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RESEARCH ARTICLE

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Symbiotic fungi alter plant resource allocation independent of water availability

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Abstract

Premise: The ability of plants to adapt or acclimate to climate change is inherently linked to their interactions with symbiotic microbes, notably fungi. However, it is unclear whether fungal symbionts from different climates have different impacts on the outcome of plant–fungal interactions, especially under environmental stress.

Methods: We tested three provenances of fungal inoculum (originating from dry, moderate or wet environments) with one host plant genotype exposed to three soil moisture regimes (low, moderate and high). Inoculated and uninoculated plants were grown in controlled conditions for 151 days, then shoot and root biomass were weighed and fungal diversity and community composition determined via amplicon sequencing.

Results: The source of inoculum and water regime elicited significant changes in plant resource allocation to shoots versus roots, but only specific inocula affected total plant biomass. Shoot biomass increased in the high water treatment but was negatively impacted by all inoculum treatments relative to the controls. The opposite was true for roots, where the low water treatment led to greater proportional root biomass, and plants inoculated with wet site fungi allocated significantly more resources to root growth than dry- or moderate-site inoculated plants and the controls. Fungal communities of shoots and roots partitioned by inoculum source, water treatment, and the interaction of the two.

Conclusions: The provenance of fungi can significantly affect total plant biomass and resource allocation above- and belowground, with fungi derived from more extreme environments eliciting the strongest plant responses.

KEYWORDS

climate change, drought, endophytes, flooding, plant-microbe interactions, root fungi, symbiosis, traits

As climate change increases global temperatures, the hydrological cycle is predicted to intensify, with wet areas becoming increasingly wet and dry ones increasingly dry (Dai, 2011; IPCC, 2023). Sessile organisms such as plants cope with changes in their environmental conditions by dispersing to new, more amenable environments, acclimating or adapting (Thomas, 2010; Reyer et al., 2013). Plant acclimatization can be expressed in numerous ways including traits linked to phenotypic or genotypic plasticity (Nicotra et al., 2010), phenology (Cleland et al., 2007), or competitive ability (Agrawal, 2001). However, many, if not the majority, of plant traits are intimately linked to symbiotic microbial partners such as fungi (Harman et al., 2021). Therefore, the ability of plants to acclimate to climate change may in part be determined by their interactions with fungal symbionts and how these fungi respond to changes in their environment (Kivlin et al., 2013; Cavicchioli et al., 2019; Rudgers et al., 2020).

Symbiotic fungi associate with all stages of plant development and all types of plant tissues (Trivedi et al., 2020). In particular, the fungi associated with healthy leaves, either as epiphytes (subsisting on leaf surfaces) or endophytes (living within leaf interiors), and those residing in and on roots have repeatedly been shown to play important roles in plant health and fitness (Porras-Alfaro and Bayman, 2011; Philippot et al., 2013; Vacher et al., 2016). These fungi can mitigate stress, including water and nutrient deficiencies and disease through direct and indirect mechanisms

(reviewed by Trivedi et al., 2020 and Harman et al., 2021). One direct mechanism by which symbiotic fungi can affect plant fitness is through eliciting changes in plant functional traits such as shoot and root growth (Friesen et al., 2011). However, the outcome of interactions between plants and their symbiotic fungi appears to be context dependent, where changes in either the abiotic environment or symbiotic communities can alter the degree of benefit received by host plants (Rúa et al., 2016; Rudgers et al., 2020; Donald et al., 2021). For example, Fitzpatrick et al. (2019) found that inoculation of *Arabidopsis thaliana* with soil microbes only increased plant fitness under drought conditions. Additionally, Bell-Dereske et al. (2017) found that biotic interactions among different guilds of symbiotic fungi and their effects on host fitness can vary depending on environmental conditions. This context dependency may in part be owed to ecotypic interactions, where populations of plants and symbiotic fungi in a given local environment optimize their interactions to their individual greatest benefit (Johnson et al., 1997; Thompson, 2014; Hawkes et al., 2020). In some circumstances, ecotypic interactions may result in improved mutualisms relative to non-local, novel partnerships (a "home advantage"), while in others, local symbiotic relationships may result in relatively more antagonistic outcomes compared to novel partnerships (an "away advantage") (Lau and Lennon, 2012; Rúa et al., 2016; Rasmussen et al., 2020).

To date, studies have conflicting results as to whether "home" versus "away" interactions with symbiotic fungi provide relatively greater benefits to host plants (Klironomos, 2003; Rúa et al., 2016; Lynn et al., 2019). One possible reason for these discrepancies is that the influence of home or away advantages is obscured by the response traits measured. In manipulative studies, a common response metric is total plant biomass, which due to asymmetric plant resource allocation to roots and shoots among treatments may have an averaging effect. Another possible confounding factor is that different plant genotypes may elicit distinct symbiotic outcomes, with some genotypes better matched to certain fungal communities relative to others, resulting in more positive or negative interactions (Fitzpatrick et al., 2019; Rudgers et al., 2020). Also, the environmental conditions of the plants and the original environments of the plant symbiotic fungal communities may determine the strength of symbiotic interactions. Studies predict that a "home advantage" should only be found when plants experience environmental stress and are partnered with symbiotic fungi adapted to these stressful conditions (Giauque et al., 2019; Donald et al., 2021), but support for this prediction is mixed (Cheplick, 2004; Bell-Dereske et al., 2017; Lynn et al., 2019). For example, the seminal study by Lau and Lennon (2012) found that plants grown with wet-adapted microbes responded more positively to well-watered conditions; specifically, plants increased flower production in wet environments but only when grown in association with microbes adapted to wet environments. Similarly, Giauque et al. (2019) found that survival and growth were greater for plants grown with drought-tolerant fungal endophytes from dry environments. Conversely, Rasmussen et al. (2020) found that plant performance was consistently worse when hosts were partnered with local arbuscular mycorrhizal fungi regardless of environmental conditions. Ultimately, interplay among all these factors likely influences our ability to detect and assess the importance of "home" versus "away" advantages.

Changes in plant resource allocation, especially to different plant organs, is a trait often associated with environmental tolerance (Nicotra et al., 2010). Under water stress, plants will allocate more energy to root growth than shoots (Chaves, 2002). However, the roles of symbiotic fungi in plant resource allocation and tolerance are understudied (Hou et al., 2021). In this study, we set out to examine the effects of shoot and root symbiotic fungi from different climates relative to uninoculated controls on host plant resource allocation when grown in either a soil moisture environment similar to their "home" or "away" conditions. We define symbiotic fungi as those inhabiting the healthy shoots, roots and rhizosphere of our experimental plants, acknowledging that these consortia may harbor a range of fungal guilds inclusive of pathogens, parasites, commensalists, mutualists, and latent saprotrophs (Rodriguez et al., 2009). We inoculated clones of a single genotype of strawberry (*Fragaria vulgaris*) as our host plant to control for any genotype × environment × fungal interactions (Sangiorgio et al., 2022). Inoculated or uninoculated plants were then grown under low, moderate or wet conditions and resource allocation measured as biomass of roots and shoots was determined at the end of the experimental period. Additionally, we monitored changes in shoot fungal communities before and after the water manipulation and compared shoot and root fungal communities at the end of the experiment. Evidence for home advantages would be significant changes in plant resource allocation when they are exposed to extreme environments and partnered with fungi derived from similar conditions, whereas away advantages would entail significant changes in resource allocation when fungal provenance and environment are mismatched. In both cases, there should be significant interactions between fungal inoculum source and environment. For example, in home advantage scenarios under the low water treatment, we predicted that dry-site fungi should increase resource allocation to roots, while under relatively higher water availability wet-site fungi should increase resource allocation to shoots. Alternatively, plants may have independent responses to the effects of inoculation and water treatment (i.e., no significant interaction between the two), indicating that inoculation affects plant tolerance independent of their environmental context. We predicted that each inoculum source would harbor a distinct symbiotic fungal community. Also, we anticipated significant shifts in fungal community composition and dispersion during the experiment due to the experimental conditions selecting for specific fungi that could persist under our water treatments.

MATERIALS AND METHODS

Experimental design and overview

Strawberry (*Fragaria vulgaris* Ehrh., Rosaceae) was chosen as the host plant for this study because clones are easily generated allowing us to control host genotype—a factor repeatedly shown to impact the outcome of plant–fungal interactions (Trivedi et al., 2020), also strawberry forms broad symbiotic associations with fungi, which have been shown to influence their fitness (Kim et al., 2019). Axenic strawberry clones were grown in the laboratory by harvesting meristem tissue from a single donor plant and culturing explants in sterile liquid and solid Murashige–Skoog (MS) growth medium (Appendix S1). Strawberry clones (ca. 6 months old) were transplanted to sterilized 1:1 sand:peat soil mixtures inoculated with size-fractionated (<100 μm) soil samples collected from three locations in Waimea Valley, O'ahu, Hawai'I, USA (21.639 N, 158.060 W) that spanned a strong elevational, temperature, and precipitation gradient (detailed below) or treated with sterile water as the control. Inoculated plants ($N = 168$) were placed into three growth chambers (84 plants per chamber) divided according to experimental water treatments: dry, moderate, and wet (detailed below). In total, 14 plants were used for each inoculum × water treatment to test the interaction of symbiotic fungi and environmental challenge on plant performance.

Inoculum collection and generation

Soils were collected in September 2020 from three sites across Waimea Valley watershed (State of Hawai'i Division of Land and Natural Resources permit number ODF-060120R). Collection sites were selected from across the length of the watershed to capture a natural environmental gradient of distance from shore (ca. 2000–12,000 m) and elevation, which generates a strong temperature gradient spanning 17.86–23.45°C mean annual temperatures (MAT), and an especially strong precipitation and spanning 1245–4562 mm mean annual precipitation (MAP) (Table 1). Change in MAP across this gradient is ~3.7-fold from driest to wettest, comparable to precipitation changes from the dry African Savannah to the Hoh Rainforest, the wettest place in the contiguous United States. For comparison, in a global analysis by Zhou et al. (2018) of microbial response to precipitation, the difference between dry and wet sites could be as low as a 2.2-fold change. Thus, while on the global scale our driest site would still be categorized as moderate-wet, locally, we captured a significant moisture gradient. Across the watershed, three increasingly wet sites were chosen to sample soil: the mouth of Kamananui Stream, the middle of the valley, and the headwaters of Kamananui Stream at the top of the watershed (Table 1). We chose to generate our fungal inocula from soil because plant-associated symbiotic fungi were previously shown to be primarily a subset of those found in their local soils (Bernard et al., 2020). At each sampling location, three replicate soil samples were collected (ca. 3 m apart). Duff and debris were removed from the soil surface, and the upper 10 cm of soils were collected (ca. 1 L) using a 70% v/v ethanol-sterilized trowel. Soils were placed into plastic bags and transported on ice to the University of Hawai'i at Mānoa and stored at 4°C.

Within 2 days of sampling, soils were processed by pooling all replicate samples ($N = 3$) from each of the three field sites (ca. 1.5 L) and homogenizing them with 1.5 L of purified water (Ultrapure, Millipore Direct-Q 3 with UV; Millipore-Sigma, Burlington, MA, USA) in a 10% v/v bleach-sterilized Waring blender. Homogenized soils were filtered through a 500-μm sieve (sterilized with 10% bleach), then a 100-μm nylon mesh filter funnel. Filtered soil samples, hereafter "inocula", were stored at 4°C until strawberry clones were inoculated the next day.

TABLE 1 Site conditions in Waimea Valley (North Shore, O'ahu, Hawai'i) where soils were collected to generate fungal inocula.

Site	Latitude	Longitude	Elevation (MASL)	MAT (°C)	MAP (mm)
Estuary	21.63696	−158.05852	21	23.45	1245.25
Upstream Drum Rd.	21.61980	−158.01286	193	21.90	2092.10
Summit	21.59013	−157.95557	692	17.86	4562.09

Note: MASL = meters above sea level, MAT = mean annual temperature, MAP = mean annual precipitation.

Plant inoculation

Inoculum prepared from one of the three sites (in 1.2 L autoclaved, distilled water) as described above or autoclaved distilled water (sterile water control) was added to a sterile sand–peat soil mixture (30 L per treatment) to produce four experimental growth media (for more details, see Appendix S1). Individual strawberry plants were removed from the MS growth medium and rinsed in purified water (MilliQ HX 7120 Type II pure water system, Millipore) to remove residual culturing media from the roots. Ten plants were destructively harvested for DNA sequencing, representing uninoculated controls, with roots and shoots excised and placed into microcentrifuge tubes, frozen (–80°C), and freeze-dried (24 h) for future processing. The remaining plants were potted in the inoculated or uninoculated control growth medium (300 mL medium) in 10-cm circular pots, the surface was covered with sterile sand to prevent cross-contamination, then plants were transferred to growth chambers. After 1 week in the growth chambers, inoculated plants received an additional inoculum dose (500 μL) added to the apical meristem and soil adjacent to the plant root–shoot axis. Plants were grown under identical conditions for 2 months (20–23°C, with 12 h light [250 μmol photons m^{-2} s^{-1}]/12 h dark, twice weekly

watering with 30 mL of UV-sterilized purified water), then temperatures were increased to 27–29°C, and 2 weeks later, water treatments were imposed.

Experimental conditions

Strawberry clones in the inocula or control treatments were grown in three replicate growth chambers (Percival Scientific, Perry, IA, USA). Each chamber represented a watering treatment, and all inocula and control treatments were represented in each chamber (additional details in Appendix S1). To account for any differences among chambers and between shelves within a chamber, we rotated and randomized plants among shelves and across chambers every 4 weeks. Watering treatments were selected to represent the natural gradient of precipitation across Waimea Valley (as mean annual precipitation): dry conditions near the estuary (500–1500 mm), moderate precipitation in the middle of the valley (2000–2500 mm), and wet at the headwaters of the watershed (> 4500 mm). With this natural precipitation gradient in mind, plants were subjected to dry (15 mL), moderate (45 mL), and wet (70 mL) watering regimes spanning a 4.7-fold moisture gradient, administered twice weekly (total: 30, 90, 140 mL wk^{-1}).

Sampling and measuring plant and fungal responses

Before the start of the water treatments and after 6 weeks of exposure to the inocula (Time = 0, T0), all plants were subsampled, whereby a single leaf from each plant was excised, placed in a microcentrifuge tube, frozen (–80°C), and freeze dried (24 h) for fungal community analyses via amplicon sequencing. Individual leaves were pooled between replicate plants ($N = 2$ leaves) within each inoculum treatment to yield ca. seven biological replicates per treatment from each of the three growth chambers ($N = 21$ leaf samples per inoculum treatment). At the end of the experiment (Time = 1, T1), that is, 151 days after inoculation with exposure to experimental water treatments for the last 47 days, plants were removed from the soil, and roots and shoots were thoroughly rinsed with tap water. Plants were then cut at the root–shoot axis to generate separate shoot an root samples for each plant. Samples were frozen (–80°C), freeze dried, then individually weighed, ground to a powder, and subsampled (10 mg) for DNA extraction.

Fungal community analyses

Plant material from three time periods were sampled for fungal community analyses: (1) shoots and roots from axenic plants before inoculation, (2) leaves from inoculated plants before water treatments (T0), (3) shoots and roots from inoculated plants at the end of the water treatments (T1). From each sample, DNA was extracted and the ITS1 spacer from the rRNA gene region was amplified using the primers described by Smith and Peay (2014). Library preparation is detailed in Appendix S1. The final library was sequenced in a single Illumina MiSeq run at the University of California Irvine Genomics, Research and Technology Hub using v.3 chemistry for 600 cycles.

Bioinformatics

Forward and reverse reads were demultiplexed in QIIME 2 version 2019.4 (Bolyen et al., 2019) using the q2-demux plugin resulting in 7,241,633 sequences being assigned sample barcodes. The ITS1 region was extracted from all sequences using ITSxpress version 1.8.0 (Rivers et al., 2018), and reverse reads were discarded to preserve sequence quality (Pauvert et al., 2019). Low-quality sequences and chimeras were removed with the DADA2 package (Callahan et al., 2016) in R version 3.6.3 (R Core Team, 2020), using a truncation length of 150 bp. Sequences passing quality checks were assembled into amplicon sequence variants (ASVs) with taxonomy assigned to ASVs using the RDP Classifier algorithm against the UNITE database version 8.3 (Abarenkov et al., 2010) with the default bootstrap threshold of 50, the recommended value for our sequence length (Wang et al., 2007). The ASVs were all assigned as kingdom Fungi and were used in the mctoolsr (Leff, 2017) package for downstream analyses.

Amplicon sequence variants with <1 read were removed resulting in 1929 ASVs in 3,911,183 reads. The prevalence method in the decontam package (Davis et al., 2018) considered 35 ASVs as contaminants and removed them from the treatments, resulting in 1894 ASVs. Potential sources of "contamination" were defined as ASVs from (1) axenic pre-inoculated plants ($N = 10$ roots and $N = 10$ shoots), DI water ($N = 2$), DNA-extraction-negative controls ($N = 13$), and water treatment plants "inoculated" with purified water ($N = 91$). We then further culled low-quality samples by removing those with <9% of the maximum read count (not considering outliers); 88 samples (leaf or root samples) with less than 1000 reads were thus removed, and 1327 ASVs remained for analysis. The vegan package (Oksanen et al., 2019) was used to generate ASV accumulation curves for each sample at T0 and T1 (Appendix S1: Figure S1).

Statistical analyses

For the plant biomass data, we built models testing both total biomass (roots plus shoot biomass) and the proportion of total biomass in leaf or roots (gram biomass/grams total biomass) as the response and the interaction of water and inoculation as fixed variables. We also tested for an effect of inoculation presence–absence on biomass using a one-way ANOVA. We ran Type II ANOVAs and used emmeans to produce pairwise Tukey comparisons (Lenth et al., 2021). Plant survivorship among inocula and water treatments

(T0 to T1) was assessed via a bias-reduced logistic regression (Kosmidis, 2020) on binary data.

For the fungal community data, we performed a Hellinger transformation on the ASV contingency table. Relative abundance of the ASVs was calculated by summing the new totals for all ASVs (the transformed number of total reads) within a sample and dividing the transformed number of reads for each ASV by this total. To visualize differences among the fungal communities by inocula and controls for T0 shoots, and T1 by root and shoot and among water treatments, we used NMDS plots. We then performed PERMANOVA analysis (Oksanen et al., 2019) to determine the effect of inoculation (dry, moderate, wet), water (low, moderate, high), the interactive effect of inoculation and water (T1 only), and time (T0 vs. T1, shoots only) using Bray–Curtis dissimilarity. We also visualized how the fungal communities changed over time from T0 (pre-water treatment) to T1 (post-water treatment) using NMDS. Further, we performed a beta dispersion analysis to assess whether the community dissimilarity within inocula changed over time.

Shoot fungal diversity was estimated for each inocula and controls at T0 using Shannon's diversity index, which was also used to estimate fungal diversity at T1 for shoots and roots among the various treatments (Feranchuk et al., 2018). Comparisons of estimated fungal diversity at T0 and T1 among the inocula and by water treatment (T1) were carried out using linear models with inocula and water treatment as main effects. Analysis of variance (ANOVA) tables using Type II sum of squares were generated using the package car (Fox and Weisberg, 2019) and post hoc comparisons in the package emmeans (Lenth et al., 2021). Venn diagrams were generated to compare overlap in fungal ASVs from T1 shoots and roots by water treatment and inocula. For all statistical tests, $a \leq 0.05$.

RESULTS

Plant resource allocation

Plant total biomass was affected by inoculum source ($P = 0.025$), but not water treatment and was greatest in plants receiving wet-site inoculum and lowest with the dry-site inoculum (Appendix S1: Figure S2, Table S1). Total biomass did not differ as a function of inoculation presence/absence ($P = 0.464$). However, there were significant effects of inoculation source and water treatments on the proportion of total biomass in shoots and roots. Shoot biomass increased with water availability and regardless of inoculum source, was significantly lower than control plants, with plants inoculated with wet-site fungi having the lowest proportional shoot biomass relative to dry-site inoculated plants and the uninoculated controls (Figure 1A; Appendix S1: Table S1). For roots, the opposite trend was observed (Figure 1B; Appendix S1: Table S1). The proportion of

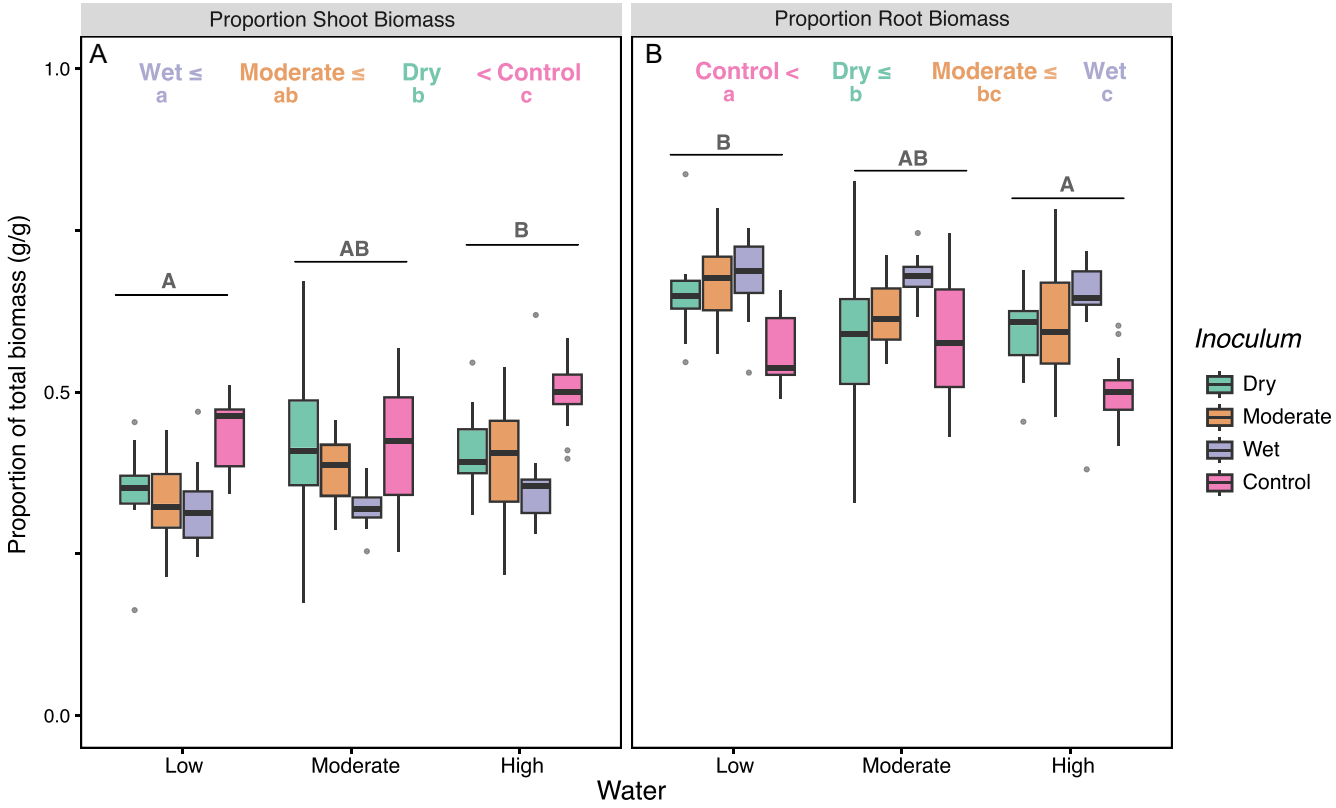

FIGURE 1 Box plots of the proportion of strawberry total biomass in shoots (A) and roots (B) after inoculation with fungal inoculum from wet, moderate or dry environments or none and exposure to three levels of water availability. Black lines indicate the median and whiskers the range of the data excluding outliers (black points). Significant differences among inoculation sources and water treatments ($P \leq 0.05$) are indicated by different letters.

biomass in roots decreased with water availability and was greatest in plants receiving wet-site inoculum and lowest in dry-site inoculum-treated plants. (Figure 1B; Appendix S1: Table S1). Plant survivorship significantly differed by inoculum source and controls ($P = 0.009$), but not water treatment or their interaction ($P \geq 0.388$). Overall, uninoculated control plants had lower survivorship relative to inoculated plants ($P = 0.011$) (83% survivorship), while inoculated plants had 90–100% survivorship across water treatments (Appendix S1: Figure S3, Table S2).

Fungal response to water treatments

Six weeks after inoculation, the fungal community composition of shoots at T0 did not partition by inoculum sources or uninoculated plants ($R^2 = 0.063$, $P = 0.159$; Appendix S1: Figure S4, Table S3). At the end of the experiment—151 days after inoculation and 47 days under differing water treatments, both shoot and root fungal communities were separately affected by inoculation and water treatments (Figure 2, Table 2; Appendix S1: Figure S5). Inoculum source explained the greatest amount of the variation in fungal community composition for shoots ($P = 0.001$, $R^2 = 0.196$), and especially roots ($P = 0.001$, $R^2 = 0.422$). While explaining substantially less of the variation in fungal community composition, water treatment was also a significant predictor for fungal composition in shoots ($P = 0.001$, $R^2 = 0.089$) and roots ($P = 0.001$, $R^2 = 0.038$), as was the interaction between water and inoculum source for shoots ($P = 0.002$, $R^2 = 0.041$) and roots ($P = 0.002$, $R^2 = 0.037$; Figure 2, Table 2). At the end of the experiment, pairwise comparisons of fungal communities in roots showed significant differences between low and moderate water treatments for all inoculum sources and the uninoculated controls ($P \leq 0.042$; Appendix S1: Table S4). The effects of the moderate and wet water treatments differed for the moderate and uninoculated control roots ($P \leq 0.009$), but not other treatments (Appendix S1: Table S4). Shoot fungal communities from each inoculum source and controls under the three water treatments significantly differed from each other for all pairwise comparisons, except the dry-site fungi under low versus moderate and high water and the moderate versus high water (Appendix S1: Tables S5, S6). Over time and in all water treatments, the shoot fungal communities of each inoculum source and controls were significantly distinct (Figure 3; Appendix S1: Table S6). Furthermore, these communities became increasingly more dispersed in NMDS space (Figure 3, Table 3).

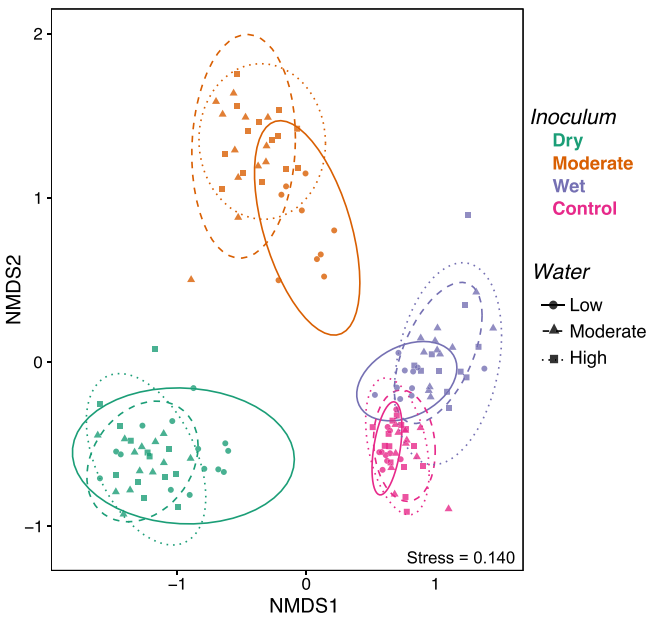

FIGURE 2 NMDS comparing fungal community composition in roots of strawberry plants inoculated with fungi originating from dry, moderate, and wet environments within the Waimea watershed and in uninoculated controls after 47-day exposure to low, moderate, or high water availability treatments. Ellipses represent 95% confidence intervals.

TABLE 2 PERMANOVA testing inoculation source and water treatment effects on plant-associated fungal communities in shoot and root biomass.

Response metric	Effect	df	SS	R^2	F	P
Shoot-associated fungi	Water	2	4.544	0.089	8.536	**0.001**
	Inoculum	3	10.005	0.196	12.530	**0.001**
	Water × Inoculum	6	2.075	0.041	1.300	**0.017**
	Residual	129	34.334	0.674		
	Total	140	50.958	1.000		
Root-associated fungi	Water	2	1.965	0.038	4.931	**0.001**
	Inoculum	3	21.907	0.422	36.648	**0.001**
	Water × Inoculum	6	1.940	0.037	1.623	**0.002**
	Residual	131	26.102	0.503		
	Total	142	51.913	1.000		

Note: PERMANOVA table generated from 999 permutations and Bray–Curtis dissimilarity. SS = sum of squares. Significant effects ($P < 0.05$) are in bold.

At 42 days after inoculation and before water treatments (T0), Shannon's diversity index for shoot fungi did not differ among inoculum source and the controls (Appendix S1: Figure S6, Table S7). However, at the end of the experiment, the fungal diversity of shoots differed significantly among inocula and controls ($P = 0.004$) as well as by water treatment ($P < 0.001$), but not their interaction ($P = 0.782$). Control shoots were significantly less diverse than fungi from the moderate-site inoculum (post hoc: $P = 0.033$), neither of which were significantly different from the dry- or wet-site inocula (post hoc: $P \geq 0.055$), which were

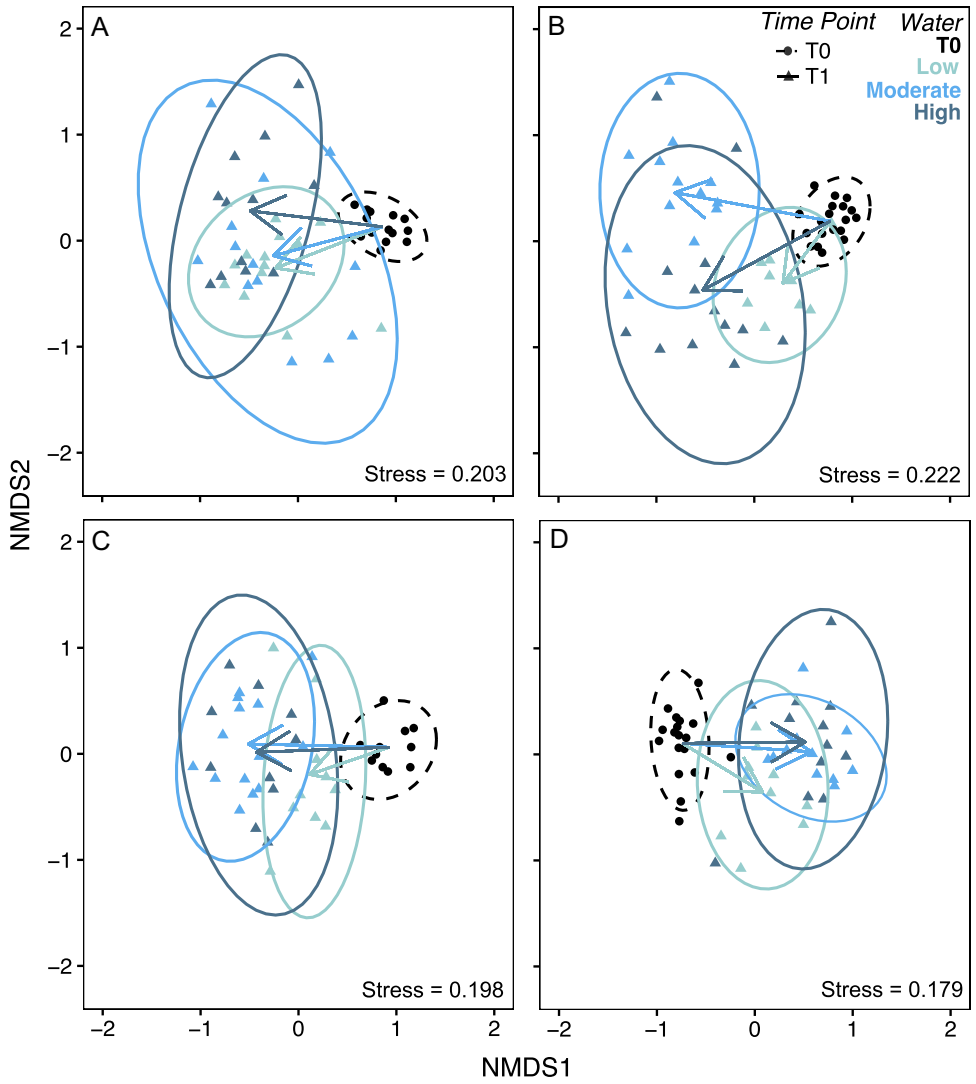

FIGURE 3 NMDS representing changes in fungal community composition in shoots of strawberry plants inoculated with fungi originating from dry (A), moderate (B), and wet (C) environments within the Waimea watershed and in uninoculated controls (D) before (T0) and 47 days after (T1) exposure to low, moderate or high water availability treatments. Vectors represent the movement of group centroids from before (T0) and after (T1) water treatments. Ellipses represent 95% confidence intervals.

TABLE 3 Beta-dispersion of fungal communities associated with aboveground plant tissues (shoots) from before water treatments (T0) to after (T1) for three inoculum sources and uninoculated control.

Inoculum source	Effect	df	SS	MS	*F*	Permutations	*P*
Dry	T0 to T1	1	0.328	0.328	32.335	999	**0.001**
	Residuals	50	0.507	0.010			
Moderate	T0 to T1	1	0.978	0.978	233.490	999	**0.001**
	Residuals	52	0.218	0.004			
Wet	T0 to T1	1	0.236	0.236	45.608	999	**0.001**
	Residuals	44	0.227	0.005			
Control, Uninoculated	T0 to T1	1	0.191	0.191	13.414	999	**0.001**
	Residuals	49	0.698	0.014			

Note: SS = sum of squares; MS = mean squares. Significant effects ($P < 0.05$) are in bold.

also not different from each other (post hoc: $P = 0.890$), (Appendix S1: Figure S6). In addition, shoot fungal diversity was greatest under high water compared to the low and moderate water treatments ($P \leq 0.002$). The diversity of root fungi differed significantly among all inoculation sources and the controls ($P < 0.001$), with the control communities the least diverse, followed by the wet, dry, then moderate (post hoc: $P < 0.001$) (Appendix S1: Figure S6). For the shoot fungi, based on ASV presence/absence, between 9% and 14% of ASVs were shared between any one inoculum source and the control under all water treatments (Appendix S1: Figure S7). For the root fungi, only 4–8% of ASVs were shared between any one inoculum source and the control under all water treatments (Appendix S1: Figure S8). Membership of each fungal community at the end of the experimental period is reported as the relative abundance of fungal families from shoots (Appendix S1: Figure S9) and roots (Appendix S1: Figure S10).

DISCUSSION

Plants altered resource allocation through interactions with symbiotic fungi and their environment

Plants' ability to respond to climate change is intimately intertwined with their symbiotic microbes and how these relationships may be leveraged to their benefit (Barnes and Tringe, 2022). Although both plants and microbes can be impacted by climate change, microbes are thought to be less dispersal-limited and to adapt faster than plants to changing environmental conditions. Therefore microbes have the potential to increase host plant tolerance to climate change (Lau and Lennon, 2012), assuming that plants can recruit and maintain beneficial microbial symbionts. Plant traits associated with stress tolerance are often expressed as phenotypic trade-offs in resource allocation such as changes in root to shoot ratios (Reyer et al., 2013; Worchel et al., 2013; Martínez-Arias et al., 2022). We predicted that if symbiotic fungi could increase plant tolerance through shifting resource allocation, then this would be most pronounced when fungi were derived from more extreme environments (in this case, low and high precipitation sites) and either exposed to relatively high or low watering regimes. Said another way, these advantages would only be observed at the far ends of the environmental stress spectrum (Donald et al., 2021).

Instead, we found support for our other predictions—plant resource allocation responds independently to the effects of inoculum source and water availability, and these effects are partially obscured by observations of total plant biomass alone. Specifically, plant resource allocation increased in shoots and decreased in roots with water availability, and inoculated plants allocated significantly less resources to shoots and more to roots than uninoculated controls. In addition, while inocula significantly increased total biomass, especially with wet-site fungi, these effects were driven by resource allocation belowground. We found greater belowground investments in root biomass in plants inoculated with wet-site fungi and least in plants receiving dry-site, with the moderate-site being intermediate. This pattern runs counter to the general trend of decreasing proportions of root biomass with increasingly water availability. Therefore, while increasing water availability shifted resources to shoot biomass, wet-site fungi drove resource allocation to roots over shoots. These findings indicate that while the interactions between soil moisture environment and fungal provenance may not lead to compounding effects on plant resource allocation, variation in these can potentially affect plant environmental tolerance. For example, wet-site fungi significantly increased plant resource allocation belowground regardless of soil moisture conditions. Also, dry-site fungi significantly increased aboveground biomass relative to the wet-site, while the opposite was true for belowground indicating that fungal inoculum source can elicit trade-offs in plant resource allocation—an important facet of environmental tolerance (Friesen et al., 2011; Giauque et al., 2019). This finding is important in light of climate change, where its effects on extant fungal communities are largely unknown (Rudgers et al., 2020), and also in the context of microbiome engineering where some facets of science and industry seek to harness the potential power of microbes to address pressing global issues ranging from crop productivity (Johansson et al., 2004; Hu et al., 2022). However, to conserve and engineer microbiomes entails knowing from where within environmental reservoirs those with unique properties exist.

Symbiotic fungal communities partitioned by environmental source, over time and under water treatment

Observations of fungal community composition in strawberry shoots at the first sampling time (T0, 42 days after inoculation) were not significantly different among inoculum sources or relative to uninoculated controls. However, at the end of the experiment (151 days after inoculation with 47 days of water treatments) shoot, and especially root fungal communities, strongly partitioned by inoculum source (explaining ~20 and 40% of community composition respectively), while also being significantly disparate from control plants' fungal communities. This finding indicates that time to colonization was greater than 5 weeks, an important consideration for future plant–fungal interaction experiments. While less of a predictor of community composition than inoculum source, water treatment and the interaction of water and inoculum explained more of the variation in shoot fungal communities than in the root. This finding, while in partial support of our predictions that environmental conditions will select

for specific fungal communities, also suggests that root symbiotic fungal communities were relatively more stable than those of the shoot under environmental change. In the cases where water treatment significantly affected root fungal communities, the low watering regime largely inflicted these shifts, indicating that similar to prior studies, a reduction in water availability is a significant factor affecting fungal community composition in roots (Lau and Lennon, 2012; Lagueux et al., 2021).

Estimates of fungal diversity for root fungi from inoculated plants were significantly higher than uninoculated controls and shoots. Furthermore, water treatment was a better predictor of estimated shoot fungal diversity than root, while inoculum source, less so. Plants inoculated with the "moderate" inoculum had the greatest fungal diversity for both roots and shoots, while the wet-site fungi were the least diverse. This finding lends further credence to our prediction of environmental conditions affecting fungal communities; in this case, the most extreme environmental conditions (low and high soil moisture) imposed the strongest filters on fungal diversity.

One possible explanation for root communities partitioning more strongly by inoculum source than shoots is that over the length of our experiment, roots were faster to recruit symbionts than shoots. Because plant microbiomes tend to be nested from the ground up (Bernard et al., 2020) and our original inoculum was generated from soil, it follows that roots were readily colonized, whereas shoots may have needed additional time or additional environmental sources such as air or precipitation to collect the specialized portions of their microbiomes (Darcy et al., 2020; Kajihara et al., 2022). This hypothesis is supported by our observation that on average, the overlap in observed ASVs shared between inoculated and uninoculated shoots was greater than in roots, indicating that while a minimal contribution to overall community membership, cross-colonization, likely via air, made up a greater proportion of shoot-associated fungi than root.

Temporal variations in shoot- and root-associated fungal communities due to changes in environment is common (Giauque and Hawkes, 2013; Hartman and Tringe, 2019; Lagueux et al., 2021; Martínez-Arias et al., 2022). In this experiment, we observed not only a significant change over time in shoot symbiotic fungal community membership when exposed to different water treatments, but also consistent and significant increases in community dispersion across all inocula and the control plants. This pattern is like that observed in other host-associated microbiomes when exposed to environmental change or disturbance. In animals, changes in microbiome dispersion have been proposed to be stochastic, with increases indicative of an "unhealthy state", a concept referred to as the Anna Karenina principle (Zaneveld et al., 2017). Here, however, regardless of their source on the strong environmental gradient, how extreme their experimental environmental perturbations, or their effects on host resource allocation, the fungal communities became significantly more dispersed over time across all treatments, including controls. Thus, rather than an indicator of dysbiosis, increased community dispersion may simply be evidence of fungi responding to change. Stated otherwise, selection may appear random or adaptive depending upon the time point at which observations are made, but eventually communities will converge upon members best suited to their environments (Rudgers et al., 2020).

CONCLUSIONS

In summary, we found that regardless of their environmental provenance, plant symbiotic fungi had significant effects on total biomass and the growth of shoots versus roots, especially under more extreme soil moisture conditions for the latter two. This observation of a shift in plant resource allocation to roots when inoculated with soil-derived fungi emphasizes the importance of measuring numerous plant functional traits beyond just total biomass. Also, changes in plant resource allocation may be an important mechanism elicited by the microbiome that aids in plant tolerance and therefore deserves further study. While root fungal communities among treatment plants clearly partitioned by inoculum source and were relatively stable across a range of water availabilities, shoot communities were relatively more influenced by water treatment. This finding highlights the perpetuating knowledge gap of understanding from where organisms recruit their most beneficial microbiome, a topic in pressing need of attention especially in this age of extinction and climate crises (Fisher et al., 2022).

AUTHOR CONTRIBUTIONS

C.B.W. helped design and execute the experiment, analyzed the data, generated figures and tables, wrote and curated the code, and helped draft the manuscript. K.K. helped generate the strawberry clones. D.Y. and S.O.I.S helped execute the growth chamber experiment and DNA sequencing. F.R., K.K., and L.V. helped with bioinformatics and data analysis. N.A.H. conceptualized the experiment, oversaw all aspects of the experiment and data analysis, and helped draft the manuscript. All authors contributed edits and approved the final version of the manuscript. Statement of inclusion: This study is part of ongoing research within the Center for Microbiome Analysis through Island Knowledge and Investigation (C-MĀIKI) a key tenent of which is acknowledgement of, and integration and engagement with traditional Hawaiian knowledge. As such, this study was designed in collaboration with local stakeholders and under the traditional Hawaiian framework of ahupua'a as natural land divisions encompassing our contemporary definition of a complete ecosystem.

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DATA AVAILABILITY STATEMENT

DNA sequences for this project can be found in the SRA under project number PRJNA1017539. Data and code can be found at https://github.com/cbwall/Plant-microbial-ecotype and are archived at Zenodo (https://zenodo.org/records/14157010).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Supplemental methods, figures, and tables.

Figure S1. ASV accumulation curves by sequencing depth.

Figure S2. Boxplot of strawberry total biomass.

Figure S3. Strawberry mortality.

Figure S4. NMDS fungal communities of shoots at T0.

Figure S5. NMDS fungal communities of shoots at T1.

Figure S6. Shannon diversity of shoot fungi at T0 and shoot and root fungi at T1.

Figure S7. Venn diagram of ASVs from shoots at T1.

Figure S8. Venn diagram of ASVs from roots at T1.

Figure S9. Relative abundance of fungal families in shoots at T1.

Figure S10. Relative abundance of fungal families in roots at T1.

Table S1. ANOVA results for strawberry biomass.

Table S2. Analysis of deviance table for strawberry mortality.

Table S3. PERMANOVA testing inoculum source effects on plant-associated fungi in shoots prior to the start of water treatments.

Table S4. Pairwise comparisons for root fungal communities of strawberry plants under differing water regimes.

Table S5. PERMANOVA testing water treatment effects for each inoculum in shoots at T0 and T1.

Table S6. Pairwise comparisons for shoot fungal communities of strawberry plants at T0 and T1, and under differing water regimes at T1.

Table S7. ANOVA results testing differences in Shannon diversity of fungi in shoots and roots by inocula and water treatments.

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