

ARTICLE

Trait relationships of fungal decomposers in response to drought using a dual field and laboratory approach

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Abstract

Decomposer fungi play a fundamental role in terrestrial ecosystem dynamics. In the southwestern United States, climate change is causing more frequent and severe droughts, which may alter fungal community composition and activity. Investigating relationships between fungal traits may improve the prediction of fungal responses to drought. In this dual field and laboratory experiment, we examine whether trade-offs occur between traits associated with drought. Specifically, we test the hypothesis that fungi sort into lifestyles specializing in growth yield, resource acquisition, and drought stress tolerance (“YAS” framework). For the field experiment, we constructed microbial “cages” containing sterilized litter and 1 of 10 fungal isolates. These cages were placed in long-term drought and control plots in a southern Californian grassland for 6 and 12 months. We measured fungal hyphal length per unit litter mass loss for growth yield, the potential activities of four extracellular enzymes for resource acquisition, and the ability to grow in the drought versus control plots for drought stress tolerance. We compared these results with a laboratory microcosm experiment constructed with the same fungal isolates and that measured the same fungal traits. The field experiment corroborated our laboratory results, in that no trade-offs were observed between growth yield and resource acquisition traits. However, in contrast to the laboratory experiment, drought tolerance was negatively related to extracellular enzyme activity and growth yield in the field, implying a trade-off. Despite this observed trade-off in the field, growth yield was not hindered by drought. We propose a modification to the YAS framework, by combining the growth yield and resource acquisition lifestyles, which may be more appropriate for this arid system. This joint laboratory and field approach contextualizes a theoretical framework in microbial ecology and improves understanding of fungal community response to climate change.

KEYWORDS

drought, extracellular enzymes, fungal traits, litter decomposition, mesocosm, microcosm, YAS framework

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INTRODUCTION

Understanding the ecology of decomposer fungi is important because they release CO₂ to the atmosphere and transform nutrients such as nitrogen and phosphorus (Jansson & Hofmockel, 2020; Treseder & Lennon, 2015; Van Der Heijden et al., 2008; Went & Stark, 1968). Climate change can alter the activities and composition of fungal communities, with potential consequences for CO₂ production and climate feedback (Jansson & Hofmockel, 2020; Kivlin & Treseder, 2014; Maynard et al., 2019; Todd-Brown et al., 2012; Xiao et al., 2018). In particular, droughts are becoming more frequent and severe in the southwestern United States (Gibson et al., 2020; Seager et al., 2007), which could shift fungal communities (Matulich et al., 2015). Investigating

relationships between fungal traits may help predict fungal responses to drought (Malik, Martiny, et al., 2020; Treseder & Lennon, 2015). For example, if drought selects for fungi with stress tolerance traits, and those traits are negatively related to decomposer ability, then shifts in community composition could coincide with less decomposition (Morrison et al., 2018; Romero-Olivares et al., 2019; Treseder & Lennon, 2015).

Previously, we conducted a laboratory experiment to test the theory that lifestyle strategies are structured by trade-offs among fungal traits (Figure 1a,b; (Alster et al., 2021). Specifically, we tested whether trade-offs exist for fungal decomposers following the newly proposed “YAS” framework (for yield–acquisition–stress tolerance lifestyles), which is a microbial modification of Grime’s competitor–stress tolerator–ruderal framework

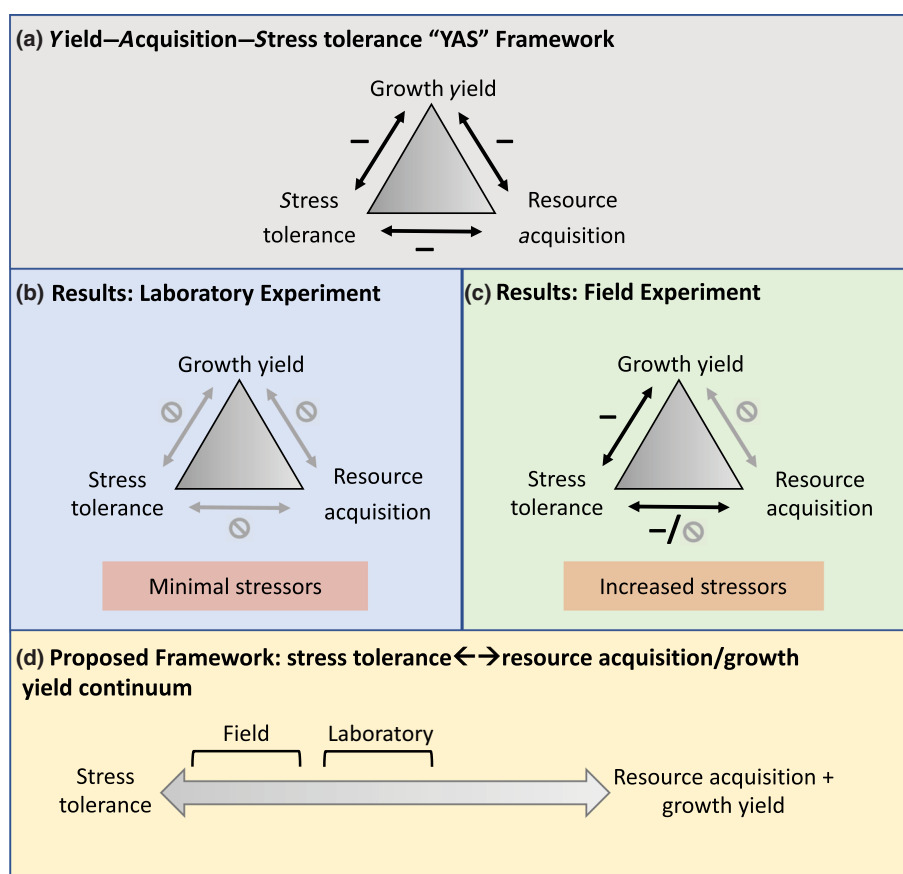


FIGURE 1 Conceptual figure summarizing (a) the growth yield, resource acquisition, and drought stress tolerance (YAS) framework (adapted from Malik, Martiny, et al., 2020) and results from our (b) laboratory experiment and (c) field experiments. Negative signs indicate trade-offs, and circle-backslash symbols indicate no significant relationship between traits. (b and c) Black lines and symbols indicate $p < 0.05$ and gray lines and symbols indicate $p > 0.05$. In the field experiment, we observed a significant trade-off between growth yield and resource acquisition with stress tolerance. (d) We propose an alternative framework, modified from the YAS framework and based on our results. The stress tolerance–resource acquisition/growth yield continuum posits that environmental stressors sort fungi into two lifestyles (stress tolerance vs. resource acquisition). Under relatively high environmental stress (e.g., our field conditions), stress tolerance is selected at the expense of resource acquisition/growth yield. When conditions are less stressful (e.g., our laboratory conditions), trade-offs are less apparent

(Malik, Martiny, et al., 2020). The YAS framework posits that microbes invest resources disparately between high growth yield versus resource acquisition or stress tolerance. In our laboratory experiment, we measured these traits on fungal isolates inoculated onto sterilized litter and incubated at different moisture levels. We predicted that trade-offs would result in negative relationships between traits. Surprisingly, we did not find evidence that these fungal isolates fit the YAS framework—all relationships between traits were nonsignificant.

Developing and testing conceptual frameworks in both the laboratory and the field may help improve understanding of microbial community and ecosystem response to climate change (Balser et al., 2006). Each setting has its advantages: Laboratory experiments can help isolate ecological mechanisms, while field experiments can account for natural variation (Morin, 1998). Yet, laboratory and field studies can yield contradictory results despite asking identical questions. For example, in a synthesis examining the effect of global warming on decomposition, laboratory and field experiments produced contrasting results for turnover times of soil carbon (C) (Conant et al., 2011). Integrating field and laboratory approaches may help researchers obtain relevant, reproducible data, and help better explain the mechanisms driving the results (Heil, 2014; Kampichler et al., 2001; Uller & Olsson, 2005). However, this solution has not historically been feasible for microbial ecologists, because microbes have proven difficult to manipulate in the field (Reed & Martiny, 2013). As a result, our ability to link mechanism-based conceptual frameworks to real-world data from the field has been limited (Prosser & Martiny, 2020). Here, we compare results from complementary laboratory and field studies of fungal traits in a southern Californian grassland to connect theory and mechanisms with natural variability.

We replicated our prior laboratory experiment in a field mesocosm experiment using the same fungal isolates as in the laboratory. Fungi were inoculated into microbial “cages,” which are essentially litterbags made of a fine-mesh nylon membrane that hinders the passage of fungi and many bacteria (Kivlin & Treseder, 2015; Reed & Martiny, 2013). We incubated the cages in drought and ambient plots in the grassland from which the isolates were derived. We then measured trade-offs between the same set of growth yield, resource acquisition, and stress tolerance traits as in the laboratory experiment. Environmental conditions such as temperature and moisture were held relatively constant in the laboratory, but varied temporally and spatially in the field. Stresses associated with this variation, including longer periods of drought and warmer temperatures, could

trigger greater investment in stress tolerance responses such as the production of osmolytes or polysaccharides to protect against desiccation (Schimel et al., 2007), or increased maintenance and repair of cellular structures (Fuchs & Mylonakis, 2009; Kultz, 2005). Additionally, the mesh size (0.45 μm) of the cages allowed water, nutrients, and extracellular enzymes to easily pass in and out of the cage (Mcguire, 2007). This contributed to a more realistic interaction of nutrients for the fungi. Overall, replicating this experiment in the field allowed for measuring fungal traits under more robust environmental conditions than could be captured in a laboratory setting.

We hypothesized that more natural conditions in the field would elicit trait trade-offs that might not have been apparent under laboratory conditions (Hypothesis 1). On the contrary, we also hypothesized that increased environmental complexity in the field would magnify variation in trait expression among replicates (Hypothesis 2). Any differences in findings between the laboratory versus field experiments could highlight the strengths and weaknesses of each setting, and help improve predictions of fungal activity and community composition under climate change.

METHODS

Experimental design

To replicate the laboratory microcosm experiment as a field mesocosm, fungal isolates were inoculated into microbial cages (litterbags) with sterilized litter. Microbial cages are effective at isolating microbial communities in the field (Allison et al., 2013; Glassman et al., 2018; Holden et al., 2013; Looby & Treseder, 2018). Thus, we used this approach to mimic a laboratory microcosm experiment in the field. Litterbags (10 $\times$ 10 cm) were constructed using nylon mesh with a pore size of 0.45 μm (Tisch Scientific, OH, USA) and gamma-irradiated for sterility. We chose a pore size of 0.45 μm to allow for the movement of water, nutrients, organic compounds, and bacteria (Glassman et al., 2018; Looby & Treseder, 2018). In addition, the small pores slow the colonization of fungi from the environment, allowing the inoculated isolates to colonize the litter first (Glassman et al., 2018; Looby & Treseder, 2018). Litterbags were filled with 5 g of roughly ground litter (~1–2 cm) that had been autoclaved at 121°C for 90 min.

The litter was collected from Loma Ridge National Landmark in southern California (33°44'13.2" N, 117°42'42.0" W, 365-m elevation), on the traditional territory of Acjachemen and Kizh/Tongva communities

(KIZH Nation, [n.d.](#); Haas, 1995). Loma Ridge is a Mediterranean grassland dominated by annual exotic grasses, *Avena*, *Bromus*, and *Lolium*; annual forbs, *Erodium* and *Lupinus*; and perennial grass, *Stipa pulchra* (Martiny et al., 2016; Potts et al., 2012). The mean annual temperature is 17°C, ranging from 0 to 44°C, and the mean precipitation is 30 cm (Kimball et al., 2014).

The litterbags were inoculated with one of 10 fungal isolates. These fungi were isolated from litter at Loma Ridge and represent eight fungal species, including both Ascomycota and Basidiomycota (<https://figshare.com/s/171c517b9ab200f1f53e>). Eight of these fungi are phylum Ascomycota, which is representative of the community in the litter at this field site (Glassman et al., 2018; Matulich et al., 2015). The isolates were previously identified via Sanger sequencing using the ITS1F/ITS4 primer set (Gardes & Bruns, 1993; White et al., 1990). Isolates were regrown on potato dextrose agar for 4 days and then transferred to potato dextrose broth and shaken continuously for 1 week. The isolates were rinsed, diluted (0.1 ± 0.01 optical density), and transferred into the litterbags (1 ml of the dilute hyphae). After inoculation, litterbags were sealed and placed for 24 h into a 35°C drying oven to remove excess moisture from the inoculation step.

After preparation, 132 litterbags were deployed into ambient and drought plots at Loma Ridge on 8 November 2017. The 132 litterbags comprise 10 isolates (plus an additional control of sterile litter with no added isolate) $\times$ 2 treatments (ambient and drought) $\times$ 3 replicates $\times$ 2 collection time points. The ambient and drought plots were established in 2007 (Allison et al., 2013). The drought plots are covered with clear polyethylene during a subset of rain events to achieve ~50% reduction in rainfall (Allison et al., 2013). Litterbags were collected from the field on 30 April and 7 November 2018 (approximately 6 and 12 months after placement in the field). These collection times correspond to the end of the rainy season and end of the dry season, respectively. During the study period, precipitation at the site totaled 82.1 mm and there were three periods of more than 40 days with no rain events. Upon collection, the litter was weighed to determine mass loss. The litter was then briefly ground to break apart the dense mass of litter and hyphae and then subdivided for trait measurements. A subsample of the litter was dried at 60°C for 48 h to determine percent moisture. The remainder was stored at –20°C for fungal hyphal counts or at –80°C for extracellular enzyme assays.

Trait measurements

Traits of growth yield (fungal hyphal length per unit litter mass loss), resource acquisition (potential activity of four

extracellular enzymes), and stress tolerance (fungal hyphal length in drought compared with ambient conditions) were estimated shortly after harvesting the litterbags from the field. These metrics were chosen to represent each trait specifically because they are quantifiable physiological variables that reflect fungal metabolic investments (Malik, Martiny, et al., 2020). To estimate growth yield, we measured fungal hyphal length (via staining and microscopy) and litter mass loss. While litter mass loss is not a perfect proxy for resource use, measuring heterotrophic respiration rates in this type of long-term litterbag experiment was not feasible. Litter mass loss represents fungal metabolism of the litter because a single fungal isolate was added to each sterilized microbial cage. The procedure for estimating fungal hyphal length is detailed in Alster et al. (2021) and was modified from Allison et al. (2013). In brief, frozen litter was mixed with a sodium hexametaphosphate solution to extract the fungal hyphae. The mixture was then vacuum-pumped through a 0.2- μ m nylon filter, which was then stained. The filters were mounted on slides, and 10 photographs were taken per sample using an imaging microscope (Axioplan 2). The fungal hyphal length was estimated from the images (AxioVision). We used the method described in Shen et al. (2016) to estimate hyphal length per gram litter. We report all trait values per litterbag, to account for the total hyphal production within the litterbag. To estimate the final growth yield, we calculated hyphal length per unit litter mass loss.

To measure resource acquisition, we estimated the potential activity of four extracellular enzymes: cellobiohydrolase (CBH) and β -glucosidase (BG) to measure cellulose degradation, β -xylosidase (BX) to measure hemicellulose degradation, and *N*-acetyl- β -D-glucosaminidase (NAG) to measure chitin degradation. These enzymes were chosen based on their relevance for degrading plant biomass (Sinsabaugh et al., 2002) and fungal cell walls (Wohl & McArthur, 2001). Sample homogenate preparation and fluorometric enzyme assays were conducted following methods described in Alster et al. (2013).

To estimate drought tolerance, we calculated fungal biomass response to drought stress via Cohen's *d* (effect size):

Cohen's *d*

$$= \frac{\text{Mean hyphal length}_{\text{Drought}} - \text{Mean hyphal length}_{\text{Ambient}}}{\text{Pooled standard deviation}}$$

Means were taken for the fungal hyphal length for each set of the three replicates from the drought plots with the same collection time and fungal isolate, and compared to corresponding means from the ambient plots with the

same collection time and fungal isolate. When Cohen's d (effect size) is more negative, the isolate displayed less drought tolerance, as there was a larger difference in fungal growth between the drought and ambient plots. By contrast, more positive Cohen's d (effect size) indicated that the isolate was more drought-tolerant. Because Cohen's d requires biomass measurements from both ambient and drought plots to calculate a single drought tolerance value for each strain, we do not have independent measurements of drought tolerance for strains in the ambient versus drought plots. Therefore, to avoid issues of statistical nonindependence, we analyze the ambient and drought plots separately in subsequent regressions involving the drought tolerance trait.

Comparison with laboratory experiment

The details of the laboratory experiment are presented in Alster et al. (2021). Here, we provide a brief comparison of the methods between the two studies. All 10 isolates from the field experiment were inoculated into amber wide-mouthed jars with 5 g of sterilized litter from Loma Ridge with the same hyphal mass as in the field experiment. (The laboratory experiment included five additional isolates, since logistics were more tractable in the laboratory.) Water was added to these jars equaling 4%, 27%, or 50% water-holding capacity (of the litter), percentages that were held constant throughout the experiment. These moisture levels are within the range of values observed in the field experiment (<https://figshare.com/s/171c517b9ab200f1f53e>). The laboratory microcosms were incubated for 5 weeks at 20°C before the trait measurements were conducted, while in the field mesocosms, trait measurements were conducted after 6 and 12 months.

Trait measurements for resource acquisition (potential extracellular enzyme activity) and growth yield (fungal hyphal length per unit litter mass loss) in the laboratory experiment were determined using methods that were identical to those of the field experiment. To standardize data between settings, we restricted our analyses to the 10 isolates that were common to both datasets. Although both the 6- and 12-month datasets are analyzed and presented in this manuscript, we only used field data from the 6-month collection time (instead of the 12-month time) for comparison with the laboratory experiment to best match the shorter laboratory incubation time. Furthermore, we selected the laboratory dataset's driest (4% water-holding capacity) and wettest (50% water-holding capacity) treatments to contrast with the field dataset's drought and ambient treatment, respectively.

Statistical analysis

To test Hypothesis 1, we first performed a fully factorial ANOVA, with the traits (growth yield, CBH, BG, BX, and NAG) as the dependent variables and fungal isolate, treatment, and field collection time as the independent variables. This portion of the analysis was conducted using only the field data. All statistics were conducted in R version 3.5.3 (R Core Team, 2020), and differences were considered significant when $p < 0.05$. Data were log-transformed to improve normality when appropriate. To check for negative relationships between pairs of traits (growth yield, drought tolerance, CBH, BG, BX, and NAG), we conducted a series of linear mixed regressions with treatment and field collection time as a random factor using the “MuMIn” package (Barton, 2020). Here, we report the marginal R^2 value to determine the proportion of variance explained by the relationships between traits alone. Hypothesis 1 would be supported if we find negative relationships between pairs of traits and rejected if we find positive or nonsignificant relationships between pairs of traits.

To test Hypothesis 2, we compared coefficients of variation (CVs) of field data (this study) versus those of laboratory data (Alster et al., 2021). A CV is the ratio of standard deviation to mean. We calculated the CV of each trait for each isolate within each combination of setting $\times$ treatment. To check for significant differences in CVs between settings, we conducted a series of fully factorial ANOVAs. The dependent variable was the CV of a given trait (growth yield, CBH, BG, BX, or NAG). The independent variables were setting (field vs. laboratory) and treatment (ambient vs. drought). Hypothesis 2 would be supported if CVs were significantly smaller in the laboratory than in the field.

RESULTS

Treatment and isolate effects

Treatment (drought vs. ambient) and collection time significantly affected all traits (Table 1; $p < 0.05$ in each case). At the end of the wet season (6 months), growth yield was significantly higher in the drought plots than in the ambient plots; by the end of the dry season (12 months), the treatment differences were no longer significant (Figure 2a). These results were driven by slower mass loss in the drought plots, compared with the ambient plots (Figure 2b), but similar hyphal growth (Figure 2c). Potential activities of CBH and BG were significantly greater in the drought plots than in the ambient plots at 12 months, but not significantly different at 6 months (Figure 2d–f). By contrast, the potential activity of

TABLE 1 Statistical results from fully factorial analyses of variance on fungal traits^a

Source of variation	df	CBH		BG		BX		NAG		Growth yield	
		F	p	F	p	F	p	F	p	F	p
Isolate	9	0.524	0.853	1.216	0.298	0.874	0.552	1.468	0.174	0.876	0.550
Treatment	1	7.491	0.008^b	5.356	0.023	3.362	0.070	0.001	0.979	4.684	0.034
Time point	1	11.998	0.001	1.287	0.260	0.001	0.975	93.276	<0.001	0.116	0.735
Isolate × treatment	9	1.459	0.178	1.098	0.374	0.828	0.592	0.966	0.474	0.541	0.840
Isolate × time point	9	1.087	0.382	0.687	0.719	1.525	0.154	1.381	0.211	1.677	0.109
Treatment × time point	1	12.159	0.001	10.139	0.002	12.240	0.001	9.511	0.003	6.19	0.015
Isolate × treatment × time point	9	1.406	0.200	0.982	0.462	1.546	0.147	1.056	0.405	0.509	0.864

^aTraits are potential enzyme activities of cellobiohydrolase (CBH), β -glucosidase (BG), β -xylosidase (BX), *N*-acetyl- β -glucosaminidase (NAG), and growth yield.

^bBold indicates significance ($p < 0.05$) or marginal significance ($p < 0.10$).

NAG did not differ significantly between treatments at either time (Figure 2e). Isolate type was not significant for any of the traits (Table 1; $p > 0.05$).

Trait trade-offs

We found partial support for Hypothesis 1—trait trade-offs with drought tolerance were evident in the field that were not detectable under laboratory conditions. There was a significant negative relationship between drought tolerance (as Cohen's *d* effect size) and growth yield ($p = 0.001$; Figure 3a) for fungi in the ambient plots. Drought tolerance was also negatively correlated with potential CBH and BX activities, which are resource acquisition traits, for fungi in the ambient plots ($p = 0.039$ and 0.020 , respectively; Figure 3c,g). However, drought tolerance was not significantly related to growth yield or any of the resource acquisition traits for fungi in the drought plots (Figure 3). Growth yield was also not significantly related to any of the extracellular enzyme activities (Figure 4). As a reminder, no relationships between any of these traits were significant in the laboratory setting (Figure 2; Alster et al., 2021).

Additionally, we found significant, positive relationships between all the extracellular enzymes (CBH, BG, BX, and NAG) ($p < 0.001$ in each case; Appendix S1: Figure S1). These positive relationships between extracellular enzyme activities also occurred in the laboratory experiment (Alster et al., 2021).

Field and laboratory comparison

There was little evidence for greater variation among replicates in the field setting than in the laboratory setting (Figure 5, Appendix S1: Table S1). For CBH,

CVs tended to be smaller in the laboratory than in the field, but only within the drought treatment and only marginally significantly (setting × treatment, $p = 0.092$). For all other fungal traits, the CVs did not vary significantly between settings. Thus, we rejected Hypothesis 2.

DISCUSSION

We conducted complementary field and laboratory experiments to test for trade-offs among YAS traits in fungal decomposers in a southern Californian grassland. In the field, drought tolerance tended to be negatively related to two of the potential enzyme activities and to growth yield. This result supports the notion that investment in stress tolerance comes at some cost to resource acquisition and growth yield, even if these trade-offs are relatively weak. These field results contrasted with the laboratory results, which detected no trade-offs between these lifestyles. We also found that growth yield was initially higher in the drought plots, which was largely driven by slower mass loss in the drought setting. Unexpectedly, the field setting did not produce significantly greater variation *within* treatments and isolates than did the laboratory setting.

In this system, trait trade-offs were more evident in a natural setting than in the laboratory. Likewise, trade-offs between stress tolerance and extracellular enzyme production have previously been observed for fungi in the field (Morrison et al., 2018). In the ambient plots, we observed a negative relationship indicative of a trade-off between drought tolerance and growth yield (Figure 3a), consistent with our initial hypothesis. However, surprisingly we also found that fungi in the drought plots had higher growth yield than fungi growing in the ambient plots (Figure 2a), in contrast to our initial prediction that

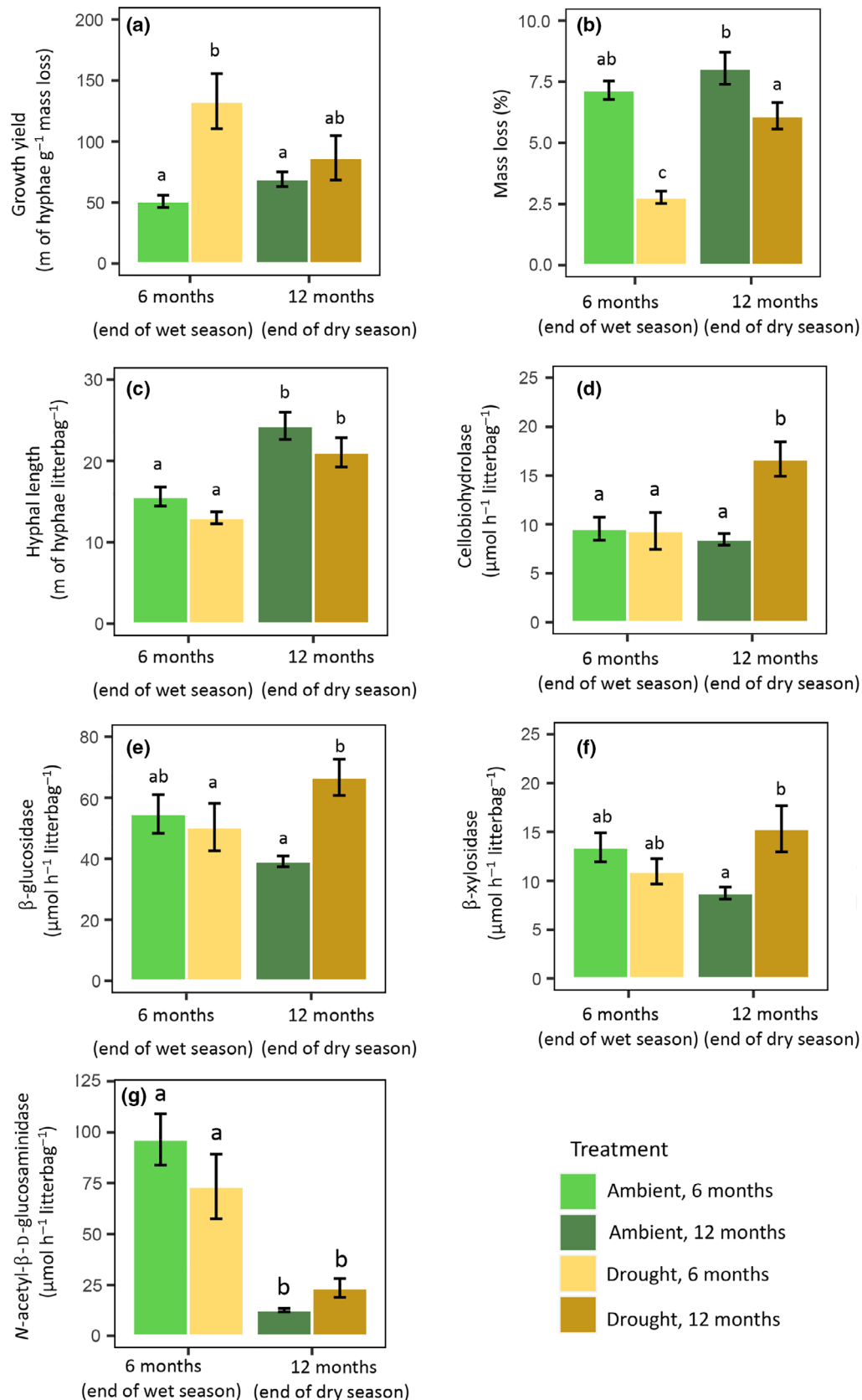


FIGURE 2 Growth yield, mass loss, hyphal length, and potential extracellular enzyme activity of fungal isolates in the field. Bars are means $\pm$ 1 SE of 30 replicates (10 isolates, each in triplicate). Groups with different letters are significantly different from one another ($p < 0.05$)

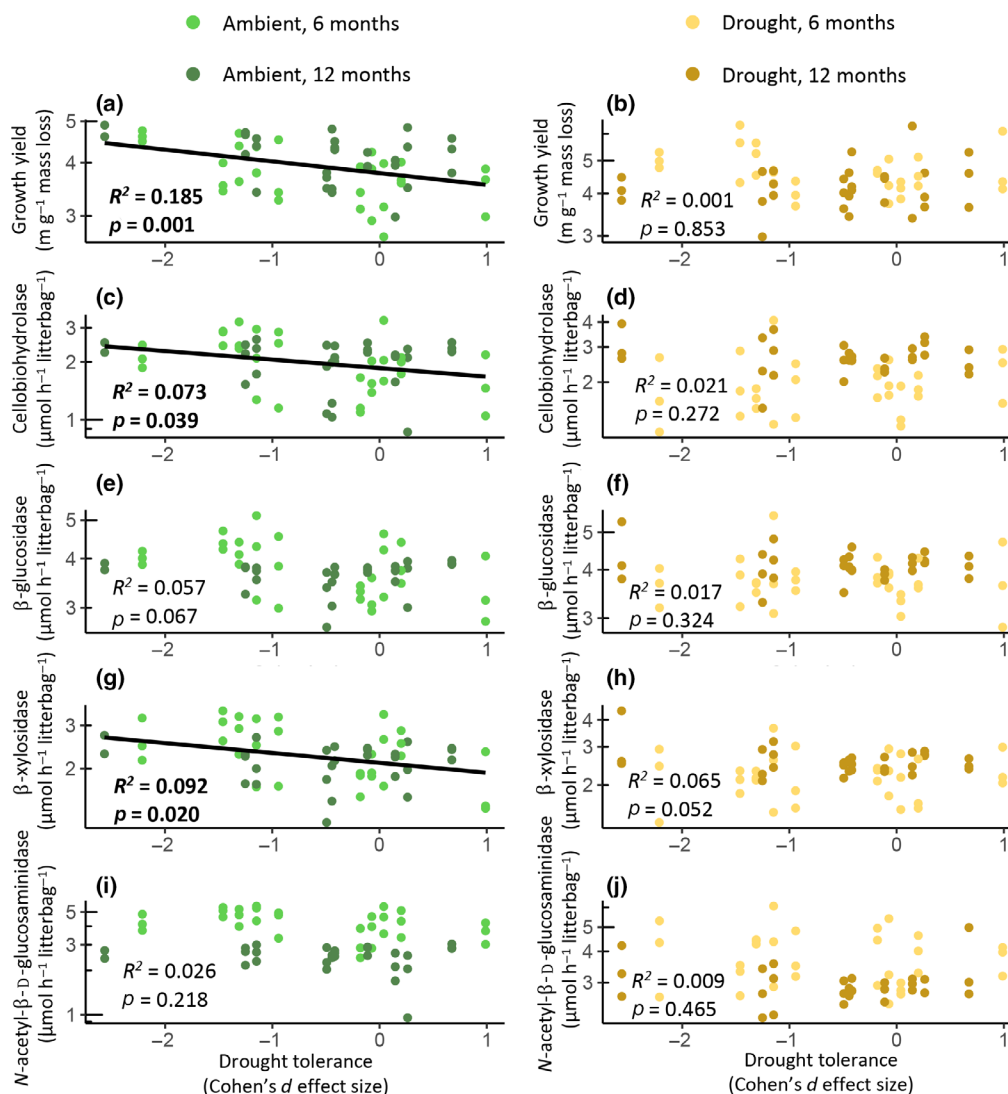


FIGURE 3 Relationships between growth yield and potential extracellular enzyme activities (cellobiohydrolase, β -glucosidase, β -xylosidase, and N-acetyl- β -D-glucosaminidase) with drought tolerance (Cohen's d effect size on hyphal length) in the field. Positive drought tolerance values indicate greater stress tolerance. Colors indicate the treatment (drought or ambient) and collection time (6 months or 12 months). Lines indicate the best fit. Significant p values are in bold and correspond to the marginal R^2

investment in drought tolerance should reduce growth yield under drought. Perhaps our fungal strains are adapted to drier conditions, for example, by producing more extensive hyphal networks to capture available water and resources, resulting in lower growth efficiency at higher moistures (Boddy, 1999; Meisner et al., 2018). Regardless of the mechanism, these findings suggest that drought in this southern Californian grassland will not hinder fungal growth and activity, although mass loss, and consequently decomposition rates, may slow down (Figure 2).

Our finding of a negative relationship between stress tolerance and growth yield in the field also appeared consistent with that of other laboratory and field studies

(e.g., Garcia et al., 2020; Gasch, 2007; Malik, Brodie, et al., 2020; Whitney, 2019; Zakrzewska et al., 2011). In fact, the Malik, Brodie, et al. (2020) study was conducted in the same field experiment as the current study. The investigators measured microbial metabolites in litterbags (1-mm mesh) incubated in the ambient and drought plots. They reported that metabolites for stress tolerance tended to be negatively correlated with growth yield for whole microbial communities. On the surface, trait trade-offs between stress tolerance and growth yield in the microbial community as a whole, in terms of metabolite production, seem to mirror those we observed for physiological traits within specific fungal isolates from this community. However, the Malik, Brodie, et al. (2020) study

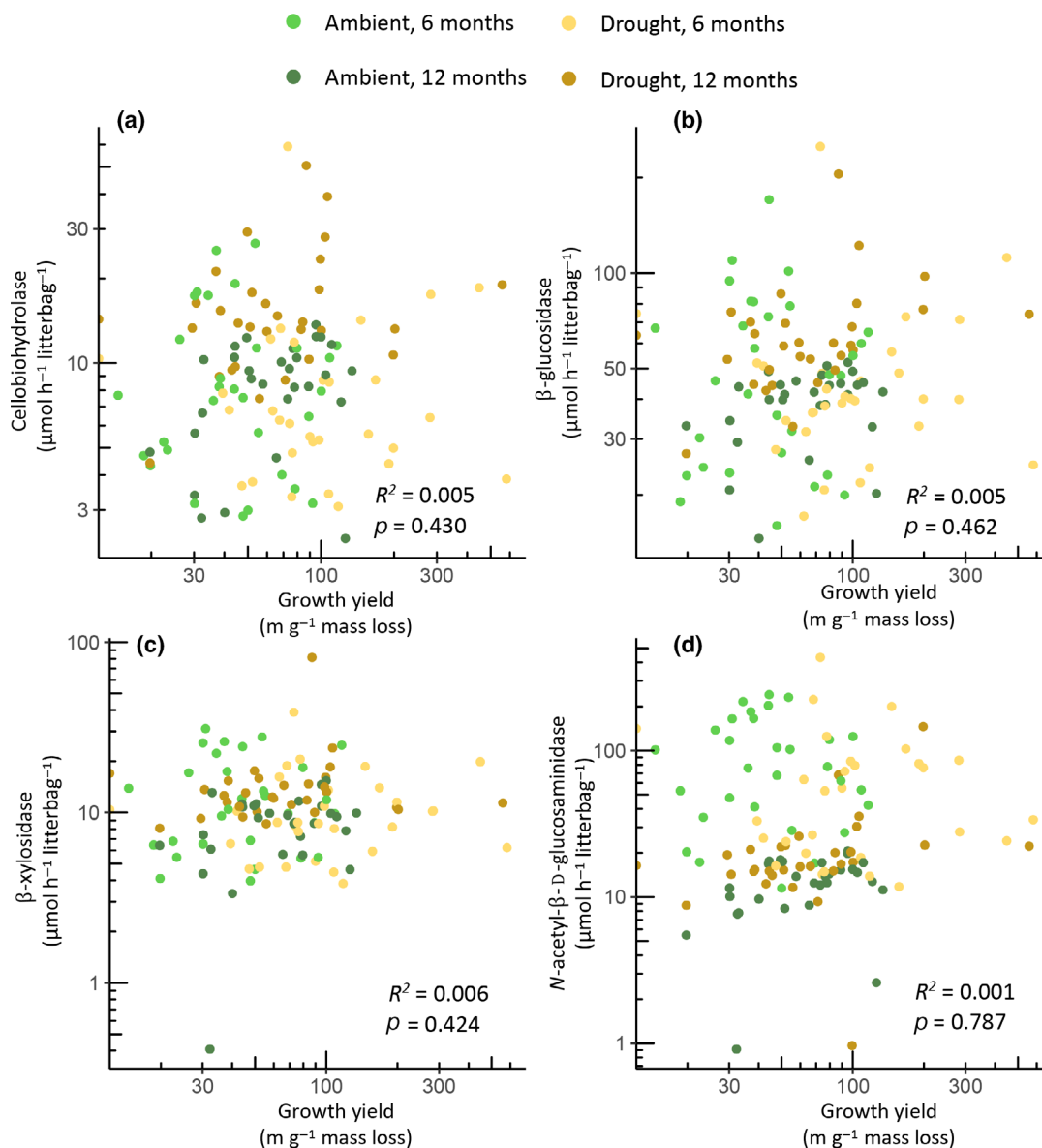


FIGURE 4 Relationships between growth yield and potential extracellular enzyme activities (cellobiohydrolase, β -glucosidase, β -xylosidase, and N-acetyl- β -D-glucosaminidase) in the field. Colors indicate the treatment (drought or ambient) and collection time (6 months or 12 months)

found lower growth yield in the drought plots—the opposite of what we observed here. These dissimilar findings could be due to differing approaches for measuring growth yield or because our study focused on fungi rather than whole microbial communities.

Our field and laboratory results agreed in one respect: There was neither documented a negative relationship between growth yield and resource acquisition. Nevertheless, two other studies have noted that growth rate and respiration are negatively related to extracellular enzyme activities for fungi in forests (Rinkes et al., 2011; Whitney, 2019). Whitney (2019) noted that Ascomycota

is not as sensitive to changes in resource availability compared with Basidiomycota. The Ascomycota taxa that dominate our field site (Glassman et al., 2018; Matulich et al., 2015) may not rely heavily on extracellular enzyme production, so trade-offs with growth yield could be slight. Alternatively, perhaps extracellular enzyme production and growth yield are positively associated. Increased extracellular enzyme production could potentially improve growth yield (Manzoni et al., 2014; Romani & Sabater, 2000), offsetting any trade-offs. Lastly, it is possible that the lack of a significant relationship between growth yield and resource acquisition

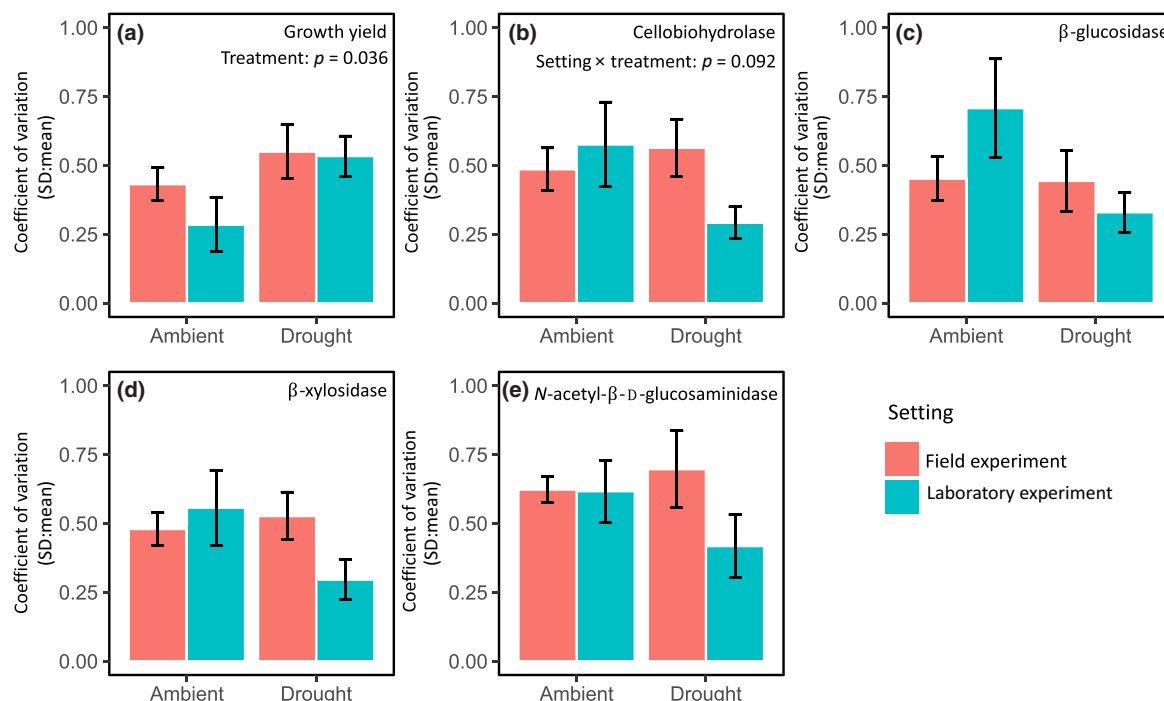


FIGURE 5 Coefficients of variation for growth yield and extracellular enzyme activity from field experiment (this study) and complementary laboratory experiment (Alster et al., 2021). The field setting included the same set of isolates as the laboratory setting. For the laboratory experiment, the 50% water-holding capacity and 4% water-holding capacity treatments represented ambient and drought conditions, respectively. For the field experiment, only data from the 6-month collection time were included. Bars are means $\pm$ 1 SE of 10 isolates. Significant or marginally significant effects of setting (field vs. laboratory), treatment (ambient vs. drought), or their interaction are noted

documented in both the field and laboratory studies is a function of the specific measures we used to represent each trait. Our measurement of growth yield may be more representative of ecosystem-scale carbon use efficiency since rates of mass loss are not perfectly equivalent to respiration rates (Geyer et al., 2016), although they are correlated (Xiao et al., 2014). It is also possible that the wetter conditions could induce higher mortality (Parker & Schimel, 2011) that would not be captured with these measurements. Future studies should determine whether these methodological differences are consequential in biasing estimates of growth yield. Using a different measurement approach (e.g., phospholipid fatty acid measurements paired with respiration measurements) could be helpful in further clarifying these trait relationships.

When characterizing fungal traits, our results from this southern Californian grassland may support an alternative to the YAS framework with two (instead of three) lifestyles: stress tolerance versus resource acquisition/growth yield (Figure 1b–d). Essentially, resource acquisition and growth yield could be collapsed into one lifestyle, since they were not negatively related to one another. Stress tolerance, on the contrary, could remain a

distinct lifestyle, since drought tolerance was negatively related to both growth yield and resource acquisition. We posit that sorting between the stress tolerance and resource acquisition/growth yield lifestyles occurred on a continuum. However, it would be useful to further test whether these findings are site-specific or representative of a broader trend. The field setting could have been relatively stressful (owing perhaps to environmental fluctuations or more extreme drought events), selecting for traits at the stress tolerance end of the spectrum (Figure 1). In comparison, the laboratory setting may not have been stressful enough to select against resource acquisition/growth yield. More fluctuations in moisture content in the ambient plots may have resulted in the most stressful environment for these drought-adapted fungi, compared with the fungi in the drought plots or laboratory setting. Differences in experimental duration (5 weeks in the laboratory vs. 6 or 12 months in the field) could have also indirectly contributed to a greater stress observed in the field. For example, despite the litterbags allowing for passage of nutrients, low moisture levels in the field may have stifled the movement of new nutrients into the litterbags. Low nutrient availability after several months in the field may have resulted in a more stressful

environment. The longer field experiment may have also increased mycoparasitism since there was more time for cell death to occur (Steyaert et al., 2003).

Conducting complementary field and laboratory experiments helped identify potential mechanisms for fungal responses to drought. Microbial cages were crucial in this approach, since they allowed us to assess activities of specific isolates in the field. Although we had expected that increased variation among replicates in the field setting might obscure relationships among traits, this was not the case. Surprisingly, variation was not necessarily higher in the field than in the laboratory (Figure 5). Few studies use this dual laboratory–field approach to directly compare variation for soil microbes (Kampichler et al., 2001; Teuben & Verhoef, 1992). However, increased variation in the laboratory compared with the field was also observed in an experiment examining freshwater planktonic communities (Wickham & Gilbert, 1991). Altogether, increased variability in field may not be a barrier to experimentation if appropriate designs and techniques such as microbial cages are used.

Our results highlight the importance of creating dual laboratory and field experiments to test conceptual frameworks in ecology. Combining these approaches may also help to improve modeling of decomposition dynamics in ecosystems (Allison & Goulden, 2017). We found that the field experiment contextualized our results from the laboratory regarding hypothesized trade-offs between fungal traits. We suggest that a modification to the YAS framework, by combining the Y and A lifestyles, may be more appropriate for this arid system. The fungi in this ecosystem appear well adapted to low moistures; drought did not hinder growth yield and only slowed mass loss, which was not fully apparent in the laboratory experiment. A dual laboratory–field approach may expose and help reconcile mechanisms and can improve our conceptual understanding of ecological systems.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data (Alster et al., 2022) are available from Figshare: <https://doi.org/10.6084/m9.figshare.16528455.v1>.

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