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Plant-microbial interactions are strong determinants of plant population and community dynamics

Y. Anny Chung 9485698

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Yan-Yi Anny Chung
Candidate

Biology
Department

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Dr. Jennifer Rudgers , Chairperson

Dr. Scott Collins

Dr. Robert Sinsabaugh

Dr. Thomas Miller

Dr. Andrea Porras-Alfaro

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**PLANT-MICROBIAL INTERACTIONS ARE STRONG
DETERMINANTS OF PLANT POPULATION AND
COMMUNITY DYNAMICS**

by

YAN-YI ANNY CHUNG

A. B., Biology and International Studies,
Washington University in St. Louis, 2011

DISSERTATION

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**PLANT-MICROBIAL INTERACTIONS ARE STRONG DETERMINANTS OF
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ABSTRACT

Plant-microbial interactions are ubiquitous and yet the consequences of these interactions on plant population and community dynamics are relatively unknown. Here, we used two different classes of plant-microbial interactions to examine their effects on key plant population and community characteristics such as commonness and rarity, competition and coexistence, as well as community stability.

Vertically-transmitted endophytes had stage-dependent effects on the population growth of two grass species *Poa sylvestris* and *Poa alsodes*, and generally increased host population growth rates. However, it was the intrinsic demographic advantage of *P. sylvestris* that allowed its population to grow at a much faster rate compared to *P. alsodes* rather than endophyte benefits.

In a greenhouse experiment, we showed that plant-soil microbial feedbacks were important in regulating the strength of self-limitation, or negative frequency dependence, of a strong competitor *Bouteloua gracilis*. These negative feedbacks increased the potential for its coexistence with *Bouteloua eriopoda*.

In a field experiment, we showed that fungal-driven plant-soil feedbacks between *B. gracilis* and *B. eriopoda* may help explain long term patterns of spatial variation in temporal stability between these two species. Negative plant-soil feedbacks for *B. gracilis* could promote locally stable plant communities, and this effect was stronger when it was at low frequency in the community.

Finally, next-generation sequencing of root-associated fungal communities from the two preceding studies revealed strong differences in composition among different growth conditions as well as cultivation periods. In addition, experimental inoculation methods in the greenhouse and field reliably altered the root-associated fungal communities of test plants.

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Fungal symbionts maintain a rare plant population but demographic advantage

drives the dominance of a common host

Chung, Y. A., Miller, T. E. X. and Rudgers, J. A. (2015), Fungal symbionts maintain a

rare plant population but demographic advantage drives the dominance of a common

host. J Ecol, 103: 967–977.

Summary

1) A potential driver of species abundance that remains understudied is the interaction between host species and their microbial symbionts. Beneficial symbionts could promote the dominance of common host species by increasing their population growth rates more than they do for rare species, and symbiont benefits could be important for maintaining rare species in communities. Alternatively, intrinsic differences in demography, independent of interactions with symbionts, could be the main driver of species' relative abundances.

2) Here, we used demographic modelling with five years of data from experimental host populations to compare how symbiotic fungal endophytes, which are vertically transmitted from parent plant to offspring, influenced the population dynamics of one pair of co-occurring, congeneric rare versus common host grasses (genus *Poa*).

3) The common plant species achieved higher population growth than the rare species. Endophyte symbiosis increased the geometric population growth rate (λ) of rare and common species by 18% and 32%, respectively, but only the rare species was predicted to decline ($\lambda < 1$) in the absence of the endophyte, demonstrating that symbiosis was essential to maintain this species in the community.

4) Endophyte symbiosis differentially affected the demographic transitions of the two hosts, increasing survival and growth for the common host, *Poa sylvestris*, and increasing survival but decreasing the probability of flowering for the rare host, *Poa alsodes*. The total contribution of the endophyte effects on host demographic rates to the overall difference in population growth between host species was small compared to the plants' intrinsic differences in demography. However, low rates of vertical

transmission in *P. sylvestris* lessened its advantage in intrinsic demography over *P. alsodes* and thus decreased the projected difference in population growth between host plants.

5) *Synthesis.* Our results highlight the importance of plant-symbiont interactions in the persistence of a rare plant population, as well as the utility of demographic models in teasing apart the relative importance of plant demographic rates versus host-symbiont interactions on the regional abundance of rare and common host plant species.

Introduction

The majority of hypotheses to explain variation in species abundance focus on differences in species traits, such as reproductive investment, dispersal, and resource requirements (Kunin and Gaston, 1997). One potential driver of variation in abundance that remains understudied is the assemblage of microbial symbionts that grow in host tissues. Symbiosis with microbes is a ubiquitous phenomenon in nature (Douglas, 1994), but has historically been overlooked as a driver of host species abundance and distribution. This topic has received increasing attention during the past decade as tools for investigating microbiomes have developed, and keystone symbioses (e.g., corals-dinoflagellates) have responded to global change (Hoegh-Guldberg, 1999). In terrestrial systems, results indicate that plant-microbe symbioses can have strong influences on individual host plant fitness (Reynolds *et al.*, 2003, Bever *et al.*, 2012), which suggests that microbial symbiosis can be a driver of species-level variation in abundance within an ecosystem. For example, effects of plant-soil microbial interactions have been shown to be more detrimental to rare than common species in an old field system (Klironomos, 2002). Alternatively, instead of being more beneficial for common species, mutualistic symbioses could allow for the persistence of a rare species in the face of superior competitors (Phillips *et al.*, 2014). The effects of symbioses on host species abundance should be stronger in symbiotic interactions that are strongly coupled, such as in vertically-transmitted symbioses, since vertical transmission is expected to select for host-symbiont mutualism. However, the role of heritable symbiosis as a driver of variation in species abundance remains poorly understood.

In vertically-transmitted (from parent to progeny) symbioses, in which the benefits and costs of cooperation ultimately feedback to affect both parties, the fitness of host and symbiont are tightly coupled (Sachs *et al.*, 2004). In such situations, not only could symbionts differentially affect the population dynamics of rare and common hosts, but the population dynamics of hosts can also feedback to determine the population dynamics of the symbiont (Yule *et al.*, 2013). Due to positive fitness feedbacks, beneficial symbionts are expected to reach fixation in host populations (e.g. Ewald, 1987). However, imperfect vertical transmission, whereby some fraction of offspring from symbiotic parents are symbiont-free, can prevent symbiont fixation (Gundel *et al.*, 2008). Therefore, both variation in the vertical transmission rate and in the benefit of symbiosis to host population growth can determine natural frequencies of symbiosis (Gundel *et al.*, 2011, Miller and Rudgers, 2014). However, the relative importance of these two pathways to the fitness, frequency, and abundance of symbionts and hosts is poorly understood for natural populations.

Quantifying the effect of symbiosis on population dynamics can be challenging. The interaction outcome between symbionts and hosts can vary in magnitude and sign throughout the ontogeny of the host (Bronstein, 1994, Rudgers *et al.*, 2010). Therefore, the effects of a symbiont at any one stage of host development may not accurately indicate its net effects over the host's life time (Palmer *et al.*, 2010). Ontogenetic shifts also provide a potential mechanism by which rare and common host species can differ in the dynamics of host-symbiont interactions. Comparisons between rare and common species often show differences in life history strategies (Kunin and Gaston, 1997), which could interact with ontogenetic shifts in host-symbiont interactions throughout host life

cycles to differentially affect rare and common species. Thus, characterizing the ecological dynamics of symbioses (and other species interactions) across host ontogeny is essential to determining how these interactions are manifest at the population level (Rudgers *et al.*, 2010, Palmer *et al.*, 2010, Rudgers *et al.*, 2012). In order to evaluate the contribution of symbiosis to variation in host species abundance and population growth, it is necessary to separate the differential outcomes of symbiosis from difference in intrinsic (symbiont-free) host demography, a goal we address here for the first time.

Demographic models are a useful but under-utilized tool for evaluating the population-level consequences of species interactions and comparing the consequences of different life history strategies (Crone *et al.*, 2011). Compared to investigations at the individual level, which evaluate individual performance at particular life stages (e.g. seed production, biomass, survival), few studies have taken a demographic modeling approach to comparing rare and common species (but see Byers and Meagher, 1997, Münzbergová, 2005, Esparza-Olguín *et al.*, 2005, Münzbergová, 2013). Here, we used size-structured demographic models to identify the demographic rates that contribute most to population growth and that are most affected by symbiosis for a pair of rare and common species. We considered symbiotic and symbiont-free members of host populations by using integral projection models (Ellner and Rees, 2006) to describe transitions between host sizes (via demography) and symbiont status (via imperfect transmission). For the first time, we specifically address the relative contributions of differences in symbiosis outcome, vertical transmission, and intrinsic demography to the differences in population growth between host taxa. We also explore the dynamic from the symbiont's perspective

by investigating how differences between hosts in their interactions with symbionts influence the persistence of symbionts in host populations.

In this study, we focused on a pair of co-occurring rare and common grass hosts (*Poa alsodes* and *Poa sylvestris*, Poaceae) that associate with vertically-transmitted fungal endosymbionts in the genus *Epichloë*. Grass-endophyte interactions are generally assumed to be beneficial to the grass host (Clay, 1990). However, the majority of evidence comes from research in agriculturally-important species (Cheplick and Faeth, 2009), and few studies have investigated native grass-endophyte interactions, particularly at the population level (but see Rudgers *et al.*, 2012, Yule *et al.*, 2013). Recent work has shown that vertically transmitted fungal endophyte symbioses can exhibit ontogenetic shifts in interaction outcome (Rudgers *et al.*, 2012, Yule *et al.*, 2013), highlighting the need for experimental studies that take a population demographic approach. To our knowledge, this study is the first to experimentally test the effects of endophyte symbiosis on grass hosts at the population level in a comparative context. The results of this study not only expand previous knowledge on the population-level consequences of grass-endophyte symbioses, but situate symbioses generally into the broader framework of studies on rarity versus commonness (e.g. Gaston, 2011).

Specifically, we asked the following questions: 1) How does endophyte symbiosis affect host demographic rates and ultimately influence rates of host population growth? 2) Do the effects of endophyte symbiosis on host demographic rates and population growth differ between rare and common species? We predicted that endophyte symbiosis could promote the dominance of the common host species by increasing its population growth rate more than for the rare species. Alternatively, the dominance of the common

species could be driven more by intrinsic demographic advantages than by the benefits of endophyte symbiosis. We also examined dynamics from the perspective of the symbiont and asked, 3) Do populations of rare and common host species differ in the expected level of symbiont persistence? Finally, throughout our investigation of questions 2 and 3, we asked 4) What is the role of imperfect vertical transmission in host population growth and symbiont persistence?

Materials and Methods

Study species

Poa alsodes (grove bluegrass) and *P. sylvestris* (woodland bluegrass) are caespitose, woodland perennials of similar stature (30-70 cm tall), which flower and fruit from early May to mid-June. Phylogenetically, the two species are grouped with five other North American species in *Poa* sect. *Sylvestres* (Barkworth *et al.*, 2007), an early diverging lineage of *Poa* (Gillespie *et al.*, 2005). In Indiana, both species are reported from mesic woods dominated by beech-sugar maple (Deam, 1929). *Poa alsodes* is state-listed as rare in Indiana (with few records for the state) and has endangered status in Illinois, which represents the western edge of its range (USDA and NRCS, 2013). Despite extensive surveys, we have found only one population of *P. alsodes* in southern Indiana with two other county records reported; however, historical records indicate multiple populations along the shore of Lake Michigan in the northern part of the state (Deam, 1929). *Poa sylvestris* reaches as far west as South Dakota and eastern Texas, and has been recorded in most counties in Indiana (USDA and NRCS, 2013). *Poa alsodes* hosts an unnamed species of *Epichloë* (formerly genus *Neotyphodium*) (Schardl *et al.*,

2007, Shymanovich *et al.*, 2013). *Poa sylvestris* is known to host *Epichloë typhina* subsp. *poae* (Moon *et al.*, 2004, Leuchtmann *et al*., 2014), but some host grasses harbor more than one *Epichloë* species (Schardl, 2010), and we have not genotyped the endophytes studied here. Both endophytes appear to be exclusively vertically transmitted through host seeds; we have not observed stromata (the sexual structures for horizontal transmission) nor have stromata been reported elsewhere (Clay and Leuchtmann, 1989).

Study sites

Seeds of both species were collected from natural populations at the Indiana University Research and Teaching Preserve at Lilly Dickey Woods, Nashville, Indiana, USA (39°14'54"N, –86°13'05"W). In two years, we randomly sampled ~30 plants each from populations of *P. alsodes* (24 May 2006, 26 Jun 2007) and *P. sylvestris* (9 June 2006, 7 June 2007). Endophyte frequency in the *P. alsodes* populations (92-100% endophyte-symbiotic) was higher than that in the *P. sylvestris* population (69-86% endophyte-symbiotic).

Experimental methods

Half of the collected seeds were heat-treated (7–10 d at 60°C in convection oven) to remove endophytes. Once the lemma and palea were removed, heat-treated and control seeds were placed in a cone of filter paper and surface sterilized by rinsing once in 1% Tween 80, three times in 1% sodium hypochlorite, then three times in sterile H_2O. Seeds were then cold-stratified on 2% water agar at 4°C for four weeks (Rudgers *et al.*, 2012). We transplanted emergent seedlings into 115 ml pots (Conetainers, Stuewe and Sons,

Canby, OR) filled with ProMix BX potting soil (Premier Horticulture, Quakertown, PA) and watered daily. Plants were split to produce equally-sized ramets planted into separate pots. To evaluate endophyte status, we applied aniline blue-lactic acid stain to thin sections of the inner leaf sheath (Bacon and White Jr, 1994). Stained tissue was examined under a compound brightfield microscope at 200-400X. Only individuals (ramets) for which the endophyte was present or effectively eliminated were planted into the field experiment.

In the field experiment, we planted two cohorts (Sep 2007 and Apr 2008) of field plots consisting of 100% endophyte-symbiotic plants (E+) or 100% endophyte-free plants (E-, endophyte-removal treatment). Plots were located at the Lilly Dickey Woods Preserve near, but not overlapping with, sites where both species occurred naturally (39°14.19'–39°14.23', –86°13.03'–86°13.12', elevation 289–294 m). Within a plot, each of the 20 plants had the same endophyte status but represented a unique genotype; thus, all plots had the same level of initial genotypic diversity. Plants were added to the natural matrix of vegetation with minimal disturbance. For each cohort, we planted 5 replicate E+ and 5 replicate E- populations, excepting that the 2008 cohort of *P. alsodes* had only 4 replicates of each treatment. Plots were positioned ~5 m apart to minimize seed dispersal between plots. Endophyte treatment was randomly-assigned to each plot.

We collected demographic data annually during peak seed production before seeds had dispersed during late May/early June of 2008-2012. For each individual plant, we recorded survival, the number of tillers, and the number of flowering culms. We estimated seed production for each plant by counting the number of spikelets per culm for three randomly chosen culms plant^{-1}; then, we multiplied the number of culms × the

number of spikelets culm^{-1} × mean number of seeds spikelet^{-1} (*P. alsodes* mean seeds per spikelet = 2.06±0.027 *s.e.*, *N* = 501 spikelets, *t*-test for endophyte effect *P* = 0.116, *P. sylvestris* mean seeds per spikelet = 1.86±0.022 *s.e.*, *N* = 558 spikelets, *t*-test for endophyte effect *P* = 0.179). Each year, we also marked all new recruits per plot using a uniquely labeled aluminum tag secured with an aluminum nail. We tracked growth and reproduction for recruits that survived beyond the first year. We calculated the probability of seedling establishment per plot as the number of recruited seedlings divided by the estimated total number of seeds produced by all parent plants in the plot in the previous year, adjusted for any seeds removed for vertical transmission trials (see next section).

To assess endophyte vertical transmission rates, we removed a subset of seeds from the originally planted individuals still surviving during May 2009, ~30 seeds from each plot. For *Poa alsodes*, we examined 179 seeds from E+ plots and 193 seeds from E- plots. For *P. sylvestris*, we examined 331 seeds from E+ plots and 289 seeds from E- plots. Batches of ~10 seeds per plot were surface sterilized as above and placed in 2% water agar on sterile, 10 cm Petri plates for six weeks of cold stratification at 4°C, then transferred to the greenhouse. To assess vertical transmission rates, we examined seedlings for endophyte presence using rose bengal stain following Belanger (1996). Vertical transmission was analyzed using a binomial generalized linear mixed model with the fixed effect of endophyte treatment and the random effect of plot nested within endophyte treatment.

Integral projection model

To obtain asymptotic population growth rates (λ) for each species with or without endophyte symbiosis, field data were used to parameterize a size-structured integral projection model (IPM). IPMs provide a framework to predict how vital rates measured at the level of individuals scale up to affect population dynamics, including λ, the geometric rate of population growth. In the context of our study, IPMs allow us to evaluate the population-level consequences of the demographic effects of endophyte symbiosis, and the contrast between host species allows us to decompose the difference in population growth into "intrinsic" demographic differences vs. differences in interactions with symbionts. Here, we provide a brief overview of the IPM structure. Further information is provided by several recent papers addressing the construction and applications of IPMs (Coulson, 2012, Metcalf *et al.*, 2013, Merow *et al.*, 2014, Rees *et al.*, 2014).

The IPM predicts the change in size structure of a population, *n(y)*, over size domain Ω, from time *t* to *t+1* as:

$$n(y)_{t+1} = \int_{\Omega} [p(y,x) + f(y,x)]n(x)_t dx$$

Eq. 1

Size-dependent survival and growth are represented by:

$$p(y,x) = s(x)g(y,x)$$

Eq. 2

$s(x)$ is the probability of survival for individuals of size *x*, and $g(y,x)$ is the probability of a surviving individual growing from size *x* to size *y*. Reproduction, *f(y,x)*, representing the production of *y*-sized plants from *x*-sized parents, is given by:

$$f(y, x) = r(x)f_n(x)pE\, d(y)$$

<div align="right">Eq. 3</div>

$r(x)$ is the size-dependent probability of flowering, and $f_n(x)$ the seed production of

plants that flowered. These two functions are multiplied by *pE,* the probability of

seedling establishment (size-independent), and $d(y)$, the probability distribution of

seedling size. These demographic functions together constitute an IPM kernel which

describes all possible transitions between sizes in a single year. Construction and analysis

of the IPM were conducted in R 3.0.0 (R Core Team, 2013).

Model parameterization

Functions describing the size-dependent growth, survival, and reproduction of

each species were fit using generalized linear models with the appropriate error

distribution. Data across all years (2008–2012) were pooled into single inter-annual (*t* to

t+1) transitions. We used the natural logarithm of tiller number (range: 1–125 and 1–93

for *Poa sylvestris* and *P. alsodes* respectively) as the dependent variable, size (*x, y*).

While tiller number is itself a discrete variable, here we use its natural log as a proxy for

continuous variation in biomass following (Yule *et al.*, 2013). Seed production $f_n(x)$

was fitted with a Gaussian linear model using the natural log number of seeds as a

response, and exponentiated to predict seed counts.

For each demographic function, endophyte symbiosis could affect vital rate

functions through the slope of the vital rate (y-axis) against size (x-axis) (size-dependent

effects), the intercept of this relationship (size-independent effects), both, or neither.

Thus, for each demographic function, we fit data to four possible models: no endophyte

effects (size-dependent only), endophyte effect on intercept, endophyte effect on slope, and endophyte effect on both slope and intercept (Table 1). We used Aikaike's Information Criterion (AIC) to evaluate model fit, and AIC weights to discriminate between models. For all fitted demographic functions, no single model AIC weight was > 0.9. Therefore, we used AIC weights to weight coefficients for model-averaging across the four models (Table 1, Burnham and Anderson, 2002). Observed rates of seedling establishment, *pE*, were *logit* transformed and analyzed using a repeated measures generalized linear mixed model with year and endophyte status as fixed effects and plot nested within endophyte status as a random effect (PROC GLIMMIX, SAS v. 9.3, Cary, NC). Seedling establishment varied widely between years for both species. Therefore, we modeled λ against the observed range of probabilities of establishment in addition to the average rates to better understand how interannual differences in recruitment affect population growth. We also separately estimated the effect of plant size on the variance of plant growth (*gvar*) for E+ and E- populations of each species.

The IPM projection kernel for each species with or without the endophyte was discretized into a matrix (Yule *et al.*, 2013), and we calculated the dominant eigenvalue of the discretized matrix to determine the asymptotic population growth rate (λ). The lower integration limit for the model was the smallest observed size (one tiller, *ln*(size) = 0), and the upper integration limit was set at 1.1 times the maximum observed size (148 tillers, *ln*(size) = 5). To avoid unintentional eviction from the model, we extended the maximum and minimum limits (by 3) and modified the kernel such that all individuals modeled that fell in extended size ranges were treated as demographically equivalent (Williams *et al.*, 2012). This essentially sets a "ceiling" and "floor" in the model by

having a class of "very big" and "very small" individuals, and includes rare instances of individuals which would otherwise be evicted from the model by exceeding the set integration limits (Williams *et al.*, 2012).

Testing the effects of endophyte symbiosis on host population growth

We generated a null expected difference in λ between E+ and E- populations $(\lambda_{E+} - \lambda_{E-})$ by randomizing endophyte status within each species and re-fitting demographic functions. This randomization procedure was repeated 10,000 times to estimate 95% confidence intervals for the distribution of the null difference. The null expectation was then compared to the observed difference in λ to determine if endophyte presence in the population significantly altered λ at a significance level of $\alpha = 0.05$.

Imperfect vertical endophyte transmission

Throughout the life cycle of the host plant, it is possible for the endophyte to be lost from maternal plant to seed through imperfect transmission (Afkhami and Rudgers, 2008). To incorporate this phenomenon in our demographic model, we can couple E+ and E- IPM kernels into a single model that includes transitions not only between sizes but also between endophyte states. This allows us to predict the equilibrium frequency of E+ hosts in the population, given the demographic effects of endophyte symbiosis and the rate of endophyte loss. Our approach is akin to the "megamatrix" approach for multiple, discrete state variables (Pascarella and Horvitz, 1998, Yule *et al.*, 2013). The combined E-/E+ model takes the form:

$$\begin{pmatrix} E^-(y)_{t+1} \\ E^+(y)_{t+1} \end{pmatrix} = \begin{pmatrix} \int [p^-(y,x) + f^-(y,x)]dx & \int [(1-\tau)f^+(y,x)]\,dx \\ 0 & \int [p^+(y,x) + \tau f^+(y,x)]dx \end{pmatrix} \begin{pmatrix} E^-(x)_t \\ E^+(x)_t \end{pmatrix} \quad \text{Eq. 4}$$

The 2x2 megamatrix represents transitions between discrete states of endophyte presence or absence, where the elements contain discretized IPM kernels as described previously, using E+ or E- functions, as appropriate. The transition between endophyte states is governed by the vertical transmission rate τ, which is the probability that a seedling from an endophyte-symbiotic parent plant is also endophyte-symbiotic (Gundel *et al.*, 2008). Because horizontal transmission has not been documented for either species, the probability of transitioning from endophyte-free to endophyte-infected was held at zero. The first eigenvector of the megamatrix predicts the equilibrium distributions of size and endophyte status. The effects of imperfect vertical transmission on host plant population growth and equilibrium endophyte frequency (expected proportion of the population that is symbiotic) were modeled using a range of *pE* based on field-observed rates.

Life table response experiment

Differences in population growth between the two host species could be caused by differences in "intrinsic" demography in the absence of symbionts, differences in the effects of symbionts on demography, or both. To quantify the contributions of these processes to the observed difference in λ between species, we used a life table response experiment (LTRE, Caswell, 1989). The between-species LTRE decomposed the total difference in λ into the contributions of intrinsic (E-) demographic coefficients and the endophyte symbiosis effects (the ratio of E+/E- for each demographic coefficient). The total difference in λ between species reflects the difference in parameter values multiplied by the parameter sensitivity and summed over all parameters that differed between *P*.

alsodes and *P. sylvestris* populations (Caswell, 2001). We calculated the midpoint

sensitivities of intrinsic demography and endophyte effect parameters using the mean

megamatrix of *P. alsodes* and *P. sylvestris* as the reference model (Caswell, 2001),

including the average *pE* for all host species across all years. Sensitivities were estimated

numerically by applying a uniform perturbation, and taking the ratio of the change in λ to

the magnitude of the perturbation (0.001) (Ellner and Rees, 2006). The LTRE included

the vertical transmission rate as a demographic parameter that differed between species.

We used vertical transmission rates measured in the laboratory experiment (*P. alsodes*

99.9 ± 7.6% *s.e.*, $N = 81$ seedlings; *P. sylvestris* 16.5 ± 4.7% *s.e.*, $N = 126$) for each host

species in the megamatrix.

Results

Endophyte effects on host life stages

        The effects of endophyte symbiosis differed between host life stages and species

(Table 2). Endophyte symbiosis increased *P. sylvestris* growth consistently across sizes,

had little effect on flowering probability or seed production, and increased survival,

especially at small sizes (Fig. 1). In contrast, *P. alsodes* in symbiosis with the endophyte

experienced no change in growth, but instead showed a size-dependent decrease in the

probability of flowering, an increase in seed production at large sizes, as well as an

increase in survival across all sizes (Fig. 2).

        Endophyte symbiosis had no significant effect on *P. sylvestris* or *P. alsodes*

seedling establishment ($X^2_{18}<0.01$, *P*=0.974, and X^2_{16}=3.40, *P*=0.065 respectively), but

establishment strongly varied among years (X^2_{43}=9.16, *P*=0.027, and X^2_{31}=19.82,

P<0.001 respectively). There was no significant endophyte status by year interaction effect on the probability of seedling establishment for either species (X^2_{43}=1.38, P=0.711, and X^2_{31}=2.38, P=0.498 respectively).

Endophyte effects on host population growth

For both host species, endophyte symbiosis significantly affected population growth: the observed difference between E- and E+ populations (at average rates of seedling establishment) fell outside the null expectation using randomized endophyte assignments ($P < 0.05$). Endophyte symbiosis increased projected stable population growth rates for both host species. At average seedling establishment probabilities, E+ populations of the common host *P. sylvestris* were projected to grow 32% faster than E- populations ($\lambda^+ = 1.43$ and $\lambda^- = 1.11$). For the rare host species *P. alsodes*, E- populations were projected to decline at average seedling establishment probabilities ($\lambda^- = 0.90$), whereas endophyte symbiosis increased population growth by 18% ($\lambda^+ = 1.08$), resulting in population persistence.

We also modeled λ across the range of possible seedling establishment rates documented in the field, and the qualitative pattern remained consistent, with projected λ's increasing as seedling establishment increased (Fig. 3). However, even under the highest observed values of seedling establishment, E- populations of *P. alsodes* were predicted to decline through time in the absence of endophyte symbiosis ($\lambda^- = 0.90$).

Life table response experiment

The LTRE analysis showed that intrinsic (E-) demographic rates and endophyte effects both contributed to the difference in λ between the rare versus common host species under the laboratory-measured vertical transmission scenario, which we measured to be *P. alsodes* 99.9 ± 7.6% *s.e.*, and *P. sylvestris* 16.5 ± 4.7% *s.e.* (Fig. 4). Summed across vital rates, the difference in λ between the two host species was more strongly driven by intrinsic differences in their demography than by differences in endophyte effects. Intrinsic survival, seed production, and seedling establishment, all greater for *P. sylvestris*, made the largest contributions to the total difference in λ between *P. sylvestris* and *P. alsodes*. Effects of endophyte symbiosis on both host species demographic rates were similar in magnitude, but occurred through different demographic rates (increased growth and flowering in *P. sylvestris*; increased survival and seed production in *P. alsodes*). These differences in endophyte symbiosis effect between host species together summed to close to zero, showing little contribution of difference in endophyte effect combined across host demographic rates to the difference in λ between hosts. The laboratory transmission rates showed low levels of vertical transmission in *P. sylvestris* and perfect vertical transmission in *P. alsodes*. The contribution of the vertical transmission rate to the difference in λ reflected the decrease in projected λ for *P. sylvestris* due to loss of endophyte symbiosis, which decreased its strong advantage in intrinsic demography.

Endophyte persistence in host populations

We found that seedling establishment interacted with endophyte transmission rate to determine equilibrium endophyte persistence (Fig. 5). For both host species, the

general pattern held that as vertical transmission rates increased, the predicted λ's and equilibrium endophyte frequencies for host populations increased. However, this relationship was not linear. Our model results showed a threshold vertical transmission rate below which the endophytes went extinct: below the threshold, λ matched the value based solely on E- demography. However, above the threshold vertical transmission rate, there was a positive, linear, relationship between equilibrium endophyte frequency and vertical transmission (Fig. 5). For both host species, the threshold transmission rate approached zero at the lowest observed levels of seedling establishment and increased as *pE* increased (Fig. 5). Thus, a greater probability of seedling establishment increased the vertical transmission rate necessary to keep endophytes in the host population. At any given the probability of seedling establishment, the projected threshold transmission rate was lower for *P. sylvestris* than *P. alsodes*, which resulted in a higher equilibrium endophyte frequency in *P. sylvestris* compared to *P. alsodes* under less than perfect transmission.

Discussion

To our knowledge, this study was the first to tease apart the effects of symbiosis and intrinsic host demography on host relative abundance.   Our results revealed several novel patterns. First, endophyte symbiosis increased host population growth, consistent with prior studies (Rudgers *et al*., 2012, Yule *et al.*, 2013). However, the net benefit of symbiosis was reflected through different vital rates in the rare versus common host species, and there was a slight cost of endophyte symbiosis to the rare host. Second, endophyte symbiosis was crucial for the rare host to maintain population growth at above

replacement, whereas the common host was projected to increase with or without endophyte symbiosis. However, intrinsic differences in demography, independent of endophyte symbiosis, contributed more to the difference in projected population growth rates than did differences between host species in the effects of endophyte symbiosis. Third, seedling establishment was a crucial demographic transition, and interacted with the vertical transmission rate of endophytes from maternal plants to seeds in nonlinear ways that affected both host and symbiont population growth. Moreover, lower rates of vertical transmission of the endophyte in the common host compared to the rare host decreased the projected difference in population growth between the two host species.

Effects of endophyte symbiosis on host demographic rates and population growth

Results for these two *Poa* species differ from prior grass-endophyte symbioses that have been examined and thus expand the range of observed demographic effects of endophyte symbiosis. Endophyte symbiosis increased survival, as well as the overall population growth of both hosts, but its effects on other host vital rates differed between the two host species we examined. Others have also found combinations of costs and benefits in vertically-transmitted endophyte-host interactions. For example, endophyte symbiosis was found to decrease survival but increase reproduction in hosts *Agrostis hyemalis* and *Cinna arundinacea* (Rudgers *et al.*, 2012, Yule *et al*., 2013), and increase reproduction and growth in *Festuca arizonica* (Faeth, 2009). These findings differ from our results, where the cost of endophyte symbiosis was to reproduction (rare host only) and the benefits to survival (both hosts) and growth (common host only). These species-

specific effects of endophyte symbiosis could be related to differences in host life history strategies and tradeoffs in the host species.

The most likely mechanism by which endophyte symbiosis enhanced host plant growth and survival in our study is herbivore deterrence. Previous work on the plants of these two species showed a 70% and 72% increase (*P. alsodes* and *P. sylvestris*, respectively) in insect herbivory on E- compared to E+ plants in laboratory trials (Crawford *et al.*, 2010). In addition, surveys of the field experimental populations studied here showed up to four times more leaf area damaged in E- *P. alsodes* plants compared to E+ plants, and over three times more in E- *P. sylvestris* plants (unpublished data, Crawford *et al.*, 2010). These herbivory studies all focused on adult plants. However, as seedling establishment did not significantly differ between E+ and E- plants in our study, it is unlikely that endophyte-mediated differences in herbivory to early seed or seedling stages drives symbiosis outcomes at the population level for these species.

Relative effects of endophyte symbiosis versus intrinsic host demography on the difference in population growth between rare and common plants

Demographic models are necessary to gain insights into the population-level consequences of symbioses due to known shifts in interaction outcomes throughout host ontogeny. However, only a handful of studies thus far have utilized this tool to compare rare and common species (e.g. Byers and Meagher, 1997, Esparza-Olguín *et al.*, 2005, Münzbergová, 2013), and none incorporated the potentially strong effects of microbial symbiosis. Using size-structured IPMs, we found that the more common species *P. sylvestris* had a higher projected population growth rate in comparison to its rarer

congener *P. alsodes*. This was true in comparing both endophyte-symbiotic and endophyte-free populations. However, when the different effects of endophyte symbiosis on each host were combined to investigate their overall contribution to the difference in host population growth, the contribution of symbiosis was small relative to intrinsic differences between the species. This result suggests that the common host is projected to grow at a faster rate, not because it benefits more from endophyte symbiosis compared to the rare host, but because of its intrinsic demographic advantage.

The higher population growth of the common species was driven by its higher probabilities of seedling establishment, seed production, and survival compared to the rare plant. Previous work comparing rare and common congeners have also found greater probability of survival to reproductive size in *Calochortus* spp. (Fiedler, 1987). Similarly, in a demographic analysis of *Neobuxbaumia* spp., the greatest contribution to the greater λ of the common species was attributed to higher recruitment (Esparza-Olguín *et al.*, 2005). Our study only focuses on a single pair of rare and common species, so the results cannot be generalized to all common and rare species comparisons. However, the results of our study add the new dimension of symbiotic interactions to this existing work. As additional demographic studies accumulate in the literature, it may become possible to detect general patterns in the critical demographic transitions and ecological interactions that determine plant species relative abundance.

Of all the vital rates, the probability of seedling establishment was the largest contributor to the higher population growth projected for the common plant, highlighting seedling establishment as a key demographic transition that determines the rarity of *Poa* in our study area. Observed seedling establishment varied widely between years in the

study, and is likely related to interannual variation in climate. For example, the lowest seedling establishment occurred in 2012, during a drought of nine consecutive months of above-average temperatures and six consecutive months of below-average precipitation in Indiana (Indiana State Climate Indiana State Climate Office, 2013). Data collection for this study is still ongoing and as more data accumulate, future modeling efforts could include stochastic demographic models to assess the effects of climate variation.

Our study of a single pair of rare and common host species supports the hypothesis that it is the intrinsic advantage in demography that drives the dominance of the common host species, but that rare species can depend on symbionts for population persistence. In our model projections, endophyte-free populations of *P. alsodes* did not reach $\lambda \geq 1$ even under the most favorable seedling establishment scenarios. Only when it was symbiotic with its endophyte partner did the model predict population growth at or above replacement ($\lambda \geq 1$). However, a worse-than-average year for seedling establishment or less-than-perfect transmission resulted in projected population decline for *P. alsodes*, which likely contributes to its rarity in the study region. In Indiana, where this study took place, *P. sylvestris* occurs commonly whereas *P. alsodes* is listed as "rare" (USDA and NRCS, 2013). The state of Indiana is situated close to the western edge of the geographic distribution of *P. alsodes*, whereas it is close to the center of the distribution of *P. sylvestris*. Our results suggest that the hypothesis that *P. alsodes* suffers in intrinsic demography compared to *P. sylvestris* due to their relative positions in their respective ranges. However, the benefits of endophyte symbiosis allowed *P. alsodes* to persist at our study site. Previous theory has predicted that facilitative interactions could expand the fundamental niche of a species (Bruno *et al.*, 2003), and others have found symbiosis to

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expand host niches (Joy, 2013). Additional demographic studies on such rare and common species pairs will be able to test the generalizability of this hypothesis for plants as well as animal hosts of beneficial symbionts.

Do rare and common hosts differ in predicted levels of endophyte persistence, and how do vertical transmission rates alter projected endophyte and host population growth?

In both host species, the probability of seedling establishment interacted with endophyte symbiosis to change equilibrium rates of endophyte frequency. In our models, increased seedling establishment led to an increase in the minimum vertical transmission rate required for persistence of endophyte symbiosis. The existence of a minimum vertical transmission rate for endophyte persistence is predicted by theory (Gundel *et al.*, 2011), and has been demonstrated in other grass-endophyte species (Yule *et al.*, 2013). The threshold that we uncovered reflects the level of association between plants and endophytes where the net fitness benefits are sufficient to compensate for endophyte loss due to imperfect transmission, resulting in increased population growth for both host and endophyte. Higher levels of seedling establishment increase the minimum vertical transmission rate, and more generally decrease equilibrium endophyte frequency, because seedling establishment enhances E- recruitment, thus amplifying the consequences of imperfect transmission.

The rare host, *Poa alsodes*, had higher minimum vertical transmission rates at any given seedling establishment probability than did the common host. In other words, a higher level of endophyte vertical transmission was required for the rarer *P. alsodes* to maintain the symbiosis, given its demographic costs and benefits. This result is in line

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with the higher costs we found of endophyte symbiosis in the rare host, which was balanced by higher levels of vertical transmission. This pattern was also reflected in our surveys of local populations where endophytes were detected in 92-100% of *P. alsodes* sampled, and only 69-86% of *P. sylvestris*. Using vertical transmission rates from maternal plant to seedling assessed in the laboratory, our models projected equilibrium endophyte frequencies of 100% in *P. alsodes* and 0% in *P. sylvestris* populations. While the discrepancy between model predictions and field surveys could be explained by disequilibria in the field populations, it is more likely that we underestimated *P. sylvestris* vertical transmission rate in the laboratory experiment. For example, it is possible that surface sterilization and laboratory conditions for germination facilitated the germination and establishment of endophyte-free seedlings, which would never be realized under field conditions, thus biasing the estimate of (realized) vertical transmission for the population. Regardless, the lower vertical transmission rates found in the laboratory and simulated by our models of the more common *P. sylvestris* subsequently decreased the projected difference in population growth rates of rare and common hosts. While we did not detect any significant demographic costs of endophyte symbiosis to the common host, endophyte symbiosis still exerts a carbon drain on host plants (Thrower and Lewis, 1973). Both the costs of endophyte symbiosis and imperfect transmission are key reasons why natural endophyte frequencies are not at 100% (Gundel *et al.*, 2008).

Conclusion

We found support for the hypothesis that rare species can be maintained in communities by their associations with beneficial symbionts. However, the different

fitness benefits of endophyte symbiosis to rare versus common host plants did not contribute substantially to the difference in host population growth rates. Instead, intrinsic demographic advantage was the main driver behind the dominance of the common host species, and asymmetry in vertical transmission rates decreased the projected difference in population growth rates between host species more than expected based on intrinsic demography alone. This is the first time that the relative effects of symbiosis and host demography in driving host population dynamics have been evaluated. We conclude that understanding the interactions among seedling establishment, vertical transmission rates, and host species demography are critical for determining population-level outcomes for both host and symbiont species. Our results demonstrate the importance of microbial symbionts in driving population dynamics of host plants, as well as the utility of a demographic perspective for generating new insights into how plant-symbiont interactions vary across host life cycles and contribute to the different life history strategies of rare versus common species.

Data Accessibility

Data deposited in the Dryad repository: http://dx.doi.org/10.5061/dryad.nf515.

Figures and Tables

Table 1

Candidate models of host species demographic functions and model fitting. Superscript + indicates endophyte effect on the intercept (subscript *b*) or slope (subscript *m*), and subscript *t* indicates year. Demographic functions follow those of Eq.2 and Eq.3.

Model	*P.alsodes*		*P. sylvestris*	
	ΔAIC	AIC Weight	ΔAIC	AIC Weight
Growth *g(y,x)*				
$size_{t+1} = g_b + g_m * size_t$	0	0.483	9.1	0.007
$size_{t+1} = g_b^+ + g_m * size_t$	2	0.178	0	0.684
$size_{t+1} = g_b + g_m^+ * size_t$	1.5	0.218	6.4	0.029
$size_{t+1} = g_b^+ + g_m^+ * size_t$	2.7	0.121	1.8	0.280
Flowering *r(x)*				
$logit(flowering_{t+1}) = r_b + r_m * size_t$	8.74	0.005	0.3	0.278
$logit(flowering_{t+1}) = r_b^+ + r_m * size_t$	0.15	0.398	0	0.317
$logit(flowering_{t+1}) = r_b + r_m^+ * size_t$	0	0.428	0.2	0.288
$logit(flowering_{t+1}) = r_b^+ + r_m^+ * size_t$	1.85	0.169	2	0.117
Seed production *f_n(x)*				
$ln(seeds_{t+1}) = f_b + f_m * size_t$	3.55	0.096	1.2	0.195
$ln(seeds_{t+1}) = f_b^+ + f_m * size_t$	3.58	0.094	0.1	0.329
$ln(seeds_{t+1}) = f_b + f_m^+ * size_t$	1.68	0.244	0	0.347
$ln(seeds_{t+1}) = f_b^+ + f_m^+ * size_t$	0	0.566	2	0.129
Survival *s(x)*				
$logit(survival_{t+1}) = s_b + s_m * size_t$	12.2	0.002	13.4	0.001
$logit(survival_{t+1}) = s_b^+ + s_m * size_t$	0	0.713	0	0.673
$logit(survival_{t+1}) = s_b + s_m^+ * size_t$	6.9	0.022	12.8	0.001
$logit(survival_{t+1}) = s_b^+ + s_m^+ * size_t$	2	0.263	1.4	0.325

Table 2

Descriptions and fitted parameter values for demographic functions, with *pE* reflecting the average rate observed in the field across years.

Parameter	*P. sylvestris*		*P. alsodes*		Description
	E-	E+	E-	E+	
s_b	-1.16	-0.81	-0.56	-0.13	Survival intercept
s_m	0.93	0.91	0.46	0.47	Survival slope
g_b	0.99	1.18	1.11	1.12	Growth intercept
g_m	0.58	0.57	0.51	0.50	Growth slope
$gvar_b$	0.93	1.19	0.95	0.71	Growth variance intercept
$gvar_m$	0	-0.12	0	0.22	Growth variance slope
r_b	-5.56	-5.46	-5.09	-5.37	Flowering intercept
r_m	2.19	2.22	2.17	2.04	Flowering slope
f_b	4.45	4.50	3.76	3.36	Seeds intercept
f_m	0.60	0.62	0.68	0.88	Seeds slope
μ	0.099	0.099	0.063	0.063	Mean seedling size
pE	0.014	0.014	0.006	0.006	Probability of establishment
τ		0 - 1		0 - 1	Transmission rate*

Fig. 1

Fitted demographic functions for *Poa sylvestris* in **A)** growth, **B)** flowering, **C)** seed production, and **D)** survival. Observed data for endophyte –symbiotic and nonsymbiotic populations are filled (E+) and open (E-) points, respectively. Fitted functions are represented in solid (E+) and dashed (E-) lines.

Fig. 2

Fitted demographic functions for *Poa alsodes* in **A)** growth, **B)** flowering, **C)** seed production, and **D)** survival. Observed data for endophyte –symbiotic and nonsymbiotic populations are filled (E+) and open (E-) points, respectively. Fitted functions are represented in solid (E+) and dashed (E-) lines.
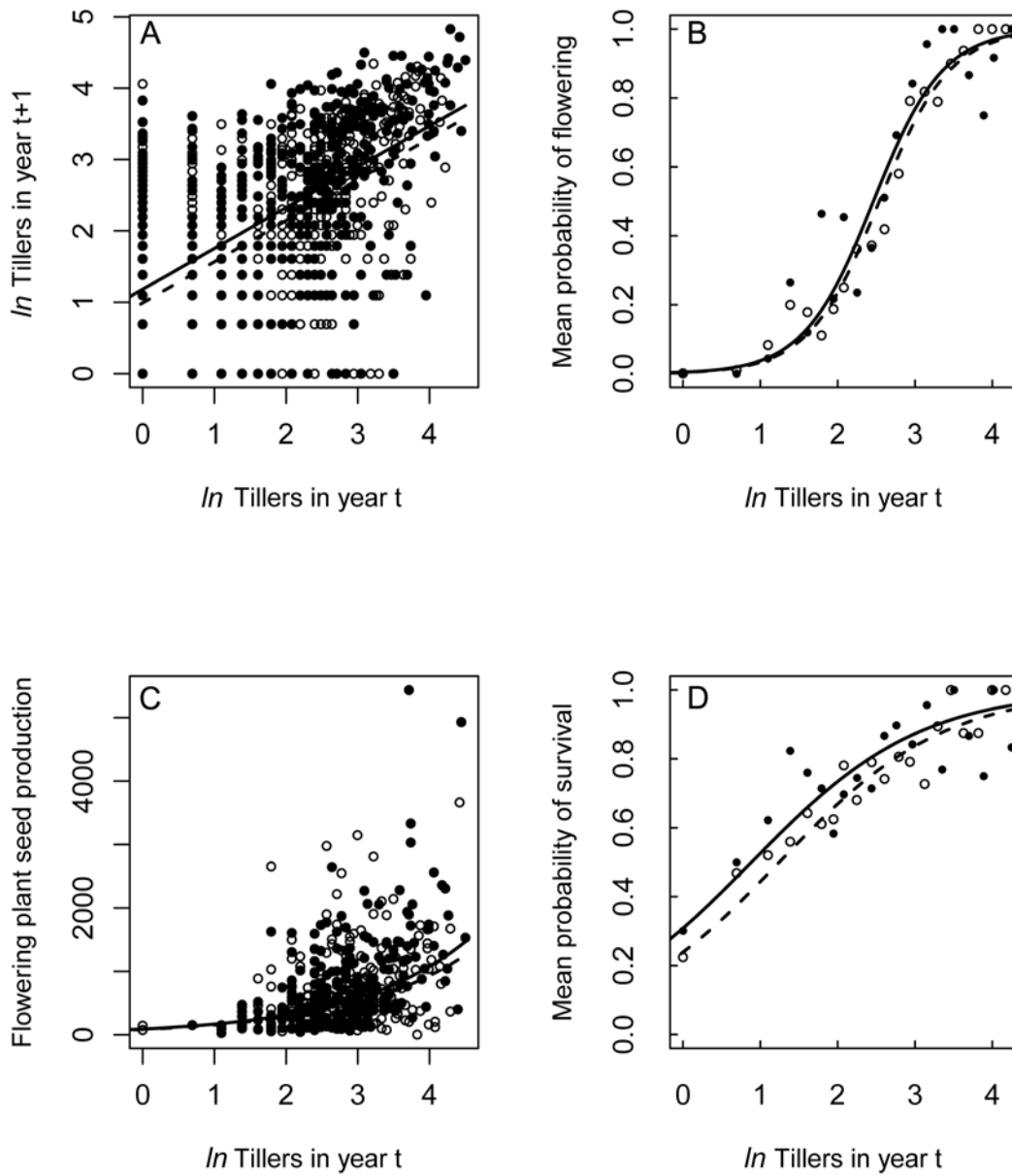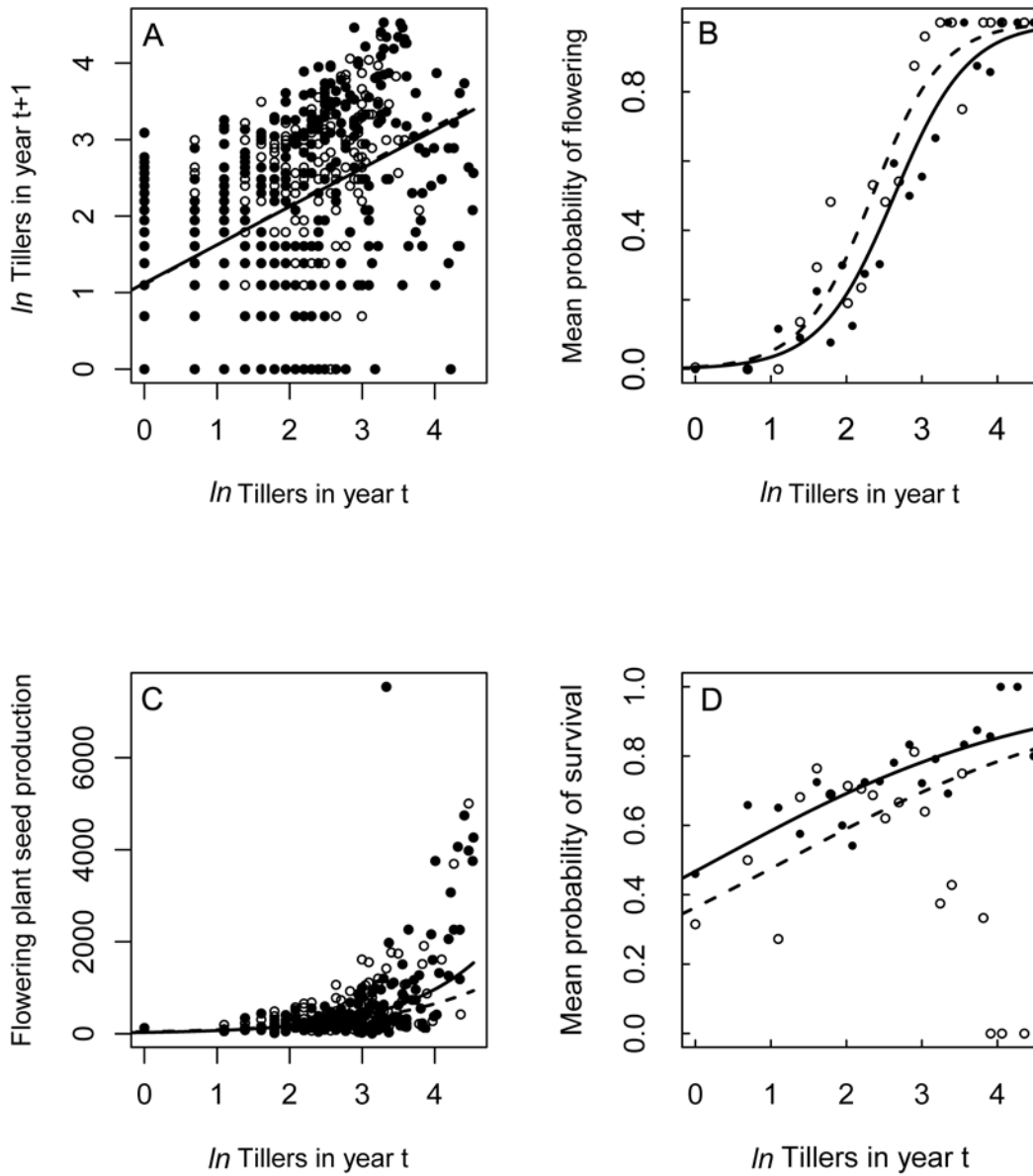
Fig. 3

Projected population growth rates (λ) for E+ (solid) and E- (dashed) for **A)** *Poa sylvestris* and **B)** *Poa alsodes* across range of observed probabilities of establishment (*pE*) for each population. Shaded areas represent 95% bootstrap CI; arrows indicate average *pE* for each species. While the range of *pE* observed in the field differed between E- and E+ populations within each species, the difference in means was not statistically significant in either species.
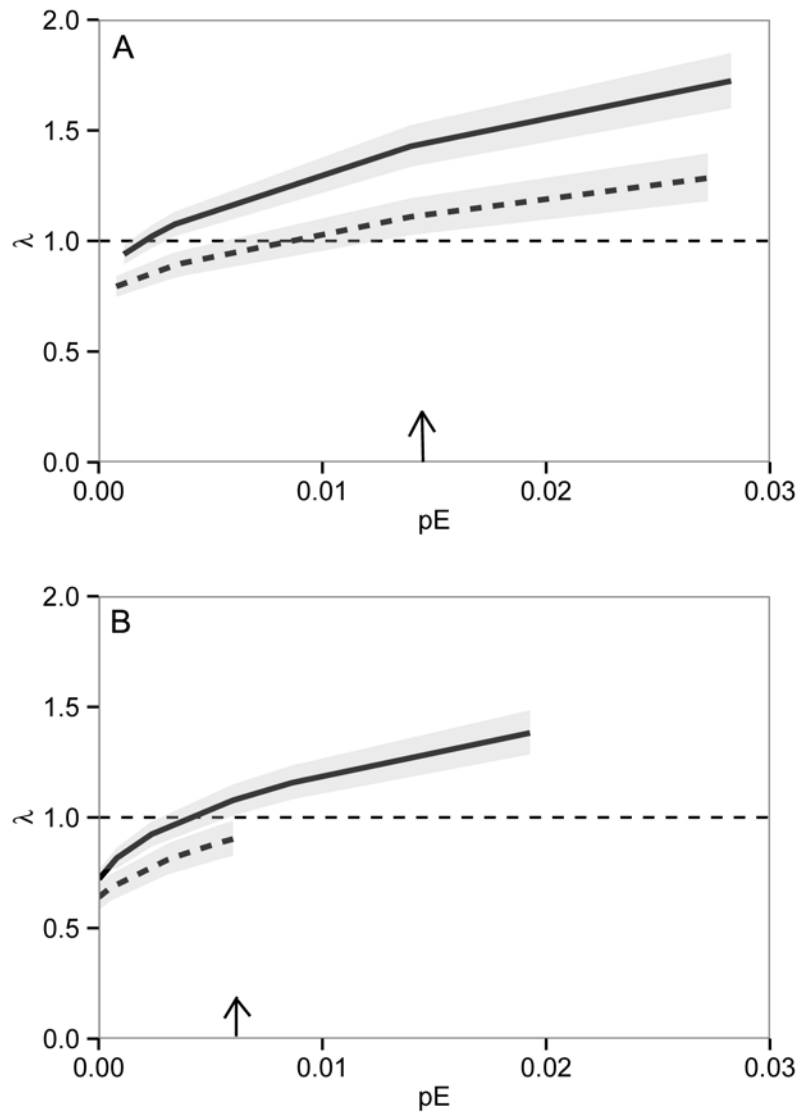
Fig. 4

Contribution of each demographic rate to the difference in λ between *Poa sylvestris* and *Poa alsodes* populations ($\Delta\lambda = \lambda_{P.sylvestris} - \lambda_{P.\,alsodes}$, in black). Sums of the subsets of contributions reflecting intrinsic demographic differences only (Σintrins.), the effect of endophyte symbiosis only (Σendo.), and the effect of different vertical transmission rates (τ) are in grey. Individual contributions through each vital rate are in white.
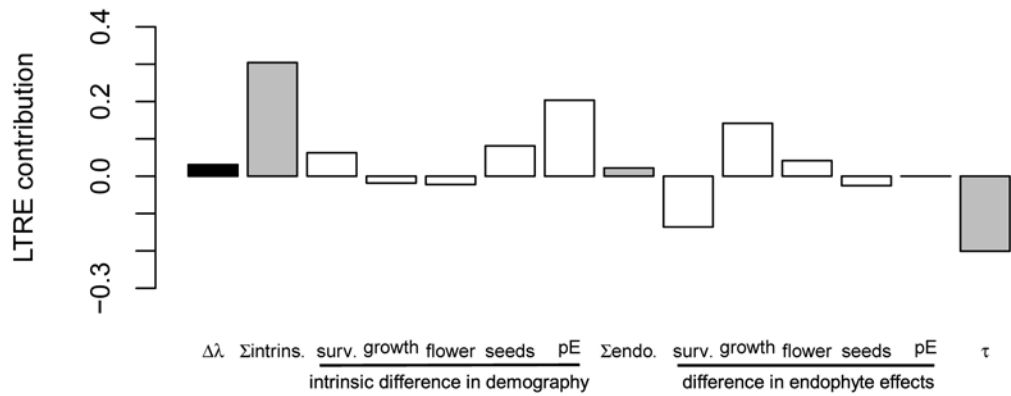
Fig. 5

The effects of imperfect transmission on **A,C)** host population growth and **B,D)** equilibrium endophyte frequency in the host population. Model projections for *Poa sylvestris* in **A,B)**, and *Poa alsodes* in **C,D)**. Effects of imperfect transmission modeled at three levels of *pE*. The low and high levels chosen are the same and biologically feasible for both host species. The middle *pE's* are different for each species and reflect the average probability of seedling establishment observed in the field.
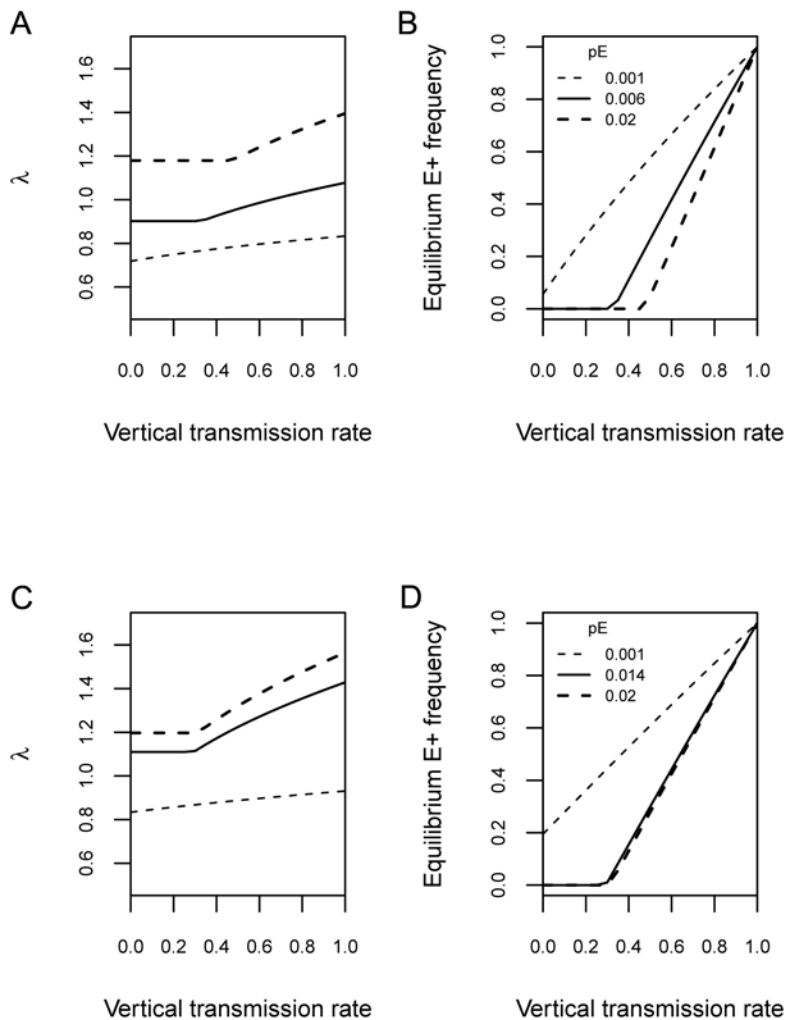
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Chapter 2

Plant-soil feedbacks promote negative frequency dependence in the coexistence of two aridland grasses

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Abstract

Understanding the mechanisms of species coexistence is key to predicting patterns of species diversity. Historically, the ecological paradigm has been that species coexist by partitioning resources: as a species increases in abundance, self-limitation kicks in, because species-specific resources decline. However, determining coexistence mechanisms has been a particular puzzle for sedentary organisms with high overlap in their resource requirements, such as plants. Recent evidence suggests that plant-associated microbes could generate the stabilizing self-limitation (negative frequency dependence) that is required for species coexistence. Here, we test the key assumption that plant-microbe feedbacks cause such self-limitation. We used competition experiments and modeling to evaluate how two common groups of soil microbes (rhizospheric microbes and biological soil crusts) influenced the self-limitation of two competing desert grass species. Negative feedbacks between the dominant plant competitor and its rhizospheric microbes magnified self-limitation, while beneficial interactions between both plant species and biological soil crusts partly counteracted this stabilizing effect. Plant-microbe interactions have received relatively little attention as drivers of vegetation dynamics in dryland ecosystems. Our results suggest that microbial mechanisms can contribute to patterns of plant coexistence in arid grasslands.

Introduction

What mechanisms allow species to coexist? Historically, species coexistence has

been ascribed to niche differentiation (Hutchinson 1959) whereby species occupy

different habitat spaces (MacArthur 1958), require divergent nutrition (Tilman 1982), or

employ different life history strategies (Grime 1977). These mechanisms promote

coexistence by causing species to be more self-limited than they are by their competitors.

The degree of self-limitation and potential for stable coexistence depends on the strength

of negative frequency dependence experienced by each competitor at high density

(Chesson 2000, Levine and HilleRisLambers 2009). More recently, the discovery of the

'unseen majority' — diverse, abundant microbial consortia associated with all macro-

organisms — has prompted evaluation of their potential to promote the coexistence of

macro-organisms. Pathogens are well-known to regulate population dynamics of their

hosts (Holt and Pickering 1985), and interactions with microbes could be particularly

important to sessile organisms, such as plants, that superficially appear to utilize identical

resources (Van Der Heijden et al. 2008). Plant species-specific interactions with

beneficial microbes could mediate access to soil resources, increasing niche

differentiation. Alternatively, the buildup of soil pathogens as the relative frequency of

the host increases can limit the performance of the plant host at high frequencies,

promoting self-limitation. Such plant-soil microbial feedbacks have been hypothesized to

be important mechanisms of plant species coexistence (Bever et al. 1997, Bever et al.

2010).

Past work has shown patterns that are consistent with the hypothesis that plant-

soil microbial feedbacks (PSF) can promote plant coexistence. First, a majority of PSF

studies report negative feedbacks (Kulmatiski et al. 2008). The pathogenic nature of most PSFs indicates that the potential for self-limitation exists, although most studies have been conducted in mesic ecosystems, limiting the geographic scope of inference. Second, in both tropical forests and temperate grasslands, the strength and direction of PSFs is correlated with plant species' relative abundances: Rare species are associated with stronger negative feedbacks than common species (Klironomos 2002, Mangan et al. 2010, but see Reinhart 2012). Third, Janzen-Connell studies, a subset of PSF research originally focused on tropical trees, have demonstrated negative effects of distance from parent or conspecific density on seedling establishment and survival (Comita et al. 2014). This could lead to negative frequency-dependence between generations: as the relative frequency of a tree species increases, so does the proportion of habitat occupied by its species-specific soil pathogens, thus decreasing the per capita fitness of a population across space. Fourth, experiments have shown that PSFs can alter the outcome of competition in 1:1 pairwise combinations (Kardol et al. 2007, Petermann et al. 2008, Pendergast et al. 2013), or under natural gradients of competition (Bagchi et al. 2014), albeit with idiosyncratic results.

However, these prior examples do not fully demonstrate PSF as a mechanism of plant coexistence because a key criterion for coexistence has not been directly assessed: Plant-soil microbial feedbacks must have higher demographic costs with increasing relative frequency of the host species in the community, causing negative frequency dependence (Mordecai 2011). For example, this could result from the increased likelihood of an individual encountering species-specific soil pathogens as its conspecific frequency increases in the community with no net change in per capita pathogen load, or

from an amplified pathogen load at high host frequency. Under the modern framework for species coexistence (Chesson 2000), this stabilizing effect of niche differentiation (negative frequency dependence) is required for long term coexistence in the absence of fluctuating temporal or spatial environments. Thus, a comprehensive test of PSF as a stabilizing mechanism of coexistence should meet the following criteria (Table 1): 1) Negative PSF occurs and is caused by soil microbiota. 2) Negative frequency dependence occurs. Stronger negative frequency dependence with higher total plant density indicates plant competition, but is not a necessary criterion. 3) Negative frequency dependence is stronger (or only occurs) in the presence of microbially-driven plant-soil feedbacks than in their absence.

Here, we tested the validity of each criterion for plant-microbe interactions to generate stabilizing mechanisms of coexistence using two common, soil microbial groups (species-specific rhizospheric microbes and biological soil crusts) and two dominant desert grasses (*Bouteloua gracilis* and *B. eriopoda*). Plants were competed in a response surface design that allowed independent investigation of frequency- and density-dependence. This is critical to capturing the frequency dependence of intra- vs. interspecific competition. In the greenhouse, we replicated the response surface under a fully reciprocal plant-soil feedback experiment, where we could control the composition of rhizospheric microbes and presence of biological soil crusts. For PSF to contribute to stabilizing mechanisms of coexistence, we expected that inoculation of live, conspecific, rhizospheric microbes would result in the strongest negative frequency dependence, and highest per capita self-limitation. By comparing frequency dependency and modeling competitive interactions and invasion growth rates across treatments, we showed that

plant-soil feedbacks caused by rhizospheric microbes can be stabilizing, whereas biological soil crusts partly offset this stabilization through their benefits to both plant species.

Methods and Materials

Study system

We investigated interactions between *Bouteloua gracilis* (Poaceae, blue grama) and *Bouteloua eriopoda* (Poaceae, black grama) with their host-specific rhizospheric microbial communities, as well as the biological soil crusts (biocrusts) that occupy plant interspaces in desert grasslands. *Bouteloua gracilis* and *B. eriopoda* are perennial, C4, grasses that naturally co-occur in the ecotone between Chihuahuan desert grasslands and the short-grass steppe (Kröel-Dulay et al. 2004). Their co-occurrence has been documented for >25 years (Collins and Xia 2015) at the Sevilleta National Wildlife Refuge (Sevilleta hereafter), where our field collections occurred. Although prior work shows that the two grass species compete and their coexistence may be facilitated through recruitment niche partitioning (Peters 2002, Peters and Yao 2012), the mechanisms promoting their long-term coexistence remain elusive.

We focused on biocrusts and rhizosphere microbiota as microbial drivers. Biocrust organisms fix N and C and engage in exchange with *Bouteloua* spp. (Green et al. 2008); they also increase soil moisture and surface stability (Belnap and Lange 2002). In arid grasslands, fungal communities in grass rhizospheres are dominated by dark septate endophytes (DSE), a polyphyletic group characterized by melanized, septate hyphae (Khidir et al. 2010, Porras-Alfaro et al. 2011). They may facilitate host water

uptake, increase plant acquisition of organic nitrogen (Newsham 2011, Kivlin et al.

2013), or act as plant pathogens (Tellenbach et al. 2011). Arbuscular mycorrhizal fungi

(AMF) also colonize grass roots at the Sevilleta (Johnson et al. 2003). While AMF are

best known for improving nutrient acquisition, their effects can span the

parasitism—mutualism spectrum (Johnson et al. 1997, Hoeksema et al. 2010). Other soil

microbial taxa associated with *Bouteloua* spp. include diverse nematodes and bacteria

(Stanton 1983, Ingham et al. 1985).

Response surface competition treatments

To quantify frequency–independent and –dependent effects, we employed a

response surface plant competition design (Law and Watkinson 1987, Inouye 2001) in

the greenhouse, where we could control microbial inoculations. We created 15

combinations of *B. gracilis* and *B. eriopoda* relative frequencies and total densities by

varying the number of individuals of each species per pot (4 levels: 0-6 individuals;

Appendix Fig. 1). This was preferred over the more commonly used replacement series

because it explicitly tests for interactions between total density (total number of plants

per pot) and the relative frequency of a given species. We expected frequency-

dependence to be stronger at higher total plant density, and our design permits that test.

Soil microbe treatments

We implemented a fully-crossed 2X2X2 factorial design of soil microbial

treatments across the response surface. Treatments included all combinations of

rhizospheric inoculum sterilization (live or sterilized), rhizospheric inoculum provenance

(rhizospheric soil from *B. gracilis* or *B. eriopoda*), and biocrust presence (presence or absence). The entire design was fully replicated three times for a total of 360 experimental communities (Appendix Fig. 1).

      Rhizospheric microbes. In late October 2012, we collected rhizospheric (immediately around root zone) soil from planted field monocultures of each species at the Sevilleta. Monocultures plots seeded at 9.07g per m^2 (2x2.5m) were established in 2007 (GPS: -106.6089, 34.406136), and weeded to maintain species composition (details: http://sev.lternet.edu/data/sev-174). From each of three plots of *B. gracilis* and *B. eriopoda* monocultures, we collected one 5.6L sample of rhizospheric soil from the root zone (rhizosphere) of 2-3 mature plants. Inocula were stored at 4°C for one week until application. To isolate the effects of the rhizospheric microbiota, half of the inocula were autoclaved (30 min at 121°C) to reduce the abundance of live biota. Inocula from a different field monoculture plot were used for each replicate to obtain biologically independent replicates.

      Biocrusts. While collections of rhizospheric microbes were designed to maximize plant species-specific differences in microbial composition by taking advantage of existing field monocultures, we had no *a priori* expectation of host-specific differences in biocrust composition. Therefore, biocrusts were collected from plant interspaces at a Sevilleta location where both *Bouteloua* spp. co-occur (GPS: -106.7358, 34.3592). We used 9cm diameter Petri dishes to excise intact biocrust (to 1cm depth), which were stored at room temperature until use (<1 week).

Greenhouse experiment and harvest

In November 2012, we filled 900ml pots with spore-free river sand and inoculated with 90ml live or sterilized soil inoculum added to the top of the pot for optimal seedling colonization. For biocrust additions, each pot received a single Petri dish sample of field-collected biocrust on the soil surface (Appendix Fig. 1). *Bouteloua gracilis* and *B. eriopoda* seeds (Curtis & Curtis Inc., Clovis, New Mexico, USA) were sown on the soil surface. After four weeks, we supplemented with additional seedlings of the same age to reach desired treatment structure. All pots were fertilized once (March 2013) with a weak liquid fertilizer (0.2% each FloraMicro and FloraGro, N:P:K=7:1:7, General Hydroponics, Sebastopol, California, USA). After six months, we recorded the number of individuals surviving, and separately harvested aboveground biomass by plant species. Because it was not possible to separate roots by plant species, washed root tissue was homogenized by pot. We collected ~0.2g root tissue for microscopic examination of fungal colonization. We also collected surface soil (biocrust, top 5mm, ~5g) from every pot. All biomass was dried at 60°C then weighed.

Microbial treatment effectiveness

To assess the treatment effect of biocrust additions, samples from 109 pots were analyzed for soil carbon and nitrogen content using an ECS 4010 CHNSO analyzer (Costech Analytical Technologies, Valencia, CA, USA). To assess the effectiveness of rhizosphere soil sterilization, collected root tissue was cleared and stained following Vierheilig *et al.* (Vierheilig et al. 1998) and scored for fungal colonization following McGonigle *et al.* (McGonigle et al. 1990) for 340 pots. Our microbial manipulation

worked: total colonization rate was 78% lower under sterile (3.3%±0.4% se) than live

soil inoculation (14%±1% se).

Statistical analysis

Microbial treatment effects on plant density and frequency dependence

For each plant species, our experimental design allowed microbial treatments to

alter plant performance regardless of plant total density/frequency, alter slopes of plant

density- or frequency-dependence, and/or alter the potential interaction between density-

and frequency-dependence. To investigate our three criteria, we evaluated six *a priori*

models, representing alternative hypotheses of microbial effects on plant performance

(Table 2). We used model selection to evaluate the likelihood of each proposed

model/hypothesis, given the data collected (Burnham and Anderson 2002). We also fit a

null model which included only an intercept (no treatment effects) to examine if

candidate models provided more information than the null, and to calculate likelihood

pseudo-r^2 values (Burnham and Anderson 2002).

Candidate models were evaluated separately for *B. eriopoda* and *B. gracilis* using

per capita aboveground biomass, *ln*-transformed to meet normality and homoscedasticity

assumptions. We used the number of *B. eriopoda* and *B. gracilis* four weeks into the

experiment (see 'Greenhouse') for total seedling density and relative frequency

predictors. In cases where additional germination occurred after four weeks, we included

these additional germinants in the initial number. Briefly, we fit each linear model to the

data, calculated log-likelihood and *AICc*, ranked candidate models by *AICc*, and

calculated model probabilities (w_i) using MuMIn (Barton 2014) in R version 3.1.2 (R

Development Core Team 2014) following Burnham and Anderson (2002). Because we took an experimental approach, we used parameter estimates from the best model; in all cases, our best models outranked alternatives by *AICc* >2.

Competition model fitting

To further investigate if soil microbial treatment effects on plant competitors altered strengths of per capita intra- and interspecific interactions, we fit simple two species competition models to the data, using biomass accumulation to approximate population growth over generations. For each plant species under each soil microbial treatment combination, we fit the modified discrete time difference logistic model (Hassell and Comins 1976, Adler et al. 2007):

$$\frac{M_i}{N_i} = \frac{\lambda_i}{1+\alpha_{ii}N_i+\alpha_{ij}N_j} \quad \text{Eq. 1}$$

M_i/N_i indicates the mean per capita biomass of individuals of species *i* in the population, where positive values indicate growth. λ is equivalent to intrinsic per capita biomass increase, and α_{ii} and α_{ij} are competition coefficients for the per capita effects of intra- and interspecific competition, respectively. *N* is the number of individuals of each species in the 'population', or experimental pot, at the beginning of the experiment. While logistic difference equations are often used to predict population size at time *t+1* from population sizes at time *t*, we used it to predict the mean per capita biomass increase of individuals in the population here. This approach assumes that biomass scales linearly with population growth at the same rate for these congener competitors, an assumption that requires future testing, but that allowed us to harness the power of the model fitting approach.

Model parameters were fit using maximum likelihood estimation, assuming

normally distributed errors, with function mle2 in R version 3.1.2 (R Development Core

Team 2014). We constrained λ's to be positive, and the absolute values of all parameters

to a maximum of 5 to avoid model fits that could not discriminate between extremely

high intrinsic growth and density dependence that was biologically implausible, or

weaker intrinsic growth and density dependence. In one model, fitted for *B. gracilis*

inoculated with live *B. gracilis* rhizospheric microbes and no biocrust, we were unable to

find a local fit. Instead, we found very strong intraspecific interactions where the

maximum likelihood parameter was estimated at the upper boundary of 5. Compared to

its converged fit at implausibly high intrinsic growth and density dependence, this

alternative boundary fit resulted in only a 1% loss in log-likelihood; thus, we used the

boundary fit, which was a conservative decision.

We evaluated coexistence by directly calculating the invasion growth rate for

each species using the fitted parameters. This expression, the per capita growth rate of

species *i* when it is rare and species *j* is at single-species equilibrium, can be solved for

analytically from Eq. 1 to be (see Appendix)(Adler et al. 2007):

$$\frac{M_i}{N_i} = \left(\frac{\lambda_i}{\lambda_j}\right)\left[\frac{\lambda_j}{1+(\alpha_{ij}/\alpha_{jj})(\lambda_j-1)}\right] \qquad \text{Eq. 2}$$

Species that coexist are mutually invasible. In this case, both species have positive

invasion growth rates. As we assumed that biomass accumulation scaled linearly and at

the same rate to population growth rate for both *Bouteloua* species, relative differences in

calculated invasion growth could be compared among microbial treatments and between

competitors. While we could not directly translate calculated invasion growth rates to

population growth because we lacked information on other vital rates (e.g., survival,

recruitment), some positive threshold "low density biomass accumulation rate" must be reached in order to reproduce and contribute to positive invasion population growth. To evaluate the effects of PSF on species coexistence, we compared invasion growth rates for each species when invading their competitor, with either live or sterile competitor soil microbial communities.

Results

Competitors showed strong fitness (frequency-independent) differences: *B. gracilis* was the competitive dominant, achieving on average 890% higher per capita shoot biomass than *B. eriopoda*. Thus, to increase potential for stable coexistence between these two plant species, inoculation with its own, live, rhizospheric microbes should increase negative frequency dependence and self-limitation for *B. gracilis*, and plant-microbe interactions should increase invasion (biomass) growth rates for both species.

Plant-soil feedbacks increased self-limitation in *B. gracilis*

Bouteloua gracilis experienced the strongest negative frequency dependence in the presence of its own rhizospheric microbes, fulfilling all three criteria to demonstrate that PSF is a viable stabilizing mechanism of coexistence. First, *B. gracilis* per capita shoot biomass was 28% lower when grown with its own live rhizosphere inocula than with *B. eriopoda* rhizosphere inocula, indicating negative PSF. This effect weakened when *B. gracilis* received sterilized inocula from its own or its competitor's rhizosphere, demonstrating that the feedback is microbially-driven (criterion 1). Second, we found

negative frequency-dependence in *B. gracilis*, which intensified at the highest plant

densities, as expected if competition is important (criterion 2). Third, negative frequency

dependence was strongest in the presence of host-specific, rhizospheric microbes

(criterion 3). The model including microbial effects on the interaction between

frequency- and density-dependence had the highest support (w=0.768, likelihood pseudo-

$r^2 = 0.23$, Table 2, Appendix Table 1). Specifically, *B. gracilis* inoculated with its own

rhizospheric microbes experienced 40%-50% stronger negative frequency dependence

(difference in slope) than when inoculated with sterilized *B. gracilis* microbes, and 75%-

115% stronger negative frequency dependence than when inoculated with live microbes

from the competitor, *B. eriopoda* (Fig. 1, Appendix Table 2).

Our competition models described similar results. Self-limitation (intraspecific

competition coefficient) was stronger for *B. gracilis* when inoculated with its own live

rhizosphere microbes compared to live *B. eriopoda* microbes (10-fold difference in

average *α*, Table 3, Appendix Fig. 2). There was no such difference between competitors

when given sterile inoculations. This effect was partially driven by the model for *B.

gracilis* with the high boundary fit ($α = 5$, no biocrusts added). However, the same

rhizosphere treatment but with biocrust added resulted in the second strongest self-

limitation for *B. gracilis* of all treatments ($α = 0.84$). Thus, we are inclined to conclude

that this is a biological result.

The subordinate competitor, *B. eriopoda*, in contrast, experienced very weak,

positive PSF (Fig. 1). The presence of its own rhizospheric microbes resulted in ~10%

higher positive frequency dependence compared to inoculation with microbes from its

competitor (best model included microbial effects on frequency-dependence: w=0.773,

likelihood pseudo-$r^2 = 0.20$, Table 2, Appendix Tables 1 & 2). While analysis revealed microbial effects on frequency dependence, the positive direction of frequency dependence did not support criteria 2 or 3 for the subordinate competitor.

Our competition modeling confirmed positive per capita intraspecific interactions among *B. eriopoda* (shown by negative α) in 5 out of 8 microbial treatments. Of the three treatments that did not result in intraspecific facilitation, standard errors of parameter estimates were large and overlapped zero (Table 3). These results suggest that there are facilitative interactions among *B. eriopoda* individuals, and that positive frequency dependence is unlikely caused solely by release from strong interspecific competition (Appendix Fig. 3). Interestingly, *B. eriopoda* experienced stronger average self-limitation when inoculated with live, rhizospheric microbes from *B. gracilis* compared to live microbes from its conspecifics. These results should be interpreted with caution, however, due to the combinations of positive and negative intraspecific interactions fitted in *B. eriopoda*.

Biological soil crusts destabilize plant species coexistence

The effects of biocrust additions were smaller than those of rhizospheric microbes, were generally positive rather than negative, and showed potential to destabilize, rather than stabilize, coexistence. Destabilization emerged through two pathways: increasing positive frequency dependence in *B. eriopoda* and decreasing negative frequency dependence in *B. gracilis*. Biocrust addition decreased negative frequency dependence of *B. gracilis* at the highest total plant densities by ~30%, and increased positive frequency dependence in *B. eriopoda* ~20% (Fig. 1, Appendix Table

2). This result was also mirrored in the competition modeling, where biocrust addition resulted in 50% more intraspecific facilitation in *B. eriopoda* and 64% less intraspecific competition in *B. gracilis* (Table 3, Appendix Fig. 2 & 3). Elemental analyses indicated that biocrust addition increased soil nitrogen by nearly 30% ($F_{1,107}$=48.93, p<0.001) and soil carbon by 20% ($F_{1,107}$=34.45, p<0.001).

Potential for plant microbial interactions to promote coexistence between plant competitors

Our results showed that negative PSF from species-specific, rhizospheric microbes strengthened negative frequency dependence and self-limitation in *B. gracilis*, the competitive dominant (Fig. 2). Positive (destabilizing) microbial effects were small compared to the microbially-driven increases in *B. gracilis* negative frequency dependence (10% increase in positive frequency dependence vs. 75-115% stronger negative frequency dependence) (Fig. 2).

Invasion growth rate calculations, best interpreted here as "low-frequency biomass accumulation rates", indicated that the presence of live PSF allowed mutual invasibility, a coexistence criterion. Particularly for *B. eriopoda*, invasion growth rates were more positive when the resident *B. gracilis* experienced live PSF compared to sterile PSF (Fig. 3A). In comparison, *B. gracilis* invasion growth rates were 1-2 orders of magnitude larger than those of *B. eriopoda*, and positive regardless of whether the resident *B. eriopoda* population experienced live PSF (Fig. 3B). We caution that our invasion growth rates only considered biomass accumulation and no other vital rates,

such as reproduction or germination. Therefore, positive invasion rates for both species under live PSF provide a foundation for coexistence, but do not ensure coexistence.

Discussion

Under the modern species coexistence framework, plant-microbe interactions can mediate plant species coexistence via two pathways: decreasing the fitness difference between plant competitors or increasing stabilization by increasing self-limitation. In this study, there were strong fitness differences between competitors: *B. gracilis* was the competitive dominant and *B. eriopoda* the competitive subordinate in all scenarios. Thus, to increase the chances of coexistence between these two plant species, plant-microbe interactions should increase self-limitation of *B. gracilis* (stabilizing), and/or decrease average fitness differences between the two species (equalizing). As our results showed little evidence of strong frequency-independent microbial effects, we focus our discussion on the former.

Plant-soil feedbacks increased self-limitation in *B. gracilis*

Our study demonstrated experimentally that negative PSFs are capable of generating negative frequency dependence in a plant species. While there may be other mechanisms (such as resource partitioning between plants) that accounted for the weakly negative frequency dependence of *B. gracilis* inoculated with sterilized rhizospheric microbes, it was the presence of its own rhizospheric microbes that resulted in the strongest negative frequency dependence. Our competition modeling focusing on per capita effects also confirmed this result, showing the strongest self-limitation in *B.*

gracilis inoculated with its own, live, rhizospheric microbes. Prior work correlating the strength of PSF with species relative abundance (Klironomos 2002, Mangan et al. 2010) as well as pairwise studies examining PSF under competitor present/absent scenarios (Kardol et al. 2007, Petermann et al. 2008, Kulmatiski et al. 2011, Pendergast et al. 2013) set the stage for PSF as a potential driver of plant community dynamics, but do not wholly demonstrate it as a stabilizing mechanism that increases self-limitation. In addition, while negative conspecific density or distance dependent effects on seedling survival found in Janzen-Connell studies may result in negative frequency dependence when considered inter-generationally, we found that soil communities are able to intensify intraspecific interactions within the same generation. Our results established the importance of PSF by comparing the strength of negative frequency dependence/self-limitation in the presence versus absence of host-specific soil microbes. In addition, our response surface design demonstrated the interactive effect of total plant density and relative species frequency, which has not yet been shown for any plant-microbe interaction, but is likely a prevalent phenomenon.

Our results suggest that rhizospheric microbes associated with *B. gracilis* plants increased self-limitation. Past work at our study site has found diverse fungal taxa associated with *B. gracilis*, including AMF species (Johnson et al. 2003) as well as putative pathogens (Khidir et al. 2010). Work from other sites has also reported bacteria and nematode species that may affect *B. gracilis* growth and performance (Stanton 1983, Ingham et al. 1985). Fungi such as AMF could potentially increase niche partitioning via alternative resource acquisition (Gustafson and Casper 2006), and species-specific phytopathogens are well-known to drive plant community dynamics (Mordecai 2011).

Future molecular work characterizing the microbial community associated with experimental populations could shed more light on potential mechanisms behind the observed phenomena.

Biocrusts: a destabilizing mechanism

Our results suggest that biocrusts generally benefited *Bouteloua* grasses. While the effects of biocrust additions were smaller than those of rhizospheric microbes, biocrusts were destabilizing through two pathways: increasing positive intraspecific interactions in *B. eriopoda* and decreasing negative intraspecific interactions in *B. gracilis*. Elemental analyses indicated that biocrust addition increased C and N in surface soils. These effects likely arose from the dominant micro-organisms found in the light biocrusts of our desert grasslands: photosynthesizing cyanobacteria such as *Microcoleus*. spp., which can contribute to soil organic carbon, and *Nostoc* spp., which can fix nitrogen (Belnap and Lange 2002, Evans and Lange 2003). Whether benefits were due directly to the presence of biocrust microbes or indirectly to their effects on edaphic characteristics remains unresolved. A greenhouse biocrust-addition experiment using the perennial grass, *Elymus elymoides,* found similar gains in biomass; moreover, *Elymus* grown with biocrust showed higher tissue nutrient concentration, a potential mechanism for increased per capita performance (Pendleton et al. 2003). Few studies have investigated biocrust mediation of plant competition and coexistence. However, a comparison of spatially paired plots that were naturally biocrusted vs. naturally bare found that biocrusted soils supported 4-9X greater percentage of exotic plant species, which likely were the stronger

competitors, lending support to potential de-stabilizing effects of biocrusts (DeFalco et al. 2001).

Can plant-microbial interactions contribute to stabilizing coexistence?

Under these experimental conditions, our results indicated that *B. gracilis* would likely have competitively excluded *B. eriopoda* under all treatment scenarios due to its much faster growth rate. However, we found that negative PSF resulted in stronger negative frequency dependence/self-limitation in the dominant competitor, *B. gracilis*, which could contribute to coexistence via stabilizing forces. In addition, positive invasion growth rates were present for both species (mutual invasibility) only when the resident species experienced live PSF. Our results suggest that while the two competitors were asymmetrically matched in their competitive abilities, feedbacks between plants and rhizospheric microbes could potentially decrease the time to competitive exclusion for species in mixture, and increase the potential for coexistence in our experimental communities. Stable, long-term coexistence in the field could require additional mechanisms. For example, others have found that plant-scale spatial heterogeneity in PSF can further promote coexistence (Burns and Brandt 2014). Temporal variance in climate, such as interannual variability in precipitation, could also promote species coexistence via fluctuation dependent mechanisms such as the storage effect (Chesson 2000, Angert et al. 2009).

Our results are likely a conservative test of coexistence. Here, we measured per capita aboveground biomass as an indicator of individual plant fitness. However, competition and coexistence outcomes are population-level phenomena, which

additionally depend on adult survival, reproduction and recruitment. In our competition modeling, we assumed that per capita biomass accumulation of both *Bouteloua* species scaled linearly to population growth at the same rate. Previous work at this site has shown that *B. eriopoda* has much higher fecundity per gram of vegetative biomass compared to *B. gracilis* (Peters 2002). Therefore, it is possible that the fitness difference between the two species at the population level is smaller than measured in this study, due to the higher reproductive capacity of *B. eriopoda*. Additional information on survival, germination, and recruitment where the two species co-occur will increase our ability to generalize our results based on plant growth.

In the Chihuahuan desert grasslands of New Mexico, USA, where their ranges overlap and field collections for this study took place, *B. gracilis* and *B. eriopoda* are the dominant species and are known to coexist based on long-term monitoring (Collins and Xia 2015). Results of experimental removal studies at this site suggest that it is likely competitive dominance of *B. gracilis* that results in *B. eriopoda* subordination, whereas other factors, such as the abiotic environment, may determine areas where *B. eriopoda* dominates and *B. gracilis* is subordinate (Peters and Yao 2012). Our greenhouse experiment uncovered a competitive hierarchy similar to that demonstrated experimentally at our field site, which provides confidence that it is applicable to this ecosystem. However, it remains to be explored under what circumstances PSF is an important mechanism in driving species coexistence in the field, relative to unexamined, alternative coexistence mechanisms including fluctuation-dependent mechanisms (Chesson 2000).

Conclusion

For the first time, we experimentally quantified the contributions of two classes of plant-microbe interactions to the mechanisms of coexistence between foundational plant species. Our study demonstrates that conspecific plant-soil feedbacks from rhizospheric microbes enhance the possibility of species coexistence through stabilizing effects that increase self-limitation of the dominant competitor. These findings also suggest a role for plant-microbe interactions in structuring plant communities in arid and semiarid ecosystems, where such interactions have not been well studied.

Data accessibility

The data supporting this article has been deposited on Dryad doi:10.5061/dryad.780hc

Competing interests

We declare that we have no competing interests

Author contributions

YAC and JAR designed the experiment and wrote the manuscript. YAC conducted the experiment and analyses under the guidance of JAR.

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Figures and Tables

Table 1
Description of necessary criteria to demonstrate that plant-soil feedbacks are a stabilizing mechanism of coexistence.

Criterion	Description	Evidence
1	Negative PSF occurs and is caused by soil microbiota	Species performance is reduced with its own soil microbes relative to those of its competitor(s).
2	Negative frequency dependence occurs	Per capita performance of a species declines as its relative frequency in the community increases; stronger effects with higher total plant density indicate strong competition.
3	Negative frequency dependence is stronger (or only occurs) in the presence of microbially-driven PSF	Declines in per capita performance as relative frequency increases are steeper in the presence of PSF than in its absence.

Table 2
Description of candidate models testing microbial effects on frequency- and density-dependence, and their relationships to coexistence criteria.

Model	Description	Parameterization*	Fulfills coexistence criteria
i	All microbial effects on frequency-, density-dependence, and their interaction	~ Freq + Dens + Dens:Freq + Crust + Inoc + Inoc:InocSPP + Crust:Freq + Inoc:InocSPP:Freq + Crust:Dens + Inoc:InocSPP:Dens + Crust:Freq:Dens + Inoc:InocSPP:Freq:Dens	Criteria 1, 2, 3
ii	Only frequency- and density-independent microbial effects	~ Crust + Inoc + Inoc:InocSPP	Criterion 1
iii‡	Only frequency-dependent microbial effects	~ Crust:Freq + Inoc:InocSPP:BOGRfreq	Criteria 1, 2, 3
iv	Only density-dependent microbial effects	~ Crust:Dens + Inoc:InocSPP:Dens	Criterion 1
v†	Only microbial effects on the interaction between frequency- and density-dependence	~ Crust:Freq:Dens + Inoc:InocSPP:Freq:Dens	Criteria 1, 2, 3
vi	Frequency- and density-dependence with no microbial effects	~ Freq + Dens + Freq:Dens	Criterion 2

*Parameter descriptions: Freq: focal species relative frequency, Dens: total plant density, Crust: biocrust presence/absence, Inoc: sterilized or live rhizosphere inoculation, InocSPP: provenance of rhizosphere inoculum. Colons (:) indicate interactions between terms.
†Best model for *B. gracilis* per capita biomass.
‡Best model for *B. eriopoda* per capita biomass.

Table 3
Maximum likelihood estimates of two species competition parameters under different microbial treatments. Standard errors included when estimation was possible. Parameters constrained to (0, 5) for λ, and [-5,5] for α.

Microbial treatment			Species i (*B. eriopoda*) fitted parameters ± SE			Model log-likelihood	Species j (*B. gracilis*) fitted parameters ± SE			Model log-likelihood
Rhizosphere soil										
Provenance	Sterilize	Biocrust presence	λi	αii	αij		λj	αjj	αji	
BOER	Live	No crust	0.016±0.004	-0.203±0.014	0.539±0.236	78.98154	0.519±0.128	0.374±0.159	0.035±0.091	40.137
BOGR	Live	No crust	0.028±0.016	0.150±0.241	0.344±0.382	65.77832	2.659±2.014	5.000±4.105	1.305±1.178	52.491
BOER	Sterile	No crust	0.048±0.027	0.190±0.383	2.322±2.134	76.88565	0.581±0.287	0.306±0.305	0.130±0.222	9.865
BOGR	Sterile	No crust	0.020±0.006	-0.106±0.026	0.360±0.232	82.20699	0.260±0.074	0.123±0.113	0.042±0.077	39.682
BOER	Live	Crust	0.078±0.028	0.195±0.295	1.691±1.307	62.18035	0.369±0.101	0.151±0.106	0.044±0.109	33.191
BOGR	Live	Crust	0.029±0.015	-0.122±0.104	0.350±0.380	48.61692	0.743±0.378	0.841±0.631	0.366±0.283	41.521
BOER	Sterile	Crust	0.051±0.016	-0.112±0.037	1.023±0.715	62.73996	0.892±0.286	0.601±0.289	0.126±0.117	36.738
BOGR	Sterile	Crust	0.011	-0.150	-0.001	47.81043	0.714±0.224	0.495±0.290	0.219±0.162	23.131

Figure 1

Each point represents the slope (±SD) of per capita biomass regressed on frequency of the plant species in the pot [*as shown in insets*], calculated for each microbial and density treatment. Frequency-dependent slopes are shown for *B. eriopoda* (top) and *B. gracilis* (bottom), with biocrust present/absent. Plants inoculated with *B. gracilis* microbes are in blue, and inoculations with *B. eriopoda* microbes are in black. Sterile inoculations are indicated by dashed lines/open symbols, and live microbial inoculations by solid lines/filled symbols. *B. gracilis* showed increasing negative frequency dependence as total density increased, whereas *B. eriopoda* showed consistent positive frequency dependence regardless of total density. The strongest negative frequency dependence was found in *B. gracilis* plants inoculated with conspecific microbes, under the highest total densities. Note the difference in *y*-axis scales between the two species.
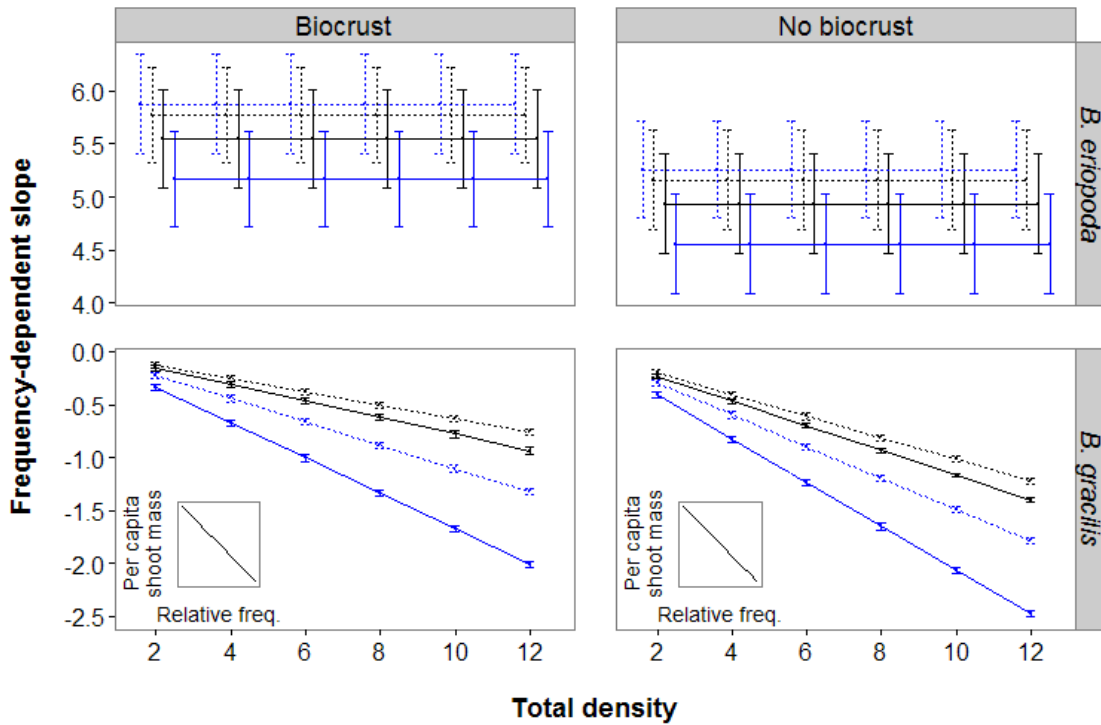
Figure 2
Frequency-dependence of fitted per capita biomass of *B. gracilis* (light blue/blue) and *B. eriopoda* (gray/black) under rhizospheric microbe treatments and A) biocrust present versus B) biocrust absent. Dashed lines with pale ribbons (SD) indicate inoculation with conspecific rhizospheric microbes, and solid lines with dark ribbons (SD) are with heterospecific microbes. Only live inoculations are shown here for clarity.
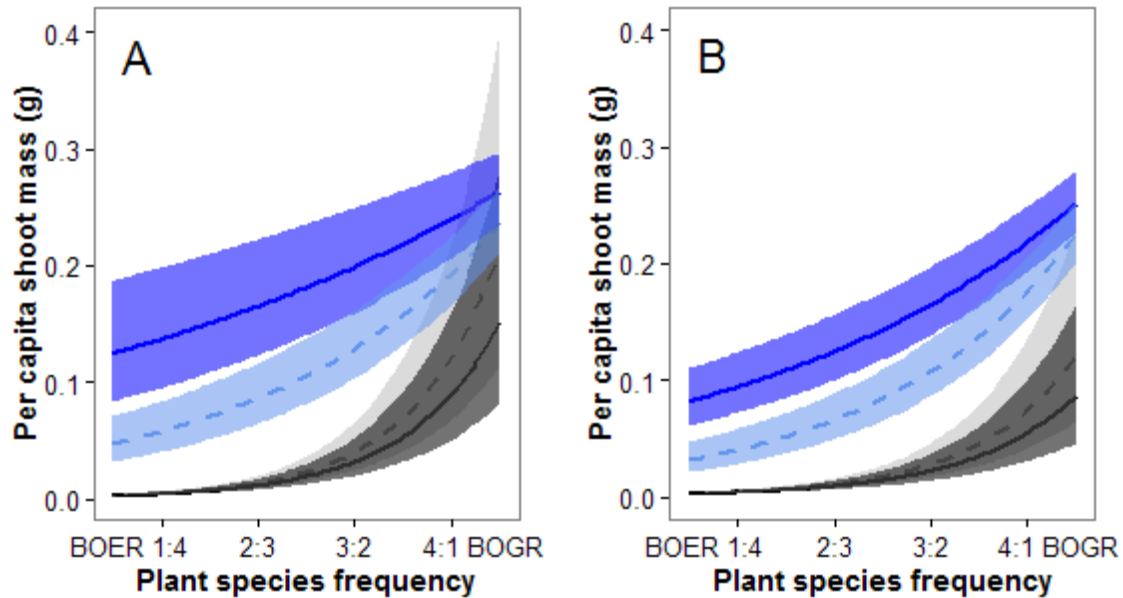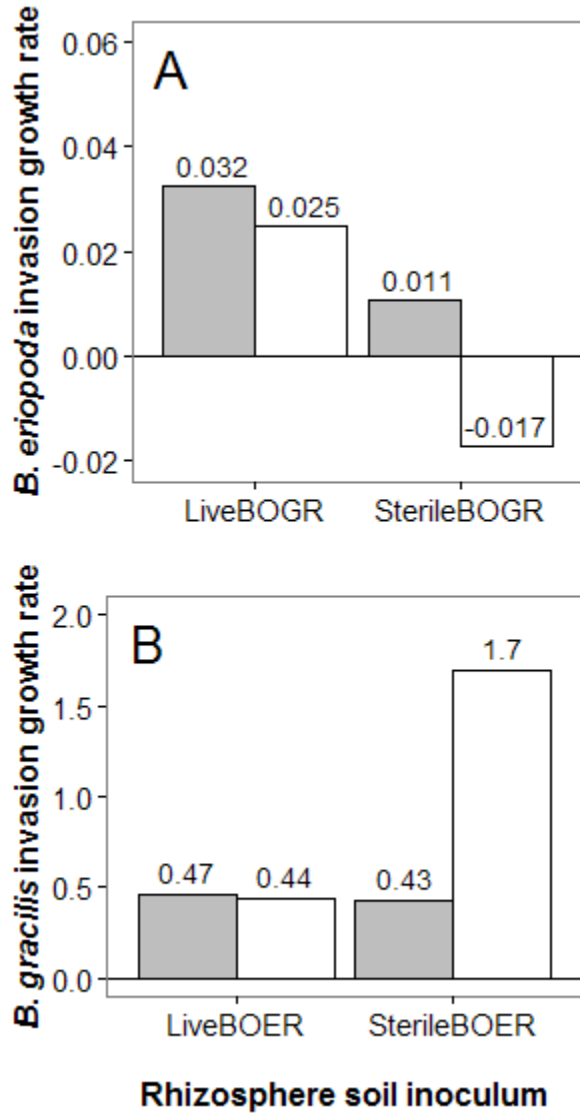
Figure 3

Calculated invasion growth rates for (A) *B. eriopoda*/BOER and (B) *B. gracilis*/BOGR with different microbial treatments. Grey bars indicate biocrust addition treatments, while white bars received no biocrust addition. Rhizosphere soil microbial inocula were live or sterilized, from the resident host plant species.

Chapter 3

Negative plant-soil feedbacks correspond with long term plant community stability in a

Chihuahuan Desert grassland

Abstract

Feedbacks between plants and their soil microbes have gained recognition as a potentially strong driver of plant community patterns. For example, plant-soil feedbacks have been found to correspond to patterns of plant commonness and rarity, alter competition and coexistence outcomes, and underlie patterns of plant diversity and productivity. Few, however, have connected plant-soil feedbacks to temporal patterns of plant community composition, and those that do have focused on directional patterns such as succession. Plant community stability, measured as the frequency and magnitude of plant composition change, arises from differential competition and coexistence among plant populations, which could be mediated by microbial symbionts. However, testing these relationships requires long-term knowledge of plant community patterns, and the ability to measure plant-soil feedbacks at ecologically-relevant spatiotemporal scales. Here, we took advantage of a long term data from a semiarid grassland that showed spatial variation in the long term patterns of temporal stability in plant community composition in a semiarid grassland, and experimentally tested plant-soil feedbacks *in situ*. We found that negative feedbacks corresponded to patches of high stability, whereas neutral to positive feedbacks corresponded to patches of low stability. In addition, the magnitude of soil microbial effects was frequency-dependent in a way that promoted invasibility when a plant species was rare, a criterion for coexistence. The relationship between plant-soil feedbacks and plant community stability differed among different plant life history stages in species-specific ways. Our results suggest that negative plant-soil feedbacks may play a key role in maintaining the stable coexistence of plant communities through time, and highlight the importance of incorporating ontogenetic dependence in studies of plant-soil feedbacks.

Introduction

Feedbacks between plants and their soil microbial communities could be crucially important in explaining plant community dynamics (Bever et al. 2010, van der Putten et al. 2013). As plant species increase in abundance, they can accumulate species-specific communities of microbial associates in their roots and nearby soils. These self-selected microbial communities are often more detrimental to the plant host compared to those cultivated by co-occurring heterospecific plants, causing a negative plant-soil feedback (PSF; Bever et al. 1997). Such feedbacks can alter competitive dynamics among plant species, potentially contributing to frequency dependence and species coexistence by altering the relative magnitudes of intra- and interspecific interactions among plant species (Burns and Brandt 2014, Chung and Rudgers 2016).

To date, the strongest evidence that PSFs explains plant community dynamics has come from studies on plant species commonness and rarity. The direction and magnitude of PSFs were correlated with the relative frequencies of plants in communities as different as tropical forests and temperate grasslands (Klironomos 2002, Mangan et al. 2010, Anacker et al. 2014, Maron et al. 2016, but see Reinhart 2012). Efforts that combine greenhouse experiments with modeling also show that PSFs can be important in diversity maintenance and density dependence at large spatial scales (Bennett et al. 2017, Teste et al. 2017). Lastly, diversity-productivity relationships have also been found to be driven partly by plant interactions with soil microbes (Maron et al. 2011, Schnitzer et al. 2011).

While these tests have contributed to understanding how plant-microbe interactions can influence plant communities, less is known about how PSFs alter temporal patterns in plant communities such as species turnover and stability. The causes of community stability have

interested ecologists for decades (e.g. MacArthur 1955, Tilman and Downing 1994, Knapp and Smith 2001, Hallett et al. 2014), yet mechanisms to explain stability have focused primarily on abiotic drivers and largely overlooked the potential role of plant-associated microbes (but see Rudgers et al. 2007). Evidence supports the potential of plant-microbe interactions, such as PSF, to shape temporal trajectories of plant communities. For example, past work has shown that early and late successional plant species differ in their feedbacks in ways that may promote directional change in plant community composition (reviewed in Kardol et al. 2013). In addition, theoretical models that consider PSFs explicitly have shown that negative PSFs can allow stable coexistence in situations where one plant would otherwise competitively exclude another by regulating the amplitude of oscillation between the relative abundance of two coexisting species (Revilla et al. 2013). However, a relationship between the strength of microbe-mediated PSF and long-term coexistence of plant species has not yet, to our knowledge, been documented for any plant community in the field.

Such work requires long-term data on community dynamics, the ability to test PSFs in the field, and an experimental period long enough to capture all plant life history stages. To date, most PSF studies to date have been conducted in the greenhouse and for relatively short timescales, which rarely reflect conditions in the field (Kulmatiski and Kardol 2008, Kulmatiski et al. 2008). Differential proliferation of microbiota from soil inocula and context-dependency of plant-microbe interactions under favorable greenhouse conditions may lead to more negative PSFs (Kulmatiski et al. 2008). In addition, evidence has shown that PSFs can undergo temporal fluctuations in greenhouse conditions (Hawkes et al. 2013), and that generally, longer studies tend to show less negative feedbacks (reviewed by Kardol et al. 2013). Thus, when PSFs can be evaluated in the field, this should maximize the ability to explain observed patterns of plant

species dynamics. Moreover, field experiments also afford the opportunity to characterize PSF effects on plant hosts that go beyond the most commonly examined vital rate: growth. Studies of other plant-microbe interactions show that the effects of these interactions can vary in magnitude and direction throughout host life history (Rudgers et al. 2012, Chung et al. 2015). To better understand how PSFs affect plant population and community dynamics, it is crucial to consider their effects within the context of the entire life history of the plant host (Kardol et al. 2013).

We conducted a field experiment to link the strength of PSF to long-term dynamics of community stability and species turnover at ecologically-relevant spatiotemporal scales. We first quantified 26 years of dynamics for two dominant grass species using observations along a 400m transect in a northern Chihuahuan Desert grassland. We identified areas along this transect that showed spatial variability in the rates of change in vegetation composition (Collins and Xia 2015). Dynamic patches showed frequent changes in species relative abundances, whereas static patches showed stable plant species composition through time. Using this information, we measured the strength of PSF in dynamic and static patches in the field for focal plant species using experimental transplants of both plants and seeds. We asked: 1) Do the strength and direction of PSF differ between dynamic and static patches? Theory predicts stable coexistence to arise under conditions when species are more self-limited than limited by others (Volterra 1926, Lotka 1932). Therefore, we predicted that static patches of high community stability would be associated with strong negative feedbacks. 2) In static patches, are soil microbial effects correlated with plant frequency? Theory predicts that the effects of conspecific and heterospecific soil microbes should increase with the relative frequency of plant hosts (Bever et al. 1997). Feedbacks that promote the establishment of a plant species most strongly when it is rare would be consistent with the negative frequency dependence that is required to promote

stable plant species coexistence. 3) Does the relationship between PSF and historical stability in plant species composition change with plant ontogeny? While it is often assumed that seedlings are most susceptible to pathogen attack and thus to negative PSF (Packer and Clay 2000), there is little evidence to demonstrate how PSF effects change across plant life history stages (e.g. Kos et al. 2015). Our goal was to provide a more complete picture of potential net effects on plant population dynamics by examining PSF at seed, seedling, and adult (up to 3 years) stages.

METHODS

Study site

The study was conducted in the Sevilleta National Wildlife Refuge, New Mexico, USA. The Sevilleta Long Term Ecological Research (LTER) program began to monitor vegetation transects in the Chihuahuan grassland ecosystem beginning in 1989. For this study, we used long-term data from the Deep Well transect (34.3591, -106.688; Collins 2016), which stretches 400m in a north-south orientation and is co-dominated by *Bouteloua gracilis* (Poaceae, blue grama) and *Bouteloua eriopoda* (Poaceae, black grama). Since 1989, the transect has been monitored twice each year in the spring and fall using the line intercept method at 1cm resolution (see http://sev.lternet.edu/data/sev-4 for detailed description). Past work on this dataset revealed spatial variation in the temporal dynamics of dominant *Bouteloua* species (Collins and Xia 2015). Dynamic patches showed frequent changes in species relative abundances, whereas static patches showed relatively stable species composition through time (Fig. 1). Using these data, we picked static and dynamic patches along the transect for the field feedback experiment.

Candidate patch selection using the magnitude and frequency of temporal dynamics

Plant cover data from the Deep Well line intercept transect from spring censuses 1989-2012 were binned into 4m patches by year. Within each section and for each year, we calculated total plant cover, *B. gracilis* cover, *B. eriopoda* cover, as well as the percentage cover of each *Bouteloua* species out of the total plant cover for that year. To identify candidate patches for the field experiment, only patches with mean total plant cover >35% across years, and for which *Bouteloua* species (combined) represented >50% of total plant cover were used (77 out of 100 met these criteria).

To quantify the *magnitude* of change in relative *B. gracilis* and *B. eriopoda* cover in each section across years, we summed the Euclidean distances between plot composition in % *B. eriopoda* and % *B. gracilis* space from year to year in each section. Patches with high interannual change in relative *Bouteloua* species cover would have high summed Euclidean distances. Bray-Curtis distances produced similar results. To quantify the *frequency* of change between *B. gracilis* and *B. eriopoda* dominance in each plot, we counted the number of switches in dominant species cover over the period 1989-2012, with dominance defined as >5% difference in cover of one species over the other. These two values (magnitude and frequency of change) were then combined into a single metric ("dynamic score") by transforming both values to a standard normal distribution (mean = 0, SD = 1) and adding them together. Patches with scores of < -1 were considered static candidates, and those >1 dynamic (Fig. 2).

Temporal dynamics of candidate patches were individually plotted and checked. This was important the transect burned in 2009, and *B. gracilis* has been faster to recover from fire than *B. eriopoda* (Collins 2016). Care was taken that the patches classified as "dynamic" were not primarily driven by the dominance switch post-fire. From these candidates, we chose 10 spatially paired dynamic and static patches to account for abiotic gradients in the field. Each patch

included the presence of both focal species, with the static patches including 5 that are more

dominated by *B. eriopoda* and 5 by *B. gracilis* (dominance defined as >10% more in percent of

total cover). In the field, one 1x2m experimental plot was established within each identified

patch <2m from the transect (*N*=20 plots). Two focal established plants, one *B. gracilis* and one

B. eriopoda, were identified in each plot (Fig. 3).

Abiotic covariates

To elucidate potential abiotic drivers of plant performance and dynamic/static co-

occurrence patterns, we measured soil texture, chemistry, temperature, and moisture at each focal

plant in each experimental plot (Fig. 3). Soil moisture and temperature were measured using an

Aquaterr T-350 probe (Aquaterr Instruments & Automation, Costa Mesa, California) to a depth

of 10-15cm in June 2014, and again following a rain event in July 2014. Two soil cores

combined to make 50g dry weight were taken near each focal plant, and soil texture determined

using a hydrometer (Bouyoucos 1962). Soil chemistry and nutrient dynamics were measured

using PRS ion exchange resin membrane probes (soil N, Ca, Mg, K, P, Fe, Mn, S, Al; Western

Ag Innovations, Saskatoon, Canada), which were installed in the field plots for 1.5 months June-

July 2014.

Field PSF transplant experiment

Our candidate plot selection methods above yielded 20 spatially-paired plots in dynamic

and static patches, with 5 of the 10 static patches more dominated by *B. eriopoda* plant cover and

5 more dominated by *B. gracilis* plant cover. To determine if differences in PSF strength

corresponded to differing histories of community stability, we used experimental transplants as

phytometers. Briefly, seedlings of each species were planted next to resident conspecific or heterospecific plants, which allowed us to calculate PSF by contrasting the performance of transplants in conspecific or heterospecific soils ('feedback plants', 4 per plot, 2 each species). A 'no fungi' seedling for each species was planted next to its conspecific resident plant (2 per plot, 1 each species) as a control to identify whether the net effect of resident fungi is mutualistic or pathogenic relative to conditions of no/reduced fungi by comparing against 'feedback plants' in conspecific and heterospecific soil environments. In total, each plot received 6 experimental transplant seedlings (Fig. 3).

Seedlings for the transplant experiment were started in April 2014. Seeds (Curtis and Curtis, Clovis, NM) of *B. gracilis* and *B. eriopoda* were germinated in autoclaved sand (sterilized 3h 121 °C) in 30cm-deep, 8x8 cm^2 conetainers (Stuewe and Sons) in the greenhouse. As seedlings germinated, they were thinned until only one seedling remained in each conetainer (*N*=120 total seedlings). Seedlings were mist watered 3 times a day for 4 mins in the first month, then 2 mins in the second month. Starting the last week of May, seedlings were fertilized once a week with a weak fertilizer solution (5ml per pot FloraGro and FloraMicro at 650 ppm, N:P:K = 7:1:7; General Hydroponics, Sebastopol, California, USA). Seedlings were moved onto an outdoor bench in late June to acclimatize to ambient temperature and light conditions, and watered twice a day to water holding capacity. Seedlings were transplanted out into the field experiment in late July, 2014, during a week of frequent rainfall.

'Feedback' plants and 'no fungi' plants differed in their exposure to the resident plant-soil feedback environment. 'Feedback' plants were transplanted enclosed in 30μm Nitex® mesh cylinders [7.6cm diam. 30cm tall] (modified from Reed and Martiny 2007). This allowed ambient microbial colonization from the rhizosphere of the resident plant, but excluded direct

root competition. In the field, a 10cm diam. × 30cm deep soil core was taken from each planting location, passed it through a 2mm sieve, and homogenized. The mesh cylinder was inserted into the ground, and filled with the homogenized field soil following methods in Casper and Castelli (2007). For the 'no fungi' control seedlings, soil cores from their planting locations were separately sterilized (autoclaved 3h gravity at 121 °C), then used to fill cylinders made of 0.45 μm nylon mesh (Ultracruz® transfer membrane, Santa Cruz Biotech, TX, USA) to limit fungal colonization. These 'no fungi' plants tested for non-fungal-mediated effects on plant growth (e.g., differences in soil resource availability). It is unlikely that the 'no fungi' plants remained free of bacterial colonization (Allison et al. 2013), or completely free of fungal colonization due to spores dispersing from aboveground. However, the comparison between 'feedback' and 'no fungi' treatments will still give a conservative estimate of the net effect of fungi. Once cylinders were installed, seedlings were transplanted directly into cylinders and watered (35ml). The height and number of tillers and leaves on each seedling were recorded at transplant.

Field PSF response variables

In the first year of establishment (2014), transplants were censused every two weeks until the end of October and survival, number of leaves, tillers, and height of tallest tiller were recorded. In the subsequent growing seasons (2015, 2016), censuses were conducted every two weeks from June through October. We recorded survival, number of tillers, basal area diameters, and height of tallest tiller. To ensure against mass mortality of transplants under high drought field conditions, transplants were watered 30ml each (equivalent to 6.6 mm rain event) during the growing season (May-Oct) when rain had not occurred for >2 consecutive weeks. These values are based on long term observations at this site, where the mean precipitation event size

1989-2015 was 4.7 mm, mean consecutive dry days was 11.3 days, and mean monsoon season precipitation was 140.6 mm. Total additional watering was equivalent to 6.6 mm in 2014 (179.7 mm ambient monsoon precipitation), 66 mm in 2014 (79.7 mm ambient monsoon precipitation), and 13.2 mm in 2016. Throughout the three years, only one individual reproduced.

On 13-15 July 2016, all live/recently-dead plants were harvest from their cylinders for above and belowground biomass (38 plants remaining of 120). Briefly, a soil knife was used to remove the buried cylinder, the contents were separated into aboveground biomass, roots (as much as could be collected), and rhizospheric soils in the field. In the lab, aboveground biomass was dried at 60C for one week. Roots were cleaned using sterile water, total biomass weighed, subsamples taken for DNA extraction and staining for microscopy, and remaining bulk biomass weighed and dried at 60°C for one week. Total dry root biomass was calculated by weighing the dry bulk biomass, and using wet:dry weight to infer dry root biomass for the part of the sample used for sequencing and microscopy. Allometric equations were then used to estimate aboveground biomass for transplants throughout the 2014-2016 censuses. From the harvested 38 plants, we fit allometric equations to estimate *ln*-transformed aboveground biomass from census variables such as transplant species identity, height, tiller number, and major and minor axes of basal area ellipse ($F_{5,32}$=16.78, p<0.0001, r^2=0.68). For censuses from 2014, when only height and tiller number were recorded, an alternative allometric equation containing only those measured variables provided a reasonable fit to predict aboveground biomass ($F_{3,34}$=4.58, p=0.019, r^2=0.18).

Rhizosphere soils and roots for DNA extraction were stored at -80 °C. We used light microscopy to estimate fungal colonization levels on host plant roots and determine fungal exclusion treatment efficacy following standard staining methods (Vierheilig et al. 1998) and the

gridline intercept scoring method (McGonigle et al. 1990). 'No fungi' treatments were successful at reducing the amount of fungal colonization: after three growing seasons, root total fungal colonization (%views colonized) was still 31.7% lower in the 'no fungi' plants than the 'feedback' plants ($F_{1,32}$=15.68, P<0.001; 88.3% ± 5.3 SE and 61.3 ± 8.0 SE).

Field and growth chamber PSF germination experiments

To investigate the effects of community stability and PSF on the recruitment life history stage in the field, we added 10 conspecific seeds to each field experimental cylinder in June 2016 and in a separate growth chamber experiment. In the field, seeds were glued onto plastic tooth picks for individual tracking using Elmer's washable school glue (Elmer's Products, High Point, NC). Seedling emergence was recorded once a month. Due to the lack of rain and thus germination activity in the field, we also conducted a germination experiment in a growth chamber (model I-30BLL, Percival Scientific, IA, USA) during Aug 2016. From the field, ~40ml of soils were collected using a soil corer to ~15cm directly from under each focal resident plant in each experimental plot (total of 40 samples). Collected soils and root fragments were transferred to 10x10 cm^2 pots, and 10 seeds of *B. eriopoda* and *B. gracilis* were added to each pot. Soils were watered to field capacity, and incubated at 27 °C daytime 15 °C night time temperatures, during 12 hour days. Germination was monitored every week for 1 month.

Data analysis

Abiotic covariates of community stability

We first investigated all measured soil texture and chemical covariates together in a MANOVA, including the effects of spatial block, plant species, community stability type, and

their interactions. We then further investigated each individual abiotic covariate in a separate ANOVA to clarify which ones drove the MANOVA results.

Calculation of PSF for plant biomass

First, we determined the maximum estimated biomass for each plant in a given year. Then for each year, maximum biomass was used to calculate PSF as a *ln* ratio (following Pernilla Brinkman et al. 2010), where PSF for a given species was *ln*(mass in conspecific soil/mass in heterospecific soil). Because survival was analyzed separately, we only calculated PSF for a pair of plants in each block in a given year if both individuals were alive. No pairwise PSF responses were calculated for survival and seedling emergence because the binomial nature of survival response does not allow for such calculations and replication of seedling emergence responses was too low to allow pairwise PSF comparisons.

Analysis of PSF and community stability (Q1)

Using the calculated PSF *ln*-ratio responses above, we compared PSFs for each species between dynamic and static patches to determine the effect of community stability on PSF. PSF biomass calculations from all three years were combined into a repeated measures analysis. Due to uneven sample sizes and variances among groups, we used a weighted-variance mixed effects model that included community stability as a fixed effect, census year and spatial block as random effects, with heteroscedasticity modeled between community stability types. This analysis was conducted for each plant species using the weights option in the lme function in package nlme (Pinheiro et al. 2016).

To infer the direction of plant-microbe interactions (pathogenic or mutualistic) that drove observed PSF, we also directly analyzed transplant growth based on *ln*-transformed biomasses throughout the census period separately by species. For each species, we modeled *ln*-transformed transplant biomass at each census in a mixed model with fixed effects of feedback environment, community stability, their interaction, as well as census number. To account for multiple observations per plant and spatial non-independence, we included plant ID and block as random effects. These analyses were conducted using the lmer function in package lme4 (Bates et al. 2016). To specifically investigate whether the effects of feedback environments were mutualistic or antagonistic, we tested pairwise differences between 'heterospecific', 'conspecific', and 'no fungi' for each plant species in static and dynamic patches separately. Pairwise comparisons were conducted using the lsmeans function in package lsmeans, with the Tukey method for p-value adjustment (Lenth 2016).

Relationship between soil microbial effects and plant frequency in static patches (Q2)

Feedbacks that promote the establishment of a plant species most strongly when it is rare would be consistent with the negative frequency dependence that is required to promote stable plant species coexistence. We were able to further examine if PSF in static patches was determined by plant frequency because candidate static patches were selected to be either dominated by *B. gracilis* or *B. eriopoda*. For those transplants in static patches only, we tested whether effects of conspecific or heterospecific soil microbes were correlated with plant frequency by directly analyzing transplant growth based on *ln*-transformed biomass. Transplant biomass for each plant species was modeled with PSF environment, conspecific frequency ('rare' vs 'common'), and their interaction as fixed effects, as well as plant ID and block as

random effects. Pairwise comparisons were similarly conducted as described in the prior section to determine pairwise differences between 'heterospecific', 'conspecific', and 'no fungi' for each plant species in patches where it was 'rare' or 'common' separately.

Analysis of PSF and community stability effects on germination and survival (Q3)

From the 2016 field germination trial, we investigated the effects of feedback environment and community stability on germination and survival of the two focal species separately. Germination data were modeled in a generalized linear mixed model, using number of germinated seeds as a response, with the effects of feedback environment, community stability, their interaction, and the random effect of block, with a Poisson distribution of errors. Seedling emergence in the growth chamber was analyzed similarly as above, except that there were no 'no fungi' soils. Survival of all transplants from the beginning of the experiment to the end of the experiment was analyzed similarly using a binomial error structure. Pairwise comparisons for survival and seedling emergence were conducted using the glht function in the multcomp package to handle nonlinear effects.

RESULTS

Abiotic covariates of community stability

Soil chemistry and texture did not covary with community stability, with no significant differences between dynamic and static patches (MANOVA *Pillai* = 0.24, $F_{12,32}$ = 0.54, p = 0.86). However, there was a significant effect of spatial block (*Pillai* = 0.64, $F_{12,32}$ = 3.11, p = 0.01). ANOVA revealed a trend of decreasing soil moisture from north to south along the transect (block, $F_{1,32}$ = 3.61, p = 0.07), higher K^+ availability near *B. eriopoda* plants that

decreased towards the south (block main effect $F_{1,32} = 8.91$, $p = 0.005$; plant main effect $F_{1,32} = 5.86$, $p = 0.02$; block X plant $F_{1,32} = 6.17$, $p = 0.02$), and decreasing Al^{3+} in the static patches (block x community stability $F_{1,32} = 5.54$, $p = 0.03$). Therefore, we included spatial block as a random variable in all subsequent analyses.

Do the strength and direction of PSF differ between dynamic and static patches?

For *B. gracilis*, PSF was more negative in static than in dynamic patches ($t = -3.17$, $df = 30$, $p = 0.004$), as we predicted. However, we found no differences in PSF between dynamic versus static patches for *B. eriopoda* ($t = -0.62$, $df = 10$, $p = 0.55$). Negative PSF for *B. gracilis* in static patches was driven by increased growth of transplants in heterospecific soils compared to conspecific soils (community stability x feedback environment $F=3.79$, $df=2$, $p=0.03$). Contrasts against the 'no fungi' treatment revealed that *B. gracilis* benefitted from mutualistic effects of soil fungi in heterospecific soil (pairwise comparison 'heterospecific soil vs. no fungi' $t = 2.49$, df $= 44.86$, $p = 0.04$). We did not find significant differences in *B. eriopoda* growth between different feedback environments or in dynamic/static patches.

In static patches, are soil microbial effects correlated with plant frequency?

In static patches where it was rare, *B. gracilis* gained 145% more biomass in heterospecific soil environments than in conspecific environments, and experienced net mutualistic fungi in heterospecific soils compared to 'no fungi' controls (pairwise comparisons *t* $= 2.96$, *df* $= 14.14$, $p = 0.03$ and $t = 2.54$, *df* $= 14.09$, $p = 0.06$, respectively; Fig. 5A). When *B. gracilis* was common, the advantage of heterospecific soil weakened to only 86.6% gain in heterospecific versus conspecific environments (pairwise comparison $t = 0.62$, *df* $= 12.73$, $p =$

0.81). In static patches when *B. eriopoda* was rare, its growth was also higher in heterospecific soils (62.6% increase; $t = 3.12$, $df = 25.43$, $p = 0.01$). However, this was driven by the net pathogenic effect of conspecific soils compared to 'no fungi' controls, causing a 32.5% decline in biomass ($t = -2.26$, $df = 27.11$, $p = 0.08$). Surprisingly, given expectations from theory, conspecific soils were more neutral for *B. eriopoda* when it was more common in the plot (compared to 'no fungi' $t = 1.10$, $df = 17.11$, $p = 0.53$), rather than increasing in their pathogenicity as conspecific frequency increased (Fig. 5B).

Does the relationship between PSF and historical vegetation community stability change with plant ontogeny?

 B. gracilis seeds showed a strong trend towards 75% higher emergence in dynamic than in static patches (main effect $X^2 = 3.34$, $df = 1$, $p=0.07$; Fig. 6A). *Post hoc* pairwise comparisons revealed that for *B. gracilis* seeds in dynamic patches, those in 'heterospecific' and 'conspecific' soils had significantly higher emergence rates than those in 'no fungi' soils, suggesting a dependence of seedling emergence on beneficial soil fungi ($z = 2.64$, $p = 0.04$, and $z = 3.13$, $p < 0.01$, respectively). In contrast, *B. eriopoda* seedling emergence did not differ, on average, between dynamic and static plots. *Bouteloua eriopoda* emergence was highest in static 'no fungi' soils compared to all other feedback environments, and the effect of feedback environment was stronger in static patches (interaction $X^2 = 6.89$, $df = 2$, $p = 0.03$). However, *post hoc* pairwise comparisons revealed no significant differences between the feedback and control plants (Fig. 6B).

 The effects of community stability and PSF on seedling emergence assessed in the growth chamber were similar to those in the field. *B. eriopoda* did not show significant

differences in germination rate among treatments, whereas *B. gracilis* germination was 30% higher in soils collected from dynamic patches (main effect $t = -3.11$, $df = 27$, $p = 0.004$, Fig. S1).

Overall, plant species survival throughout the experimental period was not affected by feedback environment or community stability (Fig. S2), a result that suggests survival responses do not follow patterns of emergence or growth life history stages.

Discussion

In a semiarid grassland, long term observations have documented spatial variability in the temporal dynamics of two co-occurring perennial grass species. Some patches showed stable coexistence through time, whereas others showed frequent change in the relative abundances of the two species. However, the causes of these patterns are unknown. We found that plant-soil feedbacks of these grasses differed depending on the past history of community stability, especially when assessed in terms of growth, and for *B. gracilis* compared to *B. eriopoda*. *Bouteloua gracilis* PSFs were negative in patches known to have higher community stability ('static'), and positive in locations with lower community stability ('dynamic'). In addition, in static patches, the benefit of heterospecific soil microbes for *B. gracilis* growth was greater when it was rare. Also, effects of feedback environment and community stability differed among plant life history stages in species-specific ways. *Bouteloua gracilis* showed negative PSF in static patches for growth, and higher seedling emergence in dynamic compared to static patches when fungi were not excluded. *Bouteloua eriopoda* showed neutral PSF for growth, and a trend towards higher seedling emergence in static patches in the 'no fungi' control. Feedback environment and community stability had no effects on survival of either species. These results

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from a multi-year field experiment point to PSFs as potential drivers of plant community turnover and stability, and highlight the importance of considering multiple stages of plant life history.

Stable patches of species coexistence are associated with negative PSFs

Theory predicts that stable coexistence occurs when a species limits itself more than it does its competitor (Volterra 1926, Lotka 1932). Negative PSFs provide a key mechanism for self-limitation and therefore stable coexistence (Bever et al. 1997, Bever 2003). That is, a plant performs worse with conspecific-cultivated soil microbial communities than with heterospecific-cultivated microbial communities. We found evidence for negative PSFs in static patches for *B. gracilis*, and a similar, but statistically insignificant, trend for *B. eriopoda.*

The source of negative PSFs could come from mutualistic or pathogenic effects of soil microbiota: negative feedback can arise when a species suffers more from its own pathogens relative to those from heterospecifics or when a species benefits from heterospecific mutualists more than from its own (Bever et al. 1997). In the case of *B. gracilis*, the negative feedback stemmed from mutualistic effects of soil fungi in heterospecific soil, whereas its growth in conspecific and 'no fungi' soils was similar. That we saw similar growth between 'conspecific soil' and 'no fungi' environments lent confidence that the two different mesh types used to construct experimental cylinders likely had minimal unintended effects in restricting water and nutrient flow. In static patches, one would expect that high plant community stability would also allow the associated establishment of stable, and perhaps more host-specific, soil microbial communities (Wardle et al. 2004). Fungal mutualists associated with plant rhizospheres are well known to show host-specificity, and differential mutualist benefits have been reported to

generate negative PSF (Bever 2002a, 2002b, Castelli and Casper 2003). Our results suggest that in static patches, fungal mutualists specific to *B. eriopoda* benefitted *B. gracilis* more than its own conspecific soil microbes.

An alternative explanation could be that these patterns were driven by differential resource use and competition. *Bouteloua gracilis* could show increased growth next to a heterospecific competitor compared to a conspecific because *B. eriopoda* does not draw down the key resources required by *B. gracilis*. While in a previous greenhouse study we showed that intraspecific competition is stronger than interspecific competition for *B. gracilis* when competing with *B. eriopoda*, we also showed that microbes increased negative intraspecific effects beyond resource competition (Chung and Rudgers 2016). In the field, the only resource measured that differed between soils occupied by the two species was K^+, which was higher near *B. eriopoda* plants. Although K addition alone has been found to increase productivity in some ecosystems with high rainfall and sandy soils (Fay et al. 2015), preliminary results from a resource addition experiment at the SNWR suggest that the abundance of *B. eriopoda* decreases in response to K addition (Carrigy et al., unpublished data). Potentially, differential utilization of K^+ may drive interspecific competition and niche partitioning between the two species, and partially explain our results.

Implications of feedbacks for long-term plant community patterns

Here, we have defined plant community stability using the magnitude of oscillations in community composition space as well as the frequency of changes in dominance on an interannual scale. Hotspots of community change along a relatively small spatial scale as seen at our site (Collins and Xia 2015) have also been documented in other ecosystems (Spotswood et

al. 2015), and hypothesized to result from combinations of spatial variation in external drivers, local variation in abiotic conditions, and patch dynamics between plant species and community types (Peters et al. 2006). The rate of turnover in plant community composition could likely affect the accumulation and succession of soil microbial communities and thus result in different PSFs. Field removal experiments at the SNWR have shown *B. gracilis* to be a superior competitor over to *B. eriopoda* (Peters and Yao 2012), have higher seed viability (Peters 2002), and be more resistant to fire and herbivory (Gosz and Gosz 1996). Despite this evidence of higher *B. gracilis* vigor, across the entire transect *B. eriopoda* cover over a 20 year period increased at a faster rate than that of *B. gracilis* (Collins and Xia 2015). Our work suggests that negative PSF for *B. gracilis* compared to neutral PSF for *B. eriopoda* may help explain this phenomenon.

What are the drivers behind observed spatial variation in PSFs, community stability patterns, and their relationship in this study? While we have shown for the first time a relationship between PSF and long term patch dynamics and stability, we cannot determine the causality. Variation in PSF may drive variation in plant community stability, or vice versa. In addition, variation in each could be determined by external factors such as abiotic conditions. In this study, none of the abiotic covariates measured differed significantly between patches that varied in community stability and could potentially explain spatial variation in plant or microbial population dynamics. Microbially-driven PSFs may exhibit spatial variability in nature due to the microscale heterogeneity of the soil environment (Bever et al. 2012). At our site, dispersal of fungal spores via dust deposition is likely high and not limiting, but fungi that reside deeper in the soil layers may not disperse easily across the landscape and may show more patchy distributions. Past work has shown variable root fungal communities from a given plant species

at the spatial scale of 5-10m (e.g. Ettema and Wardle 2002, Grünig et al. 2002, Pickles et al. 2010). Here, a combination of unmeasured abiotic covariates and stochasticity may structure the soil microbial environment, thus influencing the range of possible PSFs and driving patterns of plant community stability. Our study lays the groundwork for further experimental work that could tease apart the causal mechanisms that underlie the relationship between PSF and plant community stability, and highlights the importance of elucidating the temporal and spatial scales at which PSFs occur.

Frequency-dependent effects of soil microbes increase invasibility

Theory predicts that coexistence is maintained when competitors are mutually invasible: each is able to increase when rare and others are at equilibrium (Chesson and Ellner 1989). Our results show that in this semiarid grassland, PSFs can potentially help maintain stable plant coexistence in static patches by favoring plant performance with heterospecific soil microbes when they are rare. The presence of a greater benefit of heterospecific soil microbiota when *B. gracilis* is rare suggests that in this system, PSFs could convey an 'invasibility' advantage for *B. gracilis*. *Bouteloua eriopoda* also showed higher growth in heterospecific soils when rare, which was driven by stronger pathogenic effects of conspecific soils when rare compared to common. When considered at the whole-plot level, it makes sense that when conspecific frequency was low, and heterospecific frequency high, we saw a relatively stronger benefit of heterospecific soil. PSF theory generally assumes linear relationships between host plant frequency and the frequency of its associated microbes at the population/community level (Bever et al. 1997, Bever 2003). However, whether higher microbial frequency also results in greater per capita benefits, as shown here, has not been elucidated.

Others have shown that more negative PSFs are associated with rarer plants, inferring that these pathogenic microbial effects drive plants to rarity (e.g. Klironomos 2002, Mangan et al. 2010). Our results suggest an alternative perspective that rare species have a greater advantage in heterospecific soil allowing them to persist. In addition, we found this pattern replicated across multiple sub-communities that naturally vary in species relative abundances, which has not been shown for any other ecosystem.

Importance of field experiments in PSF research

Our study fills multiple gaps in the study of PSFs by measuring PSFs experimentally in the field, across multiple growing seasons and life history transitions, and linking PSFs directly to natural patterns in plant community dynamics at the same site. This new approach resulted in some key insights. Compared to a previous greenhouse experiment that focused on the same plant species using soils from the same field site, we found concordant results in our field experiment where *B. gracilis* experienced significant, negative, feedbacks, whereas *B. eriopoda* experienced feedbacks that were not different from neutral (Chung and Rudgers 2016). However, the types of interactions that resulted in negative feedbacks for *B. gracilis* differed between the field and the greenhouse. In the field experiment, negative PSF resulted from increased *mutualistic* interactions with heterospecific soil communities, whereas negative PSF in the greenhouse resulted from *pathogenic* interactions with conspecific soil communities. Our greenhouse experiment used an inoculum approach typical to PSF studies (Kulmatiski and Kardol 2008, Pernilla Brinkman et al. 2010). A review of these studies has shown that this approach leads to more negative PSF effect sizes than field studies, and suggests these negative greenhouse PSFs could result from larger microbial population fluctuations of the relatively

smaller subset of microbial species present and conditions that favor proliferation of pathogenic microbes (Kulmatiski et al. 2008). However, preliminary data from sequencing work on root fungal communities in our studies show that plants in our prior greenhouse experiment and these experimental field transplants had similarly diverse root fungal communities (Chung and Rudgers, in prep). In addition, long-term cultivation by perennial plants in the field is known in some cases to lead to pathogen-suppressive soils, where microbial communities develop such that detrimental effects of pathogens on plants are suppressed (Weller et al. 2002). Therefore, it is possible that the field resident plants have developed suppressive soil communities that are low in pathogenicity.

Ontogenetic shifts in PSF effects across plant life history

Our results showed that PSFs and their relationship to community stability differed among plant vital rates of seedling emergence, plant growth, and survival. Notably, effects were generally stronger for growth, moderate for seedling emergence, and negligible for survival. Past PSF studies have often focused on single vital rates, and evidence exists for significant PSF effects for each of those measured in this study, but come from different plant species and ecosystems. A comprehensive view across multiple life history stages such as ours is important because demographic transitions do not contribute equally to population growth (de Kroon et al. 1986), which ultimately is what determines the stability of coexistence. *Bouteloua gracilis* and *B. eriopoda* are perennial bunch grasses that can live for decades (Wright and Van Dyne 1976, Fair et al. 1999), and for which change in survival is expected to have the greatest impact on population growth (Franco and Silvertown 2004). However, changing patterns of community stability, as estimated by cover in our study at a ~20 year time scale, are likely more affected by

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clonal growth than recruitment. Our results suggest negative PSF in growth to be related to increased stability in static patches. In addition, increased *B. gracilis* seedling emergence in dynamic patches (effects which disappear in 'no fungi' controls) could be associated with faster rates of temporal turnover. A crucial future direction in understanding the role of PSFs in plant community dynamics is to integrate plant responses across life history stages to specifically model effects on population growth rates.

Conclusion

In a semiarid grassland, we found that the strength and direction of microbially-mediated PSF were associated with long term plant community stability patterns for two foundation grass species. Specifically, PSFs were more negative in static patches of high, long term community stability. In addition, the magnitudes of these PSFs were frequency-dependent in a way that should increase invasibility when a species is rare, a criterion for long-term coexistence. Relationships between PSF and community stability differed across multiple plant vital rates, highlighting the importance of considering ontogenetic shifts in PSF effects. Our results point to a key role for PSFs in moderating spatial variation in plant community temporal dynamics.

Acknowledgements

Figures

Figure 1 Species relative abundance change in 4m patches along a subsection of the long-term transect through time. Relative abundance of *B. eriopoda* and *B. gracilis* is shown by color of each point. "Dynamic" regions (a) were spatially paired with "static" regions (b) for the field PSF experiment.
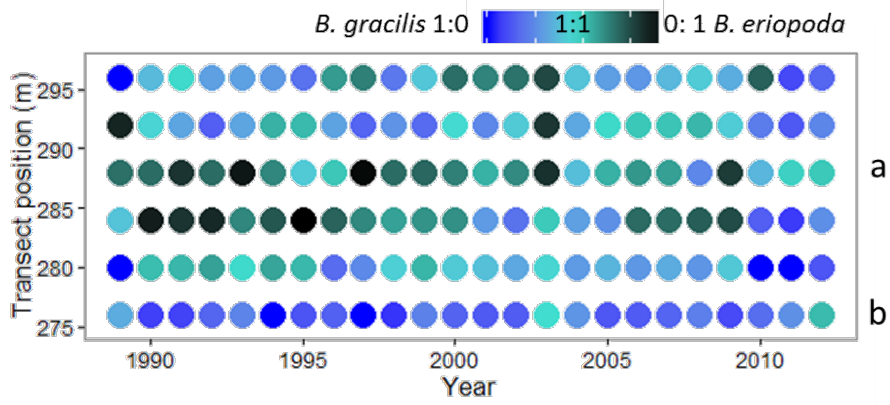
Figure 2
Variation of dynamic scores as a function of mean *B. eriopoda* and *B. gracilis* cover in the patch. Plotted dynamic score values are fitted from model based on long term data, limited to the range of observed combinations of *B. eriopoda* and *B. gracilis* cover. More orange colors indicate static patches, whereas more blue-green colors indicate more dynamic patches. Patches with a mixture of both species are more likely to be more dynamic.
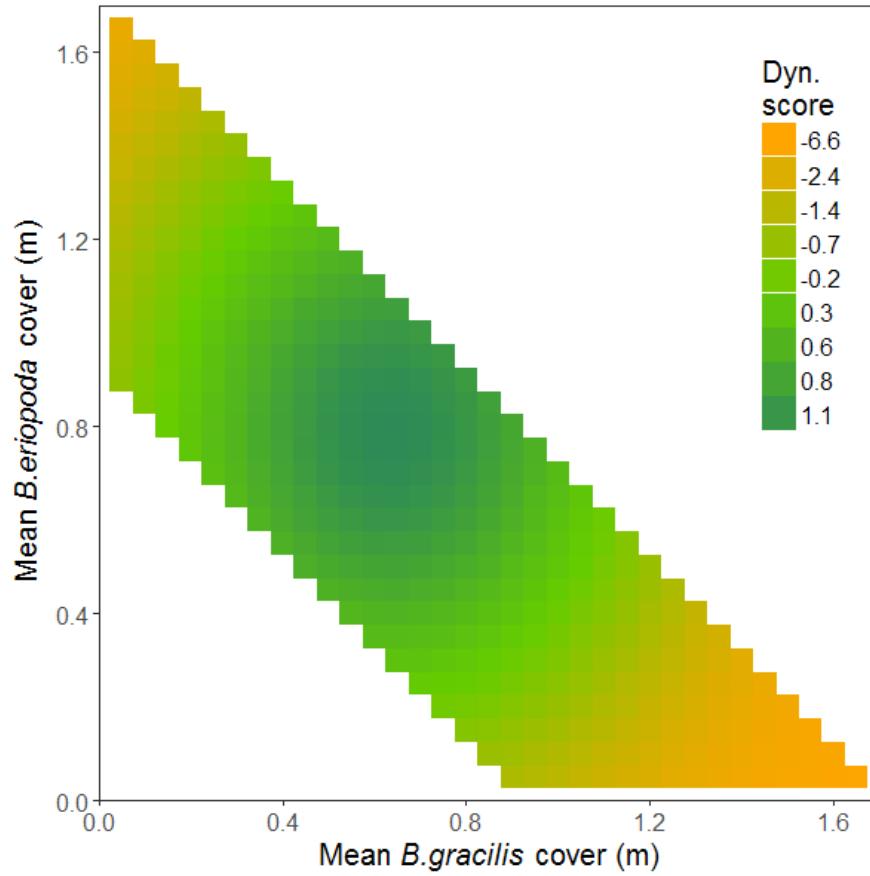
Figure 3 Field PSF experimental design. A resident *B. eriopoda* and *B. gracilis* plan were identified in each plot, and experimental transplants planted around the resident plants in cylinders that allowed hyphal colonization ('Feedback plant') or excluded fungi ('No fungi plant'). Feedback plants experienced the soil microbial community of the resident plant, indicated by the color of the cylinder. Measurements of abiotic properties such as soil chemistry using PRS nutrient probes were taken at each resident plant in each plot. Other vegetation in plot is omitted here for clarity.
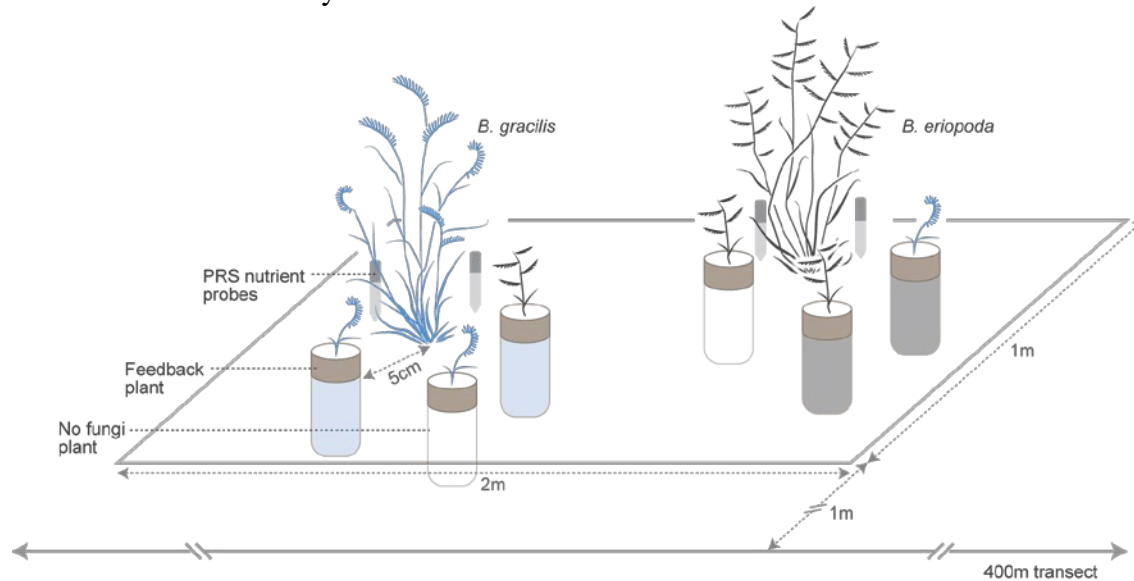
Figure 4

Plant soil feedback (PSF) of (A) *B. gracilis* and (B) *B. eriopoda* in dynamic and static patches. Each PSF measurement is calculated from pairwise *ln*-ratios of transplant biomass in conspecific vs. heterospecific soils, as shown in (C) for *B. gracilis* and (D) for *B. eriopoda*. Letters indicate significant pairwise comparisons ("n.s.": not significant). Asterisk indicates significant difference from zero.
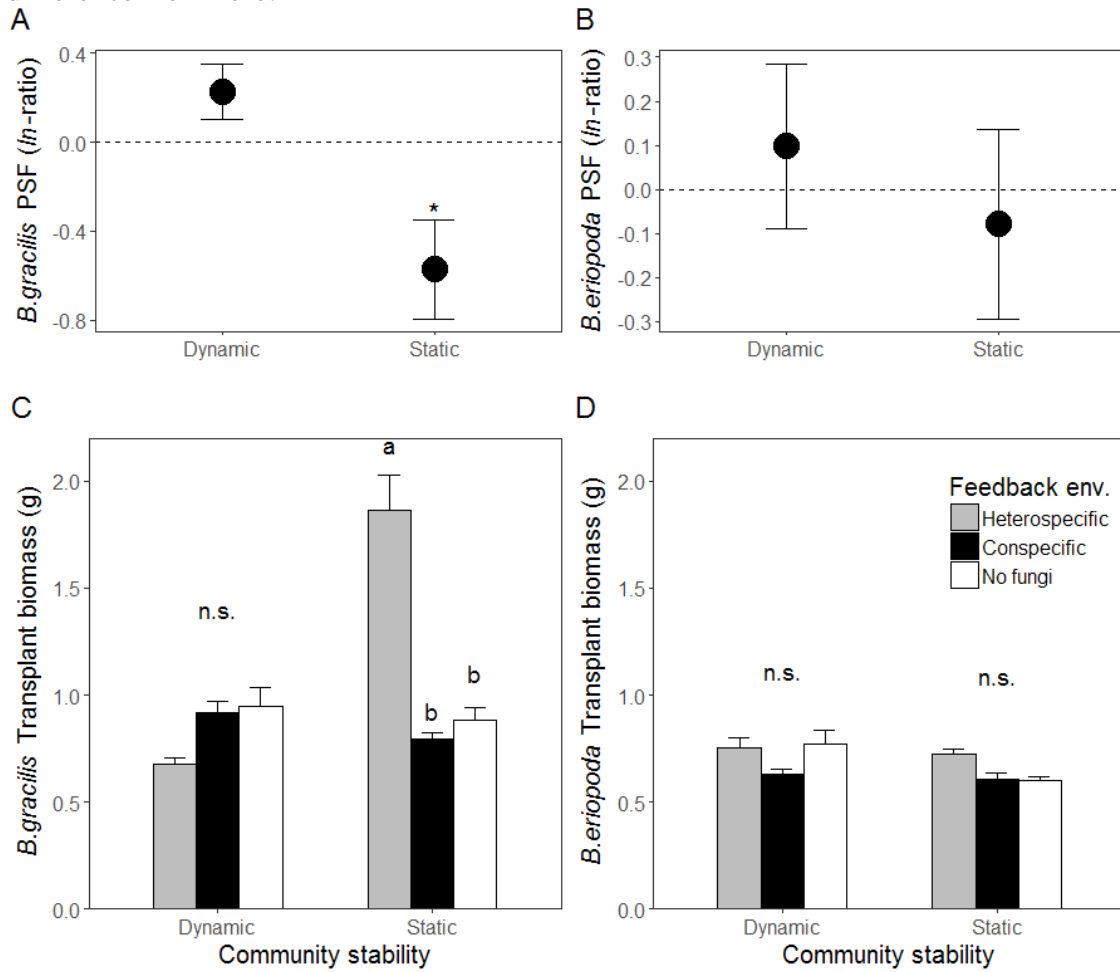
Figure 5

Effects of feedback environments on (A) *B. gracilis* and (B) *B. eriopoda* growth in static patches where each plant was either rare or common. Letters indicate significant pairwise comparisons ("n.s.": not significant).
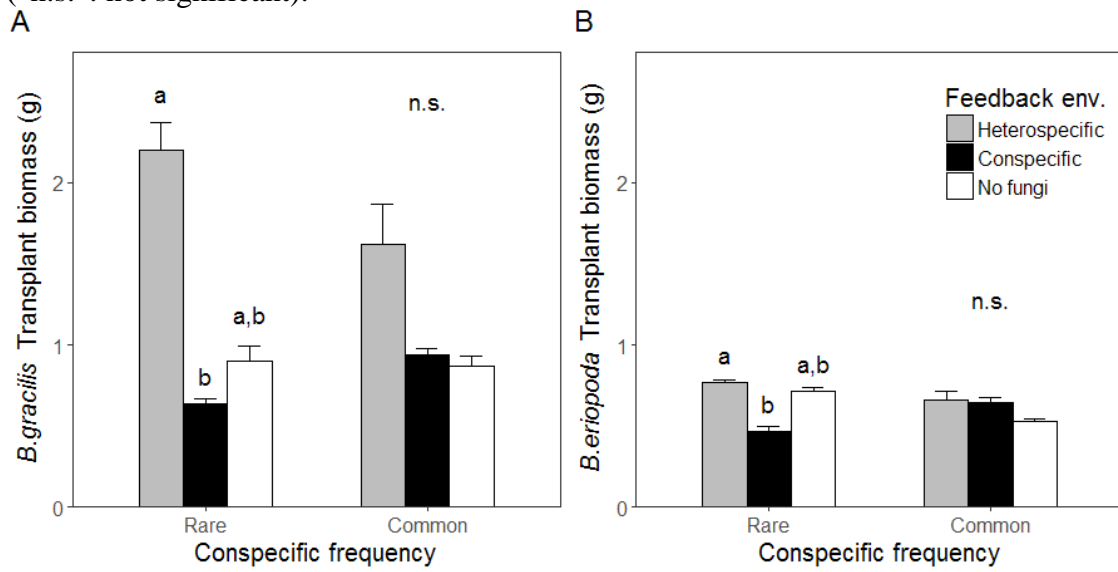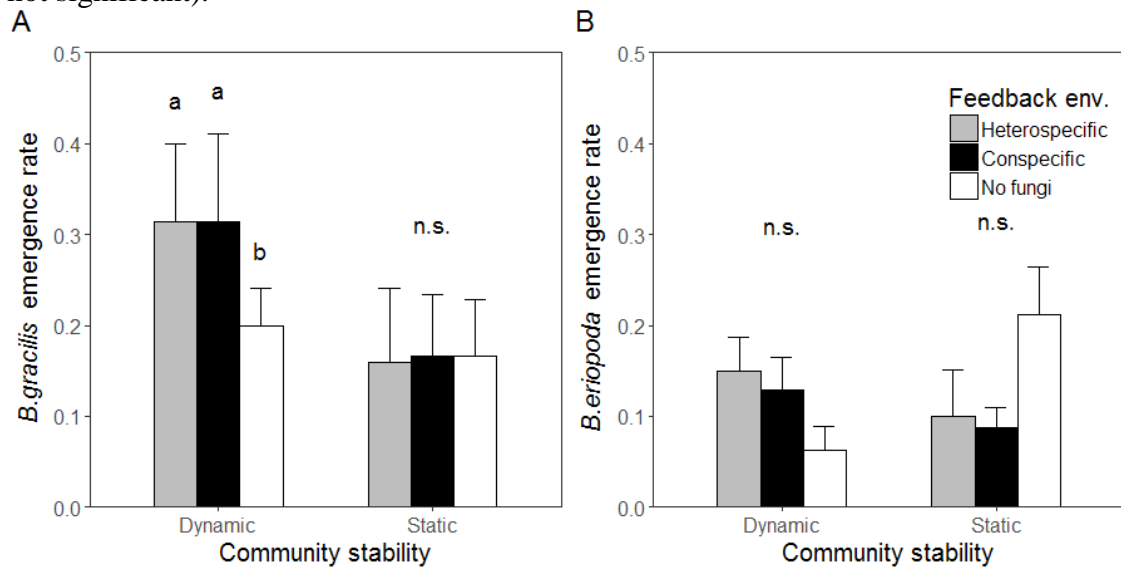
Figure 6

Effects of feedback environment on seedling emergence of (A) *B. gracilis* and (B) *B. eriopoda* in dynamic and static patches in the field. Letters indicate significant pairwise comparisons ("n.s.": not significant).

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Chapter 4

Divergent root-associated fungal community composition and diversity in the greenhouse

and field in a semiarid grassland

Abstract

Investigations of plant-soil feedbacks (PSF) and plant-soil microbial interactions often rely on experiments in the greenhouse. However, we have little understanding of how, and when, these results may be generalized to explain phenomena in nature. One way of increasing our ability to determine the generalizability of such experiments is to systematically investigate differences in the microbial community due to differing experimental conditions and methods that may undermine our ability to compare results in the field and the greenhouse. Here, we used Illumina sequencing technology to characterize the root-associated fungal communities of two foundational grasses using samples from a greenhouse PSF experiment, field monoculture stands, a field PSF experiment, and naturally-occurring resident plants in the field for which we have a 26-year record of temporal fluctuation in plant abundance. Despite similar source origins for soil inocula, root-associated fungal communities differed widely in plants across the four study types. In particular, plants from the greenhouse experiment and the field PSF experiment had much higher among-sample similarity in fungal community composition, as well as much higher root fungal diversity than those from long-term residents in the field (monocultures and natural). This result suggests that individual plants selectively filter fungal composition over decadal scales, returning lower per-plant fungal diversity and higher among-plant dispersion in fungal composition than observed in short-term inoculation studies. Host species-specific fungal communities were found in all four study types. Comparison of field and greenhouse PSF experiments demonstrated the effectiveness of two different inoculation methods and revealed that inoculation treatments overrode the effects of host species-specific selection on root-associated fungi. Finally, long-term temporal dynamics in plant community composition altered root fungal community diversity, but did not systematically change fungal community

composition. This comparison of the belowground consequences of experimental approaches when investigating plant-soil microbial interactions suggests that attempts to generalize greenhouse experiment outcomes to the field can be hampered by incomparable soil microbe communities.

Introduction

Interactions and feedbacks between plants and their soil and root-associated microbial symbionts have gained recognition as important drivers of aboveground plant population and community dynamics (Bever et al. 2010, van der Putten et al. 2013). In particular, plant-soil feedbacks (PSF), in which plant hosts cultivate species-specific soil microbiota that then differentially alter the population growth of co-occurring plant species, have been shown to play a role in promoting coexistence, succession, and patterns of species relative abundances (Kardol et al. 2006, Mangan et al. 2010, Schnitzer et al. 2011, Chung and Rudgers 2016). These advances have largely been driven by greenhouse experiments, which cultivate plants in soils with microbial communities inoculated or conditioned by con- or heterospecific plants (Kulmatiski et al. 2008). However, while ample evidence of PSFs comes from greenhouse experiments, and some from field mesocosms (Harrison and Bardgett 2010, Burns and Brandt 2014), relatively few studies have tested PSF under natural field conditions (Casper and Castelli 2007). It is unlikely that single plant responses measured in the greenhouse will translate completely to outcomes in nature (Kulmatiski and Kardol 2008, van der Putten et al. 2013).

The complexity of field conditions makes it difficult to propose *a priori* hypotheses as to how and why field-measured and greenhouse-measured PSFs may differ. Although there is a trend towards more negative PSFs measured in the greenhouse than in the field (Kulmatiski et al. 2008), the studies to date that have investigated PSFs for the same plants in the greenhouse and under field conditions returned different conclusions (Casper et al. 2008, Mangan et al. 2010, Pizano et al. 2014, Heinze et al. 2016). For example, greenhouse and field-measured PSFs showed concordance in a tropical rainforest. This is despite that the greenhouse experiment isolated the biotic component of PSF, whereas the field component measured whole-soil effects

in this study (Mangan et al. 2010). In contrast, another study found that for a temperate grassland, whole-soil PSFs were often opposite in direction when measured in the field versus in the greenhouse (Heinze et al. 2016).

There are several ways in which different study conditions may lead to different plant-soil microbial associations and thus PSF outcomes from the microbial perspective. First, different abiotic filters may result in different viable microbial species pools. For example, greenhouse environments tend to be more consistent and optimal in conditions such as temperature, nutrient, and water availability compared to field environments, which may lead to differential proliferation of members of the microbial community. Second, natural plant-microbial associations (host specificity) may change from the greenhouse to the field, as plant-microbial associations are well-known to be context dependent (e.g. Heath and Tiffin 2007). For example, past work has found that given the same starting microbial species pool, the same host plants developed different arbuscular mycorrhizal fungi communities in the greenhouse and field (Sýkorová et al. 2007, Phillips 2012, Schechter and Bruns 2013; but see van der Putten et al. 2007). Third, field and greenhouse PSF studies are likely to differ in their methods to experimentally manipulate the soil microbial environment for logistical reasons. These different methods of "inoculation" may vary in their resulting microbial communities and efficacy (Lankau and Lankau 2014). No work, however, has simultaneously examined these potential mechanisms. Knowledge of similarities and differences in rhizosphere microbial communities on the same host plants among inoculations in the field and greenhouse, and naturally-developed microbial communities in the field at multiple time scales could increase our ability to generalize PSF experiment outcomes.

An additional layer of complexity that makes it difficult to generalize greenhouse PSF studies to phenomena in nature is that unlike in the greenhouse, plant hosts in the field are not all created equal. For example, host plants could differ in their age and exhibit ontogenetic shifts in microbial community composition (Wagner et al. 2016), or differ in their ability to withstand pathogens or reward mutualists (Saunders and Kohn 2009, Newton et al. 2010). Field PSF experiments capture results based on one snapshot in time of the soil microbial community. Knowledge of long term patterns of variation in host plant dynamics could be crucially important to the interpretation of results. In this study, variation in long term plant community composition stability provided important context based on inferred plant age structure (communities with high turnover likely have younger plants) and drivers of microbial diversity (communities with high turnover could result in higher microbial diversity).

Here, we used Illumina next-generation sequencing technology to characterize the root-associated fungal community composition of two congeneric desert grasses, *Bouteloua gracilis* and *Bouteloua eriopoda*. We took advantage of archived root samples from a series of studies that spanned a greenhouse PSF experiment, field PSF experiment, field planted monocultures (7 years), and naturally-occurring plants in the field. This allowed us to assess differences in root-associated fungal community composition, host specificity, and the role of plant host community dynamics across greenhouse and field conditions. Specifically, we asked: 1) How does root-associated fungal community composition compare among observational and experimental studies across different temporal and spatial scales? 2) Does the specificity of plant-fungal interaction differ between greenhouse and field conditions? 3) How do experimental inoculations alter fungal composition in the greenhouse versus in the field? 4) Does the composition of root-associated fungal communities vary with host plant community stability?

Methods

Study sites and experiments

All studies presented here are affiliated with the Chihuahuan Dessert grassland communities at the Sevilleta National Wildlife Refuge (SNWR; 34.3591, -106.688), NM, USA. Mean annual temperature is 13.2°C, and mean annual precipitation is approximately 250mm. At this site, past and ongoing studies as a part of the Sevilleta Long Term Ecological Research (LTER) program provided us with a range of temporal and spatial scales to investigate the root-associated fungal communities of two congeneric grasses, *Bouteloua gracilis* (Blue grama) and *B. eriopoda* (Black grama), that dominate the plant community. Here, we utilized a series of four study types, briefly described below. Table 1 gives a summary of all four study types, their "treatments", and sample sizes.

Field monocultures

Field monoculture plots were established as a part of a larger grass competition and diversity experiment at the SNWR in 2005. Plants were established via seeding from southwestern U.S. regional seed stock (Curtis and Curtis, Clovis, NM; Western Native Seed, Coaldale, CO) at a rate of 90.1 kg/ha. Monoculture treatments, with 5 replicate plots per plant species (*B. eriopoda, B. gracilis*), were maintained by weeding out undesired species thrice each year (see http://sev.lternet.edu/data/sev-174 for more information). In October 2012, we collected root fragments from 1 plant growing in each monoculture plot, which were stored at -80°C ("monoculture plants").

Greenhouse PSF experiment

A greenhouse experiment was conducted during 2012-2013 to examine the effects of PSF and the presence of surface biological soil crust (biocrust) on competition and coexistence between *B. gracilis* and *B. eriopoda* (full design described in Chung and Rudgers 2016). Rhizosphere soils from the *B. gracilis* and *B. eriopoda* field monocultures (described above) at the SNWR, as well as biological soil crust (biocrust) samples were used to inoculate plants in the greenhouse in a factorial design. Inocula were collected in October 2012, at the same time the monoculture plants samples were collected. From the greenhouse experiment, we saved roots only from the highest density monoculture pots (homogenized from 4-6 plants per 8x8 cm^2 pot) for DNA extraction and sequencing ("greenhouse plants").

Field residents in high and low stability plant communities

Sevilleta LTER has included the biannual monitoring of vegetation dynamics along line intercept transects since 1989 (see http://sev.lternet.edu/data/sev-4, Collins and Xia 2015 for more information). Using the long term transect data, we identified 20 spatial patches (4 m long) with persistent differences in historical plant community stability (methods to determine stability provided in Chung and Rudgers, in prep). Ten patches were high in temporal stability of plant community composition ('static'), and ten showed low temporal stability ('dynamic'). In August 2015, roots were collected from one resident plant of each *Bouteloua* species from each patch, which were stored at -80°C until extraction and sequencing ("field residents"). Individual root samples were individual plants.

Field PSF experiment in high and low stability plant communities

A field experiment was conducted at the Sevilleta NWR site in 2014-2016 to examine the relationship between temporal plant community stability and PSF (Chung and Rudgers, in prep). We established experimental transplant seedlings around mature resident plants in areas adjacent to dynamic vs. static sections of the line intercept transect as described above. Transplant seedlings were grown in the greenhouse in sterilized sand for 3 months, and then planted into the field in mesh bags to prevent root competition in July 2014 (Chung and Rudgers, in prep). Transplant seedlings were established near conspecific or heterospecific resident plants (those sampled above) such that they experienced colonization from conspecific or heterospecific rhizospheric microbes to test PSF. A subset of seedlings was established in fine (0.45 μm) mesh bags to exclude fungal hyphal colonization from the soil near conspecific residents only. In July 2016, all surviving experimental transplants were harvested, and a subset of roots prepped for DNA extraction and sequencing ("field transplants"). Individual root samples were individual plants.

DNA extraction and sequencing

Genomic DNA was extracted from 0.2g ground root tissue in September 2016 (or smaller when limited by sample size) using MOBIO power soil DNA extraction kits following manufacturer's protocol. DNAs were quantitated using NanoDrop, and standardized to 2ng/μl for amplification. We chose to amplify the ITS2 region using forward primer fITS7 (Ihrmark et al. 2012) and reverse primer ITS4 (White et al. 1990). We amplified template DNAs from all samples in three technical primary PCR replicates with positive and negative controls using 10μl template DNA (20ng), 5μl dNTPs (0.2mM final concentration), 5μl each of forward and reverse primers (1μM final concentration), 10μl 5x Phusion Green HF Buffer containing 1.5mM MgCl₂,

14.5µl molecular grade water, and 0.5µl Phusion Green Hot Start II High-Fidelity DNA polymerase in 50µl reactions. PCR cycles included an initial denaturing at 98°C for 30s, 30 cycles of 94°C for 10s, 52°C for 10s, 72°C for 1 min, and a final extension at 72°C for 5 minutes.

For each sample, 40µl amplicons from each technical replicate of the primary PCRs were pooled, and purified using the Agencourt AmPure XP magnetic 96-well SPRIplate system (Beckman Coulter, Indianapolis, Indiana, USA) following the manufacturer's protocol with 1:1 AmPure XP solution to amplicon ratio, and one additional ethanol cleaning step. A secondary PCR was performed on the purified amplicons (10µl as template) using fITS7 and ITS4 primers with sample-specific 12bp sequencing tags for five cycles at the same temperature settings as the primary PCR. Secondary PCR products were purified again as previous, and DNA concentrations quantitated using NanoDrop. Sample amplicons were pooled together into a single library at equal molarity, and the library sequenced at the Integrated Genomics Facility at Kansas State University (Manhattan, KS, USA) as 2x300bp reads on 2/3 of a flow cell using Illumina Miseq v3.

Bioinformatics

Sequences were made into contigs, quality-filtered, trimmed to equal length, de-replicated, checked for chimeric sequences, and clustered into operational taxonomic units (OTUs) using the mothur v.1.39.0 pipeline (Schloss et al. 2009). Contigs that were below 250bp in length, contained any ambiguous bases, or contained homopolymers of more than six bases in length were discarded. This removed ~40% of total sequences. All sequences were then trimmed to the minimum length of 250bp. Chimeric sequences were checked using chimera.uchime

function with default settings, and removed. Sequences were then clustered into OTUs at a 97% similarity cutoff. OTUs with fewer than 10 sequences were discarded, leaving 2.85 million sequences and 1559 OTUs. OTUs were classified against the UNITE fungal ITS database (November 2016 release). We kept only OTUs that classified to a fungal phylum with $\geq$97% confidence. Contaminant OTUs found in negative controls were removed from the rest of the dataset by subtracting the number of sequences in the negative controls from all samples. Control samples were not otherwise included in our analyses. The final fungal OTU dataset included 1121 OTUs (see Table 2 for list of most common taxa). Rarefaction curves were saturating for the majority of samples, showing sufficient sampling to saturate OTU richness (Fig. S1).

Statistical analyses

For each subset of the entire fungal OTU table used to answer each research question, we transformed relative abundances of fungal OTUs using the Variance Stabilization method in package DESeq2 in R (following McMurdie and Holmes 2014). Community metrics and analyses were then calculated and conducted on the transformed matrices as appropriate. To investigate patterns in root-associated fungal community composition, we calculated Bray-Curtis distances between samples to represent (dis)similarity in community composition, and used PERMANOVA to test the significance of effects (9999 permutations of residuals under a reduced model with Type III sums of squares). For a list of PERMANOVA models, the data examined, and their corresponding ecological questions, see Table 3. Sample community compositions were visualized using Nonmetric Multi-Dimensional Scaling (NMDS) using 500 random starting configurations. Tests for differences among groups in community dispersion were performed using PERMDISP, including all pairwise comparisons when appropriate. All

community composition analyses and visualizations were conducted in PRIMER v6 (Clarke and Gorley 2009). Significant contrasts from compositional analyses were further investigated using indicator species analysis to determine the OTUs that were strong indicators of "treatment" groups; these analyses were conducted using the labdsv package in R 3.3.2 (R Core Team 2016).

We also analyzed the effect of treatments on fungal richness and diversity using general linear (mixed) models (Table 3). Fungal community richness was calculated using the Chao1 estimator on the untransformed OTU table, whereas diversity was calculated using the Shannon diversity index on the variance-stabilized OTU table. The only difference in model specification between these analyses and those of community composition were for question 3. It was more logical to code inoculum source not according to their species provenance, but whether they matched that of the host species. For example, instead of inoculum source "BOER" for *B. eriopoda*, we coded inocula provenance in terms of "conspecific" for that from *B. eriopoda*, and "heterospecific" for that from *B. gracilis*. We calculated diversity and richness indices and constructed rarefaction curves using the vegan package. Richness and diversity analyses were conducted in R 3.3.2 (R Core Team 2016).

Results

1) How does root-associated fungal community composition compare among observational and experimental studies across different temporal and spatial scales? And 2) Does plant-fungal interaction specificity change between greenhouse and field conditions?

Root-associated fungal communities were significantly different among all studies ($Pseudo\text{-}F_{3,67}$=5.15, P<0.001), and between the two host plant species ($Pseudo\text{-}F_{1,67}$=1.58, P=0.003; Fig. 1). However, the interaction between host plant and study type was not significant

($Pseudo\text{-}F_{3,67}$=1.06, P=0.28), suggesting similar patterns of host specificity among study types. Study type accounted for 17.7% of total variation in fungal composition and host species for a smaller fraction (1.8%). All study types differed from each other in community dispersion (pairwise tests, P<0.001) except for between monoculture plants and field residents which had similar dispersion (t=0.30, P=0.95). Greenhouse plants showed the least dispersion in composition, followed by field transplants, and then field monoculture plants and resident plants. There were no differences in community dispersion between plant species (t=0.27, P=0.78). Visualization of community composition differences using NMDS showed high stress (2D stress=0.26), likely driven by the strong divergence in composition among study types (Fig. 1). Therefore, we additionally analyzed community composition patterns within smaller subsets of studies to address further questions.

At the phylum level, fungal communities from greenhouse plants were much higher in relative abundance of Glomeromycota and lower in Basidiomycota than the other study types (Fig. S2A). This pattern was reflected in the indicator species analysis, where two of the top five indicator OTUs classified to *Paraglomus brasilianum*. Another interesting pattern from the indicator species analysis was that for field resident plants, all top five indicator OTUs classified to a *Moniliophthora* species (Table S1). Of all fungal OTUs, only 9.1% were present in all four study types, whereas 37.9% were unique to one study type only. Fungal community composition of *B. eriopoda* and *B. gracilis* differed most in their relative abundances of Basidiomycota vs. Glomeromycota, where *B. gracilis* fungal communities were higher in Glomeromycota and lower in Basidiomycota (Fig. S2B). Of the fungal OTUs for samples inoculated with conspecific soils or grown directly in the field, 23.1% were unique to *B. gracilis* plants, and 16.9% were unique to *B. eriopoda* plants.

Surprisingly, plants in the greenhouse experiment cultivated root-associated fungal communities that were highest in both richness and diversity ($F_{3,67}$=25.53, P<0.001, and $F_{3,67}$=26.48, P<0.001, respectively), followed by the field transplants, then the field monoculture plants, and finally naturally-occurring field residents (Fig. 2).

3) How do experimental inoculations alter fungal community composition in the greenhouse versus in the field?

Fungal communities differed significantly between greenhouse and field experiments (*Pseudo-F$_{1,46}$*=12.79, P<0.001), and between inocula provenances (rhizospheric soils from *B. gracilis* vs. *B. eriopoda*) (*Pseudo-F$_{1,46}$*=2.24, P<0.001; Fig. 3). The effect of inocula provenance was stronger for the greenhouse plants than for the field transplants (interaction *Pseudo-F$_{1,46}$*=2.10, P<0.001). In addition, the provenance of soil inoculum had a stronger effect on fungal community composition than the identity of the focal, living plant species from which fungi were collected. In both field and greenhouse experiments where plants were inoculated from either provenance, host species identity was not a significant predictor of root fungal community composition (*Pseudo-F$_{1,46}$*=1.18, P=0.16). However, a few OTUs showed up as significant indicators for both the host plant species group and for its inoculum. For example, two OTUs in the genus *Phaeosphaeria* were strong indicators or *B. eriopoda* host species and for *B. eriopoda* inoculum. *Claroideoglomus drummondii,* an arbuscular mycorrhizal species, was a strong indicator for *B. gracilis* host plant and for its inoculum (Table S1).

Fungal diversity was similar among study types, host plants, and inocula provenances. However, we found a trend for higher fungal richness when plants were paired with conspecific inocula than with heterospecific inocula (main effect $F_{1,46}$=3.73, P=0.06), although this effect

was present only in the greenhouse plants and not the field transplants (study type X inocula provenance interaction $F_{1,46}$=3.13, P=0.08; Fig. 4).

4) Does the composition of root-associated fungal communities vary with the rate of plant host turnover and plant community stability?

When we considered together the field resident plants and field transplants from the field PSF study, we found expected differences in community composition between the resident and transplants (*Pseudo-F$_{1,54}$*=5.15, *P*<0.001), as indicated in our analysis of all study types. We did not find any differences in fungal community composition in the roots of plants located in temporally static compared to dynamic patches (*Pseudo-F$_{1,54}$*=1.09, *P*=0.31), and neither was there a significant interaction between plant host identity and temporal stability (*Pseudo-F$_{1,54}$*=0.93, *P*=0.60; Fig. 5). However, we did recover significant indicator species for static versus dynamic patches. The best indicator species of static patches was *Monosporascus cannonballus*, a known fungal pathogen on melons but common endophyte in grasses in this ecosystem. The best indicator species of dynamic patches was *Fusarium redolens*, which is also known to be a pathogen in agricultural settings, but also exist as endophytes in natural settings (Table S1).

Despite a lack of overall compositional differences between static and dynamic patches, plants in dynamic patches had higher fungal diversity than plants in static patches ($F_{1,54}$=6.08, *P*=0.02). This effect was much stronger for *B. eriopoda* plants than *B. gracilis* plants (interaction $F_{1,54}$=5.66, *P*=0.02). A similar pattern occurred for Chao1 richness (main effect $F_{1,54}$=4.76, *P*=0.03; interaction $F_{1,54}$=4.06, *P*=0.05; Fig. 6), suggesting it was driven by higher richness than differences in evenness. That we see higher fungal richness for plants in dynamic patches but no

change in fungal composition suggests that plants in dynamic patches had higher numbers of fungal taxa in general, but the gain in fungal taxa was not systematic among plants.

Discussion

Root fungal assemblages differed among studies and plant hosts

Root fungal communities of two congener host grasses across experimental and natural conditions spanning timescales of <1 year to >20 years differed significantly in their microbial species pools in diversity and composition. The effect of host species identity on fungal community composition was similar among study types. Interestingly, the general pattern was that greenhouse experiment plants and plants raised in the greenhouse and transplanted into the field maintained more similar fungal communities across individuals and had higher diversity than those naturally-occuring or experimentally-sowed in the field. Lower divergence in the greenhouse and field transplants could be driven by plant age and establishment time. The greenhouse experiment plants were harvested after ~1 year, and the field transplants were harvested after ~2.5 years, whereas the field residents and monocultures came from plants that were likely much older. Increased time since establishment, more variable abiotic conditions that act as selection filters, and a potentially wider range of genetic diversity in host plant populations are all likely reasons that led to higher divergence in the field-grown plant fungal communities (Husband et al. 2002a, Schweitzer et al. 2008, Fujimura and Egger 2012). Individual plants could selectively filter fungal composition over decadal time scales, returning lower per-plant fungal diversity and higher among-plant dispersion in fungal composition than observed in short-term inoculation studies. This suggests a potentially strong role for plant genotype and microbial community co-evolutionary dynamics to drive PSF effects in the field (Schweitzer et al. 2014).

In our study, greenhouse plant and field transplant samples harbored root fungal communities up to four times the diversity of field monoculture and resident plants. There are a few factors that may drive this pattern in diversity. First, it is possible that higher per-sample diversity in the greenhouse plants is due to each of these samples representing 4-6 plant individuals, whereas for the other studies, each sample only came from one plant individual. However, that we saw high per-sample diversity in both greenhouse and the single individual field transplants suggests that this effect may not be the sole driver. Second, plant age or time since establishment is also known to alter plant microbiome diversity. Decreasing diversity and richness in root microbial species with increasing plant age has also been found in rhizosphere bacteria and arbuscular mycorrhiza in tropical tree seedlings (Husband et al. 2002b, Wagner et al. 2016), which is hypothesized to result from a high number of less specialized partnerships early in plant ontogeny. Third, it is possible that plants in the greenhouse experiment and field transplants that were first grown in sterilized sand in the greenhouse were additionally colonized by microbial species specific to the greenhouse environment that persisted even when the transplants were moved to the field. Of all fungal OTUs, we did find a minority (8.6%) that occurred only in samples from the greenhouse plants and field transplants, and not in other study types. These OTUs were comparatively enriched in the relative proporotion of taxa in Glomeromycota (increased from 17.5% of all OTUs to 26% of OTUs unique to transplants and greenhouse plants). The moist and cool conditions in the greenhouse may have enabled the germination of dormant AMF spores from field soil inocula, spores that are typically inactive under field conditions. This hypothesis follows from previous studies in this system that have alternately found high versus low levels of AMF colonization on *B. gracilis* in the field, potentially due to climate conditions (Johnson et al. 2003, Porras-Alfaro et al. 2008).

Across all study types, root fungal community composition significantly differed between *B. gracilis* and *B. eriopoda* host plants. Host-specificity in root microbiome composition is commonly found in naturally-occurring plants in many ecosystems (reviewed in Berg and Smalla 2009). However, our finding reassures that host-specificity in plant-fungal relationships can be recovered at multiple time scales, in the greenhouse and field, and from various methods of plant cultivation and microbial inoculation. Additionally, past work has not found significant differences in root microbial community composition specifically between *B. eriopoda* and *B. gracilis* (Rudgers unpublished data), although significantly different PSF outcomes have been reported (Chung and Rudgers 2016).

Experimental inoculations more important than host identity in determining root fungal assemblages in the field and greenhouse

While we recovered host-specificity across multiple experiments, our results showed that when experimental soil inoculations were applied, those inocula effects outweighed the influence of host plant identity both in the field and the greenhouse. This result not only validates one of the most common methods of PSF research (using field soil inoculation in the greenhouse), but also demonstrates the importance of the starting microbial species pool in the assembly of root-associated fungal communities. That is, despite potential filtering by a host species for 11 months in the greenhouse or 30 months in the field, *B. gracilis* plants and *B. eriopoda* plants that were inoculated with the same species source inoculum had more similar root fungal communities than plants of the same species inoculated with conspecific vs. heterospecific inocula. Our results dovetail with other work that has shown that legacy effects of plant species on soil microbiota persist to affect the growth of subsequent plant hosts as well as the composition of the

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subsequent microbial community (Grman and Suding 2010, Lankau and Lankau 2014). Together, these results suggest the importance of plant and microbial soil legacies in considering plant and microbial community assembly (Philippot et al. 2013).

Additionally, the effect of experimental inoculation on fungal composition was more pronounced in the greenhouse than in the field. This could result from several differences in the method of inoculation. First, plants in the greenhouse were grown in sterile sand that was inoculated once with a small volume of live rhizospheric soil from established monocultures in the field. In comparison, field transplants were "inoculated" by planting them close to a "donor" resident plant, which provided a continuous supply of soil microbe propagules throughout the experiment. While it may seem at first glance that a continuous supply of propagules in the field should lead to stronger effects of inoculation, our hypothesis is that effects were weaker because field transplants were in a matrix of natural mixed species grassland. The effects of the "donor" plant inoculum were likely diluted by the influence of multiple species' rhizospheres that were further away, but still within < 50cm radius. The spatial scale of PSF has been shown to be potentially important in structuring plant communities (Packer and Clay 2000, Mack and Bever 2014), yet spatially-explicit knowledge of the sphere of influence of a plant on the rhizosphere microbial community is lacking. Our results suggest knowledge of plant spatial neighborhood may be important in ensuring PSF treatment efficacy in designing field PSF experiments. Second, the greenhouse plants and field transplants were grown for 11 and 30 months, respectively. Past work has shown decreasing PSF effect size as the length of study increased (Kardol et al. 2013). While there were few studies beyond 12 months to substantiate this pattern, it could result from decreased host vulnerability to pathogens with increasing age for those PSFs that are pathogen-driven (reviewed in Develey-Rivière and Galiana 2007).

Fungal diversity was higher in temporally stable communities than in dynamic ones

Temporal dynamics in host plant species abundance influenced fungal diversity, but not composition. Fungal species diversity in roots was lower in plants in static patches than in dynamic patches, and this difference was stronger for *B. eriopoda* plants than *B. gracilis*. Higher diversity in dynamic patches despite the lack of significant differences in composition suggests that while per-plant fungal taxa diversity is increased, the fungal taxa gained were not similar among plants, and therefore did not drive a systematic shift in fungal composition. Higher root fungal diversity in dynamic patches may be correlated to higher rates of plant community composition change and species turnover for several reasons. First, a larger microbial species pool may be maintained in dynamic patches due to the fluctuating abundances of different plant host species, each favoring different host-specific microbial associates. Second, results from our previous analyses suggest higher root fungal community diversity in plants that are younger in age, a pattern which has been found in other systems (e.g., Husband et al. 2002b). While we do not know the age distribution of plants in dynamic versus static patches in our field experiment, it is not unreasonable to assume that due to higher rates of community composition change, the plants in dynamic patches may be younger than those in static patches, thus partially explaining the difference in root fungal diversity.

Conclusion

Distinct root-associated fungal communities formed on the same host plant species in the greenhouse and the field, even given similar starting microbial inoculua. Despite these differences, however, host-specific root fungal community composition differences were

preserved across all study types, and we confirmed the efficacy of experimental inoculation under both greenhouse and field conditions. Our work highlights the importance of experimental design and approach in driving the outcome of PSF studies, as well as sheds light on key differences between field- and greenhouse-based assessments of plant-soil microbial interactions. Caution should be taken in generalizing greenhouse plant-microbe experiment outcomes to the field, as we have shown that completely different microbial communities could develop under different conditions.

Figures and Tables

Table 1: Description of sequenced studies

Study type	Environment	Time scale	Samples	Treatments	Plant species
Greenhouse plants	Greenhouse	11 months	*n* = 2-3 (total 21)	Inocula provenance x Biocrust addition	*B. gracilis*, *B. eriopoda*
Monoculture plants	Field	7 years	*n* = 4-5 (total 9)	Plant species identity only	*B. gracilis*, *B. eriopoda*
Field transplants	Greenhouse 3 months; Field 27 months	30 months	*n* = 1-7 (total 38)	Community stability x Inoculum provenance	*B. gracilis*, *B. eriopoda*
Field residents	Field, naturally occurring	>20 years	*n* = 20 (total 40)	Community stability	*B. gracilis*, *B. eriopoda*

Table 2 List of most common taxa by sequence number. Species assignments performed using NCBI BLAST. Those OTUs that are significant indicators for certain groups are labeled.

OTU	Sequences	Sample occurrence	Phylum	Order	Family	Best assignment	Indicator status
Otu1	268340	105	Ascomycota	Pleosporales	Pleosporaceae	*Bipolaris spicifera*	
Otu2	139830	56	Basidiomycota	Agaricales	Bolbitiaceae	Panaeolus spp.	Field transplants
Otu3	127258	40	Ascomycota	Xylariales	Unclassified	*Monosporascus cannonballus*	*B. eriopoda* inocula
Otu4	115364	49	Basidiomycota	Agaricales	Bolbitiaceae	*Conocybe deliquescens*	
Otu5	105408	37	Basidiomycota	Agaricales	Marasmiaceae	*Moniliophthora* spp.	Field resident plants
Otu6	93032	36	Ascomycota	Pleosporales	Unclassified	Pleosporales spp.	
Otu7	79327	24	Ascomycota	Orbiliales	Orbiliaceae	*Arthrobotrys* spp.	Greenhouse plants
Otu8	55677	47	Ascomycota	Pleosporales	Unclassified	Pleosporales spp.	
Otu9	49391	21	Basidiomycota	Agaricales	Agaricaceae	*Coprinus silvaticus*	*B. gracilis* inocula
Otu10	48606	63	Ascomycota	Hypocreales	Nectriaceae	*Fusarium* spp.	Field transplants
Otu11	36389	51	Ascomycota	Magnaporthales	Magnaporthaceae	*Magnaporthiopsis panicorum*	Field transplants
Otu12	36370	40	Ascomycota	Sordariales	Chaetomiaceae	*Chaetomium* spp.	Greenhouse plants
Otu13	36300	67	Ascomycota	Pleosporales	Unclassified	Pleosporales spp.	Field transplants
Otu14	33528	16	Ascomycota	Xylariales	Unclassified	*Monosporascus* spp.	
Otu17	29152	53	Ascomycota	Hypocreales	Nectriaceae	*Fusarium redolens*	*B. eriopoda* inocula; Dynamic patches
Otu18	27632	23	Ascomycota	Xylariales	Unclassified	*Monosporascus* spp.	Greenhouse plants
Otu19	27561	19	Basidiomycota	Agaricales	Entolomataceae	*Clitopilus scyphoides*	
Otu20	27397	7	Ascomycota	Xylariales	Unclassified	*Monosporascus cannonballus*	Static patches
Otu21	26228	19	Ascomycota	Pleosporales	Unclassified	Pleosporales spp.	Field resident plants
Otu22	25944	18	Ascomycota	Pleosporales	Unclassified	Pleosporales spp.	
Otu23	25786	17	Basidiomycota	Agaricales	Entolomataceae	*Clitopilus giovanellae*	*B. gracilis* inocula
Otu25	25006	8	Basidiomycota	Agaricales	Psathyrellaceae	*Parasola lilatincta*	
Otu26	24399	33	Ascomycota	Pleosporales	Pleosporaceae	*Pyrenophora tritici-repentis*	Field transplants
Otu28	22654	26	Ascomycota	Xylariales	Unclassified	*Monosporascus cannonballus*	
Otu29	21492	29	Ascomycota	Magnaporthales	Magnaporthaceae	*Budhanggurabania cynodonticola*	
Otu30	20567	6	Basidiomycota	Agaricales	Bolbitiaceae	*Conocybe coprophila*	
Otu31	19509	62	Ascomycota	Dothideales	Dothioraceae	*Aureobasidium pullulans*	Field transplants
Otu32	19108	25	Ascomycota	Pleosporales	Unclassified	Pleosporales spp.	
Otu33	18797	1	Basidiomycota	Agaricales	Strophariaceae	*Leratiomyces laetissimus*	
Otu34	18646	13	Basidiomycota	Phallales	Phallaceae	*Lysurus cruciatus*	Dynamic patches

Table 3: Research questions and analysis models

Research Question	Contrasts Tested	Samples Used			
		Greenhouse	Monoculture	Field Resident	Field Transplant
(1) How does root-associated fungal community composition compare among observational and experimental studies across different temporal and spatial scales?	Study type Host plant identity Interaction	Conspecific-inoculated plants only	All	All	Plants in conspecific microbial environments only
(2) Does plant-fungal interaction specificity change between greenhouse and field conditions?					
(3) How do experimental inoculations alter fungal community composition in the greenhouse and the field?	Study type Inoculum provenance Host plant identity All two-way interactions	All			Plants in con- and heterospecific microbial environments
4) Does the composition of root-associated fungal communities vary with the rate of plant host turnover and plant community stability?	Study type Host plant identity Community stability All two-way interactions random factor: spatial block			All	Plants in conspecific microbial environments only

Figure 1 NMDS visualization of the difference in root-associated fungal composition among study types and between host plant species.
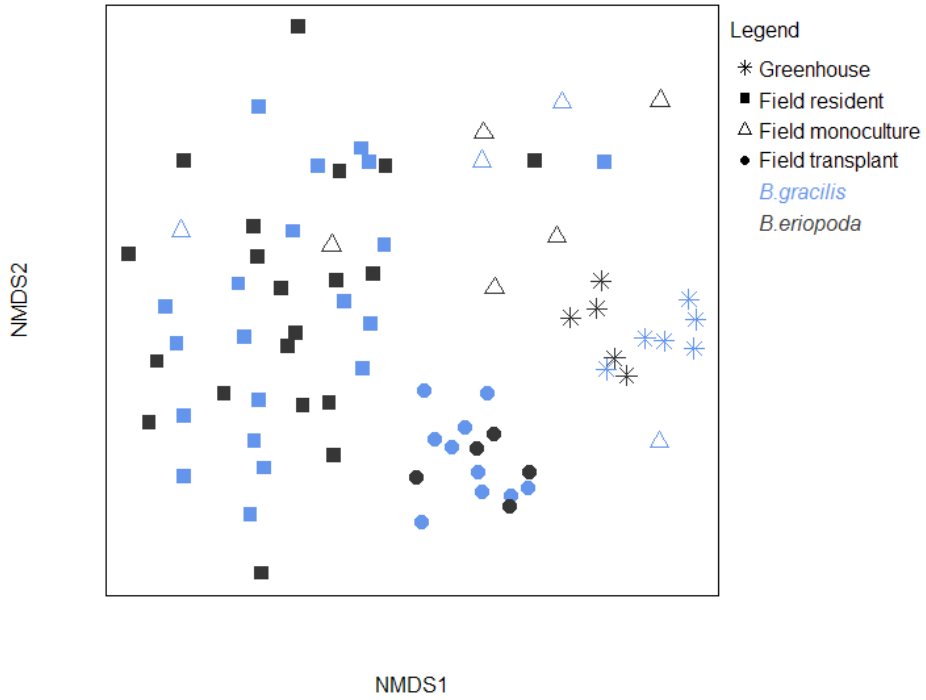
Figure 2 (A) Diversity and (B) richness of root-associated fungal communities among experiment types. Significantly different pairwise comparisons are labeled with different letters.
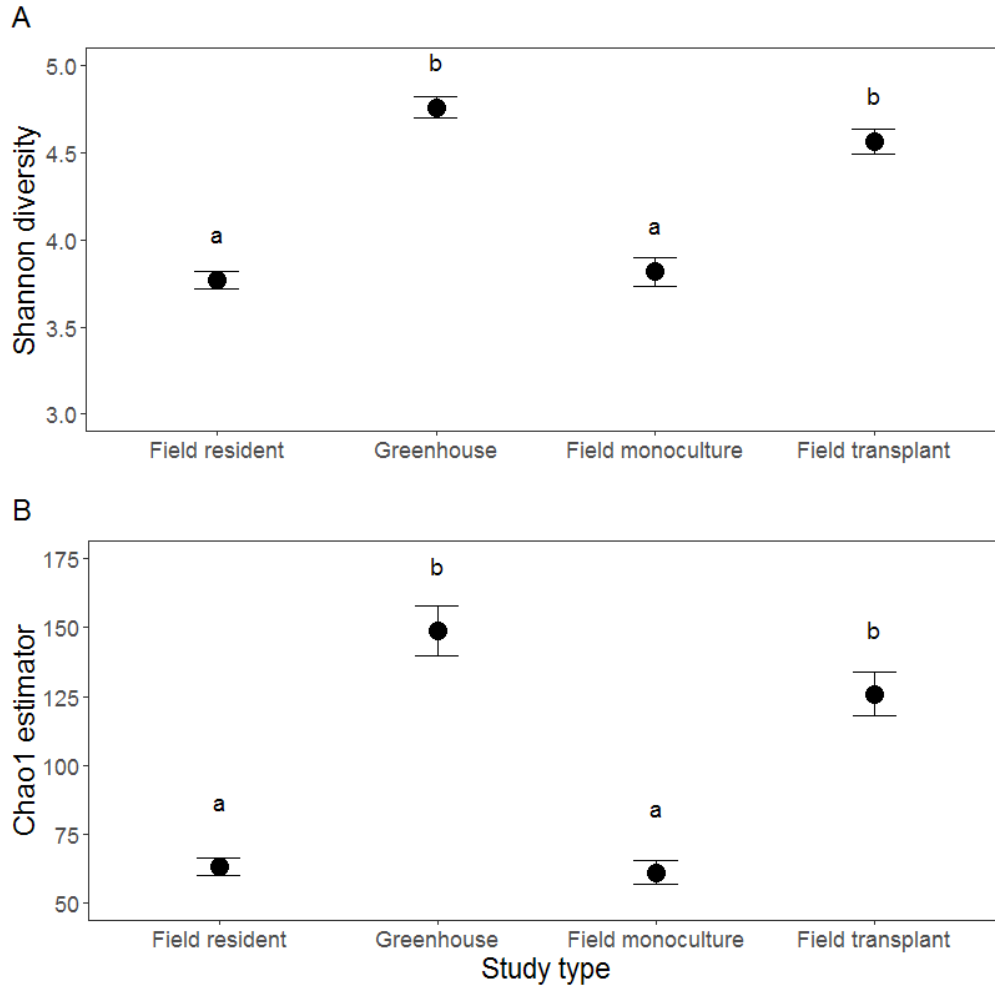
Figure 3 NMDS visualization of the difference in root-associated fungal composition among study types and inoculum provenances.
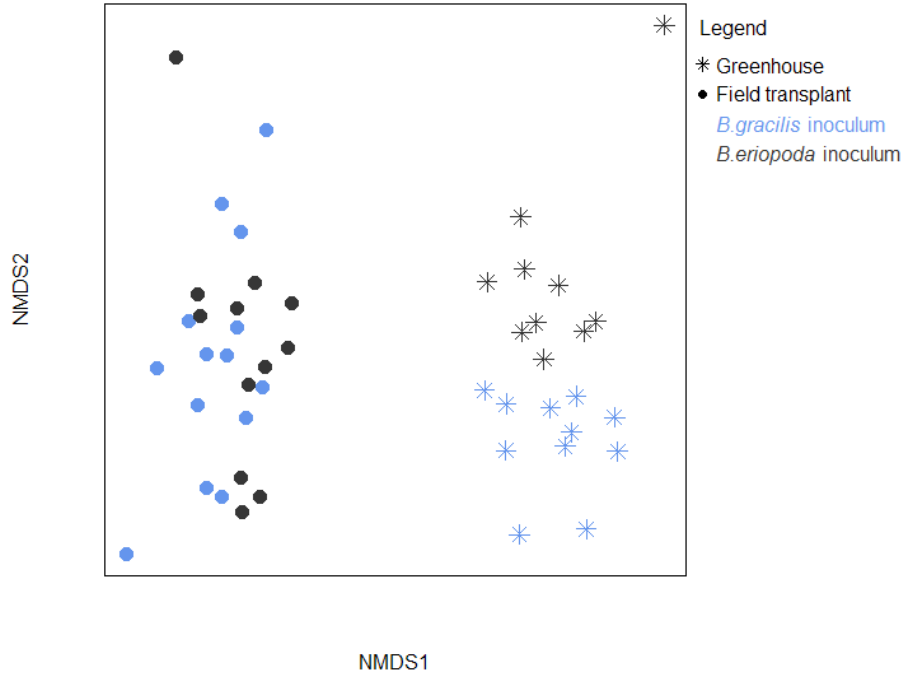
Figure 4 (A) Diversity and (B) richness differences in greenhouse and field transplant root-associated fungal communities in plants inoculated with conspecific vs. heterospecific inoculum.
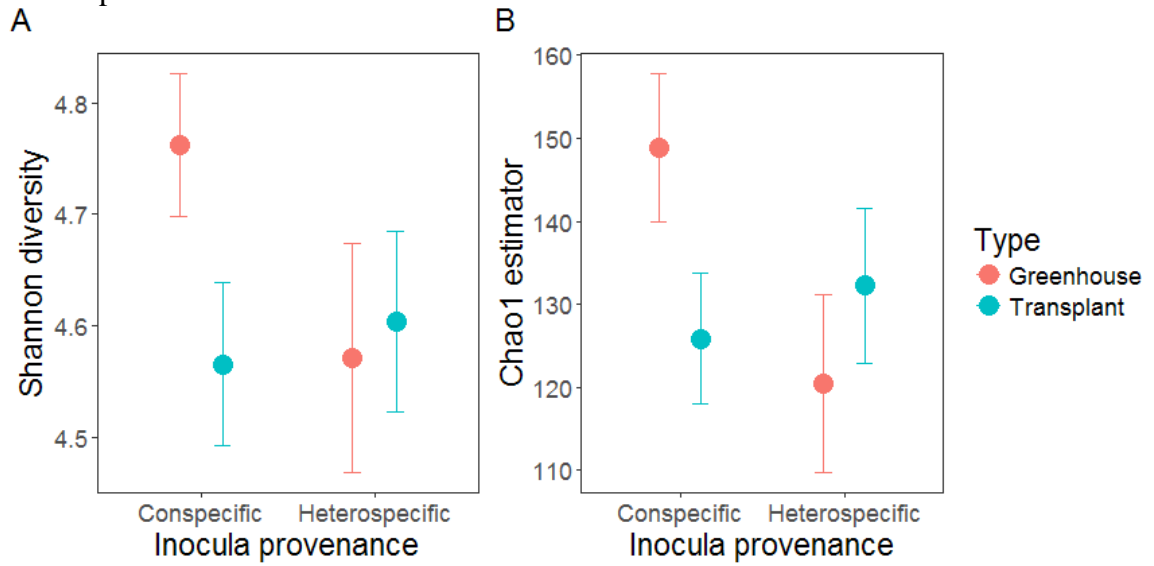
Figure 5 NMDS visualization of the effect of host community stability and turnover rate on root-associated fungal composition in the field resident ant transplants.
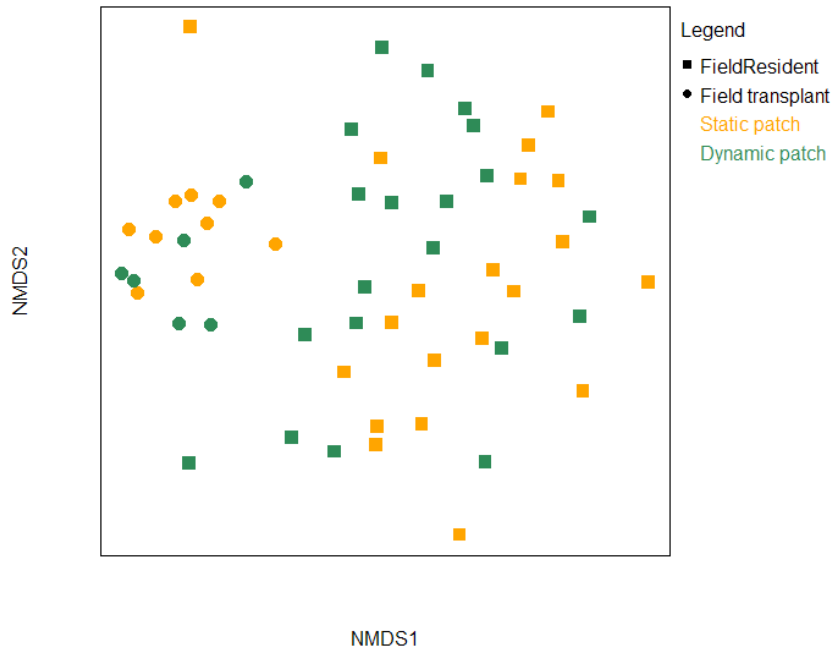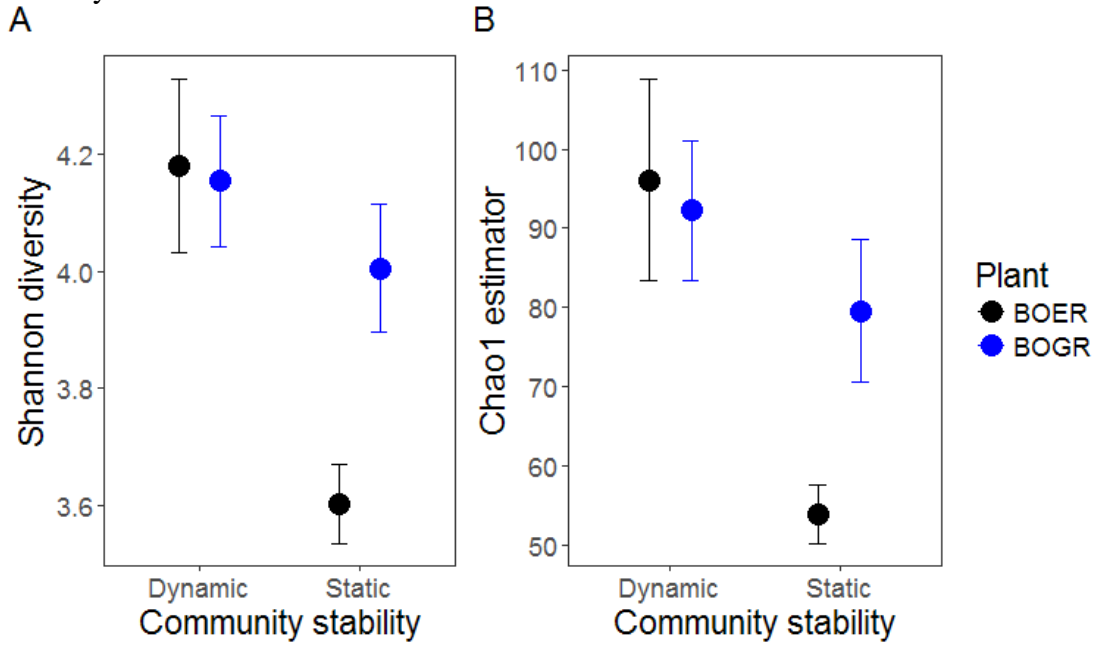
Figure 6 (A) Diversity and (B) richness differences in field resident and field transplant root-associated fungal communities in plants located in patches of different community stability.

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APPENDICES

APPENDIX TO CHAPTER 1

Citation

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The effects of heat treatment on endophyte frequency and germination rates of Poa *seeds*

In *P. alsodes*, the heat treatment reduced endophyte frequency from 88% ± 2% s.e. (control, $n = 307$ plants) to 14% ± 2% (heat, $n = 339$) ($F_{1,638} = 222.7$, $P < 0.0001$) and did not significantly reduce the proportion of seeds that germinated (mean ± s.e.; control= $0.60 ± 0.04$; heat= $0.65 ± 0.03$; $N = 727$ seeds, $F_{1,721} = 1.7$, $P = 0.20$). In *P. sylvestris*, heat treatment reduced endophyte frequency from 84% ± 3% s.e. (control, $n = 208$) to 22% ± 3% (heat, $n = 205$) ($F_{1,407} = 125.9$, $P < 0.0001$) and did not significantly reduce the proportion of seeds that germinated (control= $0.55 ± 0.03$; heat= $0.49 ± 0.03$; $N = 745$ seeds, $F_{1,739} = 2.6$, $P = 0.10$).

Fig. 1 Elasticities of λ to size transitions for **a, c)** E+ and **b, d)** E- populations of **a, b)** *P. sylvestris* and **c, d)** *P. alsodes*. Shading reflects magnitude of elasticities. Size classes include "very small" and "very large" size extensions to avoid unintentional eviction from the model.
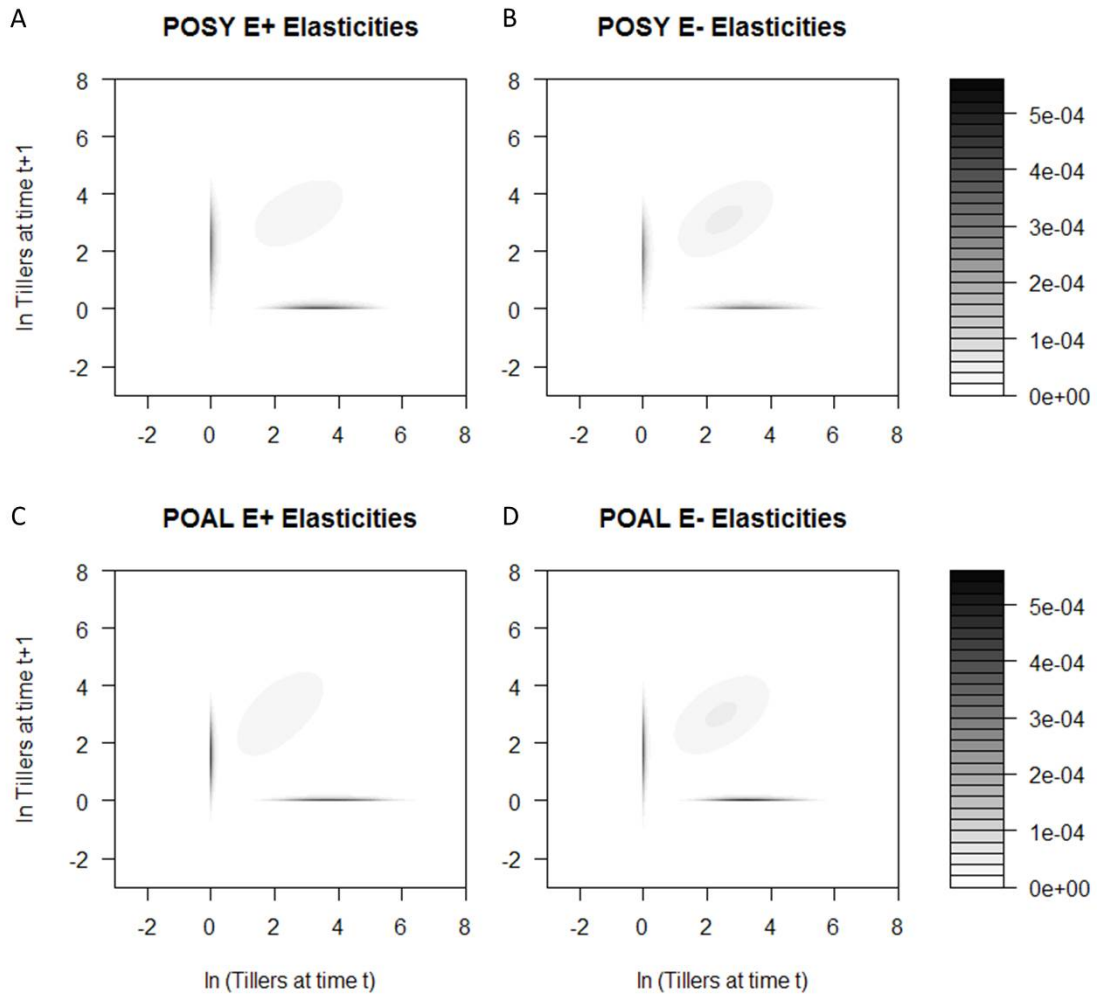
APPENDIX TO CHAPTER 2

Figure 1
A cartoon representation of the experimental design. Pots in a competition response surface array received one of 8 possible combinations of microbial treatments (biocrust addition and rhizosphere soil inoculum). Grass illustrations with permission from *Manual of grasses of the United States* ([1950](#)).
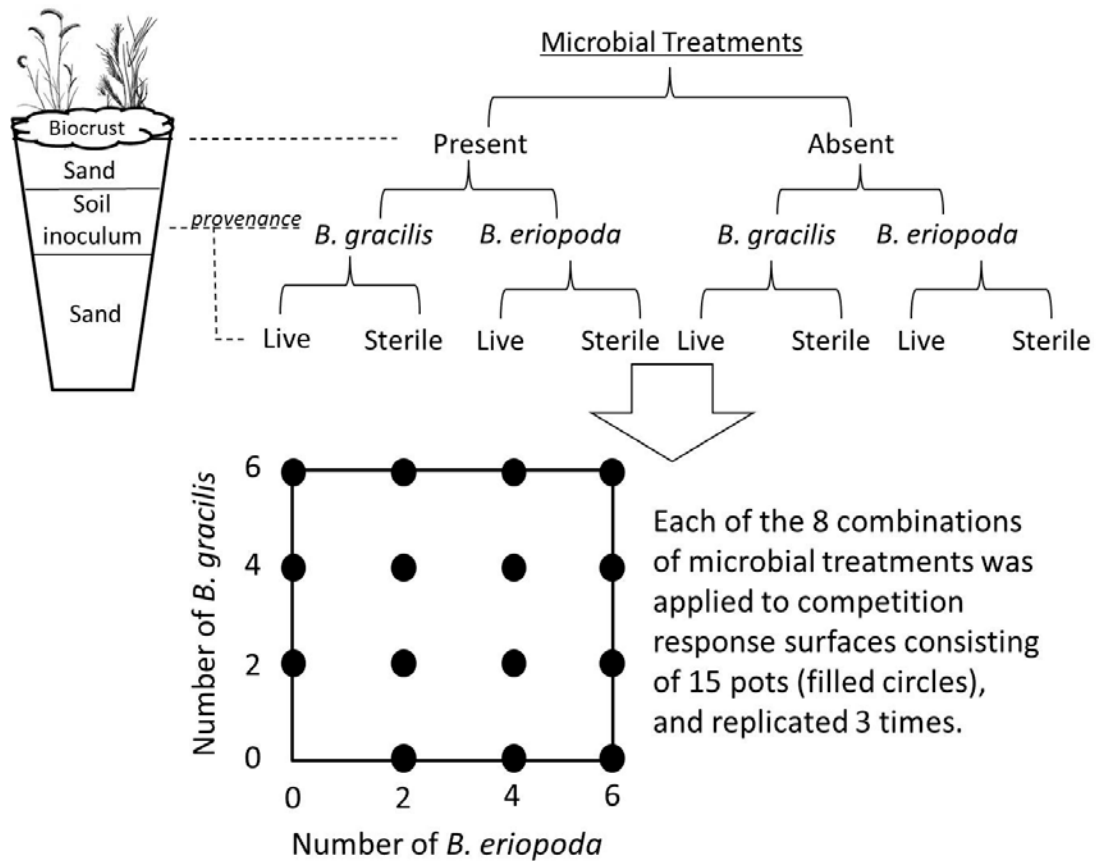
Figure 2 *B. gracilis* competition model fits. Points are observed data, and surface depicts fitted model. Microbial treatment indicated by panel titles.
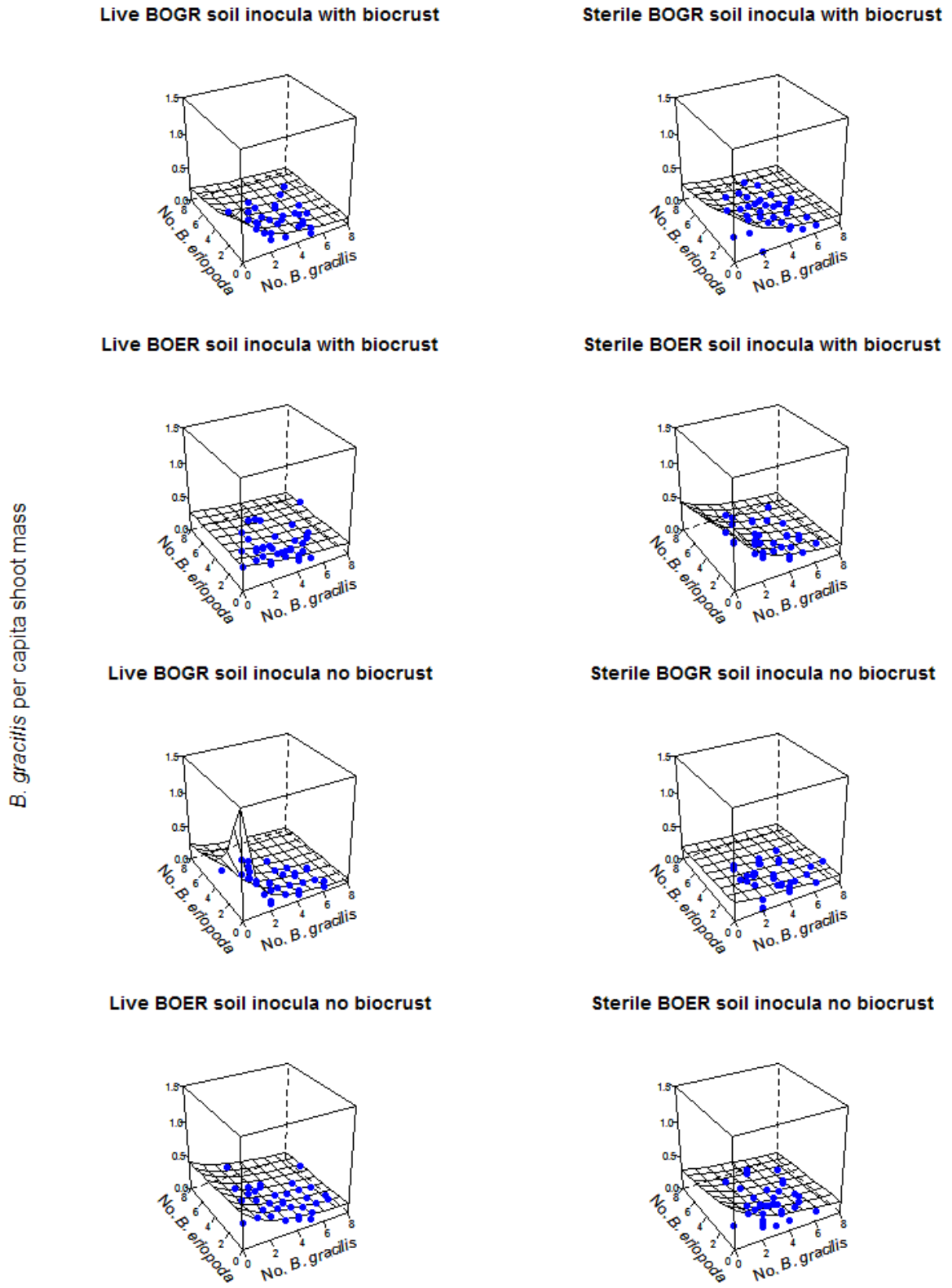
Figure 3 *B. eriopoda* competition model fits. Points are observed data, and surface depicts fitted model. Microbial treatment indicated by panel titles.
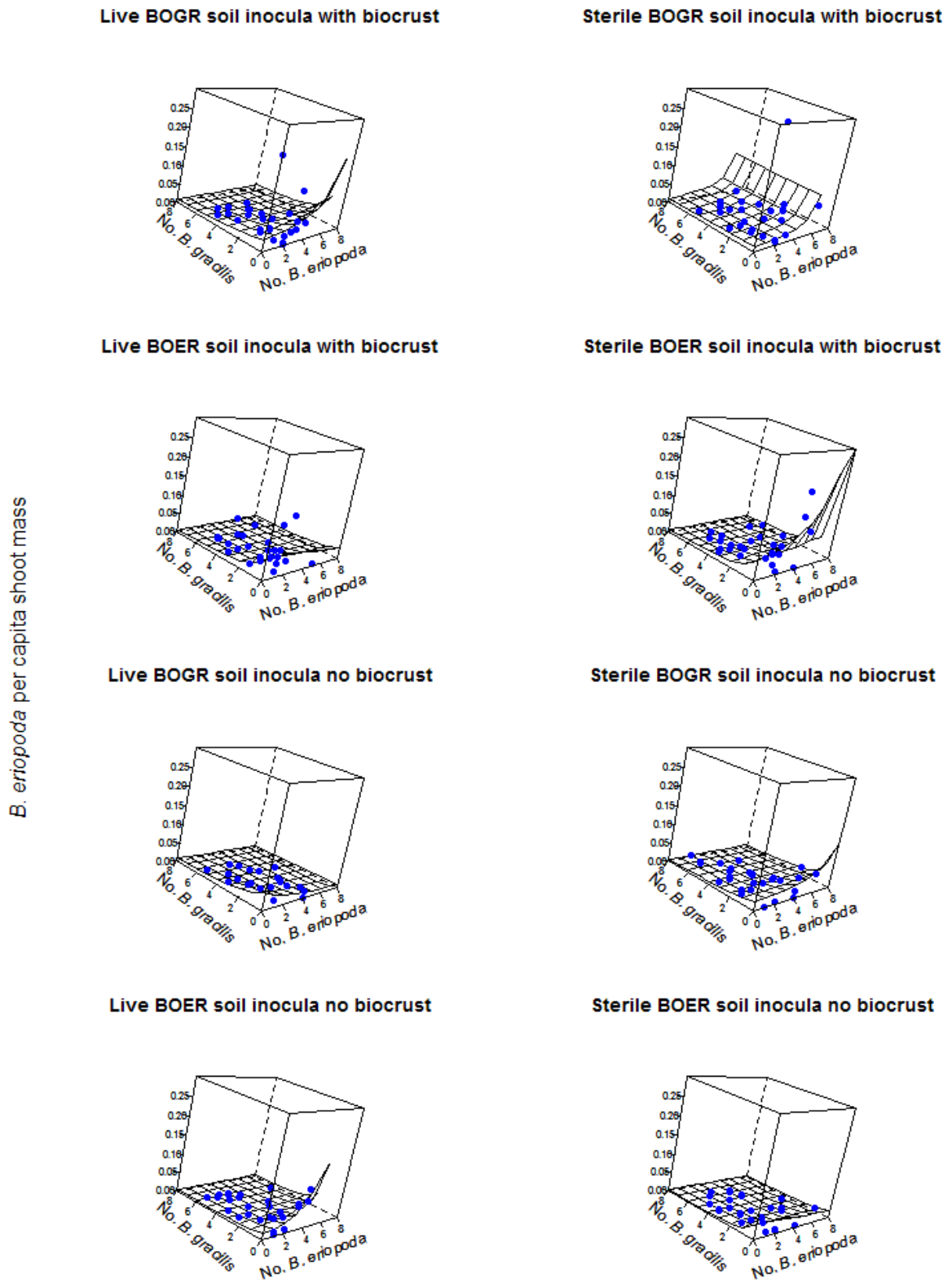
Table 1

Model selection results for each focal species. See Table 2 in main text for model parameterization.

Model	k	ΔAICc	w_i
Bouteloua gracilis			
(v) Microbial effects on Freq:Dens only	4	0	0.768
(i) All microbial effects	14	3.75	0.118
(iv) Microbial effects on density-dependence	4	3.81	0.114
(vi) No microbial effects	4	21.7	0
(ii) Frequency- and density-independent microbial effects	4	29.49	0
(iii) Microbial effects on frequency-dependence	4	31.02	0
Bouteloua eriopoda			
(iii) Microbial effects on frequency-dependence	4	0	0.773
(vi) No microbial effects	4	2.46	0.227
(i) All microbial effects	14	16.37	0
(iv) Microbial effects on density-dependence	4	37.11	0
(v) Microbial effects on Freq:Dens only	4	49.44	0
(ii) Frequency- and density-independent microbial effects	4	54.77	0

Table 2

Estimated frequency-independent (intercept) and frequency/density-dependent (slopes) effects based on best model coefficients for each microbial treatment combination. Values are estimates ± SD. Response is *ln*(per capita aboveground biomass).

Microbial treatments			B. eriopoda model		B. gracilis model	
Sterilize	Provenance	Biocrust	Intercept	BOER frequency slope	Intercept	Total Density: BOGR freq slope
Live	BOER	Crust	-6.542 ± 0.22	5.550 ± 0.46	-1.239 ± 0.08	-0.078 ± 0.03
Live	BOER	No Crust	-6.542 ± 0.22	4.933 ± 0.47	-1.239 ± 0.08	-0.117 ± 0.02
Live	BOGR	Crust	-6.542 ± 0.22	5.169 ± 0.45	-1.239 ± 0.08	-0.168 ± 0.03
Live	BOGR	No Crust	-6.542 ± 0.22	4.552 ± 0.47	-1.239 ± 0.08	-0.206 ± 0.03
Sterile	BOER	Crust	-6.542 ± 0.22	5.779 ± 0.45	-1.239 ± 0.08	-0.063 ± 0.03
Sterile	BOER	No Crust	-6.542 ± 0.22	5.162 ± 0.47	-1.239 ± 0.08	-0.102 ± 0.03
Sterile	BOGR	Crust	-6.542 ± 0.22	5.875 ± 0.47	-1.239 ± 0.08	-0.110 ± 0.03
Sterile	BOGR	No Crust	-6.542 ± 0.22	5.258 ± 0.46	-1.239 ± 0.08	-0.149 ± 0.03

Appendix S2 Deriving invasion growth rate from the population growth model

In this paper, we used a version of the discrete time difference logistic model to model per capita biomass increase of species i in competition with species j in lieu of population growth.

$$\frac{M_i}{N_i} = \frac{\lambda_i}{1+\alpha_{ii}N_i+\alpha_{ij}N_j} \quad \text{Eq. 1}$$

As in the main text, M_i/N_i indicates the mean per capita biomass of species i, where positive values indicate growth. λ is equivalent to intrinsic per capita biomass increase, and α_{ii} and α_{ij} are competition coefficients for the per capita effects of intra- and interspecific competition, respectively. N is the number of individuals of each species in the 'population', or experimental pot, at the beginning of the experiment. In a two species competition scenario, as in this study, the equation for per capita biomass increase of species j has an identical formulation but with the opposite species subscripts. Equation 1 is modified from the original discrete time logistic population model, which describes the population size of species i at time $t+1$ as:

$$N_{i,t+1} = \frac{\lambda_i N_{i,t}}{1+\alpha_{ii}N_{i,t}+\alpha_{ij}N_{j,t}} \quad \text{Eq. 2}$$

Invasion growth rates refer to the population growth rate of a species at very low numbers while its competitor is at single-species equilibrium. Here, we use the scenario of species i invading species j as an example.

To find the <u>number of individuals</u> of species j at single-species equilibrium ($\widehat{N}_j$), we use the original population model (Eq. 2) for species j. At single-species equilibrium, there is no population growth so we set $N_{j,t} = N_{j,t+1} = \widehat{N}_j$. As no competitors are present, N_i is set to zero:

$$\widehat{N}_J = \frac{\lambda_j \widehat{N}_J}{1+\alpha_{jj}\widehat{N}_J} \quad \text{Eq. 3}$$

Solving Eq. 3 for $\widehat{N}_j$,

$$\widehat{N}_J = \frac{\lambda_j-1}{\alpha_{jj}} \quad \text{Eq. 4}$$

To find the 'invasion growth rate' expressed in terms of biomass accumulation for species i, we use Eq. 1, assuming that species j is at single species equilibrium (Eq. 4), and that species i is at such a low density (invading) that there are no intraspecific effects (i.e., $\alpha_{ii}N_i = 0$).

$$\frac{M_i}{N_i} = \frac{\lambda_i}{1+\alpha_{ij}\left(\frac{\lambda_j-1}{\alpha_{jj}}\right)} \quad \text{Eq. 5}$$

Which can be re-arranged to show the effects of λ's and α's more clearly to be:

$$\frac{M_i}{N_i} = \left(\frac{\lambda_i}{\lambda_j}\right)\left[\frac{\lambda_j}{1+(\alpha_{ij}/\alpha_{jj})(\lambda_j-1)}\right] \quad \text{Eq. 6}$$

APPENDIX TO CHAPTER 3

Figure S1 Effects of community stability on PSF of A) *Bouteloua gracilis* and B) *B. eriopoda* seedling emergence in the growth chamber. Letters indicate statistically-significant comparisons.
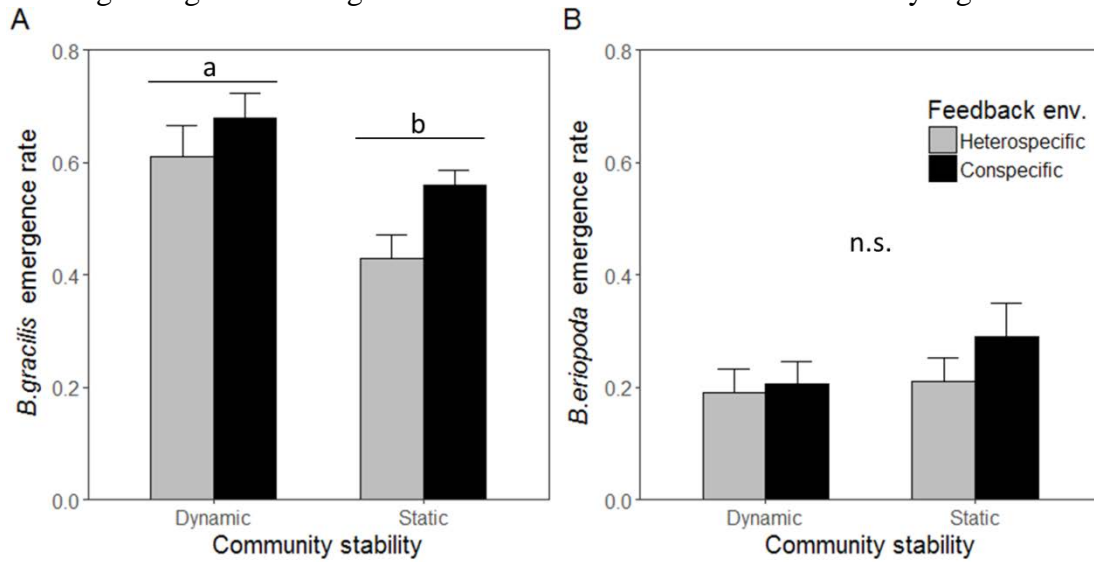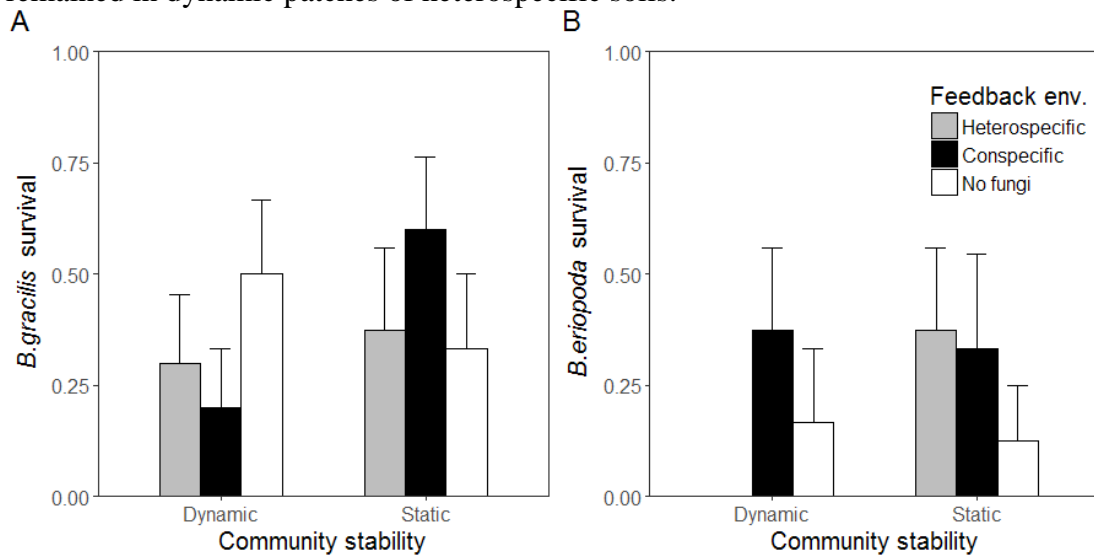

Figure S2 Effects of community stability on PSF of A) *Bouteloua gracilis* and B) *B. eriopoda* transplant survival. All effects were n.s. At the end of the experiment, no live *B. eriopoda* remained in dynamic patches of heterospecific soils.

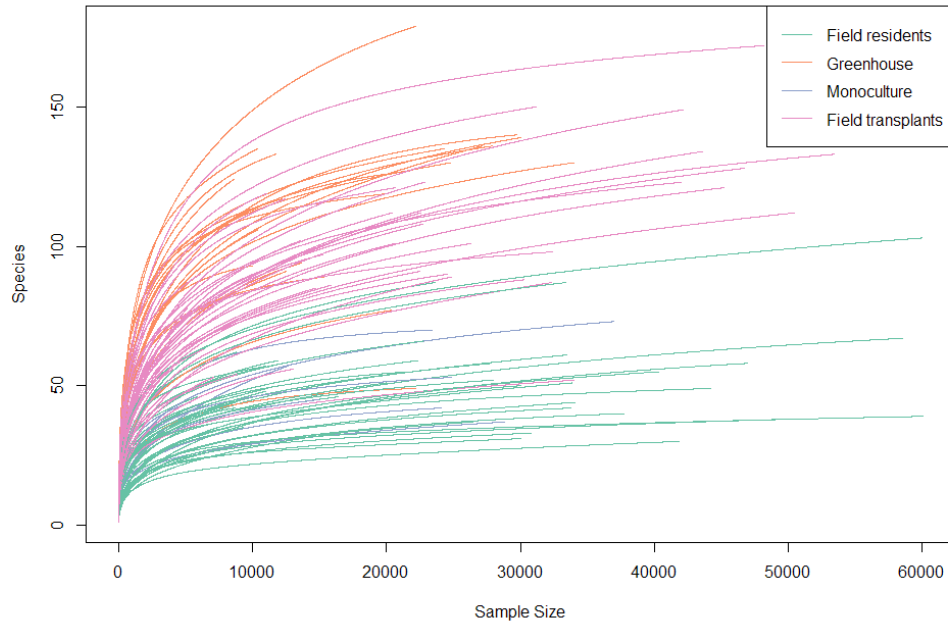Figure S1 OTU rarefaction curves of all samples, colored by study type.

Figure S2 Fungal community composition at the phylum level among (A) study types and (B) host species.
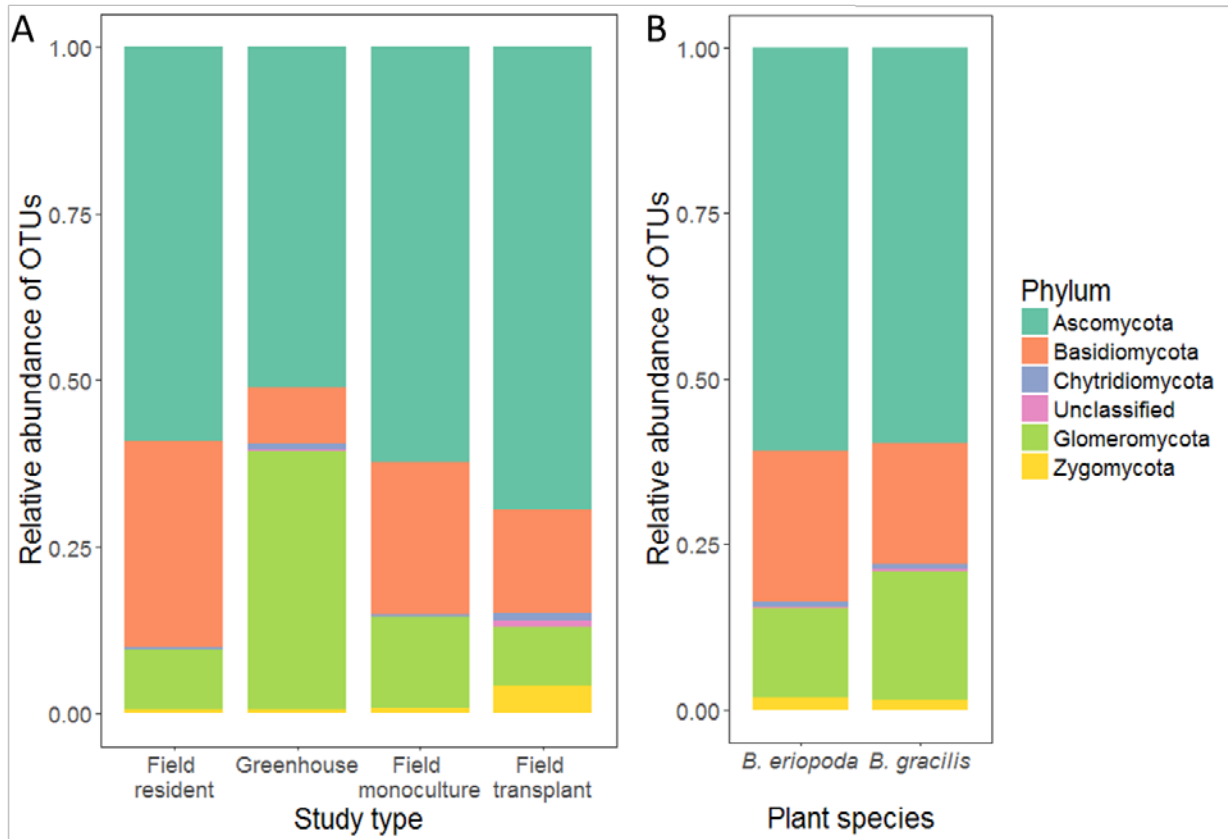
Table S1

Top five significant indicator OTUs for groups tested based on indicator value. Species assignments performed using NCBI BLAST. Indicator value calculation follows Dufrene and Legendre 1997.

OTU	Group	Indic value	Phylum	Order	Family	Best assignment
Otu7	Greenhouse	0.96	Ascomycota	Orbiliales	Orbiliaceae	*Arthrobotrys* sp.
Otu78	Greenhouse	0.83	Ascomycota	Pezizales	Pyronemataceae	*Scutellinia* sp. (Scutellinia_sp|SH230992.06FU)
Otu102	Greenhouse	0.86	Glomeromycota	Paraglomerales	Paraglomeraceae	*Paraglomus brasilianum*
Otu191	Greenhouse	0.85	Ascomycota	Sordariales	Lasiosphaeriaceae	*Cercophora vinosa*
Otu269	Greenhouse	0.73	Glomeromycota	Paraglomerales	Paraglomeraceae	*Paraglomus brasilianum*
Otu128	Field transplant	0.74	Ascomycota	Pleosporales	Tetraplosphaeriaceae	*Triplosphaeria* sp.
Otu129	Field transplant	0.73	Ascomycota	Hypocreales	Clavicipitaceae	*Beauveria felina*
Otu130	Field transplant	0.87	Ascomycota	Hypocreales	Unclassified	*Acremonium asperulatum*
Otu175	Field transplant	0.63	Chytridiomycota	Cladochytriales	Nowakowskiellaceae	*Nowakowskiella elegans*
Otu261	Field transplant	0.67	Ascomycota	Onygenales	Gymnoascaceae	*Gymnoascoideus petalosporus*
Otu222	Field monoculture	0.39	Ascomycota	Onygenales	Onygenaceaev	*Auxarthron alboluteum*
Otu243	Field monoculture	0.29	Ascomycota	Xylariales	Xylariacaea	*Arthroxylaria elegans*
Otu349	Field monoculture	0.29	Ascomycota	Eurotiales	Trichocomaceae	*Rasamsonia* sp. 775
Otu725	Field monoculture	0.29	Basidiomycota	Agaricales	Psathyrellaceae	*Coprinellus* sp.
Otu1074	Field monoculture	0.29	Ascomycota	Unclassfied	Unclassified	*Retroconis* sp. XAE_090
Otu5	Field Resident	0.57	Basidiomycota	Agaricales	Marasmiaceae	*Moniliophthora* sp. (Agaricales_sp|SH190088.06FU)
Otu104	Field Resident	0.30	Basidiomycota	Agaricales	Marasmiaceae	*Moniliophthora* sp. (Agaricales_sp|SH190088.06FU)
Otu313	Field Resident	0.33	Basidiomycota	Agaricales	Marasmiaceae	*Moniliophthora* sp. (Agaricales_sp|SH190088.06FU)
Otu388	Field Resident	0.33	Basidiomycota	Agaricales	Marasmiaceae	*Moniliophthora* sp. (Agaricales_sp|SH190088.06FU)
Otu499	Field Resident	0.30	Basidiomycota	Agaricales	Marasmiaceae	*Moniliophthora* sp. (Agaricales_sp|SH190088.06FU)

Otu224	*B. eriopoda* plant	0.20	Ascomycota	Pleosporales	Phaeosphaeriaceae	*Phaeosphaeria* sp. (Phaeosphaeria_sp|SH207014.06FU)
Otu240	*B. eriopoda* plant	0.27	Ascomycota	Pleosporales	Phaeosphaeriaceae	*Phaeosphaeria* sp. (Fungi_sp|SH207017.06FU)
Otu279	*B. eriopoda* plant	0.30	Glomeromycota	Glomerales	Glomeraceae	*Glomeromycota* sp. JB-2014f
Otu686	*B. eriopoda* plant	0.21	Ascomycota	Xylariales	Unclassified	*Monosporascus cannonballus*
Otu768	*B. eriopoda* plant	0.17	Ascomycota	Coniochaetales	Coniochaetaceae	*Coniochaeta africana*
Otu56	*B. gracilis* plant	0.28	Ascomycota	Sordariales	Lasiosphaeriaceae	*Cercophora* sp.
Otu69	*B. gracilis* plant	0.47	Ascomycota	Pleosporales	Unclassified	*Macrodiplodiopsis desmazieri*
Otu88	*B. gracilis* plant	0.23	Glomeromycota	Glomerales	Claroideoglomeraceae	*Claroideoglomus drummondii*
Otu375	*B. gracilis* plant	0.18	Ascomycota	Hypocreales	Unclassified	*Sarocladium kiliense*
Otu887	*B. gracilis* plant	0.18	Ascomycota	Pleosporales	Unclassified	*Pleosporales* sp. (Dothideomycetes_sp|SH190252.06FU)
Otu17	*B. eriopoda* inocula	0.52	Ascomycota	Hypocreales	Nectriaceae	*Fusarium redolens*
Otu173	*B. eriopoda* inocula	0.41	Ascomycota	Glomerales	Glomeraceae	*Claroideoglomus claroideum*
Otu224	*B. eriopoda* inocula	0.47	Ascomycota	Pleosporales	Phaeosphaeriaceae	*Phaeosphaeria* sp. (Phaeosphaeria_sp|SH207014.06FU)
Otu240	*B. eriopoda* inocula	0.58	Ascomycota	Pleosporales	Phaeosphaeriaceae	*Phaeosphaeria* sp. (Fungi_sp|SH207017.06FU)
Otu337	*B. eriopoda* inocula	0.41	Ascomycota	Chaetothyriales	Herpotrichiellaceae	*Phaeococcomyces catenatus*
Otu88	*B. gracilis* inocula	0.43	Glomeromycota	Glomerales	Claroideoglomeraceae	*Claroideoglomus drummondii*
Otu164	*B. gracilis* inocula	0.39	Ascomycota	Hypocreales	Cordycipitaceae	*Lecanicillium psalliotae*
Otu184	*B. gracilis* inocula	0.32	Glomeromycota	Glomerales	Glomeraceae	*Glomus* sp. 11 ZHNL-2013q
Otu230	*B. gracilis* inocula	0.38	Basidiomycota	Tremellales	Tremellaceae	*Cryptococcus* sp AL_V
Otu479	*B. gracilis* inocula	0.32	Glomeromycota	Glomerales	Glomeraceae	*Glomus* sp. 11 ZHNL-2013q
Otu17	Dynamic patches	0.36	Ascomycota	Hypocreales	Nectriaceae	*Fusarium redolens*
Otu34	Dynamic patches	0.24	Basidiomycota	Phallales	Phallaceae	*Lysurus cruciatus*
Otu240	Dynamic patches	0.26	Ascomycota	Pleosporales	Phaeosphaeriaceae	*Phaeosphaeria* sp. (Fungi_sp|SH207017.06FU)
Otu258	Dynamic patches	0.26	Glomeromycota	Glomerales	Glomeraceae	*Glomus* sp. BEG104

Otu667	Dynamic patches	0.21	Ascomycota	Pleosporales	Periconiaceae	*Periconia* sp.
Otu20	Static patches	0.21	Ascomycota	Xylariales	Unclassified	*Monosporascus cannonballus*
Otu149	Static patches	0.21	Ascomycota			BLAST 50% coverage *Gymnoascus petalosporus*
Otu252	Static patches	0.24	Ascomycota	Hypocreales	Nectriaceae	*Fusarium solani*
Otu329	Static patches	0.25	Ascomycota	Eurotiales	Trichocomaceae	*Talaromyces verruculosus*
Otu391	Static patches	0.28	Ascomycota	Hypocreales	Cordycipitaceae	*Lecanicillium primulinum*