New Phytologist

***New Phytologist* Supporting Information**

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

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

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Methods S1 Field experiment: seed sourcing

Methods S2 Field experiment: local and range adaptation contrasts

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Methods S4 Rhizosphere sampling and DNA extraction

Fig. S1 Schematic showing experimental design of field experiment. At each of the six sites, we arranged six blocks across the extent of natural *C. x. xantiana*, or, beyond the range, *C. x. parviflora* populations. Each block was comprised of 20 planting grids, spaced at least 1 m apart. Within each planting grid we sowed seeds from the three source populations [Center (C; orange below), Intermediate (I; blue), Edge (E; green)] into individual cells (5 and 4 cells per source population per grid in years 1 and 2, respectively; the actual grids had 36 cells each). Source populations were randomly assigned cell positions in each grid. Each grid received soil inocula from one of the focal within-range sites (C, I, or E), or a local control. 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; inoculum treatments were randomly assigned to grids. Within each inoculum treatment, half of the grids were caged with open-topped 0.6-m high herbivore exclosures made from 1.3-cm galvanized steel mesh (dashed outlines below); these caging treatments were randomly assigned to grids.

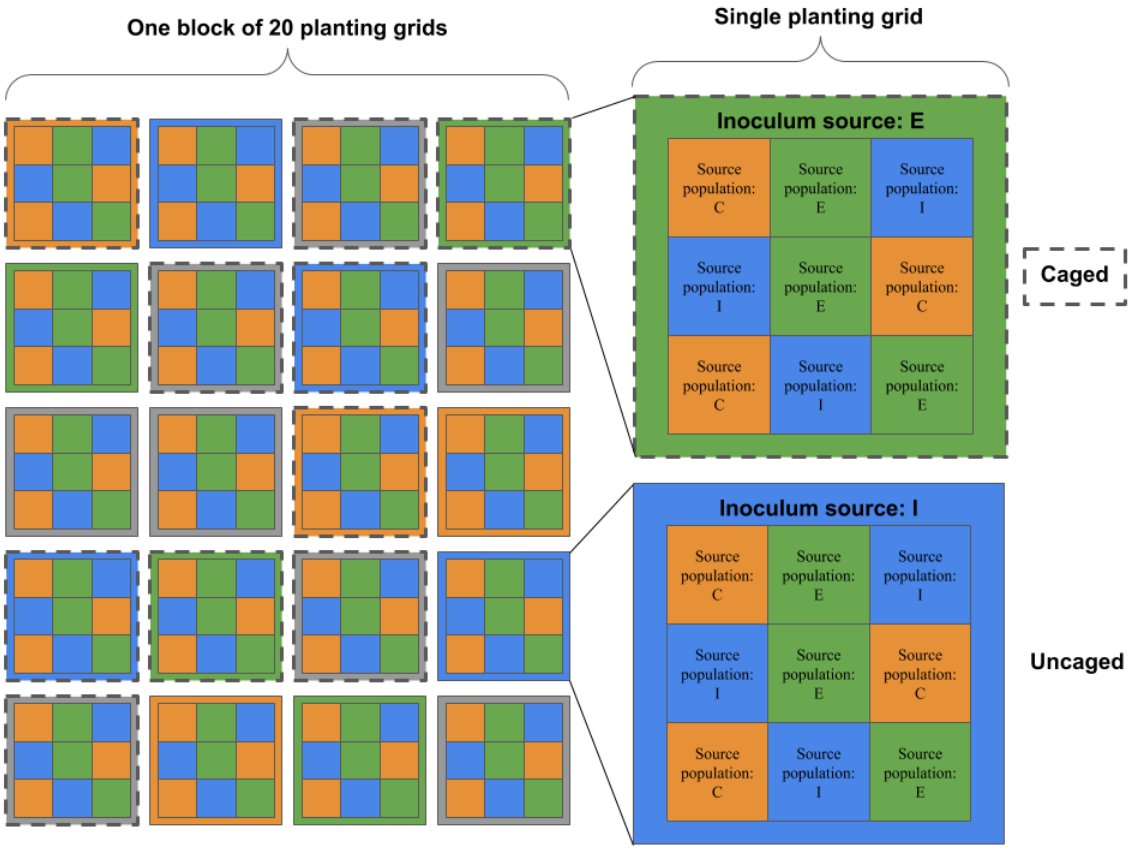

Fig. S2 Cumulative precipitation across the growing season (October - June) in the field experiment. Shown are precipitation patterns during the transplant experiment (year 1: blue lines; year 2: green lines), using data from weather stations at or near the sites. We also plotted precipitation for the years 1990 - 2017 at each site location (thin grey lines), using interpolated estimates from PRISM, to help interpret study year precipitation patterns in the context of long term trends (dashed black line shows long term trend with 95% confidence band). Precipitation data for the Beyond site in year 2 was unavailable due to a wildfire destroying our weather station.

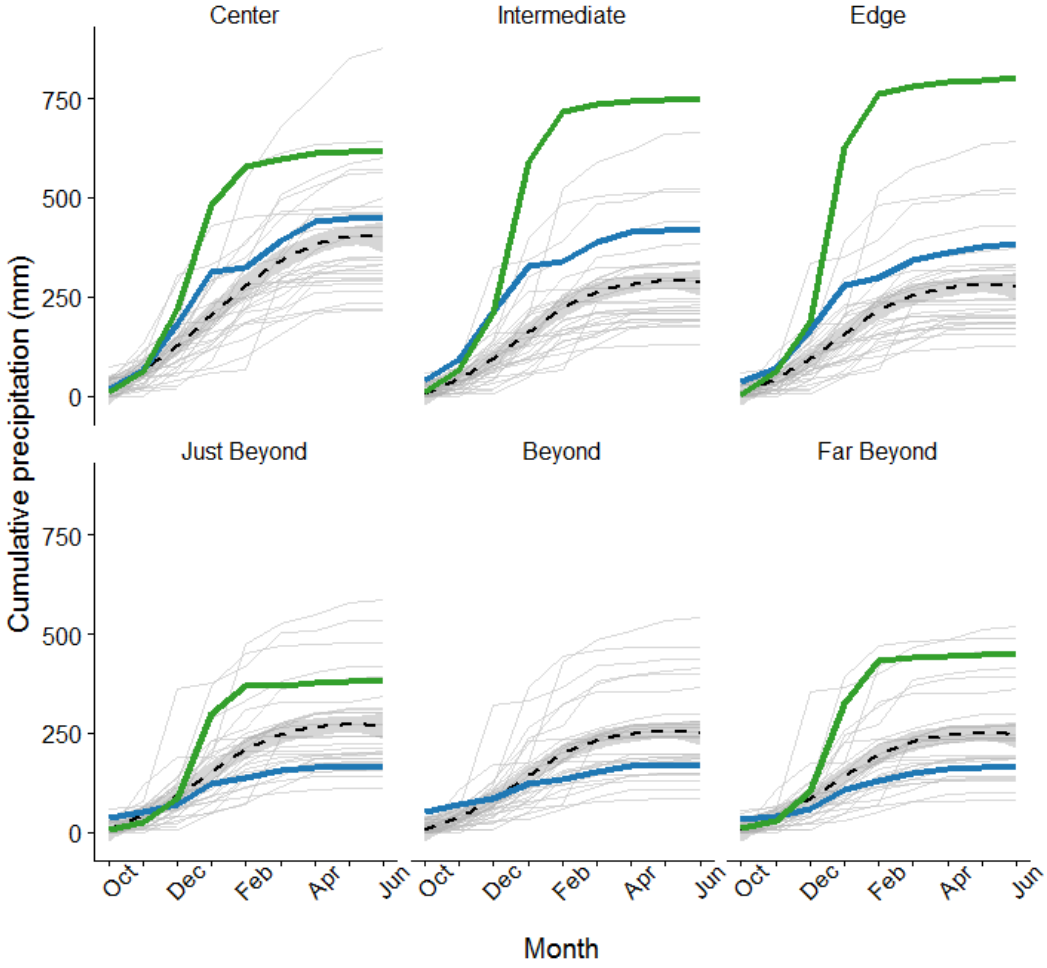

Fig. S3 Estimated mean lifetime fitness (± 95% CI) across sites, source populations, and inoculum treatments for the field experiment in year 1, as estimated from the full *aster* model. Figure shows uncaged plants in the left panel and caged plants in the right panel. Note that the Y axis is on the log scale.

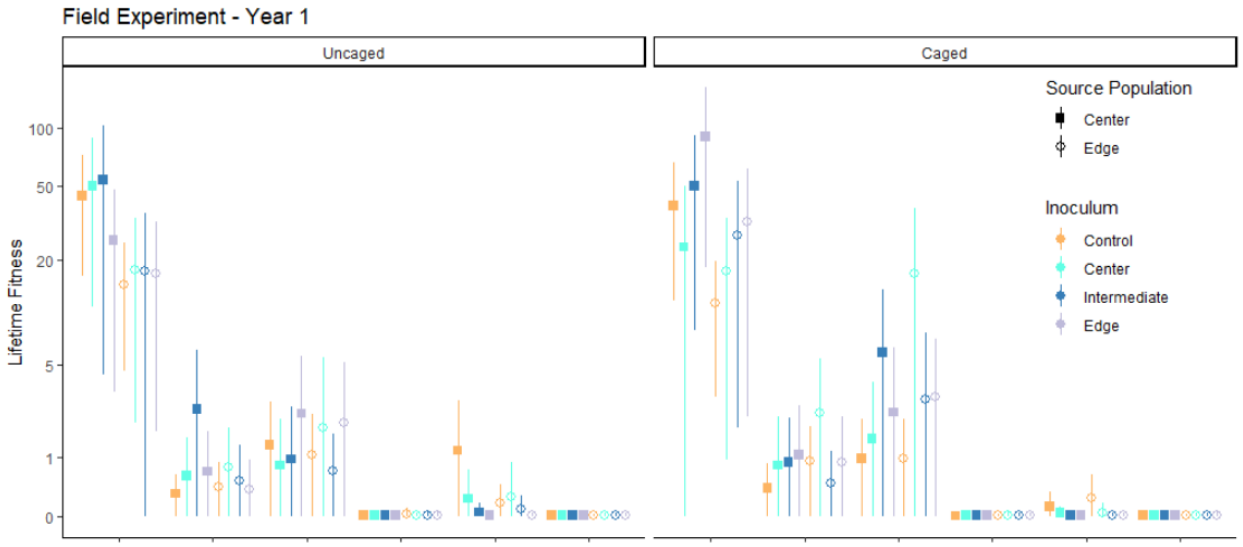

Fig. S4 Estimated mean lifetime fitness (± 95% CI) across sites, source populations, and inoculum treatments for the field experiment in year 2, as estimated from the full *aster* model. Panels are split by caging treatment (left: uncaged, right: caged) and seed planting year (top: 2015, bottom: 2016).

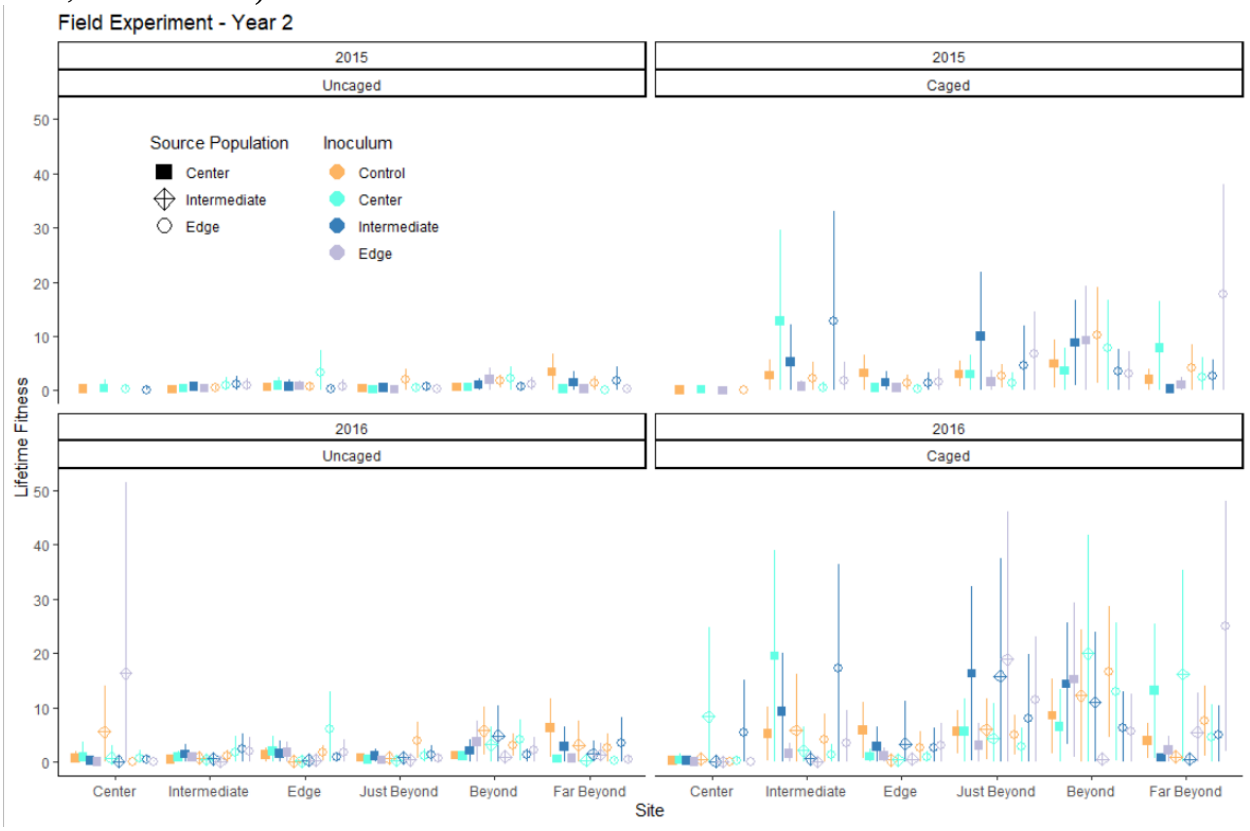

Fig. S5 Effects of site and inoculum source on seed set of fruiting plants for each source population in year 2 of the field experiment; source populations are displayed in separate panels. Estimates (± 1 SE) are estimated marginal means from the negative binomial regression of seed set on site, source population, inoculum, caging treatment, and their interactions. Estimates are averaged over caging treatment. Note Y-axis is on the log scale.

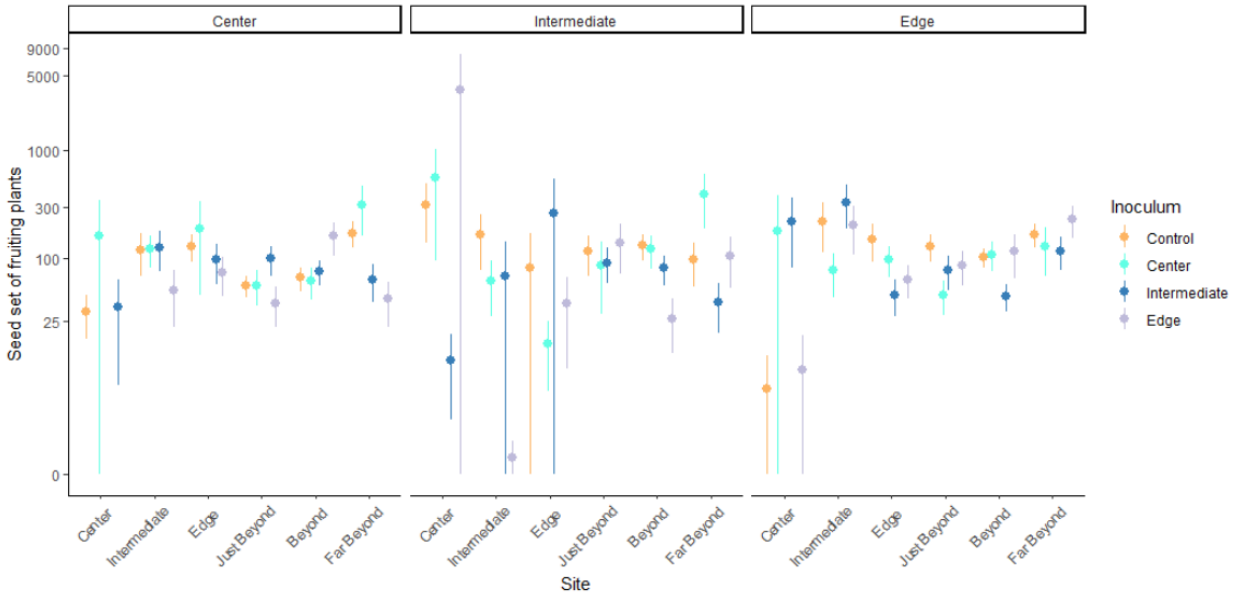

Fig. S6 Effects of source population and inoculum source on root biomass in the glasshouse experiment.

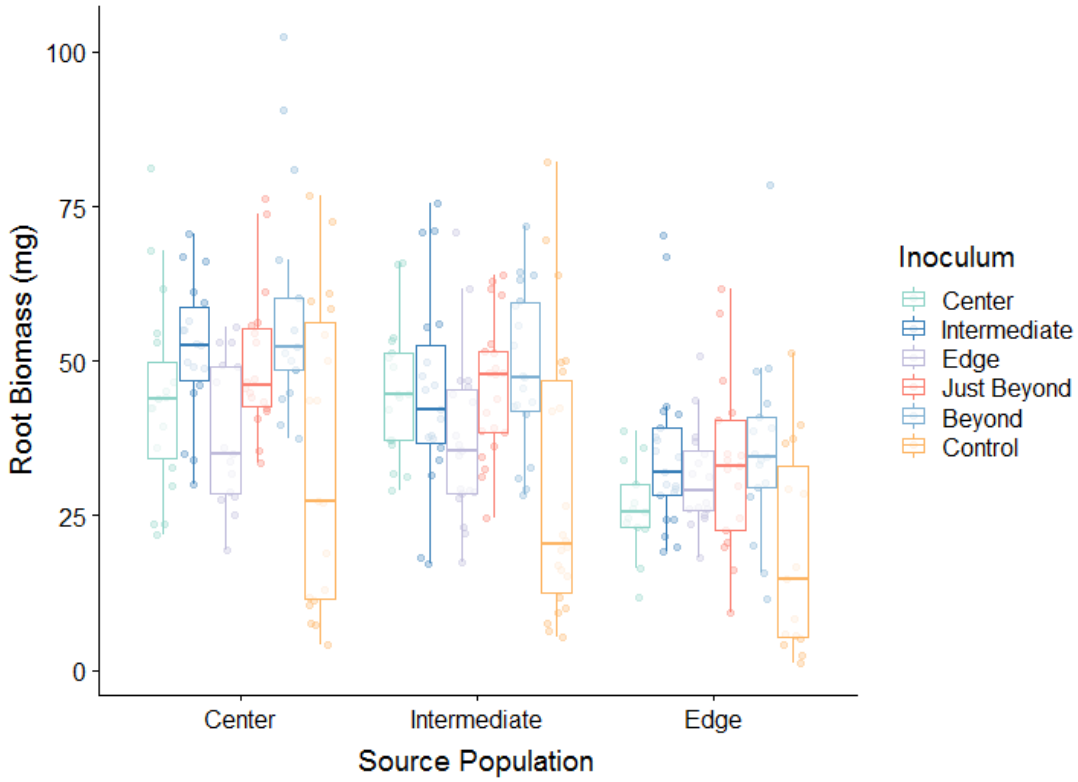

Fig. S7 Effects of source population and inoculum source on leaf number in the glasshouse experiment.

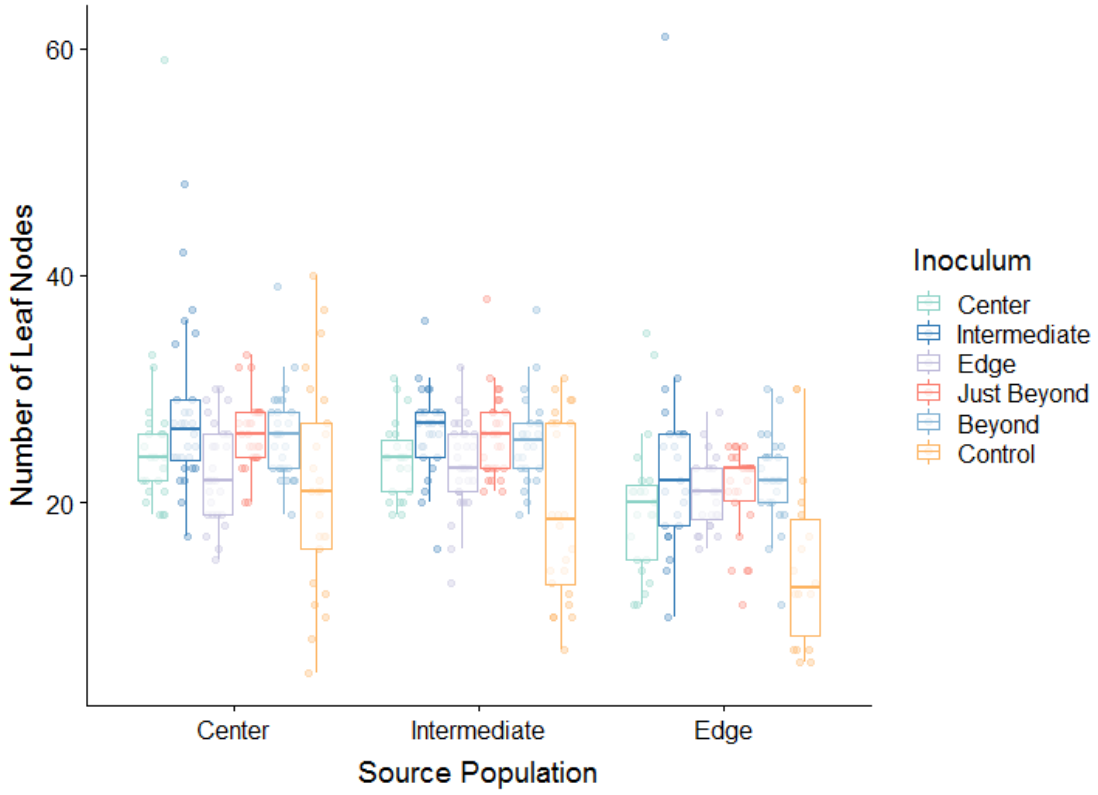

Fig. S8 Rarefaction curves for microbial ASV richness in root and rhizoplane samples from the glasshouse experiment. Each line is a separate sample. Line colors indicate sample inoculum source (black: Center, red: Intermediate, green: Edge, dark blue: Just Beyond, cyan: Beyond, pink: Control).

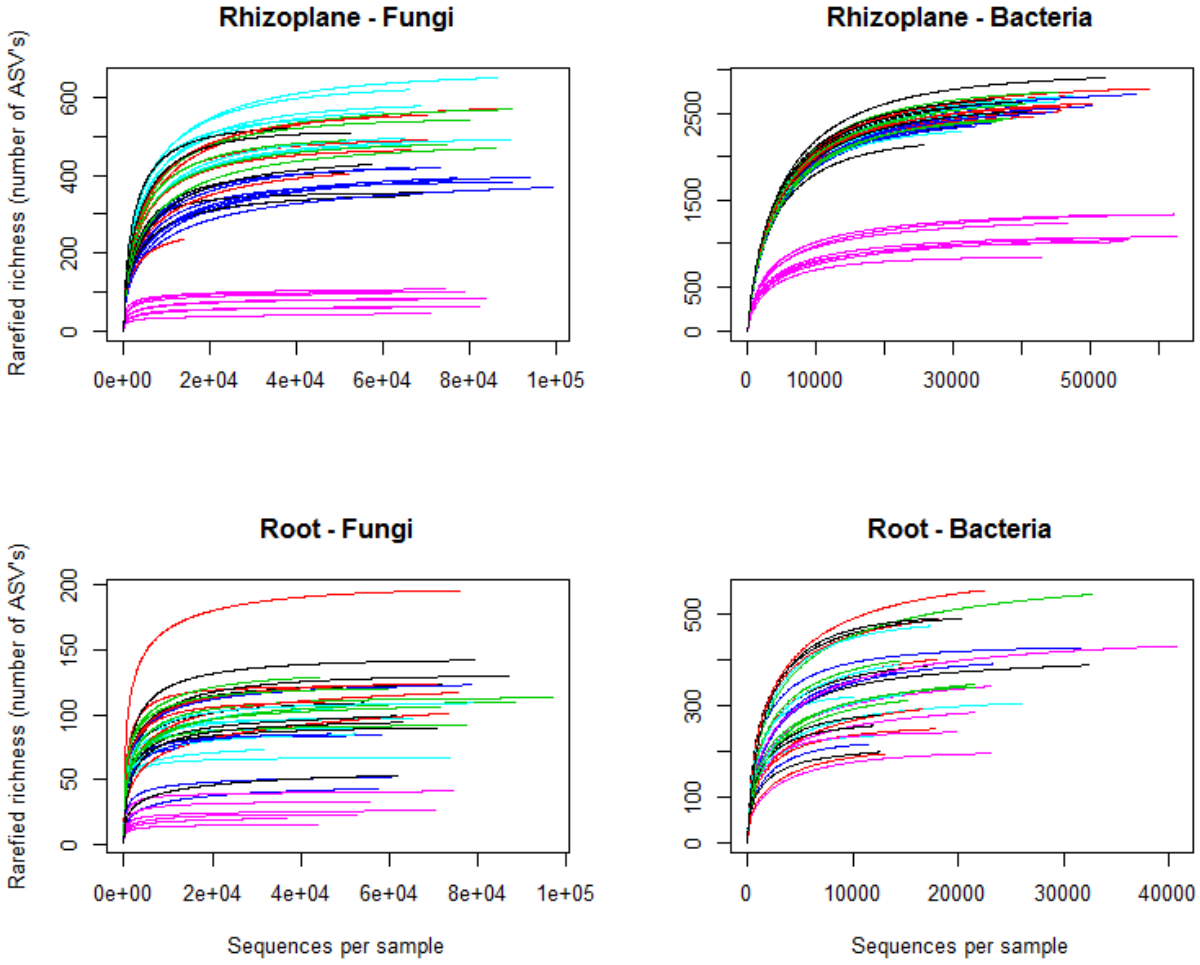

Fig. S9 Composition (by Class) of the subset of (a) bacterial and (b) fungal taxa identified as significantly more abundant in within-range or beyond-range sites. Proportional representation calculated as the number of ASV's belonging to a taxonomic class, divided by the total number of ASV's in that group [(*n*) at bottom of plots].

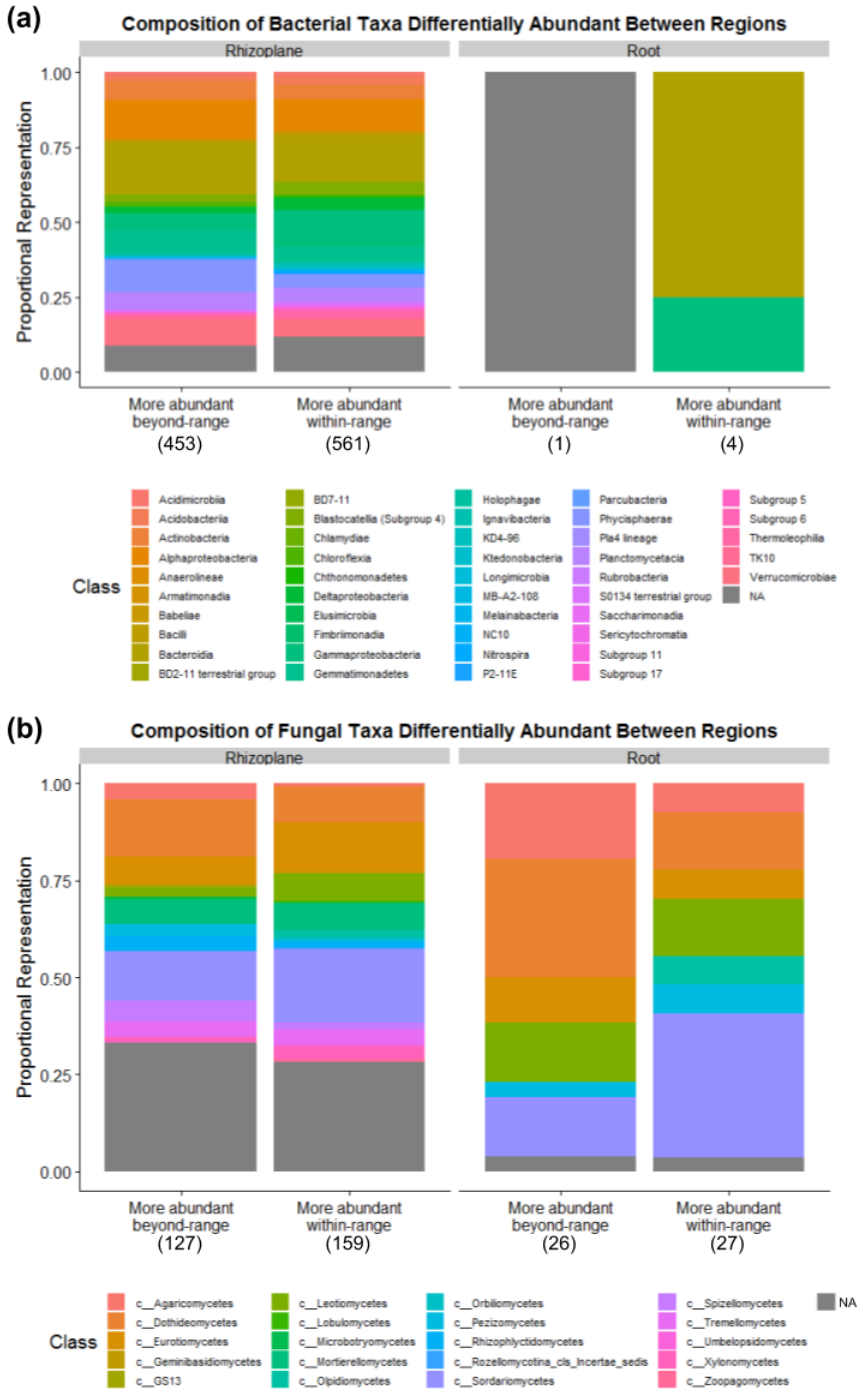

Fig. S10 PCoA for Jaccard similarity index matrices comparing bacterial and fungal community composition among inoculum sources from the glasshouse experiment.

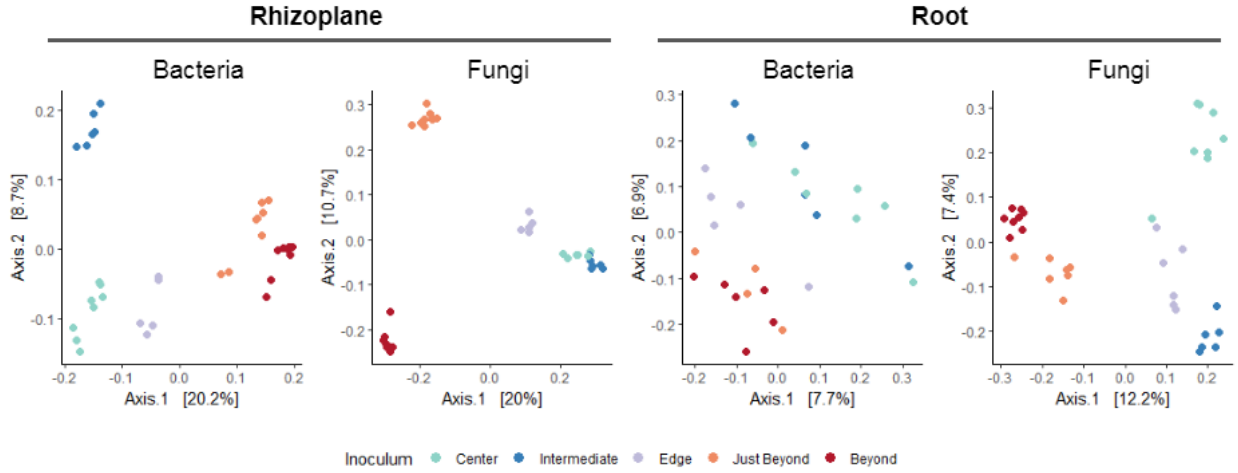

Table S1. Summary of *aster* LRT contrasts comparing lifetime fitness estimates between inoculum sources in year 2 of the field experiment. The first set of contrasts (at Center, Intermediate, and Edge sites) asks, for each source population, whether lifetime fitness differed between plants grown with control inoculum and those grown with their home site inoculum. Here, higher fitness with one's home inoculum indicates adaptation, while the reverse indicates maladaptation. The second set of contrasts (at Just Beyond, Beyond, and Far Beyond sites) asks, for each source population, whether the addition of any of the three soil inocula from within *xantiana*'s range improved lifetime fitness of plants when planted outside the range limit. Here, higher fitness with inoculum from inside the range indicates adaptation, while the reverse indicates maladaptation. Bold *P* values remain significant at $\alpha < 0.05$ after Holm adjustment. "NS" refers to "not significant" contrasts.

Site	**Source Population**	**Inoculum Contrast**	**Result**	χ^2	***P***
Center	Intermediate	Intermediate vs. Control	Maladaptation	7.1	**0.008**
	Edge	Edge vs. Control	NS	0.6	0.432
Intermediate	Center	Center vs. Control	Adaptation	8.5	**0.004**
	Edge	Edge vs. Control	NS	0.2	0.626
Edge	Center	Center vs. Control	Maladaptation	5.1	0.024
	Intermediate	Intermediate vs. Control	Adaptation	4.2	0.039
Just Beyond	Center	Center vs. Control	NS	0.03	0.874

		Intermediate vs. Control	Adaptation	17.4	**<0.001**
		Edge vs. Control	Maladaptation	3.3	0.068
	Intermediate	Center vs. Control	NS	0.4	0.528
		Intermediate vs. Control	Adaptation	2.6	0.014
		Edge vs. Control	Adaptation	1.9	0.038
	Edge	Center vs. Control	Maladaptation	4.6	0.032
		Intermediate vs. Control	NS	0.3	0.558
		Edge vs. Control	NS	1.2	0.264
Beyond	Center	Center vs. Control	NS	0.3	0.602
		Intermediate vs. Control	Adaptation	5.6	0.018
		Edge vs. Control	Adaptation	8.4	0.004
	Intermediate	Center vs. Control	NS	0.1	0.811
		Intermediate vs. Control	NS	0.2	0.631
		Edge vs. Control	Maladaptation	14.1	**<0.001**
	Edge	Center vs. Control	NS	0.3	0.607
		Intermediate vs. Control	Maladaptation	9.3	**0.002**
		Edge vs. Control	Maladaptation	7.9	0.005
Far Beyond	Center	Center vs. Control	NS	0.8	0.375
		Intermediate vs. Control	Maladaptation	5.0	0.025
		Edge vs. Control	Maladaptation	6.1	0.014
	Intermediate	Center vs. Control	Adaptation	10.9	**0.001**
		Intermediate vs. Control	NS	0.7	0.390
		Edge vs. Control	NS	2.0	0.158
	Edge	Center vs. Control	NS	3.4	0.066
		Intermediate vs. Control	NS	0.5	0.466
		Edge vs. Control	Adaptation	7.3	0.007

Table S2 Root fungal and rhizoplane bacterial ASV's overly abundant in plants grown with Intermediate inoculum compared to the four other live inocula in the glasshouse experiment. ASV taxonomy assigned with assignTaxonomy() function in the dada2 package. "NA" (not applicable) means that sample was not identifiable at a given taxonomic level.

Source	Kingdom	Phylum	Class	Order	Family
Root	Fungi	Basidiomycota	Agaricomycetes		
		Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae
			Sordariomycetes	Sordariales	Lasiosphaeriaceae
			Leotiomycetes	Helotiales	Hyaloscyphaceae
			Dothideomycetes	Pleosporales	Didymosphaeriaceae
			Sordariomycetes		
			Dothideomycetes	Pleosporales	Melanommataceae
			Leotiomycetes	Helotiales	Helotiaceae
Rhizoplane	Bacteria	Acidobacteria	Blastocatellia (4)	Blastocatellales	Blastocatellaceae
			Acidobacteriia	Solibacterales	Solibacteraceae (3)
			Holophagae	Subgroup 7	NA
			Blastocatellia (4)	Blastocatellales	Blastocatellaceae
			Actinobacteria	Propionibacteriales	Nocardioidaceae
			Actinobacteria	Micrococcales	Microbacteriaceae
			Acidimicrobiia	Microtrichales	Iamiaceae
			Actinobacteria	Kineosporiales	Kineosporiaceae
		Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae
			Bacteroidia	Cytophagales	Microscillaceae
			Bacteroidia	Chitinophagales	Chitinophagaceae
			Bacteroidia	Sphingobacteriales	Sphingobacteriaceae
			Bacteroidia	Cytophagales	Hymenobacteraceae
			Bacteroidia	Sphingobacteriales	Sphingobacteriaceae
			Bacteroidia	Chitinophagales	Chitinophagaceae
			Bacteroidia	Sphingobacteriales	Sphingobacteriaceae
			Bacteroidia	Cytophagales	Microscillaceae
			Bacteroidia	Chitinophagales	Chitinophagaceae
			Bacteroidia	Chitinophagales	Chitinophagaceae
			Bacteroidia	Cytophagales	Microscillaceae
		Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae
			Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae
			Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae
			Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae
			Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae
			Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae
		Planctomycetes	Phycisphaerae	Tepidisphaerales	WD2101 soil group
			Phycisphaerae	Tepidisphaerales	WD2101 soil group
		Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae
			Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae
			Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
			Deltaproteobacteria	Myxococcales	bacteriap25
			Gammaproteobacteria	NA	NA
			Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae
			Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae
			Alphaproteobacteria	Rhizobiales	Devosiaceae

			Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae
		Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae
			Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae
			Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae
			Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae
		NA	NA	NA	NA
		NA	NA	NA	NA
		NA	NA	NA	NA
		NA	NA	NA	NA
		NA	NA	NA	NA
		NA	NA	NA	NA

Methods S1

Field experiment: seed sourcing

In year 1, seeds used for planting were all collected from the field (30 to 70 maternal families per population). We did not include the Intermediate site in year 1 due to a seed collection error. Due to drought and thus low site productivity in 2016, we generated seed for year 2 planting in the glasshouse (26 to 30 maternal families per population). Seeds were bulked before planting (i.e., we did not keep track of maternal families).

Methods S2

Field experiment: local and range adaptation contrasts

If *aster* analyses indicated a significant three way interaction of site, source population, and inoculum, we focused on two main questions: first, within the range, how does addition of soil microbial inocula from a *xantiana* population's home site influence fitness when growing in novel environments? Thus, at each site within the range, for the two foreign populations, we asked whether lifetime fitness differed between plants grown with control inoculum and those grown with their home site inoculum. We tested this hypothesis by conducting a LRT [via anova.aster()] for two *aster* models (one with inoculum, caging treatment, and planting year terms, and the other with only caging treatment and planting year terms) built from subsets of the full data set (e.g., for the Intermediate source population growing with Intermediate or Control inoculum at the Center site). If addition of their "home" inocula consistently improved fitness of populations when planted into foreign sites, relative to plants grown with control inocula, this suggests adaptation of populations to their local soil mutualists. If addition of "home" inocula consistently depresses fitness in foreign sites, this suggests maladaptation of populations to their local soil pathogens. (Of course, soil communities will contain symbionts with both positive and negative effects, so these results capture the "net" effect of all microbes contained in an inoculum.) Second, we used LRTs as above to ask whether the addition of any of the three soil inocula from within *xantiana*'s range improved lifetime fitness of plants when planted outside the range limit. Thus, at each site beyond the range (Just Beyond, Beyond, and Far Beyond), for each population, we asked whether lifetime fitness differed between plants grown with control inoculum and those grown with each of the three within range inocula. Within the two families of pairwise contrasts (questions 1: 6 contrasts; question 2: 27 contrasts), we adjusted test *P*-values with a sequential Bonferroni (Holm) correction (SI Table S1).

Methods S3:

Field experiment: predicting mean lifetime fitness

The fitness metric of most interest was mean seeds produced per planted seed. Because we planted multiple seeds per cell, and culled extra germinants, fitness predictions from our *aster* model that includes germination as a simple bernoulli variable (the cell either contained germinant(s) or not) would be inflated relative to this metric. Thus, we obtained predicted values for lifetime fitness and their associated standard errors by taking the product of germination probabilities (estimated from the logistic regression for germination described 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. Standard errors for these products were calculated using the Delta method (Buehler, 1957).

Methods S4:

Rhizosphere sampling, DNA extraction and amplification, and bioinformatics

Rhizosphere sampling and DNA extraction

To characterize rhizosphere microbial communities, we extracted DNA from rhizoplane soil and root samples for 6-8 Center population individuals per inoculum treatment in the glasshouse experiment (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; SI Figs. S5, S6). We selected individuals from the range of plant size within each treatment. We harvested the entire root system soon after plants began flowering. For each plant sampled from the glasshouse experiment, we gently transferred the entire contents of the pot to a clean sheet of butcher paper. We used forceps to excavate the entire root system, then gently shook the roots until only ca. 1 mm of soil remained adhered to the root surface. Roots and the attached rhizoplane were then placed into sterile 50 ml tubes filled with 25 ml Phosphate Buffered Saline - Tween 0.2% (PBS-T).

We separated root and rhizoplane soil in the lab. Rhizosphere (root plus rhizoplane) samples were vortexed for 15 s, and the root mass removed and placed in a new 50 ml tube filled with 20 ml PBS-T. The remaining rhizoplane sample was vortexed for 10 s and then ca. 14 ml was immediately transferred into a 15 ml tube, filtered through sterile mesh gauze. These rhizoplane samples were centrifuged at 3200 rcf for 15 min, and all but 4 ml of supernatant discarded. The remaining pellet and supernatant were vortexed, and then 2 ml of the turbid mixture was quickly poured into a 2 ml microcentrifuge tube. These tubes were centrifuged at 10,000 rcf for 5 min, the supernatant discarded, and the remaining rhizoplane pellet lyophilized.

Roots in PBS-T were vortexed and the liquid decanted. Then roots were surface sterilized in 3% bleach + PBS for one min, and then washed twice in UltraPure water. Entire root masses were then transferred to 2 ml microcentrifuge tubes and lyophilized.

We extracted DNA from the entire lyophilized root system (ca. 40 mg) and the entire lyophilized rhizoplane soil pellet (ca. 60 mg) using Qiagen DNeasy PowerSoil kits with some modifications. We followed manufacturer protocols with a few modifications. For roots, we added 1 mm glass beads to sample tubes and disrupted samples with a TissueLyser at 30 hz for two one-min sessions, with a one-min rest between. We removed any remaining large root material, and then added the contents of the DNeasy PowerBead tubes to the sample tubes containing finely ground root material. Rhizoplane pellets were added directly to PowerBead tubes. Then, for roots and rhizoplane samples in PowerBead tubes, we disturbed samples for four two-min sessions in the TissueLyser at 30 hz, with one-min breaks between sessions. We

followed the rest of the kit protocol with three minor modifications: we performed an extra centrifugation step (one min at 10000 g) to more completely remove the C5 solution, we allowed the C6 solution to remain ca. 5 min on the filter, and we performed two 50 uL C6 washes to obtain a total of 100 uL extraction. Alongside our samples, we also extracted from both a negative control, and a positive fungal control (a fungal mock community comprising 12 synthetic "taxa", Palmer *et al.*, 2018) to aid in downstream data cleaning (Nguyen *et al.*, 2015).

Amplification and Illumina sequencing

We used amplicon sequencing to characterize rhizosphere bacterial and fungal communities. PCR amplification, library preparation, and sequencing were carried out at the University of Minnesota Genomics Center, Saint Paul, USA. Amplification and library preparation was completed using the dual-indexing protocol of Gohl *et al.* (2016). 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. Primers included Illumina adapters and multiplex barcodes. PCR products were pooled in equimolar concentrations and sequenced on an Illumina MiSeq (2 x 300 bp chemistry).

Microbial Bioinformatics

Reads were demultiplexed and adapters and primers were removed. Reads were filtered and trimmed using the filterAndTrim function in the dada2 pipeline (V4 truncated at 200bp forward, 160 bp reverse, maxEE = 2; ITS1 minimum length 50 bp; maxEE = 4), and amplicon sequence variants (ASVs) were inferred using dada2 (Callahan et al., 2016; Knight et al., 2018). Paired reads were merged, chimeras removed, and taxonomy assigned to bacteria and fungi using the Silva (Glöckner et al., 2017) and UNITE (Nilsson et al., 2019) databases, respectively.

We used phyloseq (McMurdie & Holmes, 2013), vegan (Oksanen et al., 2019), and DESEQ2 (Love, Huber, & Anders, 2014) for analysis of microbial community composition. We removed ASVs with fewer than 10 reads and reads assigned to Archaea, chloroplasts, mitochondria, or those unassignable. Abundances of non-mock ASVs in the fungal mock community were subtracted from each sample, as well as abundances of all ASVs found in the negative control (11 fungal ASVs; 27 bacterial ASVs; median ASV read abundance = 4; Nguyen et al., 2015). We removed any samples with fewer than 10,000 reads after the cleaning steps.

SI References (this Reference section is also included in the main article as Appendix A1)

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