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Plant–microbial interactions facilitate grassland species coexistence at the community level

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Abstract

Interspecific competition and plant–soil feedbacks are powerful drivers of plant community structure. However, across a range of edaphic conditions the interactive effects of these drivers on complex plant communities remain unclear. For example, plant–soil feedback studies focus on soil trained by a single plant species. We developed a method to assess effects of plant–microbial interactions (PMI) on a complex plant community. We established mesocosms with 13 grassland species, grown individually or together, in overgrazed or restored soil, with or without soil microbial inoculum collected from a productive and diverse native grassland. We assessed biomass production as influenced by edaphic conditions, interspecific competition and PMI. Furthermore, we assessed potential influences of interspecific competition and edaphic conditions on strength and direction of PMI. Our results indicate PMI drives negative growth responses for graminoids while forbs experience positive growth responses. Generally, interspecific competition did not alter the magnitude or direction of PMI-mediated growth responses. Edaphic conditions altered the influence of soil microbial communities on individual plant growth while PMI facilitated plant evenness. In plant community mesocosms, PMI-associated benefits were observed in overgrazed soil. However, interspecific competition overwhelmed plant growth benefits associated with soil microbial communities when plant communities were grown in restored soil. In mesocosms containing dominant grass species, interspecific competition had negative effects on species coexistence, but both positive and negative PMI partially counterbalanced this influence on plant species evenness. Understanding these

> mechanisms may improve our capacity to manage diverse and productive grasslands by enabling prediction of plant community composition following disturbance and subsequent restoration.

Introduction

Highly diverse plant communities are typically more stable and productive than less diverse communities (Schnitzer et al. 2011); therefore, it is critical that we understand mechanisms promoting diversity in plant communities. Alterations in soil biotic and abiotic properties driven by soil microorganisms, especially mutualists and pathogens can influence individual plant growth and species coexistence with subsequent reciprocal interactions (Reynolds et al. 2003, Kardol et al. 2006, Van Nuland et al. 2016, Vincenot et al. 2017, Mariotte et al. 2018). This phenomenon is termed plant–soil-feedback (PSF) (Bever et al. 1997, van der Putten et al. 2016). While previous studies carefully described plant–soil feedbacks between individual plant species, there is a gap in our understanding of how interactive effects and interspecific competition involving multiple plant species shape complex plant communities. Therefore, we are introducing a framework to experimentally determine plant–microbial interactions (PMI) within a multi-species plant community. Uncovering PMI within complex systems has potential to improve management of highly diverse, stable and productive grasslands.

Arbuscular mycorrhizal (AM) fungi are ubiquitous and abundant plant mutualists that can protect host–plants from pathogens while improving nutrient and water uptake (Newsham et al. 1995, Yang et al. 2014). Mycorrhizas may preferentially benefit specific host–species, resulting in positive feedbacks that can drive loss of species diversity within a plant community (Hartnett and Wilson 1999, van der Putten et al. 2013). Conversely, AM fungi can also facilitate species coexistence by promoting slow-growing species (Lin et al. 2015, Jiang et al. 2017). For example, non-N_2-fixing forbs respond more positively to mycorrhizal inoculation than C_3 grasses (Hoeksema et al. 2010). In addition, plants are under constant threat from accumulation of species-specific, soil-borne pathogens, potentially decreasing plant production while benefiting subdominant plant species (Bever 2003, van der Putten et al. 2013). These negative feedbacks are powerful drivers in numerous ecological processes, such as plant species invasion (Day et al. 2015), community succession (Kardol et al. 2006), diversity–productivity patterns (Schnitzer et al. 2011) and plant species coexistence (Bever et al. 2015). For plant communities, a meta-analysis suggested plant species with strong competitive abilities are subject to negative feedbacks, i.e. benefit less from mutualists or are more susceptible to pathogens (Lekberg et al. 2018). Therefore, dominant species, graminoids in our research site, may receive more negative effects from plant–soil feedbacks, compared to other functional groups.

Edaphic conditions, especially resource availability may influence plant performance through indirect effects on soil microorganisms, potentially altering the magnitude and direction of PMI. For example, ratios of saprotrophic, symbiotic and parasitic fungi are strongly influenced by the availability of soil nutrients (Cline et al. 2018). Mutualistic plant–mycorrhizal relationships can shift to be less beneficial or even parasitic when soil nutrients are sufficient for plant growth (Jiang et al. 2017), and plant dependency on soil microorganisms is enhanced in low nutrient soil.

Interspecific plant species competition also regulates plant community structure. Competition among plants for limited resources constrains species coexistence (Hortal et al. 2017), and species competing for the same niche are more easily excluded from the community (Harpole and Suding 2011, Harpole et al. 2016). Interspecific competition and microbial interactions simultaneously influence plant community structure in grasslands that are typically highly diverse. Positive PSF can be overwhelmed, and negative PSF can be increased by interspecific competition (Kardol et al. 2007, Shannon et al. 2012, Hol et al. 2013, Crawford and Knight 2017, Stanescu and Maherali 2017). However, previous studies focus on pairwise plant species relationships or exotic–native competition or community succession, but grasslands are typically highly diverse and comparatively stable in a short time. Therefore, previous research could not tease apart the relative importance of plant–microbial interactions from competition of diverse neighbors in a stable plant community. Our experimental design addresses this limitation by examining the impact of PMI when plants are grown in isolation and when plants are grown in the presence of multiple competitors. In our study, we examine the relative importance of interspecific competition compared with the influence of soil microorganisms at the plant community level. Thirteen plant species were grown individually or together to determine interspecific competitive effects on plant production. Soils were amended with either sterile or live inoculum to evaluate PMI. All plants were grown in soils from either long-term overgrazed grassland areas (relatively low nutrient) or restored grassland areas (previously overgrazed; relatively higher nutrient availability) to assess the influences of soil resource availability on PMI, interspecific competition and potential interactive relationships. We hypothesized that 1) interspecific competition and lower soil resource availability (i.e. overgrazed soil) would result in more beneficial microbial interactions, and 2) effects of soil microbes on plant community structure will be strengthened in resource-limited soil.

Material and methods

Study system

Experiments were conducted in a greenhouse. The thirteen selected plant species (four graminoids, nine forbs) are common in grasslands of northern China (Table 1). We selected a ratio of four graminoid: nine forb species to simulate the relative abundance of graminoid: forb in our grassland. In our grassland, graminoid species are perennially dominant. *Leymus chinensis* is the dominant species, with biomass accounting for > 40% of the entire grassland plant community. Forbs contribute little biomass but contribute substantially to overall diversity. Compositae is the most common family in our grasslands. All soil used in our experiment was collected from The National Field Station of Grassland Ecosystem (Guyuan, Hebei province, China, 41°46′N, 115°40′E) in July 2017. We selected this native soil as the inoculum because the long-term soil conditioning phase (> 10 years) occurred naturally in the field in association with a typical native grassland plant community, allowing immediate use of this soil in our greenhouse PMI experiment. Inoculum soil was collected from the top 20 cm within a productive and highly diverse 50 × 50 m^2 plant community plot, stored at −20°C, sieved (2 mm) and homogenized. For substrate soil, we selected two sites in the grassland area with differing recent management histories, one subjected to overgrazing (> 30 years), the other restored after a history of overgrazing (protective fencing, > 10 years). Soil from the top 20 cm was excavated, sieved (2 mm), homogenized, air dried and then sterilized in an autoclave (120 min, 121°C, 103 kPa). Abiotic properties varied between overgrazed and restored soils, and sterilization did not generally change the relative NO_3^--N, plant-available P, total N and total C (Table 2).

Table Table 1.. Family names, species name, abbreviation (used in Fig. 1, 2, 4), category (plant functional group), type and seedbed substrate used for germination of 13 common grassland species

Species name	Family	Abbreviation	Growth form	Type	Seedbed substrate
Leymus chinensis	Gramineae	*L. chin*	graminoids	perennial	soil
Elymus dahuricus	Gramineae	*E. dahu*	graminoids	perennial	soil
Stipa capillata	Gramineae	*S. capi*	graminoids	perennial	soil
Agropyron cristatum	Gramineae	*A. cris*	graminoids	perennial	soil
Thermopsis lanceolata	Leguminosae	*T. lanc*	forbs	perennial	soil
Taraxacum mongolicum	Compositae	*T. mong*	forbs	perennial	filter paper
Heteropappus	Compositae	*H. hisp*	forbs	annual or	filter paper

Species name	Family	Abbreviation	Growth form	Type	Seedbed substrate
Lepidium apetalum	Cruciferae	*L. apet*	forbs	annual or biennial	filter paper
Allium mongolicum	Liliaceae	*A. mong*	forbs	perennial	filter paper

Table Table 2.. Soil properties from two sites (overgrazed or restored) prior to sterilization and following sterilization. Significant differences ($p < 0.05$) between sterile and live soil, within each parameter, are indicated by an asterisk. Lowercase letters indicate significant difference ($p < 0.05$) between overgrazed and restored soil

Soil property	Live soil		Sterile soil	
	Overgrazed	Restored	Overgrazed	Restored
pH	8.25 ± 0.03^{b}	8.71 ± 0.03^{a}	$8.4 \pm 0.01^{b*}$	8.75 ± 0.03^{a}
EC[1]	155.97 ± 1.29^{b}	318.17 ± 1.56^{a}	172.83 ± 12.03^{b}	$404.7 \pm 4.65^{a*}$
NO_3^--N (mg kg^{-1})	13.97 ± 9.14	22.92 ± 5.89	13.81 ± 5.37	18.89 ± 2.63
NH_4^+-N (mg kg^{-1})	11.40 ± 0.82	11.64 ± 0.20	41.97 ± 5.81*	30.56 ± 0.54*
Plant-available P (mg kg^{-1})	4.12 ± 0.69	4.00 ± 0.57	3.81 ± 0.35	4.06 ± 0.54
Total N (g kg^{-1})	2.1 ± 0.00^{b}	2.8 ± 0.19^{a}	2.0 ± 0.10^{b}	2.8 ± 0.24^{a}
Total P (g kg^{-1})	0.27 ± 0.01^{b}	0.45 ± 0.00^{a}	$0.34 \pm 0.01^{b*}$	0.46 ± 0.01^{a}
Total C (g kg^{-1})	17.5 ± 0.4^{b}	29.6 ± 2.8^{a}	17.4 ± 1.10^{b}	26.7 ± 1.70^{a}

[a] [1] EC: electrical conductivity of soil.

Plant–microbial interactions

Plant germination

All seeds were collected at the field station in autumn of 2016. Seed were surface-sterilized first with 75% ethanol and then a 10% ‘84 disinfector’ (dominant sector: NaClO, available chlorine

5.5–7%) and placed onto wet filter paper, followed by demineralized watering. Seeds were placed in lighted growth chambers (16/8 light/dark photo regime, 26/20°C). Filter paper was substituted with 2 cm of sterilized soil for species that do not grow well on paper (Table 1). Materials used for plant germination were autoclaved for 30 min at 121°C and 103 kPa. As not all species germinated simultaneously, seedlings with 2 cm roots were placed in a lighted growth chamber (16/8 light/dark photo regime, 4°C) until transplanting (Kardol et al. 2006).

Individual plant experiment

To quantify the direction and magnitude of PMI on 13 species, one seedling from each species was transplanted into a pot (6.5 cm bottom diameter; 10 cm top diameter; 12 cm deep) that received 500 ml of a mixture of a sterilized substrate and living or sterilized inoculum (6:1 V:V). The substrate consisted of sterilized field soil and sterilized fine vermiculite (2:1 V:V) to ensure drainage. Treatments were carried out in a full factorial design with six replicates, resulting in an experimental design of 2 soil substrates (overgrazed grassland or restored grassland) × 2 soil microbial conditions (sterilized or living) × 13 plant species × 6 replicates = 312 pots. All pots were placed in the greenhouse (16-h light/8-h dark photoperiod) at approximately 20–26°C, watered with 100 ml demineralized water every five days, and randomly rearranged every week. Dead seedlings were replaced in the first week.

Plant community experiment

Mesocosms containing one seedling of each of 13 species were established to test effects of PMI on plants at the community level. Soil treatments were the same as the individual plant experiment (two substrates × two microbial conditions), and each combination had six replicates, for a total of 24 boxes (mesocosms). Rectangular boxes (34 cm length; 23 cm width; 12 cm deep) were used as mesocosm containers, and each of them received 6500 ml of mixture soil substrate, 13 times the volume of the individual pots to ensure an equivalent volume of soil per plant in each experiment to allow comparisons between these datasets. This feature of our experimental design is based on previous experiments on interspecific competition, as described by Crawford and Knight (2017) and Lekberg et al. (2018). To maximize neighbourhood and marginal effects, seedlings were randomly arranged in the centre of the mesocosm, 4 cm away from the edge. All boxes were placed in the greenhouse (16-h light/8-h dark photoperiod) at approximately 20–26°C, watered with 1300 ml demineralized water every five days, and arranged randomly with pots from the individual plant experiment. Dead seedlings were replaced in the first week.

Plant measurements

In our native grasslands, the growing season is approximately four months (June–September). Furthermore, most of the plant species in our experiment are perennial or biennial (Table 1), with slow early growth. Therefore, plants were grown for four months after transplant. All aboveground plant biomass was harvested, and shoot dry weights were measured separately for each species after drying for 48 h at 65°C. Plants that did not survive to harvest were excluded from analyses. Forbs produced relatively little biomass as they are perennial and their first-year growth are typically low. However, these plants are representative of similar aged plants in native grassland field conditions.

Data analysis

To test PMI in relation to species and soil substrates, shoot biomass was analysed using a three-way ANOVA with plant species, soil inoculum and soil substrate as fixed factors. Soil microbial effects were quantitatively represented as PMI ($\mathrm{PMI} = \ln_{\text{plant biomass in soil with live inoculum}} - \ln_{\text{plant biomass in soil with sterilized inoculum}}$). Each plant treatment has six replicates. The PMI of each replicate was calculated separately. All replicates were numbered 1–6 randomly at the beginning of the experiment and we calculated the PMI for each pair with the same number. The PMI for each species was determined by averaging the six pairwise PMIs (Mangan et al. 2010, Smith and Reynolds 2015). An index value that is greater than zero indicates a positive PMI, while a negative value indicates a negative PMI (Lepinay et al. 2018). Plant shoot biomass from living and sterilized soil was analyzed by t-test to determine the significant effects of soil microorganisms. Linear regression model analyses were performed to test the relationship of PMI measured in the individual experiment ($\mathrm{PMI}_{\text{individual}}$) with the PMI in the community experiment ($\mathrm{PMI}_{\text{community}}$). Because some plants died in the community experiment (Table 3), the $\mathrm{PMI}_{\text{individual}}$ values that had no corresponding $\mathrm{PMI}_{\text{community}}$ were not accounted for in our analyses. The effect of competition on plant growth was analyzed by a t-test with shoot biomass measured in the individual plant experiment, using the plant community experiment as reference data. For interspecific plant competition, plant biomass from individual or community experiments were used as categorical variables, and all plant biomass comparisons were within the same plant species. A t-test was used to analyze the effects of interspecific competition on PMIs. Interspecific competitive effects sizes (ln competition) were evaluated by $\ln_{\text{plant biomass in community experiment}} - \ln_{\text{plant biomass individual experiment}}$ and the correlation between competition and $\mathrm{PMI}_{\text{community}}$ were analyzed by linear regression. Evenness index of the community experiment was based on proportional shoot biomass (Wubs and Bezemer 2018). We performed three-way ANOVAs with competition, species and soil microbial condition, as fixed factors in overgrazed or restored soils to evaluate changes in plant shoot biomass. Statistical analyses were performed using the statistical software R ver. 3.3.1 ‘multcomp’, ‘car’ and ‘MASS’ packages (<www.r-project.org>, Kabacoff 2015).

Table Table 3.. Mortality rate of species grown with or without living soil inoculum on overgrazed soil or restored soil in community experiment

Species	Sterile soil		Live soil	
	Overgrazed	Restored	Overgrazed	Restored
Leymus chinensis.	0%	0%	0%	0%
Elymus dahuricus	0%	0%	0%	0%
Stipa capillata	0%	0%	0%	0%
Agropyron cristatum	0%	0%	0%	0%
Thermopsis lanceolata	83%	33%	17%	0%
Taraxacum mongolicum	33%	17%	0%	0%
Heteropappus hispidus	33%	50%	17%	0%
Saussurea japonica	0%	0%	17%	0%
Lepidium apetalum	17%	0%	0%	0%
Allium mongolicum	0%	0%	0%	0%
Sanguisorba officinalis	67%	67%	0%	33%
Erodium stephanianum	0%	17%	0%	17%
Plantago asiatica	0%	0%	0%	0%

Results

Individual experiment

In our individual experiment, all plants survived. Plant shoot biomass was strongly affected by species, soil substrates, soil microorganisms and their interactions except for microorganisms × substrates (Table 4). Plant growth was significantly influenced by soil microorganisms, as demonstrated by our calculation of PMI. The effects of PMI varied significantly among plant species ($p < 0.001$) (Fig. 1a–b). *Elymius dahuricus* and *Agropyron cristatum* grown in restored soil and *Lepidium apetalum* in overgrazed or restored soil tended to have reduced growth when microbial inoculum was included (negative PMI). Biomass of *E. dahuricus* and *A. cristatum* grown in restored soil decreased 33% and 38%, respectively, in live soil. *L. apetalum* biomass decreased 65% in overgrazed and 47% in restored soil inoculated with microorganisms. Forbs, except for *L. apetalum*, were facilitated by soil microorganisms (positive PMI). In contrast to our hypotheses, soil substrates generally did not alter the magnitude or direction of PMI (p = 0.961) (Fig. 1c).

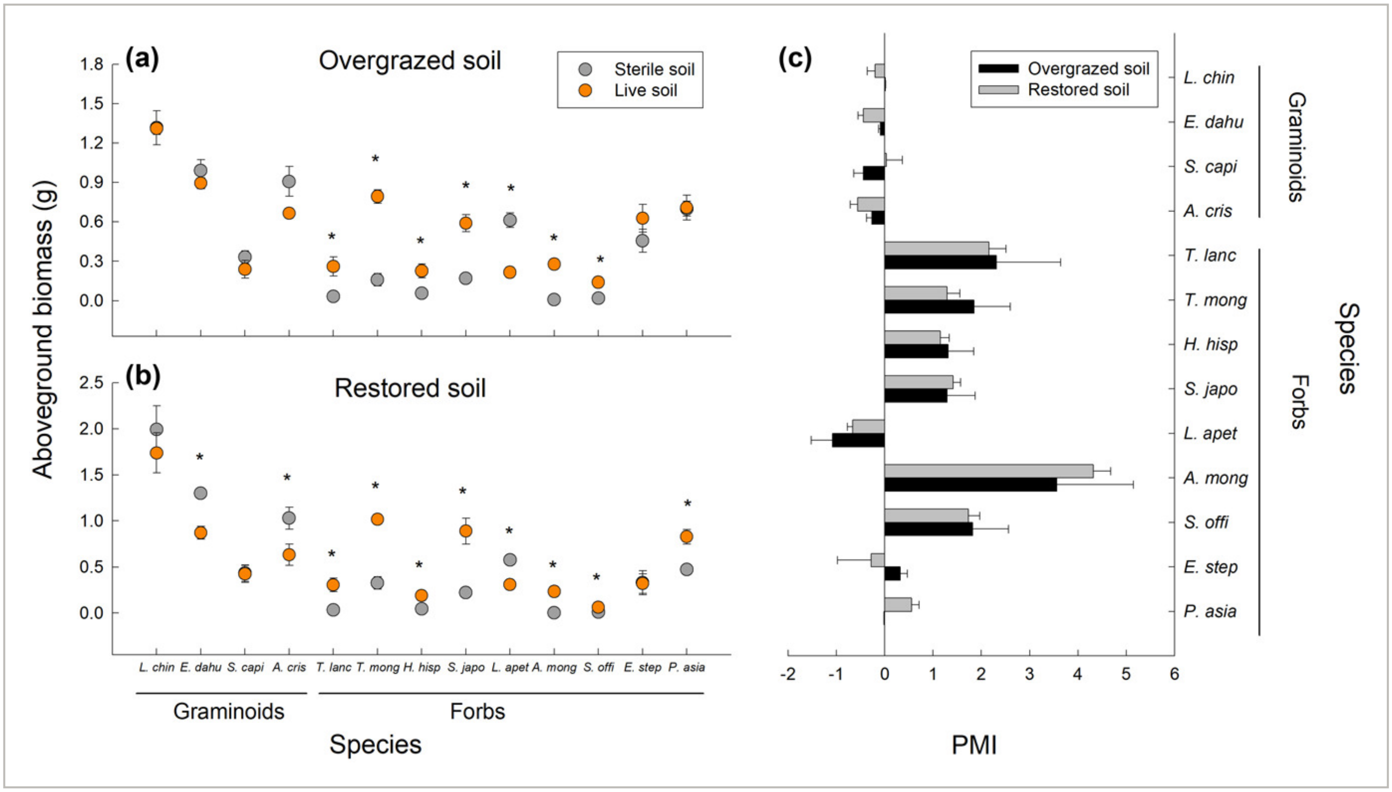

Figure 1

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Aboveground biomass (a–b) and plant–microbial interactions (PMI) (c) for 13 plant species (graminoids and forbs) grown in overgrazed (a) or restored soil (b) in our individual plant experiment. Species abbreviations are described in Table 1. Positive or negative PMI are determined by subtracting sterile soil plant biomass (ln transformed) from live (inoculated) soil plant biomass (ln transformed). Significant biomass differences between sterile soil and live soil within each species (a–b) are indicated by an asterisk ($p < 0.05$).

Table Table 4.. Three-way analysis of variance (ANOVA) for the effects of species identity, soil microorganisms, soil substrates and their interactions on plant biomass in individual experiment. Statistically significant sources of variation are in bold

Source of variation	Type III sums of squares	df	Mean square	F	p
Species	48.224	12	4.019	111.452	**<0.001**
Soil substrates (SS)	0.362	1	0.362	10.032	**0.002**

Source of variation	Type III sums of squares	df	Mean square	F	p
Soil microorganisms (SM)	0.5	1	0.5	13.854	**<0.001**
Species × SS	2.17	12	0.181	5.016	**<0.001**
Species × SM	6.453	12	0.538	14.914	**<0.001**
SS × SM	0.002	1	0.002	0.055	0.815
Species × SS × SM	0.616	12	0.051	1.424	0.156
Error	8.329	231	0.036		

Community experiment

Species mortality

In our community experiment, not all species had 100% survival (Table 3), presumably due to competitive exclusion. Five forbs had 17–83% mortality when grown in substrate soil with sterilized soil inoculum. Inoculation with live soil microorganisms partly eliminated the negative effect of community competition on species survival, with plant mortality decreasing to between 0 and 33% for all species (Table 3).

Plant–microbial interactions

The effects of PMI were observed in our community experiment (Fig. 2). *Leymus chinensis* and *E. dahuricus* were strongly negatively affected by PMI, with 42–70% reduced biomass compared to growth in sterile field soil. Aboveground biomass of six forbs grown in overgrazed soil and four forbs grown in restored soil increased significantly in substrate soil inoculated with microbes (Fig. 2a–b). Overall, soil substrates (overgrazed or restored) did not change the direction and magnitude of PMI (p = 0.345) (Fig. 2c).

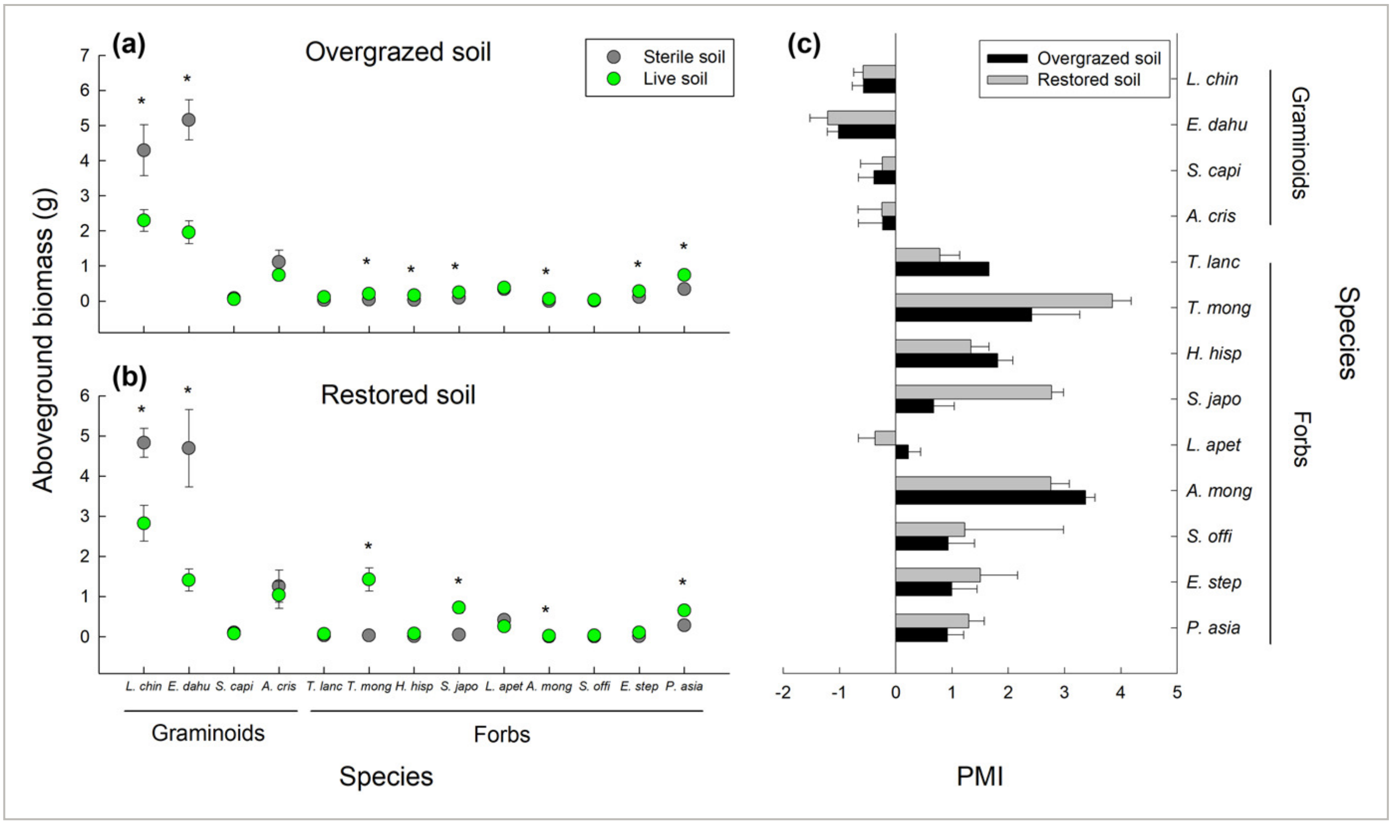

Figure 2

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Aboveground biomass (a–b) and plant–microbial interactions (PMI) (c) for 13 plant species (graminoids and forbs) grown in overgrazed (a) or restored soil (b) in our plant community experiment. Species abbreviations are described in Table 1. Positive or negative PMI are determined by subtracting sterile soil plant biomass (ln transformed) from live (inoculated) soil plant biomass (ln transformed). Significant biomass differences between sterile soil and live soil within each species (a–b) are indicated by an asterisk ($p < 0.05$).

Comparing the PMI of plants grown individually with growth in competition, interspecific competition generally had no significant effects on PMI ($p = 0.907$ in overgrazed soil; $p = 0.172$ in restored soil). Specifically, interspecific competition significantly affected the magnitude of PMI of several plant species when grown in low nutrient soil (i.e. overgrazed). Notably, direction and strength of PMI in *L. apetalum* differed between the individual and community experiment (Fig. 3a). In high-nutrient soil (i.e. restored), most forbs experienced a positive PMI, but the strength of the positive PMIs differed between the individual and community experiments (Fig. 3b). We further explored relationships between microbial effects experienced by plant species grown individually ($PMI_{individual}$) or together ($PMI_{community}$). Results indicate that $PMI_{community}$ was significantly associated with $PMI_{individual}$ regardless of overgrazed (Fig. 4a) or restored soil (Fig. 4b). A tighter and more regular linear relationship was apparent for species grown in overgrazed (low nutrient) soil (Fig. 4).

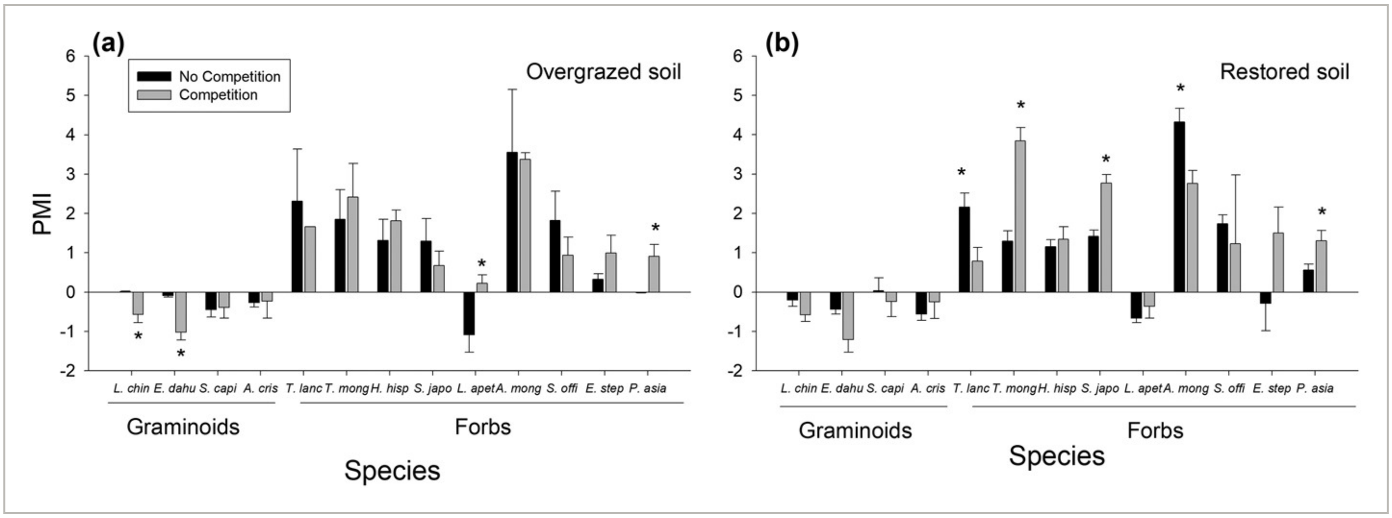

Figure 3

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Plant–microbial interactions (PMI) for 13 plant species (graminoids and forbs) grown individually (No competition) or within complex communities (Competition) in overgrazed soil (a) or restored soil (b). Significant PMI differences between No competition and Competition within each species are indicated by an asterisk ($p < 0.05$).

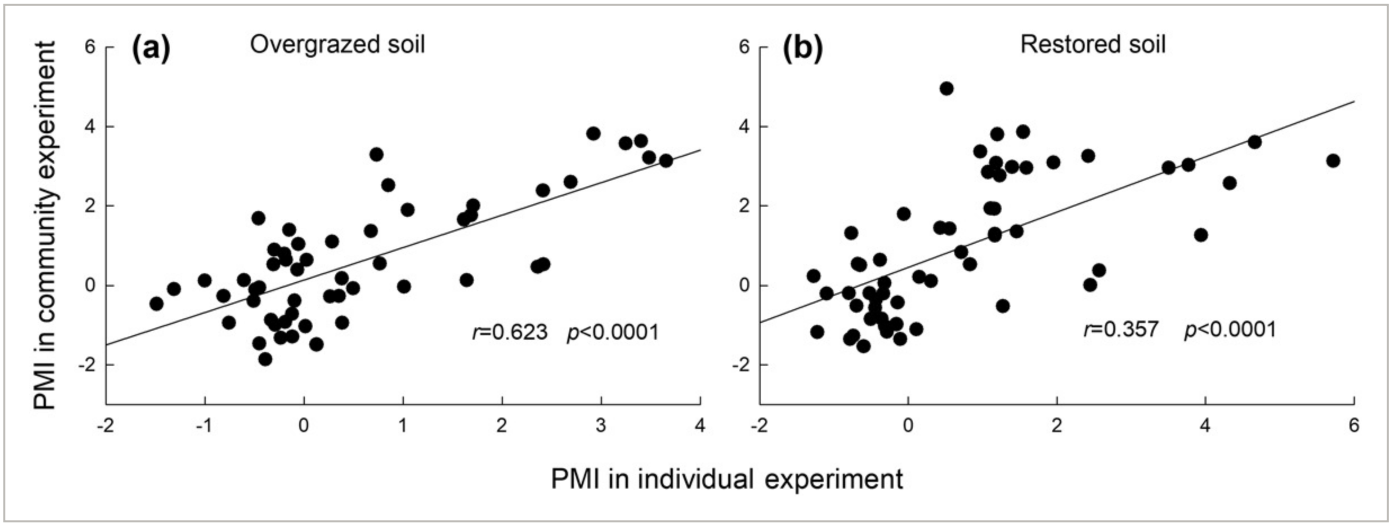

Figure 4

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Relationship between plant–microbial interactions (PMI) of species grown individually or within complex plant communities in overgrazed soil (a) or restored soil (b). Linear regression model analyses were utilized.

Interspecific competition

Aboveground biomass of several plant species was significantly influenced by interspecific competition in both soil nutrient levels (Fig. 5). Biomass of *L. chinensis* and *E. dahuricus* increased by 226% and 421%, respectively, in community mesocosms compared to individual production in overgrazed sterile soil. A reduced, but still substantial growth response of these plant species occurred in restored sterilized soil (*L. chinensis*: 142% and *E. dahuricus*: 260%). Overall, aboveground biomass was greater in the community experiment than in the individual experiment, and these differences were overall smaller in live than in sterile soil (Fig. 5). Growth of *Stipa capillata* and several forbs were significantly reduced in complex plant communities. Soil microbial effects were inversely related to the competitive strength of plant species in overgrazed but not restored soil (Fig. 6). Dominant species within the plant community were experience negative PMI in overgrazed soil (Fig. 6).

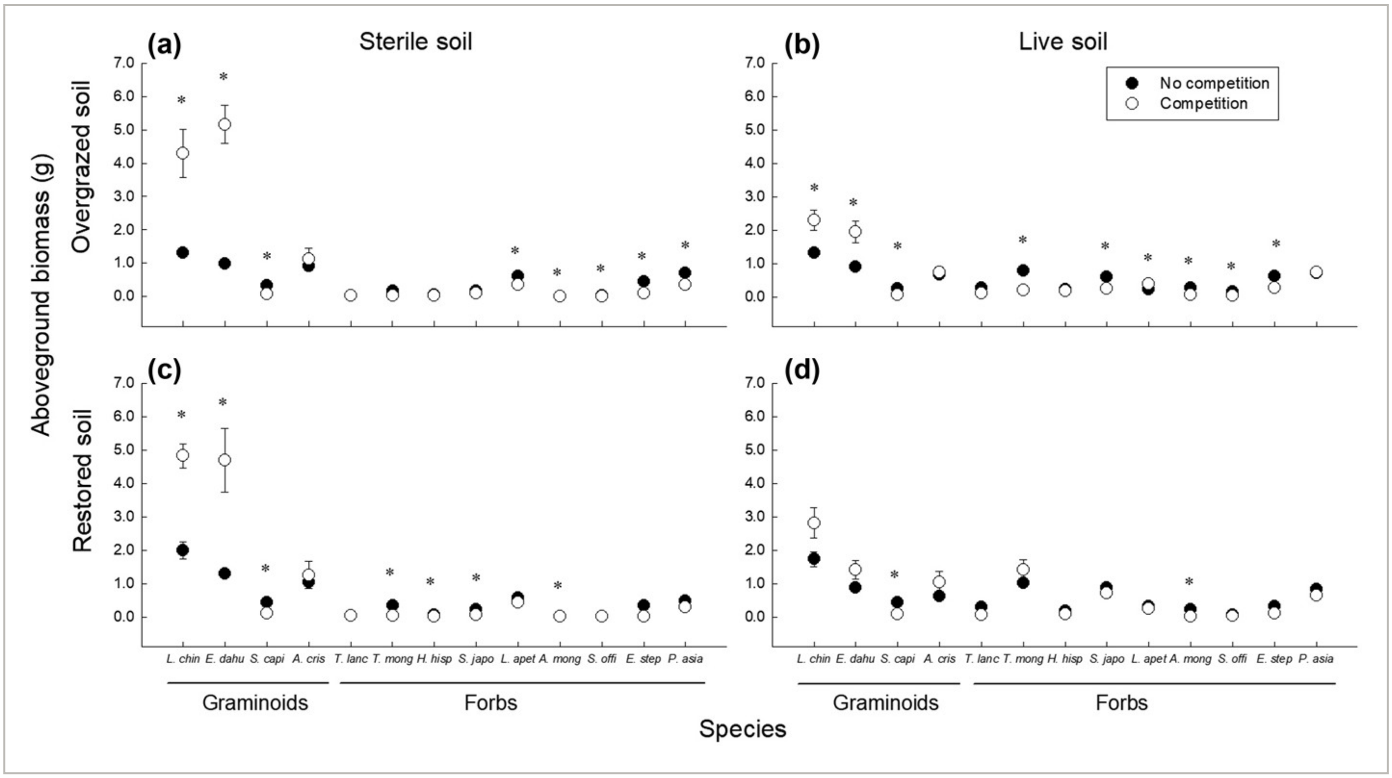

Figure 5

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Aboveground biomass for 13 plant species grown individually or within complex communities in overgrazed soil (a–b) or restored soil (c–d). Species abbreviations are described in Table 1. Soil was either sterile (a, c) or live (inoculated) (b, d). For each plant species, significant differences between individual and community experiments are indicated by an asterisk ($p < 0.05$).

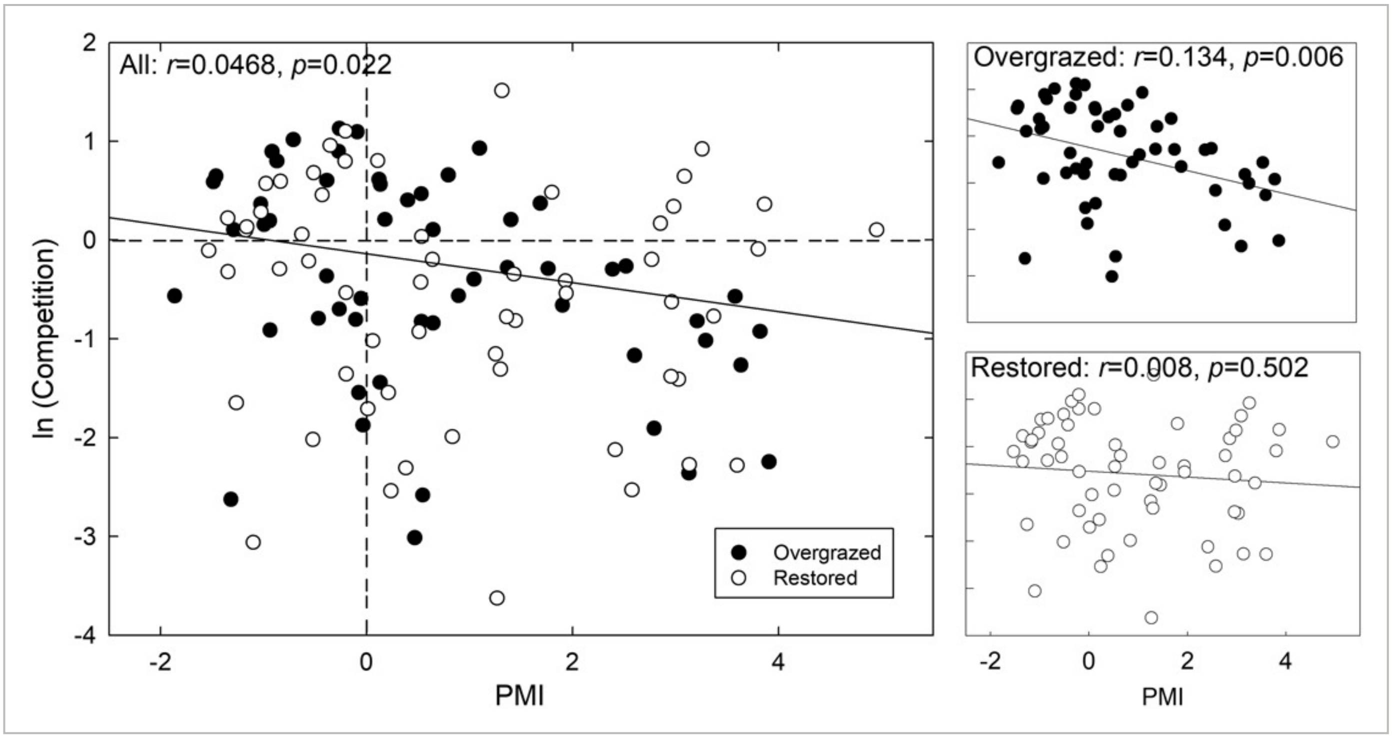

Figure 6

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Relationship of effect size of competition (ln-transformed) and plant–microbial interactions (PMI) in overgrazed and restored soil. Linear regression model analyses were utilized.

When grown in restored soil, microorganisms had no significant effects on plant biomass (Table 5). Plant biomass was primarily regulated by interspecific competition. In contrast, competition, plant species, soil microorganisms and their interactions had a significant influence on plant biomass in overgrazed soil (Table 5).

Table Table 5.. Three-way analysis of variance (ANOVA) for effects of competition, species identity, soil microorganisms and their interactions on individual plant biomass in overgrazed and restored soil. Statistically significant sources of variation are in bold

Source of variation	Overgrazed soil		Restored soil	
	F	p	F	p
Species	77.251	**<0.001**	58.899	**<0.001**
Interspecific competition (IC)	20.019	**<0.001**	12.163	**0.001**
Soil microorganisms (SM)	5.229	**0.023**	1.388	0.24
Species × IC	28.886	**<0.001**	12.662	**<0.001**

Source of variation	Overgrazed soil		Restored soil	
	F	p	F	p
IC × SM	15.958	**<0.001**	5.892	**0.016**
Species × SM	11.036	**<0.001**	11.111	**<0.001**
Species × IC × SM	8.287	**<0.001**	4.521	**<0.001**

Plant community evenness

Plant evenness was greater in soils inoculated with microorganisms compared to soil with sterile inoculum (Fig. 7). There was not a significant difference in plant community evenness between overgrazed and restored soil. These data suggest soil microorganisms play an important role in plant species diversity at the community level, regardless of soil nutrient level.

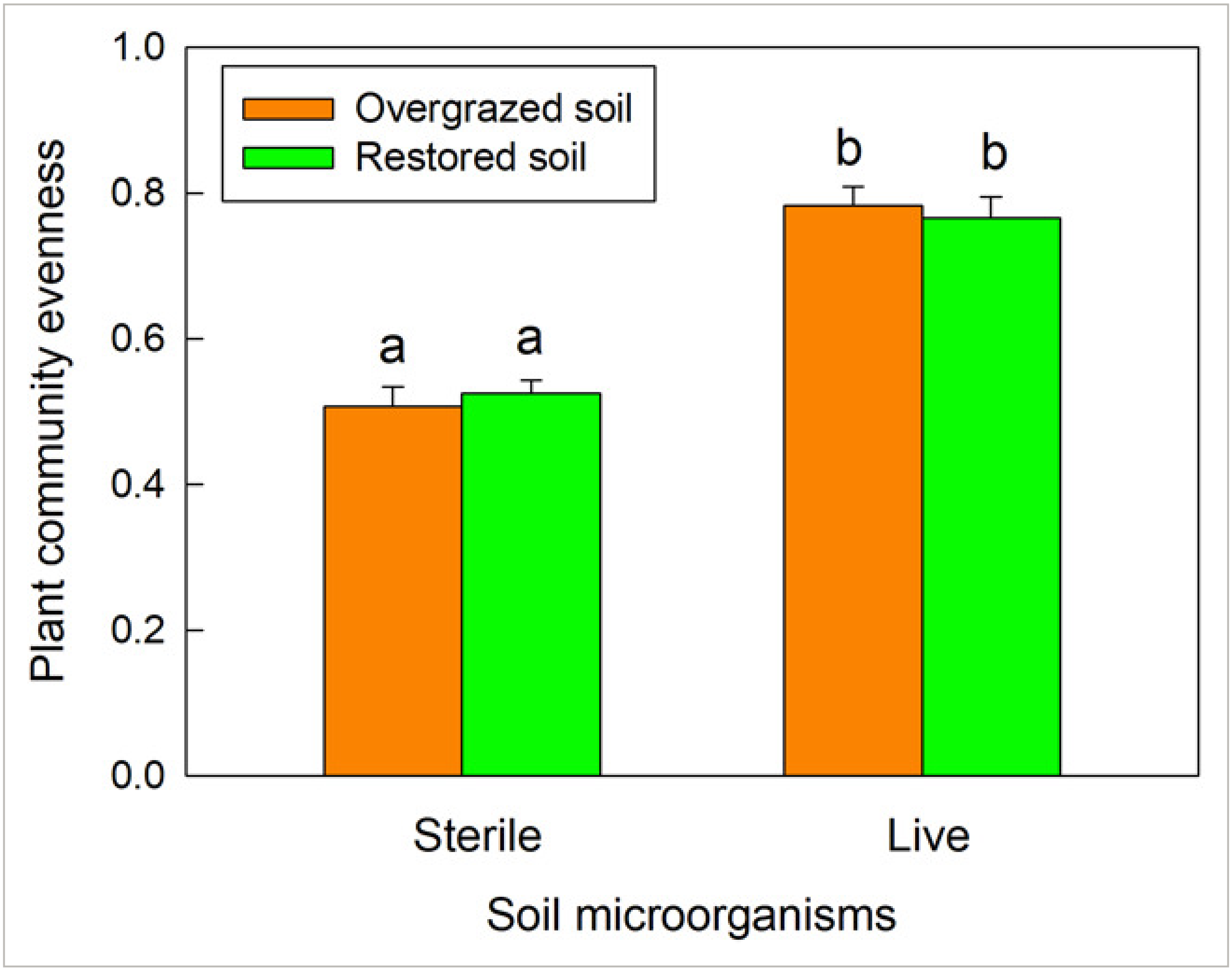

Figure 7

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Plant evenness as a function of soil substrates (overgrazed or restored) and soil microorganisms (sterile or live inoculum). Results of statistical analyses tested with one-way ANOVA are indicated by lowercase letters ($p < 0.05$).

Discussion

The most important finding of our study is that plant–microbial interactions facilitate species coexistence in complex plant communities despite substantial interspecific competition. This was presumably due to negative PMI reducing growth of dominant plant species and positive PMI enhancing growth of sub-dominate species. Effects of soil microorganisms on plant community structure were not overwhelmed by interspecific competition and microbes likely play a critical role in recovery of disturbed sites.

Many studies illustrate the important role of plant–soil feedbacks on individual plant growth and plant–plant competition (Aguilera et al. 2017, Gomez-Aparicio et al. 2017, Meiners et al. 2017). We specifically designed our study to assess complex plant community structure with soil conditioned in situ in native grasslands to provide new insight into community level interactions.

We hypothesized lower resource availability would result in more beneficial microbial interactions. In partial support of our hypothesis, we found soil microorganisms in live field soil increased biomass production of forbs, but not graminoids, regardless of soil nutrient availability. This PMI effect was plant species-dependent, with broad implications for grassland restoration and management. Meta-analytical and empirical experiments suggest plant functional group (Hoeksema et al. 2010, Cortois et al. 2016), plant traits such as root length (Cortois et al. 2016), plant nutrient-acquisition strategies (Teste et al. 2017) and AM fungal taxa (Bennett et al. 2017) govern the magnitude and direction of microbial influences observed across plant species. Plant functional group was an important category for PMI direction in our study, as graminoids overwhelmingly responded negatively to soil microorganisms and forbs generally benefitted. In many cases, graminoids benefit from soil microorganisms, particularly symbiotic mycorrhizal relationships (Kiers et al. 2011, Garcia-Parisi and Omacini 2017); however, our results are consistent with Cortois et al. (2016). Responses of grass species to AM fungi can vary substantially by photosynthetic pathway (C_3 or C_4), presumably due to differences in phenology or evolutionary biology (Wilson and Hartnett 1998). Cool-season (C_3) graminoids, such as the dominant grasses in northern China, tend to receive less benefit from AM fungi (Wilson and Hartnett 1998). Negative microbial effects may also be explained by plant–mycorrhizal interactions in high-nutrient grassland soil compared with less fertile soils (Cortois et al. 2016), such as our overgrazed (low nutrient) and restored (relatively high nutrient), because at relatively high concentrations of plant-available nutrients, mycorrhizal symbioses can shift from mutualistic to parasitic, if the cost of supporting AM fungi exceed host–plant benefits (Johnson et al. 1997, Yang et al. 2014, Jiang et al. 2017). Except for *Lepidium apetalum*, forbs tended to experience positive PMI. AM fungi typically promote plant growth in grasslands, and forbs generally receive more benefits from AM fungi than C_3 graminoids (Frouz et al. 2016, van der Heijden et al. 2016, Stevens et al. 2018). Therefore, we propose benefits received by forbs may have been driven by AM fungi in our study. This may also explain the lack of positive PMI of *L. apetalum*, as the family Cruciferae is notably non-mycorrhizal. Hoeksema et al. (2010) suggested that plant functional group is more important than nutrient availability in determining plant–mycorrhizal interactions. Similarly, our study indicates plant–microbial interactions depended more on plant functional group than soil nutrients. Plant–mycorrhizal interactions may play an important role in our observed plant–microbial correlations, although we did not specifically assess the profile of the soil microbial community structure in our current study.

An accumulating body of literature shows plant shoot production can decrease due to pathogen accumulation in soil conditioned by a single species (Diez et al. 2010, Garcia-Parisi and Omacini 2017), potentially influencing PMI direction. Since soil inoculum was field-collected in our study, microbial communities may be heavily influence by dominant graminoid species (Hortal et al. 2017). Therefore, graminoid-specific pathogens may have accumulated, potentially increasing the negative effects of PMI on graminoids in our study. In addition to shifts in mycorrhizal benefit due to soil nutrient availability, pathogen loads may increase with increasing nutrient availability, such as nitrogen (Whitaker at al. 2015, van der Putten et al. 2016). Alternatively, abundant nutrient resources mitigate the negative impacts of a pathogen on the host if plant defences and immunity are strengthened (Smith-Ramesh and Reynolds 2017). Thereby, potential negative effects of growing pathogen may be neutralized. Our experiment indicates soil nutrient availability did not significantly affect the magnitude of negative PMIs for graminoids. This is in agree with Smith-Ramesh and Reynolds (2017).

Previous studies focused on plant–microbial feedbacks in the context of competition theory (Jing et al. 2015, Ke and Miki 2015), suggesting soil microorganisms shape plant community structure (De Deyn and van der Putten 2005, Aguilera et al. 2017, Hortal et al. 2017). Some research indicates competition among plant species change the effects of soil microorganisms. However, few previous studies assess the additional influence of plant species competition on plant and microbial interactions within diverse and stable plant community. Our findings suggest the magnitude and direction of these interactions were not altered by interspecific competition at the plant community level. Microbial influences on plant growth can be altered experimentally by manipulating light (Smith and Reynolds 2015, Pfennigwerth et al. 2018), nutrient availability (Gustafson and Casper 2004, Manning et al. 2008), temperature (Olsen et al. 2016), and other environmental factors (Smith-Ramesh and Reynolds 2017) which may also influence plant interspecific competition. Most previous experiments aimed to determine the influence of one specific environmental factor on plant–soil feedbacks. We propose the consequences of interspecific plant species competition on PMI in a complex plant community cannot be predicted through a single environmental factor, as interspecific competition likely introduces interactive effects.

We hypothesized effects of soil microbes on plant community structure would be strengthened in resource poor soil (overgrazed). Our study supports our hypothesis, as microorganisms did not significantly influence biomass production of complex plant communities grown in restored soil (relatively high nutrient), even though PMI were still widely operative. In overgrazed soil, microorganisms played an important role in plant community biomass production, suggesting microbes may play a critical role on plant community structure in nutrient poor soil.

Empirical explorations of plant–microbial dynamics at the plant community level are scarce. Our data address this gap by showing interspecific competition was mitigated while PMI improved plant evenness in mesocosms containing complex grassland plant communities. While dominant graminoid species often suppress growth and survival of other plant species, a powerful driver of diversity loss, PMI can counterbalance graminoid competitive ability since soil microorganisms suppressed growth of dominant graminoids with a concomitant facilitation of forbs. Previous research suggested locally rare species are subject to negative or neutral microbial interactions (Kempel et al. 2018). Most of those conclusions were reached based on soil conditioned by a conspecific plant. In our study, forbs unexpectedly benefited from PMI when soil was collected in situ. We propose this occurred because specific pathogens may not accumulate in soil associated with complex plant communities, compared with soil conditioned by a single plant species.

A recent meta-analysis suggests microbial effects on plant community structure typically alter interspecific competition to facilitate coexistence in low nutrient soil (Lekberg et al. 2018). Our results do not support that interspecific competition increases in higher nutrient soil (restored); however, our results indicate microorganisms contribute to plant evenness in both restored and overgrazed soils, although the effects of PMI on plant biomass were overwhelmed by interspecific competition in restored soil. Negative microbial influences are key drivers of plant community structure (Mangan et al. 2010, Vincenot et al. 2017). Our study shows both negative and positive microbial influences contribute to plant evenness in complex plant communities, and illustrates the importance of soil microorganisms for sustainable restoration and management of disturbed grasslands.

Data availability statement

Data are available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.73n5tb2t1> (Li et al. 2019).

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Author contributions – JL, ST and KW conceived of the experiments. JL, SX and LG performed experiments. JL analyzed the data and wrote the manuscript. GWTW and ABC provided advice on manuscript focus and editorial guidance and edited language. BD provided experimental support.

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