

Patterns of Plant Salinity Adaptation Depend on Interactions with Soil Microbes

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Submitted July 3, 2022; Accepted March 29, 2023; Electronically published July 12, 2023

Online enhancements: supplemental PDF.

ABSTRACT: As plant-microbe interactions are both ubiquitous and critical in shaping plant fitness, patterns of plant adaptation to their local environment may be influenced by these interactions. Identifying the contribution of soil microbes to plant adaptation may provide insight into the evolution of plant traits and their microbial symbioses. To this end, we assessed the contribution of soil microbes to plant salinity adaptation by growing 10 populations of *Bromus tectorum*, collected from habitats differing in their salinity, in the greenhouse under either high-salinity or nonsaline conditions and with or without soil microbial partners. Across two live soil inoculum treatments, we found evidence for adaptation of these populations to their home salinity environment. However, when grown in sterile soils, plants were slightly maladapted to their home salinity environment. As plants were on average more fit in sterile soils, pathogenic microbes may have been significant drivers of plant fitness herein. Consequently, we hypothesized that the plant fitness advantage in their home salinity may have been due to increased plant resistance to pathogenic attack in those salinity environments. Our results highlight that plant-microbe interactions may partially mediate patterns of plant adaptation as well as be important selective agents in plant evolution.

Keywords: evolutionary ecology, local adaptation, microbial ecology, plant-microbe interactions, symbiosis.

Introduction

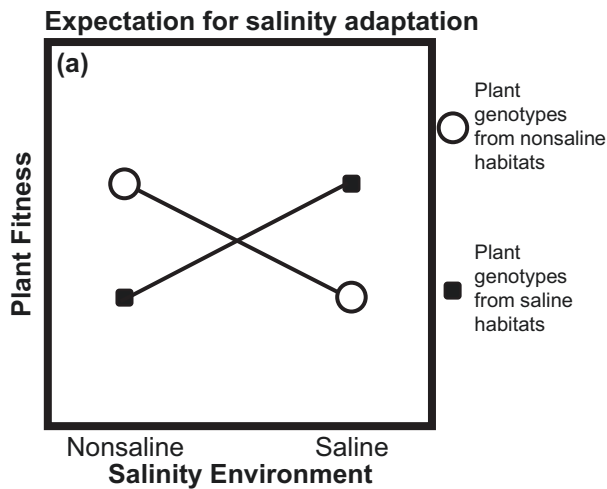
As plants can be widely distributed in space, encountering a variety of environments, it is often expected that different populations may be adapted to their local environments, with plant genotypes having higher relative fitness in their home environments than plant genotypes from foreign en-

vironments (e.g., fig. 1a; Blanquart et al. 2013). While such adaptation to the plant's local environment is relatively common (Hereford 2009), the traits underlying driving this adaptation may be unclear. For example, given that there can often be stark contrasts in the abiotic environments between populations, it may be assumed that these patterns of local adaptation are the result of the abiotic environment selecting on genetically based plant phenotypes that are adaptive to that abiotic environment. However, plants are colonized by large communities of microorganisms, and these microbes—through their various activities—can influence plant fitness and phenotype (Friesen et al. 2011; Fitzpatrick et al. 2019; Trivedi et al. 2020). In addition, plants can provide resources and act as habitats to their associated microbes (Broeckling et al. 2008; Berg and Smalla 2009), leading to potential feedbacks between microbial and plant fitness. Consequently, these interactions may facilitate both plant and microbial partners to act as significant selective agents on the other partner. Therefore, a plant's fitness and traits in a given environment are not necessarily a product of its own evolution alone (Partida-Martínez and Heil 2011) but rather reflect a series of complex ecological and evolutionary interactions between a plant's genome, abiotic environment, and associated microbes. Patterns of plant local adaptation could therefore be influenced and/or driven by these plant-microbe interactions.

Understanding the microbial contribution to these patterns of plant local adaptation will facilitate a better understanding of the evolution and impact of these microbial symbioses on their host's ecology and evolution. While host-microbe interactions are ubiquitous, with all eukaryotic organisms apparently being colonized by complex communities of microbes (Gordon et al. 2013; Simon et al. 2019), the evolutionary context behind many of the interactions is unknown, including whether these interactions are

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Hypothesis: Given a plant's associated microbes may be strong agents of selection on their host through their impact on plant fitness, patterns of plant adaptation may be dependent on interactions with their associated microbes

Prediction: Plants will be adapted to home environment only when provided with live microbes

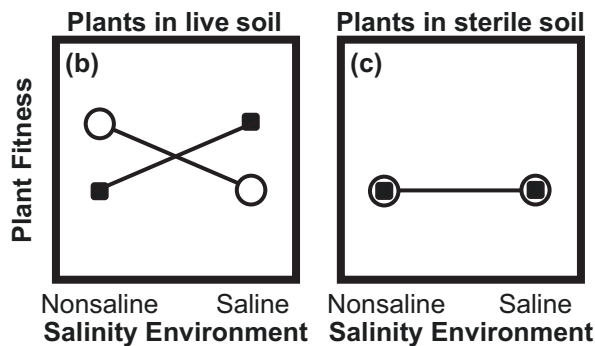


Figure 1: *a*, Expectations for patterns of plant fitness when adapted to its local environment (salinity in this case). *b*, *c*, How adaptation may be influenced by the plant's interactions with its associated microbes.

adaptive or how these interactions may shape the trajectory of each partner's evolution. By partitioning the patterns of plant adaptation to adaptation to the abiotic and biotic components, we may be able to bridge this gap and consequently provide insight into whose traits (the plant's, the microbes', or a combination of both) confer relative fitness benefits to plants between different environments and thus the evolution of and selective pressures on these plant-microbe symbioses.

Plant-microbe interactions could be important in contributing to these patterns of plant local adaptation through a number of ecological- and evolutionary-based mechanisms. For instance, patterns of plant local adaptation could be driven by plants adapting to their abiotic environment through selection on their microbial interactions. Plant-microbe interactions can have a heritable component, determining the relative abundance of specific microbial taxa, as well as those taxa's impact on plant health (Aira et al. 2010; Gehring et al. 2017; Walters et al. 2018; Bergelson et al. 2019). Consequently, there may be selection for specific plant-microbe interactions dependent on the abiotic environment. For example, the mycorrhizal colonization of root systems is a heritable trait (Wang et al. 2010; Patterson et al. 2019; Anthony et al. 2020), and increased mycorrhizal colonization can increase plant drought tolerance (Al-Karaki et al. 2004; Gehring et al. 2017; Liu et al. 2018). Therefore, droughted environments may select for plants with increased association with mycorrhizal fungi, which enhances plant drought tolerance and thus indirectly produces a plant phenotype that indirectly confers a drought advantage even though mycorrhization is the direct phenotype under selection. While this is another form of selection on the plant to the abiotic environment, here the plant depends critically on the presence of microbes to manifest the adapted trait to that abiotic environment. Patterns of plant local adaptation could also be driven by adaptation to their local microbial partners, independent of adaptation to the abiotic environment. Coevolution between plants and their microbial partners could result in plants with a "home field advantage" when interacting with mutualistic microbes that shared their historical habitat. Indeed, some prior work has suggested that a plant's fitness can be maximized when matching plants and microbes from the same habitat, which may be the result of either the microbes or the plants adapting to their respective partner (Johnson et al. 2010; Batstone et al. 2018; but see also Reinhart et al. 2003; van der Putten 2010). Thus, when a plant is grown in its home environment, it is more likely to interact with its historical microbial partners with whom local plant genotypes have recently coevolved, potentially providing a fitness benefit to the plant. These fitness benefits to the plant would be the result of plants adapting to the microbial populations from their local habitat, but in the reciprocal transplant experiments often used in examining local adaptation, this could easily be misattributed to adaptation solely of the plant to the abiotic environment.

Indeed, reciprocal transplant experiments are commonly used to study location adaptation to the abiotic environment, but their use can limit our ability to study the role of microbes in influencing these patterns of plant adaptations. A plant's local soil microbes will generally not accompany it into the transplanted environment, and this

can place these plants in an unrealistic or suboptimal microbial context. Moreover, these experiments rarely include a sterile control, where plants do not interact with any microbes, making it difficult to separate the abiotic and biotic components of plant adaptation. While these experiments inherently capture much of the abiotic variation that is important for plant adaptation, it is harder to manipulate the biotic microbial environment, and this is especially true in common-garden field trials (though see Petipas et al. 2020). To this end, to understand the evolution of these plant-microbe interactions, we examined their contributions to plant adaptation using a greenhouse experiment where the microbial inoculum could be controlled. Specifically, we collected seeds from populations of the grass *Bromus tectorum* from both high- and low-salinity habitats and generated microbial soil inocula from those same habitats. We evaluated the adaptation of these plant populations to their home salinity environments by growing plants in the greenhouse under high-salinity and nonsaline conditions, both with microbes originating from saline and nonsaline environments and in sterilized soils.

Our aim in this contribution was to isolate the interactive biotic and abiotic components of plant adaptation to their home salinity environments. We compared plant salinity adaptation between sterile and live soil conditions, where sterile conditions represent a test of solely the abiotic adaptation, while live conditions represent a test of both abiotic adaptation and biotic adaptation (fig. 1b, 1c). As such, in this approach significant impacts of the interactions between the salinity and microbe treatments on plant fitness across populations would indicate the importance of plant-microbe interactions in influencing plant adaptation through a variety of mechanisms. Moreover, by comparing plant adaptation across several live microbial inocula, with inocula that originated from either a saline environment or a nonsaline environment, we may evaluate the role of specific microbes in influencing plant salinity adaptation—specifically, the importance to plant adaptation of matching microbes from a given abiotic environment with that abiotic environment and with plants from that same abiotic environment (e.g., saline microbes with saline plants in a saline environment).

Material and Methods

Plant Material Collection

Bromus tectorum L. was introduced to North America in the late 1800s, where it subsequently invaded large portions of the American Southwest (Mack 1981; Knapp 1996). It has rapidly adapted to a variety of habitats, including highly saline habitats, such as playas and salt deserts (Leger et al. 2009; Scott et al. 2010; Merrill et al.

2012). Moreover, *B. tectorum* is an annual, cleistogamous plant with minimal outcrossing (Novak and Mack 1993), which are model traits for greenhouse work. Populations of *B. tectorum* from various salinity environments, therefore, presented an ideal natural experiment for examining the contribution of plant-microbe interactions to plant salinity adaptation.

In June 2019 we collected seeds from 10 populations of *B. tectorum* from habitats that varied in their salinity, spread throughout northern Utah (table S1; fig. S1). Of these, five populations were collected from habitats classified as saline, indicated by high-electrical-conductivity soils, and five populations were collected from habitats classified as nonsaline, indicated by low-electrical-conductivity soils, according to measurements at the time of seed collection (fig. S2). While there may be other environmental differences between these populations that contribute to plant adaptation to their local environment, the soil conductivity observed in the saline habitats is toxic to many plants and therefore likely a strong selective agent on the plants. Therefore, we chose to focus on plant adaptation to their home salinity environment as the focal treatment for our experiment.

Two saline populations were collected on a large playa surrounding the Great Salt Lake. The seeds from two additional saline populations were collected from a playa on the south side of Utah Lake. The final saline population was collected on the eastern edge of the Great Salt Lake Desert, the dry lake bed remnant from the Pleistocene-era Lake Bonneville. The five nonsaline populations were collected on mountainsides spread throughout Salt Lake Valley and Utah Valley. The two closest populations are 3 km apart, while the two farthest are 120 km apart. While there could be some concern over gene flow between populations close to each other, prior work shows very little gene flow between *Bromus* populations, even on small scales, because of rare outcrossing events (Novak et al. 1991; Schachner et al. 2008). We therefore assume that each of these populations serves as an independent replicate of populations associated with saline or nonsaline environments.

At the same time that we collected the seed material (June 2019), we also collected soil material at each population to provide inoculum for subsequent greenhouse experiments. We collected soil from the top 15 cm of soil throughout 1 m² at the center of the population using a sterilized trowel. Soils were placed in a plastic bag, returned to the lab, and then stored at 5°C until use in subsequent greenhouse experiments.

From each population, we collected seeds from 10 separate plants, from which we started 10 maternal family lines in a greenhouse environment. Seed was collected from randomly selected plants distributed across approximately 10 m². These plants were grown for a single generation in the greenhouse to equilibrate maternal effects across

populations and produce seeds for subsequent experiments. Briefly, seeds were vernalized for 8 weeks at 5°C to facilitate flowering (Meyer et al. 2004), after which they were sown in small “cone-tainer” pots and filled with approximately 120 mL of a locally produced root wash soil mix: equal parts calcined clay, torpedo sand, and field soil (University of Illinois Plant Care Facility, <https://pcf.aces.illinois.edu/soil-mixes/>). Plants were grown in the greenhouse on a 26°C/24°C day/night schedule and supplemented with 14 h of daily light. Plants began to produce seeds between 12 and 20 weeks after sowing. Upon seed ripening, seeds were collected and incubated at 30°C for an additional 6 weeks to facilitate afterripening (Christensen et al. 1996). *Bromus tectorum* is cleistogamous (self-pollinating) with minimal outcrossing (Novak and Mack 1993), and therefore the seeds collected from each maternal line were likely full-sibs, minimizing genetic variation within families. In total, we generated 100 maternal family lines, with 10 lines per population.

Greenhouse Experiment

We assessed the degree to which each family line was adapted to saline and nonsaline soil conditions, as well as the degree to which microbial interactions influenced this adaptation in a greenhouse-based reciprocal transplant experiment. In July 2020 we grew all *Bromus* lines in the greenhouse under both saline conditions and nonsaline conditions. We also crossed the salinity treatment with three microbe treatments: live microbes using soil inoculum collected from nonsaline populations, live microbes using soil inoculum collected from saline populations, and sterile inoculum. While we designed this experiment with a key comparison being between live and sterile conditions, by including live inocula from both saline and nonsaline habitats, our experiment additionally included a reciprocal microbe transplant component, which was orthogonal to the *Bromus* transplant treatment. This design provided two a priori contrasts allowing us to assess (1) whether there is any microbial role in plant adaptation to salinity by comparing live microbe treatments to sterile soil treatments and (2) whether salinity-matched plant-microbe interactions were necessary for salinity adaptation by determining whether plants paired with microbes that came from their matched salinity environments (e.g., saline plants with saline microbes) responded differently to saline versus nonsaline environments relative to plants with mismatched microbes (e.g., saline plants with nonsaline microbes). This secondary comparison allows us to evaluate whether there are specific microbes that may be facilitating plant adaptation.

We created two live microbial treatments, inoculum from saline habitats and inoculum from nonsaline hab-

itats: these were composite inocula generated by mixing equal parts of the soils from the five populations of the respective salinity habitat. Soils used for the inoculum had been stored at 5°C since collection approximately 12 months earlier. In combination with our sterilized inoculum, this resulted in three distinct microbial treatments, which we treated as a fixed effects factor in our experimental design. The pooling of inoculum does limit the inference scope, as we homogenized site-to-site variability of microbial salinity responses even while we retained replication across plant lines and populations. In particular, we cannot estimate the variance of microbial effects across sites (just across plant lines and plant populations), and we cannot estimate the effects of salinity on microbes. While ideally we would have instead included 10 live inoculum treatments, corresponding to the soils from each of the 10 *Bromus* populations, this would have expanded the experiment beyond the limits of our capacity. Instead, we constructed microbial inocula that were intended to represent microbes that would be found at the two extreme ends of the salinity gradient.

We grew our 100 maternal lines of *Bromus* in the greenhouse, on a 26°C/24°C day/night schedule, supplemented with 14 h of daily light. Plants were grown in cone-tainer pots and filled with approximately 120 mL of a root wash mix (see the previous description for details of soil mix). We sterilized soils by autoclaving (three times in 1-h cycles, with 20-min rests between cycles) shortly before the experiment. We then amended soil 10% by volume with the designated soil inoculum, mixing soils using a sterilized cement mixer. Plants assigned to the sterile treatment received sterilized inoculum at identical volumes to the live treatments (a 1:1 mix of the saline and nonsaline inocula) to control for potential chemical/nutritional differences between treatments. This sterile inoculum was autoclaved and mixed into the designated soils identically as above. For every treatment combination of population, maternal line, salinity, and microbes, there were three replicates, for a total of 1,800 plants (10 populations × 10 maternal lines/population × 2 salinity treatments × 3 microbe treatments × 3 replicates).

Before starting our greenhouse experiment, we surface-sterilized seeds by immersing them in a 2% sodium hypochlorite and 0.05% Tween 80 solution and vigorously stirring for 2 min, followed by a thorough rinsing in sterile water. We then placed seeds onto sterilized moistened filter paper to allow germination. We placed three germinated seeds into each pot, covering them with a thin layer of soil. We randomized the position of pots on the greenhouse bench to minimize environmental variation and regularly rotated their position on the bench. We thinned pots to one individual/pot in the subsequent weeks. We watered pots every 3 days to saturation to maintain soil

moisture. A small portion of the pots across all populations did not have any successful seedlings emerge in the first few weeks (~13% of all pots; fig. S3). We excluded these failed plants from all subsequent analyses.

Four weeks after planting seeds, we began salinity treatments. We continued to water plants assigned to the non-saline treatment every 3 days to saturation. On the same watering schedule, we watered plants assigned to the salinity treatment with a 0.6% NaCl solution for 1 week. After 1 week, we then began watering these same plants instead with a 1.2% NaCl solution for the remainder of the experiment. When watering, we flushed pots with either freshwater or the saline solution (according to their assigned salinity treatment) to minimize the buildup of salts in the soil over the course of the greenhouse experiment. We did not initially start our plants under saline conditions and included this short ramping of soil salinity primarily to avoid stress shocking the plants. Moreover, in this region, early spring is the wet season with frequent rainfall; as the season progresses, rainfall is less frequent, and consequently, osmotic pressure in these saline playa populations increases throughout the season as soil water slowly evaporates, leaving behind residual salts and causing increasing osmotic stress (Scott et al. 2010). Including this ramping of soil salinity thus somewhat mimics natural conditions experienced by the saline populations. We chose the 1.2% NaCl solution as the final concentration for the salinity treatment, as this concentration set soil conductivities at ~6 dS/m. A pilot experiment demonstrated that this concentration imposed significant plant stress without resulting in 100% mortality while also being on the low range of the conductivities observed in the soils of the saline populations (fig. S2). Thus, we created two greenhouse salinity environments that were reflective of these populations' likely home salinity environments. We acknowledge that this experimental design ignores the underlying variation in salinity conditions among these habitats while also ignoring other abiotic or biotic components that may be important to their adaptation. Indeed, by narrowing our test of adaptation to a single abiotic variable, it can be viewed as a conservative test of the microbial impact on plant local adaptation, with significant results potentially suggesting that we captured relevant variation.

We recorded the dates of plants' death throughout the experiment to assess population survival. Ten weeks after the initiation of the experiment, we harvested above- and belowground biomass over the course of 5 days. This timing represented 6 weeks of applying salinity stress out of the total 10 weeks of growth. As our pilot studies showed that some populations of *Bromus* completed their life cycle under greenhouse conditions in as few as 12 weeks, this length of experiment represented a significant portion of the plant's generation time. After harvest, we gently washed

root tissues to remove soil particles. All tissues were oven-dried at 75°C for 72 h and then weighed.

Data Analysis

We used total biomass (composite of above- and belowground biomass) and survival data as measures of plant adaptation to our various greenhouse treatments. We assumed that increasing biomass and survival for a given habitat represents increased fitness of the plant to that environment, as prior work has shown a strong correlation between *Bromus* reproductive fitness and aboveground biomass (Leger et al. 2014; Smull et al. 2019). While belowground biomass is not frequently used as a proxy of plant fitness, some have suggested measuring multiple traits related to plant health to fully encapsulate plant fitness (Mason et al. 2017; Younginger et al. 2017). Moreover, a prior study examining adaptation in *B. tectorum* to saline habitats found the largest evidence for adaptation in changes in root biomass (Scott et al. 2010). We did not measure reproductive fitness, as *Bromus* requires extensive periods of vernalization for flowering (Meyer et al. 2004), which in our pilots resulted in low germination rates (we speculated that this may have been due to mold issues). As we were limited in the number of seeds, we chose not to risk losing all of our seeds in a failed vernalization treatment.

Given that a significant number of our plants under the saline greenhouse treatments died because of the stressful environment, these plants had effectively zero fitness. We did not want to exclude these plants from the analysis, as they represent an important component of adaptation; however, the large number of zeros made model construction difficult. We consequently used the aster model approach of Geyer et al. (2007) to create a single composite estimate of fitness, which unified biomass measures and survival data with their appropriate statistical distribution. We modeled biomass and survival with normal and Bernoulli distributions, respectively. From here on, we will refer to this aster model as our estimated fitness; while none of the traits input into these models are a direct measure of fitness, they are effective proxies (see above).

In our aster model, we included the following as fixed effects: a plant's historical salinity environment (saline or non-saline), greenhouse salinity environment (saline or nonsaline), microbe inoculum treatment (saline microbes, nonsaline microbes, or sterile), and the interaction of these terms. To control for nonindependence among individuals from the same population, we additionally included population (nested within historical habitat) as a random effect, as suggested by Kawecki and Ebert (2004). While we attempted to include maternal line as a random effect, this failed because estimating the variance components both for all of the population effects and for maternal line effects

in aster models was too demanding of the data. Given that the maternal lines within each population were sampled fairly close to each other, they may have been close relatives. Therefore, population may statistically be the most appropriate level of sampling to consider. As a check on our work though, we do additionally include the same models but with only maternal line as a random effect (table S2; we note little difference in these models). We determined the significance of the fixed effects by using likelihood ratio tests, comparing sequentially nested models with and without the term of interest. We constructed this model using the aster package in the R environment (Geyer et al. 2007; Geyer 2021).

Given that the estimated fitness using the aster model was a composite of both survival and the component biomass (above- and belowground biomass), we additionally examined these individual components in an analysis similar to that described above to identify how each of these contributed to salinity adaptation. Specifically, we constructed models similar to those described above for survival and above- and belowground biomass. For biomass, we specifically examined biomass from plants that had not died, as this allowed us to examine whether adaptation was driven by changes in survival and/or biomass. Given that survival is a binary outcome, we accordingly analyzed survival by constructing a generalized linear mixed effects model with a binomial error distribution. Given that survival for plants under the nonsaline treatment was unsurprisingly near 100%, we exclude that treatment from the analysis. For both above- and belowground biomass, we square root transformed biomass to meet assumptions of normality and constructed mixed effects models using the lme4 and lmerTest packages (Bates et al. 2015; Kuznetsova et al. 2015).

Local adaptation to different environments is often evaluated in reciprocal transplant experiments by examining the two-way interaction between an organism's source habitat and the contemporary habitat, in this case the source habitat and the greenhouse salinity treatment (Blanquart et al. 2013). However, to determine whether plant-microbe interactions are important for an adaptive salinity response, we focused on the three-way interaction between the historical salinity environment, greenhouse salinity, and inoculum, as this term would act as an indicator of the role of microbes in influencing adaptation. To further evaluate the sign and the relative impacts of treatments, we used specific contrasts associated with these hypotheses that were chosen a priori, allowing for more powerful analyses than post hoc comparisons. Namely, within each combination of inoculum and greenhouse salinity, we compared our estimated fitness measure (aster model, biomass, and survival) between the two historical salinity habitats (see fig. 1 for predictions and planned contrasts; contrasts are between genotypes within each environment). Salinity adaptation would be

supported if, for a given greenhouse salinity treatment, we observed the highest fitness in plants whose historical salinity environment matched the greenhouse salinity treatment (e.g., saline plants with saline treatment, nonsaline plants with nonsaline treatment). We could then evaluate the importance of microbes to salinity adaptation by comparing patterns of adaptation with these comparisons within each microbial treatment.

While the primary goal of this contribution was to isolate the interactive biotic and abiotic components of plant adaptation to their home salinity environments, we additionally examined whether the identity of the microbial inoculum influenced these patterns of adaptation. To this end, we made additional comparisons with these estimated fitness measures, comparing the impact of matching a plant with its matched inoculum (e.g., saline genotypes with saline inoculum) versus the mismatched inoculum (e.g., saline genotypes with nonsaline inoculum). The inclusion of the sterile microbe treatment was not required for these comparisons.

Results

The three-way interaction between habitat, greenhouse salinity, and inoculum significantly influenced our estimated fitness (aster model using total biomass and survival; table 1; $P < .001$; see also table S2). This significant interaction term suggested that any adaptation to the home salinity environment was significantly influenced by the microbial treatment; however, from this interaction alone, it is not clear how microbes may be influencing adaptation. We therefore used pairwise comparisons to parse this interaction, as described below.

On the basis of the estimated fitness aster model, our pairwise comparisons suggested that plants from saline habitats were better adapted to high-salinity soils than plants from nonsaline habitats but only when they were grown with live microbes (fig. 2). Under saline conditions, saline genotypes had significantly higher relative fitness than nonsaline genotypes when paired with a live microbial inoculum (fig. 2a, 2b; table S3). However, when grown with a sterile inoculum, saline genotypes did not have statistically different fitness estimates from nonsaline genotypes (fig. 2c).

Similarly, our pairwise comparisons suggested that plants from nonsaline habitats were better adapted to nonsaline soils than plants from saline habitats but only when grown with live microbes (fig. 2). Under nonsaline conditions, nonsaline plants had significantly higher relative fitness than saline genotypes when paired with the saline inoculum, though not when paired with the nonsaline inoculum (fig. 2a, 2b; table S3). However, when grown with a sterile inoculum, nonsaline genotypes did

Table 1: Model evaluating drivers of plant adaptation

Model term	df	Estimated fitness (total biomass + survival) deviance	Aboveground		Belowground		Survival χ^2
			MS	<i>F</i>	MS	<i>F</i>	
Habitat source	1	2.20	1.47	.35	10.5	1.53	2.23
Salinity	1	500.08***	160.22	37.55***	3,943.4	579.05***	NA
Inoculum	2	102.28**	65.47	15.35***	1,312.6	192.73***	13.85***
Habitat $\times$ salinity	1	7.20**	28.87	6.76**	99.5	14.62***	NA
Habitat $\times$ inoculum	2	4.98 ⁺	22.39	5.25**	17.3	2.54 ⁺	11.27**
Salinity $\times$ inoculum	2	38.59***	67.06	15.72***	215.5	31.64***	NA
Habitat $\times$ inoculum $\times$ salinity	2	28.62***	26.25	6.15**	175.1	25.72***	NA

Note: Model evaluating the effects of the plant's habitat of origin (saline and nonsaline populations), greenhouse salinity treatment (saline and nonsaline), the inoculum provided (saline microbes, nonsaline microbes, and sterile), and their interactions on the estimated fitness (total biomass and survival, using aster models), with population as a random effect. An analogous model using maternal line as a random effect can be seen in table S2. Separate models were also built for the components going into this measure of estimated fitness, including above- and belowground biomass and survival. For survival, terms including the salinity treatment were excluded, as there was no variance in survival under nonsaline treatments (near 100% survival). For estimated fitness, we display the deviance from the aster model for each model term. For above- and belowground biomass models, we display both the *F* value and the mean square (MS) for each model term. For survival, we display the χ^2 value for each model term. Terms with an associated *P* value less than .05 are in bold.

⁺ $P \leq .10$.

^{**} $P \leq .01$.

^{***} $P \leq .001$.

not have statistically different fitness estimates from saline genotypes (fig. 2c).

Similar patterns of salinity matching in the presence of microbes could be found in both the survival data and the component biomass data. For example, under saline conditions, saline genotypes had both higher survival rates and higher above- and belowground biomass than nonsaline genotypes, but only when paired with a live microbial inoculum (figs. S4, S5; table S3). Under nonsaline conditions, nonsaline genotypes had significantly higher belowground, but not aboveground, biomass than saline genotypes, but only when paired with a live microbial inoculum.

We found minimal effect of matching a plant with its salinity-matched inoculum on our estimated fitness aster measure (e.g., saline genotypes with saline inoculum; fig. 3). In one case, we found that nonsaline genotypes had significantly higher estimated fitness when paired with their mismatched microbes (nonsaline microbes); however, this was only when they were in a foreign salinity environment (saline). Conversely, we found that saline genotypes had significantly lower estimated fitness when paired with their matched microbes (saline microbes) than when paired with their mismatched microbes (nonsaline microbes) while under saline conditions. All other comparisons had non-significant impacts on estimated plant fitness.

Discussion

Overall, our results suggest that the apparent adaptation of *Bromus tectorum* populations to their historical salinity environment was significantly influenced by interactions with soil microbes, but those interactions did not depend on

matching live microbes with their historical salinity environment. Specifically, under saline conditions with any live inoculum, *Bromus* genotypes from saline habitats had higher biomass and survival than genotypes from nonsaline habitats, while under nonsaline conditions with any live inoculum, genotypes from nonsaline habitats had higher biomass than genotypes from saline habitats. This result is consistent with the expectation that populations should be locally adapted to their home salinity environment (fig. 1a; i.e., higher relative fitness in their home habitat than foreign genotypes). However, under sterile soil conditions, these patterns disappeared. Moreover, while the fitness of nonsaline plants and saline plants did not statistically differ across salinity treatments under both saline and nonsaline environments in these sterile soils, plants appeared maladapted to their home salinity conditions, with plants trending toward having higher relative fitness estimates and biomass in their foreign salinity environment (figs. 2c, S5C, S5F). Taken together, these results suggest that the observed patterns of plant salinity adaptation may have been partially influenced by plant interactions with soil microbes. However, on average, plants had higher fitness in sterile soils than in soils with live microbes, which may imply that microbial interactions may have been a net negative for *Bromus* and significant drivers of the fitness patterns.

Through what mechanisms are these plant-microbe interactions influencing plant salinity adaptation? Prior work in resource mutualisms, such as rhizobia and mycorrhizal symbioses, has shown that plants may have the highest fitness when paired with microbial genotypes that have a shared evolutionary history (Johnson et al. 2010; Batstone et al. 2020). In adapting specifically to

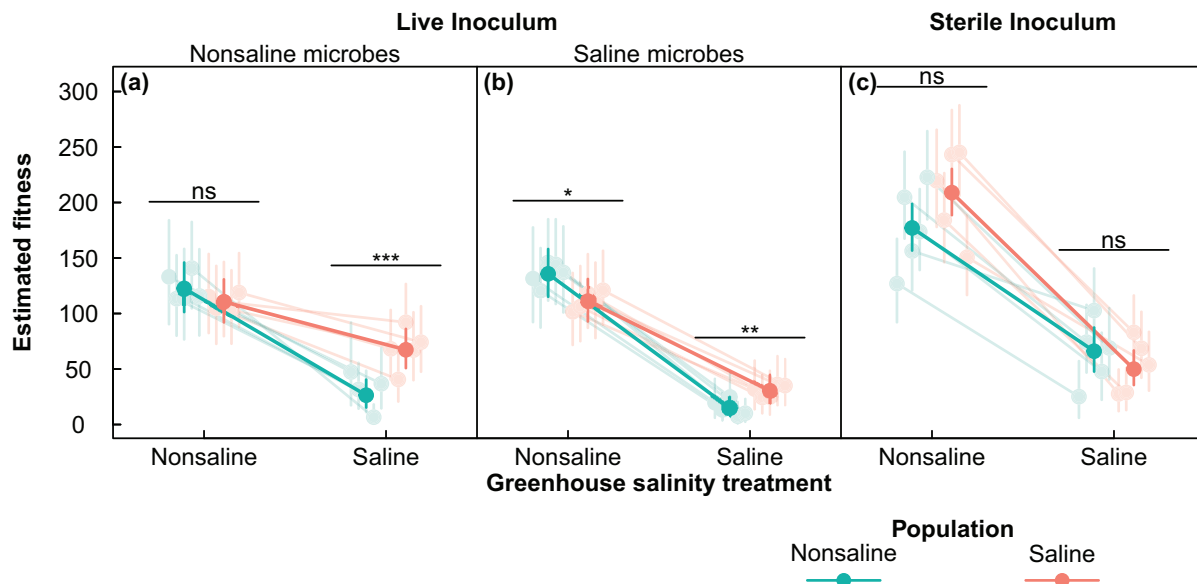


Figure 2: Estimated fitness from our greenhouse experiment, using aster models to combine total biomass (mg) and survival data into one composite fitness measure. Data are grouped by the plant's habitat of origin (saline and nonsaline populations), greenhouse salinity treatment (saline and nonsaline), and the inoculum provided (saline microbes, nonsaline microbes, and sterile). We display data for both the individual populations and the average of populations based on their habitat of origin. Individual populations are semitransparent, while averages of habitat are solid. We chose specific orthogonal contrasts a priori to evaluate salinity adaptation; namely, we compare the habitat of origin under each salinity and microbial combination, as indicated in the figure, as is appropriate to evaluate local adaptation. Bars represent 95% confidence intervals of the mean generated from the standard error. The statistical significance of each comparison is indicated. * $P \leq .05$; ** $P \leq .01$; *** $P \leq .001$.

their biotic environment, such coevolutionary dynamics between plants and their associated microbes could generate patterns expected in local adaptation (fig. 1a). While this experiment was not necessarily designed to address this hypothesis, we can conduct a post hoc examination to determine whether there is support for it by comparing plant fitness across the two microbial inocula. For plant-microbe coevolution to drive salinity adaptation, we should have found plants having the highest relative fitness in their home salinity environment when grown with microbial inoculum that was matched to the historical salinity environment, where plant and microbial genotypes would have been most likely to share an evolutionary history. However, there is no support that these coevolutionary dynamics facilitated the observed microbial benefit in these plants' home salinity environment, as overall salinity-matched inocula were not better than mismatched inocula (fig. 3). In one case, there was a small positive benefit to plant fitness when matching nonsaline plants with their home inoculum; however, this was in a salinity treatment foreign to the plant. In another case, saline plants were more fit in foreign inoculum, which could be due to the release from one's own specialized pathogens (i.e., enemy release hypothesis; Liu and Stiling 2006) or pathogens from saline environments being adapted to saline conditions. It may be the case

that our pooling of soil inocula from multiple sites with the same salinity conditions could have obscured any signal of coevolution by disrupting particular plant-microbe interactions between site-specific combinations of plants and microbes. However, given that we observed patterns of plant salinity adaptation across both live microbial treatments and our inability to detect a salinity-matching effect, this likely indicates that the fitness patterns we found here resulted from generalized plant interactions with microbes functionally redundant across both live microbial communities.

We therefore speculate that these patterns of the salinity adaptation in *Bromus* may be generated by plant immune responses adapted to their home salinity environments to broadly available antagonistic/pathogenic microbial activities. We have noted that plants grown under sterile conditions on average had higher survival and biomass than plants inoculated with live soils, suggesting that the soil may harbor microbes that are detrimental to *Bromus* or that *Bromus* is particularly susceptible to pathogen pressure. Moreover, negative microbial effects were always more severe when plants were grown in their nonnative salinity environment, while release from microbial interactions in sterile soil resulted in similar fitness measures between saline- and nonsaline-adapted populations. We suggest that the observed patterns of *Bromus* local adaptation may be

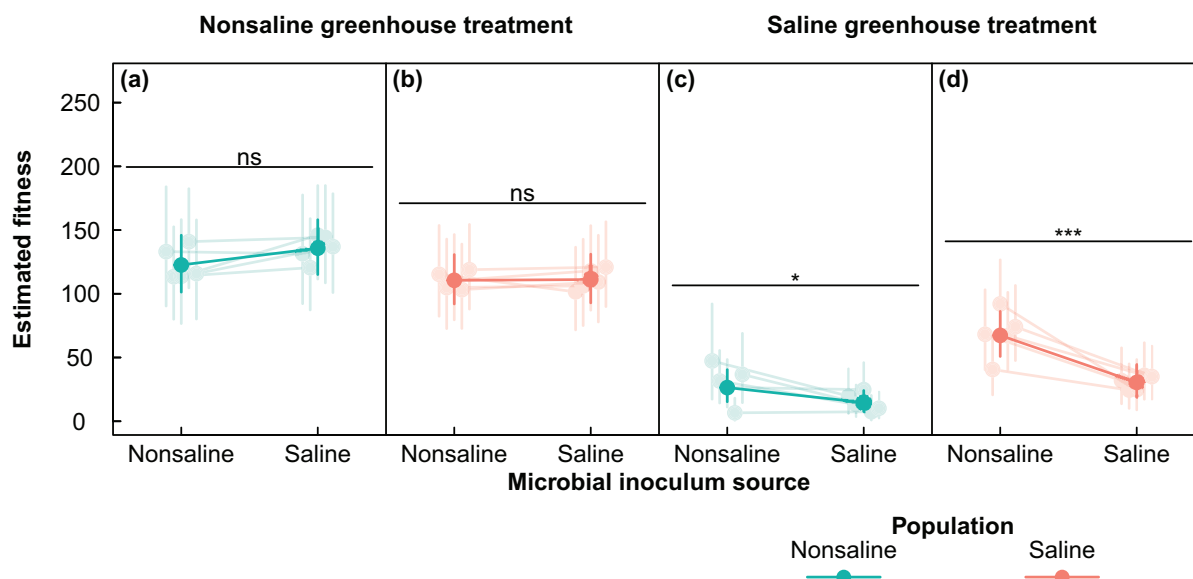


Figure 3: Estimated fitness from our greenhouse experiment, using aster models to combine total biomass (mg) and survival data into one composite fitness measure. Data are grouped by the plant's habitat of origin (saline and nonsaline populations), greenhouse salinity treatment (saline and nonsaline), and the inoculum provided (saline microbes and nonsaline microbes). We note that this figure utilizes the same data from figure 2, except that the panels display different treatment pairs and the sterile treatment is excluded. Specifically, we wanted to assess the impact of matching a plant population with its home inoculum. For example, for saline populations the saline microbes are the home inoculum, while for the nonsaline populations the nonsaline microbes are the home inoculum. We display data for both the individual populations and the average of populations based on their habitat of origin. Individual populations are semitransparent, while averages of habitat are solid. Bars represent 95% confidence intervals of the mean generated from the standard error. The statistical significance of each comparison is indicated. * $P \leq .05$; *** $P \leq .001$.

driven by the fact that plant fitness may be colimited by salinity and pathogen pressure. While it may seem counterintuitive that net negative microbial effects could cause a plant population to appear to be better adapted to its home salinity environment, these plants have evolved in the presence of microbes (vs. under sterile conditions), so plant genotypes that had less detrimental interactions with the soil microbiota would have a competitive advantage that could be selected on, even if microbial interactions were still overall negative. For example, in foreign environments an organism's immune system may be weakened and less able to fend off pathogenic microbes compared with when it is in its home environment (Karl et al. 2010), exacerbating the stress of the foreign environment (David et al. 2018). Consequently, if there are widely distributed generalist pathogens of *Bromus*, plants may appear locally adapted to their home salinity environment as a result of immune systems optimized to these salinities. Similarly, given that there may also be overlapping molecular mechanisms in plants responsible for tolerance to both osmotic stress and pathogens (Asselbergh et al. 2008; Ranty et al. 2016), plant adaptation for tolerance to pathogens or saline environments may incidentally facilitate adaptation to the other. Overall, under this pathogen-driven hypothesis, microbes are not necessarily mediating adaptation to these salinity en-

vironments. Rather, their presence alters the relative fitness difference of plants in a comparison of local versus transplanted genotypes that produce patterns identical to what is expected for salinity adaptation.

There have unfortunately been few studies investigating the *Bromus* rhizosphere communities, so it is difficult to determine whether pathogenic interactions are common in this species and therefore likely drivers of our observed patterns of fitness. While there have been several specialized pathogens identified associated with *Bromus* (Meyer et al. 2016), this is not necessarily indicative of it being particularly susceptible to pathogens, as these were identified for the potential application as a biocontrol of invasive *Bromus*. Future work may therefore attempt to connect specific plant-associated microbial communities with their impact on plant adaptation.

While we have thus far focused on how negative and pathogenic interactions may be driving these patterns of plant adaptation, we acknowledge that these patterns could alternatively be the result of beneficial microbial interactions lurking in this net negative effect. These hidden beneficial interactions could underlie the reduced antagonism we found when plants were grown in their home salinity condition. Such hidden beneficial interactions could emerge as the product of selection on heritable plant traits that facilitate

beneficial interactions under a high- or low-salinity condition (Aira et al. 2010; Gehring et al. 2017; Walters et al. 2018; Bergelson et al. 2019).

Without detailed microbiome-level descriptions of microbial communities, it is not possible to pinpoint specific microbial taxa, whether with positive or negative impacts on the plant, that may be influencing our measures of plant fitness or how these contribute to patterns of plant salinity adaptation. We have estimated the net effects of the microbial inocula in our experiment, and a more detailed investigation of the resultant microbiomes could identify species-specific effects. Similarly, without plant genomic data, we cannot pinpoint particular plant genes responsible for these effects; even with such genomic data though, it would likely be difficult to identify potentially relevant genes, as there would likely be a suite of genomic changes in these populations not associated with the plant's microbial interactions. This level of detail for both plant genomics and soil microbiome is outside the scope of this study. Nevertheless, the broad patterns of plant responses across the salinity and microbial conditions do suggest several directions for future mechanism-focused research.

We briefly note that there is significant variation in soil conductivity between our saline populations (fig. S2). If these plants are adapting to saline environments, we might expect those populations from the higher conductivities to have the highest fitness. While under saline greenhouse conditions and with live microbes, there broadly was higher fitness in the saline-adapted populations compared with the nonsaline populations, and variation in these saline populations' fitness was not related to their home soil conductivity (fig. S6). This may be a result of sampling design, as we had only five populations from the saline habitats, limiting inference.

Conclusion

This work highlights that host-microbe interactions may be key in shaping the patterns of plant adaptation. Similarly, several recent studies have provided results similar to our own, wherein host populations were only locally adapted to their home environment when paired with live microbes (Gehring et al. 2017; Macke et al. 2017; Henry et al. 2020). While our results cannot point to an exact mechanism, they, as well as those of others, emphasize the significant impact of microbes on their host's fitness, likely acting as a significant selective agent on their host, and overall suggest that these host-microbe interactions may play a role in shaping the observed patterns of host adaptation to their local environment. Indeed, many traits long associated with the host, including development, morphology, and physiology, are being linked with the host's associated microbes (Friesen et al. 2011; Theis et al. 2016; Haag 2018). Host-microbe inter-

actions may be altering host evolutionary potential (Henry et al. 2021) by acting as significant targets for natural selection and as the traits underlying host adaptation. We call for further integrating the microbial component into the host's traits and selective environment, as such work will lead to further insights into the drivers of the evolution of host-microbe symbioses as well as their adaptation.

Acknowledgments

This research is a contribution of the Genomics and Eco-evolution of Multi-Scale Symbioses (GEMS) Biology Integration Institute, funded by the National Science Foundation Division of Biological Infrastructure Integration Institutes Program award 2022049. It was additionally supported by the Cooperative State Research, Education, and Extension Service, US Department of Agriculture, under project ILLU 875–952, as well as by the School of Integrative Biology and the Graduate College at the University of Illinois Urbana-Champaign. We additionally thank Sierra Pérez for numerous comments on the manuscript.

Statement of Authorship

K.D.R. and A.C.Y. designed the study, with input from N.J.R.; N.J.R. collected plant and soil samples; K.D.R. conducted the greenhouse experiments, carried out the statistical analysis, and wrote the first draft of the manuscript; all authors contributed to revising the manuscript.

Data and Code Availability

The data supporting this contribution have been archived in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.4f4qrfdq>; Ricks et al. 2023).

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Associate Editor: Rachel B. Spigler
Editor: Daniel I. Bolnick



“View on Caroline Island.” From the review of *Memoirs of the National Academy of Sciences* (*The American Naturalist*, 1885, 19:780–781).