Host-specific soil microbes contribute to habitat restriction of closely related oaks (*Quercus* spp.)

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Abstract

Habitat divergence among close relatives is a common theme in ecology. While recent studies have frequently found that the abundance and diversity of plant species are regulated by soil microbes, little is known whether soil microbes can also affect the habitat distributions of plants. To fill in this knowledge gap, we investigated whether interactions with soil microbes restrict habitat distributions of closely related oaks (*Quercus* spp.) in eastern North America. We performed a soil inoculum experiment using two pairs of sister species that show habitat divergence: *Quercus alba* (local species) vs. *Q. michauxii* (foreign), and *Q. shumardii* (local) vs. *Q. acerifolia* (foreign). To test whether host-specific soil microbes are responsible for habitat restriction, we investigated the impact of local sister live soil (containing soil microbes associated with local sister species) on the survival and growth of local and foreign species. Secondly, to test whether habitat-specific soil microbes are responsible for habitat restriction, we also examined the effect of local habitat live soil (containing soil microbes within local sister's habitats, but not directly associated with roots of local sister species) on the seedlings of local and foreign species. We found that local sister live soil decreased the survival and biomass of foreign species' seedlings while increased those of local species, which supports the roles of host-specific microbes in mediating habitat exclusion. In contrast, local habitat live soil did not differentially affect the survival or biomass of the local vs. foreign sister species, providing no support for the roles of habitat-specific microbes. Our study indicates that soil microbes associated with one sister species can suppress the recruitment of the other host species, contributing to habitat partitioning of the closely related oaks. Our findings emphasize that considering the complex interactions with soil microbes is essential for understanding habitat distributions of closely related plants.

Keywords:

Habitat distributions, Habitat divergence, Host specificity, Plant–soil (below-ground) interactions, *Quercus*, Soil microbes

1 | INTRODUCTION

Understanding the mechanisms underlying species habitat distributions has been a long-standing issue in ecology, biogeography, and evolution (MacArthur 1972, Rabinowitz 1981, Bazzaz 1991, Sexton et al. 2017). Habitat specialization among closely related species is frequently observed in a wide range of taxa, especially in species-rich clades, such as monkeyflowers (*Mimulus*), oaks (*Quercus*), and silver-sword (*Argyroxiphium*, *Dubautia* and *Wilkesia*) (Cavender-Bares et al. 2004, Sobel 2014, Blonder et al. 2015). Traditionally and intuitively, researchers associate abiotic variables, such as resource levels, microclimates, soil conditions, and light intensity, to divergent habitat distributions among close relatives. On the other hand, biotic interactions can also limit geographic distributions of a host species. Particularly, the roles of seed predators, herbivores, and soil microbes on species distributions is an active area of research (Gaston 2009, McCarthy-Neumann and Ibáñez 2012, Alexandre et al. 2018, Benning and Moeller 2020). However, most of these studies have not linked these biotic interactions among close relatives with habitat restriction, *i.e.*, limited occurrence of a species to certain habitat(s) within its geographic range. More empirical evidence is needed to answer the question: how do biotic interactions restrict habitat distributions and promote habitat partitioning among closely related species?

Recent research has found that biotic interactions can mediate habitat exclusion among closely related plant species. For example, in multiple plant taxa in Amazonian rainforests, herbivores drive clay-soil specialist plants to occur only in clay-soil forests because of their low tolerance to herbivory in white-sand forests, while their close relatives, white-sand specialists, withstand the intensive herbivory better and remain occupying white-sand forests (Fine et al. 2004, Fine et al. 2013). Consistently, numerous studies have found that herbivores limit plant distributions by restricting hosts to a smaller subset of habitats within their physiological tolerance, and consequently, the specialization to marginal habitats helps the disadvantaged host escape from intensive herbivory that otherwise they would have encountered in the primary habitat of the other host (Parker and Root 1981, Rand 2003, Pizano et al. 2011, Benning et al. 2019).

The roles of soil microbes in regulating plant species abundance and diversity are coming to the surface in recent years (Comita et al. 2010, LaManna et al. 2017, Marden et al. 2017).

Theoretically, microbial communities can also mediate mutual exclusion of habitats and range distributions of plants (Bever et al. 2015, Holt and Bonsall 2017), given that they can be transmitted and infect among closely related hosts in a similar fashion as generalist herbivores. Most examples supporting this hypothesis involve the introduction of exotic species that are carriers of novel pathogens, which decrease populations of native close relatives (Tompkins et al. 2000, Paillet 2002, Tompkins et al. 2003, Engelkes et al. 2008). One text-book example is that of the introduced Japanese Chestnut (*Castanea crenata*), which transmitted a canker fungus, *Cryphonectria parasitica*, and devastated populations of the native American Chestnut (*Castanea dentata*) in eastern North America (Rhoades and Park 2001). Limited evidence suggests that soil microbes from native species can constrain distributions of native close relatives as well. For instance, range-restricted plant species typically are more susceptible to soil negative feedback when grown in the live soil from closely related species, while widespread species are much less affected by this feedback from native close relatives (Liu et al. 2012, Kempel et al. 2018). These results suggest that habitat specialists might be suppressed by soil microbes from the widespread congeneric relatives. Other studies found that habitat segregation among closely related species is caused by local adaptation to arbuscular mycorrhizal fungi found in their own soil habitats: transplanted ecotypes/species show poorer performance due to maladaptation to the fungal communities in a novel habitat, making them less competitive compared to the local host (Pizano et al. 2011, Osborne et al. 2018). While hinted, these studies have not directly tested whether and how soil microbes contribute to habitat restriction among closely related hosts.

To fully reveal the roles of soil microbes in habitat restriction of host plants, two distinctive mechanisms should be considered. The first mechanism is that soil microbes associated with one host plant exclude the other host species from invading its habitat. This mechanism assumes that the composition and functions of soil microbes are host-specific, even among closely related plants. Additionally, this mechanism suggests that soil microbes associated with one species might be harmless or beneficial to the coevolved host, while they are parasitic and harmful to the novel host. Indeed, a host tree effect on soil microbial communities has been found in congeneric species (Morris et al. 2008, Morris et al. 2009), yet it is unknown whether the differences in the association with soil microbes would translate to habitat exclusion of close relatives. The second mechanism is that soil microbes associated with the local habitat

of one host species exclude the other host from expanding to the new habitat. This mechanism assumes that soil microbial communities are habitat-specific, and that host plants are negatively affected by cross-habitat soil microbes. Supporting this assumption, previous literature reports that soil microbial communities vary with habitat types (Yang et al. 2016, Wang et al. 2021); additionally, transplanted host plants are negatively affected by soil microbes of novel habitats (Pizano et al. 2011, Osborne et al. 2018). While the first mechanism emphasizes host-specific composition and function of soil microbes, the second one emphasizes habitat specificity. Noticeably, these two mechanisms are not mutually exclusive: the habitat limits of host species might be reinforced by both mechanisms.

We suggest that these two mechanisms can be vigorously tested using habitat-divergent sister species in a soil inoculum experiment, as explained below (Fig. 1). If soil microbes directly associated with sister species limit habitat distribution (the host-specificity mechanism), one would predict that live soil associated with one sister species (Fig. 1; hereafter "local sister live soil") should decrease the fitness of foreign sister's seedlings from a different habitat. This is because soil pathogens from the local sister can be parasitic to the foreign sister, and/or foreign sister is inherently more susceptible to local sister's pathogens. In addition, live soil associated with local sister should support higher fitness of its own seedlings due to specialized soil mutualists and higher tolerance of local sister to its own pathogens (Fig. 1b; Prediction 1). Thus, we would expect a strong interaction effect between host habitat origin (local sister vs. foreign sister) and the soil treatments (local sister live soil vs. sterilized soil). Sterilization of local soil would cancel both of these effects. A lack of interaction effect, or an interaction effect opposite to the predicted direction, would lead us to reject the host-specificity mechanism. Similarly, we can test the habitat-specificity effect (Fig. 1c; Prediction 2): if cross-habitat soil microbes constrain habitat distribution, one would predict that general microbes from local sister's native habitat (which are not directly associated with the roots of local species, hereafter "local habitat live soil") should decrease the fitness of foreign sister's seedlings, while increasing the fitness of the local sister species. By experimenting with two different types of live soils, namely local sister live soil and local habitat live soil, we can distinguish the contributions of these two mechanisms in maintaining habitat partitioning of host plants.

In this study, we used two sister-species pairs of oaks (*Quercus* spp.) in a soil inoculum experiment to test the role of soil microbes in constraining species habitats. By testing the host-specificity vs. habitat-specificity mechanisms, we revealed the biological processes of microbe-mediated habitat restriction. This study has practical implications for planning conservation of native habitat specialists, since rare species conservation requires us to understand how local biotic interactions affect population dynamics (DeCesare et al. 2009, Recart et al. 2012, Flores-Tolentino et al. 2020).

2 | METHODS

2.1 | Study system

We used two oak sister-species pairs (*Q. alba*-*Q. michauxii*, *Q. shumardii*-*Q. acerifolia*) in the soil inoculum experiment (Fig. S1). In the sister pair *Q. alba*-*Q. michauxii*, *Q. alba* grows on dry upland slopes to well-drained loam and is widely distributed throughout the eastern U.S., while *Q. michauxii* is adapted to wet bottomlands and is abundant in the southeastern U.S. (Stein et al. 2003). In the sister pair *Q. shumardii*-*Q. acerifolia*, *Q. shumardii* is restricted to well-drained soils along streams and rivers and is widely distributed in the southeastern U.S. (Stein et al. 2003). In contrast, *Q. acerifolia* is adapted to xeric soils on mountain ridges and occurs at only four known locations where *Q. shumardii* has not been found in close proximity (pers. obs. by the first author and communications with knowledgeable local botanists; Fig. S1d). A recent genomic analysis by Hipp et al. (2018) confirmed their sister-species relationships. Hereafter, we use the term "foreign sister" for *Q. michauxii* and *Q. acerifolia*, in relation to our experimental sites within or close to St. Louis, MO (38.64°N, 90.24°W), which are beyond the natural habitats of these two species (Fig. S1). In contrast, we use the term "local sister" for *Q. alba*, *Q. shumardii*.

Oak species encounter many taxa of soil pathogens, including soil fungi (Rizzo et al. 2002, Balci et al. 2007, Haavik et al. 2015), root-parasitic nematodes (Maboreke et al. 2016), and ectomycorrhizal fungi that occasionally turn parasitic depending on external environments and host species (Johnson et al. 1997, Ibáñez and McCarthy-Neumann 2016, Nash et al. 2020). Despite the high diversity of soil pathogens, previous research found positive conspecific soil feedback in oaks (McCarthy-Neumann and Ibáñez 2012, Bennett et al. 2017), providing support

for our Prediction 1 that seedlings of local sister grow better in conspecific live soil (Fig. 1b). For our study species, we did not directly test the underlying assumption that soil microbes can be transmitted among sister species, but this assumption is probably true because phylogenetically related host plants share similar root-associated pathogens (Liu et al. 2012, Schroeder et al. 2019).

2.2 | Acorn collection

Acorns were collected from early October to early November 2018 from the Shaw Nature Reserve (Gray Summit, MO; 38.48°N, 90.82°W), the Missouri Botanical Garden (St. Louis, MO; 38.61°N, 90.26°W), and the campus of University of Missouri–St. Louis (St. Louis, MO; 38.71°N, 90.31°W), depending on the availability of each species' acorns at each location. Specifically, for foreign sister species, we collected acorns from two mature trees of *Q. michauxii* in the Missouri Botanical Garden, and from one mature tree of *Q. acerifolia* in the Shaw Nature Reserve (see Note S1 for provenance). For local sister species, we collected acorns from two trees per species. To ensure that seed source and maternal effects (Fort et al. 2021) did not confound the treatment effect, we used the same seed source composition for each treatment within the same species.

We selected healthy acorns by visually inspecting and excluding acorns with damages, and then used float tests to further exclude floating acorns that are non-viable (Morina et al. 2017); only the healthy "sinkers" were kept and stored in bags with moist and sterilized sphagnum moss at 4°C for stratification. All seeds were stratified until early April 2019, when most acorns showed radicals. We only used acorns with radicals for the experiment, since acorns that did not show radicals were likely non-viable.

2.3 | Soil inoculum experiment

We set up a soil inoculum experiment in a climate-controlled greenhouse at the University of Missouri–St. Louis from April to August 2019. Deep tree pots (10.16 cm diameter, 35.56 cm depth) were cleaned carefully using 10% bleach before the experiment. We used commercial soil (Berger BM7 35% Bark HP; Berger Company, QC, Canada) for the background soil, which

made up 90% of the soil in all the pots; this ensured that the nutrition levels and soil structure in all pots were consistent. This background soil was sterilized in an autoclave twice with a 24-hr interval, at 121°C for 75 min each time; double sterilization prevents growth of any heat-resistant strains.

Two types of live soil were collected from two natural forests: the Shaw Nature Reserve and the Tyson Research Center (Eureka, MO; 38.53°N, 90.56°W), in late March 2019. The first type of live soil was associated with the mother trees of local sister (corresponding to green dashed circles in Fig. 1a), representing the local sister live soil. We collected the live soil from the bases of two mature trees from each of the local species, *Q. alba* and *Q. shumardii*, from three locations within the Shaw Nature Reserve. We collected the soil in cores of 20 cm depth and 10 cm radius, at three points 1—1.5 m distant from the tree trunk. Thus, local sister live soil consisted of live soil from three trees for each sister pair. The live soils were mixed within the host species to allow maximum statistical power in the experiment, especially when sampling intensity of soils is low in our study (Cahill Jr et al. 2017). While we are aware of the debate regarding issues of soil sample pooling (Reinhart and Rinella 2016, Rinella and Reinhart 2019), a recent meta-analysis found no evidence that soil sample pooling systematically biases estimates of plant–soil feedback direction, magnitude, or variance (Allen et al. 2021).

The second type of inoculum was live soil containing general microbes that the foreign oak species have not encountered whereas the local sister have encountered in their own habitats (corresponding to the brown dashed circles in Fig. 1a), representing the local habitat live soil. This live soil was randomly collected from 10 locations within 1—1.5m from the base of other tree species (listed in Note S2) within the Tyson Research Center, and the samples were then combined into a soil mixture.

We set up four soil treatments in the greenhouse. 1) Sterilized soil, which included 10% sterilized general local soil in addition to the 90% sterilized background soil. 2) Local sister live soil (green circles in Fig. 1a), which included 10% live soil from the mother trees of the local species *Q. alba* (for pots containing seeds of *Q. alba* and *Q. michauxii*), or from the mother trees of the other local species *Q. shumardii* (for pots containing seeds of *Q. shumardii* and *Q. acerifolia*). 3) Local habitat live soil (brown circles in Fig. 1a), which included 10% local habitat live soil collected from the base of other host plants. 4) Local habitat live soil plus fungicide

treatment, which had the same soil mixture as treatment 3), to which we applied Ridomil Gold MZ WG fungicide (Syngenta Crop Protection, Greensboro, NC) on the soil surface every two weeks following manufactures' instructions. This fungicide, generally used to eliminate soil pathogens, has reportedly limited effects on ectomycorrhizal fungi (Bell et al. 2006, Norghauer et al. 2010, Maron et al. 2011). We applied this fungicide to examine whether the elimination of soil pathogens from local habitat live soil had an impact on the seedlings; specifically, if we found a significant increase in performance of foreign sister under treatment 4) compared to soil treatment 3), it would suggest that soil pathogens from local habitat live soil can effectively suppress foreign sister, lending support to Prediction 2.

Live soils were added to the pots within four days after field collection. These soil mixtures were manually homogenized before potting. To minimize soil splashing across pots, we filled soils only to 30.5 cm deep for all tree pots (35.56 cm-deep pots). Each soil treatment mentioned above had 10 replicates (pots) per species, resulting in a total of 160 pots in the greenhouse. In each pot, one viable acorn was planted immediately beneath the soil surface. Seed source, seed length, and seed width were documented for each pot to statistically control for potential effects of mother tree and seed size on seedling survival and growth (Bonfil 1998, Shi et al. 2019). In our experiment, seed size was not differentiated among soil treatments nor host habitat origin ($P > 0.80$); thus, it should not confound the main effect of soil treatments or habitat origin. Pots were randomly distributed within the greenhouse so that spatial variation of environmental variables did not confound experimental results. Pots were spaced at least 15 cm apart to minimize cross-over of soil microbes. We watered the pots every five to six days with a water hose serving one pot at a time to avoid soil splashing. A shade cloth with 40% light penetration was hung in the greenhouse to mimic the light environment within natural forests.

Seedling survival, height, diameter of the widest aboveground part, and leaf number were recorded in August 2019. At the end of August 2019, we harvested surviving seedlings to measure the aboveground biomass and belowground biomass. Aboveground biomass was measured as the seedling dry weight above the emergence point from the acorn. Roots were carefully separated from soil and were washed to remove all attached soil particles, and the belowground biomass was measured as the dry weight of the roots. Total biomass was the sum of the above and belowground biomass.

2.4 | Data analyses

To test the effects of soil microbes on seedling survival and growth, we first fitted full models for separate response variable using maximum-likelihood models as implemented in the R package *lme4* (Bates et al. 2014): we used 1) a generalized linear mixed model (GLMM) with a binomial distribution for survival rate, 2) linear mixed-effect models (LME) for the total, aboveground, belowground biomass as well as seedling height and diameter, and 3) a GLMM with a Poisson distribution for leaf number. Seedling biomass and height were log transformed to meet the requirement of a normal distribution. For each model, we first defined the full model and then perform model selection. In the full model, we included soil treatments, host habitat origin (local vs. foreign sister), and their interaction term as the fixed-effect factors; we also included seed length and seed width as fixed-effect factors to account for possible effects of seed size. Species identity and species pairs were also included as fixed-effect factors, instead of random-effect factors because they only have two levels (Crawley 2002). Mother tree was included as a random-effect factor. We then used "dredge" function from the R package *MuMIn* (Barton 2010) to generate a set of models with combinations of fixed-effect terms from the full model, and used the corrected Akaike Information Criterion (AICc) to identify the best model (Table S1). Since testing our hypotheses requires testing the significance of the interaction term between soil treatment and host habitat origin (as illustrated in Fig. 1b, c), we kept soil treatments, host habitat origin, and their interaction term during model selection.

After identifying the best model (Table S1), we then obtained distribution of each parameter within a Bayesian framework with Markov chain Monte Carlo (MCMC) in Stan as implemented in the R package *rstanarm* (Goodrich et al. 2018). Specifically, we used the "stan_glmer" functions for generalized linear mixed-effect model, or "stan_lmer" functions for linear mixed-effect model. This Bayesian inference method is a simulation technique to obtain the distribution of each parameter in a model (Zuur and Ieno 2016), which is suited for the small sample size in our study. We focused on interpreting the Bayesian inference also because the maximum-likelihood models mentioned above, implemented in package *lme4*, occasionally reported singular fits due to small sample size. We set the model prior as a Cauchy distribution with center 0 and scale 2.5 for each model, which is a weakly informative prior recommended by

(Gelman et al. 2008). Each model ran for 2,000 iterations (1,000 "burn-in" iterations followed by 1,000 sample iterations) in each of four chains. We used the default "adept_delta" (target average proposal acceptance probability) = 0.95 during Stan's adaptation period, or when necessary, we increased it to 0.99 until no divergent transitions were detected. Model convergence of the Bayesian models was evaluated by examining *Rhat* (the ratio of between-chain variance to within-chain variance) and the effective number of simulation draws (Gelman and Rubin 1992). Statistical significance of the effects is indicated when 90% credible interval (CIs) or 80% CIs of the Bayesian point estimates do not include zero. Using the 90% CIs is a conservative threshold, while using the 80% CIs is a slightly more liberal threshold (Gomes et al. 2021). When significant interaction term was detected, results were visualized using the estimated marginal means of the best Bayesian model, which was implemented with the "emmeans" function in the R package *emmeans* (Lenth et al. 2019). All statistical analyses were performed in R version 3.5.0 (R Core Team 2018).

3 | RESULTS

The results for greenhouse seedling survival were consistent with Prediction 1, that is, local sister live soil reduced survivorship of the foreign sister species, but not of the local sister (Fig. 2, 3). Consistent with Prediction 1 (Fig. 1b), we detected a significant interaction between host habitat origin and the treatment of local sister live soil in the direction that we predicted (90% CI does not overlap zero; Fig. 2a, 3a, Table S2). The results were consistent for both species pairs (*Q. alba*-*Q. michauxii*, and *Q. shumardii*-*Q. acerifolia*). Specifically, when planted in the soil inoculated with conspecific species' live soil, seedlings of the local sister survived better than in sterilized soil, while seedlings of the foreign sister survived less well in local sister live soil than in sterilized soil (Fig. 3a). Contrary to Prediction 2 (Fig. 1c), we did not find significant interaction effect between host habitat origin and soil treatment of local habitat live soil on seedling survival (Fig. 2a, Fig. 3b, Table S2).

The results of the greenhouse experiment for seedling biomass were also consistent with Prediction 1, and again held for both species pairs (Fig. 2, 3). When planted in the soil inoculated with the local sister live soil, seedlings of the foreign sister had significantly lower aboveground biomass compared to seedlings of the local sister (90% CI of the interaction term does not

overlap zero; Fig. 2c, Fig. 3c, Table S3). Inconsistent with Prediction 2, soil inoculation with local habitat live soil did not differentially impact the aboveground biomass for local sister vs. foreign sister, as compared to the sterilized soil (Fig. 3d, Table S3). Results for total biomass and belowground biomass were similar to that of aboveground biomass (Fig. 2b, d).

For seedling height, diameter and number of leaves, we did not detect a significant interaction between host habitat origin and soil treatment of local sister live soil (Fig. S2; Table S4). Seed size was positively related to seedling biomass, height, diameter, and number of leaves (Table S4).

When comparing the effects of the fungicide treatment vs. no fungicide in local habitat live soil, we did not find a significant increase in performance of foreign sister under the fungicide treatment, indicating that soil pathogens from local habitat live soil did not suppress seedlings of the foreign sister (Table S2—S4). This is inconsistent with our Prediction 2. Rather, the fungicide treatment increased the aboveground biomass and seedling diameter of only local sister (Table S3, S4).

4 | DISCUSSION

While abiotic conditions have been considered the main drivers of species distributions, recent research has increasingly emphasized the roles of biotic interactions in mediating plant performance and species distributions (Pigot and Tobias 2013, reviewed by Wisz et al. 2013). We used a carefully designed experiment to investigate whether and how soil microbes could limit species habitat distributions in an ecologically dominant and diverse clade—oaks (*Quercus* spp.) in North America. We identified and tested two separate mechanisms through which soil microbes can restrict host habitat: the first mechanism is that sister species have host-specific soil microbes that can inhibit the growth and survival of the other sister species; the second mechanism is that sister species are adapted to habitat-specific soil microbes, and perform poorly when encountering soil microbes from novel habitats.

We found that host-specific soil microbes (the first mechanism), but not habitat-specific microbes (the second mechanism), contribute to habitat restriction of sister species. Specifically, when seedlings of foreign sister species (*Q. michauxii*, *Q. acerifolia*) grew in the live soil of the

local sister (*Q. alba*, *Q. shumardii*), the probability of survival and biomass decreased compared to when growing in sterilized soil (Fig. 3a, c); in contrast, local sister species did not show decreased survival or reduced biomass when growing in their own live soil, but increased performance, compared to growing in sterilized soil. This suggests that soil microbes associated with one sister species can inhibit the other sister species from occupying the habitat by decreasing seedling survival and growth. In other words, our experiment shows that host-specific soil microbes can promote habitat partitioning between the hosts.

Previous studies have found that plant-soil interactions can limit species distributions. For instance, when the annual plant *Clarkia xantiana* ssp. *xantiana* was transplanted beyond its habitat, soil microbes decreased lifetime fitness of the transplanted individuals while the home-range live soil improved the fitness (Benning and Moeller 2020). Other transplant experiments also found survival of the transplanted species to be restricted by the presence of soil fungal pathogens or the absence of soil mutualists (Brown and Vellend 2014, Carteron et al. 2020). Notably, our result differs from these previous experiments that tested maladaptation to the general microbes beyond the range or habitats of the transplanted host; in those studies, the live soil inoculum was not associated with sister species or close relatives of the target host. In fact, our experiment indicated that general soil microbes beyond the foreign sister's habitats did not suppress the survival or growth of the seedlings (Fig. 3b, d), suggesting that maladaptation to general microbes of novel habitats does not restrict habitat distributions of our study species. Instead, we found that host-specific soil microbes explained their poor performance when growing in the soil microbial environments of their sister species (Fig. 3a, c). This could be because that habitat-specific microbes collected from non-sister species are less effective in transmitting to the foreign species, given that phylogenetical relatedness of host species correlates positively with the proportion of shared microbes (Liu et al. 2012, Schroeder et al. 2019).

Consistent with our finding and Prediction 1, Kempel et al. (2018) found that soil microbes from widespread and possibly habitat-generalist hosts more strongly suppressed the growth of the regionally rare close relatives than their widespread relatives. The same pattern was found in Amazonian plants: herbivores specific to a forest type prevent confamilial relatives from coexisting together within the same forest habitat (Fine et al. 2004). Indeed, this mosaic co-

existence through niche partitioning, or a checkerboard pattern of close relatives produced through the effects of shared biotic interactions, is consistent with the Janzen-Connell hypothesis in a phylogenetic context (Liu et al. 2012, Araújo and Rozenfeld 2014). Although some argue that species habitat distributions are determined more by inherent environmental tolerance than by biotic interactions (Manthey et al. 2011), the effects of soil microbes on host plants can be perceived as extended phenotypes of the hosts. Our findings support the concept that plant habitat distributions are affected by their responses to specific fungi groups (Singh et al. 2011, Afkhami et al. 2014, Gerz et al. 2018).

Several mechanisms might explain the effects of host-specific microbes on habitat restriction, as observed in our study. First, different host plants co-evolve with, and adapt to, their local pathogens, and when sister species come into contact, transmission of novel pathogens can reduce the fitness of the foreign sister species (Petipas et al. 2021). Second, the lack of microbial mutualists in novel soil habitats might assist pathogen invasion by allowing faster transmission rates. Specifically, ectomycorrhizal fungi are host-specific soil mutualists in oaks (Morris et al. 2009, Aponte et al. 2010), and the association with beneficial ectomycorrhizal fungi assists host defense against root pathogens (Mohan et al. 2015, Vishwanathan et al. 2020). Without the protection of host-specific ectomycorrhizal fungi, pathogens transmitted from close relatives might invade faster into the roots of the foreign sister species. Third, from a genetic perspective, genes related to disease resistance (R-genes) might lead to specialized recognition of, and defense against, only a small subset of pathogens (Marden et al. 2017). Maintaining multiple defense pathways is likely costly when a species mostly encounters few pathogens in a limited range of habitats, resulting in reduced defense against pathogens in novel habitats (Laine 2006, Stump et al. 2020). In extreme cases, a habitat specialist is too isolated to encounter any pathogens, leading to the loss of pathogen defense (Gibson et al. 2010). Once hosts disperse beyond native habitats, the limited diversity of R-genes allows novel pathogens from close relatives to invade more easily (Marden et al. 2017).

Additionally, we found that the soil of local species increased the survival and growth of the conspecific seedlings, relative to the sterilized soil treatment. This suggests that mutualistic soil microbes coevolved with the local species facilitate the self-recruitment and growth of conspecific seedlings. This finding is concordant with previous plant-soil feedback studies,

404 which show that conspecific soil feedback is generally positive for temperate woody species
405 (including oaks of eastern North America used in our study) (LaManna et al. 2017). In the case
406 of temperate oak species, soil microbes from adult trees indeed show positive feedback to
407 conspecific seedling survival and growth, as compared to growing in heterospecific or sterile soil
408 (McCarthy-Neumann and Ibáñez 2012, Bennett et al. 2017).

409 This positive conspecific feedback is likely linked to ectomycorrhizal association.
410 Ectomycorrhizal fungi, a fungal group commonly associated with oaks, often generate positive
411 plant-soil feedback and thus facilitate the self-recruitment of the locally abundant species
412 (Connell and Lowman 1989). Consistent with our support for the host-specificity mechanism,
413 previous research did find host-specific ectomycorrhizal fungi associated with different oak
414 species (Morris et al. 2008, Morris et al. 2009, Aponte et al. 2010), suggesting that the
415 mutualistic effect through fungi is determined by host identity. This specificity might explain
416 why we observed a positive effect of local sister live soil only on local species seedlings, but a
417 negative effect on foreign sister species. It is worth noting that in tropical ecosystems, such
418 positive conspecific feedback is often weakened and even replaced by negative conspecific
419 feedback (Comita et al. 2010, LaManna et al. 2017). Therefore, the roles of soil microbes in
420 maintaining habitat restrictions of plants might be weakened or not supported for tropical
421 species. We encourage future studies to utilize our experimental design (Fig. 1) and to further
422 compare habitat restriction through soil microbes in temperature vs. tropical plant species.

423 Some limitations of the experiment should be recognized. Firstly, interactions with soil
424 microbes should be regarded as a partial factor contributing to species habitat restriction, but not
425 the full explanation for why the two foreign species (*Q. michauxii* and *Q. acerifolia*) were not
426 found beyond their habitats. Habitat restriction can be affected by a combination of other factors,
427 including microclimatic differences, soil chemistry, and other forms of biotic interactions related
428 to host habitats. It is possible that multiple abiotic and biotic processes limit habitat distributions
429 simultaneously and even synergistically (Lau et al. 2008, Rajakaruna 2017). Another caveat of
430 this experiment is the limited representation of genetic diversity of seed sources, since we used
431 seeds from a small number of *ex-situ* or cultivated individuals (Note S1) instead of gathering
432 seeds representative from multiple wild populations across target species' ranges. A soil
433 inoculum experiment that uses representative wild seeds will be needed to more accurately

measure the effects of soil microbes in our study system. Lastly, we did not test the other direction of plant-soil interactions by introducing foreign sister's live soil to the seedlings of local sister species. Without this treatment, we cannot determine whether the habitat exclusion is symmetrical (*i.e.*, equal strength of negative suppression from each host species) or asymmetrical. A reciprocal soil inoculum experiment will be needed to test whether the effect of soil microbes is bidirectional.

5 | CONCLUSIONS

The role that biotic interactions play in constraining species habitat distribution is just coming to the forefront (Sexton et al. 2009, Hargreaves et al. 2014, Katz et al. 2017). Using a well-designed soil inoculum experiment, we found that host-specific soil microbes contribute to habitat restriction of closely related oaks. Our finding implies that species habitat distributions are more than a simple function of abiotic constraints. Particularly, we demonstrate that considering the effects of soil microbial communities and the phylogenetic relationships among host plants will be essential to fully capturing the factors determining fine-scaled plant distributions (McCarthy-Neumann and Ibáñez 2012, Kempel et al. 2018, Pither et al. 2018, Benning and Moeller 2020, Benning and Moeller 2021). We encourage future studies to account for the effects of belowground biotic interactions to advance our understanding of habitat preferences and habitat partitioning.

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AUTHOR CONTRIBUTIONS

Y.W. and R.E.R. conceived of the study. Y.W. designed the experiments, collected and analyzed the data, and wrote the manuscript. A.B. assisted in collecting and analyzing the data. R.E.R. assisted in experimental design and in major revisions of the manuscript. All authors agreed on the final manuscript.

CONFLICT OF INTERESTS

None declared.

DATA AVAILABILITY STATEMENT

Raw data and codes used in this study are available in Dryad Data Repository (doi:10.5061/dryad.fqz612jt0) (temporary link during peer review: https://datadryad.org/stash/share/L1fmCjcqTrgStoJUO-e83IznKzFm0eK5UtuJtwlYkls)

FIGURES AND CAPTIONS

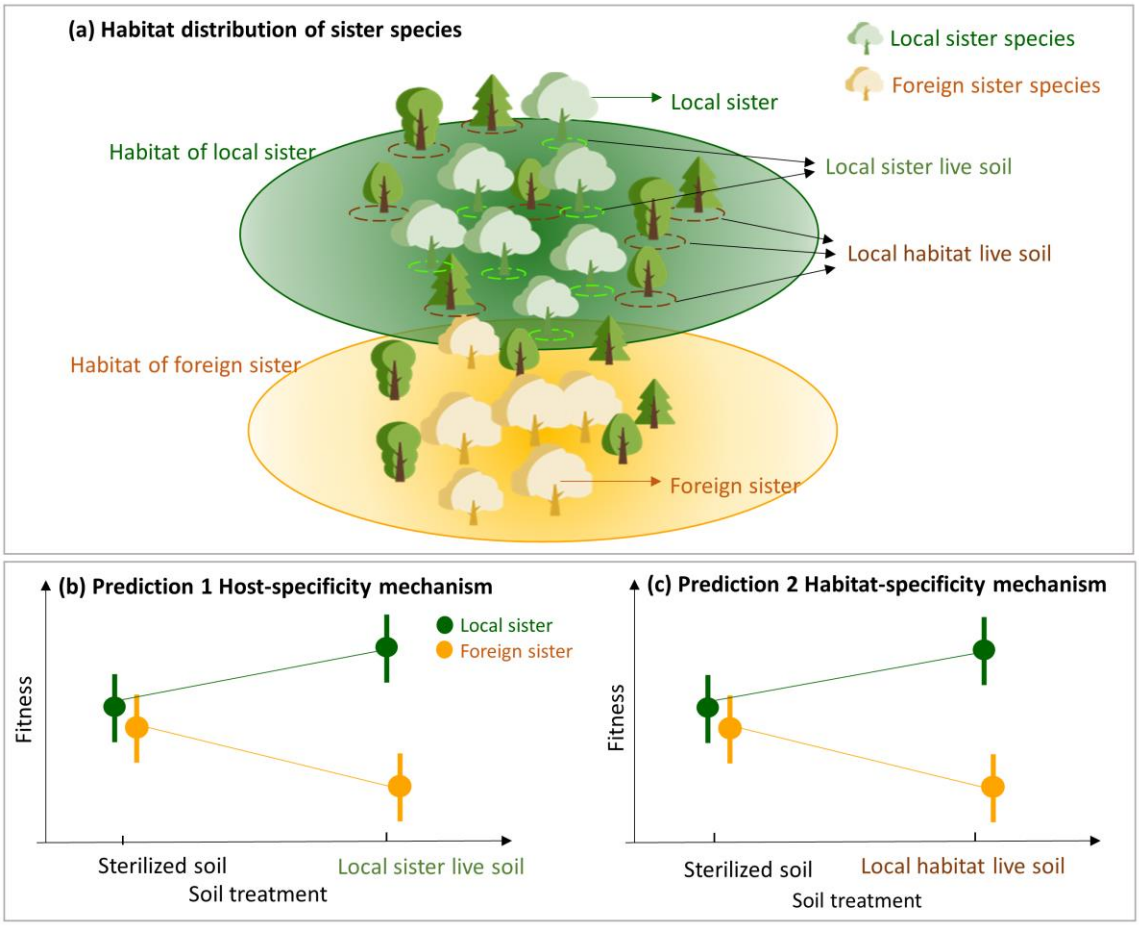
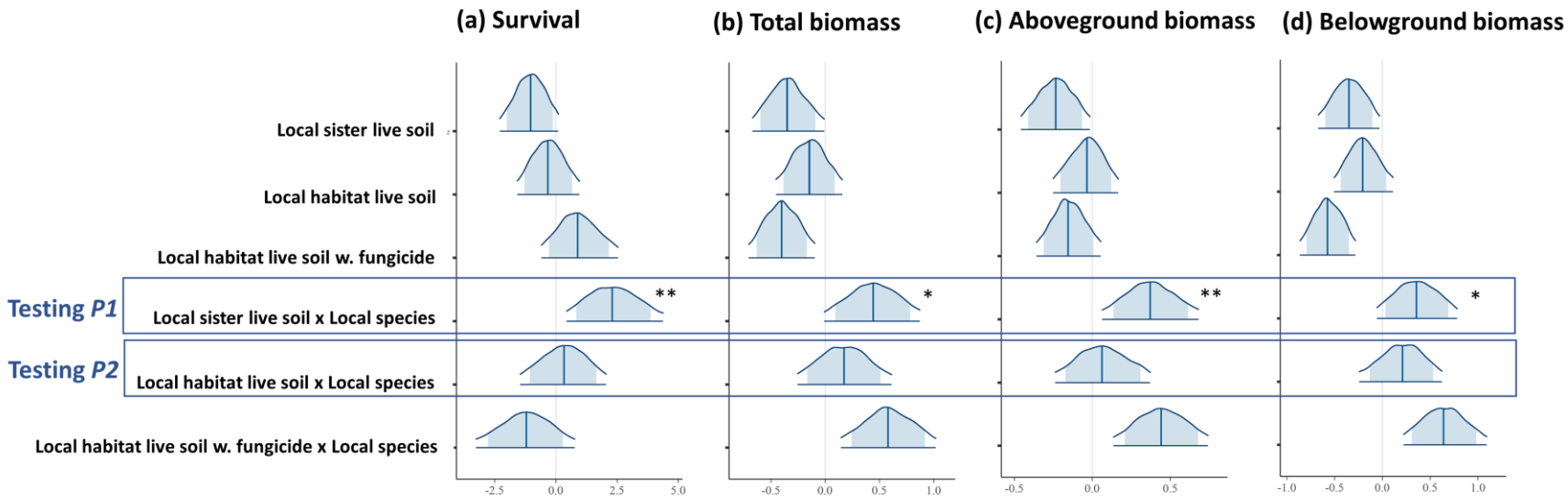


Figure 1 Hypothesis of soil microbe-mediated habitat restriction of sister species. This diagram visualizes the predictions that soil microbes of a local sister constrain habitat distribution of its foreign sister species. (b) Prediction 1 – host-specificity mechanism: local sister live soil collected from adult trees of local sister species (green dashed circles in panel a) increases the fitness of conspecific seedlings due to specialized soil mutualists and tolerance of its own pathogens, while the same soil decreases the fitness of foreign sister's seedlings due to soil pathogens parasitic to the foreign sister and foreign sister's susceptibility. (c) Prediction 2 – habitat-specificity mechanism: local habitat live soil collected from other species co-occurring within local sister's habitat (brown dashed circles in panel a) differentially affects the fitness of local sister's and foreign sister's seedlings.



491

492 **Figure 2 Bayesian estimates of the effects of soil treatments and host habitat origin (local species vs. foreign species) on oak seedling**
493 **survival and biomass in a soil inoculum experiment.** Sterilized soil is used as a reference level for soil treatment, and foreign species is used as
494 a reference level for host habitat origin. Blue vertical lines represent median estimates of the coefficients derived from the Bayesian models. The
495 truncated distribution outline represents 90% credible intervals (CIs), while the shaded-light blue region represents 80% CIs. A light-grey vertical
496 line marks $x = 0$ in each panel. The tests for Prediction 1 (*P1*) and Prediction 2 (*P2*) are highlighted with rectangles. Statistical significance is
497 highlighted with asterisks: ** indicates that 90% CIs of the posterior estimates of the coefficient do not overlap with zero, while * indicates that
498 the 80% CIs do not include zero.

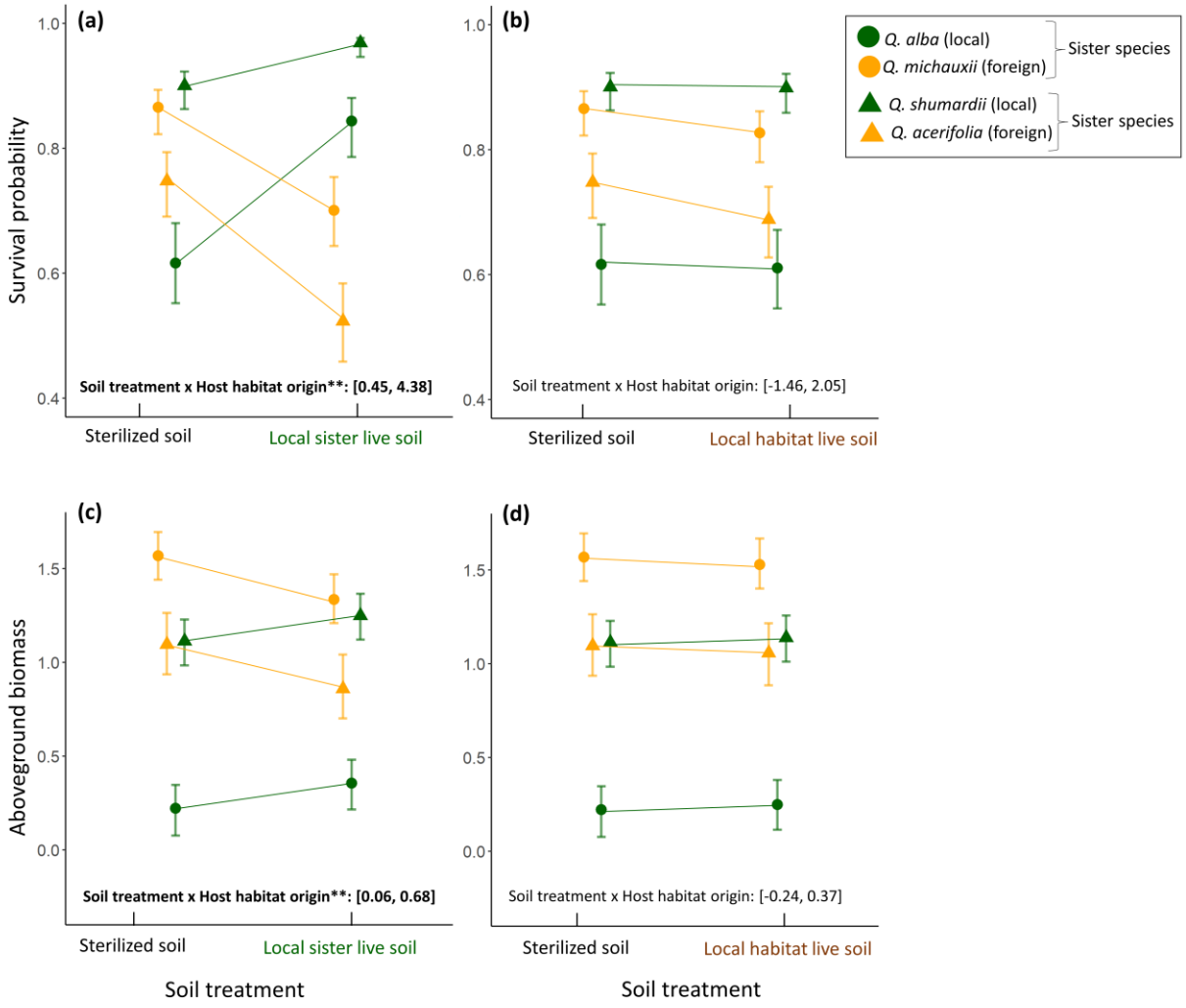


Figure 3 Seedling survival probability and aboveground biomass of the local vs. foreign sister species in different soil treatments. Values were derived from the best Bayesian model, using estimated marginal means. Panels (a, c) compare the survival probabilities and aboveground biomass of local sister (green points) when grown in sterilized soil vs. in local sister live soil, and the survival of foreign sister (yellow points) in these two treatments. Panels (b, d) compare the survival probabilities and aboveground biomass of local sister (green points) when grown in sterilized soil vs. in local habitat live soil that does not associate specifically with one host, and the survival of foreign sister (yellow points) in these two treatments. Error bars represent one standard error. Statistical significance, as tested using Bayesian models, is highlighted with asterisks: ** indicates that 90% credible intervals of the posterior distribution of the model coefficient do not overlap with zero. The 90% credible intervals are marked on each panel.

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