Genetic diversity and species diversity weaken plant-soil feedback-mediated coexistence

Running Title: Genetic and species diversity weaken PSF

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ABSTRACT

Theory suggests that genetic diversity may influence species coexistence and that species diversity may influence genotype coexistence by altering competitive outcomes among species and genotypes, respectively. However, other coexistence mechanisms such as microbe-mediated plant-soil feedbacks (PSF), may also contribute. Interspecific PSF promotes species coexistence when plants grow better with heterospecific soil microbes than with conspecific microbes, and similarly, intraspecific PSF promotes genotype coexistence when plants grow better with heterogenotypic than with congenotypic microbes. Here, we tested whether genetic diversity influences the strength or direction of interspecific PSF and whether species diversity influences the strength or direction of intraspecific PSF. We found that genetic diversity reduced the capacity for interspecific PSF to promote species coexistence, and, for one study species, species diversity reduced the capacity for intraspecific PSF to promote genotype coexistence. These results suggest that genetic diversity and species diversity may weaken the ability of PSF to promote coexistence.

Genetic diversity and species diversity weaken plant-soil feedback-mediated coexistence

INTRODUCTION

Species coexistence is fundamental to ecology because it in part determines the maintenance of species diversity, and similarly the coexistence of competing genotypes is fundamental to evolution because it determines the maintenance of genetic diversity. However, a growing body of theoretical work has proposed how genetic diversity could influence species coexistence, and how species diversity could influence genotype coexistence, potentially linking these two fundamental levels of biodiversity (Vellend & Geber 2005; Vellend 2006, 2008; Eck *et al.* 2019). For example, competition could cause genetic diversity to positively influence species coexistence, and species diversity to positively influence genotype coexistence, if different species have a competitive advantage against different genotypes of a common focal species and if different genotypes have a competitive advantage against different species (Vellend & Geber 2005; Vellend 2006, 2008). Alternatively, competition could cause genetic diversity to negatively influence species coexistence, and species diversity to negatively influence genotype coexistence, if high genetic diversity reduces available niche space for heterospecifics, and if high species diversity reduces available niche space for genotypes within species (Vellend & Geber 2005).

While the effects of diversity on the strength of coexistence have so far been considered primarily through the mechanism of competition, other types of species interactions, like the interaction between plants and their soil microbiota, also may contribute. Microbe-mediated plant-soil feedback (PSF hereafter, although we note that PSF can also be mediated by abiotic factors [Ehrenfeld *et al.* 2005]) is a ubiquitous mechanism for promoting plant species coexistence whose effects on plant growth are similar in magnitude to those of competition (Lekberg *et al.* 2018). PSF occurs when different plant species attract and encourage the growth of different soil microbial communities, and these microbial communities then feed back to differentially affect the fitness of plant species through species-specific pathogens or mutualists. Negative PSF occurs when plant species have *lower* fitness in their own soil microbial community relative to other members of the plant community, leading to negative density-

77 dependence and the promotion of species coexistence (Bever *et al.* 1997). Positive PSF, on the other
78 hand, occurs when species have *higher* fitness when grown with their own soil microbial community
79 relative to other members of the plant community, reducing the likelihood of coexistence and potentially
80 leading to species diversity declines (Bever *et al.* 1997).
81 While most work has focused on microbe-mediated *inter*specific PSF as a mechanism for
82 promoting species coexistence, some studies have found significant *intra*specific PSF that can affect the
83 coexistence of genotypes within species (Bever *et al.* 1997; Felker-Quinn *et al.* 2011; Bukowski &
84 Petermann 2014; Liu *et al.* 2015; Bukowski *et al.* 2018). For example, when pathogens differentially
85 affect genotypes of a species, as has been found in both agricultural (Neupane *et al.* 2015; Croll &
86 McDonald 2017; Walters *et al.* 2018) and natural (Laine 2004; Laine *et al.* 2011; Eck *et al.* 2019)
87 populations, then the pathogens most harmful to a common genotype will accumulate and reduce the
88 fitness of that genotype in the next generation. As with interspecific PSF, these dynamics lead to negative
89 density-dependence and the promotion of genotype coexistence through negative intraspecific PSF. On
90 the other hand, if mutualists differentially affect genotypes of a species, then mutualists most beneficial to
91 a common genotype may accumulate and increase the fitness of that genotype in the next generation.
92 Such positive intraspecific PSF, which reduces the likelihood of genotype coexistence and potentially
93 leads to the erosion of genetic diversity, has also been demonstrated (Bever *et al.* 1996; Bukowski &
94 Petermann 2014; Bukowski *et al.* 2018).
95 Analogous to theory regarding competition as a mediator of diversity effects on coexistence, we
96 may expect PSF to cause genetic diversity to positively influence species coexistence, and species
97 diversity to positively influence genotype coexistence, if: (1) some species perform better with the
98 microbial communities associated with one genotype of a focal species, while other species perform
99 better with the microbial communities associated with different genotypes, and (2) some genotypes of a
100 focal species perform better with the microbial communities associated with one species, while other
101 genotypes perform better with the microbial communities associated with different species. These
102 dynamics could occur, for example, if a legume species that is highly dependent on rhizobia (nitrogen-

fixing bacteria) benefits from co-occurring with a heterospecific legume genotype that strongly promotes rhizobia growth, while another plant species may benefit instead from the microbes associated with a different genotype of the legume, perhaps through associational resistance to an enemy.

On the other hand, PSF may cause genetic diversity to negatively influence species coexistence and species diversity to negatively influence genotype coexistence if: (1) high genetic diversity in a focal species dilutes conspecific pathogens that would otherwise promote negative interspecific PSF, and (2) high species diversity dilutes the pathogens specialized on a common genotype that would otherwise promote negative intraspecific PSF. Recent theoretical work found support for the dilution of conspecific pathogens, showing that when pathogens were genotype-specific, simulated communities with low genetic diversity resulted in more negative interspecific PSF relative to simulated communities with high genetic diversity (Eck *et al.* 2019). The reciprocal effects of species diversity on genotype coexistence may also occur, although this was not tested in the Eck and coauthors (2019) model.

Here, we tested empirically whether genetic and species diversity can influence PSF-mediated coexistence of species and genotypes, respectively. We manipulated both genetic diversity and species diversity using two populations of each of two prairie plant species, and measured the resulting impact on the strength and direction of interspecific and intraspecific PSF. Specifically, we asked:

1) Does genetic diversity alter the strength of interspecific PSF?

2) Does species diversity alter the strength of intraspecific PSF?

If genetic diversity causes interspecific PSF to become more negative (or less positive) and species diversity causes intraspecific PSF to become more negative (or less positive), then genetic diversity will promote species coexistence, and species diversity will promote genotype coexistence, potentially promoting positive linkages between species and genetic diversity. On the other hand, if genetic diversity causes interspecific PSF to become more positive (or less negative) and species diversity causes intraspecific PSF to become more positive (or less negative), then genetic diversity will inhibit species coexistence and species diversity will inhibit coexistence among genotypes, potentially promoting negative linkages between species and genetic diversity.

METHODS

Experimental Design Overview

To test how genetic diversity influences interspecific PSF and how species diversity influences intraspecific PSF, we conducted a two-generation PSF experiment (Bever 1994) in the greenhouse in the fall of 2017. In this method, a plant or group of plants conditions the soil microbial community in the first generation ("Phase I"), and the effects of those microbial communities on plant growth are then assessed in a second generation ("Phase II"). We calculated PSF as the net-pairwise feedback, I_s, a measure that allows us to make predictions about whether PSF will promote or inhibit the coexistence of species or genotypes (Bever *et al.* 1997). Additionally, we estimated the ln-response ratio for each species and each population, which is a direct measure of that species' or population's relative growth in its own soil microbial community vs. heterospecific or -population soil.

We manipulated genetic diversity and species diversity in Phase I to test their influence on the strength and direction of inter- and intraspecific PSF, respectively. We manipulated species diversity by planting pairs of plants in each pot that were either two individuals of the same species or one individual of each of two species, and we manipulated genetic diversity within each species in a similar way, except we planted two individuals from the same population or one individual from each of two populations (note that each population includes numerous genotypes; Fig. 1). While we acknowledge that a pot containing two species or two genotypes would not typically be considered high diversity, for simplicity we refer to our two-species and two-population treatments as "high species diversity" and "high genetic diversity" respectively, and our single-species and single-population treatments as "low species diversity" and "low genetic diversity". We used these two levels of diversity within each pot because including more levels would have made the experiment unfeasibly large, and the largest effects of increasing species or genotype richness are often observed at relatively low richness (*e.g.,* Tilman *et al.* 1997).

Feedback Experiment

Phase I: conditioning soil — We used two commonly co-occurring perennial prairie plant species - *Echinacea purpurea* (*Asteraceae; "Echinacea"* hereafter) and *Coreopsis lanceolata* (*Asteraceae; "Coreopsis"* hereafter). For each of these species we used two populations - one from the southern Midwestern United States ("South"; *Coreopsis*, Missouri Wildflower Nursery, originally collected from Joplin Co., MO, USA; *Echinacea*, Hamilton Native Outpost, cultivated in Putnam Co., MO, USA but likely originating from populations in Iowa) and one from the upper Midwestern United States ("North"; *Coreopsis*, Agrecol, originally collected from Kenosha, WI, USA; *Echinacea*, Agrecol, originally collected from Madison, IA, USA). These populations differ in several traits, including relative growth rate (Lau *et al.* 2019; Zirbel & Brudvig 2020a, b; Table S1).

We inoculated all pots with a common field soil inoculum at the time of planting. The inoculum was comprised of soils collected from beneath *Echinacea* and *Coreopsis* plants in August 2017, at each of four restored prairie sites at the W.K. Kellogg Biological Station (Hickory Corners, MI, USA). These sites were sown with twelve common prairie species in Fall, 2015, and two sites were planted with South populations of *Echinacea* and *Coreopsis* while two were planted with North populations. Specifically, we used a 1.9 cm diameter core to collect soil to a depth of 15 cm from beneath the *Echinacea* and *Coreopsis* individual located closest to every five-meter mark along two 30-meter transects, except for one site where we used the first twelve plants we could find because our species were rare at this site. We sieved the soil (2 mm mesh) to remove rocks and roots, then homogenized it to create a single inoculum that we stored at 4°C until Phase I was planted (max. 20 days).

We sterilized and filled 656 mL Deepots™ (Stuewe and Sons, Tangent, OR, USA) with a sterile base soil composed of a 9:1 mixture of untreated sand (Quickrete All Purpose Sand, Atlanta, GA, USA - included for drainage) and sifted, sterilized (autoclaved at 121°C for two periods of 45 min with a 48-hour rest between) field soil that we collected from a restored prairie that received a similar seed mix and management as the experimental prairies described above. We then inoculated each pot with a 40 ml layer of the common inoculum and topped with a thin layer of sterile base soil to reduce contamination between

pots. We planted four seeds into each pot, later thinning to two seedlings, in the combinations described above (Fig. 1). We planted 30 replicates of each treatment, but due to poor germination of the *Echinacea* South population we ended up with 15 replicates of the *Echinacea* low genetic diversity treatments and 28 replicates of the *Echinacea* high genetic diversity treatment (Phase I $N = 178$).

We harvested all plants after 18 weeks and dried and weighed aboveground biomass to account for Phase I productivity in our analyses (see below). Roots of the plant pairs could not be separated, and so were discarded. We stored conditioned soil at 4°C until we planted Phase II (approximately one week).

Phase II: quantifying plant growth effects of conditioned soil — To test for the effect of each soil microbial community from Phase I on plant growth, we inoculated single individuals of each population of each species with soils from each Phase I treatment in a full factorial design (Fig. 1; $N = 384$, 5-15 replicates of each Phase I inoculum × 10 Phase I inocula × 2 species × 2 populations per species). In the low genetic diversity treatments where we were testing the growth of a species in heterospecific soil, we only planted five replicates of each population because the two populations were combined to represent the species. For example, to represent the growth of *Coreopsis* in soil conditioned by *Echinacea*, we planted five replicates each of *Coreopsis* South in soil conditioned by *Echinacea* South, *Coreopsis* South in soil conditioned by *Echinacea* North, *Coreopsis* North in soil conditioned by *Echinacea* South, and *Coreopsis* North in soil conditioned by *Echinacea* North, for a total of 20 plants (see Tables S2, S3 for additional replication details).

We filled pots with a sterile base soil as described for Phase I above (except we sterilized the sand for Phase II) and inoculated them with a 40 ml layer of conditioned soil originating from a single Phase I pot (soil from each Phase I pot was inoculated onto 2-4 Phase II pots). Maintaining independence of replicates in this way is essential because mixing soils from multiple pots produces falsely precise estimates of PSF (Reinhart & Rinella 2016). We then sprinkled a thin layer of twice-autoclaved Turface (calcined clay, Profile Products, IL) on top of the inoculum layer to improve drainage and reduce contamination between pots. To improve germination, we cold-stratified *Echinacea* seeds in wet quartz

sand in a plastic bag for four weeks. We surface sterilized all seeds for two minutes in 5% bleach, followed by three rinses in DI water, and then planted three seeds into each pot, which were later thinned to one seedling. We planted *Coreopsis* as seeds but transplanted *Echinacea* as seedlings at the two-leaf stage because of germination concerns. After 14 weeks above- and belowground biomass were harvested, dried at 65°C for at least seven days, and weighed.

Statistical Analyses

Does genetic diversity alter the strength of interspecific PSF?

To determine whether genetic diversity (two populations vs. one) affects the strength or direction of interspecific PSF, we fit linear mixed models using the lme4 package (Bates *et al.* 2015) in R, version 3.5.2 (R Core Team 2018). Phase II biomass (the sum of aboveground and belowground biomass) was the response variable; and Phase II species (*Echinacea* or *Coreopsis*), Phase I species (*Echinacea* or *Coreopsis*), Phase I genetic diversity (high or low), and all interactions were included as fixed effects. Phase I pot and Phase II greenhouse bench were included as random effects. To control for potential confounding effects of microbial density or nutrient drawdown being greater in soil conditioned by larger plants, Phase I pot biomass (the sum of aboveground biomass for both individuals) was included as a covariate. Interspecific PSF is calculated as the coefficient I_s of a significant interaction between Phase II species and Phase I species ($I_s = G(A)_\alpha - G(A)_\beta - G(B)_\alpha + G(B)_\beta$, where α is soil conditioned by species A and β is soil conditioned by species B, and G represents growth; Bever *et al.* 1997); therefore, a significant Phase II species × Phase I species × Phase I genetic diversity interaction would indicate that interspecific PSF differs between high and low genetic diversity treatments. We assessed statistical significance using Type III ANOVA with Satterthwaite's approximation of denominator degrees of freedom using the package lmerTest (Kuznetsova *et al.* 2015).

We then estimated interspecific PSF at each genetic diversity level as the coefficient for the Phase II species × Phase I species interaction (Bever *et al.* 1997) in two separate models: one for high genetic

diversity and one for low genetic diversity. Phase I pot and Phase II greenhouse bench were again included as random effects, and Phase I pot biomass was again included as a covariate. For the low genetic diversity treatment, our estimation of a species' growth in conspecific soil only included treatments where each population was grown in soil conditioned by its own population (*e.g.,* growth of *Echinacea* North in soil conditioned by *Echinacea* North, but not by *Echinacea* South).

To quantify plant growth responses to con- vs. heterospecific soil, we estimated the ln-response ratio for each species within each level of genetic diversity using identical models to those described above, but on ln-transformed total biomass (Bates et al. 2019). We then calculated the ln-response ratio for each species by subtracting the model estimate in heterospecific soil from the estimate in conspecific soil. Because we included the interaction between main effects in our model, we needed to do an additional adjustment to propagate the standard error estimates, which we did according to Hedges *et al.* (1999).

Does species diversity alter the strength of intraspecific PSF?

To test for the effect of species diversity (two species vs. one) on intraspecific PSF, we fit linear mixed models separately for each species. We built both models as described above, except the fixed effects were Phase II population (North or South), Phase I population (North or South), and Phase I species diversity (high or low). Here, a significant Phase II population × Phase I population × Phase I species diversity interaction would indicate that intraspecific PSF differs between high and low species diversity treatments.

Using methods similar to those above, we estimated intraspecific PSF separately for each level of species diversity as the coefficient I_s for the Phase II population × Phase I population interaction (Bever *et al.* 1997) for each species. For the low species diversity treatment, we only included low genetic diversity treatments to avoid confounding species diversity with genetic diversity (*i.e.*, because all high species diversity treatments included only one population within a species, we only included low species diversity treatments that also included only one population).

To quantify plant growth responses to con- vs. heteropopulation soil, we estimated the ln-response ratio for each population of each species within each level of species diversity using the model fitting method as described above (Bates et al. 2019).

RESULTS

Does genetic diversity alter the strength of interspecific PSF?

Interspecific PSF was significantly more positive (or less negative) in the high genetic diversity treatment (conditioned by two populations) than in the low genetic diversity treatment (conditioned by one population), indicating that genetic diversity may weaken the ability of interspecific PSF to promote species coexistence (Phase II species × Phase I species × Phase I genetic diversity: $F_{1,143} = 5.73$, $P = 0.02$; Fig. 2). Interspecific PSF was negative in the low genetic diversity treatment (Phase II species × Phase I species: $F_{1,90} = 5.40$, $P = 0.02$) but did not significantly differ from zero in the high genetic diversity treatment (Phase II species × Phase I species: $F_{1,84} = 1.00$ $P = 0.33$; Fig. 2A), suggesting that PSF would contribute to the coexistence of these two species only at low levels of genetic diversity. The negative interspecific PSF in low genetic diversity treatments was driven by *Coreopsis* producing substantially more biomass when grown in soil conditioned by *Echinacea* than in soil conditioned by conspecifics (Fig. 2B, D). *Coreopsis* was also more responsive to the genetic diversity treatments, which drove the difference in interspecific PSF between low and high genetic diversity (comparing *Coreopsis* ln-response ratios in Fig. 2B, C). Note that the greater absolute growth of *Coreopsis* relative to *Echinacea* may have contributed to the negative PSF observed in the low genetic diversity treatment (Fig. 2A, D), but it should not influence the effects of genetic diversity on interspecific PSF. There was no relationship between Phase I pot biomass and Phase II biomass (Phase I Pot biomass: $F_1 = 2.1$, $P = 0.15$; Fig. S1), suggesting that these effects cannot be explained by differences in plant biomass (which could affect things like nutrient drawdown) among Phase I treatments.

Does species diversity alter the strength of intraspecific PSF?

For *Coreopsis*, intraspecific PSF tended to be more positive (or less negative) in the high species diversity treatment (conditioned by both *Coreopsis* and *Echinacea*) than in the low species diversity treatment (conditioned by *Coreopsis* alone), indicating that species diversity may weaken the ability of intraspecific PSF to promote genotype coexistence (Phase II population × Phase I population × Phase I species diversity: $F_{1,54} = 3.91$, $P = 0.052$; Fig. 3A-E). This effect was primarily driven by the North population, which tended to produce more biomass in heteropopulation (South) soil when species diversity was low but switched to producing more biomass in conpopulation (North) soil when species diversity was high (Fig. 3D, E). For *Echinacea,* species diversity did not influence intraspecific PSF (Phase II population × Phase I population × Phase I species diversity: $F_{1,54} = 0.43$, $P = 0.52$; Fig. 3F-J).

In our model for *Coreopsis*, we detected a four-way interaction with the Phase I pot biomass covariate (Phase II population × Phase I population × Phase I species diversity × Phase I pot biomass: $F_{1,51} = 5.29$, $P = 0.03$). Including the interaction with the covariate in our models caused three terms to shift from non-significant to significant (Fig. S2). Here we present results from the more conservative model that excludes interactions with the covariate, but we provide results and an interpretation of the model with covariate interactions in the supplement (Fig. S2, S3, Table S4).

Estimated intraspecific PSFs did not differ significantly from zero in any treatment for either species, despite being similar in magnitude to those of interspecific PSF. However, intraspecific PSF for *Coreopsis* was significantly negative when the 4-way interaction with the covariate was included (as described above), suggesting that intraspecific PSF might act to promote the coexistence of these two populations (Fig. S2A).

DISCUSSION

Theory suggests that genetic diversity may influence species coexistence and that species diversity may influence genotype coexistence (Vellend & Geber 2005; Vellend 2006, 2008; Eck *et al.* 2019). Here, we empirically tested whether microbe-mediated plant-soil feedback (PSF) can mediate the

effects of diversity on coexistence. We found that genetic diversity (two populations vs. one) reduced the capacity for interspecific PSF to promote species coexistence, and that species diversity (two species vs. one) reduced the capacity for intraspecific PSF to promote the coexistence of competing *Coreopsis* genotypes. Together, these results suggest that genetic diversity and species diversity may weaken the ability of PSF to promote coexistence.

Potential Mechanism of genetic diversity and species diversity weakening PSF-mediated coexistence

Recent theoretical work has shown that when pathogens are genotype specific, PSF more strongly promotes species coexistence when plant genetic diversity is low (Eck *et al.* 2019). Using a simulation model that included four species and three levels of genetic diversity, Eck and coauthors (2019) found that plant genetic diversity weakened negative interspecific PSF through a pathogen dilution effect. When plant genetic diversity was low, seeds of a focal species were more likely to land in soil previously occupied by their same genotype, resulting in strong negative interspecific PSF that strengthened species coexistence and promoted species diversity. On the other hand, when genetic diversity was high, seeds more often landed in heterogenotypic soil, resulting in less negative interspecific PSF and the dominance of a single plant species. By contrast, if mutualists were mediating these patterns, then low genetic diversity would cause seeds to frequently land in soil containing their genotype-specific mutualists, leading to more *positive* interspecific PSF that would weaken species coexistence and erode species diversity. Thus, while we did not test whether genetic diversity-mediated shifts in interspecific PSF were caused by pathogens vs. mutualists, our results are consistent with a pathogen dilution effect.

The Eck *et al.* (2019) simulation model did not test the effect of species diversity on intraspecific PSF, but we would expect by analogy that the dilution of species-specific pathogens by the presence of other species would have a similar effect as the dilution of genotype-specific pathogens had on interspecific PSF, leading to less negative intraspecific PSF (provided that genotype-specific pathogens are generating intraspecific PSF). Our results were consistent with such a pathogen dilution effect in *Coreopsis*, but not in *Echinacea*.

334

335 *Relative Strengths of Intra- and Interspecific PSF*

336 Although we did not detect any significant intraspecific PSF, the parameter estimates of
337 intraspecific PSF rivaled those of interspecific PSF (the strongest estimate for both was $I_s = -0.13$ while
338 weaker estimates were likewise similar, ranging from $I_s = 0.005$ to 0.06; Figs. 3A, 4A, 4F), indicating that
339 the effects of intraspecific PSF on genotype coexistence may be similar in magnitude to the effects of
340 interspecific PSF on species coexistence. In contrast, a prior study that measured both inter- and
341 intraspecific PSF in four plant species (but that did not investigate how genetic diversity influenced
342 interspecific PSF or how species diversity influenced intraspecific PSF) found that interspecific PSF was
343 five times stronger than the strongest estimate of intraspecific PSF (Bever *et al.* 1996). While the
344 difference in outcome between studies could be due to species-specific differences in interspecific PSF, it
345 may also reflect differences in the degree of trait divergence among studied genotypes (Crawford *et al.*
346 2019). Bever *et al.* (1996) compared genotypes co-occurring within a small field, so these genotypes were
347 likely closely related and therefore also likely similar in their traits. By contrast, our results suggest that
348 divergence in relevant PSF traits between our geographically distant populations rival the divergence of
349 those traits between our two species, leading to intra- and interspecific PSF's similar in strength.

350 Our interspecific PSF results were driven primarily by one of our two species, *Coreopsis*. There
351 are several possible reasons for this asymmetric soil response. First, *Echinacea*-specific pathogens may
352 have been less abundant than *Coreopsis*-specific pathogens in these recently established prairie
353 restorations, resulting in low host-specificity between plants and soil pathogens which often weakens
354 negative interspecific PSF (Cortois *et al.* 2016). Second, transplanting *Echinacea* into conditioned soil as
355 seedlings, rather than as seeds, could have protected *Echinacea* from the negative effects of pathogens
356 given that plants are generally more susceptible to pathogens earlier in development (Develey-Rivière &
357 Galiana 2007). Lastly, species differences could be related to successional dynamics as later successional
358 species like *Echinacea* generally experience weaker negative PSF than early successional species (Kardol

359 *et al.* 2006; Bauer *et al.* 2015) due to being more defended against pathogens and more responsive to
360 mutualists (Reynolds *et al.* 2003; Van der Putten 2003; Koziol & Bever 2015).
361 Similarly, *Coreopsis'* reduction in intraspecific PSF from high species diversity to low species
362 diversity was driven primarily by one population, although neither population exhibited significant
363 growth responses to con- vs. heteropopulation soil (Fig. 3B, C). Intraspecific PSF strength has been
364 shown to differ among populations (Felker-Quinn *et al.* 2011), families (Eck *et al.* 2019), and genotypes
365 (Bever *et al.* 1996; Bukowski & Petermann 2014; Bukowski *et al.* 2018), so it is not surprising that our
366 populations responded differently to soil microbes. While we do not know what caused these differences,
367 intraspecific variation is common in plant traits that are likely to contribute to PSF, including plant
368 defenses (Moore *et al.* 2014) and root exudation (Binns *et al.* 2002; Micallef *et al.* 2009).
369
370 *Caveats*
371 We have shown that genetic diversity can reduce the capacity for PSF to promote species
372 coexistence, and that species diversity can reduce the capacity for PSF to promote genotype coexistence,
373 but we do not know how these patterns generalize to different plant species or populations, especially
374 given that the effects were primarily driven by one species, *Coreopsis,* and one population. Was
375 *Coreopsis'* strong response the exception or the norm? And were our intraspecific PSF results driven by
376 species diversity and the dilution of genotype-specific pathogens, or were they a function of unique
377 properties of *Coreopsis* and *Echinacea*? In this preliminary work we set out to test whether genetic
378 diversity and species diversity could influence PSF-mediated coexistence in principle, and we showed
379 that it is possible, at least with these species. While it is heartening that the Eck and coauthors (2019)
380 model found similar results with four species and three levels of genetic diversity, we hope that future
381 empirical work will further explore the generality of these findings.
382
383 *Implications*

If our findings apply to more diverse communities and to a variety of species, then our result that genetic diversity can influence the strength of interspecific PSF may explain variation in the strength and direction of PSF observed across species (Klironomos 2002; Bezemer *et al.* 2006; Kardol *et al.* 2006; Crawford *et al.* 2019). While there are certainly true differences among species in their PSF, estimates of interspecific PSF may be artificially increased (*i.e.,* measured as more positive) for species that are experimentally represented by a genetically diverse population, while estimates may be artificially decreased (*i.e.,* measured as more negative) for species represented by a less genetically diverse population.

If stronger stabilizing coexistence leads to greater diversity, then our findings that genetic diversity weakened species coexistence and that species diversity weakened genotype coexistence suggest that genetic and species diversity may be negatively linked through PSF. In the scenario we documented, PSF would cause diversity at one level of biological organization to reduce diversity at the other level (Fig. 4A). Theory has predicted negative linkages between genetic and species diversity, as well as positive linkages such that diversity begets diversity (Fig. 4B), again with a primary focus on competition as the mediator (Vellend & Geber 2005; Vellend 2006). While this body of theory often assumes that stronger species or genotype coexistence result in greater species or genetic diversity, respectively, this assumption may not always be met because diversity is determined by additional mechanisms beyond just coexistence. However, in cases where coexistence does result in greater diversity, and where PSF is a major driver of coexistence, the dynamics we observed point to two contrasting outcomes: a plant community with few species but high genetic diversity within those species, or a community with many species but low genetic diversity within species. While community diversity is certain to depend on other ecological and evolutionary processes, our results suggest that PSF may dampen the negative effects of diversity loss by promoting diversity at other levels of biological organization.

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Data availability

Should the manuscript be accepted, the data will be archived in Dryad and the data DOI will be included at the end of the article.

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Figure 1. Experimental design. Small colored circles represent the soil microbial communities conditioned by different plant pairs in Phase I, which were inoculated onto a second generation of plants in Phase II. We planted 30 replicates of each of seven treatments (top row, left to right): A–D = low species diversity & low genetic diversity (*Echinacea* South, *Echinacea* North, *Coreopsis* South, *Coreopsis* North); E–F = low species diversity & high genetic diversity (*Echinacea* South + North, *Coreopsis* South + North); and G = high species diversity (all possible pairwise combinations of *Echinacea* and *Coreopsis* populations). The number next to each pot represents the final number of replicates for each soil inoculum, which were reduced in the low species diversity *Echinacea* treatments because of low germination.

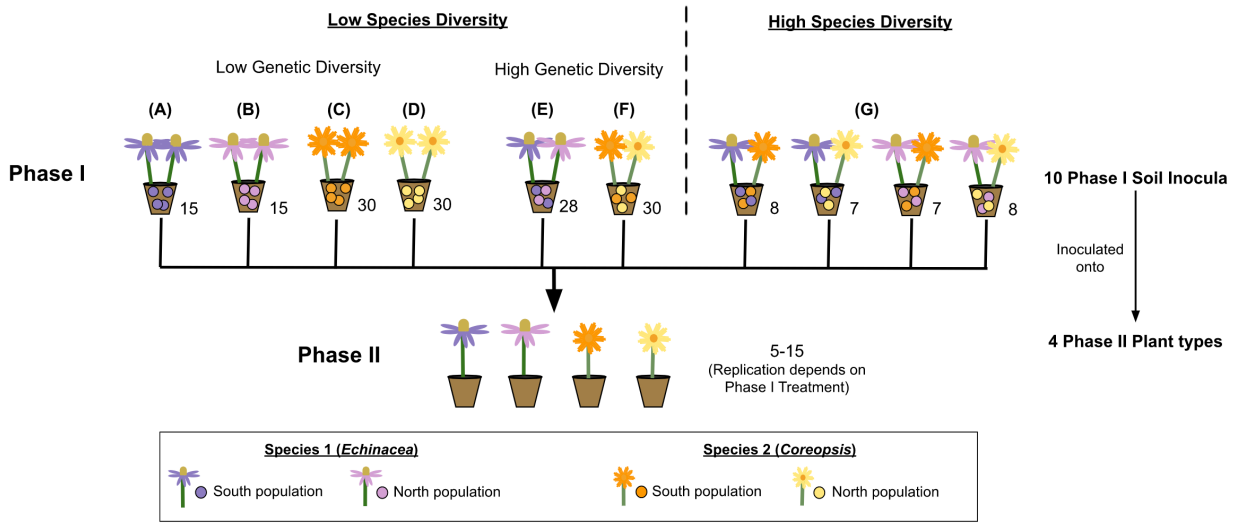

Figure 2. Interspecific microbe-mediated plant-soil feedback (PSF) between *Echinacea* and *Coreopsis* grown in soil conditioned by pairs of plants representing low (left column) or high (right column) genetic diversity. The top panel shows the net pairwise interspecific PSF (I_s) between *Echinacea* and *Coreopsis* (A). The middle row shows the ln-response ratio of biomass produced in conspecific soil compared to biomass produced in heterospecific soil [(B), (C)], and the bottom row shows the biomass produced by each species when grown in soil conditioned by each species [(D), (E)]. The interaction coefficient of the biomass plots in (D) and (E) are depicted as the interspecific PSF for each level of genetic diversity in (A) (I_s, Bever *et al.* 1997). Error bars are fitted SE. Asterisk above bar in (A) indicates significant difference between groups; asterisk above treatment indicates that (A) PSF or (B) ln-response ratio differs significantly from zero; * P < 0 .05, *** P < 0.001.

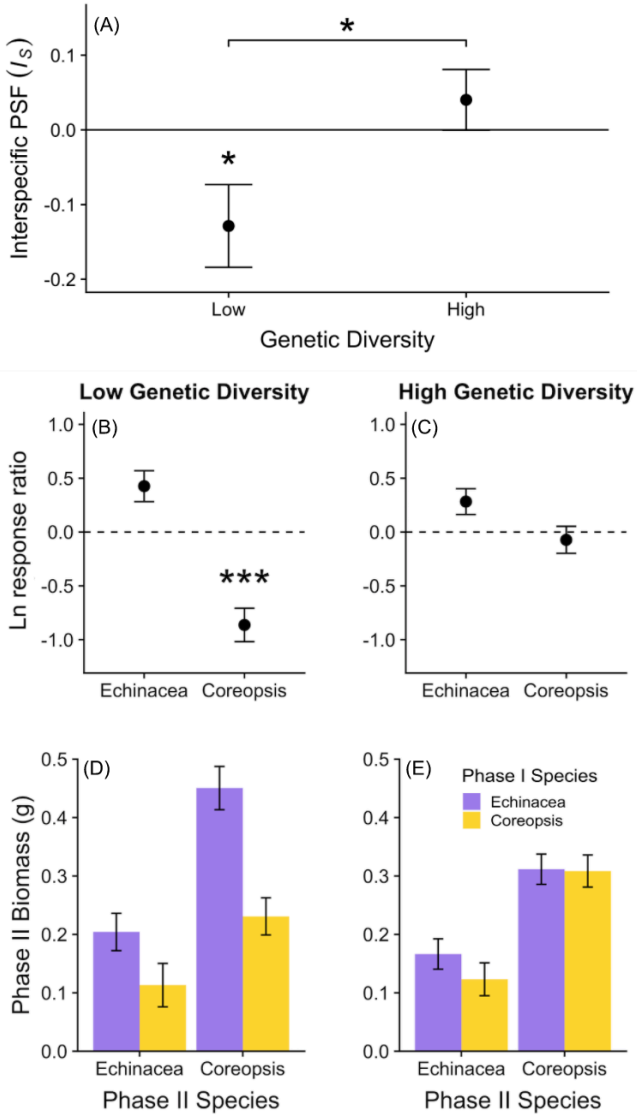

Figure 3. Intraspecific microbe-mediated plant-soil feedback (PSF) between South and North populations of *Coreopsis* (left) and *Echinacea* (right) grown in soil conditioned by pairs of plants representing low (left column under each species) or high (right column under each species) species diversity. The top row shows the net pairwise intraspecific PSF (I_s) between South and North populations [(A), (F)]. The middle row shows the ln-response ratio of biomass produced in conpopulation soil compared to biomass produced in heteropopulation soil [(B), (C), (G), (H)], and the bottom row shows the biomass produced by each population when grown in soil conditioned by each population [(D), (E), (I), (J)]. The interaction coefficient of the biomass plots in (D) and (E) are depicted as the intraspecific PSF for each level of genetic diversity in (A) and (F) (I_s, Bever *et al.* 1997). Error bars are fitted SE.

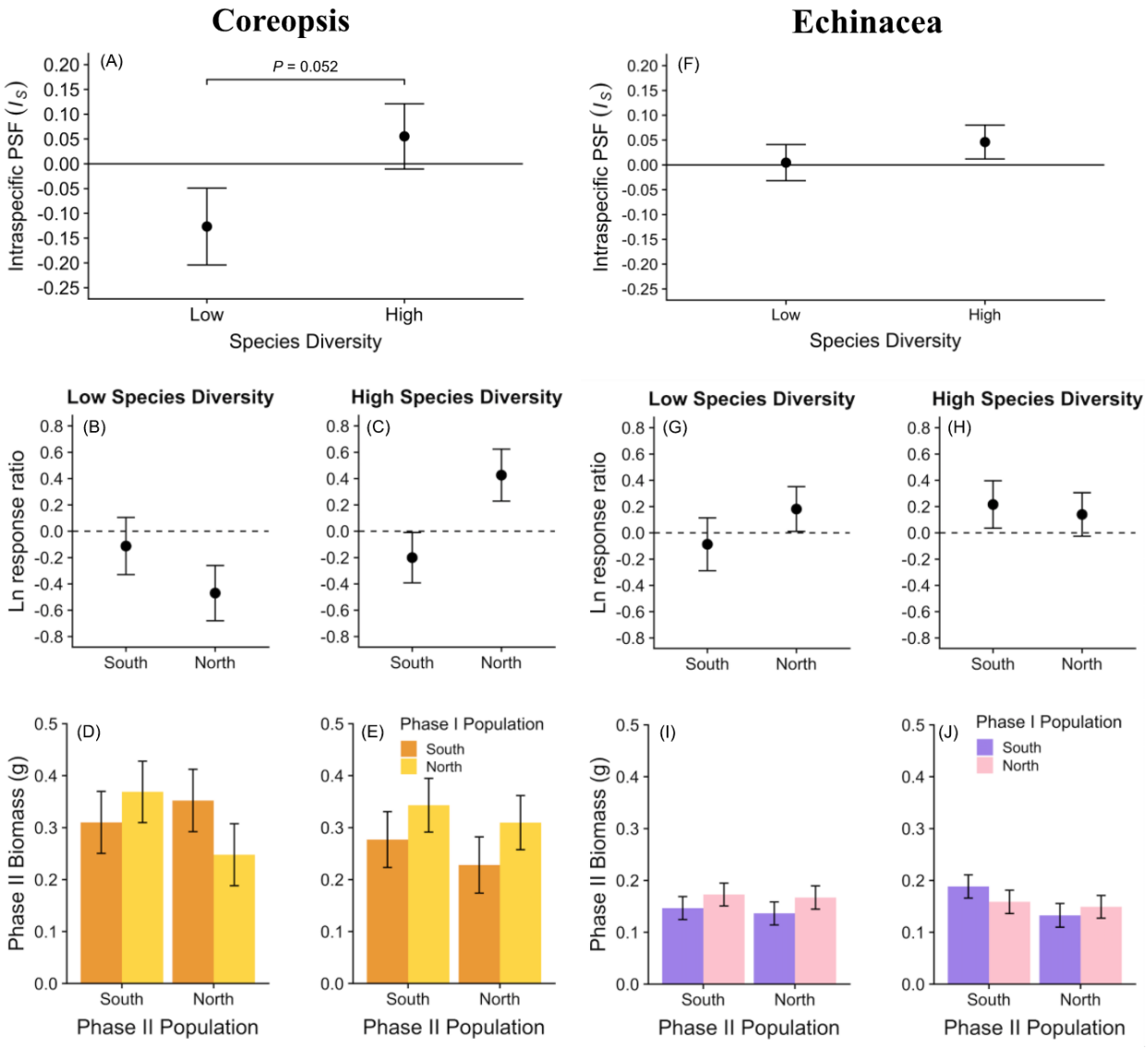

Figure 4. Conceptual diagram showing how the effects of genetic and species diversity on coexistence have the potential to lead to **(A)** negative and **(B)** positive linkages between genetic diversity and species diversity that are mediated by plant-soil feedback (PSF). **(A)** If genetic diversity causes interspecific PSF to become more positive (or less negative) (A1), and species diversity causes intraspecific PSF to become more positive (or less negative) (A4), then genetic diversity and species diversity will reduce the potential for PSF-mediated coexistence of competing species and genotypes, respectively. If weaker coexistence leads to a loss of diversity, this could then cause genetic and species diversity to negatively influence each other such that diversity at one level of biological organization reduces diversity at the other level. **(B)** Reciprocally, if genetic diversity causes interspecific PSF to become more negative (or less positive) (B1), and species diversity causes intraspecific PSF to become more negative (or less positive) (B4), then genetic diversity and species diversity may positively influence each other such that diversity begets diversity. The resulting effect of each level of diversity on the other is summarized by the vertical dotted lines. Note that here we assume that more positive inter- and intraspecific PSF always inhibit species coexistence and genotype coexistence, respectively (A2, A5, B2, B5), and coexistence always promotes the maintenance of species and genetic diversity, respectively (A3, A6, B3, B6).

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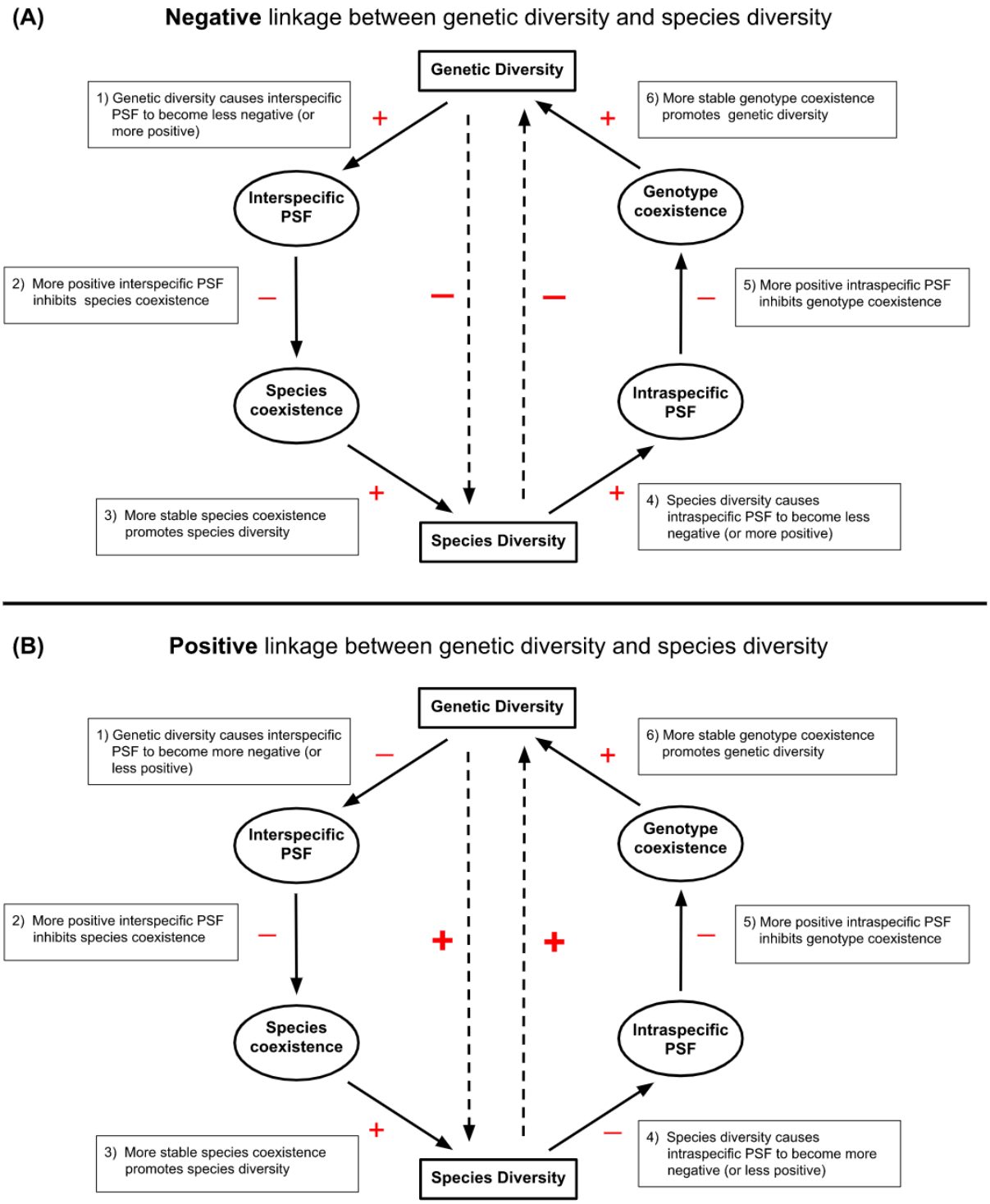
(A) Negative linkage between genetic diversity and species diversity
Genetic Diversity
1) Genetic diversity causes interspecific PSF to become less negative (or more positive)
6) More stable genotype coexistence promotes genetic diversity
Interspecific PSF
Genotype coexistence
2) More positive interspecific PSF inhibits species coexistence
5) More positive intraspecific PSF inhibits genotype coexistence
Species coexistence
Intraspecific PSF
3) More stable species coexistence promotes species diversity
4) Species diversity causes intraspecific PSF to become less negative (or more positive)
Species Diversity
(B) Positive linkage between genetic diversity and species diversity
Genetic Diversity
1) Genetic diversity causes interspecific PSF to become more negative (or less positive)
6) More stable genotype coexistence promotes genetic diversity
Interspecific PSF
Genotype coexistence
2) More positive interspecific PSF inhibits species coexistence
5) More positive intraspecific PSF inhibits genotype coexistence
Species coexistence
Intraspecific PSF
3) More stable species coexistence promotes species diversity
4) Species diversity causes intraspecific PSF to become more negative (or less positive)
Species Diversity

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