Invasion in a Community Context: Genetic Variation in the Strength of Direct and Indirect Effects

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4 direct and indirect ecological interactions
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Abstract

Predicting invasion success requires understanding how abiotic and biotic factors in the local environment interact with the particular traits of potential invaders. Relative to studies of direct antagonistic effects, fewer studies have examined how positive species interactions, such as facilitation or mutualism, or indirect interactions in multispecies communities, can affect invasion success. We examined the effects of drought and mutualisms with rhizobia bacteria on the performance of a widely invasive legume, *Medicago polymorpha*. In a greenhouse experiment, we found that watering regime affected plant performance, but non-linear patterns in response to decreasing water were dependent on the specific plant genotype. In a second experiment, we found that the effects of drought on plant performance were dependent on the presence of rhizobia, particularly for genotypes collected from the invasive range. This suggests that indirect ecological effects may have important consequences for invasion success. We contextualize the strength of these direct and indirect effects by comparing this study to effect sizes in other studies of the same species. In this species, predicting invasion into a natural community context will require understanding multiple direct and indirect effects in the local environment, as well as their effects on the specific genetic composition of the invading population.

Key words: Drought, Evolution, Indirect Effects, Invasive Species, Mutualism

34 **Introduction**

35 Invasive species are an ongoing and increasing threat to ecosystems and economies
36 around the world (Moser et al., 2009; Paini et al., 2016). Predicting and preventing invasion
37 success requires understanding the abiotic and biotic factors in the local environment that
38 influence invader performance and the invader traits that allow individuals to overcome
39 barriers to invasion. Habitats can exhibit abiotic resistance to plant invasion through drought
40 and disturbance (Bever et al., 1997; Duchicela et al., 2012; Bayliss et al., 2017), nutrient
41 availability (Davis et al., 2000; McGlone et al., 2011; Zefferman et al., 2015), or other factors
42 that decrease invader performance. Further, communities can confer biotic resistance to
43 invasion through antagonistic species interactions between native and non-native species,
44 such as competition (Bever, Platt, and Morton 2012; Pages and Michalet 2003), herbivory
45 (Hillebrand et al., 2008; Bayliss et al., 2017), or negative effects of the soil bacterial
46 community on plant performance (Bever, 2015; Revillini et al., 2016; Hansen et al., 2020).
47 Fewer studies have examined how positive species interactions, such as facilitation or
48 mutualism, or indirect interactions in multispecies communities, can affect invasion success
49 (Aslan, 2019; Cavieres, 2021).

50 The consequences of direct abiotic effects on native plant communities and non-
51 native plant invaders, particularly nutrient level, drought, and disturbance, have been well-
52 studied. The availability of nutrients such as nitrogen and phosphorous can be altered by both
53 native and invasive plant species, which can affect invasion success or create deleterious
54 effects on native plant communities (Huenneke et al., 1990; Kulmatiski et al., 2006;
55 Rosenblatt, 2018). Prolonged drought influences reproduction and growth of many plant
56 species, and many plants will not set seed in drought conditions (Thalmann & Santelia, 2017;
57 Rosenblatt, 2018). However, when invasive species are more tolerant of drought than their
58 native counterparts, they may have a competitive advantage that increases invasion success

(Everard et al., 2010). Fire or other natural disturbances can physically damage plants, affect seed banks, and create available space in plant communities that differentially affects species adapted to such disturbances (Kinnaird & O'Brien, 1998; Lentile et al., 2007; Knutson et al., 2014). Other anthropogenic effects, such as agriculture, land use changes, or climate change can also create disturbances in natural communities that can affect invasion success (Kuhman et al., 2013; González-Moreno et al., 2013; Csecserits et al., 2016). Such disturbances often affect soil nutrient levels, and different plant responses to nutrient availability can alter the likelihood of plant invasion success (Huenneke et al., 1990; Kulmatiski et al., 2006).

Negative species interactions can increase biotic resistance to invasion (Maron & Vilà, 2001; Levine et al., 2004; Mitchell et al., 2006). Antagonistic effects from natural enemies, such as herbivores, can physically damage leaves and roots, cause reallocation of resources away from reproduction, or stress plants such that invader performance is reduced (Edwards et al., 2000; Rand, 2003; Gianoli et al., 2009). Competition from other plants can also decrease invader performance by limiting access to light and nutrients (Weber & Kolb, 2013; Shabbir et al., 2020). Positive species interactions may also play a role in determining biotic resistance to invasion. Plant mutualists, such as pollinators, fungal mycorrhizae, or rhizobial bacteria may be required for reproduction or access to limiting resources in low-nutrient habitats (Marschner et al., 2001; Berg et al., 2014; Fang et al., 2019) and thus their absence may be considered a form of biotic resistance. Some plant species are only able to invade after the introduction of their mutualist partners (Halliday & Somasegaran, 1983; Richardson et al., 2000; Dickie et al., 2017).

Although many studies have identified abiotic and biotic factors that contribute to resistance to invasion, fewer studies have considered the complexities that arise when we account for multiple factors. Potential invaders face a complex web of novel abiotic and biotic interactions when they attempt to invade a community and these ecological interactions

rarely occur in isolation. Predicting invasion success in natural habitats requires understanding the consequences of multiple direct effects, as well as indirect ecological interactions. Biotic interactions may ameliorate or intensify the effects of abiotic stressors, resulting in indirect ecological interactions that affect plant performance (Gianoli et al., 2009; Rosenblatt, 2018; Szczepaniec & Finke, 2019). For example, the presence of mycorrhizal symbionts can increase performance in plants exposed to water stress (Jayne & Quigley, 2014). Increased abundances of invasive plants can increase local herbivore abundances, which can indirectly affect native plant performance (Rand, 2003). Allelopathy by either native or invasive plants may affect invader performance by chemically altering soil composition or changing the composition of the microbial community (Cipollini & Cipollini, 2016; Algandaby & El-Darier, 2018). Such indirect ecological effects have proven to be important in a variety of systems, often rivaling the strength of direct interactions (Strauss, 1991; Wootton, 1994; Dill et al., 2003).

If a single direct ecological interaction is the primary driver of invasion success in a community, then we may already have a firm grasp on successful management practices for many non-native species. However, if indirect ecological interactions are similarly important, then we cannot understand invasion resistance in a community context without accounting for direct and indirect effects. Invasive species management approaches that combine multiple factors, such as competition and nutrient levels, can have a greater affect than managing either factor in isolation (Edwards et al., 2000).

Study Species

Medicago polymorpha is a legume native to the Mediterranean that has invaded every continent in the world, except Antarctica (Bullitta et al., 1994). It is frequently used in agriculture as a cover crop, but can escape agricultural settings to surrounding environments and beyond (Bullitta et al., 1994; Porter & Simms, 2014). Once established in natural

environments, *M. polymorpha* can compete with native plants, and is difficult to eradicate because the burrs are easily dispersed by animals and can remain in the soil for years. Work on invasive *M. polymorpha* populations has focused on direct ecological effects like herbivory (Jack & Friesen, 2019), mutualism with rhizobia (Porter & Simms, 2014), and competition (Algandaby & El-Darier, 2018), alongside others. Less work has examined indirect effects involving *M. polymorpha*. The presence of *M. polymorpha* increased herbivory on native *Acmispon* (formerly *Lotus*) *wrangelianus* (Lau & Strauss, 2005), presumably because insect herbivores cue in on or find the invasive species more easily, but then switch to consume the native species as well. Disturbance and insect herbivory interact, in that disturbance increased *M. polymorpha* flowering, but only in the absence of insect herbivores (Bayliss et al., 2017). In another study, rhizobia were found to increase *M. polymorpha* tolerance of interspecific competition and effects on competitor biomass (terHorst et al., 2018).

The consequences of ecological interactions with *M. polymorpha* may largely depend on intraspecific variation within this species. For example, strains of *M. polymorpha* originating from arid environments have higher drought tolerance than strains from more humid climates (Yousfi et al., 2015; Badri et al., 2016). The extent to which rhizobia affect competition with *M. polymorpha* depended on the plant genotype and the location from which they were collected (terHorst et al., 2018). Indirect effects of rhizobia on competition were relatively small in plants collected from the native range, but plants collected from the broad invasive range of this species showed highly variable indirect effects, ranging from rhizobia greatly decreasing the effects of competition in some genotypes, to rhizobia increasing the negative effects of competition in other genotypes (terHorst et al., 2018). Presumably, genotypes collected in the native range reflect adaptive traits in a relatively narrow range of environmental conditions, whereas genotypes from the invasive range reflect

traits adaptive in the invasive range, which encompasses a broad array of different environmental conditions. This suggests that invasion success depends not only on the combination of direct and indirect effects at a potential invasion site, but also on the particular genetic composition of the introduced population.

In this paper, we seek a more comprehensive approach to understanding plant invasions that accounts for the community context in which invasions occur and intraspecific variation in responses to potential barriers to invasion. We ask whether such a framework provides more insight into how plant invasions occur, or whether our existing understanding of individual direct effects is sufficient. We use *Medicago polymorpha* as a model species to understand barriers to invasion more broadly and to better understand the role of community context in understanding how species expand their ranges, taking both direct and indirect effects into account. Here we present the results of two experiments that examined direct and indirect effects of drought and mutualisms with rhizobia on *M. polymorpha* fitness. Then we contextualize these results by comparing the magnitude of direct and indirect effects in these experiments to previously published direct and indirect effect sizes of *M. polymorpha* ecological interactions.

Methods

Direct effects of drought

We used a greenhouse study to test the effects of drought on different genotypes of *M. polymorpha*. We used 5 haphazardly selected *M. polymorpha* ascensions from the U.S. National Plant Germplasm System. Because this species reproduces almost entirely through selfing, resulting in homozygous clones, we refer to these ascensions as genotypes. These genotypes were collected from different parts of the species range, with three from the native range (292427 from Israel, 469263 from Egypt, and 543039 from Spain) and two from the

invasive range (478530 from Peru and w65527 from Australia). Genotypes were categorized as invasive or native using the CABI Invasive Species Compendium (<https://www.cabi.org/ISC>). All genotypes were grown in a common garden greenhouse environment for one generation prior to the experiment to ensure that any observed differences among genotypes were due to genetic differences, rather than maternal or epigenetic effects. Seeds collected from this common garden generation were used in the experiment.

M. polymorpha seeds were scarified and planted in 164 mL conetainers (Stuewe and Sons, Tangent, OR, USA) containing low-nitrogen LP5 Grower Mix soil (SunGro, Agawam, MA, USA), composed of 90% topsoil, 9% sand (DecoRock All Purpose), and 1% perlite. Plants were grown in the California State University, Northridge greenhouse with conetainers haphazardly distributed among racks, with racks rotated weekly to reduce any microclimate effects. We planted three seeds of each of the five genotypes in replicate conetainers and after two weeks, thinned the seedlings to one per pot. We transplanted removed seedlings to new conetainers when possible.

Plants were watered daily for 28 days and watering treatments were imposed thereafter. We watered plants in all treatments approximately every two days, with some adjustments based on the local temperature. On the few occasions where the outside temperature exceeded 29°C, plants were watered daily. All plants were watered with the same frequency, but with different volumes: Level 1 = 100 mL, Level 2 = 50 mL, Level 3 = 25 mL, Level 4 = 12.5 mL per conetainer. These volumes were based on preliminary data showing that plants experienced signs of water stress after two days in the smallest volume treatment, but did not die. The ecological relevance of these watering regimes differs depending on the potential invasion site and the origin of each genotype.

We started the experiment with 20 replicates per treatment combination (400 containers = 5 genotypes x 4 watering treatments x 20 replicates), but differential germination success among genotypes, seedling deaths, and transplantation of excess seedlings led to 14-26 replicates per treatment combination (N=411). In June 2016, 29 days after the watering levels were imposed, we harvested the plants and separated above- and belowground biomass of each plant and dried these at 50°C for three days, and then weighed dry biomass using an analytical balance. We used total plant biomass (aboveground + belowground biomass) as a measure of plant size and performance and the ratio of above:belowground biomass to determine if biomass allocation to shoots and roots differed across the four watering levels or plant genotype.

Direct and indirect effects of drought and rhizobia

We conducted a similar study in 2015 with 43 haphazardly chosen *M. polymorpha* genotypes (18 collected from the native range and 25 collected from the invasive range; Table S1). We manipulated watering level at only two treatment levels in this experiment and also manipulated the availability of rhizobia in the soil. Seedlings were established in containers as above, with three replicates per genotype, and were grown in the outdoor botanical garden at California State University, Northridge. This experiment included 516 plants (43 genotypes x 2 watering treatments x 2 rhizobia treatments x 3 replicates).

We randomly distributed replicates among two rhizobia treatments. In the enhanced rhizobia treatment, we supplemented the soil with rhizobia collected from the nodules of *M. polymorpha* plants in the botanic garden at California State University, Northridge in December 2014. *M. polymorpha* associates almost exclusively with the bacterium *Ensifer medicae* in laboratory and field conditions (Ballard et al., 2004; Porter et al., 2011). We collected nodules from plant roots and surface sterilized them with sequential rinses in 10% bleach, 95% ethanol, and sterile deionized water (adapted from Somasegaran & Hoben,

1985). We crushed the surface-sterilized nodules in sterile deionized water and plated the solution on tryptone yeast (TY) agar plates. We incubated plates at 25°C and found only colonies with the same morphology as *E. medicae* (Somasegaran & Hoben, 1985). We used multiple colonies to inoculate a heat-sterilized TY nutrient broth. We simultaneously started a sterilized broth of TY media without the rhizobia inoculant. Both media were cultured at 25°C for 24 hours before use. We added 1 mL of media (either rhizobia-inoculated or the sterile control) around the base of 2-week-old *M. polymorpha* seedlings (terHorst et al., 2018). We assumed rhizobia to be absent or in low abundance in the commercial soil prior to inoculation and did not control for colonization of rhizobia from the environment during the experiment. We did not quantify rhizobia abundance in the soil, but assume that the addition of rhizobia greatly increased their abundance. We refer to these as Enhanced or Background rhizobia treatments.

We randomly distributed replicates among two watering treatments that we imposed six days after rhizobia inoculation. All plants received 100 mL of water each time they were watered. Plants in the high watering treatment were watered approximately four times more often than those in the low watering treatment. Plants in the high water treatment were watered approximately every two days, but occasionally daily when the temperature was particularly high; watering was delayed a day following rare precipitation events. In the low watering treatment, we only watered plants following signs of water stress, such as leaf folding or mild wilting. In April 2015, 28 days after the watering treatments were imposed, we harvested the plants. Plant biomass was divided into above- and belowground components and weighed as dry mass, as above.

Statistical analysis

For the greenhouse experiment, we fit generalized linear mixed models using R to examine the fixed effects of watering level, plant range (native or invasive), and their

interaction on total biomass and above:belowground biomass ratio. We included genotypes nested within range, and the interaction between watering level and genotype as random effects in the models. We found the best-fit model and error distribution for each dependent variable (gamma distribution in each case) by comparing Akaike Information Criteria (AIC) values. Interaction terms and random effects were dropped from the model if their inclusion did not result in a lower AIC value. Genotype was retained in all models to avoid pseudoreplication in the experimental design. We performed likelihood ratio tests to determine the significance of random factors. We conducted similar analyses for the botanical garden experiment, but included rhizobia treatment as a fixed effect and the genotype*rhizobia interaction as a random effect.

Comparing direct and indirect effect sizes to previous studies

Papers were selected for inclusion in this meta-analysis based on a Google Scholar, Web of Science, and JSTOR search for keywords “*Medicago polymorpha*”, “*M. polymorpha*”, and “*Medicago*”. We selected papers with experimental manipulations of ecological interactions with *M. polymorpha*, and if sample size and variance could be determined from the paper. Standardized effect size for direct effects and confidence intervals were calculated using Cohen’s d (Ziegel et al., 1995).

Direct effects represented the standardized difference between a treatment and control. For example, the direct effect of rhizobia in the second experiment was calculated as the difference in a variable (X) between the Enhanced rhizobia treatment (X₂) and the Background rhizobia treatment (X₁), standardized by the pooled standard deviation and multiplied by a corrector for sample size (J):

$$\frac{X_2 - X_1}{\sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}} * J$$

We calculated the additive effect of the control treatment (X_1) and both other treatments (X_2 and X_3) as in terHorst et al. (2015).

$$X_{\text{additive}} = X_1 + (X_2 - X_1) + (X_3 - X_1) = X_2 + X_3 - X_1$$

We also calculated standardized indirect effect sizes using an equivalent of Cohen's d. We calculated the difference between the observed variable in the presence of both interactions (X_4) and the additive effect of both interactions based on direct effects (terHorst et al. 2015).

$$X_{\text{indirect}} = X_4 - X_{\text{additive}}$$

We standardized this indirect, or non-additive, effect by the pooled standard deviation, resulting in this standardized indirect effect comparable to direct effects:

$$\frac{X_4 - X_2 - X_3 + X_1}{\sqrt{\frac{(n_1 - 1)s_1^2 + ((n_2 - 1)s_2^2 + (n_3 - 1)s_3^2 + (n_4 - 1)s_4^2)}{n_1 + n_2 + n_3 + n_4 - 4}}}$$

Similarly, we calculated direct and indirect effect sizes from previous studies on *M. polymorpha* in response to various ecological interactions. We took the absolute value of each effect size and grouped these as direct effects of drought, disturbance, herbivory, nutrient level, competition, and mutualism, as well as indirect effects (Table S2). We further distinguished the studies by their use of native or invasive range genotypes of *M. polymorpha*, categorized by origin in the Mediterranean or outside of that region, using the reported genotypes, strains, or collection sites identified in each study. Because there were relatively few studies within each category, we did not conduct a formal statistical analysis.

Results

In the greenhouse experiment, 95% of replicate plants survived the experiment, resulting in an average sample size of 20 (range = 11-26) across treatment*genotype combinations. The size of *M. polymorpha* plants was determined by the interaction between

genotype and watering treatment ($X^2 = 26.1$, $P < 0.001$). Genotypes exhibited variation in both the direction and magnitude of their responses (Fig. 1A). The genotype from Australia (w65527), on average, grew to the largest size, but was the most affected by decreasing water to the lowest level. In contrast, the genotype from Spain (543039) had the smallest plants, but was relatively unaffected by different watering levels. The genotype from Israel (292427) grew largest with intermediate watering, but the genotype from Egypt (469263) grew largest at the highest and lowest watering levels.

Plant genotypes allocated above- and belowground biomass differently in response to the watering treatments. On average, genotypes from the native range allocated more resources toward aboveground growth relative to genotypes from the invasive range (Range: $F_{1,4} = 22.5$, $P = 0.001$), although there was also significant variation among genotypes within range ($X^2 = 22.6$, $P < 0.001$, Fig. 1B). Each genotype also displayed different growth allocation responses to different watering levels. Two genotypes had the highest allocation toward belowground biomass at the lowest watering level, but the remaining genotypes had variable responses (Fig 1B).

In the botanical garden experiment, survival was similar to the greenhouse experiment (95%). The effect of the watering treatment was dependent on the rhizobia treatment and the range from which genotypes were collected ($F_{1,396} = 4.68$, $P = 0.031$). Invasive genotypes grown in the high water and enhanced rhizobia treatment produced the most biomass (Fig. 2A). Although native genotypes grew larger with more water, there was no added benefit of enhanced rhizobia (Fig. 2A). However, native genotypes grew larger with enhanced rhizobia in the low water treatment (Fig. 2A). Conversely, invasive genotypes grown in the low water treatment were actually smaller in the enhanced rhizobia treatment, but benefitted from rhizobia in the high water treatment. Beyond the effects of collection range, different genotypes responded differently to watering treatments ($X^2 = 11.6$, $df = 1$, $P < 0.001$). In the

background rhizobia treatment, most genotypes responded negatively to the low water treatment, albeit to different extents, but nine genotypes had positive average responses to the low water treatment (Fig. 3A). However, in the enhanced rhizobia treatment, the rank-order of effects of watering on *M. polymorpha* biomass was quite different (Fig. 3B), reflecting the fact that different genotypes responded to the watering and rhizobia treatments differently.

Biomass allocation to above- and belowground biomass depended on the interaction between watering treatment and plant genotype ($X^2 = 7.37$, $df = 1$, $P = 0.007$). The effects of genotype on changes in biomass allocation with different watering treatments was not significantly related to collection range of those genotypes (Range*Water: $F_{1,41} = 0.43$, $P = 0.516$). The range*water*rhizobia interaction also had no significant effect on the above:belowground biomass ratio ($F_{1,372} = 0.28$, $P = 0.595$). On average though, genotypes collected from the native range allocated more energy toward aboveground biomass than invasive genotypes ($F_{1,41} = 5.98$, $P = 0.019$, Fig. 2B).

In our second experiment, drought had similarly large direct effects on the size of plants from the native and invasive range (Cohen's d : native = 0.43, invasive = 0.45). Rhizobia had much smaller direct effects on plant size, but were twice as high on plants from the native range compared to invasive range plants (native = 0.17, invasive = 0.08). Indirect effects between drought and rhizobia were nearly as strong as direct effects for plants from the invasive range (0.34), but much smaller for plants from the native range (0.04). To contextualize these effects, we also compared these results to other studies of ecological interactions with *M. polymorpha*. Our direct and indirect effects were relatively weak compared to previous studies on this species (Fig. 4). In decreasing order of effect size, mutualism, nutrients, competition, drought, disturbance, and herbivory had greater effects on *M. polymorpha* abundance than those observed in this study. However, consistent with previous studies, effect strengths were dependent on whether genotypes originated from the

native or invasive range. Native range genotypes were affected more by interactions with mutualists, whereas invasive range genotypes were affected more by nutrients, drought, and herbivory. In past studies, indirect effects were smaller in magnitude than direct effects, inconsistent with the results observed in this study.

Discussion

In our two experiments, we found that the effects of water on plant performance were dependent on the presence of rhizobia and the genotype of the plant, suggesting that understanding invasion success by *Medicago polymorpha* will require understanding abiotic, biotic, and evolutionary factors. Water availability affected *M. polymorpha* performance in our greenhouse and botanical garden experiments, suggesting that the ability of this species to invade a particular habitat may depend on the spatial and temporal availability of water. Adding rhizobia to the soil tended to increase plant size, indicating that the presence of mutualists in the soil microbial community may increase the chances of invasion by this legume. However, we found strong indirect effects of water and rhizobia on plant size, meaning that the total effects of water and rhizobia cannot be predicted in isolation of one another. Further, the direct and indirect effects of water and rhizobia on plant size varied widely depending on whether plant genotypes were collected from the native or invasive range of this species; plant genotype altered the direction and magnitude of direct and indirect ecological effects. In this species, predicting invasion into a natural community context will require understanding multiple direct and indirect effects in the local environment, as well as their effects on the specific genetic composition of the invading population.

Direct effects of water

We found that the amount of water provided to plants in both experiments affected plant performance (total biomass), as well as growth allocation to above- and belowground

biomass. In the greenhouse experiment, the effects of watering level were generally non-linear (Fig. 1), suggesting that certain genotypes of *M. polymorpha* may perform best at intermediate water levels. Previous work found that piñon pine (*Pinus edulis*) genotypes originating in dry habitats performed better in drier soils (Mitton & Duran, 2004). Similarly, the genetic differences we observed could be the result of different evolutionary histories. We minimized the effects of recent ecological environments using a common garden generation, suggesting that the observed differences are not the result of phenotypic plasticity or maternal effects, but rather inherited genetic differences. It is possible that the effects of water on our different *M. polymorpha* genotypes may depend on long-term historic soil moisture in the environments in which these genotypes evolved. Unfortunately, we do not have sufficiently precise information about the original collection sites to obtain historic records to test this hypothesis.

Previous studies suggest that plants grown under water stress will allocate more energy toward belowground growth to increase root biomass and acquire more water resources (Matsui & Singh, 2003; Goh et al., 2013; Saeed et al., 2016). Although we did not observe a consistent response to watering across genotypes in the greenhouse experiment, we found that plants in the botanical garden experiment had the opposite response than expected—plants grown in the low water treatment had relatively less root biomass. It is possible that this is the result of an annual life history in plants native to a Mediterranean climate, in which plants must grow as large as possible during a short, wet winter before soils dry in the summer. Drier soils that signal the approach of summer may trigger a reproduction response, rather than a long-term investment in water capture in longer-lived plants. Regardless of the mechanism, this result suggests that allocation to above- or belowground growth is somewhat plastic in this species, although the variation among genotypes suggests that it is also partially genetically determined.

Indirect effects of rhizobia on plant responses to water

Plants grew larger in the enhanced rhizobia treatment, but only under certain water conditions. Although rhizobia may directly enhance plant growth by providing nitrogen, such increased nutrients may only be valuable if no other resources are limiting. The exact mechanism of how rhizobia affect responses to watering treatment are unclear, but previous work demonstrates that bacteria may often affect plant stress responses. For example, the plant traits that rhizobia alter in response to nitrogen limitation, such as changes to lateral root density, root length, and shoot:root ratio (Goh et al., 2013; Kunert et al., 2016), may also alter plant responses to watering by increasing root surface area and the depth to which roots can grow to access water (Goh et al., 2013). Bacteria have also been observed to contribute to the resistance of *Arabidopsis thaliana* and *Zea mays* to abiotic stress by altering plant stress signal transduction (Chen et al., 2017). This can cause plasticity in the production of secondary metabolites, such as reactive oxygen species, which plants use as signals of environmental stress (Bailey-Serres & Mittler, 2006), thereby indirectly increasing resistance to stress (Pandey et al., 2012; Goh et al., 2013). In addition, compounds secreted by some rhizobia increase the expression of plant genes whose products reduce sodium toxicity by sequestering Na⁺ into vacuoles (Chen et al., 2017). Other non-rhizobia bacteria species (e.g. *Pseudomonas aeruginosa*) can increase salt tolerance and increase performance under low water conditions (Pandey et al., 2012).

The interaction between rhizobia and legumes varies along a spectrum from mutualistic to parasitic (Friesen et al., 2011; Jones et al., 2015) and the exact nature of the interaction likely depends on the evolutionary history of the plant, as well as the specific environmental context in which the interaction takes place. Although previous studies have shown that the local availability of nitrogen affects the nature of the plant-rhizobia interaction (Kiers et al., 2007; Oono et al., 2009; Weese et al., 2015), our results suggest that the

availability of water may be important as well. The evolutionary history of the rhizobia may be important as well (Heath & Tiffin, 2007). Although we only enhanced the soil with rhizobia collected locally from a single plant, we do not know the genetic diversity of the rhizobia in this treatment. Although *M. polymorpha* associates almost exclusively with *Ensifer medicae* (Porter et al., 2011), some plants can contain many strains of this rhizobia species (Djedidi et al., 2011). The genetic diversity of bacteria collected from a single plant, is certainly a very conservative subset of that found around the globe (Bromfield et al., 2010).

Selection for traits during invasion

We compared genotypes from the native and invasive range because we assumed that invasive genotypes would represent *M. polymorpha* traits that either facilitate invasion to new habitats around the globe, or are more adaptive in the invasive range relative to traits in the native range. However, because this species has invaded such a wide variety of locations around the globe, the traits that facilitate invasion may vary widely. Despite this expected variation among invasive genotypes, we still found some general trends in trait differences between genotypes collected from the invasive and native range. In both experiments, genotypes from the invasive range had, on average, lower shoot:root ratios. Size is often used as a proxy for competitive ability in invasive species (Blossey & Notzold, 1995; Joshi et al., 2014), and if so, this suggests that belowground competitive ability for resources like water or nutrients increases *M. polymorpha* invasion success. This result is consistent with a previous study that showed greater investment in belowground biomass by invasive *M. polymorpha* genotypes (Getman-Pickering et al., 2018). However, that same study showed that size does not necessarily increase competitive ability against three co-occurring species in invaded California (USA) fields. It is possible that biomass allocation in this species may be in response to other factors besides competition, such as water, searching for rhizobia mutualists, or acquiring nitrogen in the absence of mutualist partners.

The benefits from rhizobia in our experiment varied widely among genotypes (Fig. 3). Although the average effect of rhizobia on plant size was positive, different genotypes experienced various magnitudes of positive and negative effects, depending on the watering treatment. Native genotypes tended to benefit from rhizobia under high water conditions, but invasive genotypes tended to benefit from rhizobia under low water conditions. The mechanism underlying this difference is not yet clear, but the pattern suggests that invasive and native genotypes interact with their environments in different ways. Given sufficient water, invasive genotypes can grow large, even in the absence of partnerships with rhizobia, and generally receive less overall benefit from rhizobia (terHorst et al., 2018). The ability to invade without mutualists may give certain genotypes an advantage over others in novel habitats. Perhaps invasive genotypes increased investment in belowground biomass allows them to obtain sufficient nitrogen in the absence of nitrogen-fixing partners.

Relative importance of direct and indirect effects

Across studies of *M. polymorpha* that measured the effects of different ecological interactions on various aspect of plant performance and fitness, we found that direct effects of mutualism, nutrient level, and competition had the biggest effect on plants. The strong effects of competition are perhaps not surprising, as many studies have demonstrated the importance of competition with native plants on invasive plant species success (Boyden et al., 2005; Jones & Gomulkiewicz, 2012; Bever et al., 2012). Many studies focus on the role of natural enemies in preventing invasion into novel habitats (Levine et al., 2004; Mitchell et al., 2006; Duan et al., 2015) and how escaping natural enemies may allow increased investment in competitive ability (Blossey & Notzold, 1995). Similarly, many studies examine how increased anthropogenic disturbances favor exotic species over native species that may not be adapted to such disturbance regimes (Hobbs & Huenneke, 1992; Seabloom et al., 2003; Thomson & Leishman, 2005; Price et al., 2011). However, the effects of drought or water

availability on invasion success have been considered somewhat less and may stand out as important in this particular species that is historically adapted to a Mediterranean climate, where precipitation and water availability are highly seasonal. We were surprised to see that mutualisms ranked so highly in importance, but the generality of this result should be interpreted cautiously. Because we focused our short review only on this selfing legume species, all of the mutualist studies focused on soil microbes, and not on pollinators that may be especially important for other potential plant invaders. Nevertheless, the availability of mutualists, or lack thereof, has been historically understudied in invasive species and the increased attention such interactions have received in the past decade seems warranted.

Although we did not conduct a formal statistical analysis, in comparing abiotic and biotic direct effects on invasive and native genotypes of *M. polymorpha*, some general patterns emerged. Although mutualists have the strongest effects on this species overall, effects are much stronger on genotypes collected from the native range. Those genotypes collected in the invasive range seem to be relatively less affected by mutualists, suggesting that perhaps the strong effects of mutualists on this species do not play an equally large role in invasion success. In contrast, invasive genotypes are most affected by nutrient levels, drought, and herbivory. If invaders rely less on rhizobia for nitrogen, this may explain the increased importance of soil nitrogen for their success.

In the botanical garden, we found indirect effect sizes due to interactions between mutualists and drought rivaled those of the direct effects of drought, at least for invasive range genotypes. Even in the absence of strong direct effects, abiotic and biotic factors can have important influences on invasion success because of their involvement in indirect effects. For example, terHorst and Lau (2015) found no significant direct effects of herbivores and competitors on *M. polymorpha* performance, but strong indirect effects because herbivores reduced fruit production only in the presence of competitors.

Given the data in our experiment, we would conclude that it is essential to incorporate indirect ecological interactions in order to predict invasion success in natural diverse communities. However, in our review of other studies, indirect ecological effects were almost trivial relative to the strong direct effects observed. These few studies of indirect interactions are not a random sample, but rather come entirely from two lab groups, including ours, and were conducted only in greenhouses or field sites in California. Estimates of direct interactions from our lab group were among the lowest in our review of other studies, suggesting that perhaps our manipulations are less extreme than in other studies. The relatively few estimates of indirect effects and limited scope suggest that further studies are needed to determine the importance of indirect effects on invasion success more broadly. Further, individual indirect effects may be weaker, on average, but the number of potential indirect interactions can greatly outnumber direct interactions in diverse communities, as simple and more complex indirect effects increase exponentially with increasing direct interactions. In other systems, indirect effects have been found to increase (Suwa & Louda, 2012) or decrease (Adams et al., 2003) invasion success in a community context. This suggests that the total effect of any ecological interaction would be under- or overestimated unless measured in the broader community context in which the interaction naturally occurs. In both our system and in others, there are many potential indirect effects on invasion success that have yet to be quantified.

Overall, these findings suggest that the relative magnitudes of direct and indirect effects are context dependent. Though general trends emerge, including the importance of mutualism, nutrient level, and competition, the order and magnitude of these effects are dependent upon the genetic composition of the invading *M. polymorpha* population. Our review of previous studies suggests that indirect are unimportant relative to direct effects in predicting invasion success of this species. However, the relatively large role that indirect

effects play for some genotypes, particularly those that have already successfully invaded other habitats, suggests that more work is needed to determine the role that indirect plays in invasion into complex natural habitats.

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Figure captions

Figure 1. Effects of increasing watering levels on means ($\pm$ SEM) of different genotypes of *M. polymorpha* (A) total biomass and (B) above:belowground biomass in the greenhouse experiment.

Figure 2. Effects of watering levels on (A) total biomass and (B) above:belowground biomass (means $\pm$ SEM) of *M. polymorpha* genotypes collected from the invasive or native range in the botanical garden experiment.

Figure 3. The log response ratio of each genotype (color coded by their collection range) to decreased watering in rank order in the background rhizobia treatment (A). The same order of genotypes along the x-axis is presented in the enhanced rhizobia treatment (B).

Figure 4. Effect size of different direct effects and pooled indirect effects ($\pm$ SEM) on *M. polymorpha* distinguished by native or invasive range.

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B

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806 Figure 1.

Figure 2.

Figure 3.

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843 Figure 4.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementalmaterial.docx](#)