Soil fungal responses to experimental warming and drying in a Mediterranean shrubland

Running head: Soil fungi respond to climate change in MTEs

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Abstract

Implications of a drying and warming climate have been investigated for aboveground vegetation across a range of biomes yet below-ground effects on microorganisms have received considerably less attention, especially in Mediterranean Type Ecosystems (MTE) that are predicted to be negatively impacted by climate change. We experimentally reduced rainfall and increased temperature across two contrasting study sites (deep sand dune vs shallow sand swale) to test how projected future climate conditions may impact soil fungal composition, richness and diversity. We also assessed fungal OTU warming responses and putative functions of 100 most abundant OTUs and 120 OTUs that either increased or decreased based on their presence/absence across treatments. We found a significant effect of study site, treatment and canopy species on fungal composition. Soil fungal diversity increased under warming treatment in swale plots as compared to control plots indicating a positive effect of warming on fungal diversity. In dunes, significantly more OTUs responded to drought than warming treatment. Among the most abundant soil fungal putative functional groups were endophytes, ericoid mycorrhizas, yeasts and ectomycorrhizas consistent with previous studies. Plant pathogens were found to increase across dunes and swales, while ericoid mycorrhizae decreased. In summary, our study revealed that it is critical to understand belowground microbial patterns as a result of climate change treatments for our ability to better predict how ecosystems may respond to global environmental changes in the future.

Keywords: biodiversity hotspot, shrubs, endophytes, soil, Western Australia

1. Introduction

Plant communities and their interactions with belowground soil microbes strongly shape ecosystem diversity and functioning by controlling organic matter turnover and nutrient cycling (Bardgett and van der Putten, 2014, Wood et al., 2017, Jackson et al., 2007). Microbial communities in soil are influenced by a number of biotic and abiotic factors, including soil pH, organic matter content, soil moisture and vegetation type (Delgado-Baquerizo et al., 2016, Tedersoo et al., 2016, Kaisermann et al., 2015, Zhang et al., 2016, Egidi et al., 2019). Understanding which factors regulate microbial diversity and abundance in soil is therefore important for predicting the effects of climate change on ecosystem functioning (Haugwitz et al., 2014). Whilst many climate change studies have focused on plant aboveground responses (e.g. productivity and community composition), responses of belowground microbial communities have received much less attention (Wilcox et al., 2017, Wilcox et al., 2015).

Climate change is forecast to cause widespread changes in precipitation regimes which will cause longer and more intense droughts in many regions globally (McLaughlin, 2014). Droughts are predicted to have negative impacts on the diversity and abundance of soil microbial communities (Wu et al., 2011) and to decrease soil microbial and soil enzyme activity, resulting in reduced organic matter turnover and nutrient availability for plants (Selsted et al., 2012, Sardans et al., 2008, Allison and Treseder, 2008). Warming has been reported to cause structural changes in fungal communities and increase the fungal to bacteria ratio (Zhang et al., 2005, Fry et al., 2016).

Overall, soil fungi are expected to have better adaptability to cope with drought events than other microorganisms, e.g. bacteria, partly due to their ability to transport water through the hyphal network (Yuste et al., 2011, Khalvati et al., 2005, Kaisermann et al., 2015). For

instance, Castro *et al*. 2010 found soil fungal abundance to increase under warming treatments (+3°C), as did Allison and Treseder, 2008 and Clemmensen *et al*. 2006. Yu *et al.* 2019 found that soil fungal diversity increased after seven years of warming in the alpine meadows of Northern Tibet. However, Yuste *et al*. 2011 showed that fungal diversity did not change with changing temperature and moisture levels, and fungal communities displayed great adaptability to manipulated changes in their study in Southern Catalonia (North-Eastern Iberian Peninsula). Treseder *et al*. 2016 found that free-living filamentous fungi and ectomycorrhizal fungi responded positively to experimental warming, and that warming also induced shifts in fungal community composition in a boreal forest system. Others have reported a decrease in the abundance of free-living filamentous fungi as compared to yeasts, as they have less genetic capacity for stress tolerance (Treseder and Lennon, 2015). Thus, despite fungi being overall more resilient to climate change compared to other soil microbial functional groups (e.g. bacteria), their composition and associated ecosystem services might be disrupted due to climate change and therefore require our improved understanding of these ecological processes in the context of climate change (Bellard et al., 2012, Ochoa-Hueso et al., 2018).

Mediterranean type ecosystems (MTEs) are predicted to be among the most 'at risk' to the future impacts of climate change worldwide (Enright et al., 2014). For example, southwestern Australia (SWA) is identified as one of the most vulnerable of the world's biodiversity hotspots and may lose more than 2000 plant species over the next 100 years as the climate warms and dries (Malcolm et al., 2006). The MTE of SWA is predicted to become hotter (i.e. temperature increase 1–3 °C across all seasons of the year) and drier (i.e. 10-20 % reduction in rainfall largely in winter) based on the 2070 climate change predictions (Enright et al., 2012, Enright et al., 2014, Hopper and Gioia, 2004, IPCC, 2018). However, our understanding of climate change effects on belowground soil fungal communities in MTEs

remains elusive as there have been very few studies that have assessed such effects (but see Hopkins et al. 2018).

To assess how warming and drying conditions may shape soil fungal communities in MTEs in southwestern Australia, we took advantage of a climate change experiment that manipulated warming (+2.9 °C mean daily maximum) and drying (rainfall reduced by 30 %) for four years (2010-2014) using open topped chambers (OTCs) and rain-out shelters (Williams, 2014). Soil samples were collected by digging vertically underneath two common shrub species within five cm from the roots of *Beaufortia elegans* and *Melaleuca leuropoma* (both Myrtaceae), and in bare sand patches away from plants (i.e. no vegetation), across two treatments (warming, drying) with adjacent controls, and for two contrasting study sites (deep sands of dune tops, shallow sands of swales). We predicted that: 1) soil fungal community composition will be affected by study sites (i.e. dune vs swale) that have been previously reported to differ in soil moisture (Williams, 2014), 2) soil fungal composition, richness and diversity in warming and drying treatments will be altered compared to control plots and 3) soil fungal community composition will be affected by microsite location, i.e. being either adjacent to plant roots versus being away from plant roots, as soil microbial communities tend to accumulate around living plant roots and thus we expect greater soil fungal diversity near roots.

2. Materials and Methods

2.1. Study site

The study area was located near Eneabba (29°44’73”S 115°13’54”E), Western Australia, approximately 280 km North of Perth (Fig. 1a). The study area experiences a Mediterranean-

type climate with mean annual rainfall of 494 mm, 80% of which falls in the winter period from May to September, and mean daily temperature ranging from 19.6 °C in July (mid-winter) to 36.2 °C in February (late summer; Eneabba climate station No. 008225, Australian Bureau of Meteorology). The area experiences regular disturbance by fire with a mean fire return period of around 20 years (Enright *et al*., 2012). Vegetation consists of shrublands on dunes and low heaths in swales dominated by perennial woody species from the families Proteaceae, Fabaceae, Myrtaceae and Ericaceae, and tussock and rhizomatous monocots from the Cyperaceae and Restionaceae (Enright *et al*. 2012).

The region is characterised by nutrient poor soils in a landscape of undulating sand dunes and intervening swales over lateritic clays and gravels (Hopper, 1979). They have very high sand content and are extremely low in nutrients, with phosphorus generally regarded as the most limiting (Beadle, 1966; Cowling *et al*., 1996), and little variation between dune and swale (Table S1, Perry *et al*. 2008). Changes in sand depth to the underlying gravel-clay layer are accompanied by shifts in species composition and stature and are attributed to variations in water availability where low heaths on shallow sands in swales (sand depth ~0.5-1.0 m) become water limited early in summer whereas tall shrublands on dunes (sand depth ~5-15 m) may have access to soil water throughout the year (Enright & Lamont 1992a). Vegetation in swales is generally <1.0 m in height whereas dunal vegetation ranges from 1-4 m in height.

2.2. Experimental set-up and study species

Experimental infrastructure was built in 2010 and was designed to create changes in rainfall (reduction by 30 %; drying) and temperature (increase by ~2.9 °C; warming) in accordance with projected 21st century climate change scenarios for southwest Australia (Williams, 2014). Passive permanent rain-out shelters (ROS) and open top chambers (OTC) were placed

in both dune and swale sites (Fig. 1b and 1c). ROS were constructed following Yahdjian and Sala (2002) with some modifications to suit local field conditions. The ROS height was 1.3 m and 1.7 m in swale and dune sites, respectively, to avoid interference from the canopies of the shrub vegetation (Williams, 2014). Each shelter consisted of a 5 x 5 m steel frame centred over a 4 x 4 m vegetation plot, with seven clear strips of polycarbonate sheeting (© SUNTUF, > 90% photosynthetic active radiation penetration) equally spaced across the roof of the shelter (for more details see Williams, 2014).

Open top chambers (OTCs) have mostly been used in colder (e.g. alpine) environments to study the effects of global warming (Williams, 2014), but have been used also in warm climate regions, including South Africa (Musil et al. 2005). The International Tundra Experiment (ITEX) design OTCs (following Molau and Molgaard (1996)) were constructed using six panels of fibreglass sheeting (© Sun Lite HP) joined to form a hexagon chamber shape which sloped inwards towards the open top of the chamber (Williams, 2014) creating a passive daytime temperature increase. The diameter of the chambers was 168 cm at the base and 110 cm at the top, with a height of 58 cm (Fig. 1c) (Williams, 2014). At each site (i.e., dune and swale), three replicate ROS plots, each with a paired control (Fig. 1d), and six OTCs were established, giving a total of N = 24 plots (i.e. 3 ROS + 6 OTC + 3 controls x 2 sites). The ROS controls acted also as OTC controls. The experimental infrastructure successfully mimicked projected climate changes for southwest Australia with the drying treatment receiving ~28% less rainfall and the warming treatment 1.8 – 3.8℃ higher ambient day time temperatures, depending on season (Williams, 2014).

The effectiveness of the warming treatment was assessed using Hydrochron iButtons (© Maxim Integrated) to record temperature and humidity 30 cm above ground in OTCs, under rainout shelters, and in control plots, across seasons. The effectiveness of the drying treatment was assessed by measuring soil moisture using a Delta-T PR2/6 Profile Probe

which measures soil moisture based on electromagnetic permittivity (Delta-T Devices Ltd (2016) User Manual Version: PR2-UM-5, Cambridge, U.K.). Probe access tubes were installed to 100 cm depth in the centre of each of the 24 4 x 4 m vegetation plots. For each access tube, three replicate soil moisture measurements (probe rotated 3 x 120° between measurements) were taken at 10, 20, 30, 40, 60 and 100 cm depth on a monthly basis from December 2011 until April 2013. In addition, a hand-held theta probe (Delta-T Devices Ltd (2017) User Manual for the ML3 Theta Probe. Cambridge, U.K.) was used each month to measure average soil moisture in the top 6 cm of the soil profile for both rainfall (rain-out shelter) and temperature (OTC) treatments.

Two widely distributed and abundant woody species that occurred on both dunes and swales were chosen as focal species; *Beaufortia elegans* Schauer (Fig. 1e) and *Melaleuca leuropoma* Craven (Fig. 1f). Both are small-leaved shrubs of similar size, but have contrasting regeneration strategies, *B. elegans* is a non-sprouter (i.e., is fire-killed) and *M. leuropoma* resprouts (i.e. regrows after fire from below-ground buds), so that the latter is characterised by a long-lived root system that persists through multiple fire cycles. In Australia, many trees and shrubs in the Myrtaceae have dual ECM (Ectomycorrhiza)/AMF (Arbuscular mycorrhizal fungi) associations (Brundrett, 2008). *Melaleuca leuropoma* has been reported to be ECM in a study based in Jurien Bay, Western Australia (Hayes *et al*., 2014). To our knowledge there are no published reports of the mycorrhizal associations for *B. elegans*.

2.3. Soil collection

Soil samples were collected in August 2014 to separately assess the effect of experimental drying and warming on soil fungal communities. We were not able to assess the interactive effects of warming and drying because these treatments were not applied in the field

simultaneously. In each plot, five soil samples (each approximately 100 grams) were collected using hand trowel from the top 5 cm of soil underneath the canopies, as close to the roots as possible, of *B. elegans* and *M. leuropoma* and bulked at plot level for each shrub species. Additionally, five soil samples per plot were collected from bare soil patches away from canopies, and bulked. Although *B. elegans* and *M. leuropoma* were chosen because of their wide distribution across the study sites, *B. elegans* was less common in the swale plots. Across treatments, we sampled soils under 17 *B. elegans* and 23 *M. leuropoma*, respectively. Soil sampling equipment was sterilized with 80% ethanol between replicates. Samples were kept in a cooler in the field before being transferred to storage at -20°C within 8 hours of collection. Each bulked soil sample was thawed and passed through a 2-mm sieve to remove leaves and other coarse material and to homogenize samples, and 100g of soil per sample retained for analysis.

Soil chemical properties for dune (n=5) and swale (n=3) sites were assessed. Samples were taken using a standard cylindrical container (6.5cm diameter x 8cm height). Leaf litter and other surface organic material was removed prior to the samples being taken. Each soil sample was dried at 45^{o}C for 24 hours and, a 10g sample sent to CSBP Soil and Plant Analysis Laboratory (Bibra Lake, Western Australia) for soil nutrient analysis.

2.4. Molecular analysis

Total DNA was extracted from 64 soil samples using the PowerSoil Pro DNA kit (MoBio, Carlsbad, USA) following the manufacturer's protocols. The ITS2 region of the ribosome encoding genes was amplified from soil DNA using the primer fITS7 (GAACGCAGCRAAIIGCGATA; specific for higher fungi) (Ihrmark et al., 2012) and the general primer ITS4 (White *et al*., 1990) with adapters attached. Amplification was

performed following the protocols outlined in Ihrmark et al. (2012) using the HotStarTaq (Qiagen, Valencia, CA), 30 amplification cycles and an annealing temperature of 57˚C. The ITS4 primer contained a ten base pair tag specific to each sample. Two replicate PCRs were performed per sample and these were combined following amplification. The products were visualised on a 1% agarose gel and then mixed in similar concentrations before being cleaned using an Agencourt AMPure® XP PCR purification kit (Beckman Coulter Inc., MA, USA). The final library was then subjected to 454 sequencing on a Roche GS Junior at the Western Australian State Agricultural Biotechnology Centre located at Murdoch University.

2.5. Bioinformatics

Sequences were analysed using the Sequence Clustering and Analysis of Tagged Amplicons (SCATA) pipeline (scata.mykopat.slu.se) with settings according to Ihrmark *et al*. (2012) and a clustering threshold of 98.5% similarity. We chose this sequence similarity as the accepted standard for ITS (Nguyen et al., 2017, Ottosson et al., 2015, Lindahl et al., 2013). SCATA is a bioinformatics pipeline specially developed for processing fungal ITS data sets derived from 454-sequencing (Lindahl et al., 2013). Prior to clustering, all sequences were trimmed to 300 bp length and the primers removed.

Sequence data was subsampled without replacement (329 sequences per sample) to ensure equal representation across all samples using 'phyloseq' (McMurdie and Holmes, 2013) package in R software (Weiss et al., 2017). Representative cluster sequences (operational taxonomic units or OTUs) were identified where possible by searching against internally curated SCATA databases and by blasting against NCBI's sequence database GenBank (Altschul *et al*., 1990) through Geneious (version R8.0.2, http://www.geneious.com, Kearse et al., 2012). Potentially similar OTUs were aligned in Geneious to confirm identification. OTUs were ranked by the number of reads in the dataset and for the one hundred most

abundant OTUs across the entire dataset, which represented ~78% of all reads. We performed NCBI blastn searches for identification and literature searches to assign putative function to 100 most abundant OTUs, where possible. Additionally, we assessed the function of five most abundant OTUs for each treatment (warming, drying) by location (dune, swale) and under canopy of *B. elegans* and *M. leuropoma* as well as away from canopy yielding 60 OTUs per category (i.e., 5 × 2 × 2 × 3, N=120 total). Functional categories were assigned based on their putative life history following ecological guild assignment *sensu* FUNGuild (Nguyen et al., 2016).

2.6. OTU warming and drying responses

We calculated treatment responses for all 826 OTUs in our dataset. Warming and drying responses were defined as the change in prevalence of the OTU in response to warming/drying (Treseder et al., 2016):

$$\text{Response } (\%) = [(T_p/N_p) - (C_p/N_p)] \times 100$$

Where T_p is the number of treatment plots (calculated separately for warming and drying treatments) in which a given OTU is present, and C_p is the number of control plots occupied by the same OTU. N_p is the number of plots for each treatment or control. The possible response value ranged from +100% to -100%. Positive response indicated an increase of an OTU and negative response, a decrease. Responses were calculated based on OTU presence/absence matrix.

2.7. Statistical analyses

To assess whether soil fungal community composition was affected by treatments, site and by canopy species, non-metric dimensional scaling (NMDS) based on Cao dissimilarity index (Cao et al., 1997) was carried out on subsampled data generated from the OTU species matrix after removing global singletons and doubletons. Cao index (Cao et al., 1997) has been suggested as an appropriate index for high beta diversity and variable sampling intensity (i.e. unbalanced Permanova) (Anderson and Millar, 2004, Krebs, 1999). This allowed visual inspection to identify compositional difference in soil fungal composition between sites (dune, swale), under canopy species (*B. elegans*, *M. leuropoma*, and away from canopy species) and treatments (controls, drying, warming). Ellipses were added to NMDS plots using stat_ellipse function with default settings using ggplot2 package (Wickham, 2009). To support the NMDS analysis and quantify differences in fungal community composition among groups, Permutational multivariate analysis of variance (Permanova) (Anderson, 2001) was used (9999 permutations) from R package 'vegan' to test for differences in soil fungal community composition between sites, treatments and canopy species within each site (Oksanen et al., 2017). Complementary, we also implemented PERMDISP2 procedure (Anderson, 2006) for the analysis of multivariate homogeneity of group dispersions (variances) using 'betadisper' function in R.

To assess if warming and drying affect soil fungal diversity, the Shannon diversity index was calculated for each sample using the 'diversity' function in 'vegan' package. Expected species richness and its standard deviation was calculated using 'specaccum' and 'rarefy' functions (method = rarefaction) in 'vegan' package. Observed OTU richness and Shannon diversity between sites, within sites and treatments under and away from canopy species, and OTU warming responses between treatments were analysed using one-way Anova and 'aov' function in R. TukeyHSD test was used for pairwise comparisons when Anova model result

was significant. To determine indicator species from each treatment we used 'multiplatt' function in 'indicspecies' package (Cáceres and Legendre, 2009). Venn diagrams were built using 'VennDiagram' package in R (Chen, 2018). One-way ANOVA was used to assess differences in relative abundances between the functional groups of 100 most abundant OTUs and additional 120 OTUs. All analyses were performed in the R 3.4.3 programming language (R Core Team, 2018).

3. Results

A total of 1815 OTUs were identified, comprising 155,783 sequences. After removing singletons and doubletons 1073 OTUs remained (a total of 150,815 sequences). Following subsampling 826 OTUs remained. The subsampled data matrix was used for all following statistical analyses. Fungal community composition was significantly different between the dune and swale sites (Table 1, Fig.2a). However, OTU richness ($F_{(1,63)} = 0.06$, $P = 0.81$) and Shannon diversity index ($F_{(1,63)} = 0.68$, $P = 0.41$) were similar between dune and swale. Dune and swale sites shared 435 OTUs, but the number of unique OTUs was higher in dune than swale; 170 and 125, respectively (Fig. 3a).

Global Permanova showed a significant interaction between site and canopy species (Table 1). Treatment also had a significant effect on overall soil fungal composition (Table 1). When dune and swale matrices were analysed separately with Permanova, treatment and canopy species had a significant effect on soil fungal composition in dune plots (Table 1, Fig.2b-d). The multivariate dispersions were similar between treatments in dunes (Fig.S1a). The observed fungal OTU richness (Species x Treatment: $F_{(4,25)} = 0.5$, $P = 0.74$) in the soil and Shannon diversity index (Species x Treatment: $F_{(4,25)} = 0.5$, $P = 0.71$) were similar suggesting no effect of drying and warming on soil fungal richness or diversity in dunes

(Fig.4 a-f). Rarefied species richness followed similar trends as observed OTU richness (FigureS2 a-c). Rarefaction curves did not reach asymptote. The number of unique OTUs was higher in warming than drying treatment compared to control (Fig. 3b) and very similar under *B. elegans* and *M. leuropoma* canopies (Fig. 3c).

In swale plots, canopy species and treatment, had a significant effect on soil fungal composition (Table 1, Fig. 2e-g).). The multivariate dispersions were similar between treatments in swales (Fig.S1b). Observed OTU richness was significantly affected by canopy species ($F_{(2,22)}$ = 3.9, P = 0.03), but not treatment ($F_{(2,22)}$ = 1.5, P = 0.23) which was driven by significantly higher OTU richness under *M. leuropoma* canopy as compared to away from canopy (88.6 (± 3.28 S.E) vs 72.9 (± 4.84 S.E), respectively, P = 0.02) across the three treatments in swale plots. Rarefied species richness was higher in warming treatments away from canopy (FigureS1 d) and under *B. elegans* canopy (FigureS1 e) as compared to drought and control treatment. Under *M. leuropoma* canopy, rarefied species richness was similar between warming and control treatments and higher than in drought treatment (FigureS1 f). Rarefaction curves did not reach asymptote. Shannon diversity index was significantly affected by species ($F_{(2,22)}$ = 3.9, P = 0.03) and treatment ($F_{(2,22)}$ = 3.8, P = 0.04), but not their interaction ($F_{(4,22)}$ = 1.85, P = 0.15) (Fig.4j-l). Shannon diversity index was significantly higher in warming treatment as compared to control plots (3.78 (± 0.04 S.E) vs 3.36 (± 0.22 S.E), respectively, P = 0.03) and marginally higher under *M. leuropoma* canopy as compared to *B. elegans* canopy (3.84 (± 0.07 S.E) vs 3.43 (± 0.17 S.E), P = 0.054) across all three treatments. The number of unique fungal OTUs was 2.5 times higher in warming than drying treatment and under *M. leuropoma* canopy compared to *B. elegans* canopy (Fig. 3d and e).

3.1. Indicator species analysis

Indicator species analysis revealed that 13 and 18 OTUs significantly ($P < 0.05$) associated with treatments in dune and swale sites, respectively (Table 2). Fewer OTUs associated significantly with treatments in dune sites as compared to swale sites. Of these 31 OTUs 13 were Ascomycota, 4 Basidiomycota and 13 unknown fungi (Table 2). *Rhytidhysteron rufulum* (Ascomycota) associated significantly with control treatment in dunes and with drought treatment in swales.

3.2. OTU warming response and fungal functional groups

OTU warming responses (change in % plots present) were highly variable across treatments within dune and swale sites ($F_{(3,122)} = 0.73$, $P = 0.53$, Fig.5a). However, the number of OTUs responding to warming was significantly different between treatments ($F_{(3,118)} = 5.83$, $P = 0.001$, Fig.5b). In dunes, significantly more OTUs responded (i.e. negative or positive) to drought than warming treatment (estimate = -38.84 (±11.41 S.E), t-value = -3.4, $P = 0.005$), whereas in swales this pattern was reversed but not significant (Fig.4b). Across dunes and swales, ~50% of total OTUs response to warming was neutral (Table S2).

There was no significant difference in the relative abundance between functional groups of hundred most abundant OTUs ($F_{(6,20)} = 0.98$, $P = 0.46$). The one hundred most abundant fungal OTUs comprised of 82% Ascomycota and 12 % Basidiomycota (Table S3). We were able to assign a putative function to a total of 21.4 % of most abundant OTUs comprising of unidentified root endophytes (6.6 %), ericoid mycorrhizal fungi (5.3%), ectomycorrhizal fungi (3%), yeasts (4.1%), plant pathogenic fungus (1.4%), wood saprotrophic fungus (0.6%)

and lichenized fungus (0.4%) (Fig. 6, Table S3). For 120 OTUs that either increased or decreased we assigned a putative function to 18.3% and 50% of OTUs, respectively (TableS4 and TableS5, respectively). In dunes, treatment did not have a significant effect on soil functional groups that increased ($F_{(1,6)} = 3.11$, $P = 0.13$, Fig. 7a) or decreased ($F_{(1,9)} = 1.52$, $P = 0.23$, Fig. 7b). Ectomycorrhizaes (ECM) and endophytes increased in abundance (based on presence/absence) in drought treatments only, while plant pathogens and yeast had similar relative abundance in both drought and warming treatments (Fig.7a). Ericoid mycorrhizae (EM) that decreased in dunes had the highest mean relative abundance in drought treatment and were not identified from warming treatment (Fig.7b).

Similarly, in swales treatment did not have a significant effect on soil functional groups that increased ($F_{(1,1)} = 0.17$, $P = 0.75$, Fig. 8a) but had marginal effect on soil functional groups that decreased ($F_{(1,13)} = 4.31$, $P = 0.058$, Fig. 8b). Among the OTUs that increased in their abundance, ECMs and plant pathogens were identified only from drought treatment, while EM were identified only from warming treatment (Fig.8a). Among the OTUs that decreased in their abundance, EMs and endophytes were identified only from drought treatment while ECMs and plant pathogens were present in both treatments, and lichenized fungus and wood saprotroph were present only in warming treatment (Fig.8b).

4. Discussion

Our study provides one of the first characterisations of soil fungal composition for MTE shrublands in response to climate change manipulations (but see Hopkins et al. 2018). The results suggest that changes in future climate conditions, i.e. lower precipitation and higher temperatures, as projected by climate-change models, will affect soil fungal composition, but not necessarily reduce soil fungal richness or diversity, as also shown by some other studies (Toberman et al., 2008, Christiansen et al., 2017).

Here, we predicted that soil fungal community composition would be affected by study site (i.e. dune vs swale). Dunes and swales differ considerably in soil depth, with dunes having sandy soils up to 10 m deep, whereas swales have a shallow sand layer (<1m) underlain by impermeable clays (Williams, 2004). Williams et al. (2014) found that swales at Eneabba had consistently higher soil moisture levels than dunes in winter-spring and that drought treatment had significant effects on soil moisture in the dunes but not in the swales. Consequently, these two topographic contexts differ in their hydrological regimes (Enright & Lamont, 1992). Some authors have found that soil moisture may function as an abiotic filter affecting AMF assembly (Deepika and Kothamasi, 2015), while others have suggested that fungal community composition may show high plasticity when soil moisture fluctuates (Kaisermann et al., 2015). We found several differences between dune and swale sites; i) significant differences in soil fungal composition, ii) higher number of significant fungal indicator OTUs in swales, iii) higher number of unique fungal OTUs in dunes, and iv) significantly more OTUs responding to drought in dunes, suggesting that differences in soil moisture may underpin these observed patterns since other edaphic factors (e.g. total N, P, K, organic C, pH, NH_4^+) were overall similar between the two sites (Table S1, see also Table 1 in Perry *et al.* 2008, Herath *et al*. 2009a, 2009b).

In MTEs, where water availability is already the most significant environmental constraint because of combination of high summer temperatures and low rainfall (Specht et al., 1983, Larcher, 2000), drought has been reported to limit the physiological performance of microbes (Harris, 1981) and therefore submit the microbial communities to a strong adaptive force (Yuste et al., 2011). In view of this assumption, we predicted that richness and diversity in warming and drying treatments would be lower than in control plots. However, we found that soil fungal diversity increased under warming treatment in swale plots as compared to control plots indicating a positive effect of warming on fungal diversity. Recent studies have found that soil fungal diversity responses to climate warming may be largely fungal taxa- and functional group specific (Geml et al., 2015, Looby and Treseder, 2018, Treseder et al., 2016). For example, Looby and Tresder (2018) found that richness of wood saprotrophs, yeasts, and fungal pathogens increased following warming in a tropical montane cloud forest. In this study, we assessed putative functions of 100 most abundant OTUs and additional 120 OTUs response to the warming and drying treatments, although for majority of these OTUs it is challenging to assign a putative function due to lack of information and thus these results should be interpreted with caution. Of the one hundred most abundant OTUs in our data set we were able to assign a putative function to a total of 21.4 % revealing overall a high abundance of fungal endophytes, ericoid mycorrhizas, yeasts and ectomycorrhizas. For 120 OTUs across dunes and swales that either increased or decreased we assigned a putative function to 18.3% and 50% of OTUs, respectively. In dunes, soil fungal putative functional groups that increased in their abundance and were only identified from drought treatment were ectomycorrhizaes (ECMs) and endophytes whereas plant pathogens and yeasts increased in both treatments and had the highest mean relative abundance. This suggests that drying and warming climate may facilitate plant pathogens in dunes, which warrants further investigation. The most abundant functional group that decreased in dunes was ericoid

mycorrhizas (EMs). EMs associate predominantly with members of Ericaeae family that are abundant in MTE in south-western Australia (Cowling et al., 1994). EMs also contribute to many important ecosystem processes such as plant productivity (0-50%) (Grelet et al., 2009), plant nitrogen acquisition (0-80%) (Hobbie and Hobbie, 2008, Zijlstra et al., 2005) and plant phosphorus uptake (0-80%) (Smith and Read, 1997). Thus, the loss of EMs in this ecosystem may have negative consequences for plant species diversity and key ecosystem processes. In swales, ECMs and plant pathogens were found to increase in drought treatments while EM increased in warming treatment only. Similarly to dunes, EMs and endophytes had highest mean relative abundance and both groups decreased in drought treatment only suggesting that drought conditions may have negative effects on key soil fungal functional groups. Taken together, better understanding the response of fungal functional groups to climate warming in MTEs in SWA is certainly an area that requires further research and has important implications for elucidating future plant diversity patterns and the provision of ecosystem services.

We found that site had a significant interaction effect with canopy species in the global Permanova model suggesting that canopy species effects on soil fungal communities were site specific. Across both dune and swale plots soil fungal composition was consistently affected by the canopy species which was mediated via site specific conditions that *B. elegans* and *M. leuropoma* experience. Therefore, we found support for our hypothesis that soil fungal community composition will be affected by canopy species. Notably, we found that in swale plots the fungal OTU richness and Shannon diversity under *M. leuropoma* canopy was significantly higher compared to away from canopy and under *B. elegans* canopy, respectively. In addition, the number of unique fungal OTUs was 2.5 times higher under *M. leuropoma* canopy compared to under *B. elegans* canopy in swale plots. These results suggest that soil fungal community composition under *M. leuropoma* canopy is more

diverse compared to *B. elegans* canopy or away from canopy in swale plots. *Melaleuca leuropoma* is a resprouter that has long-lived root system that persists through multiple fire cycles, is less vulnerable to change or slower to respond to changes in the environment (Enright et al., 2011, Williams, 2014), and thus it can retain its diverse soil fungal communities across seasons whereas *B. elegans* is a fire-killed non-sprouter (Williams, 2014) that has to re-establish its soil after fire which may explain the lower fungal diversity in its soil. Thus, better understanding the aboveground effects of warming and drying and their interaction with site specific abiotic conditions on *B. elegans* and *M. leuropoma* may assist in understanding the belowground soil fungal responses as well. This clearly warrants further investigation.

In conclusion, we found evidence for site, treatments and canopy species affecting soil fungal composition with soil fungal diversity increasing under warming treatment in swale plots as compared to control plots indicating a positive effect of warming on fungal diversity. We found strong site specific differences in soil fungal composition indicating that it is important to consider soil abiotic conditions when interpreting microbial responses to climate change treatments in the field studies. In terms of soil fungal putative functional groups, we found that plant pathogens presence increased while common ericoid mycorrhizae decreased as a response to treatments across both sites. Future studies should focus on describing the soil fungal functional groups in MTEs to better predict changes in ecosystem services provisioned by soil fungi.

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Figure 1. Clockwise from top left: (a) study location in Eneabba, Australia, (b) rain-out shelter with drum at a shallow sand site (i.e. swale), c) open top chamber at a deep sand site (i.e. dune), d) control plot, e) *Beaufortia elegans* and f) *Melaleuca leuropoma*. Photo credits: Amity Williams, Joe Fontaine and Phil Ladd.

Figure 2. Non-metric multidimensional scaling ordination for soil fungal communities based on extracted DNA from soils associated with *Beaufortia elegans* and *Melaleuca leuropoma*, as well as bare soil patches away from hosts (–Host), for dune and swale sites at Eneabba, Western Australia. A – soil fungal communities by site (i.e. dune or swale), by treatment and species in dune sites (B-D) and treatment and species in swale sites (E-G).

Figure 3. Venn diagrams showing the number of shared and unique OTUs by study site (A), and separately by treatment and host species within dune (B-C) and swale plots (D-E). (See figure attached separately.)

Figure 4. Barplots (±S.E.) showing observed OTU richness and Shannon diversity index for dune (A-F) and swale sites (G-L) by treatment (Control, Drought, Warming) and by host species (*Beaufortia elegans* and *Melaleuca leuropoma*) as compared to away (-Host) from host plant species. Standard error could not be calculated for *B. elegans* in drought treatment in swale sites due to low replicate numbers.

Figure 5. Mean treatment response of fungal OTUs in treatments across dunes and swales (A) and number of OTUs responding to treatments (drought/warming) across dunes and swales (B) in Eneabba, Western Australia. Bars are means with standard errors. Treatment response for each OTU was calculated as change in percentage of plots occupied by that OTU in the drought/warming versus control treatments (A). In B panel, only OTUs that responded positively/negatively to treatments are shown. OTUs that did not respond (i.e. neutral) to treatments are not shown. For all OTUs responses (positive, neutral, negative) please see TableS2. N.S. – not significant difference between drought and warming treatments in swales. Asterisk indicates significant difference between drought and warming treatment in dunes.

Figure 6. Mean relative abundance (±S.E where possible to calculate) of soil fungal functional groups identified from 100 most abundant OTUs across entire dataset. For full descriptions and references, please see Table S2. ECM – Ectomycorrhizae, EM – Ericoid mycorrhizae. Standard errors could not be calculated for lichenized fungi, plant pathogens and wood saprotroph because n=1.

Figure 7. Mean relative abundance (±S.E where possible to calculate) of dune soil fungal functional groups that increased (A) or decreased (B) in drought and warming treatments in Eneabba, Western Australia based on assessment of 60 most abundant OTUs. For more details see TableS4. ECM – Ectomycorrhizae, EM – Ericoid mycorrhizae.

Figure 8. Mean relative abundance (±S.E where possible to calculate) of swale soil fungal functional groups that increased (A) or decreased (B) in drought and warming treatments in

Eneabba, Western Australia based on assessment of 60 most abundant OTUs. For more details see TableS5. ECM – Ectomycorrhizae, EM – Ericoid mycorrhizae.

Tables

Table 1. Permanova results for the global model, and dune and swale sites separately, showing the effect of site, host species and treatment, as well as their interaction, on soil fungal composition at Eneabba, Western Australia. Values in bold indicate significant values where $P < 0.05$.

Factor	Df	SS	MS	F-value	P-value
Global					
Site	1	8.75	8.75	6.84	**0.0001**
Species	2	6.22	3.11	2.43	**0.0001**
Treatment	2	4.05	2.03	1.58	**0.002**
Site:Species	2	3.46	1.73	1.35	**0.02**
Site:Treatment	2	3.06	1.53	1.19	0.09
Species:Treatment	4	4.53	1.13	0.88	0.89
Site:Species:Treatment	4	5.03	1.26	0.98	0.52
Residuals	47				
Total	64				
Dune					
Species	2	4.04	2.02	1.58	**0.001**

Treatment	2	3.28	1.64	1.28	**0.03**
Species:Treatment	4	4.58	1.14	0.89	0.88
Residuals	25				
Total	33				
Swale					
Species	2	5.78	2.89	2.20	**0.0001**
Treatment	2	3.59	1.79	1.37	**0.02**
Species:Treatment	4	5.11	1.28	0.97	0.57
Residuals	22				
Total	30				

Table 2. Indicator species analysis (alpha = 0.05) output showing OTUs associated significantly to treatments as well as their combinations (i.e. shared OTUs) across dunes and swales. Names determined following NCBI nucleotide BLAST search. GenBank accession numbers to closest match are given.

Site	Treatment	OTU number	Indicator value	*P* value	Accession number	Name	Phylum and family (if known)
Dunes	Drought	OTU228	0.584	0.04	AY112917	*Capronia sp.*	Ascomycota (Herpotrichiellaceae)
	Warming	OTU193	0.559	0.047	NR_155177	*Hawksworthiomyces hibbettii*	Ascomycota (Ophiostomataceae)
	Control	OTU261	0.667	0.005	KP162181	*Rhytidhysteron rufulum*	Ascomycota (Patellarioaceae)
		OTU136	0.577	0.033	KP687491	*Geastrum austrominimum*	Basidiomycota (Geastraceae)
		OTU296	0.577	0.03	AB846975	*Mucoromycotina sp.*	Unknown
		OTU222	0.53	0.049	KU354831	Unknown fungal strain	Unknown
Drought +Warming		OTU84	0.804	0.003	LC317453	*Aspergillus lentulus*	Ascomycota (Trichocomaceae)
Drought + Control		OTU58	0.854	0.015	KT834985	*Talaromyces sp.*	Ascomycota (Trichocomaceae)
		OTU67	0.827	0.002	AB986359	*Eurotiales sp.*	Ascomycota
		OTU65	0.758	0.01	KX193618	Uncultured fungus	Unknown
		OTU141	0.667	0.012	JX243798	*Mucoromycotina sp.*	Unknown
		OTU206	0.653	0.031	JX987756	Uncultured fungus	Unknown
Warming + Control		OTU133	0.693	0.032	NR_111163	*Cryptotrichosporon anacardii*	Basidiomycota (Trichosporonaceae)
Swales	Drought	OTU195	0.736	0.003	MF671990	*Xylogone sphaerospora*	Ascomycota
		OTU27	0.673	0.005	NR_120038	*Inocybe redolens*	Basidiomycota (Inocybaceae)
		OTU263	0.647	0.028	KY228684	*Mucoromycotina sp.*	Unknown
		OTU261	0.593	0.036	KP162181	*Rhytidhysteron rufulum*	Ascomycota (Patellarioaceae)
		OTU320	0.576	0.032	KY228684	*Mucoromycotina sp.*	Unknown
		OTU184	0.535	0.04	FN557563	Uncultured ectomycorrhizal fungus	Unknown

		OTU664	0.535	0.042	KU189091	Uncultured fungus	Unknown
		OTU183	0.535	0.042	MG198621	*Arthrinium arundinis*	Ascomycota (Apiosporaceae)
	Warming	OTU39	0.827	0.011	AF050278	*Phaeococcomyces nigricans*	Ascomycota (Herpotrichiellaceae)
		OTU89	0.667	0.035	KU164637	Uncultured fungus	Unknown
		OTU94	0.612	0.05	KY228749	*Sordariales sp*.	Ascomycota
	Control	OTU35	0.699	0.027	MF772497	*Talaromyces atroroseus*	Ascomycota (Trichocomaceae)
		OTU73	0.695	0.025	MF976233	Uncultured fungus	Unknown
		OTU897	0.612	0.025	KM116464	*Pabia signiensis*	Chlorophyta (Trebouxiaceae)
		OTU232	0.594	0.027	KU063522	Uncultured fungus	Unknown
Drought +Warming		OTU20	0.877	0.035	KJ869136	*Phaeococcomyces rothmanniae*	Ascomycota (Herpotrichiellaceae)
Drought + Control		OTU29	0.911	0.001	DQ875350	*Moreaua fimbristylidis*	Basidiomycota (Anthracoideaceae)
		OTU91	0.667	0.047	KJ827618	Uncultured fungus	Unknown

Highlights

- Effects of drying and warming climate on soil fungi were studied using metagenomics
- Four years of climate change manipulations show effects on soil fungal communities
- Significant effect of study site on soil fungal composition
- No evidence found for reduced soil fungal richness and diversity

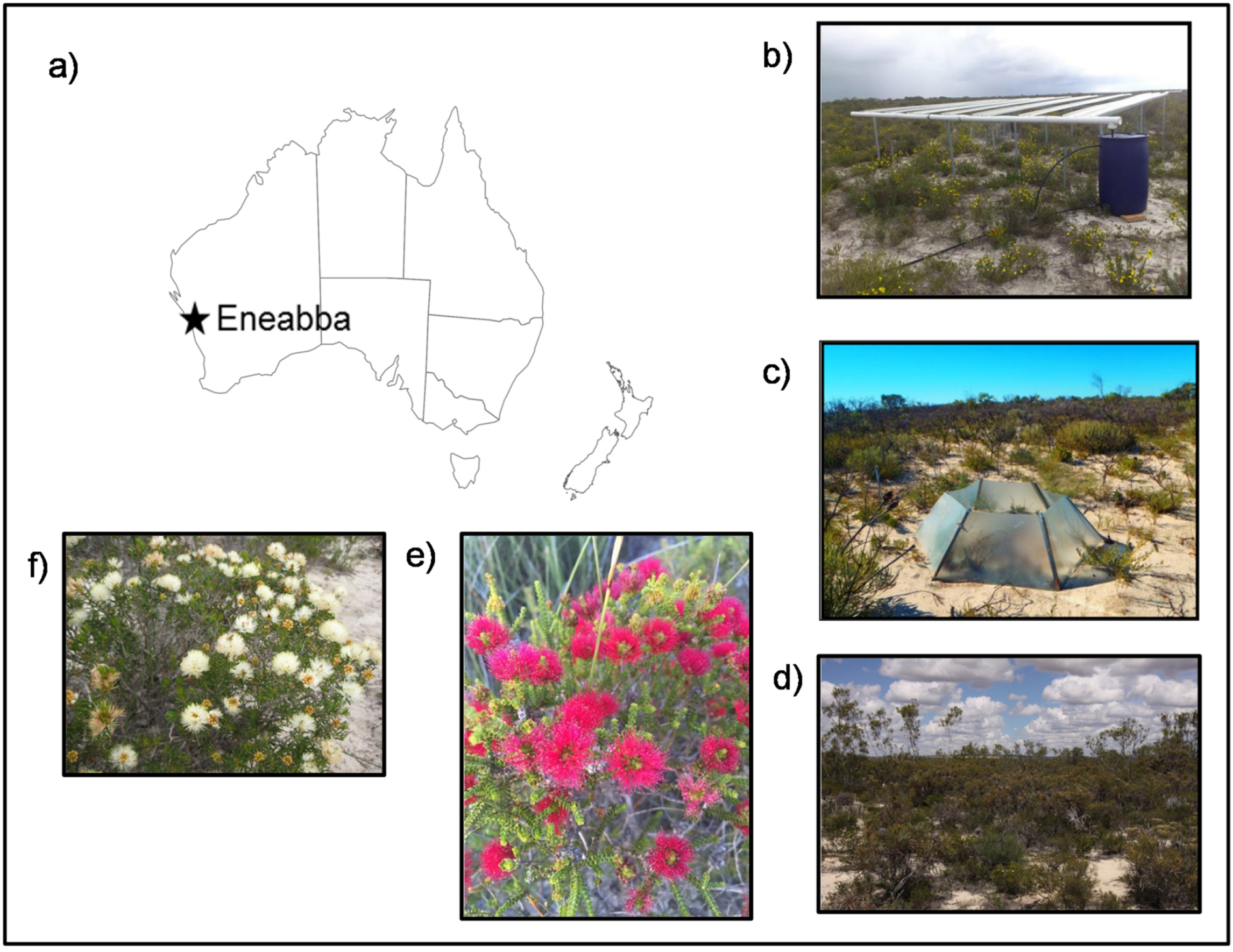

Figure 1

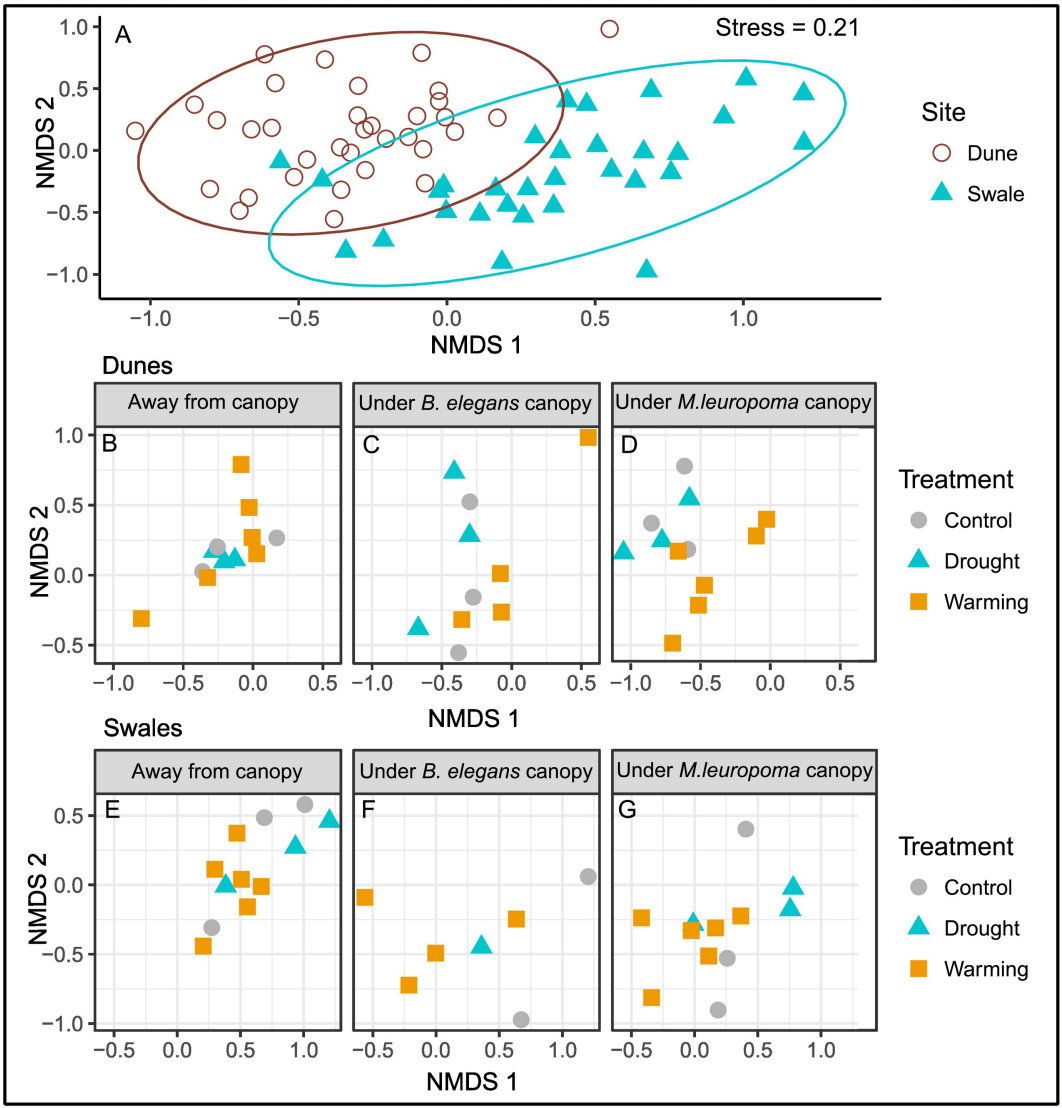
A
Stress = 0.21
NMDS 2
NMDS 1
Site
Dune
Swale
Dunes
Away from canopy
Under B. elegans canopy
Under M.leuropoma canopy
B
C
D
Treatment
Control
Drought
Warming
Swales
E
F
G

Figure 2

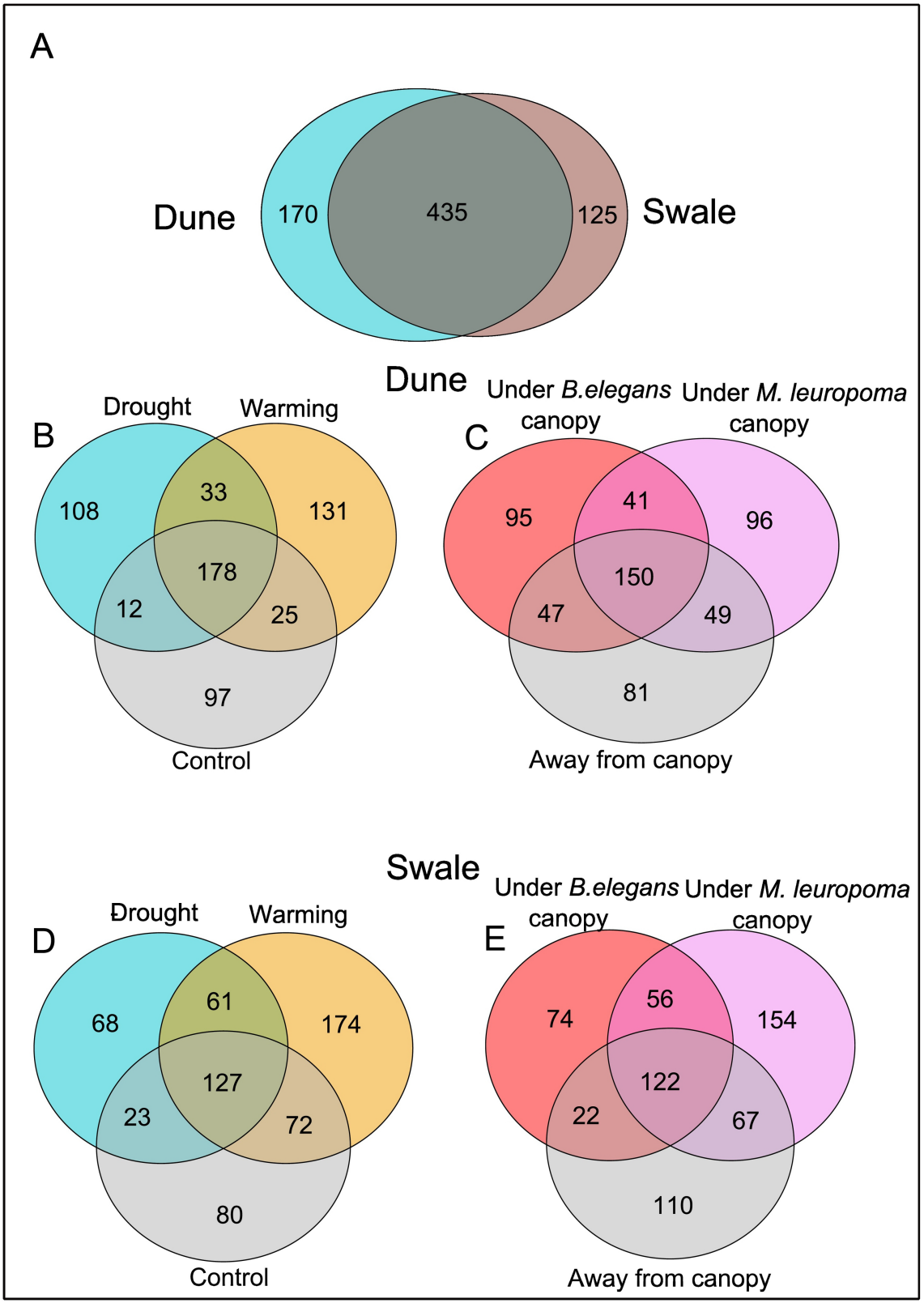
A
Dune
170
435
125
Swale
B
Drought
Warming
108
33
131
178
12
25
97
Control
Dune
C
Under B.elegans canopy
Under M. leuropoma canopy
95
41
96
150
47
49
81
Away from canopy
Swale
D
Drought
Warming
68
61
174
127
23
72
80
Control
Swale
E
Under B.elegans canopy
Under M. leuropoma canopy
74
56
154
122
22
67
110
Away from canopy

Figure 3

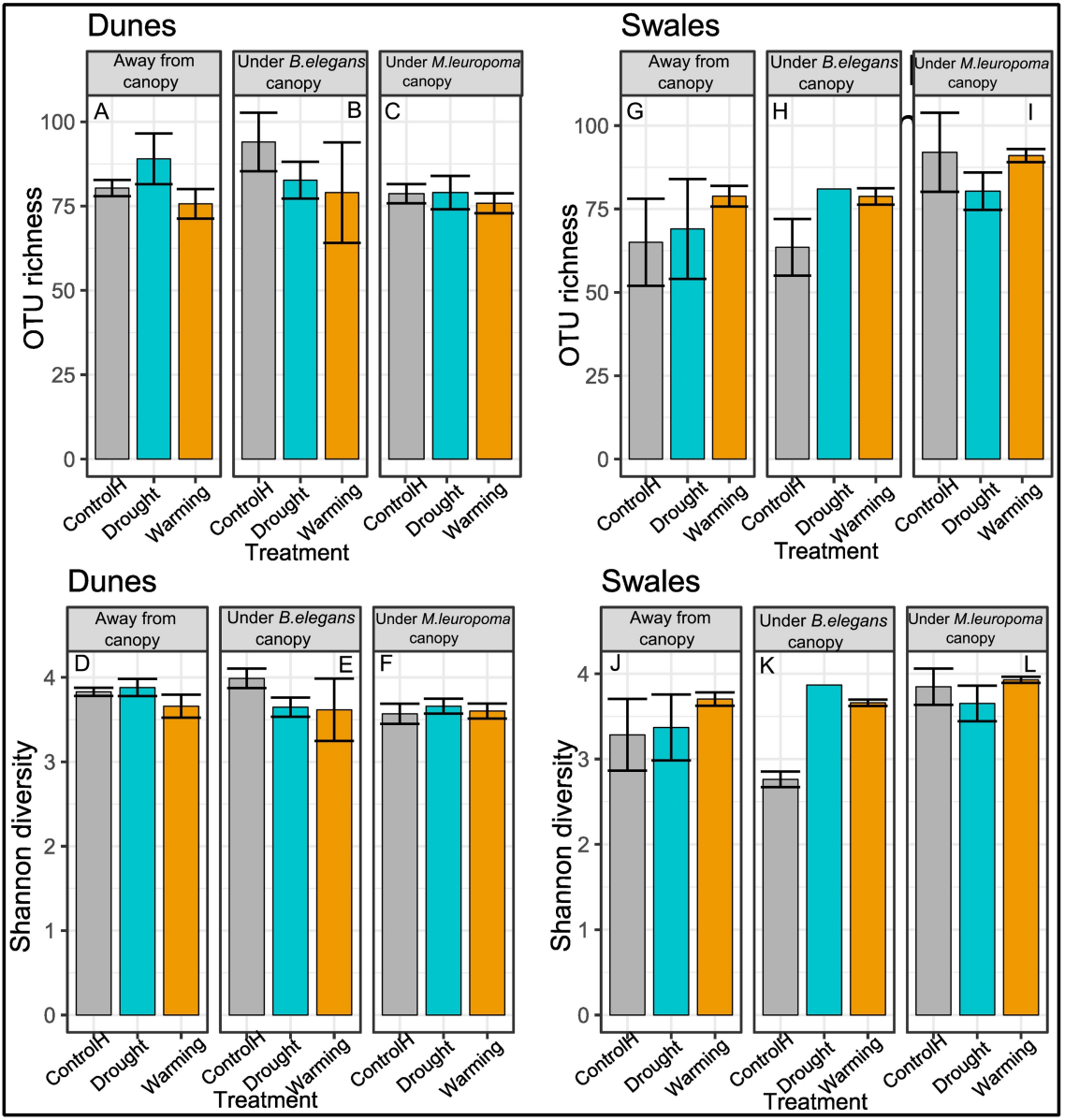
Dunes
Swales
Away from canopy
Under B.elegans canopy
Under M.leuropoma canopy
A
B
C
G
H
I
D
E
F
J
K
L
OTU richness
Shannon diversity
100
75
50
25
0
4
3
2
1
ControlH
Drought
Warming
Treatment

Figure 4

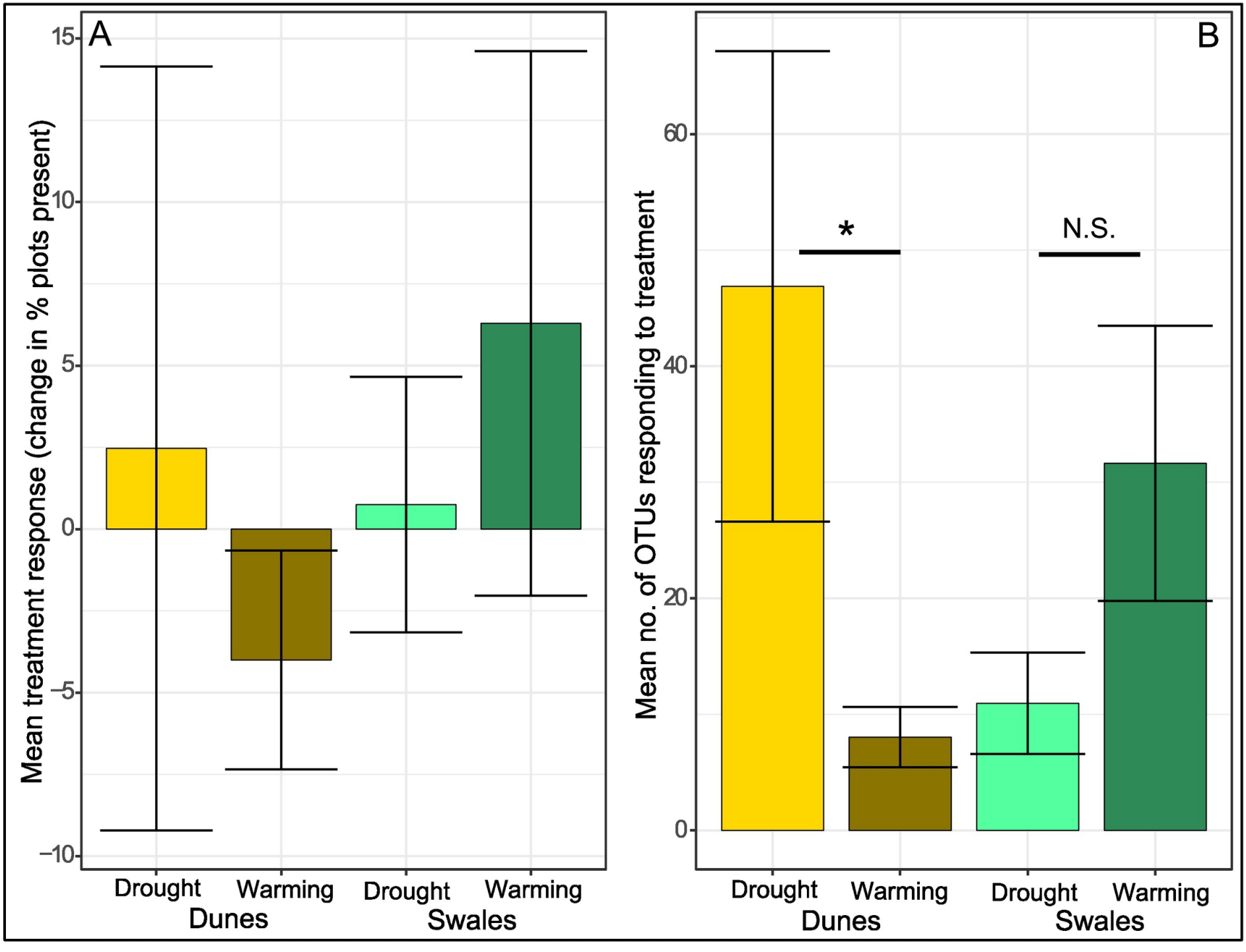
A
B
15
10
5
0
−5
−10
Mean treatment response (change in % plots present)
60
40
20
0
Mean no. of OTUs responding to treatment
*
N.S.
Drought
Warming
Drought
Warming
Dunes
Swales
Drought
Warming
Drought
Warming
Dunes
Swales

Figure 5

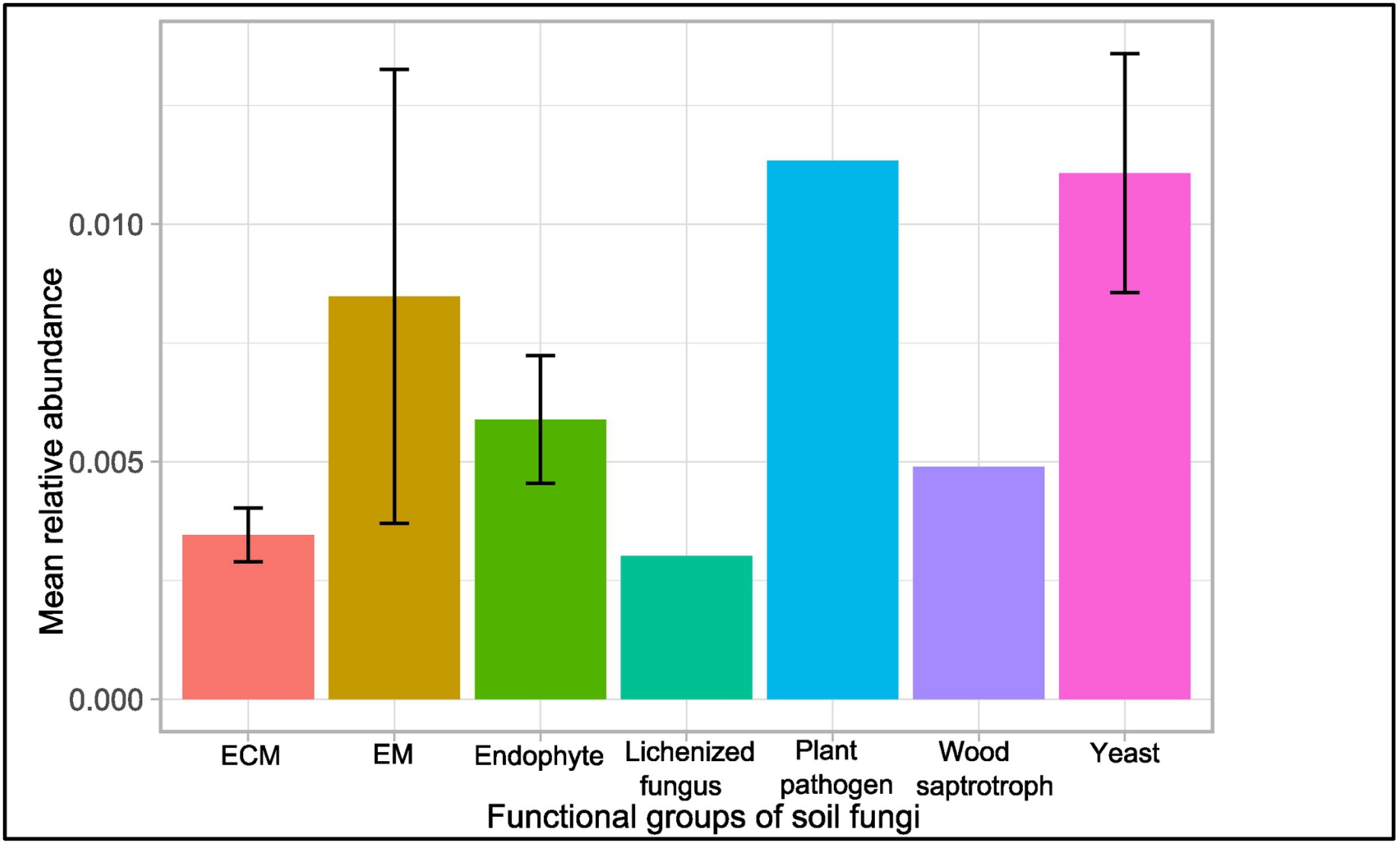
Mean relative abundance
0.010
0.005
0.000
ECM
EM
Endophyte
Lichenized fungus
Plant pathogen
Wood saptrotroph
Yeast
Functional groups of soil fungi

Figure 6

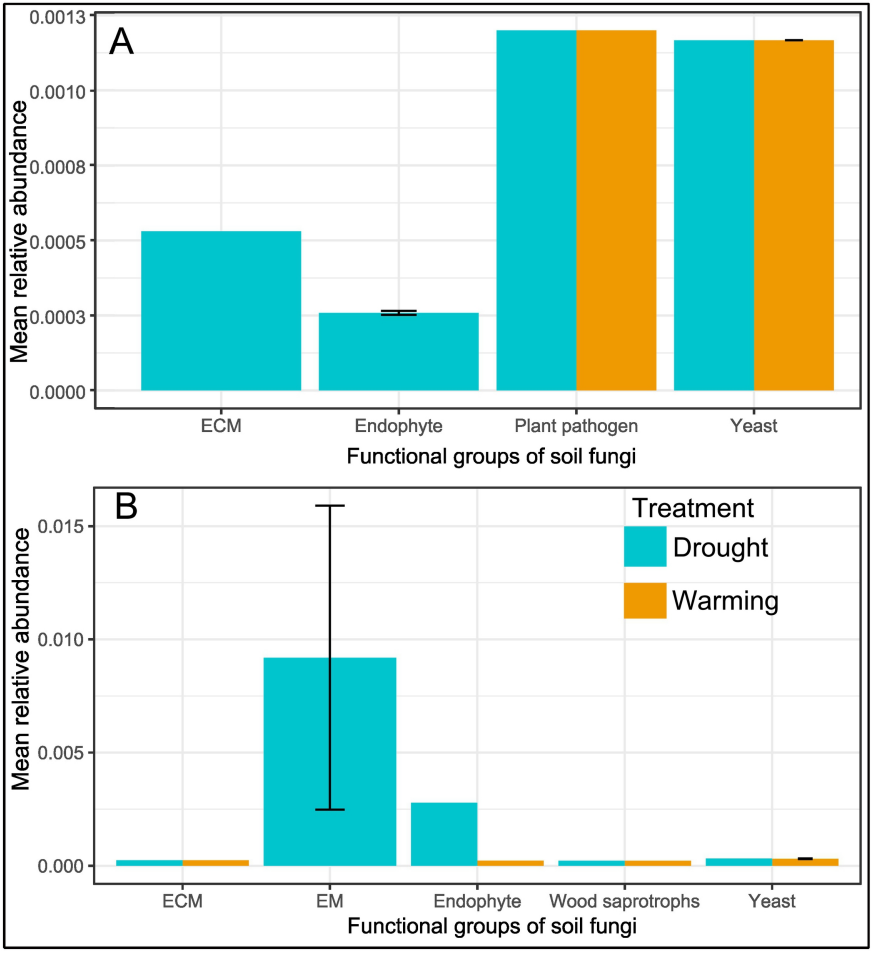
A
Mean relative abundance
0.0013
0.0010
0.0008
0.0005
0.0003
0.0000
ECM
Endophyte
Plant pathogen
Yeast
Functional groups of soil fungi
B
Mean relative abundance
0.015
0.010
0.005
0.000
Treatment
Drought
Warming
ECM
EM
Endophyte
Wood saprotrophs
Yeast
Functional groups of soil fungi

Figure 7

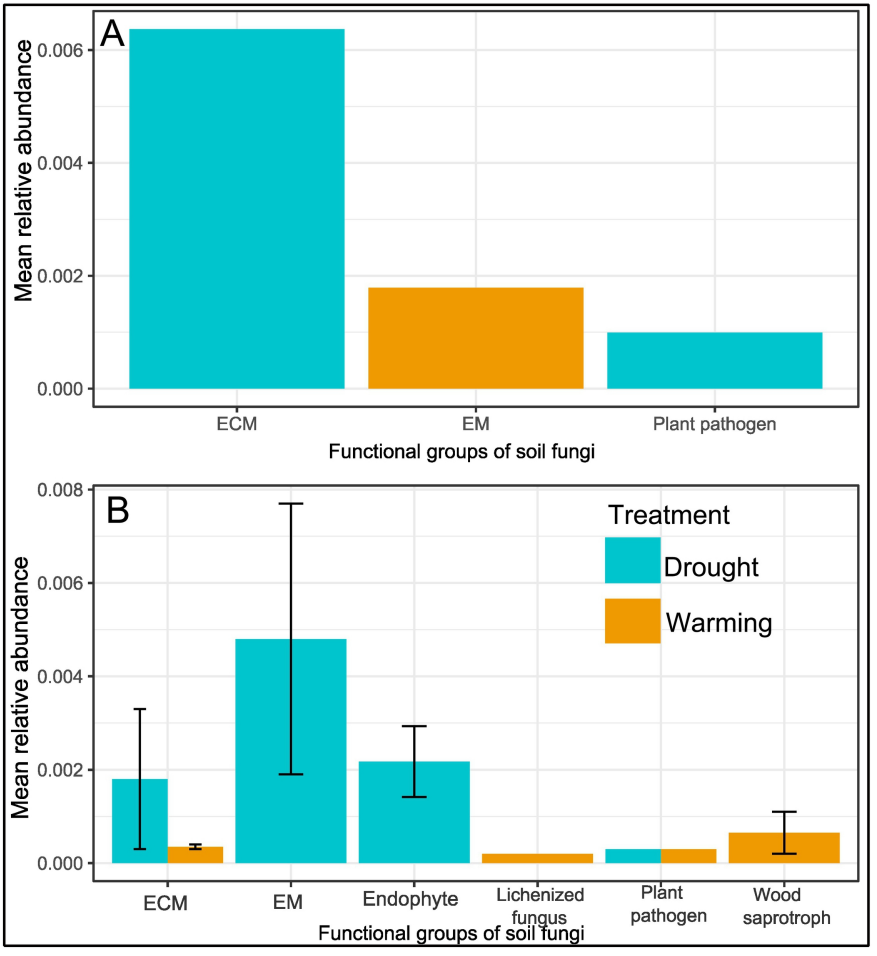
A
Mean relative abundance
0.006
0.004
0.002
0.000
ECM
EM
Plant pathogen
Functional groups of soil fungi
B
Mean relative abundance
0.008
0.006
0.004
0.002
0.000
Treatment
Drought
Warming
ECM
EM
Endophyte
Lichenized fungus
Plant pathogen
Wood saprotroph
Functional groups of soil fungi

Figure 8