig. 5). Similar patterns were observed for Shannon diversity (Fig 5).



**Table 2.** Linear model results of the effects of plant identity, treatment, and their interaction on soil microbial diversity. PERMANOVA results of the effects of plant identity, treatment, and their interaction on soil bacterial/archaeal and fungal compositional dissimilarity.

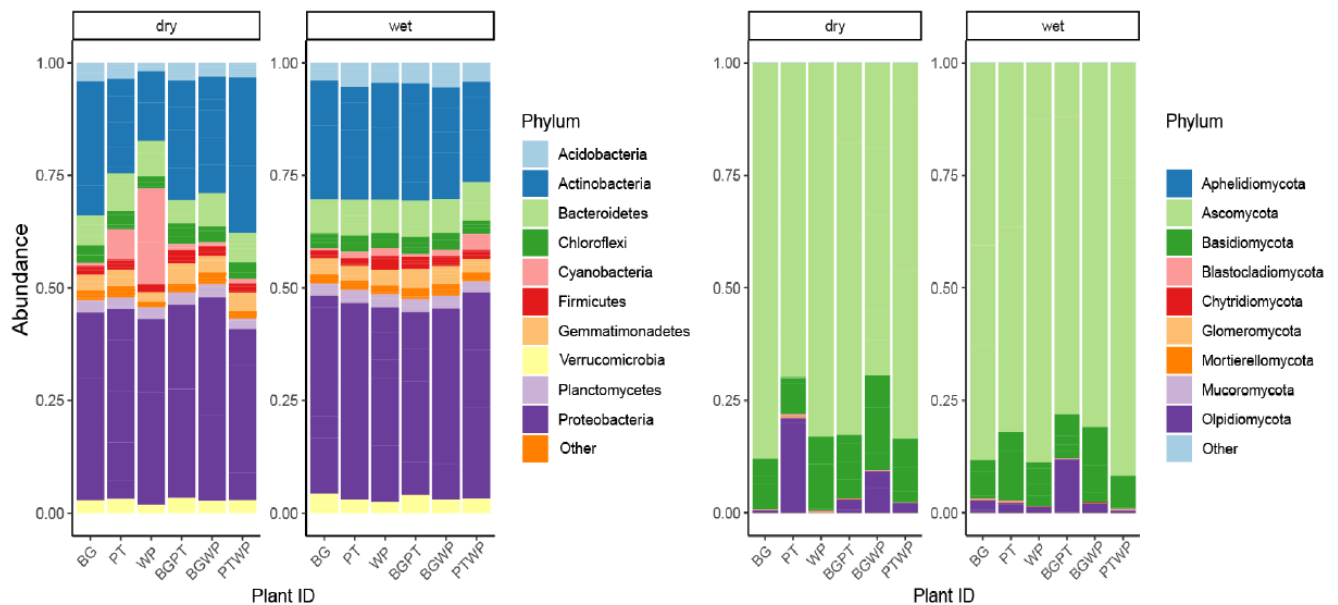
|                                       |             | F    | P              | R <sup>2</sup> |
|---------------------------------------|-------------|------|----------------|----------------|
| Bacterial/archaeal richness           | Plant       | 7.26 | < <b>0.001</b> | 0.477          |
|                                       | Drought     | 1.44 | 0.234          |                |
|                                       | Interaction | 1.23 | 0.309          |                |
| Bacterial/archaeal Shannon            | Plant       | 6.64 | < <b>0.001</b> | 0.466          |
|                                       | Drought     | 2.35 | 0.131          |                |
|                                       | Interaction | 1.27 | 0.288          |                |
| Fungal richness                       | Plant       | 2.22 | 0.067          | 0.301          |
|                                       | Drought     | 1.42 | 0.237          |                |
|                                       | Interaction | 1.63 | 0.169          |                |
| Fungal Shannon                        | Plant       | 2.27 | 0.061          | 0.282          |
|                                       | Drought     | 1.85 | 0.179          |                |
|                                       | Interaction | 1.11 | 0.363          |                |
|                                       |             | F    | P              | R <sup>2</sup> |
| Bacteria/archaea Bray Curtis distance | Plant       | 2.37 | <b>0.001</b>   | 0.16           |
|                                       | Drought     | 4.50 | <b>0.001</b>   | 0.06           |
|                                       | Interaction | 1.54 | <b>0.001</b>   | 0.10           |
| Fungi Bray Curtis distance            | Plant       | 1.96 | <b>0.001</b>   | 0.14           |
|                                       | Drought     | 2.30 | <b>0.001</b>   | 0.03           |
|                                       | Interaction | 1.42 | <b>0.001</b>   | 0.10           |



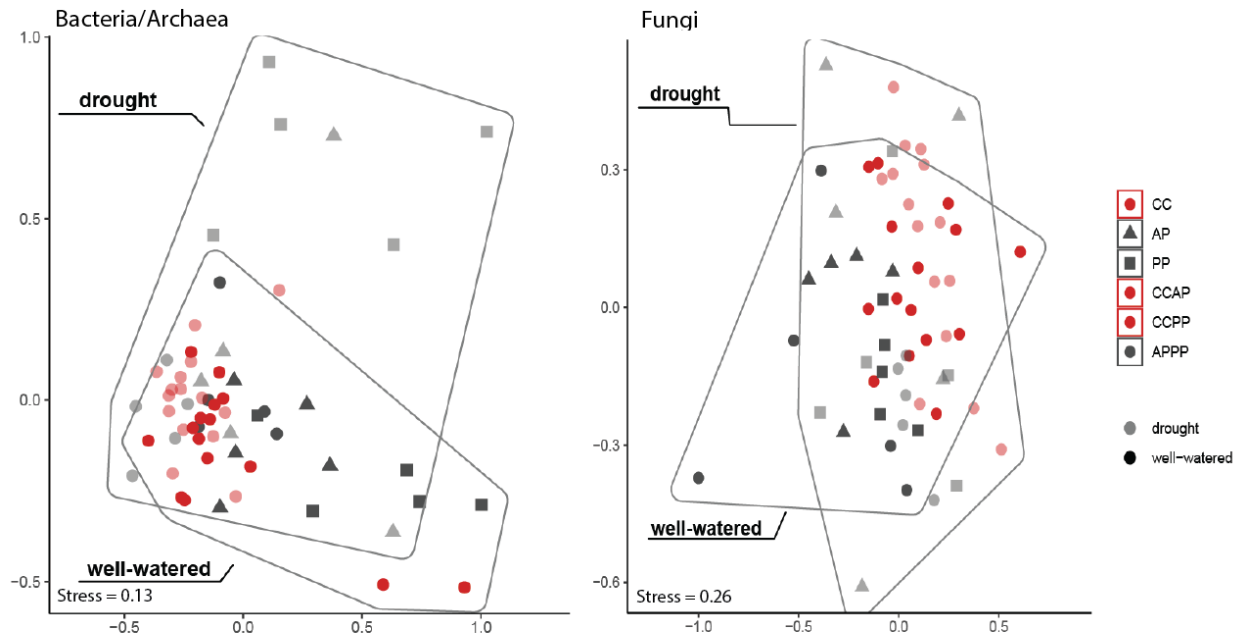
**Figure 5.** Soil microbial Richness and evenness for Bacteria/Archaea and Fungi across plant identities and different treatments. Point ranges show model means and standard errors.

Soil bacterial/archaeal communities were dominated by Proteobacteria (42.43%), Actinobacteria (25.44%), Bacteroidetes (7.34%), Acidobacteria (3.95%), and Gemmatimonadetes (3.71%). Soil fungal communities were dominated by Ascomycota (80.73%), Olpidiomycota (9.42%), and Basidiomycota (9.28%) (Fig 6). Overall soil bacterial/archaeal and fungal

compositions were significantly different across plant treatments, watering treatments, and their interaction (Table 2; Fig 7). For both Bacteria/Archaea and Fungi, differences across plants explained a larger part of the variation (Table 2; Fig 7). As expected from the results of overall microbial composition, the abundance of most soil microbial functional groups varied significantly across different plant treatments (except for denitrifiers, chitinolytic and cellulolytic bacteria; Table 3). For instance, the abundance of fungal plant pathogens under well-watered conditions increased compared to the native monocultures when different species were planted together (Table 3; Fig. 8). The abundance of nitrogen fixers was higher when native plants were planted with buffelgrass only under drought conditions (Table 3; Fig. 8). The abundance of nitrifiers was generally higher in the well-watered treatment (Table 3; Fig. 8).



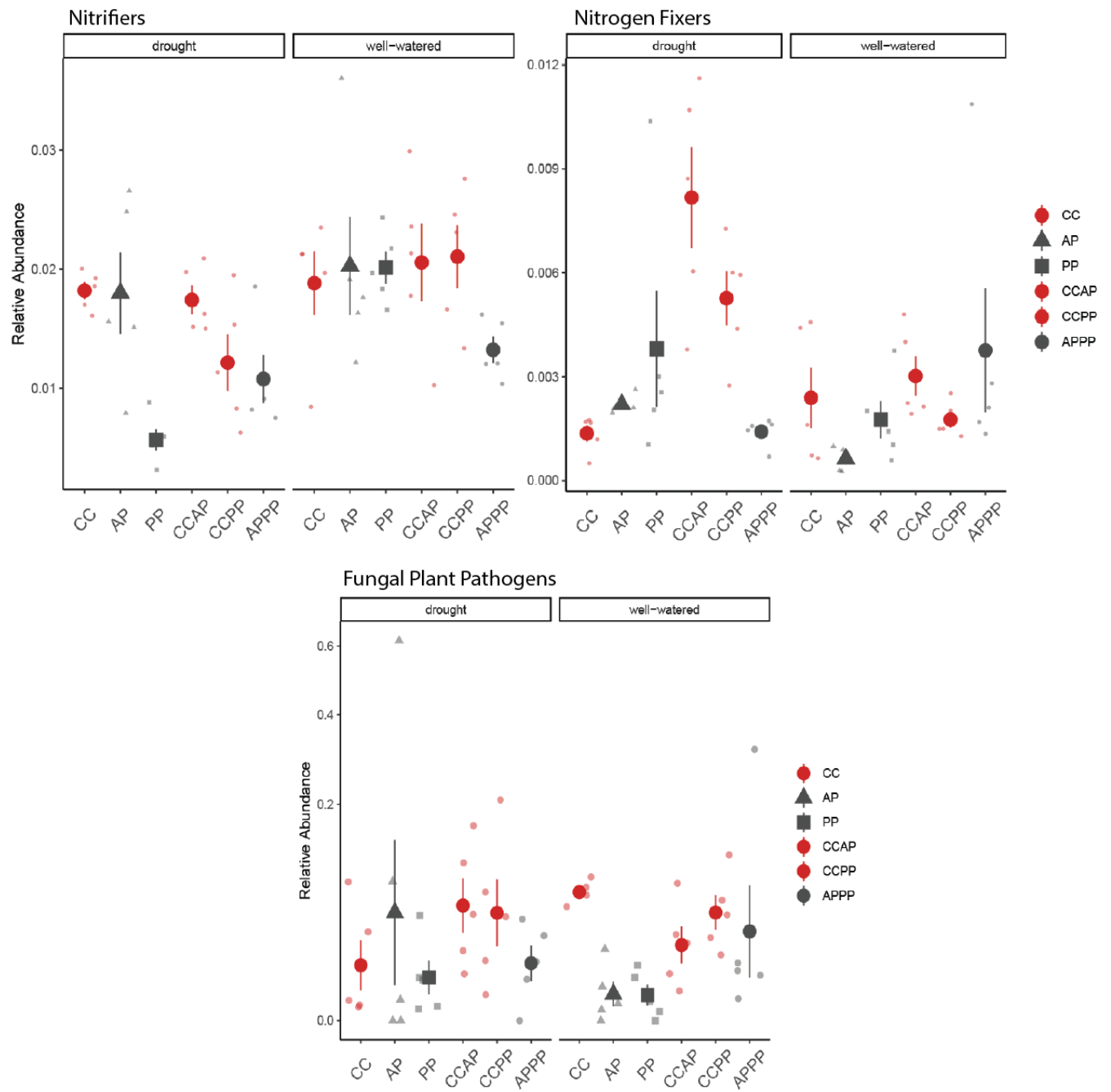
**Figure 6.** Soil microbial taxonomic composition across plant identity and treatment.



**Figure 7.** Non-metric multidimensional scaling (NMDS) ordination plot of microbial community dissimilarity (Bray-Curtis) for Bacteria/Archaea and Fungi. Figure shows all samples plotted together to visualize clustering based on plant identity and wet/dry treatment (stress = 0.12 & 0.26, respectively).

**Table 3.** Taxonomic variations among plant identity, treatment, and their interaction results of generalized linear models with negative binomial distributions. Bold values indicate significant effects ( $P < 0.05$ ).

|   |             | X <sup>2</sup> | P              | R <sup>2</sup><br>(Nagelkerke) |
|---|-------------|----------------|----------------|--------------------------------|
| <b>Bacteria/archaea</b>                     |             |                |                |                                |
| Methylotrophic bacteria                     | Plant       | 26.934         | < <b>0.001</b> | 0.528                          |
|   | Drought     | 0.072          | 0.789          |                                |
|   | Interaction | 5.506          | 0.357          |                                |
| Nitrifiers                                  | Plant       | 23.445         | < <b>0.001</b> | 0.805                          |
|   | Drought     | 23.850         | < <b>0.001</b> |                                |
|   | Interaction | 30.151         | < <b>0.001</b> |                                |
| Denitrifiers                                | Plant       | 4.017          | 0.547          | 0.226                          |
|   | Drought     | 0.996          | 0.318          |                                |
|   | Interaction | 5.439          | 0.365          |                                |
| Chitinolytic bacteria                       | Plant       | 7.872          | 0.163          | 0.478                          |
|   | Drought     | 4.544          | <b>0.033</b>   |                                |
|   | Interaction | 15.724         | <b>0.007</b>   |                                |
| Nitrogen Fixers                             | Plant       | 44.437         | < <b>0.001</b> | 0.856                          |
|   | Drought     | 10.043         | <b>0.002</b>   |                                |
|   | Interaction | 40.449         | < <b>0.001</b> |                                |
| Cellulolytic bacteria                       | Plant       | 4.636          | 0.462          | 0.356                          |
|   | Drought     | 0.974          | 0.324          |                                |
|   | Interaction | 12.649         | <b>0.027</b>   |                                |
| Fermenters                                  | Plant       | 17.870         | <b>0.003</b>   | 0.593                          |
|   | Drought     | 2.655          | 0.103          |                                |
|   | Interaction | 18.873         | <b>0.002</b>   |                                |
| Ureolytic bacteria                          | Plant       | 13.459         | <b>0.019</b>   | 0.534                          |
|   | Drought     | 0.802          | 0.370          |                                |
|   | Interaction | 18.536         | <b>0.002</b>   |                                |
| <b>Fungi</b>                                |             |                |                |                                |
| Saprotrophs (Dung, Soil, Wood, & Undefined) | Plant       | 16.026         | <b>0.006</b>   | 0.525                          |
|   | Drought     | 1.132          | 0.287          |                                |
|   | Interaction | 16.314         | <b>0.006</b>   |                                |
| Fungal plant pathogens                      | Plant       | 13.050         | <b>0.023</b>   | 0.481                          |
|   | Drought     | 0.004          | 0.950          |                                |
|   | Interaction | 17.407         | <b>0.004</b>   |                                |



**Figure 8.** Soil bacterial/archaeal functional groups with  $p$ -value  $< 0.05$  for Plant ID.

## 4. DISCUSSION

Co-occurring stressors such as climate disturbances and biological invasions are significant threats to biodiversity and ecosystem functioning (Gornish & Miller 2015; Fahey et al. 2020). For instance, drought can modulate plant-plant interactions via resource competition, and plant-soil interactions, which, in turn, can also mediate plant competition as plants depend on microbial activity to make soil nutrients bioavailable (Suttle et al. 2007; Pugnaire et al. 2019). Moreover, soil microorganisms can play important roles in facilitating or hindering plant invasion (van der Putten et al. 2007). Finally, drought can interact synergistically or antagonistically with invaders. For example, competitive interactions between native and invasive plants can either be disrupted during intense environmental stress (Davis et al. 2000), or invasive plants may mitigate drought effects on natives via habitat modification (Rodriguez 2006). Arid ecosystems in the southwest USA might be particularly susceptible to the interactive effects of these stressors as water availability is the most critical factor regulating biological activity (Schwinning & Sala 2004), and in recent decades non-native grasses are spreading and transforming the fire regime (Fusco et al. 2019).

### **Effects of drought and competition on buffelgrass' growth and biomass allocation**

Although invasive plants are expected to disproportionately succeed in high resource environments, some invaders have specific traits associated with higher resource use efficiency or faster recovery after a stress pulse than native species, allowing them to become dominant in low resource environments such as arid ecosystems (Van Kleunen et al. 2010; Funk 2013).

In our greenhouse experiment, buffelgrass maintained a high growth rate even under the drought watering treatment, similar to other studies (Valliere 2019; Farrell et al. 2022). In addition,

buffelgrass produced a larger number of flowering culms in drier conditions, a typical drought escaping strategy (Kooyers 2015; Shavrukov et al. 2017). Surprisingly, when buffelgrass was grown with native neighbors (particularly under the drought watering treatment), buffelgrass responded by growing more although its biomass allocation did not change. Natives' growth was similar across all treatments, including when grown with buffelgrass. Hence, in our controlled greenhouse conditions, we did not observe buffelgrass' negative interspecific competition effects on natives as reported in other greenhouse studies (Stevens & Fehmi 2011; Farrell et al. 2022), or dehydration symptoms and reduced photosynthetic rates in neighboring vegetation as reported in the field (Alexander Eilts & Huxman 2013; Castellanos et al. 2016). While *A. purpurea* reduced its root allocation in competition under drier conditions but increased its root allocation in the well-watered treatment, *P. patagonica* biomass allocation was similar across treatments adding evidence to the large variability of responses to competition and drought (Lozano et al. 2020; Asefa et al. 2022).

Although it has been documented the ability of buffelgrass to adjust its strategy depending on its neighbor's strategy (Farrell et al. 2022), our findings show that, as most invasive species, buffelgrass employs the strategy of rapid growth and accelerated lifecycle whereas native plants tend to be more conservative (Van Kleunen et al. 2010).

### **Effects of drought and plant competition on soil microbial communities**

While water availability regulates soil microbial ecophysiology (Schimel 2018) and total biomass (Evans & Wallenstein 2012), in general, drought has little impact on soil microbial diversity (Naylor & Coleman-Derr 2018). Correspondingly, we did not observe a significant effect of watering treatments on soil bacterial or fungal diversity. Bacterial and fungal richness have been shown to be disproportionately affected by water availability wherein bacterial richness is



decreased and fungal richness increases (Fahey et al. 2020). On the other hand, watering treatments weakly but significantly shifted soil microbial composition. As observed in previous studies, bacterial communities were more responsive to drought than fungi (Evans & Wallenstein 2012; Barnard et al. 2013; Ochoa-Hueso et al. 2018). Soil communities associated with buffelgrass were dominated by bacterial/archaeal phyla consistent with previous findings (Gornish et al. 2020; Jara-Servin et al. 2023) and with the overall composition of arid regions (Maestre et al. 2015; Naylor & Coleman-Derr 2018). Specifically, nitrifiers were more abundant in well-watered treatments, while the proportion of nitrogen fixers was higher in drier conditions. These results are expected because low water availability inhibits nitrification in soil (Stark & Firestone 1995), and association with nitrogen fixers alleviates plant water stress (Pellegrini et al. 2016). Other symbiotic microorganisms such as arbuscular mycorrhizal fungi can also buffer the negative effects of drought (Porter et al. 2020), but as we used barren local soil and perlite (i.e., without any presence of vegetation) as soil medium, mycorrhizal fungi were essentially undetectable in our samples.

Although plant traits and resource use strategies can partially explain the success of invasive species (Van Kleunen et al. 2010), more and more studies show how invasives modify soil microbial communities, with consequences for ensuing plant growth, development and survival (Reinhart & Callaway 2006; van der Putten et al. 2007; Meisner et al. 2013; Fahey et al. 2020). For instance, soil biota richness generally increases following plant invasion as plant species drive the diversity of soil resources and microhabitats through complementarity in root structures and root exudate profiles (Pyšek et al. 2012; Lamb et al. 2011). In our greenhouse experiment, buffelgrass grown with neighboring natives increased soil bacterial richness but not fungal richness. Alternatively, Gornish et al. (2020) did not observe significant changes in soil

community richness between buffelgrass invaded and uninvaded sites in arid southeastern Arizona. Potential reasoning for this discrepancy could be related to effects of natural versus artificial communities. Previous field studies reported increases in soil fungal richness with plant invasion (Si et al. 2013; Fahey et al. 2020). Although we hypothesized that the competition treatment would promote the abundance of fungal saprotrophs by providing a larger diversity of resources, our results show high variability across treatments and no consistent trend with plant competition.

Invasive plants may benefit through decreased pathogen exposure, increased mutualistic interactions relative to native plants or by disrupting the nitrogen cycle (Reinhart & Callaway 2006; Liao et al. 2008; Pei Zhang et al. 2019). In the well-watered treatment, the proportion of fungal plant pathogens was higher in pots with buffelgrass alone and pots with a combination of two plant species. In drier conditions, the abundance of fungal plant pathogens was higher when buffelgrass was grown with a native neighbor. Thus, although these results suggest that fungal plant pathogens' abundance increase in the presence of two plant species, no large and consistent trend was observed in relationship to buffelgrass, reinforcing meta-analytical studies that show that plant-soil feedbacks may not be generally important as a mechanism for plant invasion (Suding et al. 2013; Meisner et al. 2014; Crawford et al. 2019).

The proportion of nitrogen fixers was the highest when buffelgrass was grown with a native neighbor only in drier conditions. Many successful invasive plants benefit from mutualisms with nitrogen-fixing bacteria (Reinhart & Callaway 2006; Liao et al. 2008), and our results indicate that this might also be the case with buffelgrass under the conditions tested. Although invasive plants tend to be generally associated with higher nitrification rates (Hawkes et al. 2005; Liao et al. 2008), and in particular, soils from buffelgrass-invaded areas in the Sonoran Desert had a larger

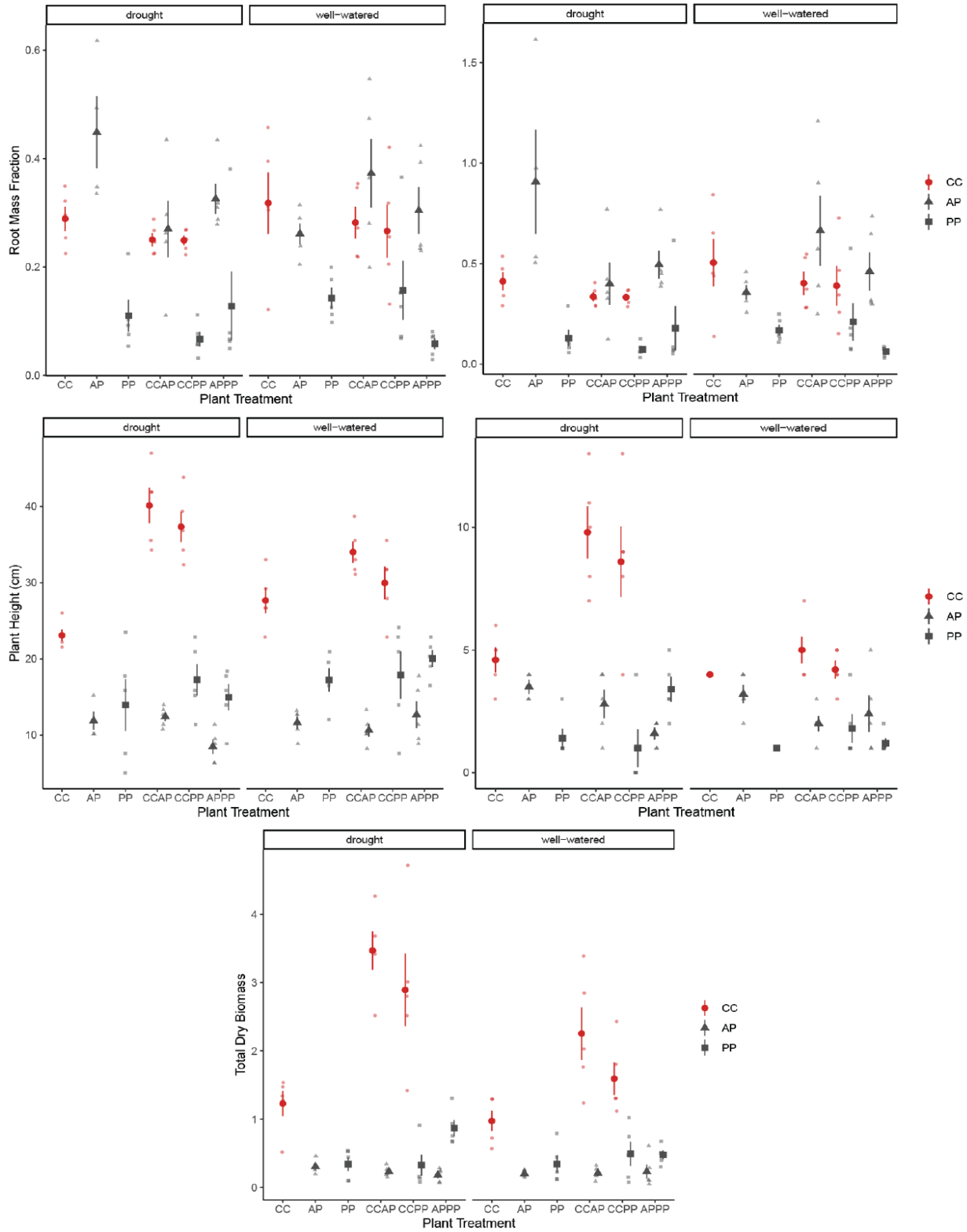
proportion of nitrifiers than uninvaded soil (Gornish et al. 2020), we did not observe any clear association between the presence of buffelgrass (solo or in combination) and the abundance of nitrifying microorganisms.

## 5. CONCLUSION

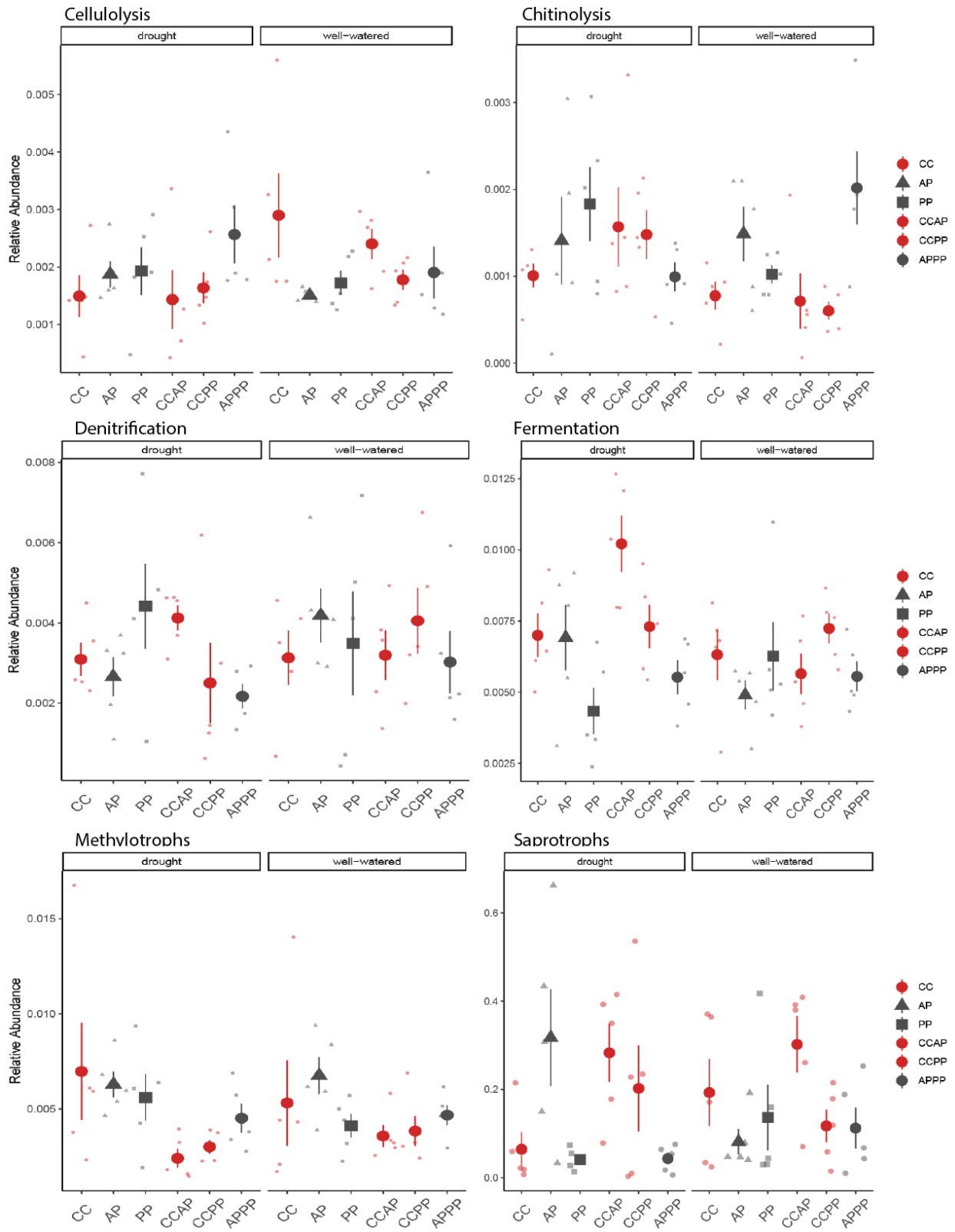
Understanding the interactive effects of plant invasions and drought is essential for predicting future ecosystem changes (Gornish & Miller 2015). Studying plant competition and plant-soil interactions in a greenhouse setup enabled us to interpret potential mechanisms without the limitations of environmental heterogeneity in the field (Gibson et al. 1999). However, experiments conducted in the field or in the greenhouse can yield different (and even opposite) plant growth and plant-soil responses (Forero et al. 2019). For example, in this greenhouse study we observed a positive effect of buffelgrass when grown with a native on soil bacterial richness but no effects whatsoever on fungal richness, while a similar field experiment with the invasive C<sub>4</sub> grass *Imperata cylindrica* (cogongrass) found that soil fungal richness was affected by both invasion and drought but bacterial richness was unaffected by invasion (Fahey et al. 2020).

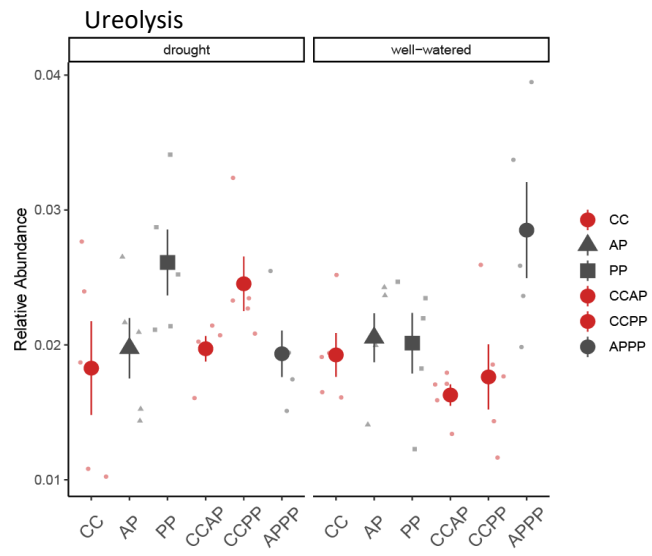
Buffelgrass' ability to spread in low resource habitats, outcompete native species for resources, alter fire regimes, and resist eradication is a major threat to arid ecosystems (Farrell & Gornish 2019). Our results showed that buffelgrass benefitted from growing in the vicinity of a native, particularly in drier conditions, and our high-throughput sequencing approach suggest that this effect might be mediated by soil bacterial richness and an increased proportion of nitrogen-fixing bacteria. Overall, these findings solidify the idea of incorporating plant-soil interactions in the fields of invasion science and restoration ecology in order to tease apart the intricacies of interactive effects of abiotic and biotic anthropogenic stressors.

## APPENDIX A – SUPPLEMENTARY DATA



**Figure 1.** Plant functional traits along planting identities and watering treatments.





**Figure 2.** Relative abundance of functional groups by plant ID and treatment.

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