DROUGHT RESPONSE OF PLANT COEXISTENCE ARE MEDIATED BY ARBUSCULAR MYCORRHIZAL FUNGI

by

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An Abstract of the Thesis of

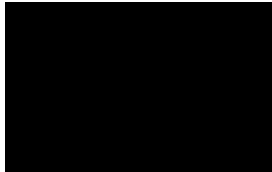Austyn Tavernier   for the degree of   Bachelor of Science

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Climate change is predicted to alter interannual precipitation patterns and increase drought severity and frequency. Although plant ecologists are actively investigating the impacts of climate change, we still lack robust and generalizable predictions of future plant community dynamics in light of novel precipitation regimes. One of the most pressing obstacles for making these predictions is understanding how the many species in these communities, as well as the interactions between them, respond to drought. Among the more important biotic players in structuring herbaceous plant communities are arbuscular mycorrhizal fungi (AMF) which have been shown to contribute to plant responses to drought at both an individual and community level. However, the mechanisms of drought-dependent mycorrhizal effects on plant competition have yet to be empirically assessed. To explore how AMF may modify the effect of drought on plant competitive dynamics, we conducted a greenhouse competition experiment within a two-by-two factorial manipulation of soil moisture and AMF presence. We used the data from this experiment to parameterize population dynamic models for each species (*Clarkia amoena, Collinsia grandiflora, and Plectritis congesta)* within each treatment combination. We used posterior distributions of our parameter estimates to explore how mycorrhizae and drought singularly and interactively influenced species vital rates, interaction coefficients, and the niche and fitness differences underlying pairwise species coexistence. We found that intrinsic growth rates of species generally decreased with drought and increased with AMF inoculation. The effects of drought and AMF were highly variable between species pairs. Overall, we found evidence of a three-way interaction between drought, mycorrhizae, and species pair on niche and fitness differences and the probability of coexistence. Most species pairs exhibited the highest probability of coexistence when both inoculated with AMF and exposed to drought conditions. The results of this study suggest that drought and AMF singularly and/or interactively influence the demographic parameters and interaction coefficients underlying plant community dynamics. Differences in coexistence probability between our treatments may implicate AMF as a key factor in whether species are able to

coexist during projected increases in drought frequency. Thus, AMF could prove to be a potentially useful tool for managing plant community resilience to climate change. However, variable species responses to both drought and mycorrhizae suggest that under these conditions competitive outcomes might be affected by the strength of species-specific mycorrhizal mutualisms. Future studies on the interactive effects of mycorrhizae and droughts on plant competition should focus on identifying axes for plant species variation which may underlie the complex, species-specific interactions between mycorrhizal mutualisms and water availability.

Introduction

Global climate change is causing more frequent and severe droughts (Romanello, 2021) which may lower biodiversity and ecosystem stability (Aurore Kaisermann et al., 2017) Finding ways to alleviate these impacts among plant communities is increasingly important for ecological restoration and species conservation as climate change progresses. This goal starts with understanding how these types of communities are anticipated to change. Though we can make some guesses about what types of plants are likely favored to outcompete in these shifting drought regimes, we do not have a strong grasp of how different communities will respond. Though the effects of shifting drought regimes on biological communities are not fully understood, they are expected to alter plant community dynamics in favor of species better adapted to lower moisture environments.

Environmental context influences plant community structure both directly, by altering a given plant species' performance, and indirectly, by altering the interactions between species (Mastias et al., 2019). Context-dependent interactions such as competition between plant species influences can affect species' response to drought. Further, positive interactions between species such as the presence of mutualistic symbionts might affect plant response to drought as well (Koffel et al., 2021). Biotic and abiotic factors such as these offer additional layers of complexity in making accurate predictions on plant species responses and community structuring. Given that mutualisms between species can sway competitive outcomes, these interactions are likely to be altered in some way by environmental context such as drought.

One important interaction that has been shown to mediate plant response to drought is arbuscular mycorrhizal fungi (AMF), which acts as a ubiquitous mutualist for most lineages of plants (van der Heijden et al. 2008 & Smith & Read 2008). AMF have been shown to mediate the impacts of drought on plants at the individual level. AMF accomplishes this by attaching to plant roots and increasing nutrient and water uptake via its fungal hyphae (Mathimaran et al., 2017). While they have been shown to affect community dynamics (Hartnett & Wilson, 2002; Jiang et al, 2016; Yang et al., 2018). Additionally, it is not well known how these potential drought dependent effects of mycorrhizae scale up to a community level and if they influence coexistence between plant species under such conditions.

Recently, ecologists have utilized advances in theoretical ecology to better understand how environmental context and inter-trophic species interactions influence community dynamics (Bimler et al., 2018; Koffel et al., 2021; Ke & Wan, 2019). Thus, adopting ideas from coexistence theory may provide a theoretical framework for identifying the role that mycorrhizae play in mediating the effects of drought on plant community dynamics. Modern Coexistence Theory (MCT) suggests that the outcome of competition is a function of niche differences and fitness inequalities (Chesson, 2000). Niche differences describe how differently two species utilize resources and interact with their environment, whereas fitness inequalities describe how differently two species are in their baseline demographic performance. Based on MCT, coexistence is more likely when fitness inequalities are low and niche differences are high. Drought has been shown to raise fitness inequalities between competing plant species (Wainwright et al., 2018) which led to a decrease in coexistence probability (Van Dyke et al., 2022). While AMF have been shown to alter and often increase niche differences between competition species (Van Nuland et al., 2022) and in this sometimes made coexistence more likely (Veresoglou et al., 2018). However, not much is known on how AMF mutualisms are affected under the presence of a drought and how these both effect plant community structuring.

In this paper we aimed to identify the role that AMF and droughts play on mediating coexistence singularly and interactively. To explore this topic, we formulated three hypotheses: 1) Drought decreases the probability of plant coexistence by increasing the fitness inequalities between species pairs, exacerbating existing inherent differences between individual species response to drought as capability to withstand drought is variable by species. 2) Arbuscular mycorrhizae decrease the probability of plant coexistence by increasing fitness inequalities between species pairs by favoring species that benefit more so from mycorrhizae. However, this hypothesis assumes that AMF are predominately generalists. If AMF tend to be specialists, then they might help enable plants to partition resources, thus increasing niche differences and subsequently increasing coexistence chances. 3) AMF ameliorates the effect of drought on plant coexistence via increasing plant host water availability which would lower species' fitness inequalities. To test this, we conducted a greenhouse experiment that factorially manipulated soil moisture and AMF presence over a competition gradient. We measured individuals' seed output, which was used to estimate species' intrinsic growth rates and competition coefficients. These parameters were used to calculate fitness inequalities and niche differences between species pairs to then assess the singular and interactive effects of drought and AMF on competitive dynamics.

Materials and Methods
Experimental Design
We conducted a greenhouse experiment at the University of Oregon in Eugene, OR. Climate control in the greenhouse kept temperatures between 4.44 and 15.56°C. From early November to late June, we grew three annual plant species known to co-occur in upland prairies throughout the Willamette Valley (*Clarkia amoena, Collinsia grandiflora,*

Plectritis congesta). To quantify both intra- and interspecific competition, we grew plants in *size* pots alone, with conspecifics, and heterospecifics. Competition pots contained a focal individual and either one or three competitor individuals to represent a competitor density gradient. Early in the experiment, we transplanted extra individuals to account for variation in germination and ensure the correct competitor densities within each pot. We repeated this design across two soil moisture treatments (control and drought) and two AMF treatments (sterile and inoculated).

For the control soil moisture treatment, we watered the plants daily, whereas the drought treatment was watered every two and a half days. Soil moisture in both treatments were monitored using soil moisture probes in spare pots *(Were monitored using a soil moisture probe from that has yet to be done). All soil was a 50/50 sand and soil mix sterilized via three rounds of autoclaving for 40-minute liquid cycles at 121° C. Pots in the inoculation treatment were inoculated with MycoApply Ultrafine Endo Mycorrhizae, which contained multiple species of AMF (*Glomus intraradices, Glomus mosseae, Glomus aggregatum,* and *Glomus etunicatum).* A seed sterilization procedure was performed on all seeds by submerging seeds in 3% hydrogen peroxide for 10 minutes and immediately rinsed five times with DI water. In the second month of growing, all plants were treated once a week for five weeks with the insecticide treatment Endeavor) to prevent aphid infestation.

Data Collection
To quantify individual fitness, we estimated seed output for all plants in the experiment. We randomly sampled 25 pots for each species over the two soil moisture treatments to find the average number of seeds per fruit or inflorescence. We counted the total number of fruits or inflorescence per individual. We then multiplied the total number of fruits/inflorescences per individual by the moisture treatment specific averages to estimate total seeds per each plant. Seed output data and competitor counts were collected from all individuals which allowed us to estimate and parameterize variables used to assess species' competitive interactions.

Data Analysis
We used the measured fecundity of plant species across the competition gradient and treatment types to estimate the three species' intrinsic growth rates (λ) and the full three by three competition matrix (α) in each of the four treatment levels. We fit three alternate forms of competition in Stan (Carpenter et al. 2017; Stan Development Team, 2024): the Lotka-Volterra, Beverton-Holt, and Ricker, and then conducted model comparison based on the ELPD (Vehtari et al. 2017). The Beverton-Holt model had the best predictive performance, so we used its parameter estimates for all downstream analyses. We used these parameter estimates to calculate niche differences and fitness inequalities between species pairs across the four treatment types using the equations from Godoy et al. (2014). These values were used to assess coexistence probability and the outcomes of competition between species pairs across treatment types. In order to quantify the singular and interactive effects of treatment types on species and species pairs, contrasts for the posterior distributions of species'

intrinsic growth rates, competition coefficients, and niche & fitness differences were assessed.
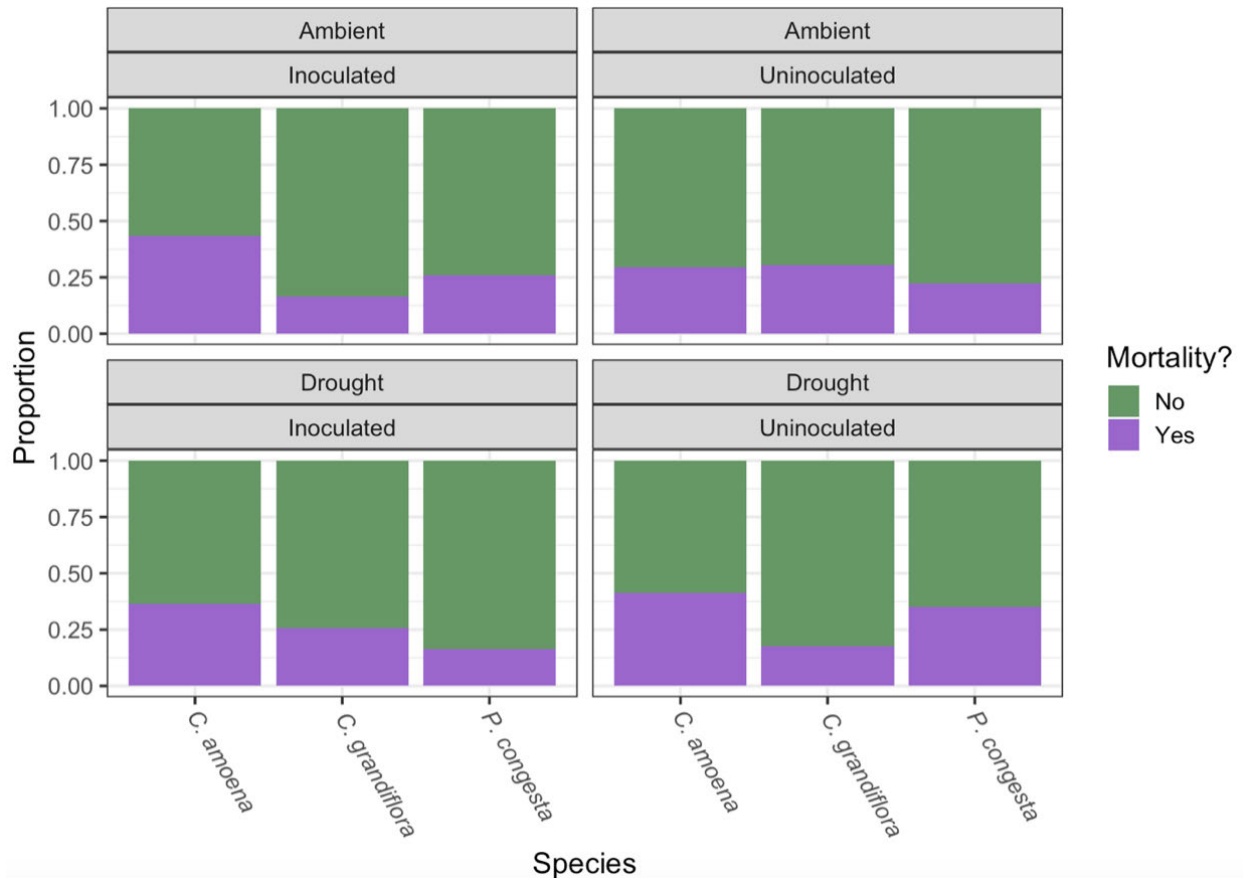
Results

Figure 1: Total proportion of individuals which died before reproducing (purple) vs those that set seed (green) by species and faceted by treatment.

Intrinsic Growth Rates

Overall, we found some evidence that drought, inoculation, and their interaction affected species' intrinsic growth rates, but the magnitude and direction of these effects were species dependent (Figure 3). We found that drought decreased intrinsic growth rates among species (Median Contrast = 43.07, CI [-56.16, 229.63]). We found some evidence (x% of distribution above 0) suggesting fungal inoculation increases intrinsic growth rates (Median = -14.31, 95% CI [-195.84, 39.29]), but the magnitude of this effect was species-dependent. We found some evidence of a three-way interaction among species, drought and inoculation. *Clarkia amoena* showed no evidence of an interaction between the two treatments (Median = 8.33, CI[-55.61, 73.37]) Inoculation tended to dampen the negative effect of drought on *Collinsia grandiflora's* λs, whereas

we found strong evidence that inoculation exacerbated the negative effect of drought on *Plectritis congesta's* λs (Median = 15.06, CI [-41.82, 71.12]).
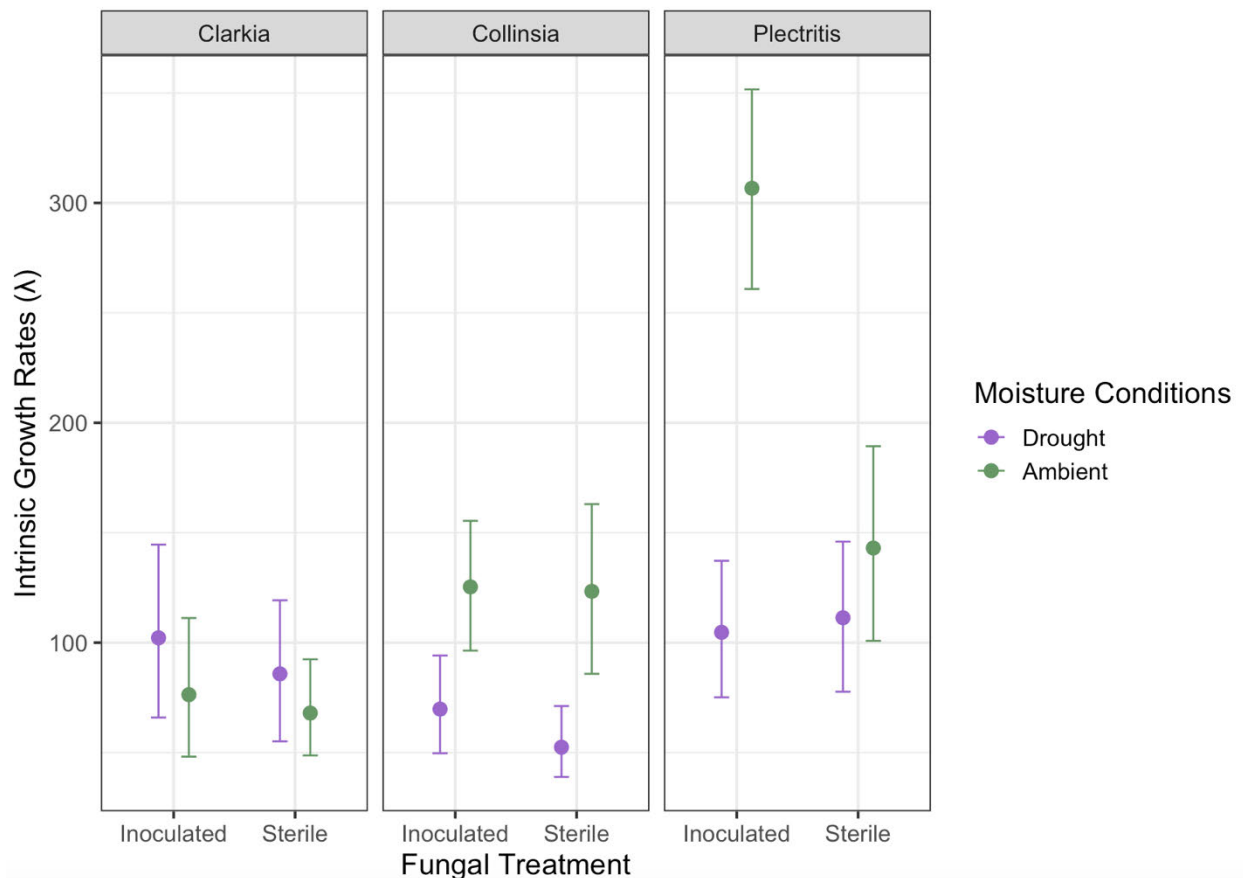

Figure 2: Species intrinsic growth rates across fungal treatment types (inoculated & sterile), colored by moisture conditions (drought in purple and ambient in green), and faceted by species. Points represent median parameter estimates, and error bars represent the lower and upper 95th credible intervals.

Competition Coefficients

Generally, we did not find evidence to suggest drought or inoculation affected competition coefficients, however there we did notice species-pair specific interaction effect on competition coefficients (**Figure 4**). Overall, we found no consistent evidence of drought affecting competition coefficients (Median = 0.38, 95% CI [-1.92, 2.20]). Similarly, when looking at all species pairs, there was no clear evidence of fungal inoculation affecting competition coefficients (Median = 0.36, 95% CI [-2.12, 1.52]). However, we did see some indication of a positive interaction between fungal inoculation and drought as x% of the contrast distribution was... (Median = -0.78, 95% CI [-3.33, 1.44]). Generally, the interaction between treatments suggests that if inoculated, the differences in median competition coefficients between mesic and xeric conditions is greater than when not inoculated. More specifically, when inoculated, the

median values for competition coefficients increase in both ambient and drought treatments, but the ambient conditions had a much greater degree of increase in median values.
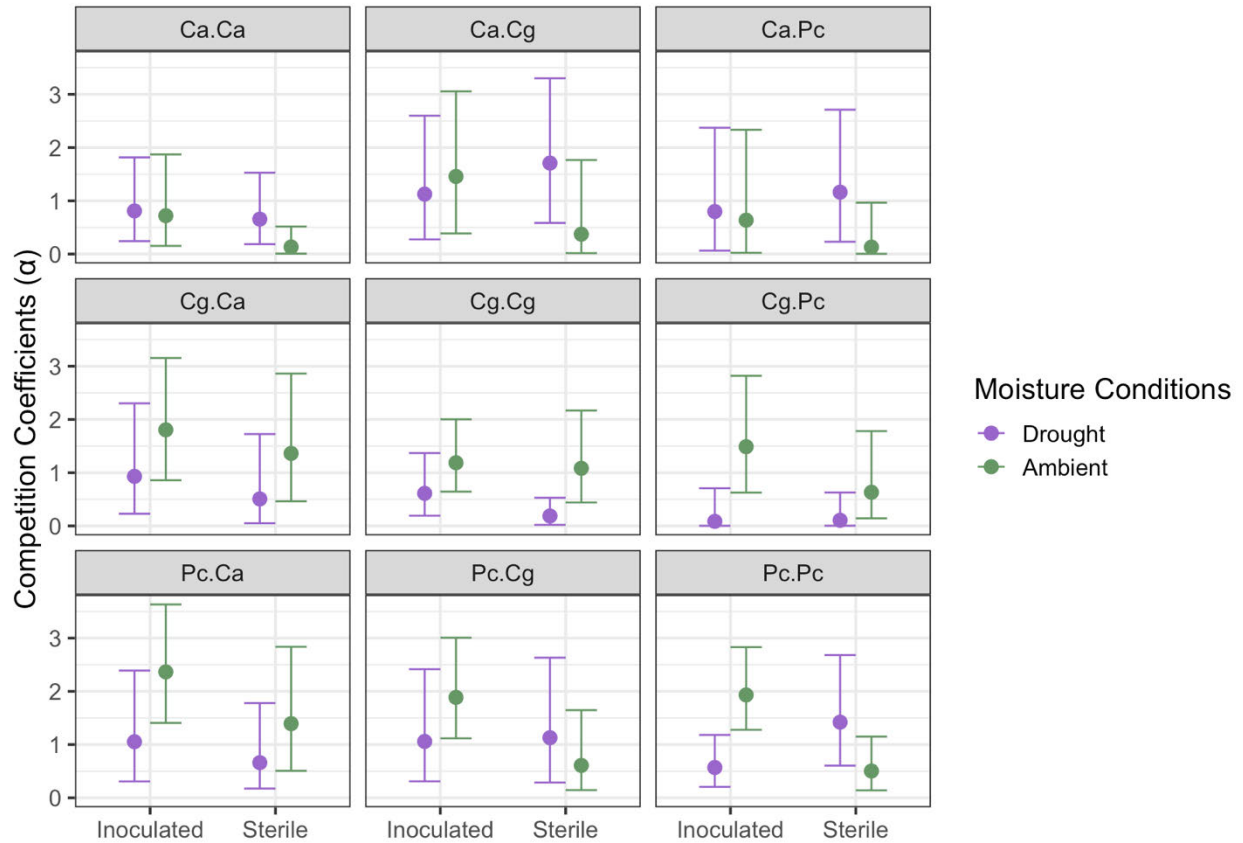

Figure 3: Competition coefficients across fungal treatments (inoculated & sterile), colored by moisture conditions (drought in purple & ambient in green), and faceted by species pairs. Coefficients represent the per capita effect of the second species in the label on the first species (e.g. "Cg.Ca" represents the per capita effect of *Clarkia amoena* on *Collinsia grandiflora*). Points represent median parameter estimates, and error bars represent the lower and upper 95th credible intervals.

Niche Differences
We found no evidence of drought or fungal inoculation affecting the niche differences between competing species (Median = -0.23, 95% CI [-4.27, 3.37], Median = -0.098, 95% CI [-5.69, 1.97]) (**Figure 5)**. Despite these results, we did find some indication of a positive interaction effect that inoculation had on the drought treatments (Median = -0.86, 95% CI [-3.26, 1.49]). The effects of the possible interaction vary by species pairing (e.g., increasing or decreasing median values, alternating the positional trends when compared to their sterile counterparts).
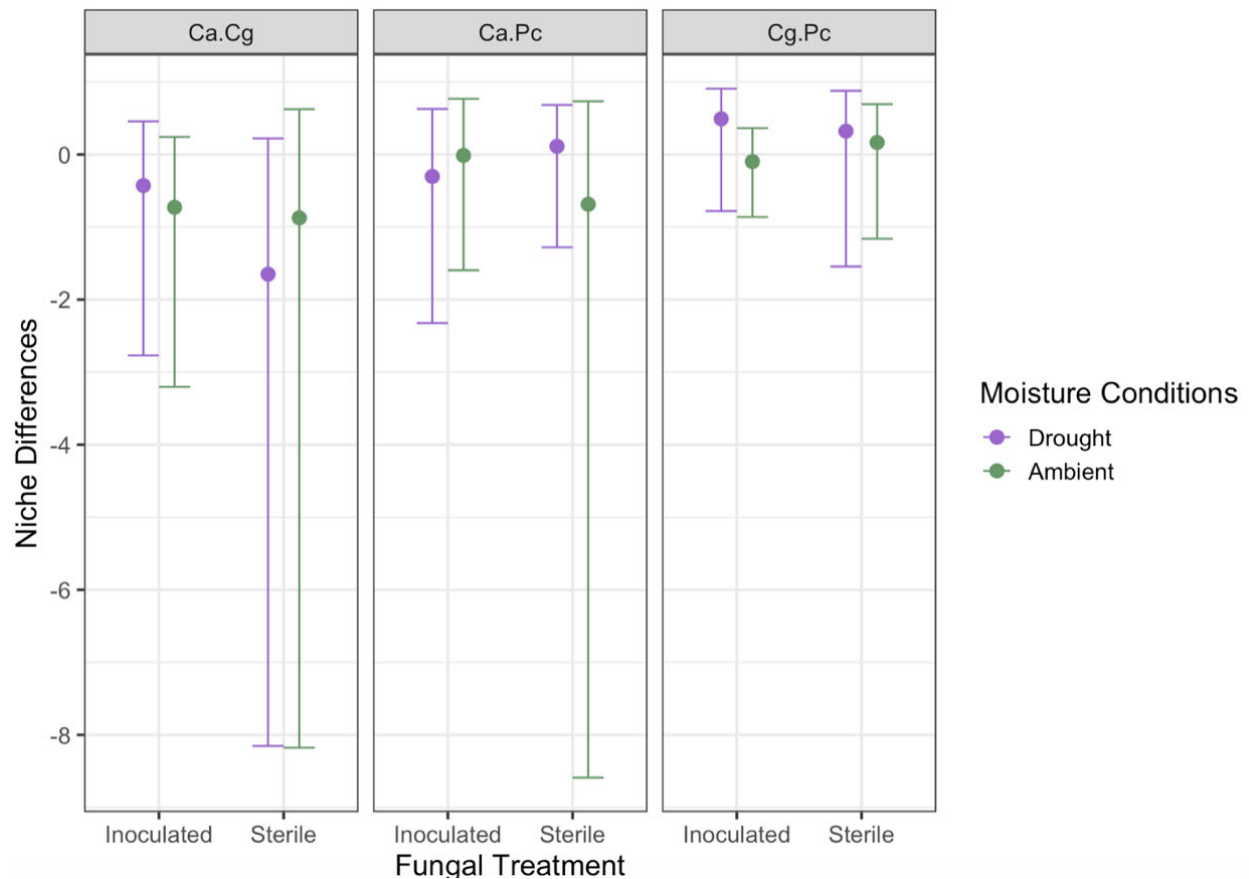
Figure 4: Niche differences across fungal treatments (inoculated & sterile), colored by moisture conditions (drought in purple & ambient in green), and faceted by species pairs. Points represent median values, and error bars represent the lower and upper 95th credible intervals.

Fitness Inequalities

We found no evidence of drought affecting fitness inequalities among species pairs (Median = -0.01, 95% CI [-11.01, 10.73]). Our results do suggest there might be fungal effects on fitness inequalities that are tending negatively (Median = 0.61, 95% CI [-2.40, 16.10]). We found some evidence suggesting that the effects of drought on fitness inequalities might be negatively affected by fungal inoculation (). In species pairs Ca.Cg and Ca.Pc, fitness inequalities were overall lower and the difference between median values for ambient and drought conditions was noticeably reduced. Alternatively, for species pair Cg.Pc, the disparity between the median values of ambient and drought conditions was increased and their values had an overall increase when compared to the sterile treatments. Our findings suggest that the effects of drought on species' intrinsic growth rates might be ameliorated by fungal inoculation (Figure).
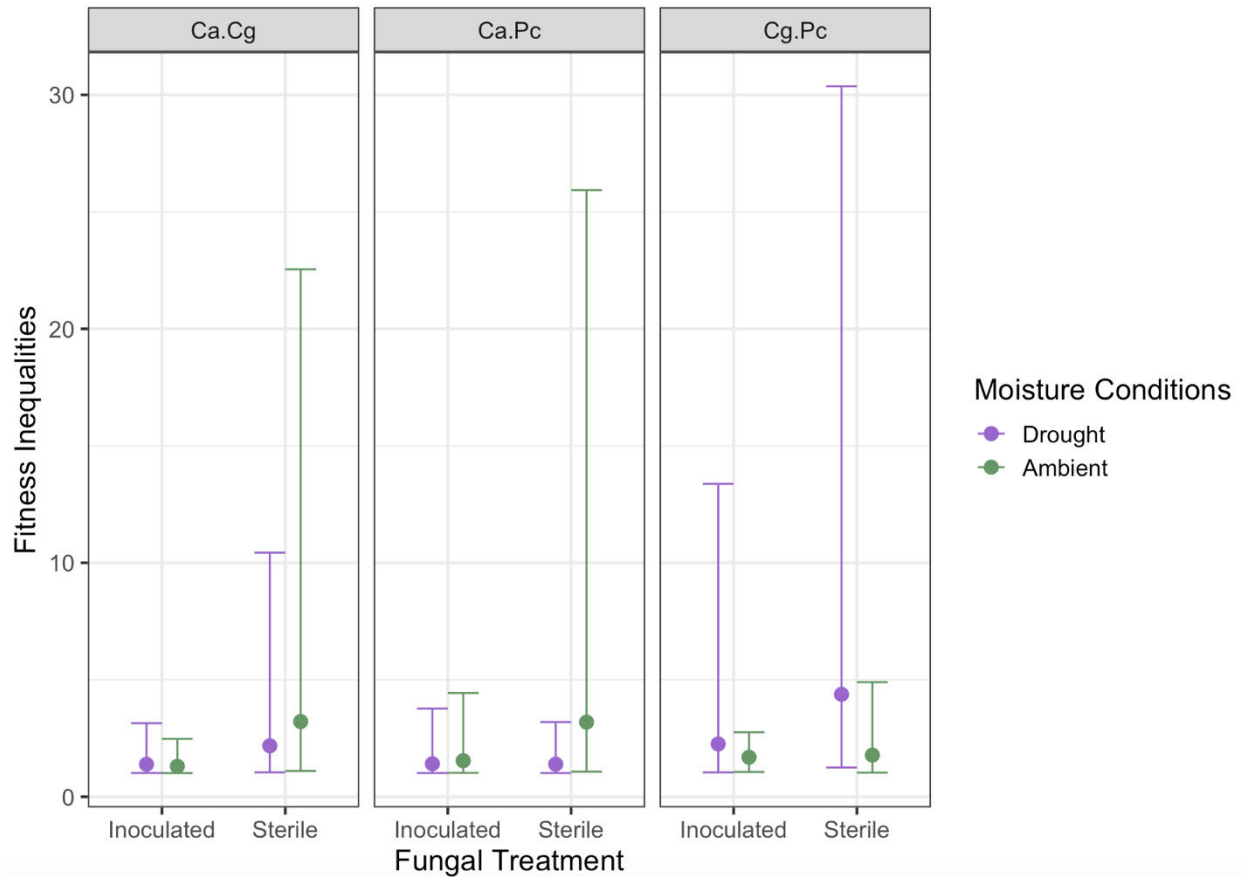
Figure 5: Fitness inequalities across fungal treatments (inoculated & sterile), colored by moisture conditions (drought in purple & ambient in green), and faceted by species pairs. Points represent median values, and error bars represent the lower and upper 95th credible intervals.

Competitive Outcomes

Our results suggest that the effects of drought on species competition and coexistence are dependent on AMF presence. With inoculated drought pots for two out of the three species pairs (Ca-Cg and Cg-Pc) being the most likely conditions where coexistence would occur among the four treatment types. While the species pair Ca-Pc, had coexistence most likely under sterile drought conditions, inoculated pots led to a decrease in likelihood of coexistence and an increase in coexistence outcomes under ambient moisture conditions (Figure 6).
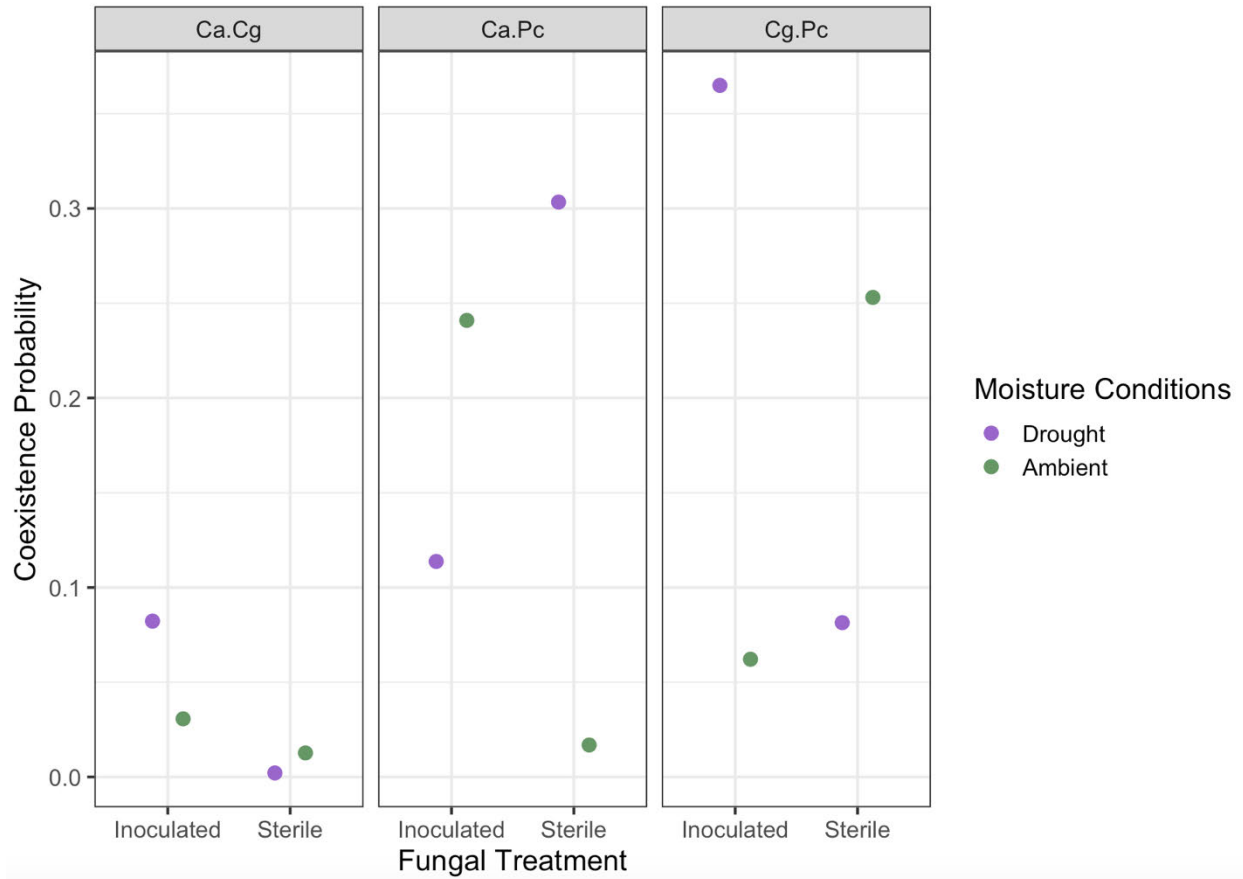
Figure 6: Coexistence probability across fungal treatments (inoculated & sterile), colored by moisture conditions (drought in purple & ambient in green), and faceted by species pairs.
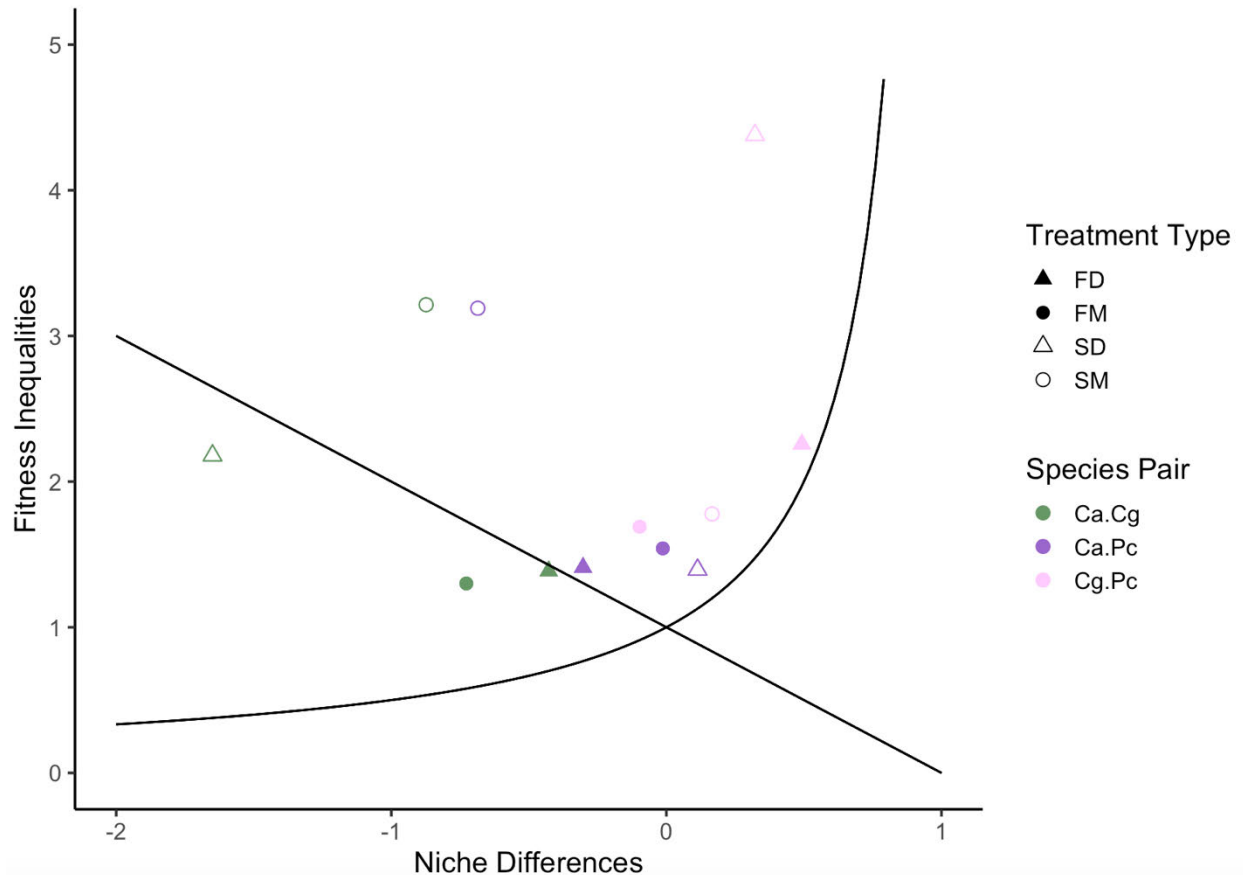
Figure 7: Fitness inequalities and niche differences for each species pair across treatment types indicated by shape (drought) and fill (fungi). Triangles represent drought conditions whereas circles represent ambient moisture conditions. Open points represent sterile conditions whereas filled points represent inoculated conditions. Color represents species pair. Black curves represent the theoretical constraints around coexistence, exclusion, and priority effects based on values of niche and fitness differences. Values in the rightmost quadrant represent coexistence, values in the top or bottom quadrants represent exclusion, and values in the leftmost quadrant represent exclusion.

Our results suggest that median competitive outcomes are mostly unchanged, (Figure 7) however, two out of three pairings (Ca.Cg and Cg.Pc) did have the most movement towards coexistence space under fungal drought conditions. Additionally, ambient Ca.Cg moved from exclusion space to priority effects space when inoculated. While the effects of the treatment types on species pairs were idiosyncratic, it appears that both the fitness inequalities and niche differences were affected by either drought, inoculation, or both.

Discussion

The unpredictable response of plant communities to drought is a perennial problem in the field of ecology, and the role of AMF in mediating community-level drought responses is poorly understood. To address this, we tested how drought and arbuscular mycorrhizal fungi separately and interactively affect plant species coexistence. We hypothesized that 1) drought decreases likelihood of coexistence by increasing fitness inequalities, 2) AMF decreases likelihood of coexistence by increasing fitness inequalities, and 3) AMF lessens the effects of drought on coexistence by increasing plant water availability.

Based on our findings, our first hypothesis was not well supported as drought had inconsistent effects on fitness inequalities (depending on species pair, drought positively or negatively affected it). Our findings also suggest that AMF tends to lower fitness inequalities which may increase coexistence between species pairs which does not support our second hypothesis. We did find some evidence that AMF ameliorates the impacts of drought as AMF tended to decrease fitness inequalities, which was in line with our final hypothesis.

Although we did not find consistent effects of drought on niche and fitness differences, other studies Van Dyke et al. (2022) found a potential explanation for this discrepancy is that these studies can find more generalizable results given their larger species pools. Regardless, our results highlight how species-specific drought responses are an important factor in determining community dynamics. Although very few studies look at biotic (AMF) and abiotic variables (drought). Other studies have found that drought tends to decrease coexistence between species (Matias et al., 2018). Further, in some studies, drought has been shown to lead to an increase in fitness inequalities (Wainwright et al., 2018). Additionally, some studies have found that AMF has contrasting effects on species fitness inequalities and coexistence (Jiang et al., 2016; Van Nuland et al., 2022). Thus, we are still unsure of the exact role these biotic and abiotic factors play on species fitness inequalities, niche differences, and in turn coexistence. Though, based on our findings and the current literature, it seems likely that species specific traits are the determining factor for whether these types of environmental interactions promote coexistence between a given species pair.

In addition to species-specific drought effects, we also identified species-specific effects of mycorrhizae on competitive dynamics. Complex and variable mechanisms of mycorrhizal interactions may underlie the idiosyncratic nature of these results. Specifically, because our second hypothesis hinged on assumptions about host specificity, this might suggest that AMF vary in the degree to which they specialize. Studies such as Tsiknia et al. (2021) found that AMF root colonization was characterized by strong biotic filtering that suggested the importance of co-evolution between beneficial symbionts and their hosts. Given the recent literature on the mycorrhizal impacts on plants, our inconsistent mycorrhizal effects may be caused by species-specific responses of plants to mycorrhizal colonization or variation among AMF taxa in the specificity of their interactions.

While we did find some evidence that showed consistent effects of treatment on intrinsic growth rates, the magnitude of response was variable between species. Additionally, we had very inconsistent effects of treatments on competition coefficients, niche differences, fitness inequalities, and coexistence probability. However, we did see that these all tended to be affected by treatment in some way. Our research highlights the need for further study on how community dynamics are shaped by mutualisms and how these mutualists are often dependent on environmental context and host species. Given the mixed results of AMF ameliorating drought impacts, a larger species pool should be used to make more confident assessments of the role these factors play in plant species coexistence.

Our highly variable species response to AMF treatment highlights the need for future studies on how inoculum affects plant competition. We did find some evidence that AMF helped lessen the impact of drought at the community level. This can provide a potential avenue for restoration ecologists that seek to increase community resilience to climate change. While responses varied considerably, we did find evidence that the presence of AMF may be an important factor in determining competitive outcomes under drought conditions for some portion of species within a community and should be considered when attempting to predict these future community dynamics.

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