Full Paper

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Microbes, mutualism, and range margins: testing the fitness consequences of soil microbial communities across and beyond a native plant's range

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Summary

- Interactions between plants and soil fungi and bacteria are ubiquitous and have large effects on individual plant fitness. However, the degree to which spatial variation in soil microbial communities modulates plant species' distributions remains largely untested.
- Using the California native plant *Clarkia xantiana* ssp. *xantiana* we paired glasshouse and field reciprocal transplants of plant populations and soils to test whether plant–microbe interactions affect the plant's geographic range limit and whether there is local adaptation between plants and soil microbe communities.
- In the field and glasshouse, one of the two range interior inocula had a positive effect on plant fitness. In the field, this benefit was especially pronounced at the range edge and beyond, suggesting possible mutualist limitation. In the glasshouse, soil inocula from beyond-range tended to increase plant growth, suggesting microbial enemy release beyond the range margin. Amplicon sequencing revealed stark variation in microbial communities across the range boundary.
- Plants dispersing beyond their range limit are likely to encounter novel microbial communities. In *C. x. xantiana*, our results suggest that range expansion may be facilitated by fewer pathogens, but could also be hindered by a lack of mutualists. Both negative and positive plant–microbe interactions will likely affect contemporary range shifts.

Introduction

Soil microbial communities can greatly influence plant growth, phenology, and reproduction (e.g. Klironomos, 2003; Wolfe *et al.*, 2005; Lau & Lennon, 2012) and have been shown to exhibit high turnover rates at small (e.g. Wolfe *et al.*, 2007) and large spatial scales (e.g. Fierer & Jackson, 2006; Tedersoo *et al.*, 2014). Increasingly, experiments suggest that such spatial variation in soil microbe communities may affect patterns of local adaptation in plants (e.g. Lankau, 2013; Pickles *et al.*, 2015) and have suggested axes of abiotic environmental variation that interact with microbe communities to affect plant fitness (e.g. soil nutrients, Johnson *et al.*, 2010; aridity, Lau & Lennon, 2012). Because of rapid climate change and the ubiquity of plant–microbe symbioses, it has been of particular interest whether interactions with soil microbes may influence the location of plant species' geographic range margins and predictions of contemporary range shifts (Van Grunsven *et al.*, 2007; Van der Putten, 2012).

Within a plant species' range, geographic environmental variation may result in local adaptation of plant populations to their home soil microbial communities (Johnson *et al.*, 2010; Pickles *et al.*, 2015; Revillini *et al.*, 2016). Alternatively, plant populations may be maladapted to their local soil microbes, for instance due to pathogen specialization on local plant genotypes (McCarthy-Neumann & Ibáñez, 2012). In testing for local adaptation of plants to soil microbes, researchers either isolate specific groups of microbes (e.g. mycorrhizal fungi, rhizobia) to use in experimental inocula, or take a 'whole community' approach. The benefit of experiments with whole soil communities is that they can capture the complex web of positive and negative plant–microbe interactions that occur in nature, which may be especially important if microbial effects are dependent on microbial community context (e.g. Hoeksema *et al.*, 2010). Glasshouse experiments focusing on whole soil microbial communities have shown both adaptation and maladaptation of plant populations to their local whole soil communities (Sherrard & Maherali, 2012; Smith *et al.*, 2012; Pickles *et al.*, 2015; Lankau & Keymer, 2018), and it is unclear whether either pattern predominates in nature.

The environmental variables that structure adaptation *within* a species' range may or may not be those that contribute to the taxon's geographic range limit. Maladaptation to environments outside their distributional limit likely prevents range expansion in many species (Angert & Schemske, 2005; Geber & Eckhart, 2005; reviewed in Lee-Yaw *et al.*, 2016), but it is difficult to

know which abiotic or biotic factors cause a range limit to occur where it does (Gaston, 2009). Compared to abiotic factors, few experiments have tested the idea that species interactions contribute to a geographic range boundary (but see Stanton-Geddes & Anderson, 2011; Afkhami *et al.*, 2014; Baer & Maron, 2018; Benning & Moeller, 2019). Variation in microbial communities across a range boundary could influence plant population dynamics in three main ways. First, low abundance of important mutualists at or beyond the range edge has the potential to influence the location of plant species' boundaries (e.g. Nuñez *et al.*, 2009; Stanton-Geddes & Anderson, 2011; Lankau & Keymer, 2016; Osborne *et al.*, 2018). Second, novel pathogen taxa or genotypes, or relatively high pathogen abundance, at or beyond the range edge could depress peripheral population growth rates or prevent colonization outside the range (Parker & Gilbert, 2004; Lankau & Keymer, 2018). Third, plants dispersing outside their species' current range limit may encounter relatively benign microbial communities due to fewer specialist and/or generalist pathogens being present ('enemy release'; Keane & Crawley, 2002; Van Grunsven *et al.*, 2007; Reinhart *et al.*, 2010; Ramirez *et al.*, 2019), or even form novel mutualisms (Nuñez & Dickie, 2014). This previous work suggests that plant–soil microbe interactions have the potential to influence plant distributional limits, and highlights the need for more research, especially experiments that test the influence of complex, whole soil microbial communities on plant fitness in the field.

Most experimental work with plant–soil microbe dynamics takes place in the laboratory or glasshouse (but see Johnson *et al.*, 2001; Parker *et al.*, 2006; Stanton-Geddes & Anderson, 2011). There have been calls for more realistic field experiments (e.g. Dawson & Schrama, 2016) because of recognition that plant phenotype can vary strongly between field and glasshouse (e.g. Poorter *et al.*, 2016), and that many plant–microbe interactions are context dependent (e.g. Hoeksema *et al.*, 2010); however, to date few have been executed. Although controlled conditions aid in isolating the effects of pairwise interactions between plant and microbial taxa (e.g. Klironomos, 2003), the lifetime fitness consequences of plant–microbe interactions will remain poorly understood without experiments in natural environments. In this study, we used field and glasshouse reciprocal transplants of a well-studied California annual plant, *Clarkia xantiana* ssp. *xantiana* (Onagraceae), to test for plant–soil microbe local adaptation and the potential for soil microbes to contribute to the plant's geographic range limit.

Clarkia. x. ssp. *xantiana* is endemic to the southern Sierra Nevada foothills, a region with high biological diversity and fine-scale heterogeneity in climate and soils. Transplant experiments have demonstrated that its geographic range is likely limited by adaptation, not dispersal (Geber & Eckhart, 2005), and high environmental heterogeneity has driven population divergence in important phenotypic traits such as phenology and size (Gould *et al.*, 2014). A key environmental driver in this system is precipitation, with a spatial trend of increasing aridity and greater drought stress toward the eastern range edge (Eckhart *et al.*, 2010, 2011). Because soil microbial communities can directly influence plant water relations (e.g. Augé, 2001; Lau & Lennon, 2012) and/or affect traits related to drought avoidance (e.g. phenology: Wagner *et al.*, 2014), we were especially interested in how soil microbes may modulate local and range adaptation across and beyond the precipitation gradient that underlies the subspecies' geographic distribution.

Our core objective in the current study was to investigate whether geographic variation in soil microbial communities across and beyond the range of *C. x. xantiana* may influence plant fitness and the likelihood of range expansion. In the field, we transplanted multiple plant populations into six sites within and beyond the range, where plants grew with one of three soil microbial inocula sourced from the sites inside the range, or a local control. We paired this field transplant with a glasshouse experiment in which we grew those same plant populations with soil microbial communities sourced from sites inside and outside the geographic range limit, in a full factorial design. We also used amplicon sequencing to characterize rhizosphere bacterial and fungal communities of glasshouse plants grown with the various experimental inocula. We used this combination of approaches to answer three main questions: First, is there geographic variation in rhizosphere microbial communities across and beyond the range of *C. x. xantiana*? We predicted that differentiation between within- and beyond-range sites would be stronger than differentiation among sites within-range. Second, is there evidence that range expansion is limited by mutualists, limited by pathogens, and/or facilitated by enemy release outside the plant's geographic range margin? Mutualist limitation would be evidenced by beneficial effects of inoculation with within-range microbial communities when plants are transplanted beyond-range, and/or increased growth with within-range microbes, relative to beyond-range microbes, in the glasshouse. Pathogen limitation would be supported by negative effects of beyond-range microbes on plant fitness relative to within-range microbes. Enemy release would be suggested by a pattern of increased fitness of plants growing with beyond-range microbes, especially if combined with evidence of decreased microbial colonization of plant roots. And third, are plant populations locally adapted to their home microbial communities? If so, plant source populations should perform best when paired with their home soil microbial communities.

Materials and Methods

Study system

Clarkia xantiana ssp. *xantiana* A. Gray is a winter annual native to the Southern Sierra Nevada foothills of California, USA (Eckhart & Geber, 1999). Populations are distributed across an aridity gradient (mean spring precipitation decreases by *c.* 30%, and interannual variability in precipitation more than doubles, going from western to eastern populations) that contributes to reduced performance at the eastern range edge and beyond (Eckhart *et al.*, 2010, 2011; Fig. 1a). Seeds germinate in winter (November–December) and adults set seed in June. Central populations are found in relatively mesic oak woodlands and edge populations in drier pine woodlands (Eckhart *et al.*, 2011). Most populations,

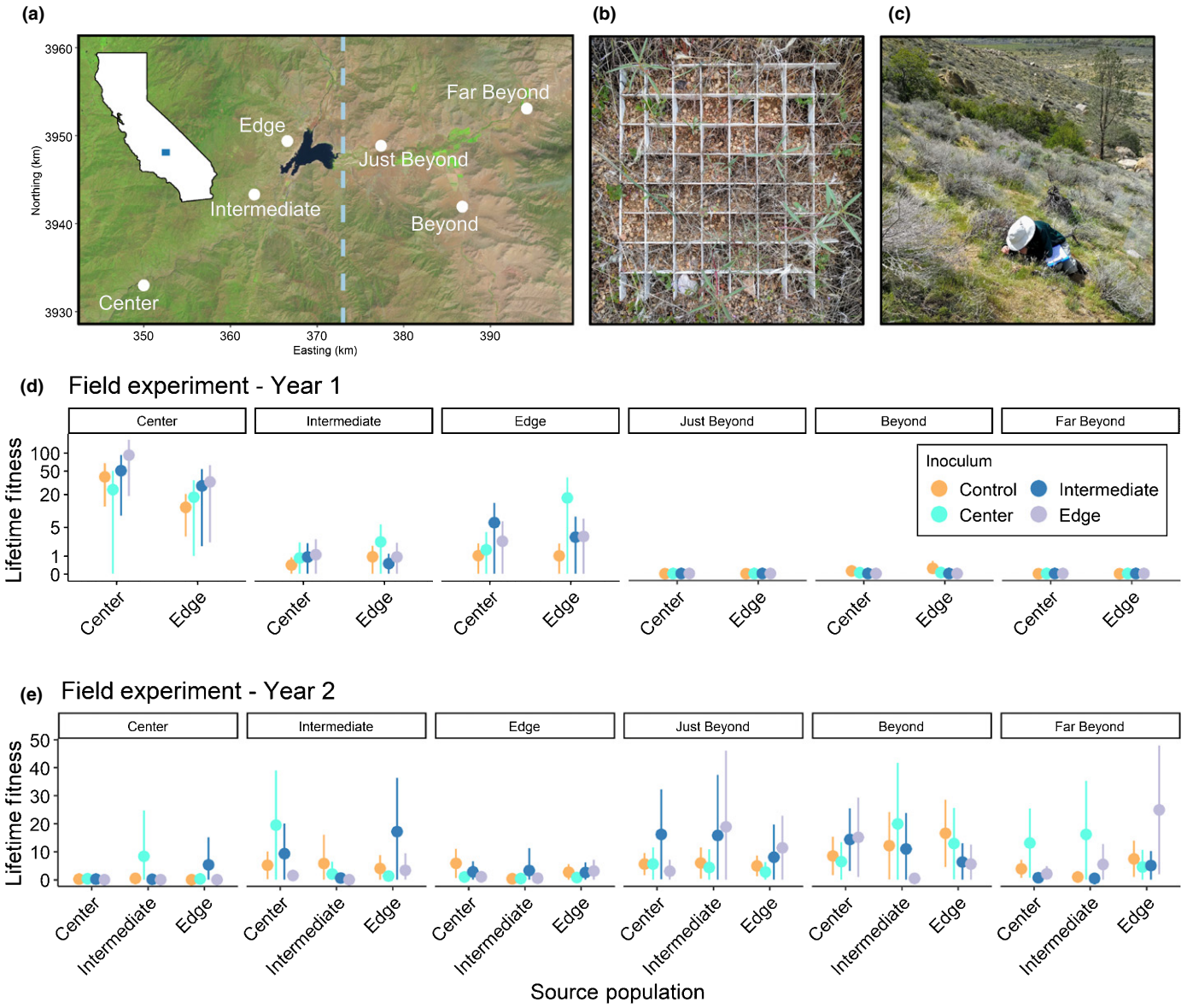

Fig. 1 Study area and lifetime fitness in the field experiment. (a) Overview of study area in Southern California and the locations of sites used in the glasshouse and field experiments. The dashed blue line marks *Clarkia x. xantiana*'s eastern range limit. Background image is 19 April 2016 LANDSAT imagery of study area. Axes are Universal Transverse Mercator (UTM) coordinates; Zone 11 S. (b) One of the planting grids used in the field experiment. (c) Censusing the experiment at the Far Beyond site in March 2017. (d) Estimated mean lifetime fitness (± 95% CI) of *C. x. xantiana* across sites, source populations, and inoculum treatments for the field experiment in year 1 (only caged plants shown) and (e) year 2 (only caged plants planted in year 2 shown), as estimated from *aster* models. Mean fitness was generally lower for uncaged plants and plants planted in the prior year (Supporting Information Figs S3, S4). In year 1, n = 57–122 planting cells per source population × inoculum × caging treatment combination at each site; in year 2, n = 30–244 (Intermediate source population had lower replication due to exclusion from year 1 planting). Note that the y-axis in (d) uses a logarithmic scale.

including all in this study, occur on steep slopes of sandy soil (Eckhart *et al.*, 2010). The eastern range edge is stark, and extensive searching over the past 20+ yr has uncovered no populations beyond this limit.

Field transplant experiment

We used a transplant experiment to estimate the effects of soil microbial communities, geography, and plant source population on plant lifetime fitness. We planted three plant source populations into six sites: at the range center (Center), between the center and range edge (Intermediate), near the range edge (Edge), and at three locations beyond the range (5 km beyond, Just Beyond; 14 km, Beyond; 22 km, Far Beyond, Fig. 1a). The three beyond-range sites harbour populations of *C. x. xantiana*'s sister subspecies, *Clarkia xantiana* ssp. *parviflora*; these taxa diverged in allopatry and are now in secondary contact (Pettengill & Moeller, 2012). We manipulated soil microbial communities via the addition of inocula from one of the three sites within the range, or a local control (Supporting Information Fig. S1). The

experiment was conducted in 2015–2016 and 2016–2017; hereafter, years 1 and 2. We compared precipitation between the two years, and in relation to long-term trends, using data from weather stations located at or near our sites (see Fig. S2).

Seeds were sourced from the Center and Edge sites in year 1, and Center, Intermediate, and Edge sites in year 2 (Methods S1). At each of the six sites, we installed 120 plastic grids (American Louver, Des Plaines, IL, USA) arranged into six blocks set into natural vegetation (20 grids per block; blocks spaced *c.* 10–60 m apart) so as to span the main extent of resident populations of *C. xantiana* (Fig. S1). Grids comprised a 6 × 6 matrix of 3.5 cm × 3.5 cm cells (2-cm high walls) and were set at least 1 m apart (Fig. 1b). These grids allowed us to follow individual seeds while maintaining a natural growing environment for the experimental plants. Source populations were randomly assigned to cells within grids using three randomized planting schemes (Fig. S1). Two seeds were planted per cell in October of each year (five and four cells per source population per grid in years 1 and 2, respectively). In year 2, the experiment included newly planted seeds as well as seeds that were planted in year 1 and did not germinate (year 1 $n = 7387$ cells; year 2 $n = 14\,735$).

We manipulated soil microbe communities by adding whole soil inocula to the planting grids. This treatment adds microbial communities (including mutualists, commensalists, pathogens, and organisms not directly interacting with the plant) to the growing environment (rather than replacing *in situ* soil environments entirely), and thus mimics dispersal of within-range microbes to sites beyond-range. After removing the top 5 cm to prevent seed contamination, soil was collected in October 2015 from the top 30 cm at multiple points within the three within-range *C. x. xantiana* populations. Each grid within a block was randomly assigned one of four inoculum treatments – soil from Center, Intermediate, Edge, or control. Soil samples were homogenized before applying 750 ml of soil to grids. Inoculum applied to each grid represented a minute fraction of the total soil environment experienced by each plant; thus, we assume any abiotic effects of inoculum addition would be minimal. For control grids, we collected soil from beside each block in the same manner as we did for inoculum treatments. We followed all treatments with a thin top layer of control soil to equalize soil depth within grids and minimize inoculum dispersal between grids; seeds were sown on top of the soil as described in the previous paragraph. In each block of 20 grids, control inoculum was applied to eight grids, and each of the other three inocula was applied to four grids.

Thus, inside the range we fully reciprocally transplanted plant populations and soil inocula. Outside the range, we fully crossed all plant source populations with soil inocula from inside the range (but did not transplant soil inocula from outside the range into sites within the range). The experiment also included a caging treatment (where half of the grids within each treatment combination at each site were surrounded by wire caging) to test the effects of fatal mammal herbivory on lifetime fitness (reported in Benning & Moeller, 2019).

We scored germination and early season survival in March and April and late season survival, growth, and total seed set in May/June. We estimated seed set in each fruit using a linear model that predicted seed set as a function of individual fruit weight (Benning & Moeller, 2019). A plant's lifetime fitness was equal to the total number of seeds contained in all its fruits.

Statistical analyses

Individual life history components All analyses were conducted in R (R Core Team, 2013). We tested the influence of experimental treatments on plants at four life history stages (germination, early survival (March), survival to fruit set, and total seed set), analyzing years 1 and 2 separately. Analyses were conditional on survival to the previous stage; e.g. analyses of early survival only included plants that germinated. We used logistic regressions to test the effects of site, source population, inoculum, caging treatment, and first and second order interactions, on our Bernoulli life history components (germination through survival to fruit set). Using the same model structure, we used negative binomial regression to model seed production. For germination, we included planting year in analyses of year 2 to account for seed age (planted in year 1 or year 2). The model for seed production including all second order interactions would not converge; thus, we built a model with all first order interactions and the site × source population × inoculum interaction (the three-way interaction of interest). If Type II analysis of deviance (anova() function in the CAR package (Fox & Weisberg, 2019)) indicated a significant main effect of inoculum or a significant interaction of inoculum with other terms, differences between treatment levels were tested with Tukey HSD tests via the emmeans() function in the EMMEANS package (Lenth, 2020). We used Type II tests, as opposed to Type III, to respect the principle of marginality (Hector *et al.*, 2010). For these contrasts, we dropped three-way interaction terms from the model if they were not significant. Across life history components within years, we adjusted term *P* values from ANOVA with a Holm adjustment, but readers should note that these components are not independent.

Lifetime fitness We also used *aster* life history models (Geyer *et al.*, 2007; Shaw *et al.*, 2008) to evaluate the effects of treatments on plant lifetime fitness. Our *aster* model incorporated the four components of lifetime fitness analyzed separately in the previous paragraph (*nodes* in the graphical model). The first three components were modeled as Bernoulli variables (0,1), and seed set as a zero-truncated negative binomial variable:

$$1 \rightarrow \underset{(0,1)\text{Bernoulli}}{\text{Germination}} \rightarrow \underset{(0,1)\text{Bernoulli}}{\text{Early survival}} \rightarrow$$

$$\underset{(0,1)\text{Bernoulli}}{\text{Survival to fruit set}} \rightarrow \underset{\text{zero}-\text{truncated negative binomial}}{\text{Seeds produced}}$$

We built *aster* models with site, source population, caging treatment, inoculum treatment, and all first and second order interactions as predictors; response variables are those associated with each component of fitness. The model for year 2 also included a term to account for cohorts planted in 2015 and

2016. To estimate the effects of each predictor on lifetime fitness, each predictor was fit at the level of total seed set (Shaw *et al.*, 2008). We used likelihood ratio tests (LRTs via the anova() function) comparing submodels to fuller models to test each term of interest. When terms involving *inoculum* were significant, we explicitly tested relevant contrasts within sites using LRTs of reduced models (the EMMEANS package cannot be used with *aster* models). Because few plants survived outside the range in year 1 and at the Center site in year 2, we could not model lifetime fitness at all sites simultaneously due to limitations of maximum likelihood estimation (Geyer, 2009). To circumvent this issue, we added two 'pseudo-records', producing one and zero seeds, respectively, to each treatment combination in each year.

A significant site × source population × inoculum term indicated that the effects of inoculum on fitness of source populations differed among sites. For a significant three-way interaction, we used specific contrasts (see Methods S2 for details) to ask two questions. First, within the range, how does addition of inocula from a population's home site influence fitness in novel environments? Second, how does the addition of any of the three inocula from within-range affect fitness beyond-range?

Predicting mean lifetime fitness We obtained predicted values for lifetime fitness (seeds produced per planted seed) and their associated standard errors by taking the product of germination probabilities (estimated from the logistic regression for germination described in the sub-section 'Individual life history components', above, which incorporated information on multiple seeds per cell) and unconditional parameter estimates from a full aster model that did not include a germination node (Methods S3; Appendix A1).

Glasshouse experiment

We conducted a fully factorial glasshouse experiment with the three plant source populations (Center, Intermediate, and Edge) and soil microbial inocula from those three within-range sites, two beyond-range sites (Just Beyond and Beyond), and a control. We collected soil from the top 30 cm at five or more locations per site in November 2016, and kept it at 4°C until the experiment (December 2016).

We implemented six inoculum treatments, each comprising equal amounts of soil from all inoculum sources, but with different 'live' inocula. Each experimental inoculum consisted of 20% live focal inoculum and 80% of an even mix of the other four inoculum sources, which were autoclaved. This approach controls for differences in abiotic properties of the different soil sources (Johnson *et al.*, 2010). We autoclaved field soil for 1 h at 121°C, allowed it to rest overnight, and autoclaved for another 1 h at 121°C. The control inoculum consisted of all five (autoclaved) inoculum sources. All experimental inoculum mixtures were homogenized before filling pots.

We planted two seeds into each 983 cm^3 D60 Deepot (Stuewe & Sons, Oregon, USA), which were steamed for 2 h at 80°C before filling. The soil mix for each pot comprised 400 cm^3 of the mixed inoculum, with 270 cm^3 of sand (twice steamed at 80°C for 2 h). We poured inoculum and sand into pots simultaneously so that they were distributed throughout the entire pot. We completely randomized treatment combinations across the glasshouse. We culled one germinant if both seeds germinated. Final sample sizes were 18–30 (mean = 25) replicates per treatment combination ($n = 452$); unequal sizes were mainly due to unequal germination.

To simulate the limited soil moisture conditions in the field, each plant received 30 ml of reverse osmosis water per wk. We measured root biomass and the total number of leaves produced until flowering (a proxy for aboveground growth, as plants begins to senesce leaves before flowering). Leaf number is well correlated with seed production of surviving plants in the field ($r = 0.8$ in the field experiment).

Statistical analyses

We tested for the effects of plant population, inoculum, and their interaction on leaf number and root mass using linear fixed effect models with Type II ANOVA. We also included a term to account for glasshouse bench position. We adjusted *P* values from ANOVA with a Holm adjustment. We tested for differences among treatment levels using Tukey's HSD and calculated estimated marginal means using the emmeans() function.

We tested for local adaptation to soil microbial communities using pre-planned contrasts from our model of leaf number, using both the *local* vs *foreign* and *home* vs *away* criteria (Kawecki & Ebert, 2004). Using this same approach, we tested whether there was an overall effect of microbial communities from inside vs outside the geographic range on plant performance for each source population.

Rhizosphere sampling, microbial DNA extraction and amplification, and bioinformatics

Full methodological details for microbial community sampling and analyses are provided in Methods S4 and summarized here. To characterize rhizosphere microbial communities, we extracted DNA from rhizoplane soil and root samples for six to eight Center population individuals per inoculum treatment in the glasshouse experiment, soon after plants began flowering (we did not sequence soil from the field experiment). We limited extractions to Center population individuals because populations responded similarly to inoculum treatments (Table 3; Figs S5, S6). In the lab, we separated root and rhizoplane soil, and extracted DNA from the entire lyophilized root system (*c.* 40 mg) and the entire lyophilized rhizoplane soil pellet (*c.* 60 mg) using Qiagen DNeasy PowerSoil kits. We used amplicon sequencing to characterize rhizosphere bacterial and fungal communities, sequencing PCR products on an Illumina MiSeq (2 × 300 bp chemistry). For bacteria, we sequenced the V4 hypervariable region of the 16S rRNA gene, using the 515-F/ 806-R primer pair (Caporaso *et al.*, 2011). For fungi, we sequenced the ITS1 region of the rRNA gene using the ITS1F (Gardes & Bruns, 1993)/ITS2 (White *et al.*, 1990) primer pair. Reads were

filtered and trimmed, amplicon sequence variants (ASVs) inferred, and taxonomy assigned using the DADA2 pipeline (Callahan *et al.*, 2016; Knight *et al.*, 2018). We used PHYLOSEQ (McMurdie & Holmes, 2013), VEGAN (Oksanen *et al.*, 2019), and DESEQ2 (Love *et al.*, 2014) for analysis of microbial community composition.

We calculated bacterial and fungal ASV richness and diversity (Shannon's H index) for each inoculum source for rhizoplane soil and root samples. We calculated Bray–Curtis distance (based on proportional read abundance) and Jaccard similarity (based on presence/absence, after rarefaction), and visualized community distance using principal coordinates analysis (PCoA). Bray–Curtis distances were also used for cluster analysis (unweighted pair group method with arithmetic mean; UPGMA), and trees were visualized using ITOL v.3 (Letunic & Bork, 2016). We used PERMANOVA to test for differences in fungal and bacterial community composition between within- and beyond-range sites in root and rhizoplane soil. We used the DESEQ2 package to test for differentially abundant ASVs in rhizoplane soils and roots between within- and beyond-range inocula. Based on results from the field and glasshouse, we also tested for differentially abundant ASVs between the Intermediate inoculum and the other four live inocula. We filtered ASVs using a False Discovery Rate of $\alpha = 0.01$ to correct for multiple tests. We note that because the inocula used in both the field and glasshouse experiments are drawn from 'bulked' soil samples at each site, our experimental and microbial community composition results reflect site-wide microbial pools (Reinhart & Rinella, 2016).

Results

Field experiment

Here we focus on results regarding inoculum treatments but preface each section by highlighting the main results from Benning & Moeller (2019) regarding geography, caging treatment, and source populations for context.

Year 1 In year 1, precipitation was near or above the 27-year average within-range, and considerably below average outside (Fig. S2), resulting in mean fitness near zero outside the range edge (Fig. 1d). There was evidence of local adaptation of the Center population to the Center site, and a positive effect of caging at the Edge site (Figs 1, S3).

The effects of inoculum source on lifetime fitness differed among sites (inoculum × site, $P = 0.02$; Table 1) and were driven by inoculum effects at the Edge and Beyond sites (inoculum $P = 0.01$ and 0.002 at these sites, respectively; Fig. 1d). At the Edge site, plants grown with Center inoculum had the highest lifetime fitness. At the Beyond site, plants grown with Control inoculum had the highest lifetime fitness. Inoculum treatment did not have a significant effect on any of the four components of lifetime fitness in conditional analyses (Table 2).

Year 2 In year 2, precipitation was high within- and beyond-range (Fig. S2). Herbivory was substantial at the three sites outside the range margin and the Intermediate site (12% to 37% of uncaged adult plants eaten); caging treatment greatly increased fitness at these sites. Seeds planted in year 2 had higher

Table 1 Summary of results from *aster* likelihood ratio test (LRT) model comparisons testing effects of site, source population, caging treatment, inoculum treatment, and all first and second order interactions, on *Clarkia x. xantiana* lifetime fitness, in both years of the experiment.

	Year 1			Year 2		
Term	Model parameters	Test df	χ^2	Model parameters	Test df	χ^2
Full	84			118		
Site × caged × inoculum	69	15	7.3	103	15	**43.4*** **
Pop × caged × inoculum	81	3	1.1	112	6	5.6
Site × pop × caged	79	5	2.3	108	10	**22.5***
Site × pop× inoculum	69	15	8.4	88	30	**76.0*** **
First order interactions	46			57		
Caged × inoculum	43	3	4.5	54	3	5.3
Caged × pop	45	1	0.7	55	2	1.0
Inoculum × pop	43	3	1.7	51	6	7.3
Site × caged	41	5	**11.1***	52	5	**32.5*** **
Site × inoculum	31	15	**28.5***	42	15	24.8†
Site × pop	41	5	4.4	47	10	**60.0*** **
Main effects only	14			16		
Plant year				15	1	**12.0*** **
Inoculum	11	3	2.9	13	3	0.8
Caged	13	1	1.0	15	1	**78.2*** **
Pop	13	1	**14.9*** **	14	2	2.1
Site	9	5	**690.7*** **	11	5	**37.3*** **

Year 2 models include a term for seed planting year.
***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; †, $P < 0.1$.

germination rates, and thus lifetime fitness, than seeds planted in year 1 (Table 1; Fig. S4). Averaging over treatments, mean lifetime fitness was higher outside the range than inside (4.3 ± 0.5 SE vs 2.2 ± 0.4 SE seeds per planted seed, respectively).

There was no main effect of inoculum treatment on lifetime fitness, but there was a significant second order interaction of inoculum × site × source population (Table 1; Fig. 1e). The addition of a population's home inoculum did not consistently increase (local adaptation) or decrease (local maladaptation) fitness relative to controls at sites within-range (Fig. 1e; Table S1). Outside the range edge, source populations did not have consistent responses to inocula sourced from inside the range (Fig. 1e; Table S1). There was also a significant second order interaction of inoculum × site × caging treatment, where responses of plants to the caging treatment at the Center and Far Beyond sites differed somewhat among inoculum treatments (Table 1; Fig. S4).

In conditional analyses, there was a significant effect of inoculum treatment on a plant's survival to fruit set, given early survival ($P = 0.005$; Table 2; Fig. 2a). Plants grown with Intermediate inoculum were 68% more likely to survive to fruit set than those grown with Edge inoculum (Tukey $P = 0.005$), and *c.* 25% more likely than those grown with Control or Center inoculum ($P = 0.1$ and $P = 0.38$). This effect was especially pronounced at the Edge, Just Beyond, and Beyond sites (Fig. 2a). There were also significant site × inoculum, and site × inoculum × source population effects on seed set (Table 2), but there was no indication that this reflected local mal/adaptation of source populations to their home inocula or within-range inocula overall (Fig. S5).

Glasshouse experiment

Growth Above and belowground growth differed strongly among source populations and inocula; the effect size of the two factors was roughly equal (Table 3; Figs 2b,c, S5, S6). Center plants were largest (most leaves and largest root biomass), and Edge plants were smallest (*c.* 20% smaller than Center; Figs S5, S6). Plant source populations responded similarly to inoculum treatments (source population × inoculum $P > 0.7$; Table 3; Fig. S7). Plants grown with Control inoculum were, on average, smaller above- and belowground than those grown with any live inocula, and plant size in Control inoculum was highly variable. The inoculum source with beneficial effects in the field, Intermediate, also increased growth in the glasshouse. For within-range inocula, plants grown with Intermediate inoculum produced 14% more leaves and 15 and 23% more root biomass than those grown with Center and Edge inocula, respectively (Fig. 2b,c). Plants grown with inoculum from outside the range (Just Beyond and Beyond) tended to be larger than those grown with Center

Table 2 Summary of Type II Analysis of Deviance for logistic regressions (germination through survival to fruit set) and negative binomial regression (seed set) testing effects of site, source population (pop), inoculum, and their interactions, on sequential components of *Clarkia x. xantiana* lifetime fitness in years 1 and 2.

	Lifetime fitness components									
	Year 1					Year 2				
Term	df	Germination Res. df = 7114	Early survival Res. df = 1800	Survival to fruit set Res. df = 1269	Seed set Res. df = 249	df	Germination Res. df = 14 332	Early survival Res. df = 2449	Survival to fruit set Res. df = 1803	Seed set[a] Res. df = 466
Site	5	**494.1*** **	**54.3*** **	**394.0*** **	**178.8*** **	5	**1292.9*** **	**374.4*** **	**44.5*** **	**34.9*** **
Pop	1	2.3	0.6	**7.7** **	**6.1***	2	**39.8*** **	5.1†	2.2	4.9†
Inoculum	3	6.9†	5.5	0.2	7.2†	3	1.5	9.2*	**12.7** **	2.6
Caged	1	0.8	0.7	**7.2** **	0.0	1	**5.5***	0.8	**100.3*** **	**29.8*** **
Plant year						1	**353.7*** **			
Site × pop	5	8.3	8.1	6.5	**13.2** **	10	**119.6*** **	8.6	6.3	19.1*
Site × inoculum	15	23.1†	17.7	16.6	14.8†	15	23.7†	17.2	23.7†	**34.7** **
Pop × inoculum	3	1.3	2.3	0.6	8.1*	6	4.6	2.6	3.2	6.4
Site × caged	5	9.0	1.8	7.8	6.9†	5	**23.1*** **	4.1	**23.2*** **	9.3†
Pop × caged	1	0.0	0.4	0.4	1.1	2	1.6	0.1	0.3	0.9
Inoculum × caged	3	3.5	2.0	4.2	7.1†	3	7.3†	1.4	0.3	5.7
Site × pop × inoculum	15	24.6†	10.1	6.6	6.2	30	41.4†	45.4*	28.3	**66.6*** **
Site × pop × caged	5	6.0	8.2	5.4	2.1	10	16.9†	12.3	6.1	
Site × inoculum × caged	15	19.4	14.9	10.8	12.0	15	10.1	27.7*	17.1	
Pop × inoculum × caged	3	1.55	8.1*	6.7†	2.7	6	4.4	3.5	9.3	

Values are likelihood ratio χ^2 statistics. Year 2 models include a term for seed planting year. Bolded values remain significant ($\alpha < 0.05$) after Holm adjustment.

[a]For year 2, a negative binomial model of seed production including *all* second order interactions would not converge; thus, for seed production we built a model with all first order interactions and the site × source population × inoculum interaction (the main three-way interaction of interest).

***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; †, $P < 0.1$.

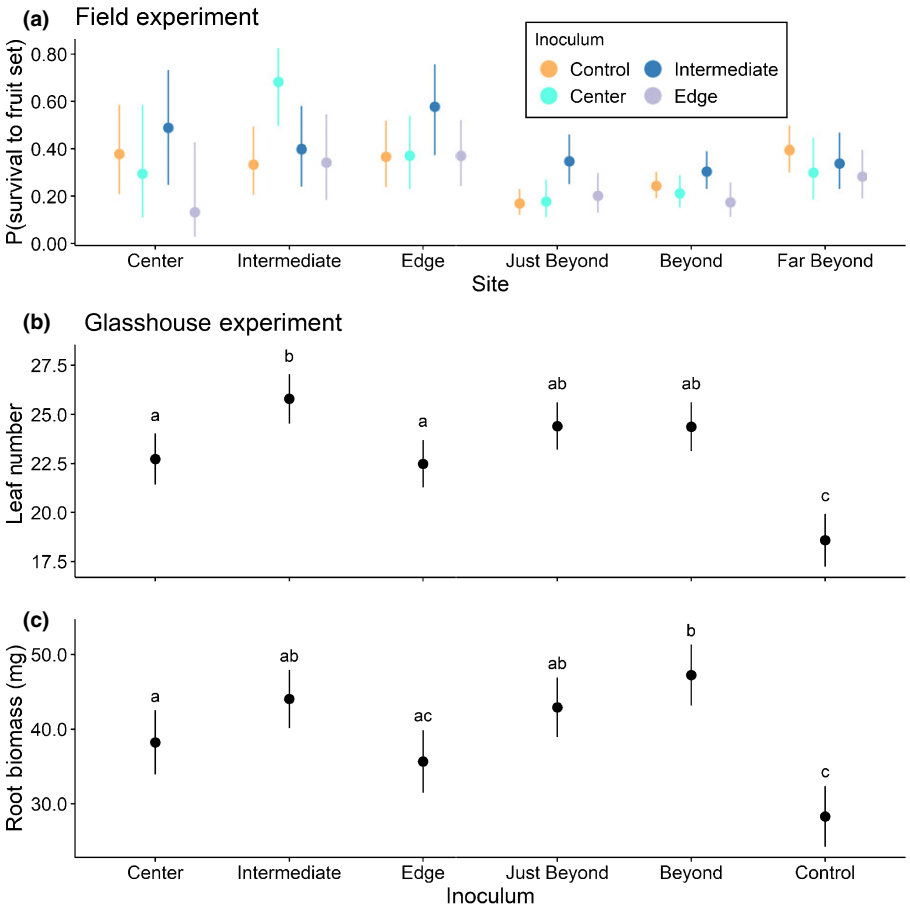

Fig. 2 Effects of inoculum treatments on plant fitness in the field and glasshouse. (a) Probability of *Clarkia x. xantiana* survival to fruit set, given early survival, for each inoculum treatment in year 2 of the field experiment. Values (± 95% CI) are estimated marginal means from the logistic regression of survival to fruit set on site, source population, inoculum, caging treatment, and their interactions, averaging over source populations and caging treatment. Inoculum *n* = Center, 14–27; Intermediate, 30–53; Edge 27–62; Just Beyond, 98–217; Beyond, 122–284; Far Beyond, 50–98. (b, c) Effect of microbial inocula from within and beyond *C. x. xantiana*'s range on (b) leaf number (*n* = 67–82) and (c) root biomass (*n* = 49–58) in the glasshouse. Values (± 95% CI) are estimated marginal means from linear models of each response on source population, inoculum, and their interaction, averaging over source populations and benches. Letters indicate Tukey groupings at α = 0.05.

Table 3 Summary of Type II ANOVAs testing effects of source population, inoculum, and their interaction, and bench position, on *Clarkia x. xantiana* growth in the glasshouse.

Term	df	Root mass Res. df = 301	Leaf number Res. df = 433
Population	2	**31.1*** **	**27.8*** **
Inoculum	5	**11.3*** **	**14.0*** **
Bench	3	0.6	**9.7*** **
Population × inoculum	10	0.7	0.7

Values are *F* ratios, with asterisks indicating significance of term in Type II tests. Bolded values remain significant after adjusting for multiple tests with the Holm method. Root mass was measured on *c.* 70% of the experimental plants, hence the lower residual degrees of freedom.
***, $P < 0.001$.

or Edge inocula, but on par with those grown with Intermediate inocula.

Local and range adaptation There was no evidence of local adaptation to soil microbial communities among populations (Table 3) under either the *home* vs *away* or *local* vs *foreign* criteria (Fig. S7). There was also no evidence that performance differed between plants grown with within- vs beyond-range inoculum (contrast $P > 0.15$ for all source populations; Fig. 2b,c).

Soil microbial communities in root and rhizoplane

A total of 8945 ASVs were recovered across all glasshouse samples (6166 bacterial, 2779 fungal; Table 4) and rarefaction curves were nearly saturated for most samples (Fig. S8). Overall, bacterial and fungal ASV richness and diversity was higher in the rhizoplane soil than root, and similar across the five live inocula. The exception was root fungal communities – beyond-range sites tended to have fewer fungal ASVs than within-range sites (mean richness beyond-range: 84 ASVs, within-range: 115 ASVs), and also had lower average read abundances. Control inoculum had lower bacterial and fungal richness and diversity than all live inocula.

Composition of both bacterial and fungal communities clustered foremost by sample origin (rhizoplane soil vs root; Fig. 3). In the rhizoplane soil, within- and beyond-range sites differed significantly in community composition for bacteria ($F_{1,33} = 9.8$; $P < 0.001$) and fungi ($F_{1,31} = 11.4$; $P < 0.001$). The PCoA showed the separation between within- and beyond-range samples as well as among all sites individually; the first and second PCoA axes explained *c.* 25% and 13% of variation (Fig. 3a). There were no readily observable patterns in relative abundance of bacterial or fungal classes among inocula, indicating samples were clustering based on differences at finer taxonomic scales (Figs 3b, S9). The one exception was the rhizoplane fungal community of plants grown with Control inoculum, which was

Table 4 Summary of amplicon sequence variant (ASV) richness (number of observed ASVs), diversity (Shannon's *H*), and number of reads (in thousands) per sample for fungal and bacterial communities in roots and rhizoplanes of *Clarkia x. xantiana* grown with various field inocula in the glasshouse experiment.

	Rhizoplane						Root					
	Bacteria			Fungi			Bacteria			Fungi		
Inoculum	*n*	*ASVs*	*H*	*Reads (k)*	*n*	*ASVs*	*H*	*Reads (k)*	*n*	*ASVs*	*H*	*Reads (k)*	*n*	*ASVs*	*H*	*Reads (k)*
Center	8	2547 ± 81	7.0 ± 0.03	38.0 ± 2.7	6	429 ± 30	4.0 ± 0.08	57.1 ± 4.8	7	355 ± 43	4.3 ± 0.17	17.9 ± 2.7	8	105 ± 10	2.1 ± 0.19	68.7 ± 3.8
Intermediate	6	2613 ± 48	6.9 ± 0.03	8.4 ± 2.3	6	454 ± 50	3.9 ± 0.08	59.9 ± 10.2	6	363 ± 57	4.3 ± 0.27	17.6 ± 1.2	6	128 ± 14	3.1 ± 0.18	66.1 ± 5.2
Edge	5	554 ± 60	6.9 ± 0.05	40.2 ± 1.4	5	510 ± 19	4.0 ± 0.03	76.5 ± 6.8	5	388 ± 41	4.3 ± 0.21	21.0 ± 3.3	6	112 ± 5	2.7 ± 0.19	73.0 ± 7.8
Just Beyond	8	2537 ± 41	6.8 ± 0.03	43.9 ± 2.8	8	389 ± 9	3.6 ± 0.04	78.1 ± 5.3	4	352 ± 47	4.1 ± 0.22	20.2 ± 4.5	7	78 ± 10	2.6 ± 0.25	48.5 ± 8.5
Beyond	8	2525 ± 43	7.0 ± 0.01	40.9 ± 1.9	8	553 ± 22	4.0 ± 0.07	68.2 ± 5.2	6	341 ± 33	4.3 ± 0.11	15.9 ± 2.2	8	90 ± 5	2.8 ± 0.14	59.3 ± 5.4
Control	8	1123 ± 60	5.4 ± 0.16	53.7 ± 2.4	8	80 ± 8	2.1 ± 0.27	75.9 ± 2.5	5	299 ± 41	4.0 ± 0.16	25.6 ± 3.8	6	26 ± 4	1.5 ± 0.17	55.6 ± 6.0

Values are mean ± SE.

distinct in composition from those grown with live inocula and was dominated by Pezizomycetes.

In the roots, within- and beyond-range inocula also differed, but less strongly so, for bacteria ($F_{1,26} = 1.9$; $P = 0.003$) and fungi ($F_{1,33} = 5.3$; $P < 0.001$; Fig. 3). Bacterial communities did not group by inoculum type, except for Control inoculum, where samples were characterized by a lower relative proportion of Actinobacteria and Alphaproteobacteria compared to plants grown with live inocula. By contrast, root fungal communities largely clustered by inoculum type. Plants grown with beyond-range inocula had roots with relatively more reads from Agaricomycetes and fewer from Sordariomycetes than those grown with within-range inocula. Root fungi that were relatively over abundant within-range were largely Sordariomycetes, but the single most differentially abundant taxon was identified as *Olpidium brassicae* (Olpidiomycota) (Fig. S9b). Plants grown with Intermediate inoculum had root fungal communities that largely clustered separately from other inocula (Fig. 3b) and had relatively large proportions of reads from Eurotiomycetes and Leotiomycetes. For microbial communities in both root and rhizoplane soil, ordinations based on the Jaccard index were similar to those based on Bray–Curtis distances (Fig. S10).

The Intermediate inoculum conferred benefits to plant fitness in both the field and glasshouse, and root fungal and rhizoplane bacterial communities of plants grown with the Intermediate inoculum largely clustered separately from other samples (Fig. 3a). Thus, we were interested in the root fungal and rhizoplane bacterial ASVs that were significantly more abundant in plants grown with the Intermediate inoculum. For the Intermediate inoculum, differential abundance analyses indicated that there was a diverse set of 47 bacterial ASVs overabundant in rhizoplane soils and nine fungal ASVs overabundant in roots (Table S2). Five of these fungal ASVs were in Helotiales (Leotiomycetes) and Pleosporales (Dothideomycetes), two were in Sordariomycetes, one was an unknown Agaricomycete, and another an unknown Ascomycota.

Discussion

Although microbes are well known to affect plant performance, we have a poorer understanding of how spatial variation in soil microbial communities influences larger scale patterns in plant fitness and the location of geographic range limits. We found strong spatial structure among microbial communities within and outside *C. x. xantiana*'s range, and this variation affected components of plant fitness in the glasshouse and field. In the field, there was a three-way interaction between site, source population, and inoculum treatment in predicting lifetime fitness, but specific contrasts showed little support for plant local adaptation, or maladaptation, to soil microbial communities. However, inoculum from one site, Intermediate, increased the probability of survival to fruit set. This same site increased plant growth in the glasshouse, where there was also evidence supporting the hypothesis that beyond-range microbial communities were less pathogenic than those within-range. Overall, our results indicate the potential for both enemy release and mutualist limitation

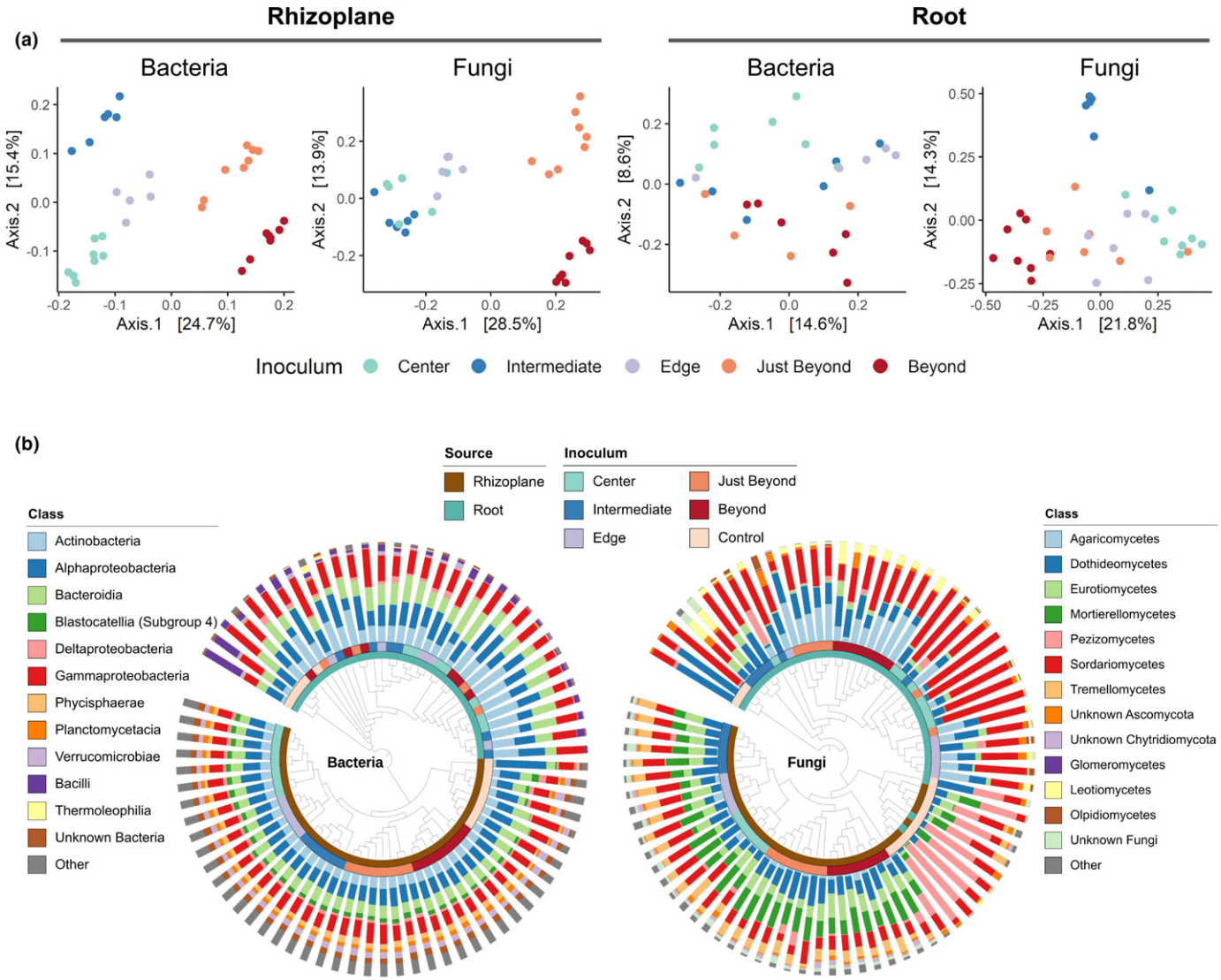

Fig. 3 Microbial community composition in the rhizosphere. (a) Principal coordinates analysis (PcoA) of Bray–Curtis distance matrices comparing bacterial and fungal community composition among rhizoplane soils and roots of *Clarkia x. xantiana* plants grown with different inocula in the glasshouse. (b) Clustering of bacterial and fungal communities via unweighted pair group method with arithmetic mean (UPGMA); each tree tip represents a separate root or rhizoplane soil sample from the glasshouse experiment. Sample source compartment (rhizoplane soil vs root) is shown on the innermost ring beyond the tips of the tree; inoculum treatment is shown in the second ring. The outer bar charts show the relative abundance of the top 10 most abundant bacterial and fungal classes. ('Top 10' abundance was assessed within root and rhizoplane separately; thus, there are more than 10 classes shown in the legends in order to capture the top 10 classes from each source compartment).

outside the range limit. This work highlights how the interplay of mutualistic and antagonistic microbial effects influence plant fitness inside and outside the range, and demonstrates the capacity for plant–microbe interactions to influence plant distributions.

Enemy release and mutualist limitation outside the range boundary

In the glasshouse, plants grown with beyond-range inocula tended to be larger than those grown with Center and Edge inocula, and on par with plants grown with Intermediate inocula. In addition, plants grown with inocula from beyond-range had fewer fungal taxa and fewer fungal reads from root tissue, compared to those grown with within-range inocula. Together, these results suggest partial release from fungal root pathogen pressure outside the range. Furthermore, in the field in year 2, when precipitation was not limiting, mean fitness of plants was higher outside the range than inside (especially when protected from herbivory). Although this fitness increase was likely influenced by lower competition outside the range, microbial enemy release likely also contributed (even with inoculum added from inside the range, the majority of the soil environment experienced by all experimental plants would be local soil). Enemy release beyond the range could reflect negative plant–soil feedbacks within the range, lower productivity outside the range (e.g. low host abundance leads to fewer generalist enemies), or both. The

most differentially abundant root fungal ASV between within- and beyond-range inocula was identified as *O. brassicae*, a generalist root pathogen, supporting the notion that more productive sites within the range harbor more generalist pathogens than the arid, less productive sites beyond the range. It is also possible that some microbial taxa switch from being functionally pathogenic to mutualistic in the more abiotically stressful sites beyond-range (context-dependency *sensu* Johnson *et al.*, 1997; Hoeksema *et al.*, 2010).

Although pathogen loads may be smaller beyond-range, our results are also consistent with the potential for mutualist limitation at and outside the range edge. Addition of inoculum from the Intermediate site increased a plant's probability of survival to fruit set (given early survival) by 25–50% in sites at and beyond the range edge (except the Far Beyond site). In the glasshouse, soil microbes from this same site also increased growth. (Interestingly, this benefit was more apparent in comparison with other within-range inocula as opposed to beyond-range inocula, which our results suggest is likely due to the growth benefits that enemy release afforded in the latter). Though the field experiment measured components of fitness directly, while the glasshouse experiment measured a fitness proxy, these results in aggregate suggest the possibility that there are soil mutualists present at the Intermediate site that can increase growth and/or survivorship, and that presence of these beneficial microbes may be especially helpful in the stressful environments at and beyond the range margin. Most of the fungal taxa disproportionately abundant in plants grown with the Intermediate inoculum were in Helotiales and Pleosporales, orders that contain many dark septate endophytes, which have been found in > 600 plant species and shown to increase plant biomass (Newsham, 2011). Our results further suggest that beneficial microbial communities may be patchily distributed in *C. x. xantiana*'s range interior, as the Center inoculum did not produce the growth benefits produced by the Intermediate inoculum.

Local adaptation and maladaptation within the range

There is increasing evidence that spatial variation in soil microbial communities could contribute to the heterogeneous performance of plant populations (in growth rate, extinction probability, etc.) across a species' range (e.g. David *et al.*, 2019). Our study adds support to this hypothesis by demonstrating that one site, Intermediate, hosted a particularly beneficial microbial community in relation to other sites within-range. By contrast, local adaptation of plant populations to their home microbial communities could dampen variability in plant population performance across the range. There was no strong evidence that plant populations were locally adapted to their home soil microbial communities in our study. Plant–microbial community local adaptation may be rare due to the accumulation of specialized microbial pathogens within sites (*sensu* Janzen, 1970), and even if plant populations were locally adapted to certain microbial mutualists, negative pathogen effects could mask positive mutualist effects. Indeed, the vast difference in generation time between plants and their microbial pathogens may often tip the balance of any coevolutionary dynamics toward rapidly reproducing microbes. However, in this study plant populations did not appear to be maladapted to their local microbial pools, either, which we might expect if specialized pathogen effects dominate local plant–microbe interactions. Because microbial communities consist of thousands of distinct microbial populations spanning the parasite–mutualist continuum, clear patterns in adaptation of individual plant populations to their home microbial communities may be rare (Thrall *et al.*, 2007; Lankau & Keymer, 2018; Briscoe Runquist *et al.*, 2020).

Range limits in complex environments

Previous work has demonstrated that drought stress, herbivory, and pollen limitation all reduce mean fitness outside *C. x. xantiana*'s range margin (Geber & Eckhart, 2005; Eckhart *et al.*, 2011; Moeller *et al.*, 2012; Benning *et al.*, 2019; Benning & Moeller, 2019). In this experiment we observed strong differentiation in soil microbial communities across the range boundary, and asked, does this microbial variation further contribute to *C. x. xantiana*'s range limit? Our work suggests that both enemy release and, perhaps to a lesser extent, mutualist limitation are likely beyond the range limit. Thus, it is not entirely clear whether the novel microbial communities we observed outside the range would, overall, facilitate or hinder range expansion. Notably, the relative effects of these two contrasting mechanisms on a colonizing *C. x. xantiana* population would likely vary according to abiotic context. Interannual variation in precipitation, which is especially high outside the range (Eckhart *et al.*, 2011), means that some years will be strongly limited by the abiotic environment (e.g. year 1 in this experiment) while others will not (e.g. year 2). Our results suggest that biotic interactions have relatively small effects in years when abiotic conditions are harsh. However, when precipitation is higher, biotic interactions play a larger role in mediating plant fitness outside the range, as seen in year 2 in regard to mammal herbivory (Benning & Moeller, 2019) and soil microbial communities (current results). With plants 'released' from limiting precipitation in year 2, lower pathogen loads likely increased fitness outside the range, and there was some evidence of mutualist limitation at and outside the range edge. In years of more average precipitation, mutualist limitation may be more important if microbes help mediate plant–water relations (Augé, 2001; Lau & Lennon, 2012).

Implications for shifting plant distributions

Soil microbial populations may respond very differently to climate change than their host plant populations. Many factors structuring microbial distributions (e.g. soil temperature, soil nutrient concentrations, substrate) will most often not change synchronously with air temperature (Gehrig-Fasel *et al.*, 2008; Zamin *et al.*, 2014). If microbial populations do not migrate at the same speed as their plant hosts, shifting plant populations will likely encounter novel soil microbial environments (e.g. Ramirez *et al.*, 2019). This decoupling of historical plant–microbe interactions could have consequences for plant population dynamics

and may help explain the large variation in the responses of plant distributions to climate change (e.g. Chen *et al.*, 2011; Rumpf *et al.*, 2018). As we have shown, novel microbial communities outside the current range could have both positive (via enemy release) and negative (via mutualist limitation) effects on plant fitness, with the overall effect varying with environmental context. A greater understanding of aboveground–belowground interactions, especially the response of plant populations to novel microbial communities, is needed to accurately forecast species distributions as the climate changes (Van der Putten, 2012).

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Author contributions

JWB and DAM planned and designed the research. JWB performed experiments, conducted fieldwork, and analyzed data, with assistance from DAM. JWB wrote the manuscript, with DAM contributing substantially to revisions.

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Data availability

All data and code needed to replicate these analyses are available at https://doi.org/10.6084/m9.figshare.c.5209067. Raw sequence data were deposited in the NCBI Sequence Read Archive (PRJNA612331).

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Schematic showing experimental design of field experiment.

Fig. S2 Cumulative precipitation across the growing season (October – June) in the field experiment.

Fig. S3 Estimated mean lifetime fitness across sites, source populations, and inoculum treatments for the field experiment in year 1.

Fig. S4 Estimated mean lifetime fitness across sites, source populations, and inoculum treatments for the field experiment in year 2.

Fig. S5 Effects of site and inoculum source on seed set of fruiting plants for each source population in year 2 of the field experiment.

Fig. S6 Effects of source population and inoculum source on root biomass in the glasshouse experiment.

Fig. S7 Effects of source population and inoculum source on leaf number in the glasshouse experiment.

Fig. S8 Rarefaction curves for microbial ASV richness in root and rhizoplane samples from the glasshouse experiment.

Fig. S9 Composition (by Class) of the subset of bacterial and fungal taxa identified as significantly more abundant in within-range or beyond-range sites.

Fig. S10 PCoA for Jaccard similarity index matrices comparing bacterial and fungal community composition among inoculum sources from the glasshouse experiment.

Methods S1 Field experiment: seed sourcing.

Methods S2 Field experiment: local and range adaptation contrasts.

Methods S3 Field experiment: predicting mean lifetime fitness.

Methods S4 Rhizosphere sampling and DNA extraction.

Table S1 Summary of LRT contrasts comparing lifetime fitness estimates between inoculum sources in the field experiment.

Table S2 Root fungal and rhizoplane bacterial ASV's overly abundant in plants grown with Intermediate inoculum compared to the four other live inocula.

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Appendix A1

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