Resistance and resiliency of soil microbiomes under climate change

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Abstract

Soil microbiomes play key roles in plant productivity and nutrient cycling, and we need to understand whether and how they will withstand the effects of global climate change. We exposed *in situ* soil microbial communities to multiple rounds of heat, drought, or both treatments, and profiled microbial communities with 16S rRNA and ITS amplicon sequencing during and after these climatic changes, and then tested how domain and symbiotic lifestyle affected response. Fungal community composition strongly shifted due to drought and its legacy. In contrast, bacterial community composition resisted change during the experiment, but still was affected by the legacy of drought. We identified fungal and bacterial taxa with differential abundance due to heat and drought and found that taxa affected during climate events are not necessarily the taxa affected in recovery periods, showing the complexity and importance of legacy effects. Additionally, we found evidence that symbiotic groups of microbes important to plant performance respond in diverse ways to climate treatments and their legacy, suggesting plants may be impacted by past climatic events like drought and warming even if they do not experience the event themselves.

Introduction

Anthropogenic climate change is increasing both global temperature and the frequency and severity of weather events like drought (Thuiller 2007). Soil microbial communities are among the largest and most diverse ecosystems on earth (Singh et al. 2009) and their response to climate change will affect elemental cycling, greenhouse gas production, plant-soil interactions, and ecosystem services and agriculture (Dubey et al. 2019; Jansson & Hofmockel 2020; ASM Report 2022). Here, we examine the effect of repeated heat and drought events on a soil

microbiome across time, characterizing how fungal and bacterial communities resist, and recover from, heat and drought events.

Resistance in a microbial community represents how quickly and by what magnitude a community responds to shifting environmental conditions, whereas recovery and resilience can be measured by whether community composition reflects historical conditions (Bissett et al. 2013; Hartmann et al. 2013; Martiny et al. 2017). Microbial communities can resist more or less depending on the type and duration of environmental change (Schimel et al. 2007; Zhou et al. 2020), with evidence of microbes being more resistant to short-term than long-term changes (Pec et al. 2021). After an environmental shift, microbial communities are often assumed to have high resilience due to short microbial generation times (Allison and Martiny 2008), but empirical evidence is mixed, as discussed below. Soil ecosystems exhibit differing microbial resistance and resilience to environmental change (Waldrop & Firestone 2006). Ecosystems characterized by natural fluctuations, such as northern temperate environments that experience large differences between winter low and summer high temperatures, provide an interesting opportunity to test microbial resistance and resilience because microbes are expected to be adapted to both temperature extremes.

Microbial communities display highly variable responses to warming and drought. Microbial biomass can increase (Castro et al. 2010) or decrease (Allison & Treseder 2008) in response to heat, with a decrease in microbial biomass accompanying a loss of overall soil carbon (Melillo et al. 2017). Heat can increase species richness (Allison & Treseder 2008) or decrease it (Sheik et al. 2011), yet a meta-analysis of studies found no overall trend of warming or altered precipitation on microbial alpha diversity (Zhou et al. 2020). Instead, both heat and drought often shift microbial community composition (Allison & Treseder 2008; Castro et al.

2010; Zhou et al. 2020; Pec et al. 2021). Drought and heat combined can have more of an effect on microbes than drought alone (Bérard et al. 2011), highlighting the importance of studying multiple climatic factors and their interactions; yet multifactor studies remain rare (Zhou et al. 2020). Furthermore, studies measuring the persistence of these effects, i.e., a community's resilience, show mixed results; some find no persisting legacy effects from climate events on soil community composition (Rousk et al. 2013; Dacal et al. 2022), while others find lasting legacy effects (Averill et al. 2016; Martiny et al. 2017; de Nijs et al. 2019). More empirical evidence on how resilient microbial communities are to climate events is needed (Jansson & Hofmockel 2020) and we need to investigate factors that underlie microbial resilience itself, given that microbes are a major contributor to global nutrient cycling of carbon, phosphorus, and nitrogen (ASM Report 2022).

Differences in resistance and resilience are possible among component members of the microbiome, due to the vast taxonomic and functional diversity of soil microbes (Singh et al. 2009). For example, bacteria are generally fast-growing, with short generation times and the ability to horizontally transfer potentially adaptive genes (Ochman et al. 2000), while fungi show a myriad of life history strategies, many of which require multiple stages (Andrews 1992) and thus longer generation times. In the soil food web, bacteria are considered a 'fast' energy channel that cycles nutrients rapidly, while fungi are considered a 'slow' energy channel that cycles nutrients slowly (Rooney et al. 2006; Bardgett & Caruso 2020). Consistent with this classification, turnover times of soil fungi are estimated to be an order of magnitude longer than those of soil bacteria (Rousk & Bååth 2011). Slow fungal turnover times mean that it could take longer to observe a shift in fungal than in bacterial community composition, but that the effect of

climate stressors on fungi may persist. We therefore hypothesized that fungal communities may be more resistant but less resilient than bacterial communities to climatic perturbations.

Microbial lifestyle (e.g., symbiosis propensity and type) could have implications for how well a microbe can resist or recover from climatic events. In the soil, microbes can live symbiotically with (and generally derive a benefit from) host organisms like plants, animals, and macroscopic fungi (Berendsen et al. 2012), with the outcome of symbiosis ranging from negative to positive for the host. For example, a polyphyletic group of soil bacteria known as rhizobia can facultatively live inside specialized legume root structures called nodules, where the rhizobia provide biologically available nitrogen for the plant in return for carbon (Beringer et al. 1979). Rhizobia genomes are large and contain symbiotic plasmids required for nodule formation (MacLean et al. 2007), and this extra genetic material could hamper growth rates and select for genome streamlining under warming (Hessen et al. 2010; Sabath et al. 2013; Simonsen 2021). The bacterial plant pathogen *Agrobacterium tumefaciens* also carries large plasmids that contain the tumor-inducing genes required for its lifestyle (White & Winans 2007), which could slow replication times. In addition, plants have a ubiquitous, often mutualistic, symbiosis with mycorrhizal fungi wherein mycorrhizae provide mineral nutrients to plants in return for carbon and improve plant tolerance of water stress by extending the root system (Kakouridis et al. 2022). However, mycorrhizal fungi are obligately symbiotic (Smith & Read 1997), and thus the fate of a mycorrhizal fungus under climate stress may depend on the performance of their plant partner and vice versa, to positive or negative effect (Stachowicz 2001; Kiers et al. 2010). In sum, there are clear pathways through which the life history and symbiotic nature of a microbe could underlie facets of the microbe's resistance or resilience to climate events.

We used a factorial experiment to examine the response of soil microbial communities to the interacting climate factors of heat and drought, with an emphasis on measuring resiliency and legacy effects. We identify microbial groups and lifestyles particularly altered under climate change, and discuss why they may be differentially affected.

Methods

Site description and sampling

We conducted this study at the Koffler Scientific Reserve (KSR, www.ksr.utoronto.ca) in Ontario, Canada (latitude 44°01'48”N, longitude 79°32'01”W) in an old field environment (Figure 1A). The average annual precipitation is 767 mm, with the average high temperature reaching 27°C in July and the average low temperature reaching -11°C in January (Koffler Scientific Reserve 2023).

The temperature-free-air-controlled enhancement experiment at KSR was established following Kimball et al. (2008). Plots are 3m in diameter, with six plots having six 100W infrared heaters each (Mor Electric Heating Association) and six plots having equivalent dummy infrared heaters. In 2017, we excavated twelve plots 30 cm deep and filled them with a 1:1:1 mixture of local soil, peat moss, and sand; then, we planted white spruce seedlings (*Picea glauca*, provided by Natural Resources Canada, Laurentian Forestry Centre, Quebec QC) in the plots. A top layer of commercially supplied cedar bark mulch was added in 2020 and 2021, and weeding occurred biweekly to keep plant cover constant. Starting in 2020, we applied climate treatments to plots: control, heat, drought, or heat and drought (Figure 1B). The heaters were activated in the heated plots to increase leaf temperature by 5°C day and night, while the drought plots had concave-up (U) slotted rainout structures that covered ~50% of the plot surface area and drained rain out and away from all plots. To control for the effects of the structures

themselves, non-drought plots had the same rainout structures except in the concave-down position (⋂), allowing all water to pass off the structure and into the plot. There were two periods of active treatment (Table S1): rainout structures were present July-November 2020, and June-October 2021, for a total of 8 months; heaters were active in heated plots August-December 2020, and August 2021-January 2022, for a total of 9 months. Heat and drought treatments were set up in this way to study the physiological and molecular responses of different white spruce genotypes to climatic stressors through the growing season and into early winter.

On June 11th 2021, September 14th 2021, and June 5th 2022, we sampled from the first 10 cm of topsoil from three locations ~50 cm apart in each plot. We collected the June 2021 and 2022 samples when there were no active climate treatments being applied (plots in a recovery period) to test microbial resilience, and the September 2021 samples when climate treatments were being actively imposed to test resistance.

Sequencing and QIIME2

We extracted soil microbial DNA using the QIAGEN DNeasy PowerSoil Pro Kit and sequenced samples at Genome Quebec (Montréal, Canada) using Illumina MiSeq PE 250bp 16S rRNA amplicon sequencing on the conserved hypervariable V4 region (primer pair 515F-806R) and ITS region (primer pair ITS1FP2-58A2RP3). Samples from 2021 and 2022 were sequenced separately. We used Quantitative Insights Into Microbial Ecology 2 (QIIME2) v.2022.2 (Bolyen et al. 2019) to trim sequences and then denoised sequences with DADA2 (Callahan et al. 2016). Using QIIME2, we removed ASVs that had fewer than 10 reads across all samples, and assigned taxonomy using the ‘sklearn’ feature classifier (Pedregosa et al. 2011); we used Greengenes 16S V4 region reference for bacteria (McDonald et al. 2012), and UNITE (Nilsson et al. 2019) version 9.0 with dynamic clustering of global and 97% singletons for fungi.

We then filtered out reads assigned as cyanobacteria and mitochondria to remove plant and animal DNA. Finally, for each of the bacterial and fungal datasets we constructed a phylogeny using QIIME2's MAFFT (Katoh & Standley 2013) and FastTree (Price et al. 2010) functions to obtain a rooted tree.

Statistical Analysis

We performed analyses in R v4.2.0 (R Core Team 2022), with the 'tidyverse' (Wickham et al. 2019), 'phyloseq' (McMurdie & Holmes 2013), and 'microbiome' (Lahti & Shetty 2019) packages. For statistical tests, we merged the subsamples of plots such that each plot had one set of reads for each time point and domain, then rarefied samples to the minimum read number for that sampling time point (Table S2). We analyzed the data from each time point separately unless otherwise stated. All models followed the same general structure, with heat treatment, drought treatment, and the heat × drought interaction as predictors. We tested for differences in Shannon diversity using the rarefied datasets and linear models, then used type 3 ANOVAs from the 'car' package (Fox & Weisburg 2019) on the linear models to calculate F values. We used weighted Unifrac principal coordinates analysis (PCoA) on the rarefied relative abundance data. Next, we tested for differences in rarefied relative community composition using adonis2 permutational analysis of variance with the Bray-Curtis method and 9999 permutations ('vegan' package, Oksanen et al. 2022); and results did not change based on the order of factors. To detect differentially abundant taxa we used the analysis of compositions of microbiomes with bias correction ('ANCOMBC') package (Lin & Peddada 2020). We performed the analysis at the genus level with Holm's method p-value adjustment.

To assess treatment effects in a group of known symbiotic bacteria, we subsetted the rarefied datasets to include only rhizobia genera (Lajudie et al. 2019) and tested for differences

in Shannon diversity and community composition as described above. To assess treatment effects in symbiotic fungi, we first classified fungi into guilds using FUNGuild annotation (Nguyen et al. 2016) and kept classifications only when 'Probable' or 'Highly Probable.' We concatenated classifications across years and relativized each guild's raw read number to sample read number, then used repeated measures ANOVAs with time included as a factor and plot as the error term. The response variables were the relative abundance of guilds of interest: parasites, pathogens, endophytes, and mycorrhizae; response variables were natural log transformed as needed to improve the normality of error distributions.

Results

Fungal communities

Active treatment and recovery periods

During active treatment, fungal community composition was not resistant; it was strongly shaped by drought ($F_{1,8}$=1.24, p<0.05; Appendix S1: Table S4), but not heat or heat × drought. Shannon diversity was not significantly affected by any active experimental treatment (Appendix S1: Table S3).

Fungal communities were not resilient, as they were slow to recover after experiencing climate treatments. In the first recovery period, there was reduced Shannon diversity in the treatments with drought and heat × drought legacies ($F_{1,8}>7.19$, p<0.05 for all effects; Figure 2A, Appendix S1: Table S3). Then, in the second recovery period, the legacy of drought also predicted fungal community composition ($F_{1,8}$=2.35, p<0.05; Figure 2B, Appendix S1: Table S4).

Taxonomic and guild responses

Alongside whole community shifts, we identified differentially abundant genera due to both heat and drought. The full list of differentially abundant fungal genera is shown in Figure 4. Notably, Saccharomycetales (yeasts) showed strikingly higher relative abundance in drought plots (Figure 3), driven by the *Babjevia* genus (~3x log fold increase in drought, Holm's adjusted $p<0.05$; Figure 4). We also saw interactions of seasonality and climate treatments; for example, Pezizales visually increased in relative abundance in our September samples (Figure 3), but drought significantly dampened this increase compared to control plots, leading Pezizales to have one of the largest negative log fold changes among fungi (Holm's adjusted $p<0.05$; Figure 4).

Our analysis of symbiotic fungal guilds showed the mycorrhizal guild significantly decreased in relative abundance due to heat ($F_{1,8}=6.15$, $p<0.05$; Appendix S1: Table S5) and had a non-significant trend of lower abundance in drought. Most of the mycorrhizal reads were ectomycorrhizal, which is not surprising given that the spruce saplings in the plots are ectomycorrhizal, and mycorrhizal relative abundance was much greater during September samples compared to June samples ($F_{2,22}=13.2$, $p<0.001$; Appendix S1: Table S5). Our differential abundance analysis found two ectomycorrhizal genera, *Wilcoxina* and *Suillus* (Dahlberg & Finlay 1999; Yu et al. 2001), that associate with Pinaceae tree saplings both had decreased relative abundance due to legacy of drought, but differing responses during active drought, with *Wilcoxina* being decreased while *Suillus* was increased (Holm's adjusted $p<0.05$; Figure 4). Additionally, our guild analysis showed the relative abundance of parasitic fungal guilds (parasitic on other fungi, lichen, plants, or insects) was increased by drought ($F_{1,8}=13.0$, $p<0.01$; Figure 5, Appendix S1: Table S5). Pathogens and endophytes were not significantly affected by the climate treatments (Appendix S1: Table S5).

Bacterial communities

Active treatment and recovery periods

Bacteria communities were more resistant to the climate events than fungi, with no significant shifts due to active treatments (Appendix S1: Table S6). Shannon diversity was also not affected during active treatment (Appendix S1: Table S7).

Bacteria diversity and composition were also very resilient to climate treatments, especially heat. The first recovery period suggested no significant legacy effects from treatment. In the second recovery period, only the legacy of drought increased bacterial Shannon diversity ($F_{1,8}=9.30$, $p<0.05$; Figure 2C, Appendix S1: Table S7).

Taxonomic and guild responses

Despite whole bacterial communities having strong resistance to climate treatments, we identified differentially abundant bacterial genera due to heat and drought during active treatment and recovery periods (Holm's adjusted $p<0.05$; Figure 4), though fewer genera were found to be significantly affected compared to fungi. Drought and legacy of drought not only increased the abundances of some rhizobia (*Burkholderia*, *Cupriavidus*, *Methylobacterium*, *Rhizobium*, *Phyllobacterium)*, but also increased other plant growth-promoting rhizobacteria in the soil, for example *Variovorax* spp., and *Pseudoxanthomonas* spp., which can help plants tolerate abiotic stresses such as drought (Kang et al. 2014; Kaushal 2019; Singh et al. 2021). In contrast, a potentially pathogenic genus *Agrobacterium* decreased in abundance due to drought, and a human pathogen, *Legionella*, significantly increased due to the legacy of heat.

The rhizobia guild's Shannon diversity significantly increased due to active heat ($F_{1,8}=9.78$, $p<0.05$, Appendix S1: Table S8) and active drought ($F_{1,8}=5.29$, $p=0.05$, Appendix S1:

Table S8), and remained significantly increased due to legacy of drought in the second recovery period ($F_{1,8}$=5.94, p<0.05, Appendix S1: Table S8, Figure 5). While rhizobia communities visually clustered into treatment groups in PCoAs (Figure 5), only the first recovery period was significantly predicted by the heat × drought interaction ($F_{1,8}$=1.78, p<0.05, Appendix S1: Table S9); community composition was not significantly affected by heat and drought factors in the second recovery period or the active treatment (Appendix S1: Table S9).

Discussion

Our results suggest that climate change, and especially drought, will have more persistent effects on soil fungi than on soil bacteria. In fungal communities, drought shaped community composition, decreased diversity, and altered the relative abundance of symbiotic fungi important to plant communities. In contrast, bacterial communities were largely resistant to climate treatments, although drought tended to increase diversity and increased the relative abundance of some bacteria beneficial to plants. We discuss the implications of the differences between these members of the microbial community, and the effects of climatic events on symbiotic microbes below.

Fungi are less resistant and resilient

Microbial communities experienced legacy effects from the climate treatments, with the effect of drought being stronger than the effect of heat. The study site regularly experiences extreme annual swings in temperature, potentially conditioning microbes to resist and recover faster from heat (Hawkes & Keitt 2015). In contrast to some studies (de Vries et al. 2018; Pec et al. 2021), even relatively short-term climate treatments had strong immediate and lasting impacts on fungal communities; droughted fungal communities did not resemble control communities

during or after treatment. Moreover, fungal community resilience was lower than bacterial resilience to drought. Our prediction that fungi would be more resistant but less resilient to climate change than bacteria was therefore only partially supported; their more complex life history strategies and longer generation times may contribute to fungi's lack of resilience under climate stress, but do not prevent immediate shifts in relative abundance or presence. In contrast to our prediction, the bacterial community composition in our plots was very resistant to climate treatments, despite literature predicting high turnover rates (Rousk & Bååth 2011). These results are consistent with climate conditions affecting slow-growing microbes more, and fungal communities having a higher proportion of very slow-growing microbes compared to bacteria, thus showing greater change. While some literature suggests slow-growing microbes have lower relative fitness under more extreme climate conditions (Sabath et al. 2013), slow-growing microbes may also benefit from climate change relative to faster-growing microbes, especially if heat and drought make resources scarce or reduce the cost of slower growth (Konstantinidis & Tiedje 2004; Abreu et al. 2023).

The life histories of bacteria and fungi likely affected their resistance and resilience to climatic shifts, although differences in dispersal could have also played a role. It is possible that climate treatments may have influenced local bacterial populations, but dispersal was high enough among bacterial metacommunities to mask these effects. If bacteria could disperse easily between our plots, their community compositions would converge more readily and the strength of signal would be smaller (Gianuca et al. 2017), while if fungi cannot disperse as easily, shifts in composition would be more evident. Actual rates and distances of microbial dispersal are not well described, but there is evidence that fungi are more dispersal-limited than bacteria (Zhang et al. 2021) meaning fungi may be less able to escape a poor environment. While we cannot rule

out the role of dispersal, dispersal did not mask the significant effects of treatment on rhizobia and other specific bacterial genera. While surprising, highly resistant bacterial communities are not uncommon; for example, Waring & Hawkes (2018) also observed no direct compositional changes to altered precipitation. Despite no significant changes in community composition, the bacteria in our plots likely experienced physiological changes and costs (Schimel et al. 2007) as well as turnover in allele frequencies and gene functions that we could not detect with 16S rRNA sequencing, which may have then contributed to drought's legacy effects on bacterial diversity.

The community-wide shifts in composition and diversity we detected are likely a product of both the direct effects of heat and drought on microbes and the indirect effects of climate treatment on microbes mediated through species interactions, such that microbes not directly affected by climate may still have increased or decreased in relative abundance. Microbes that interact strongly with other microbes may have a linked response if any of their network is disturbed by climate (Bardgett & Caruso 2020). Indirect effects of climate may also be mediated through plant-microbe or animal-microbe interactions, where stressed organisms may alter the soil environment in some way, for example plants may secrete different root exudates (Karlowsky et al. 2018; Bakhshandeh et al. 2019), or, stressed organisms may themselves be an altered environment for symbionts due to factors like growth or immune responses (Nejat & Mantri 2017). While examining differential abundance trends over time, it became apparent that microbes affected during active treatment were not necessarily the ones affected in recovery periods (Figure 4). This observation demonstrates the complexity of legacy effects and resiliency, and suggests a strong role of persisting direct and indirect effects of climate on microbial communities.

Fungal communities showed low resistance and resilience, indicating that the ecosystem services they provide may change under future climate conditions. Fungi are major decomposers of organic material, and therefore unlock a large supply of nutrients for higher soil trophic levels (Boddy & Watkinson 1995). Mycorrhizal fungi in particular contribute to nutrient cycling and mineral weathering (Courty et al. 2010), as well as carbon sequestration (Clemmenson et al. 2013). Despite their importance to global elemental cycling and other ecosystem processes, most research on fungal stress responses focuses solely on yeasts (Branco et al 2022). In this experiment, yeasts were common in drought plots and did not represent the average fungal response to treatments. Although yeasts thrived under drought, overall fungal diversity was lower after drought and mycorrhizal abundance was reduced after heat, suggesting the breadth of ecosystem services provided by fungal communities will be decreased under future climatic conditions (Mori et al. 2016). Additionally, low resilience in soil communities means that plants that recruit into a population following a climatic shift may interact with an altered pool of microbes even if they do not experience the climate event themselves. Soil microbiomes affect plant performance and fitness (Berendsen et al. 2012; Rubin et al. 2018; Lu et al. 2018; Fitzpatrick et al. 2019) through mechanisms such as nutrient provisioning (Beringer et al. 1979) and altering plant gene expression profiles (Lu et al. 2018). For plants, associations with microbes can result in improved stress tolerance (Gehring et al. 2017; Allsup et al. 2023) or even broadened geographic range size (Afkhami et al. 2014), which can be adaptive under climate change. Therefore, soil microbes modulate how plants respond to climate events (Classen et al. 2015; Rudgers et al. 2020) and soil legacy effects may have far-reaching ecological and environmental consequences.

Climate events on symbiotic microbes

Free-living and symbiotic microbes may respond differently to climate change, for example if living inside host cells or tissue buffers microbes against changes to the abiotic environment. Plant symbionts sometimes responded differently to climate treatments than the average response of bacterial and fungal communities. We do not attribute these results to a shifting plant community, because it was held constant throughout the experiment, however results may be influenced by shifts in plant traits under different climate treatments.

The rhizobia guild showed a similar post-drought response compared to the overall bacteria community with an increase in diversity; however, because we also saw significantly higher diversity when heat and drought were being actively applied, this indicates rhizobia positively responded more to active treatment than the overall community. Since rhizobia fix biological nitrogen through legume symbiosis (Beringer et al. 1979), their high abundance bodes well for global nitrogen cycles under climate change. This was unexpected, as we predicted rhizobia would be a worse soil competitor under climate treatments due to their relatively large genomes and thus longer replication times. However, if slower-growing microbes are more sensitive to changes in climate, as we saw with our domain comparison, then this rhizobia result is consistent. At higher temperatures, slow-growing marine bacteria cells retain more normal membrane functions and have a lower mortality burden than fast growers (Schaum & Collins 2014; Abreu et al. 2023), which may apply to slow-growing soil bacteria too. Given our results, we might predict the likelihood of symbiosis between specific plant-rhizobia partners to differ in future climate conditions. A change in the availability of rhizobia partners matters because rhizobia-plant interactions vary in specificity (Chen et al. 2021), which could lead to shifts in plant communities and legume ranges (Harrison et al. 2018). The increased abundance and

diversity of rhizobia could also shape evolution in the rhizobia-legume system through increased bacterial competition (Burghardt & diCenzo 2023).

While beneficial bacteria increased with our climate treatments, mutualistic fungi were negatively affected. Mycorrhizae, mainly composed of ectomycorrhizae, had significantly lower relative abundance in heated plots, suggesting mycorrhizae were less resistant to heat than the fungal community overall (which showed no major responses to heat). We also observed significant decreases in the abundance of ectomycorrhizal genera in the bulk soil due to active drought and its legacy, and possibly as a result of this decreased abundance, drought has been shown to reduce ectomycorrhizal root colonization (Erlandson et al. 2022). A recent study by Fu et al. (2022) found that drought lowered the diversity of field arbuscular mycorrhizae, and they suggested this shift was mediated by plant communities. In our experiment, because the ectomycorrhizae obligately associated with the spruce saplings in our plots, it is likely there were negative indirect climate effects on the mycorrhizae mediated through changes in the physiology of the spruce, indirect microbe-microbe interactions, as well as the direct effects of climate treatment. Ectomycorrhizal fungi have a narrow climatic tolerance and thus are predicted to be disproportionately affected by climate change (Baldrian et al. 2023), which our results support.

Mutualistic and parasitic fungi both derive nutrients from hosts, however while heat decreased mycorrhizae, drought increased parasites in recovery periods. If parasitic fungal species become more prevalent in post-drought conditions this could increase the likelihood of soil dwelling organisms hosting a parasite. Drought and heat may be especially problematic in temperate forests, where most trees are ectomycorrhizal (Baldrian et al. 2023), since trees may simultaneously lack their main mutualist partners and experience increased parasite pressure. Organisms hosting parasites may have reduced growth and fitness, leading to reduced ecosystem

productivity. Additionally, parasitized hosts may experience more negative effects from climate events than non-parasitized hosts (Baldrian et al. 2023). In positive feedback, pathogens and parasitic microbes are predicted to increase in heated environments in part due to increased susceptibility of stressed hosts (Pritchard 2011; Altizer et al. 2013; Baker et al. 2018). While we saw limited evidence of heat differentially affecting parasites, our results did suggest drought may also play a role in moderating parasite abundances under future climate conditions.

Conclusions

Soil microbiomes are tightly linked to global nutrient and chemical cycling, and there is a rich literature describing how microbes buffer plants against the abiotic stresses of climate change (e.g. Yang et al. 2009; Lau & Lennon 2012; Rudgers et al. 2020; Marro et al. 2022; Allsup et al. 2023). Given the soil microbiome's important role in ecosystems, it is important to understand how microbial populations respond to climate events and the factors underlying variation in resistance and resilience. We found that fungi were less resistant and less resilient to drought and its legacy compared to bacteria, while heat had limited overall effect on both domains. Symbiosis generated variation in resistance and resilience within the domains, but did not have a universal effect; for example notable mutualistic bacteria performed well under climate stress while mutualistic fungi did not. Our results suggest that slower-growing microbes will be more affected by climate change. Low resistance and resilience of soil fungal communities will have far-reaching consequences for ecosystems under climate change.

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Conflict of Interest Statement

The authors declare no conflicts of interest.

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Figure captions

Figure 1. Experimental design at Koffler Scientific Reserve. (a) The temperature-free-air-controlled enhancement plots showing the infrared heaters and rainout structures, as well as the water drainage system. Photo by Julia Boyle. (b) Schematic diagram of the experimental plots (numbered) and their climate treatments. Dots represent sampled sites within plots.

Figure 2. Community structure of fungal and bacterial soil microbes across climate conditions and time. Boxplots of Shannon diversity across time in fungal (a) and bacterial (c) microbial communities. Weighted Unifrac PCoA plots of fungal (b) and bacterial (d) microbial communities. When significant, main effects of heat, drought, and their interaction are included with panels.

Figure 3. Community composition over time for each subsample in each treatment. (a)-(c) Fungi relative abundances at the order level. (d)-(f) Bacteria relative abundance at the order level. Orders were aggregated and included when they were at least 1% of the compositional abundance, and found in at least 30% of samples. Plot order within treatment groups remains consistent, with 2-3 samples per plot.

Figure 4. Differentially abundant microbe genera due to drought (a) and heat (b) across all sampling times, compared to control plots. Taxa shown are significant ($p<0.05$) with Holm's method p-value adjustment, grouped by domain, and ordered by descending average log fold change.

Figure 5. Symbiotic microbes under climate treatments. (a) Rhizobia Shannon diversity. (b) Rhizobia community weighted Unifrac PCoAs. (c) and (d) Fungal parasite and mycorrhizae relative abundance across treatment and time. When significant, main effects of heat, drought, and their interaction are included with panels.

Figures

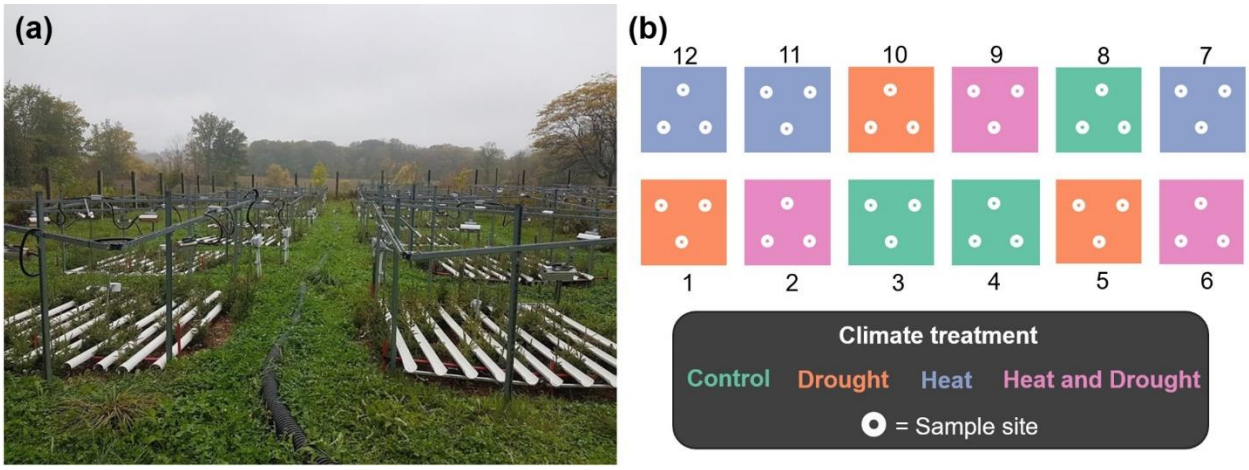

(a)
(b)
12
11
10
9
8
7
1
2
3
4
5
6
Climate treatment
Control
Drought
Heat
Heat and Drought
= Sample site

Figure 1

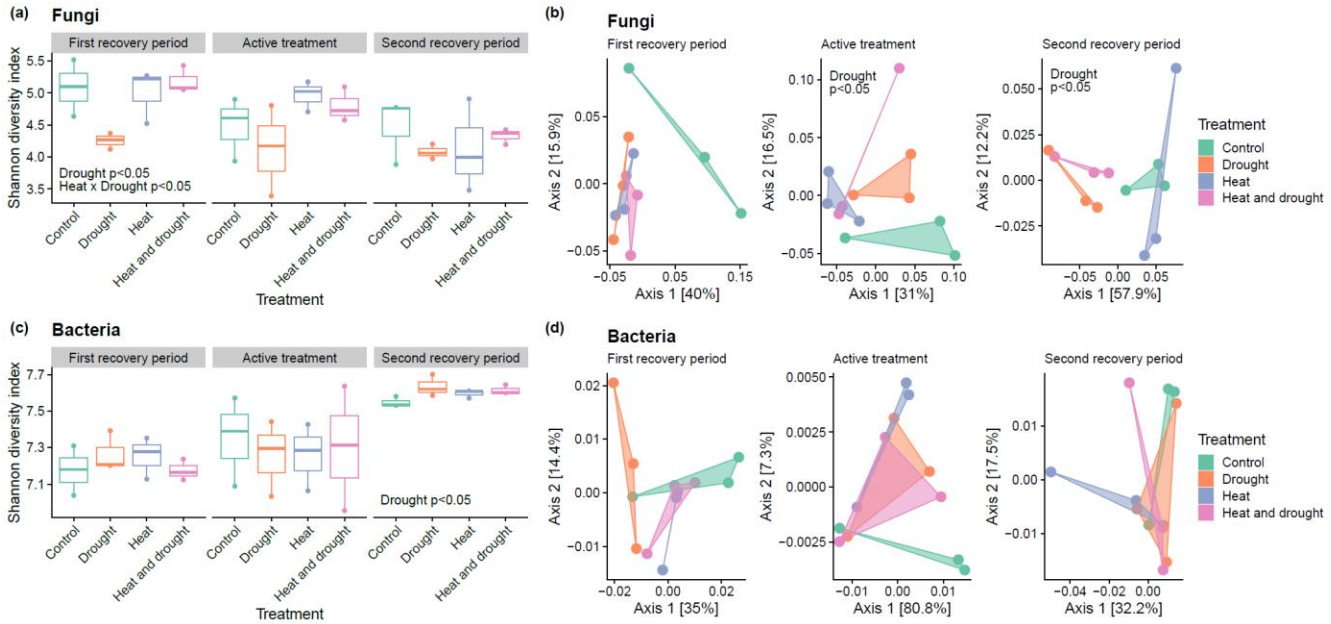

(a) Fungi
First recovery period
Active treatment
Second recovery period
Shannon diversity index
Drought p<0.05
Heat x Drought p<0.05
Control
Drought
Heat
Heat and drought
Treatment
(b) Fungi
First recovery period
Active treatment
Second recovery period
Axis 2 [15.9%]
Axis 1 [40%]
Axis 2 [16.5%]
Axis 1 [31%]
Axis 2 [12.2%]
Axis 1 [57.9%]
Drought p<0.05
Treatment
Control
Drought
Heat
Heat and drought
(c) Bacteria
First recovery period
Active treatment
Second recovery period
Shannon diversity index
Drought p<0.05
Control
Drought
Heat
Heat and drought
Treatment
(d) Bacteria
First recovery period
Active treatment
Second recovery period
Axis 2 [14.4%]
Axis 1 [35%]
Axis 2 [7.3%]
Axis 1 [80.8%]
Axis 2 [17.5%]
Axis 1 [32.2%]
Treatment
Control
Drought
Heat
Heat and drought

Figure 2

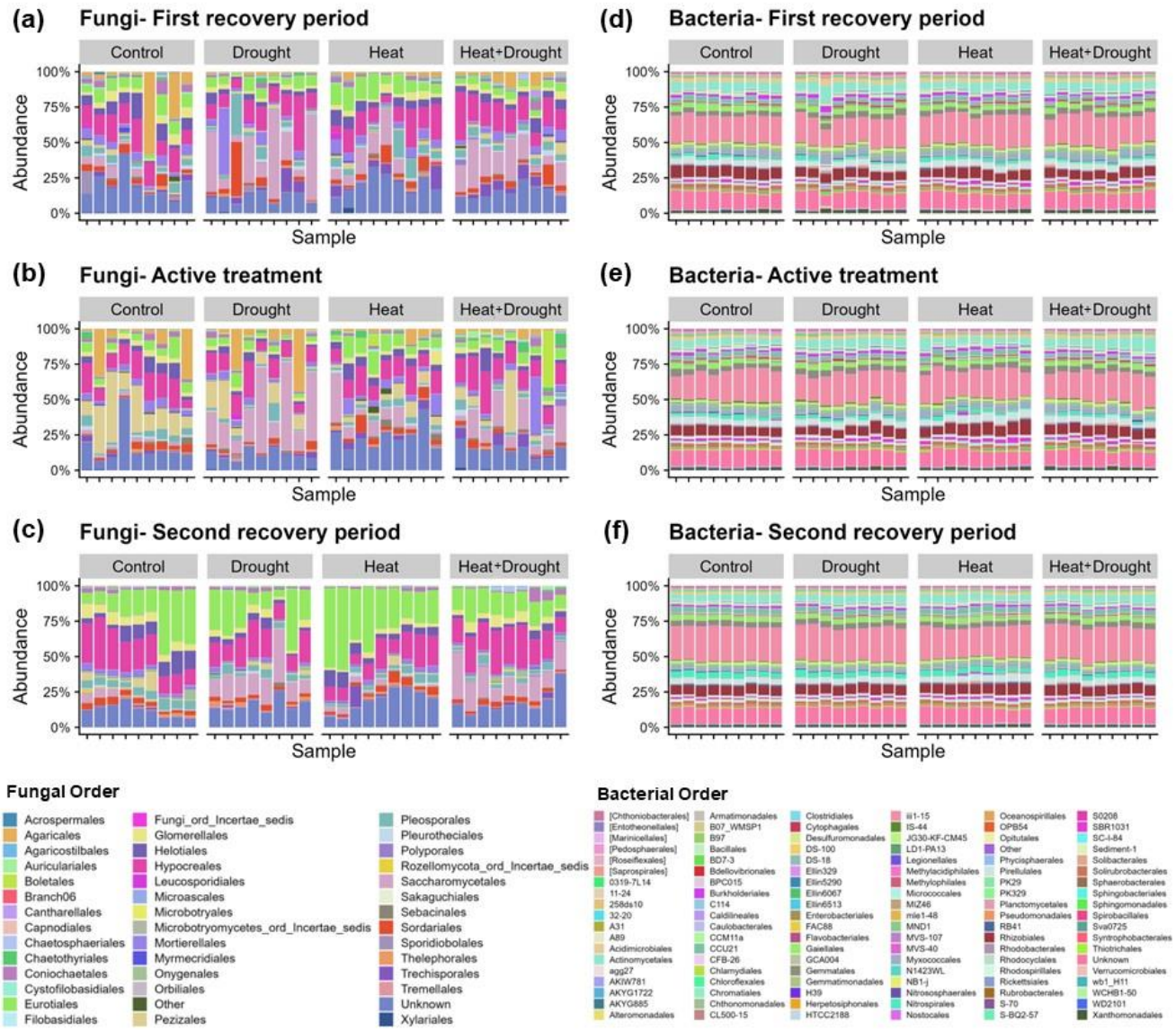

(a) Fungi- First recovery period
(b) Fungi- Active treatment
(c) Fungi- Second recovery period
(d) Bacteria- First recovery period
(e) Bacteria- Active treatment
(f) Bacteria- Second recovery period
Control
Drought
Heat
Heat+Drought
Abundance
100%
75%
50%
25%
0%
Sample
Fungal Order
Acrospermales
Agaricales
Agaricostilbales
Auriculariales
Boletales
Branch06
Cantharellales
Capnodiales
Chaetosphaeriales
Chaetothyriales
Coniochaetales
Cystofilobasidiales
Eurotiales
Filobasidiales
Fungi_ord_Incertae_sedis
Glomerellales
Helotiales
Hypocreales
Leucosporidiales
Microascales
Microbotryales
Microbotryomycetes_ord_Incertae_sedis
Mortierellales
Myrmecridiales
Onygenales
Orbiliales
Other
Pezizales
Pleosporales
Pleurotheciales
Polyporales
Rozellomycota_ord_Incertae_sedis
Saccharomycetales
Sakaguchiales
Sebacinales
Sordariales
Sporidiobolales
Thelephorales
Trechisporales
Tremellales
Unknown
Xylariales
Bacterial Order
[Chthoniobacterales]
[Entotheonellales]
[Marinicellales]
[Pedosphaerales]
[Roseiflexales]
[Saprospirales]
0319-7L14
11-24
258ds10
32-20
A31
A89
Acidimicrobiales
Actinomycetales
agg27
AKIW781
AKYG1722
AKYG885
Alteromonadales
Armatimonadales
B07_WM5P1
B97
Bacillales
BD7-3
Bdellovibrionales
BPC015
Burkholderiales
C114
Caldilineales
Caulobacterales
CCM11a
CCU21
CFB-26
Chlamydiales
Chloroflexales
Chromatiales
Chthonomonadales
CL500-15
Clostridiales
Cytophagales
Desulfuromonadales
DS-100
DS-18
Elin329
Elin5290
Elin6067
Elin6513
Enterobacteriales
FAC88
Flavobacteriales
Gaiellales
GCA004
Gemmatales
Gemmatimonadales
H39
Herpetosiphonales
HTCC2188
iii1-15
IS-44
JG30-KF-CM45
LD1-PA13
Legionellales
Methylacidiphilales
Methylophilales
Micrococcales
MIZ46
mle1-48
MND1
MVS-107
MVS-40
Myxococcales
N1423WL
NB1-j
Nitrososphaerales
Nitrospirales
Nostocales
Oceanospirillales
OPB54
Opitutales
Other
Phycisphaerales
Pirellulales
PK29
PK329
Planctomycetales
Pseudomonadales
RB41
Rhizobiales
Rhodobacterales
Rhodocyclales
Rhodospirillales
Rickettsiales
Rubrobacterales
S-70
S-BQ2-57
S0208
SBR1031
SC-I-84
Sediment-1
Solibacterales
Solirubrobacterales
Sphaerobacterales
Sphingobacteriales
Sphingomonadales
Spirobacillales
Sva0725
Syntrophobacterales
Thiotrichales
Unknown
Verrucomicrobiales
wb1_H11
WCHB1-50
WD2101
Xanthomonadales

Figure 3

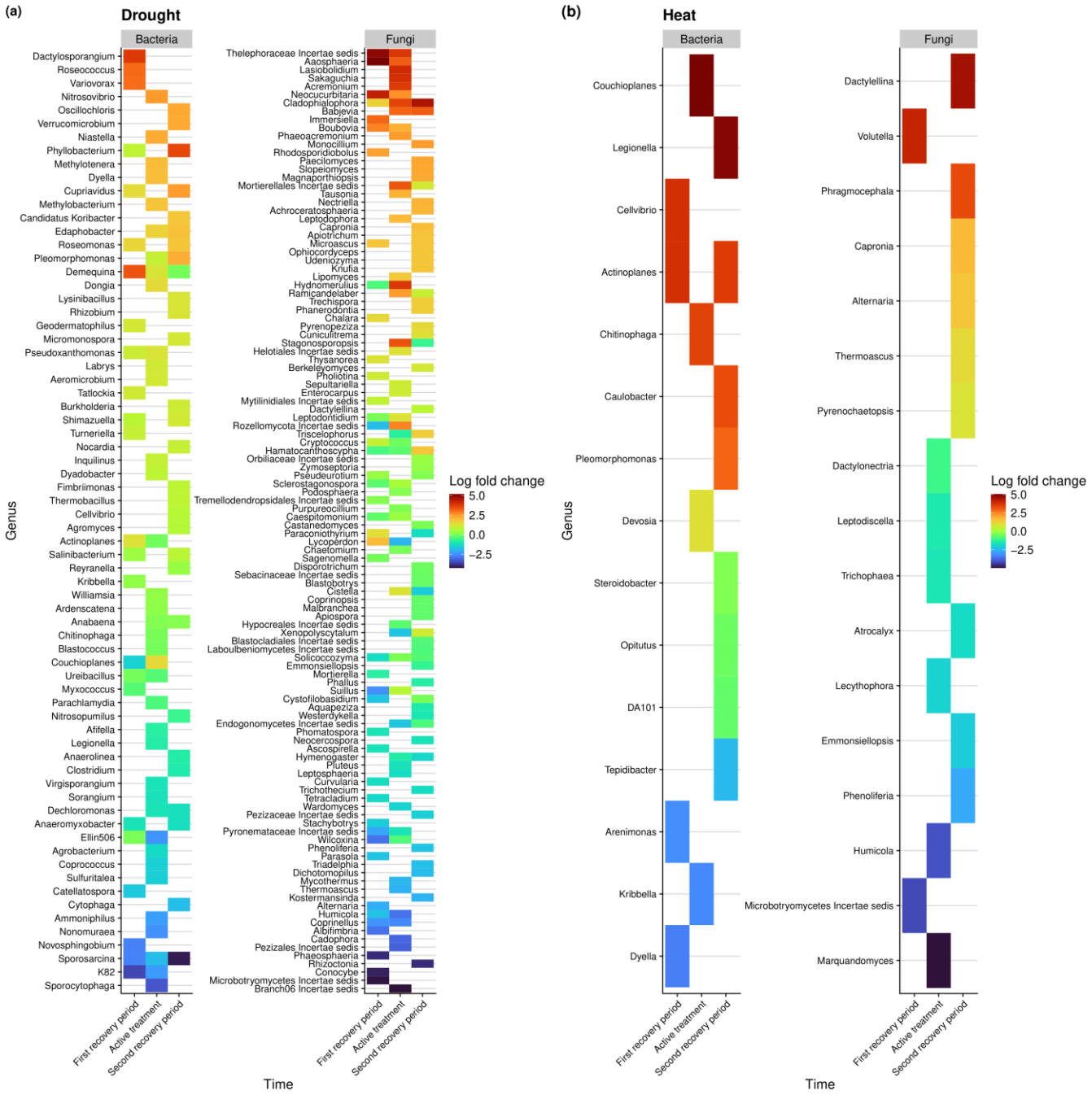

(a)
Drought
Bacteria
Fungi
Genus
Time
First recovery period
Active treatment
Second recovery period
Log fold change
5.0
2.5
0.0
−2.5
(b)
Heat
Bacteria
Fungi
Genus
Time
First recovery period
Active treatment
Second recovery period
Log fold change
5.0
2.5
0.0
−2.5

Figure 4

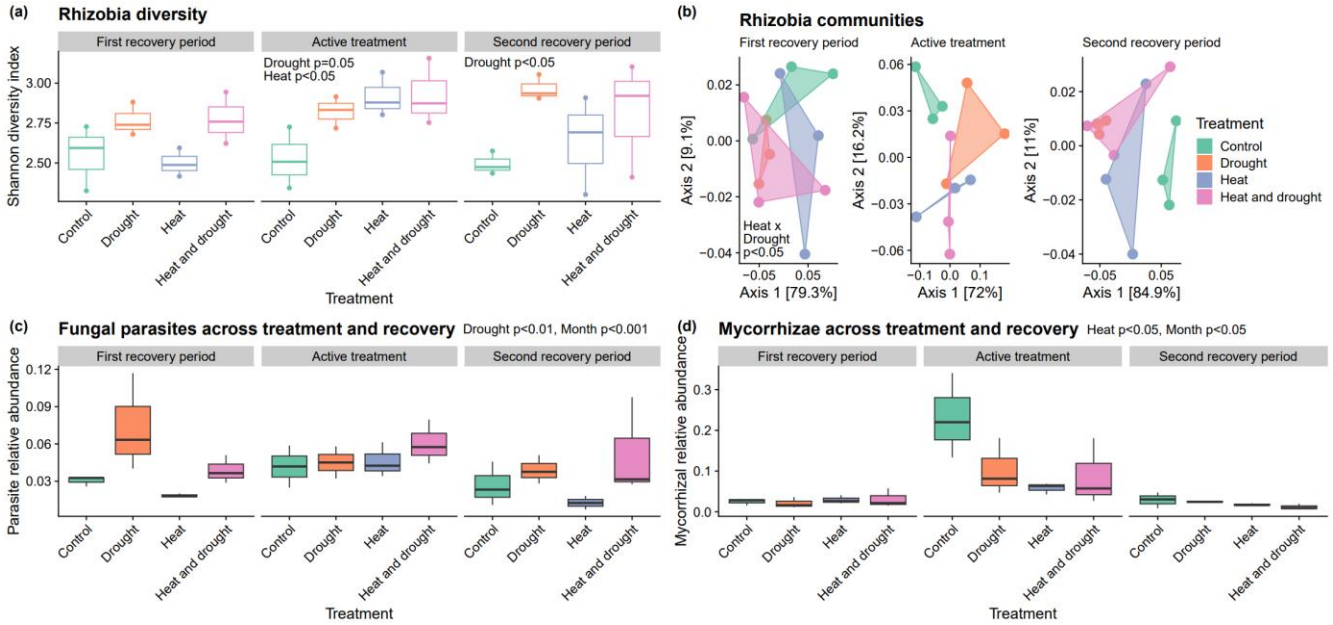
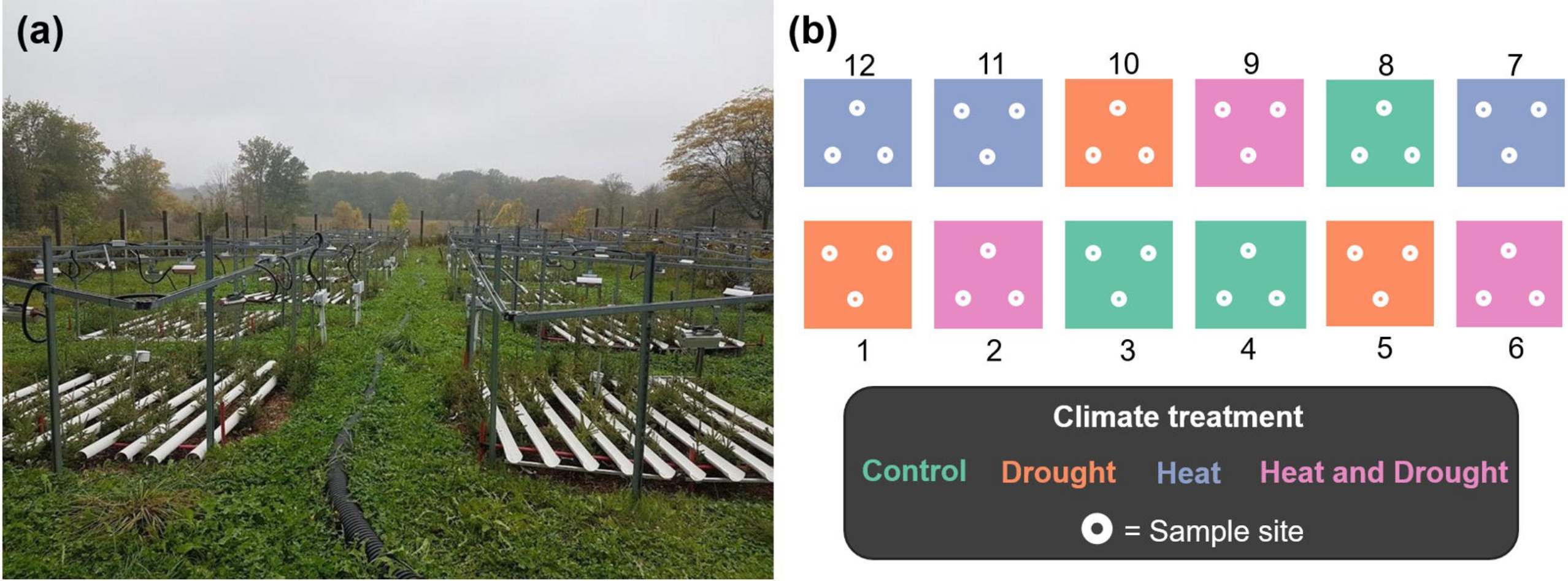
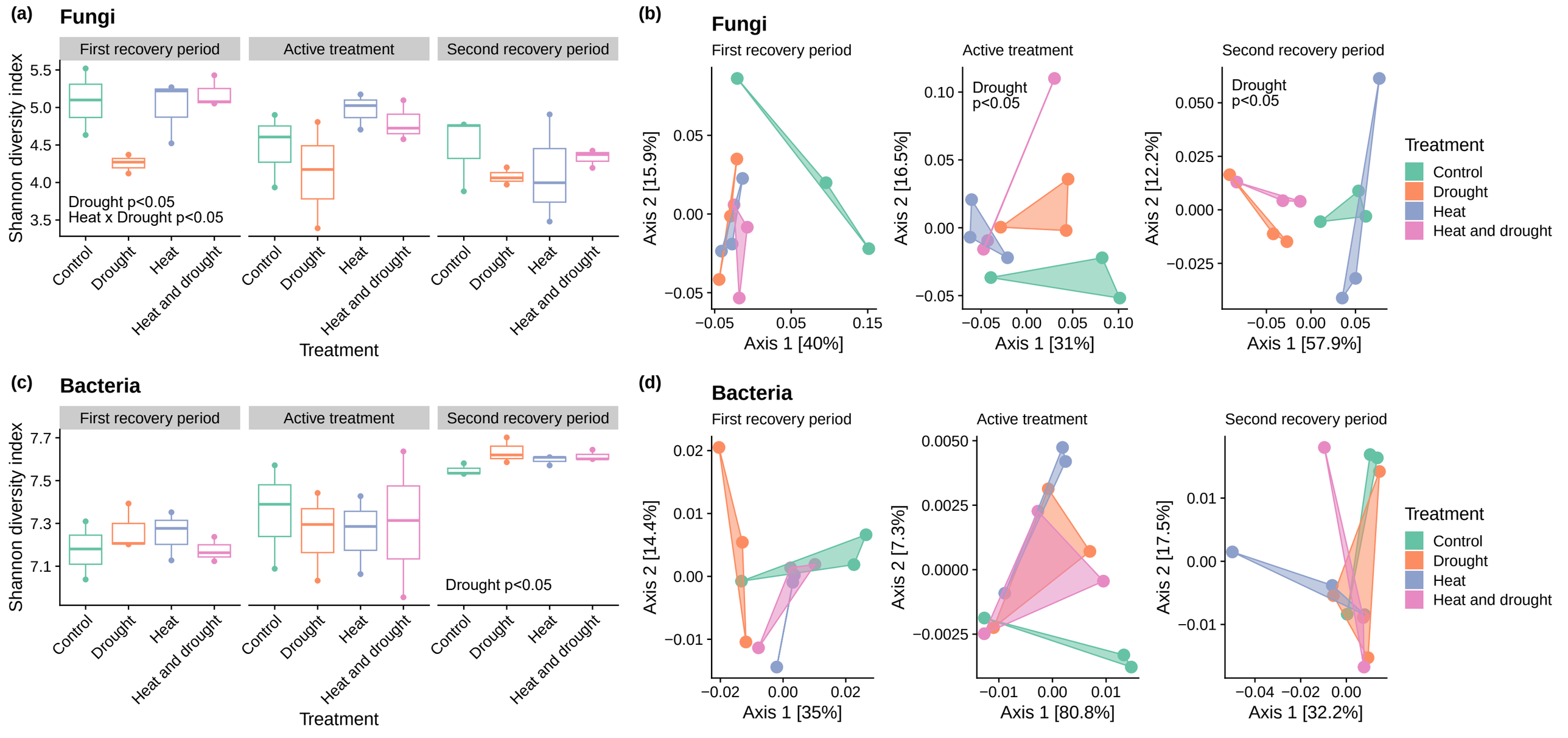
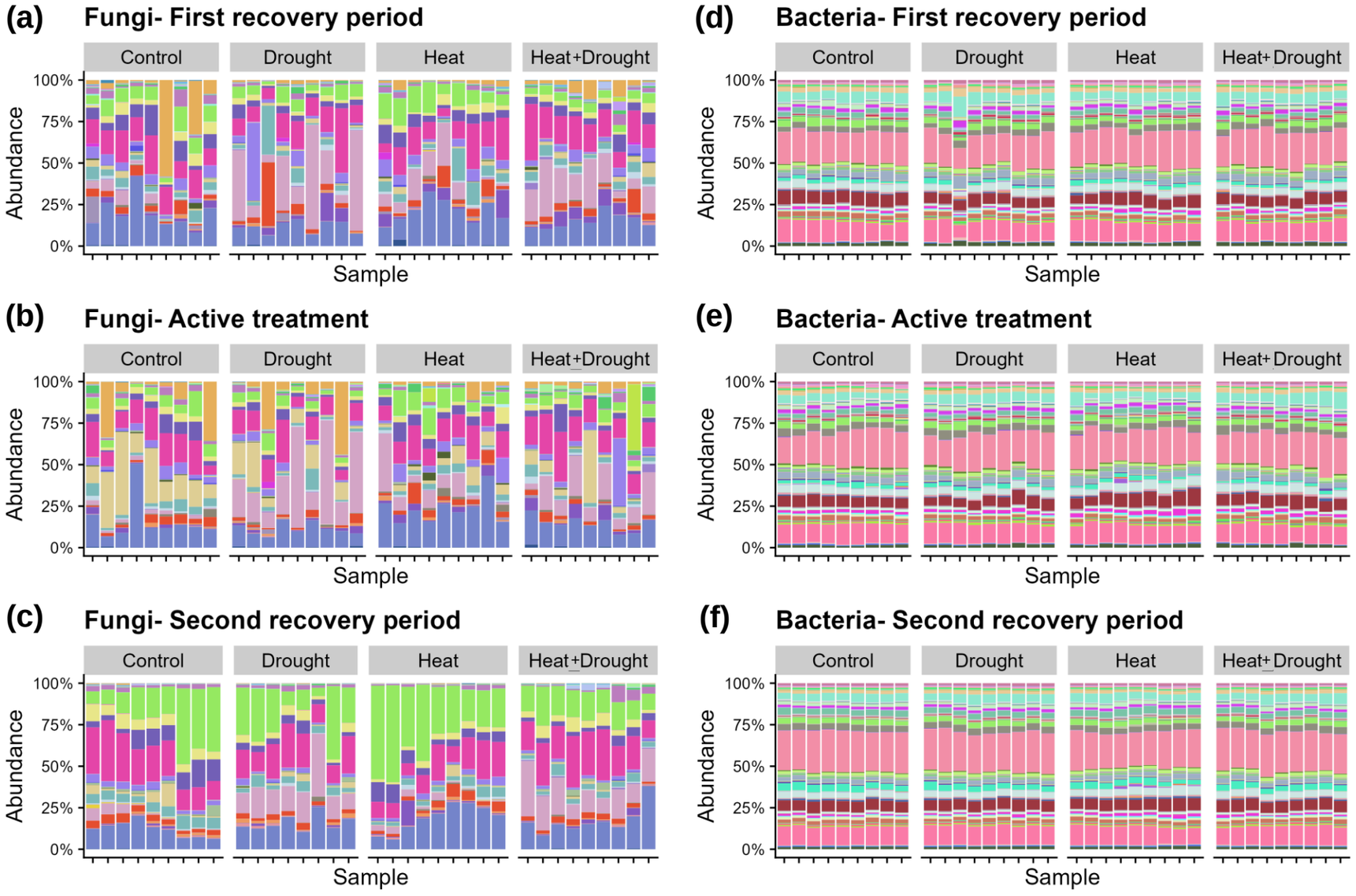

(a)
Rhizobia diversity
First recovery period
Active treatment
Second recovery period
Drought p=0.05
Heat p<0.05
Drought p<0.05
Shannon diversity index
Control
Drought
Heat
Heat and drought
Treatment
(b)
Rhizobia communities
Axis 2 [9.1%]
Axis 1 [79.3%]
Heat x Drought p<0.05
Axis 2 [16.2%]
Axis 1 [72%]
Axis 2 [11%]
Axis 1 [84.9%]
Treatment
Control
Drought
Heat
Heat and drought
(c)
Fungal parasites across treatment and recovery
Drought p<0.01, Month p<0.001
Parasite relative abundance
(d)
Mycorrhizae across treatment and recovery
Heat p<0.05, Month p<0.05
Mycorrhizal relative abundance

Figure 5


(a)
(b)
12
11
10
9
8
7
1
2
3
4
5
6
Climate treatment
Control
Drought
Heat
Heat and Drought
= Sample site


(a)
Fungi
First recovery period
Active treatment
Second recovery period
Shannon diversity index
5.5
5.0
4.5
4.0
3.5
Drought p<0.05
Heat x Drought p<0.05
Control
Drought
Heat
Heat and drought
Treatment
(b)
Fungi
First recovery period
Active treatment
Second recovery period
Axis 2 [15.9%]
Axis 1 [40%]
Axis 2 [16.5%]
Axis 1 [31%]
Drought
p<0.05
Axis 2 [12.2%]
Axis 1 [57.9%]
Drought
p<0.05
Treatment
Control
Drought
Heat
Heat and drought
(c)
Bacteria
First recovery period
Active treatment
Second recovery period
Shannon diversity index
7.7
7.5
7.3
7.1
Drought p<0.05
Control
Drought
Heat
Heat and drought
Treatment
(d)
Bacteria
First recovery period
Active treatment
Second recovery period
Axis 2 [14.4%]
Axis 1 [35%]
Axis 2 [7.3%]
Axis 1 [80.8%]
Axis 2 [17.5%]
Axis 1 [32.2%]
Treatment
Control
Drought
Heat
Heat and drought

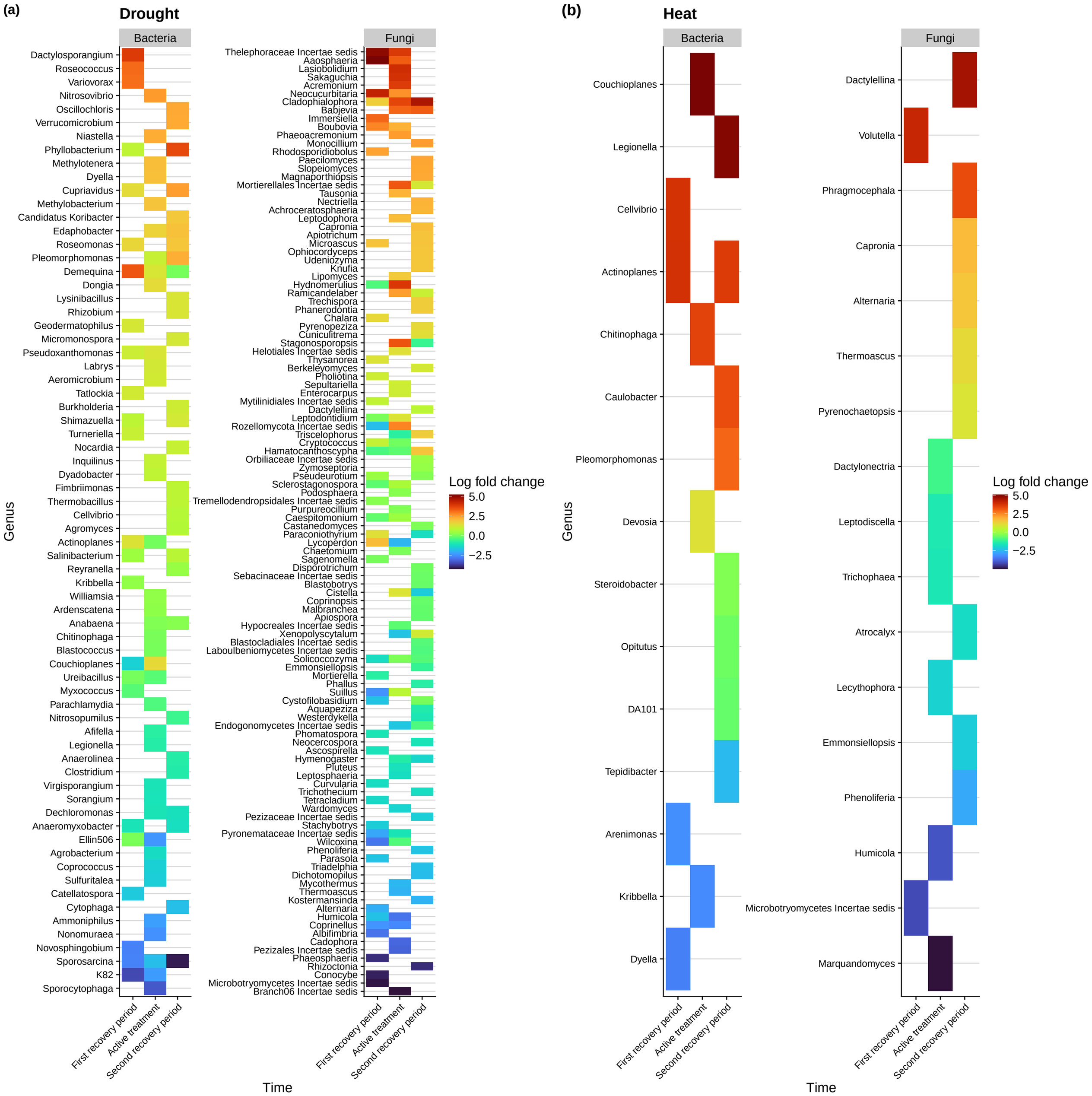


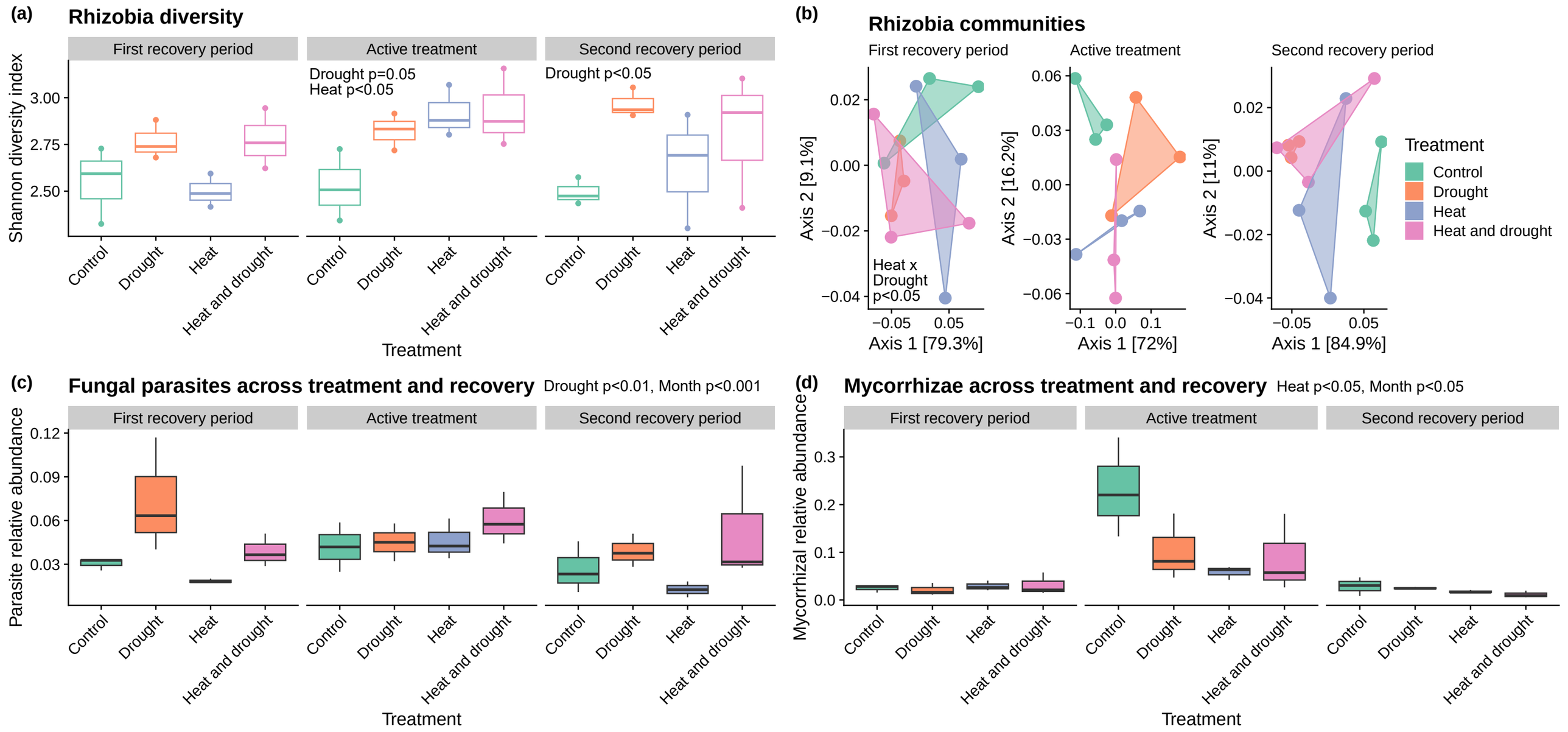
Fungal Order

Acrospermales, Agaricales, Agaricostilbales, Auriculariales, Boletales, Branch06, Cantharellales, Capnodiales, Chaetosphaeriales, Chaetothyriales, Coniochaetales, Cystofilobasidiales, Eurotiales, Filobasidiales, Fungi_ord_Incertae_sedis, Glomerellales, Helotiales, Hypocreales, Leucosporidiales, Microascales, Microbotryales, Microbotryomycetes_ord_Incertae_sedis, Mortierellales, Myrmecridiales, Onygenales, Orbiliales, Other, Pezizales, Pleosporales, Pleurotheciales, Polyporales, Rozellomycota_ord_Incertae_sedis, Saccharomycetales, Sakaguchiales, Sebacinales, Sordariales, Sporidiobolales, Thelephorales, Trechisporales, Tremellales, Unknown, Xylariales

Bacterial Order

[Chthoniobacterales], [Entotheonellales], [Marinicellales], [Pedosphaerales], [Roseiflexales], [Saprospirales], 0319-7L14, 11-24, 258ds10, 32-20, A31, A89, Acidimicrobiales, Actinomycetales, agg27, AKIW781, AKYG1722, AKYG885, Alteromonadales, Armatimonadales, B07_WMSP1, B97, Bacillales, BD7-3, Bdellovibrionales, BPC015, Burkholderiales, C114, Caldilineales, Caulobacterales, CCM11a, CCU21, CFB-26, Chlamydiales, Chloroflexales, Chromatiales, Chthonomonadales, CL500-15, Clostridiales, Cytophagales, Desulfuromonadales, DS-100, DS-18, Ellin329, Ellin5290, Ellin6067, Ellin6513, Enterobacteriales, FAC88, Flavobacteriales, Gaiellales, GCA004, Gemmatales, Gemmatimonadales, H39, Herpetosiphonales, HTCC2188, iii1-15, IS-44, JG30-KF-CM45, LD1-PA13, Legionellales, Methylacidiphilales, Methylophilales, Micrococcales, MIZ46, mle1-48, MND1, MVS-107, MVS-40, Myxococcales, N1423WL, NB1-j, Nitrososphaerales, Nitrospirales, Nostocales, Oceanospirillales, OPB54, Opitutales, Other, Phycisphaerales, Pirellulales, PK29, PK329, Planctomycetales, Pseudomonadales, RB41, Rhizobiales, Rhodobacterales, Rhodocyclales, Rhodospirillales, Rickettsiales, Rubrobacterales, S-70, S-BQ2-57, S0208, SBR1031, SC-I-84, Sediment-1, Solibacterales, Solirubrobacterales, Sphaerobacterales, Sphingobacteriales, Sphingomonadales, Spirobacillales, Sva0725, Syntrophobacterales, Thiotrichales, Unknown, Verrucomicrobiales, wb1_H11, WCHB1-50, WD2101, Xanthomonadales


(a)
Drought
Bacteria
Fungi
Genus
Time
First recovery period
Active treatment
Second recovery period
Log fold change
5.0
2.5
0.0
−2.5
(b)
Heat
Bacteria
Fungi
Genus
Time
First recovery period
Active treatment
Second recovery period
Log fold change
5.0
2.5
0.0
−2.5


(a)
Rhizobia diversity
First recovery period
Active treatment
Second recovery period
Drought p=0.05
Heat p<0.05
Drought p<0.05
Shannon diversity index
3.00
2.75
2.50
Control
Drought
Heat
Heat and drought
Treatment
(b)
Rhizobia communities
First recovery period
Active treatment
Second recovery period
Axis 2 [9.1%]
Axis 1 [79.3%]
Heat x
Drought
p<0.05
Axis 2 [16.2%]
Axis 1 [72%]
Axis 2 [11%]
Axis 1 [84.9%]
Treatment
Control
Drought
Heat
Heat and drought
(c)
Fungal parasites across treatment and recovery
Drought p<0.01, Month p<0.001
First recovery period
Active treatment
Second recovery period
Parasite relative abundance
0.12
0.09
0.06
0.03
Control
Drought
Heat
Heat and drought
Treatment
(d)
Mycorrhizae across treatment and recovery
Heat p<0.05, Month p<0.05
First recovery period
Active treatment
Second recovery period
Mycorrhizal relative abundance
0.3
0.2
0.1
0.0
Control
Drought
Heat
Heat and drought
Treatment