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Effects of interspecific competition on plant-soil feedbacks generated by long-term grazing

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ABSTRACT

Grazing by large herbivores leads to changes in soil properties which can in turn modify plant performance. However, little is known about how competition among plant species alters the strength and direction of grazing-induced plant-soil feedbacks (PSFs). In a previous monoculture experiment, we found that the intensity of sheep grazing generated consistent abiotic feedback effects and species-specific biotic feedback effects. To test if and how interspecific plant competition modifies the PSFs observed in our previous monoculture experiment, five naturally-occurring plant species (*Artemisia capillaris*, *Dodartia orientalis*, *Lespedeza davurica*, *Oxytropis racemosa*, and *Stipa bungeana*) were grown in mixed communities in sterilized and unsterilized soils from plots of four grazing intensities (0, 2.7, 5.3, and 8.7 sheep/ha). Further, the five plant species were grown in mixtures in treatment-specific soil following inoculation with arbuscular mycorrhizal fungi (AM) and/or a mixture of pathogenic *Fusarium* species (FU), to test the contribution of these two fungal groups to biotic PSFs. All plant species experienced net neutral to positive abiotic and biotic PSFs. Compared with our previous monoculture experiment, we found that the presence of interspecific competition did not change the abiotic PSF effects generated with increasing grazing intensity, but shifted the direction and strength of the biotic PSFs generated within each grazing intensity. The presence of pathogenic *Fusarium*-only significantly decreased the proportional biomass of the dominant species *A. capillaris* and *S. bungeana*, and the presence of AM fungi-only significantly increased the proportional biomass of the subordinate species *D. orientalis* and *O. racemosa*. Our study strongly suggests that in grazed ecosystems interspecific competition and biotic PSFs interact to drive plant community dynamics, and also that soil mutualists such as AM fungi have the potential to promote plant species coexistence by facilitating the performance of subordinate species.

1. Introduction

Plant-soil feedbacks (PSFs) are plant-induced changes in abiotic and biotic soil properties that in turn influence the performance of the same or other plant species (Ehrenfeld et al., 2005; van der Putten et al., 2013). Biotic PSFs play an important role in driving species composition and plant diversity (Kulmatiski et al., 2008; Bever et al., 2015), community succession (Kardol et al., 2006; Castle et al., 2016), and plant invasions (Klironomos, 2002; Inderjit and van der Putten, 2010). Soil pathogens often lead to negative feedback effects that potentially reduce plant competitive abilities and as such could promote species coexistence (Mangan et al., 2010; Bever et al., 2015). In contrast, symbiotic mutualists, such as arbuscular mycorrhizal fungi (AM), commonly generate positive feedback effects that could increase species abundance (Klironomos, 2002; Lin et al., 2015).

Our understanding of the mechanisms and drivers of PSFs in nature has been substantially advanced since the pioneering work of van der Putten et al. (1993) and Bever (1994). However, one key issue that remains unclear is how PSFs function in diverse, mixed plant communities (Kulmatiski and Kardol, 2008; van der Putten et al., 2016). In natural ecosystems, plant species typically grow in mixed communities and thus can facilitate or inhibit the performance of neighbouring species through their effects on soil properties (Bonanomi et al., 2005; van der Putten, 2009), i.e., heterospecific PSFs. Although several

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studies on PSFs have been performed at the community level (e.g., Casper and Castelli, 2007; Pendergast et al., 2013; Crawford and Knight, 2016; Maron et al., 2016), most PSF studies ignore interspecific competition by investigating the effects of single species grown in monoculture. Moreover, studies quantifying the interactive effects of interspecific competition and PSFs on plant performance have yielded mixed results. Bever (1994) found no effect of negative PSFs on competitive interactions, whereas other studies have found compelling evidence of the contrary (Casper and Castelli, 2007; Kardol et al., 2007).

In grasslands, large aboveground herbivores are key drivers of plant community dynamics (Bardgett and Wardle, 2003; Bakker et al., 2006). Selective grazing by large herbivores directly modifies plant competition by reducing the dominance of some preferred species through consumption (Anderson and Briske, 1995; Hartley and Amos, 1999). In addition, aboveground herbivores can lead to changes in abiotic and biotic soil properties (Hamilton and Frank, 2001; Mikola et al., 2009) and soil food web interactions (Neilson et al., 2002; Andrés et al., 2016), and as such alter plant competitive interactions (Wardle et al., 2004; van der Putten et al., 2013). Meanwhile, the effects of grazing on soil properties are strongly dependent on the duration and intensity of grazing (Bardgett et al., 2001). In spite of the widespread recognition that grazing can modify soil microbial communities and rates of nutrient cycling (Bardgett et al., 1999; Hamilton and Frank, 2001), little is known about the indirect effects of grazing on plant-plant interactions through its effects on soil properties. A study by Medina-Roldán et al. (2012) has demonstrated that grazing-induced changes in soil nutrients can modify plant competitive interactions, but we are not aware of any studies exploring how grazing and interspecific competition interact to affect soil biota and subsequent feedbacks to plant performance.

Semiarid grasslands in northwest China occupy vast areas and are sensitive to grazing disturbance (Christensen et al., 2004). Recent studies in this area have shown that the intensity of grazing by sheep can alter the composition of soil microbial communities (Hu et al., 2017), and root-associated fungi (Chen et al., 2017b), and also affects rates of nitrogen mineralization (Liu et al., 2011). In a previous PSF experiment, we found that long-term sheep grazing subjected to different intensities creates consistent positive abiotic PSFs but strongly species-specific biotic PSFs when plants were grown in monocultures (Chen et al., 2017a). However, it is not clear whether the PSFs measured in monoculture experiments would also be realized in mixed plant communities, since both experiments and meta-analysis strongly suggest that the expression of PSFs depend on the competitive environment (Kardol et al., 2007; Kulmatiski et al., 2008).

In the present study, we examined if and how competition among plant species grown in mixtures alters the strength and direction of grazing-induced PSF effects we observed in our previous monoculture experiment (Chen et al., 2017a). To achieve this goal, we assembled plant communities with five plant species that naturally co-occur in the semiarid grasslands of northwest China and performed two greenhouse experiments. First, the five plant species were grown in mixtures in soil from plots of four grazing intensities crossed with a soil sterilization treatment. Here, we tested how plant competition and grazing intensity interact to affect plant performance through grazing-induced changes in soil biota and nutrients. Second, the five plant species were grown in mixtures in treatment-specific sterilized soil inoculated with AM fungi and/or pathogenic fungi. The second experiment allowed us to further examine how these two groups of fungi interact to affect plant performance and plant community assembly at different grazing intensities. Investigating how grazing-induced changes in abiotic and biotic soil properties feed back to plant performance in the context of interspecific competition will allow us to better predict community dynamics in grazed ecosystems.

2. Materials and methods

2.1. Study site and plant species

The study site is located near the Tian Shui Grassland Research Station, Huan County, Gansu Province, northwestern China (37.12°N, 106.82°E, 1650 m in elevation). This area has a typical semi-arid monsoon climate. The mean annual temperature is about 7.1 °C and the average annual rainfall is approximately 360 mm, with more than 80% occurring from June to September. In 2001, 12 experimental plots that had similar vegetation composition and cover were fenced to establish a grazing experiment. The details of this grazing experiment have been described by Chen et al. (2017a). Briefly, 0, 4, 8, and 13 sheep were rotationally grazed in three replicated 0.5 ha plots of each grazing treatment, representing stocking rates of 0, 2.7, 5.3, and 8.7 sheep/ha, respectively. The plots were arranged in a completely randomized design.

Five naturally co-occurring plant species in this area were selected for use in the experiments based on seed availability: *Artemisia capillaris* Thunberg (Asteraceae), *Dodartia orientalis* Linn. (Scrophulariaceae), *Lespedeza davurica* (Laxm.) Schindl (Fabaceae), *Oxytropis racemosa* Turcz. (Fabaceae), and *Stipa bungeana* Trin. (Poaceae). *Artemisia capillaris*, *S. bungeana*, and *L. davurica* are dominant species in this grassland ecosystem with an average percentage cover of 45%, 18%, and 7%, respectively (Chen et al., 2017a); *D. orientalis* and *O. racemosa* are two subordinate species with a percentage cover less than 3% (Chen et al., unpublished data). In 2014, seeds of each species were collected from a field site approximately 2 km from the study site. The seeds were brought to the laboratory where they were air-dried, cleaned, and stored at 4 °C.

2.2. Soil sampling and processing

Soils for the experiments were collected from the grazing plots in June 2015. Approximately 90 kg of fresh soil was sampled from the top soil in each plot at nine positions randomly along a W-shaped transect, yielding a total of 1080 kg of soil (90 kg per plot × 4 grazing intensities × 3 replicates). Soil was collected to a depth of approximately 10 cm and the distance between adjacent sampling positions within each plot was about 10 m. The shovel was sterilized between plots to prevent cross-contamination of soil samples. Soil samples were immediately taken to the laboratory, where they were sieved through a 5-mm mesh to remove large roots and plant residues. Soil samples collected from the same plot were bulked and thoroughly mixed, resulting in three replicated soil samples for each grazing intensity. The homogenized soils were divided into two parts. A small fraction of each soil was used as living soil inoculum and to extract AM fungal spores (see below). The remaining part of each soil was autoclaved three times for 2 h at 121 °C and used as background soil for the greenhouse experiments. The efficiency of autoclaving was evaluated with serial dilutions and plate techniques (Berns et al., 2008). The soil samples were considered to be sterile when there was no fungal growth after an incubation period of 15 days at 20 °C. Prior to the setup of the greenhouse experiment, all soil samples were stored at 4 °C.

2.3. Experiment 1: plant growth in unsterilized and sterilized soil

To test whether plant interspecific competition modifies the strength and/or direction of the grazing-induced PSF effects observed in our previous monoculture experiment (Chen et al., 2017a), we performed an experiment in which plants of five species (*A. capillaris*, *D. orientalis*, *L. davurica*, *O. racemosa*, and *S. bungeana*) were grown in mixtures in sterilized and living (unsterilized) soils obtained from plots of four grazing intensities (4 grazing intensities × 2 soil treatments = 8 soil treatment combinations). The living and sterilized soils of each grazing intensity were mixed with a treatment-specific sterilized

background soil at a ratio of 1:10. We did not directly compare plant growth in sterilized versus unsterilized soil because in our previous monoculture experiment we found that autoclaving increased soil organic carbon content (Chen et al., 2017a). The approach of adding a small amount of living or sterilized soil to sterilized background soil can effectively dilute the effects of nutrient release caused by autoclaving (Brinkman et al., 2010). Also, four soil subsamples were obtained from each replicate of the 8 soil treatment combinations to determine soil nutrient concentration, and the results showed that there was no significant difference between sterilized and unsterilized soil in the concentration of soil nutrients (Table S1).

Seeds of each species were surface-sterilized (70% ethanol for 1 min, followed by 1% NaClO for 2 min, and rinsed with distilled water), and germinated on sterile glass beads. One-week old seedlings (one plant per species for a total of five plants per pot) were transplanted into plastic pots (20 cm in diameter and 18 cm deep) containing approximately 3 kg of soil (dry weight). To maximize interactions among the five plants, the first randomly-selected plant was transplanted in the center of the pot and then the remaining four plants were randomly arranged around the first plant in a circular pattern 5 cm away from the edge of the pot. The bottom of each pot was filled with 500 g of sterilized sand, followed by 2000 g of treatment-specific sterilized background soil mixed with either 200 g of sterilized or living soil inoculum, topped off with 300 g of sterilized sand. There were three replicates per soil treatment combination for each grazing intensity, matching the three bulked soil samples from each grazing intensity. For each replicated soil treatment, four pots were established. Thus, the design of Experiment 1 involved 4 grazing intensities × 2 soil treatments (sterilized and unsterilized) × 3 replicates × 4 pots, for a total of 96 pots. The pots were randomized on greenhouse tables at 60% relative humidity, a day/night cycle of 16/8 h, and a day/night temperature regime of 21/16 °C. The pots were watered every other day to bring the soil moisture content to 25% (w/w) by weighing. Natural daylight was supplemented by metal halide lamps (225 μmol s^{-1} m^{-2} photosynthetically active radiation, 1 lamp per 1.5 m^2). Seedlings that died during the first week were replaced the same day. The pots were re-positioned randomly once a week to minimize the effects of potential greenhouse microclimate variation.

After 12 weeks, the plant communities were harvested by clipping the shoots at the soil surface and sorting them into species. Roots were washed from the soil with tap water and carefully separated into species. The shoots along with the roots were then oven-dried at 70 °C for five days and weighed.

2.4. Experiment 2: plant growth in response to fungal inocula

To test the contribution of different groups of soil fungi to PSFs observed in Experiment 1, we performed a second experiment in which plants of the same five species were grown in mixtures in treatment-specific sterilized soil of each grazing intensity inoculated with AM fungi and/or pathogenic fungi. The two groups of fungi were prepared as follows. As for the preparation of AM fungi, five 500-g soil subsamples from each plot were used to extract AM fungal spores using a wet-sieving method (Klironomos, 2002). Each soil subsample was dispersed in water and passed through a series of sieves (500, 250 and 45 μm). We collected fungal spores on a 45-μm sieve and surface-sterilized them using 10% NaClO to remove any potential pathogens. Spores of all soil samples were bulked and thoroughly mixed to obtain one composite inoculum. A suspension containing approximately 400 a.m. fungal spores/mL was prepared in sterile water for use as inoculum (Rillig et al., 2014).

Many members of the fungal genus *Fusarium* are common plant pathogens (Booth, 1971). A previous trial in our study area investigating the effects of sheep grazing on the composition of root-associated fungi of dominant plant species showed that three *Fusarium* species, i.e., *F. tricinctum*, *F. oxysporum* and *F. redolens* were the most dominant pathogenic fungi and can attack all tested plant species (Chen et al., 2017b). We therefore used a mixture of *F. tricinctum* (from *A. capillaris*), *F. oxysporum* (from *L. davurica*) and *F. redolens* (from *S. bungeana*) (hereafter referred as FU) as representative of pathogenic fungal communities. For the preparation of the FU communities, actively growing mycelium of each *Fusarium* species was transferred onto water agar plates to obtain large numbers of spores. After 3 weeks, spores were scraped from the agar surface with a sterile scalpel and a mixture of spore suspension containing approximately 6×10^3 spores/mL was prepared (2×10^3 spores of each *Fusarium* species; determined by direct counting using a haemocytometer) following Rillig et al. (2014).

Seeds of each plant species were treated, germinated, and transplanted into pots exactly as in Experiment 1. Here, the bottom of each pot was filled with 500 g of sterilized sand, followed by 2200 g of treatment-specific sterilized background soil, and topped off with 300 g of sterilized sand. Fungal inocula were added to the pots three weeks after the seedlings were transplanted. For AM fungi-only and FU-only treatments, 1 mL of AM fungi (400 spores/mL) or 1 mL of FU (6×10^3 spores/mL) spore suspension was injected to the rhizosphere of each seedling in each pot. For AM + FU treatment, each seedling received 0.5 mL of AM fungi plus 0.5 mL of FU spore suspension. Seedlings in control treatments received 1 mL of autoclaved water. The design of Experiment 2 involved 4 grazing intensities × 4 fungal treatments (Control, AM, FU, AM + FU) × 3 replicates × 4 pots, for a total of 192 pots. After 16 weeks, shoots and roots of each pot were harvested, dried and weighed as in Experiment 1.

To demonstrate the effectiveness of fungal inoculations, we measured root colonization by AM fungi and non-AM fungi as follows. A small amount of dried roots from each pot was rehydrated, rinsed with tap water and cut into 5-cm fragments (Hilbig and Allen, 2015). The cleaned root fragments were then cleared for 30 min at 90 °C in 10% KOH, and stained with 0.05% trypan blue, and assessed at 250× magnification using the magnified intersections method (McGonigle et al., 1990). The two groups of fungi were distinguished based on their morphological characteristics, i.e., by the presence of arbuscules/vesicles/coenocytic hyphae vs. the presence of regularly septate hyphae and the absence of arbuscules and vesicles (Klironomos et al., 1996). A total of 150 intersections were checked for each replicate, and the colonization percentage was calculated as the number of intersections in which AM or non-AM fungi were observed divided by total intersections.

2.5. Statistical analysis

All data analyses were performed using GenStat version 17.1 (VSN International Ltd., UK). In Experiment 1, the concentrations of soil nutrients, aboveground biomass, belowground biomass, total community biomass, and the belowground:aboveground biomass ratio were analyzed using two-way ANOVA, with grazing intensity, soil treatment and their interactions as fixed factors. In Experiment 2, aboveground biomass, belowground biomass, total community biomass, the belowground:aboveground biomass ratio, and root colonization by AM and non-AM fungi were also analyzed using two-way ANOVA, with grazing intensity, fungal treatment and their interactions as fixed factors. Individual comparisons among grazing intensities were performed using Tukey's tests at $p < 0.05$, and individual comparisons between unsterilized and sterilized soil were performed using independent sample *t*-tests at $p < 0.05$. If necessary, biomass data were log-transformed and proportional data were arcsin square root-transformed to meet normality assumptions of ANOVA.

All multivariate statistical analyses were conducted using the *vegan* package in R Program (R Core Team, 2016). Permutational multivariate analysis of variance (PERMANOVA) was conducted using the *adonis* function with 4999 permutations to evaluate the effects of grazing intensity, fungal treatment and their interactions on plant community

Table 1
Results from two-way ANOVA testing effects of grazing intensity (GI) (0, 2.7, 5.3, and 8.7 sheep/ha) and soil treatment (ST) (sterilized or unsterilized), and their interactions on aboveground biomass (AB), belowground biomass (BB), total biomass (TB) and the belowground:aboveground biomass ratio (BAR) of five plant species (*Artemisia capillaris*, *Dodartia orientalis*, *Lespedeza davurica*, *Oxytropis racemosa*, and *Stipa bungeana*) grown in mixtures in the greenhouse. Significant *p*-values ($p < 0.05$) are bolded.

Source	Df	AB[a]		BB[a]		TB[b]		BAR[a]	
		F	*p*	F	*p*	F	*p*	F	*P*
GI	3	398.33	< **0.001**	14.74	< **0.001**	259.90	< **0.001**	19.18	< **0.001**
ST	1	132.86	< **0.001**	2.46	0.139	79.95	< **0.001**	7.74	**0.015**
GI × ST	3	1.44	0.272	1.01	0.418	1.65	0.223	0.79	0.521

[a] Data ln(x+1)−transformed before analysis.
[b] Data ln(x)− transformed before analysis.

composition. Pairwise comparisons were conducted using the Bonferroni correction. Distance-based redundancy analysis (db-RDA) was conducted using the *capscale* function with 4999 permutations to further assess how grazing intensity and fungal treatment affected plant community composition. In the db-RDA ordination plot, each group of fungal treatment or grazing intensity was indicated by confidence ellipses using the *ordiellipse* function. Finally, vector analysis was conducted to determine the relationship between the biomass of each plant species and the fungal and grazing treatments using the *envfit* function based on multivariate regression.

3. Results

3.1. Experiment 1: plant growth in unsterilized and sterilized soil

3.1.1. Community biomass and biomass allocation

At the community level, aboveground biomass, total biomass, and the belowground:aboveground biomass ratio of plants grown in mixtures were significantly affected by grazing intensity and soil treatment, but there were no significant interactive effects (Table 1). Grazing intensity had the strongest effect: aboveground biomass, belowground biomass, and total community biomass significantly increased as the grazing intensity increased, independent of soil sterilization (Fig. 1a–c). Within each grazing intensity, aboveground biomass and total community biomass were significantly greater in unsterilized than in sterilized soil (Fig. 1a and c). The belowground:aboveground biomass ratio decreased significantly as the grazing intensity increased, but did not show significant differences between the sterilized and unsterilized soil within each grazing intensity (Fig. 1d).

3.1.2. Species-specific feedback effects

Individual plant biomass in unsterilized soil relative to sterilized soil subjected to different grazing intensities differed among plant species (Table S2). Biomass of *A. capillaris*, *S. bungeana* and *L. davurica*, the three dominant species in the community, increased significantly with increasing grazing intensity (Table 2). Biomass of the two subordinate species, *D. orientalis* and *O. racemosa*, did not significantly increase with increasing grazing intensity (Table 2). Within each grazing intensity, feedback effects (i.e., plant biomass in unsterilized soil relative to sterilized soil) were strongly species-specific (Table 2). Biomass of *A. capillaris* and *S. bungeana* did not differ between unsterilized and sterilized soil, generating neutral feedback effects (Table 2). By contrast, biomass of *D. orientalis*, *L. davurica*, and *O. racemosa* was significantly greater in unsterilized than in sterilized soil for all grazing intensities, generating positive feedback effects (Table 2).

3.2. Experiment 2: plant growth in response to soil fungal communities

3.2.1. Root colonization

Root colonization by AM fungi in response to fungal treatment and grazing intensity differed among plant species (Table S3). Indeed, roots were not colonized by AM fungi in the treatments without AM fungi inoculation (Fig. 2a). There were interactive effects of plant species, grazing intensity, and fungal treatment on AM fungi colonization of individual plant species (Table S3). Root colonization by AM fungi in *L. davurica* and *O. racemosa* was significantly higher than in *S. bungeana* (Table S4). Root colonization by AM fungi in *A. capillaris*, *D. orientalis*, *L. davurica* and *O. racemosa* increased significantly as the grazing intensity increased (Table S4). In contrast, root colonization by AM fungi in *S. bungeana* did not differ significantly among grazing intensities (Table S4).

Root colonization by non-AM fungi in response to fungal treatment differed among plant species, but was not affected by grazing intensity (Table S3). The overall non-AM fungal colonization in the treatments with FU inoculation was significantly higher than in the treatments without FU inoculation (Fig. 2b). There were interactive effects of plant species and fungal treatment on non-AM fungal colonization of individual plant species (Table S3). In the treatments without FU inoculation, root colonization by non-AM fungi did not differ among the five plant species (Table S5). In the treatments with FU inoculation, root colonization of *A. capillaris* by non-AM fungi was significantly higher than in the other four plant species (Table S5). Root colonization of *A. capillaris* and *S. bungeana* by non-AM fungi in the treatment with inoculation of AM and FU was significantly lower than in the FU-only treatment (Table S5).

3.2.2. Community biomass and biomass allocation

At the community level, aboveground biomass, belowground biomass, total biomass, and the belowground:aboveground biomass ratio of plants grown in mixtures were significantly affected by grazing intensity and fungal treatment, but as in Experiment 1, no significant interactive effects were found (Table 3). Regardless of the fungal treatment, aboveground biomass, belowground biomass, and total biomass significantly increased as the grazing intensity increased (Fig. 3a–c). Within each grazing intensity, aboveground biomass, belowground biomass, and total biomass in the treatments without AM fungi inoculation were significantly lower than in the treatments with AM fungi inoculation, and were typically lowest in the FU-only treatment (Fig. 3a–c). The belowground:aboveground biomass ratio in soils from plots without sheep grazing was significantly higher than in soils from grazed plots, but was not significantly different among fungal treatments within each grazing intensity, except for the 2.7 sheep/ha treatment in which the belowground:aboveground biomass ratio in the FU-only treatment was significantly lower than in other fungal treatments (Fig. 3d).

3.2.3. Species-specific effects

Plant species differed markedly in their responses to fungal treatment and grazing intensity (Table S6). There were interaction effects of plant species with either grazing intensity or fungal treatment on biomass of individual species, but no significant interactive effects of grazing intensity and fungal treatment were found (Table S6). Similar

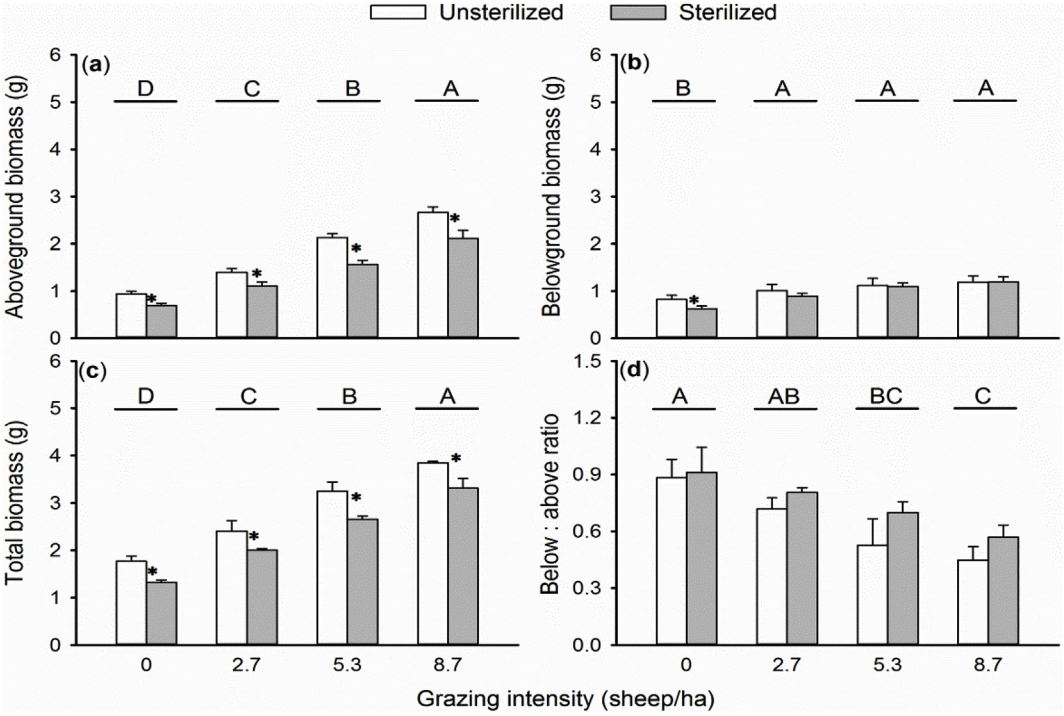

Fig. 1. Aboveground biomass (**a**), belowground biomass (**b**), total biomass (**c**) and the belowground:aboveground biomass ratio (**d**) of five plant species (*Artemisia capillaris*, *Dodartia orientalis*, *Lespedeza davurica*, *Oxytropis racemosa*, and *Stipa bungeana*) grown in mixtures in sterilized soil from plots with four different grazing intensities (0, 2.7, 5.3 and 8.7 sheep/ha) inoculated with either living (unsterilized) or sterilized soil. Data presented are mean values of three replicates and the bars indicate standard errors. Different uppercase letters indicate significant differences among grazing intensities ($p < 0.05$). Within each grazing intensity, asterisks indicate significant differences between sterilized and unsterilized soils ($p < 0.05$).

to Experiment 1, biomass of the dominant species *A. capillaris*, *S. bungeana* and *L. davurica* increased significantly with increasing grazing intensity (Table 4). Within each grazing intensity, individual plant biomass in inoculated soils relative to sterilized soil was strongly species-specific (Table 4). Compared to the control, AM fungi-only inoculation significantly increased biomass of *D. orientalis*, *L. davurica* and *O. racemosa*; FU-only inoculation significantly decreased biomass of *A. capillaris* and *S. bungeana*, but increased biomass of *D. orientalis*; and, the inoculation of both AM and FU significantly increased biomass of *D. orientalis* and *L. davurica* (Table 4). Biomass of *A. capillaris*, *L. davurica* and *S. bungeana* in soils inoculated with both AM and FU was significantly higher than in the FU-only treatment (Table 4).

3.2.4. Plant community composition

The PERMANOVA showed that plant community composition was significantly affected by grazing intensity and fungal treatment, explaining 58% and 29% of the total variation in plant community composition (Table 5). Pairwise comparisons between each level of fungal treatment indicated that the plant community composition significantly differed between the AM fungi-only treatment and the control (Table 5; Fig. 4a). In addition, the community composition in FU-only treatments significantly differed from AM-only and AM + FU treatments (Table 5; Fig. 4a). In contrast, the plant community composition significantly differed among all grazing intensity treatments (Table 5; Fig. 4b). Vector analysis showed that total community biomass increased significantly as the grazing intensity increased (Fig. 4b).

Proportional biomass of individual plant species in response to the

Table 2
Individual biomass (g) of plant species (*Artemisia capillaris*, *Dodartia orientalis*, *Lespedeza davurica*, *Oxytropis racemosa*, and *Stipa bungeana*) grown in mixtures in unsterilized (US) and sterilized (S) soil from plots with four different grazing intensities (0, 2.7, 5.3, and 8.7 sheep/ha) in the greenhouse. Data shown are mean values of three replicates (with SE in brackets).

Plant species	Soil treatment	Grazing intensity (sheep/ha)			
		0	2.7	5.3	8.7
A. capillaris	US	0.47 (0.09)^{c}	0.78 (0.12)^{b}	1.19 (0.08)^{a}	1.44 (0.12)^{a}
	S	0.48 (0.04)^{d}	0.75 (0.12)^{c}	1.09 (0.09)^{b}	1.40 (0.14)^{a}
D. orientalis	US	0.11 (0.02)*	0.12 (0.03)*	0.15 (0.05)*	0.14 (0.02)*
	S	0.01 (< 0.01)	0.04 (0.02)	0.04 (0.01)	0.04 (0.02)
L. davurica	US	0.49 (0.12)^{c*}	0.73 (0.10) ^{bc*}	0.96 (0.13) ^{ab*}	1.16 (0.11)^{a*}
	S	0.24 (0.05)^{c}	0.51 (0.07)^{b}	0.68 (0.10)^{a}	0.84 (0.06)^{a}
O. racemosa	US	0.09 (0.02)*	0.10 (0.01)*	0.11 (0.02)*	0.13 (0.02)*
	S	0.05 (0.01)	0.05 (0.01)	0.06 (0.01)	0.07 (0.01)
S. bungeana	US	0.60 (0.11)^{c}	0.68 (0.13) ^{bc}	0.83 (0.03) ^{ab}	0.97 (0.08)^{a}
	S	0.55 (0.04)^{c}	0.66 (0.09) ^{bc}	0.78 (0.09) ^{ab}	0.96 (0.12)^{a}

Different lowercase letters indicate significant differences among grazing intensities ($p < 0.05$); Asterisks indicate significant differences between sterilized and unsterilized soils ($p < 0.05$).

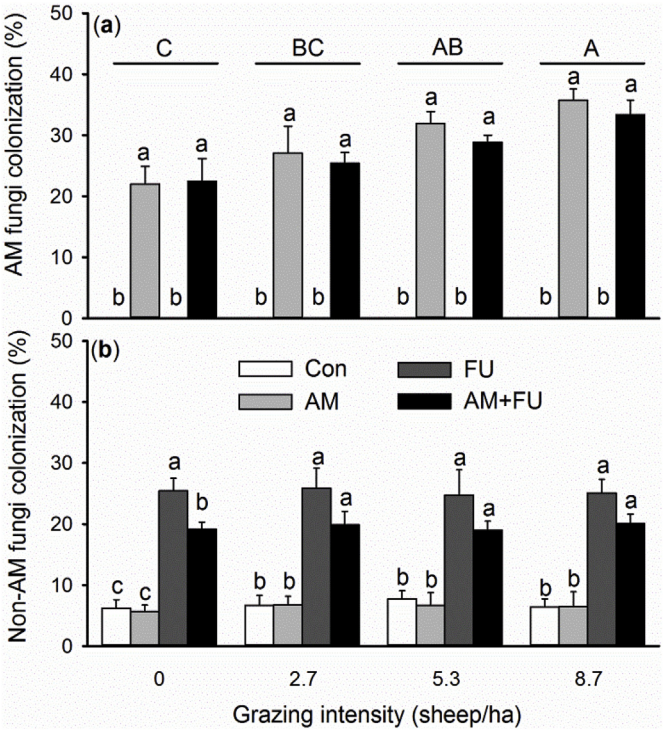

Fig. 2. Percentages of root colonization by arbuscular mycorrhizal fungi (AM) (**a**) and non-AM fungi (pathogenic/saprophytic fungi) (**b**) of five plant species (*Artemisia capillaris*, *Dodartia orientalis*, *Lespedeza davurica*, *Oxytropis racemosa*, and *Stipa bungeana*) grown in mixtures in sterilized soil from plots with four different grazing intensities (0, 2.7, 5.3, and 8.7 sheep/ha) inoculated with different fungal communities (control (Con), arbuscular mycorrhizal fungi (AM), a mixture of pathogenic *Fusarium* spp. (FU), AM + FU). Data presented are mean values of three replicates across the five plant species, and the bars indicate standard errors. Different uppercase letters indicate significant differences among grazing intensities using Tukey's tests at $p < 0.05$. Within each grazing intensity, different lowercase letters indicate significant differences among fungal treatments using Tukey's tests at $p < 0.05$.

fungal treatments differed markedly among plant species (Table S6). Compared to the control, inoculation with AM fungi-only increased the proportion of *L. davurica*, *D. orientalis* and *O. racemosa*, but decreased the proportion of *S. bungeana*. The inoculation with FU-only decreased the proportion of *A. capillaris* and *S. bungeana*, but increased the proportion of *D. orientalis* and *O. racemosa*. The inoculation of both AM and FU significantly increased the proportion of *D. orientalis*, *O. racemosa* and *L. davurica*, but decreased the proportion of *A. capillaris* (Table 6).

4. Discussion

Our main objectives were to evaluate if and how interspecific competition alters the strength and direction of grazing-induced PSF effects observed in our previous monoculture experiment, and to disentangle the potential role of different soil fungal communities (AM fungi and/or pathogenic fungi) in structuring plant communities under different grazing intensities. In our Experiment 1, community biomass increased as the grazing intensity increased, regardless of soil sterilization. For individual species grown in mixture, biomass of three dominant species (*A. capillaris*, *L. davurica*, and *S. bungeana*) increased significantly with increasing grazing intensity. This is consistent with our previous work in which biomass of the three dominant species increased significantly with increasing grazing intensity when they were grown in monocultures (Chen et al., 2017a). It has been recognized that grazing by large herbivores can speed up nutrient cycling and increase soil fertility (Bardgett and Wardle, 2003), with positive effects on plant performance (McNaughton et al., 1997). In our grazing plots, soil inorganic N (NH_4^+–N and NO_3^--N) was found to increase as the intensity of sheep grazing increased, probably due to increased input of dung and urine, which therefore generate positive effects on the three dominant plant species.

Medina-Roldán et al. (2012) found that *Nardus stricta*, a typically abundant species under high sheep grazing pressure, performed better when grown in grazed relative to ungrazed soil, independent of interspecific competition; *Eriophorum vaginatum*, a subordinate species whose abundance was diminished greatly under grazing, only grew better in grazed soil when it did not experience interspecific competition with *N. stricta*. A subsequent yield density model showed that the indirect effects of sheep grazing increased intraspecific competition in both species, but the indirect effects of sheep grazing on interspecific competition were species-specific and favoured the performance of the superior competitor *N. stricta* (Medina-Roldán et al., 2012). In our mixture trial, biomass of two subordinate species (*D. orientalis* and *O. racemosa*) only increased to some extent as the grazing intensity increased. We did not evaluate the indirect effects of grazing intensity on *D. orientalis* and *O. racemosa* in monocultures, so whether the indirect effects of grazing on the performance of the two subordinate species is affected by interspecific competition remains unclear. Based on our monoculture and mixture experiments and the results of Medina-Roldán et al. (2012), we strongly suggest that grazing-induced changes in soil properties create positive, nutrient-mediated, abiotic PSF effects for dominant plant species which become stronger with increased grazing intensity, but the direction of these abiotic PSFs does not vary with respect to interspecific competition.

However, within each grazing intensity, community biomass in unsterilized soil was greater than in sterilized soil, and we generally found the same pattern for biomass of individual species, generating net neutral or positive biotic feedback effects on plant growth. By contrast, in our monoculture trial we observed strong negative feedback effects for *A. capillaris* and *S. bungeana* grown in soil from ungrazed plots and in soil from plots with the highest grazing intensity, respectively (Chen et al., 2017a). Given that soil sterilization effectively kills soil organisms and that our approach of adding living soil inoculum to treatment-

Table 3
Results from two-way ANOVA testing effects of grazing intensity (GI) (0, 2.7, 5.3, and 8.7 sheep/ha) and fungal treatment (FT) (control (Con), arbuscular mycorrhizal fungi (AM), a mixture of pathogenic *Fusarium* spp. (FU), AM + FU), and their interactions on aboveground biomass (AB), belowground biomass (BB), total biomass (TB), and the belowground:aboveground biomass ratio (BAR) of five plant species (*Artemisia capillaris*, *Dodartia orientalis*, *Lespedeza davurica*, *Oxytropis racemosa*, and *Stipa bungeana*) grown in mixtures in the greenhouse. F-values (*F*) and p-values (*p*) were given. Significant *p*-values ($p < 0.05$) are bolded.

Source	df	AB[a]		BB[a]		TB[b]		BAR[a]	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*P*
GI	3	143.64	< **0.001**	86.61	< **0.001**	147.09	< **0.001**	12.82	< **0.001**
FT	3	41.95	< **0.001**	81.51	< **0.001**	74.54	< **0.001**	13.24	< **0.001**
GI × FT	9	0.42	0.912	0.28	0.974	0.57	0.809	1.52	0.185

[a] Data ln(x+1)– transformed before analysis.
[b] Data ln(x) – transformed before analysis.

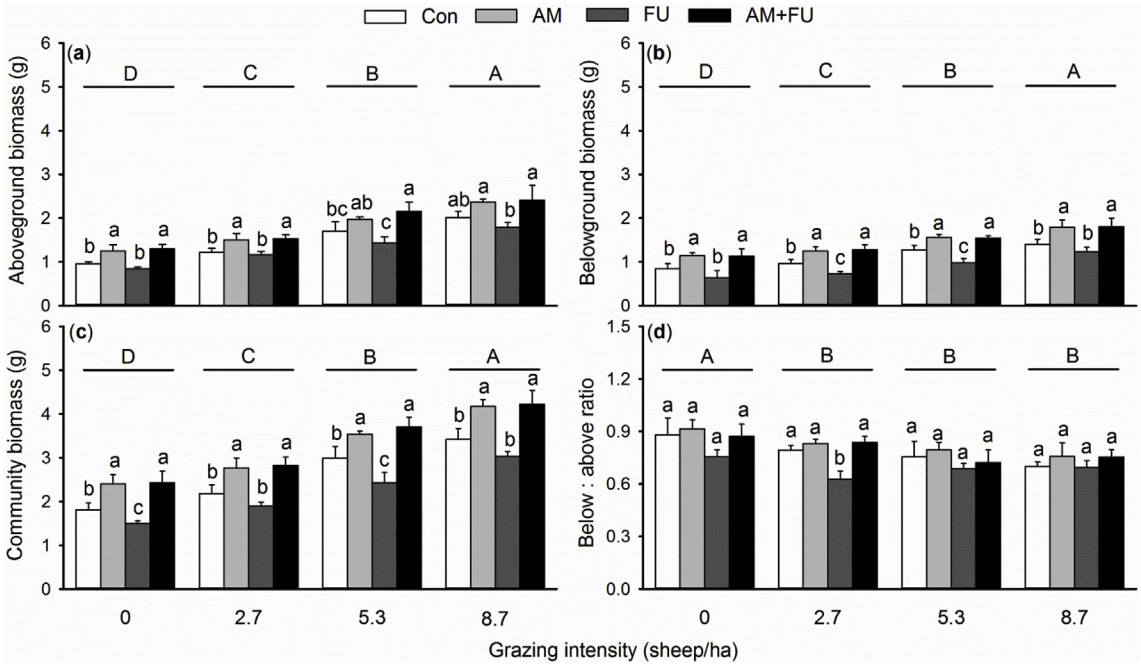

Fig. 3. Aboveground biomass (**a**), belowground biomass (**b**), total biomass (**c**) and the belowground:aboveground biomass ratio (**d**) of five plant species grown in mixtures in sterilized soil from plots with four different grazing intensities (0, 2.7, 5.3 and 8.7 sheep/ha) inoculated with different fungal communities (control (Con), arbuscular mycorrhizal fungi (AM), a mixture of pathogenic *Fusarium* spp. (FU), AM + FU). Data presented are mean values of three replicates and the bars indicate standard errors. Different uppercase letters indicate significant differences among grazing intensities ($p < 0.05$). Within each grazing intensity, different lowercase letters indicate significant differences among fungal treatments ($p < 0.05$).

Table 4
Individual biomass (g) of the five plant species grown in mixtures in sterilized soil from plots with four different grazing intensities (0, 2.7, 5.3, and 8.7 sheep/ha) following inoculation with different fungal communities (Control (Con), arbuscular mycorrhizal fungi (AM), a mixture of pathogenic *Fusarium* spp. (FU), AM + FU) in the greenhouse. Data shown are mean values of three replicates (with SE in brackets).

Plant species	Fungal treatment	Grazing intensity (sheep/ha)			
		0	2.7	5.3	8.7
A. capillaris	Con	0.63 (0.09) ^{Ab}	0.77 (0.20) ^{Ab}	1.37 (0.19) ^{Aa}	1.68 (0.16) ^{Aa}
	AM	0.70 (0.16) ^{Ac}	0.86 (0.12) ^{Ac}	1.42 (0.10) ^{Ab}	1.85 (0.21) ^{Aa}
	FU	0.41 (0.07) ^{Bc}	0.57 (0.08) ^{Bc}	0.97 (0.12) ^{Bb}	1.38 (0.10) ^{Ba}
	AM + FU	0.65 (0.11) ^{Ab}	0.81 (0.12) ^{Ab}	1.47 (0.20) ^{Aa}	1.78 (0.16) ^{Aa}
D. orientalis	Con	0.07 (0.01)^{B}	0.06 (0.02)^{B}	0.08 (0.02)^{B}	0.09 (0.02)^{B}
	AM	0.21 (0.03)^{A}	0.21 (0.04)^{A}	0.25 (0.05)^{A}	0.26 (0.01)^{A}
	FU	0.18 (0.01)^{A}	0.19 (0.03)^{A}	0.20 (0.02)^{A}	0.21 (0.03)^{A}
	AM + FU	0.17 (0.04)^{A}	0.18 (0.02)^{A}	0.21 (0.06)^{A}	0.22 (0.05)^{A}
L. davurica	Con	0.35 (0.04) ^{Cc}	0.57 (0.07) ^{Bb}	0.75 (0.10) ^{Bab}	0.90 (0.11) ^{Ba}
	AM	0.75 (0.03) ^{Ac}	1.00 (0.10) ^{Ab}	1.19 (0.15) ^{Aab}	1.33 (0.12) ^{Aa}
	FU	0.30 (0.06) ^{Cc}	0.50 (0.10) ^{Bb}	0.70 (0.12) ^{Bab}	0.86 (0.05) ^{Ba}
	AM + FU	0.61 (0.08) ^{Bc}	0.88 (0.03) ^{Ab}	1.08 (0.10) ^{Aab}	1.21 (0.13) ^{Aa}
O. racemosa	Con	0.05 (0.01)^{B}	0.05 (0.01)^{B}	0.07 (0.03)^{B}	0.08 (0.02)^{B}
	AM	0.13 (0.02)^{A}	0.13 (0.02)^{A}	0.18 (0.03)^{A}	0.20 (0.02)^{A}
	FU	0.09 (0.02) ^{AB}	0.10 (0.01)^{A}	0.13 (0.02) ^{AB}	0.16 (0.03)^{A}
	AM + FU	0.12 (0.01)^{A}	0.11 (0.01)^{A}	0.13 (0.01) ^{AB}	0.17 (0.03)^{A}
S. bungeana	Con	0.56 (0.07) ^{Bc}	0.72 (0.05) ^{Ab}	0.81 (0.10) ^{ABb}	1.01 (0.06) ^{ABa}
	AM	0.46 (0.04) ^{BCc}	0.55 (0.07) ^{ABbc}	0.61 (0.05) ^{BCb}	0.86 (0.15) ^{BCa}
	FU	0.36 (0.02) ^{Cb}	0.52 (0.09) ^{Bb}	0.53 (0.05) ^{Cb}	0.75 (0.06) ^{Ca}
	AM + FU	0.72 (0.07) ^{Ac}	0.84 (0.11) ^{Abc}	0.92 (0.10) ^{Ab}	1.17 (0.10) ^{Aa}

Within columns, different uppercase letters indicate significances among fungal treatments ($p < 0.05$); Within rows, different lowercase letters indicate significances among grazing intensities ($p < 0.05$).

specific sterilized background soil avoids potentially confounding effects of nutrient release by soil sterilization (Brinkman et al., 2010), our findings strongly suggest that soil biota were the primary agents causing positive biotic PSF effects within each grazing intensity. One possible explanation for the differences in individual biotic PSFs between monocultures and mixtures is that plants grown in monoculture may experience greater competition for soil mutualists than plants grown in mixture, thus resulting in more positive feedback effects with interspecific competition (Hilbig and Allen, 2015). Another possible explanation is that the negative effects of soil pathogens accumulate faster when plants are grown in monocultures, and the presence of interspecific competition may "dilute" the negative effects of soil pathogens which may operate in a frequency-dependent manner (Maron et al., 2016). Here, the accumulation of soil pathogens may result in negative feedbacks reducing plant performance when plants are grown in monocultures. In mixtures, on the other hand, the negative effects of

Table 5
Results from permutational multivariate analysis of variance (PERMANOVA) testing effects of grazing intensity (GI) (0, 2.7, 5.3, and 8.7 sheep/ha) and fungal treatment (FT) (control (Con), arbuscular mycorrhizal fungi (AM), a mixture of pathogenic *Fusarium* spp. (FU), AM + FU), and their interactions on the composition of the plant communities grown in the greenhouse. Significant *p* values ($p < 0.05$) are bolded.

Source	Df	F	R^2	*P*
FT	3	30.60	0.29	< **0.001**
GI	3	61.68	0.58	< **0.001**
FT × GI	9	0.94	0.03	0.527
FT pairwise comparisons				
Con vs AM	1	7.37	0.25	**0.028**
Con vs FU	1	5.28	0.19	0.110
Con vs AM + FU	1	4.34	0.16	0.213
AM vs FU	1	7.48	0.25	**0.035**
AM vs AM + FU	1	2.32	0.10	0.652
FU vs AM + FU	1	8.44	0.28	**0.025**
GI pairwise comparisons				
0 vs 2.7	1	5.15	0.19	**0.049**
0 vs 5.3	1	25.87	0.54	< **0.001**
0 vs 8.7	1	50.24	0.70	< **0.001**
2.7 vs 5.3	1	9.90	0.31	**0.001**
2.7 vs 8.7	1	26.03	0.54	< **0.001**
5.3 vs 8.7	1	5.83	0.21	**0.029**

soil pathogens could be mitigated by the presence of neighbouring species, or outweighed by the positive effects of soil mutualists (Liang et al., 2015). This idea is supported by the finding that root systems of *A. capillaris* grown in monoculture in unsterilized soil from ungrazed plots developed poorly and had conspicuous root blackening, probably due to high pressure of soil pathogens (e.g., pathogenic fungi, nematodes, and other invertebrates). We did not determine the number of nematodes and other invertebrates from the field soil, but we found that the occurrence of the pathogenic fungus *F. tricinctum* was high in the unsterilized soil from the control plots (Chen et al., 2017a), strongly suggesting that pathogenic fungi play a key role in causing the root blackening. In contrast, in our mixture trial, we did not observe any lesions in the roots of *A. capillaris* grown in unsterilized soil from ungrazed plots. Taken together, our findings indicate that grazing also induces biotic feedback effects, and the direction and strength of these feedbacks vary greatly with regard to competitive environment.

Soil fungal pathogens have been suggested to promote species coexistence by lowering the performance of dominant species (Bonanomi et al., 2005; Petermann et al., 2008). In support of this assumption, in our Experiment 2, we found that the presence of pathogenic *Fusarium* spp. impaired the growth of the dominant species *A. capillaris* and *S. bungeana*. However, the inoculation with pathogenic *Fusarium* spp. facilitated the growth of the two subordinate species, *D. orientalis* and *O. racemosa*. In our study, we did not test the mechanisms underlying the differences among plant species in their responses to fungal pathogens. But, one possible explanation is that the *Fusarium* species that we used exhibited strong host-specificity; they were not directly obtained from *D. orientalis* and *O. racemosa* (Errasti et al., 2010). However, inoculation with *Fusarium* spp. only had weak negative effects on the performance of *L. davurica*, even though one of them was isolated from roots of *L. davurica*. It has been hypothesized that plants with highly-branched root systems should be more susceptible to pathogen infection because of increased numbers of meristems and lateral roots where pathogenic fungi can attack (Newsham et al., 1995). In our study, root architecture of the mixed plant community was dominated by root systems of *A. capillaris* and *S. bungeana*, which have dense root hairs and thus increase the root's surface area (Hodge et al., 2009). It is therefore likely that the inoculated *Fusarium* preferentially infected the highly-branched roots of *A. capillaris* and *S. bungeana* and by that, created more available niche space for the poorly-branched roots of *D. orientalis* and *O. racemosa*. Furthermore, considering that we used a mixture of three *Fusarium* species, fungal-fungal interactions could also have affected the

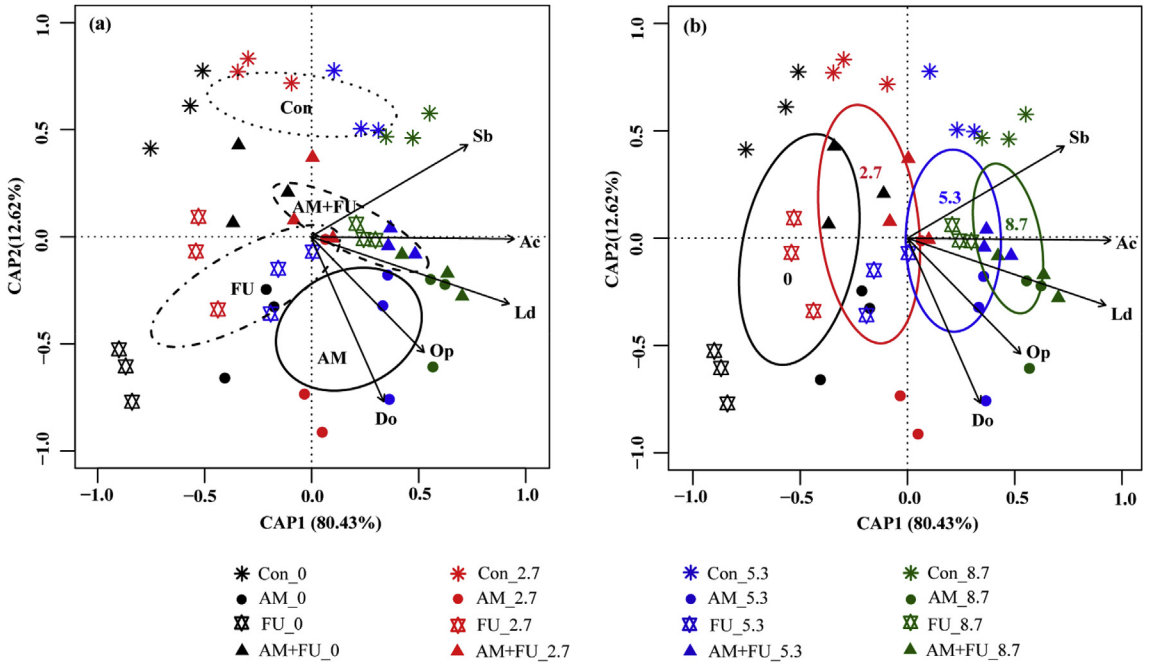

Fig. 4. Ordination plots from distance-based redundancy analysis (db-RDA) testing (a) effects of fungal treatment (control (Con), arbuscular mycorrhizal fungi (AM), a mixture of pathogenic *Fusarium* spp. (FU), AM + FU) and (b) grazing intensity (0, 2.7, 5.3 and 8.7 sheep/ha) on plant community composition. Fungal inoculation treatments are indicated by different symbols while grazing intensities are indicated by different colors. Ellipses indicate the confidence areas for fungal treatments (a) or grazing intensities (b). The relationship between the five plant species (*Artemisia capillaris* (Ac), *Dodartia orientalis* (Do), *Lespedeza davurica* (Ld), *Oxytropis racemosa* (Or), and *Stipa bungeana* (Sb) with fungal treatment and grazing intensity were further explored using vector analyses. The vector arrow points in the direction of most rapid change in the plant biomass. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 6
Proportional biomass of individual plant species grown in mixtures in sterilized soil from plots with four different grazing intensities (0, 2.7, 5.3, and 8.7 sheep/ha) following inoculation with different fungal communities (Control (Con), arbuscular mycorrhizal fungi (AM), a mixture of pathogenic *Fusarium* spp. (FU), AM + FU) in the greenhouse. Data shown are mean values of three replicates (with SE in brackets).

Fungal treatment	Grazing intensity (sheep/ha)	Plant species				
		A. capillaris	*D. orientalis*	*L. davurica*	*O. racemosa*	*S. bungeana*
Con	0	0.38 (0.02)	0.04 (0.01)	0.21 (0.02)	0.03 (0.01)	0.34 (0.01)
	2.7	0.35 (0.07)	0.03 (0.01)	0.26 (0.02)	0.02 (0.01)	0.33 (0.04)
	5.3	0.44 (0.03)	0.03 (0.01)	0.24 (0.02)	0.02 (0.01)	0.26 (0.04)
	8.7	0.45 (0.01)	0.02 (0.01)	0.24 (0.02)	0.02 (0.01)	0.27 (0.01)
	Mean	0.41 (0.05)^{A}	0.03 (0.01)^{C}	0.24 (0.02)^{C}	0.02 (0.01)^{C}	0.30 (0.04)^{A}
AM	0	0.31 (0.05)	0.09 (0.01)	0.33 (0.02)	0.06 (0.03)	0.20 (0.01)
	2.7	0.31 (0.06)	0.08 (0.01)	0.37 (0.06)	0.05 (0.01)	0.19 (0.11)
	5.3	0.39 (0.02)	0.07 (0.01)	0.33 (0.04)	0.05 (0.02)	0.17 (0.03)
	8.7	0.41 (0.06)	0.06 (0.00)	0.30 (0.05)	0.05 (0.00)	0.19 (0.06)
	Mean	0.36 (0.05)^{AB}	0.07 (0.01)^{B}	0.33 (0.03)^{A}	0.05 (0.01)^{AB}	0.19 (0.02)^{C}
FU	0	0.31 (0.04)	0.13 (0.00)	0.22 (0.05)	0.07 (0.02)	0.27 (0.02)
	2.7	0.30 (0.05)	0.10 (0.01)	0.27 (0.04)	0.05 (0.00)	0.28 (0.05)
	5.3	0.39 (0.06)	0.08 (0.02)	0.28 (0.02)	0.05 (0.01)	0.21 (0.04)
	8.7	0.41 (0.03)	0.06 (0.01)	0.26 (0.01)	0.05 (0.01)	0.22 (0.02)
	Mean	0.35 (0.06)^{B}	0.09 (0.03)^{A}	0.26 (0.02)^{BC}	0.05 (0.01)^{A}	0.24 (0.01)^{B}
AM + FU	0	0.29 (0.02)	0.08 (0.02)	0.27 (0.01)	0.05 (0.01)	0.32 (0.01)
	2.7	0.29 (0.02)	0.06 (0.01)	0.31 (0.01)	0.04 (0.01)	0.30 (0.04)
	5.3	0.38 (0.04)	0.05 (0.02)	0.28 (0.01)	0.03 (0.00)	0.24 (0.04)
	8.7	0.39 (0.09)	0.05 (0.01)	0.27 (0.04)	0.04 (0.00)	0.26 (0.05)
	Mean	0.34 (0.06)^{B}	0.06 (0.01)^{B}	0.28 (0.02)^{B}	0.04 (0.01)^{B}	0.28 (0.03)^{AB}

Different uppercase letters indicate significant differences among fungal treatments ($p < 0.05$).

outcomes of plant-fungal interactions (Wagg et al., 2011). However, the mechanisms underlying plant community responses to multiple fungal pathogens is still largely unexplored and should be a focus of future research.

Arbuscular mycorrhizal fungi typically benefit plant growth by enhancing plant resource uptake, especially phosphorus (Laliberté et al., 2015). In our Experiment 2, the presence of AM fungi generally increased biomass of *D. orientalis*, *L. davurica* and *O. racemosa* but not *S. bungeana* and *A. capillaris*. This is probably because *D. orientalis*, *L. davurica* and *O. racemosa* with poorly-branched root systems are more strongly dependent on AM fungi for nutrient acquisition than *A. capillaris* and *S. bungeana* with highly-branched root systems (Newsham et al., 1995; Yang et al., 2015). This assumption was further supported by the finding that biomass production of *D. orientalis*, *L. davurica* and *O. racemosa* showed a positive relationship with the percentage of root colonization by AM fungi as the grazing intensity increased. In addition, biomass production of *A. capillaris* and *S. bungeana* was greater when both AM fungi and pathogenic fungi were present than when only pathogenic fungi were present. The root colonization by non-AM fungi for the two species was strongly decreased by the presence of AM fungi, suggesting that AM fungi may also benefit plants by exerting a protective role against fungal pathogens (Sikes et al., 2009).

Although it is generally recognized that the accumulation of soil mutualists such as AM fungi generates positive PSFs that can potentially reduce local plant diversity by allowing the dominance of early-arriving species (Callaway, 2000; Bever et al., 2012), several recent studies suggest that feedbacks with AM fungi also play an important role in maintaining species coexistence (Bennett et al., 2017; Teste et al., 2017). For example, using empirical studies and model simulations, Teste et al. (2017) showed that the maintenance of plant diversity by soil biota cannot be explained solely by negative feedbacks with soil pathogens but also by positive feedbacks with mycorrhizal fungi. In support of this theory, we found that the presence of AM fungi significantly increased the proportional biomass of the subordinate species *D. orientalis* and *O. racemosa*, suggesting that AM fungi may also promote species coexistence by facilitating the performance of subordinate species.

Negative soil feedbacks are thought to predominate in semiarid grasslands (Kulmatiski et al., 2008; Reinhart, 2012). However, based on the combined results of our previous monoculture work and our present work using mixed plant communities, we show that most species actually experienced positive biotic feedback effects, largely independent of interspecific competition and grazing intensity. This is possibly because we directly used field-conditioned soils rather than greenhouse-conditioned soils which have been argued to favor negative soil feedbacks (Kulmatiski et al., 2008). Alternatively, considering that the vast semiarid grasslands of northwest China are strongly nutrient-limited (Bai et al., 2010), it is likely that soil mutualists such as AM fungi may favor plants to acquire the limiting soil nutrients, thus yielding net positive biotic PSFs. An important next step would be to investigate how different plant species with contrasting nutrient-acquisition strategies respond to the intensity of grazing, and how the type of mycorrhizal association mediates the strategy-dependent feedback since mycorrhizal type is an important factor regulating PSFs (Bennett et al., 2017).

Although our study illustrates the importance of plant competition in modifying the direction and strength of biotic PSFs generated by long-term grazing, we acknowledge a few limitations. First, in Experiment 2, we did not observe any interactive effects of fungal treatment and grazing intensity on plant performance. This is probably because we used common AM fungi and *Fusarium* spp. inocula, which do not actually reflect the effects of grazing intensity on soil fungal communities in our grassland ecosystem. In a preliminary experiment, we sadly failed to obtain the representative pathogenic fungal communities of each grazing intensity, and we therefore used a common fungal inoculum consisting of three pathogenic *Fusarium* species. For consistency, we then also used a common AM fungal inoculum. Second, apart from soil fungi, other agents such as bacteria (Weidner et al., 2015), nematodes (Olff et al., 2000), and other invertebrates (De Deyn et al., 2003) also play a role in biotic feedback effects, but it has been suggested that in soil, different taxonomic groups of organisms may have similar effects on plant performance (Kardol et al., 2007). Further, grazing involves a combination of several factors, including the removal of plant shoot tissue, dung and urine return to the soil, and trampling, and these factors simultaneously affect soil properties (Mikola et al., 2009; Liu et al., 2015). Previous studies indicate that trampling by large herbivores can severely alter soil structure, leading to changes in soil compaction (Habeck, 1960; Frank and Groffman, 1998), which in turn

feed back to plant performance (Kardol et al., 2014). In our study, we performed greenhouse experiments in field-conditioned soil subjected to different grazing intensities to study the indirect effects of sheep grazing on plant performance and plant community assembly. This approach does not account for the effects of trampling on soil properties since the processing of field-conditioned soils for use in the greenhouse experiments disrupted the soil structure with potential consequences for bulk density. Future work is needed to further disentangle the pathways through with grazing affects plant-soil feedbacks.

In conclusion, our study advances the understanding of how abiotic and biotic soil properties structure plant communities under different grazing regimes. Numerous studies have focused on the direct impacts of grazing by herbivores on grassland ecosystems (e.g., Olff and Ritchie, 1998; Bakker et al., 2006; Liu et al., 2015), but the indirect effects of grazing on plant performance and community dynamics through modifying soil properties are less explored. By performing two PSF experiments under controlled greenhouse conditions, we show that interspecific competition plays an important role in shifting the strength and direction of biotic PSF, and also that AM fungi have the potential to promote species coexistence by facilitating the performance of subordinate species. Several recent studies suggested that PSF effects are more likely to be found under greenhouse conditions, and PSFs seem to play a relatively minor role in the field (Heinze et al., 2016; Schittko et al., 2016). Thus, future work on PSFs should be performed under natural conditions and in natural ecosystems, which is often overlooked in PSF research (Kulmatiski and Kardol, 2008).

Acknowledgements

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.soilbio.2018.08.029.

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