Plant Soil (2017) 413:303–317
DOI 10.1007/s11104-016-3103-y

REGULAR ARTICLE

The effects of different intensities of long-term grazing on the direction and strength of plant–soil feedback in a semiarid grassland of Northwest China

Tao Chen · Michael Christensen · Zhibiao Nan · Fujiang Hou

Received: 16 April 2016 / Accepted: 4 November 2016 / Published online: 10 November 2016

Abstract
Aims Plant–soil feedbacks (PSFs) and grazing drive community dynamics in grasslands. We examined how the intensity of grazing and PSF interact to affect plant growth and explored what drives the observed feedback effects.
Methods Three dominant perennial plant species; *Artemisia capillaris*, *Lespedeza davurica*, and *Stipa bungeana* were grown in field-conditioned soil (sterilized or unsterilized) collected from four grazing intensities in a semiarid grassland of northwest China. Soil nutrient concentrations and root fungal communities were determined.
Results Plant biomass increased with grazing intensity for the three plant species. Within each grazing intensity, plant growth in sterilized soil relative to unsterilized soil differed markedly among species. Soil inorganic nitrogen (N) concentration tended to increase with increasing grazing intensity. Arbuscular mycorrhizal fungi (AMF) colonization was high for all grazing intensities for *L. davurica*. *Fusarium tricinctum,* the most common pathogenic *Fusarium* species, had the highest frequency from the control for *A. capillaris* and tended to increase with increasing grazing intensity for *S. bungeana*.
Conclusions Our results suggest that in grasslands plant growth can be modified by the intensity of grazing via grazing-induced changes in soil nutrient availability and fungal communities. Additional studies are needed to determine how grazing intensity affects species co-existence through PSFs to mixed communities.

Keywords Plant–soil interactions · Grasslands · Grazing intensity · *Fusarium* · Arbuscular mycorrhizal fungi · Species–specific interactions

Introduction

In grassland ecosystems, grazing by large herbivores plays a crucial role in driving plant community dynamics (Olff and Ritchie 1998; Bakker et al. 2006). Grazing modifies species co-existence by decreasing the abundance of some preferred species through consumption (Collins et al. 1998) or grazing-induced changes in soil properties (Bardgett and Wardle 2003). However, predicting and understanding the effects of grazing on soil properties is difficult because grazing involves a combination of several factors, including the removal of plant shoot tissue, dung and urine return, and trampling, and these factors simultaneously affect soil conditions (Mikola et al. 2009). For example, the removal of plant shoot tissue reduces the aboveground litter input into the soil, which decreases the activity of soil fungi (Hamilton and Frank 2001). However, the return of

T. Chen · Z. Nan (✉) · F. Hou
The State Key Laboratory of Grassland Agro-ecosystems; College of Pastoral Agricultural Science and Technology, Lanzhou University, PO Box 61, Lanzhou, Gansu 730020, China
e-mail: zhibiao@lzu.edu.cn

M. Christensen
Retired scientist of AgResearch, Grasslands Research Center, Private Bag 11-008, Palmerston North 4442, New Zealand

dung and urine creates patches in the upper layers of soil that are rich in nitrogen and organic matter, providing resources for soil fungi (Stark and Kytöviita 2006). In addition, the duration and intensity of grazing further complicates its effects on abiotic and biotic soil properties (Bardgett et al. 2001). For example, fungal communities are the dominant decomposers of organic matter in submontane regions of the UK exposed to grazing at low intensities, whereas bacterial communities play a prominent role in high-intensity grazing systems (Bardgett et al. 2001). Despite the widespread recognition that grazing can strongly modify soil conditions (Hamilton and Frank 2001; Bardgett and Wardle 2003), little is known about the indirect effects of grazing on plant performance via its effects on soil properties.

Plants change the biology, chemistry, and structure of the soil they grow in, which in turn, influences the performance of plants that grow later in the soil. This process is referred to as plant–soil feedback (PSF) (Bever 2003; van der Putten et al. 2013). PSF is negative when conditioned soil decreases the performance of a plant species and is positive if the opposite is true (Bever 1994). Negative PSFs typically reduce the abundance of plant species (van der Putten et al. 1993; Petermann et al. 2008; Kardol et al. 2013), whereas positive PSFs often increase the abundance of species (Callaway 2000; Inderjit and der putten WH 2010). The process of PSF can be mediated by biotic soil conditions, such as pathogenic and/or beneficial fungi (Klironomos 2002; Kardol et al. 2007; Bezemer et al. 2013), as well as by abiotic soil conditions, such as nutrient availability (Ehrenfeld et al. 2005; Manning et al. 2008; Kos et al. 2015).

The effects of PSF depend on direct interactions between plants and soil conditions, but the direction and strength of these interactions can be modified by shifts in external environmental conditions (Gustafson and Casper 2004) and at temporal scales (Kardol et al. 2013). The direction of PSF, for example, can shift from negative to positive with decreasing light in forest understory species (Smith and Reynolds 2015). While PSF is increasingly recognized as a powerful framework for understanding how ecosystems function (Bever 2003; Kulmatiski et al. 2008; van der Putten et al. 2013), very little is known about how PSFs vary with respect to grazing intensity, and more specifically whether shifts in these interactions are driven by abiotic and biotic soil properties. Although recent studies have reported that grazing-induced changes in soil can modify plant competitive interactions (Medina-Roldán et al. 2012) and alter plant biomass allocation (Veen et al. 2014), no study to date has explored how changes in abiotic and biotic soil conditions at different grazing intensities modify the direction and strength of PSF.

In the present study, we explored how abiotic and biotic soil conditions interact with long-term grazing to affect plant performance within a well-established grazing trial site where four levels of grazing intensity had been maintained for 13 years in a semiarid grassland of northwest China. We performed a greenhouse experiment using field-conditioned soil from the four grazing intensities. Half of the soil sampled from each grazing intensity was heat-sterilized and the remainder was not sterilized, to test the following two hypotheses. First, we hypothesized that plant biomass production will increase with increased grazing intensity. This is based on the assumption that the return of dung and urine will increase with grazing intensity, resulting in positive effects on plant growth via increased nutrient availability (Ehrenfeld et al. 2005; Manning et al. 2008; Kos et al. 2015). The second hypothesis was that within each grazing intensity, plants grown in sterilized soils will have higher biomass production than plants grown in unsterilized soils. This is because negative feedbacks are thought to predominate in semiarid grasslands (Kulmatiski et al. 2008; Reinhart 2012), and soil sterilization can eliminate most fungal pathogens that are key drivers of the negative feedback effects (Kardol et al. 2007; Petermann et al. 2008). By testing the two hypotheses in a controlled greenhouse experiment, we expected to further our mechanistic understanding of how changes in abiotic and biotic soil conditions by grazing at various intensities affect plant growth in semiarid grasslands.

Materials and methods

Study site

The study site is located in a semiarid grassland of the Tian Shui Grassland Research Station, in Huan County, Gansu Province, northwest China (37.12°N, 106.82°E; 1650 m in elevation). The grassland is a typical semiarid and arid ecosystem, and is sensitive to climate change and grazing disturbance (Christensen et al. 2004). The mean annual temperature is about 7.1 °C and the average annual rainfall is approximately 360 mm, with more

than 80 % falling from July to September. The soil is classified as Cambisol based on the FAO soil classification (Liu et al. 2011). The vegetation of heavily-grazed grassland was characterized by the disappearance of rhizomatous grasses and the dominance of undershrubs and small bunch grasses (Ren 1998). In the semiarid grassland where our trial site is located, there are no rhizomatous grasses and the grassland is dominated by the forb *Artemisia capillaris* Thunberg (Asteraceae), semishrub *Lespedeza davurica* (Laxm.) Schindl (Fabaceae) and bunch grass *Stipa bungeana* Trin. (Poaceae) (Hou et al. 2002). This is consistent with the characteristics of heavily-grazed grassland and hence we inferred that the grassland of the trial area had experienced a long period of moderate to heavy grazing.

In 2001, 12 adjacent experimental plots within the semiarid grassland with a visually similar botanical composition, slope, and aspect were selected and fenced to establish a grazing trial. Four grazing intensities were established as follows: 0, 4, 8, and 13 lambs were rotationally grazed in three replicated 0.5 ha plots of each treatment, representing the stocking rates of 0, 2.7, 5.3, and 8.7 sheep/ha, respectively. All plots were arranged in a completely randomized design. Lambs of the 'Tan' breed weighing approximately 20 kg were purchased from local farmers in the spring of each year. Each plot is rotationally grazed three times per year (10 days each time with a rotation interval of 30 days) from early June to early September. Prior to the onset of our experiment, the four grazing intensities were maintained for 13 years from 2001 to 2014.

Field survey and seed collection

A vegetation survey was conducted in June 2014, prior to the beginning of the grazing trial. Plant community composition, coverage, and the number of plants of each species were recorded in five 1 × 1-m quadrats per plot. In each plot, a W-shaped transect (2 m from the edge of the plots to avoid edge effects) was established and vegetation recordings were obtained at the five outer-ends of the W-shape (van de Voorde et al. 2012). The frequency of a particular plant species in each plot was recorded in 20 random 0.5-m diameter wire frames (2 m from the edge of the plots to avoid edge effects). The abundance of a plant species in each plot was estimated as the importance value (IV) of a plant species in the community. The IV was calculated as follows: IV = (relative cover + relative frequency + relative density)/3 (Gilland and Mccarthy 2014). In 2013, seeds of *S. bungeana*, *L. davurica,* and *A. capillaris* were collected from a roadside 2 km from the grazing plots within the grassland in the middle of June and early and middle October, respectively, when seeds ripened. Mature inflorescences were harvested manually from many plants and brought to the laboratory, where the seeds were air-dried, cleaned, and stored at 4 °C.

Soil sampling and soil analyses

Soil samples were obtained when the vegetation survey was completed. Approximately 40 kg of fresh soil was sampled from the top soil in each plot at ten positions randomly along the W-shaped transect, resulting in a total of 480 kg of soil (40 kg per plot × 4 grazing intensities × 3 replicates). The depth of soil sampling was about 10 cm and the distance between adjacent sampling sites within each plot was about 10 m. The soil was passed through a 5-mm mesh sieve to remove large roots and plant residues. Soil samples from the same plot were merged uniformly to obtain a composite soil sample. The homogenized soil was divided into two equal parts. One part was heat-sterilized for 20 min at 120 °C twice in two consecutive days and the other was kept intact (unsterilized), yielding 8 treatment combinations (4 grazing intensities × 2 soil treatments). Both halves were stored at 4 °C prior to the greenhouse experiment. Four soil subsamples were obtained from each replicate of 8 soil treatment combinations, air-dried (< 30 °C) and ground to pass a 2-mm sieve, to determine soil nutrient concentration. Soil inorganic N was analyzed by adding 50 mL of 2 M KCl to a 10-g soil subsample and shaking for 2 h. After shaking, the soil suspensions were filtered through Whatman No. 1 filter paper. Soil NH_4^+-N and NO_3^--N were determined colorimetrically in the KCl extract on a FIAstar 5000 Analyzer (Foss Tecator, Höganäs, Sweden). Soil total phosphorus (P) and total N were determined by adding 3.3 g of catalyst (K_2SO_4 vs. $CuSO_4$ at a ratio of 10:1) and 10 mL of concentrated sulfuric acid to a 0.5-g soil subsample, digesting for 2 h at 420 °C, and analyzing on a FIAstar 5000 Analyzer. The percentage of soil organic carbon (C) was determined by the Walkley–Black method as described by Nelson and Sommers (1982). Briefly, 0.5 g of soil was digested with 5 mL of 1 N $K_2Cr_2O_7$ and 10 mL of concentrated sulfuric acid at 185 °C for 5 min, followed by titration of the digests with standardized $FeSO_4$.

Greenhouse experiment

The growth of three dominant species in the grassland; *A. capillaris*, *L. davurica,* and *S. bungeana*, was examined in field-conditioned soil subjected to the four intensities of grazing in pot trials under greenhouse conditions, which was methodically similar to some PSF studies (De Long et al. 2014; Veen et al. 2014). Seed of each plant species were selected randomly, surface-sterilized (2 min in a 1 % chloride solution and rinsed), and germinated on sterile glass beads. Five one-week old seedlings of each species were transplanted into plastic pots (15 × 15 × 15 cm deep) containing approximately 1500 g of sterilized or unsterilized soil (dry weight). There were three replicates per treatment, matching soil samples from the three replicated plots per grazing intensity. The greenhouse experiment was duplicated to allow us to independently determine plant biomass production and fungal colonization. Hence, the design of the greenhouse trial involved 3 species × 4 grazing intensities × 2 soil treatments × 3 replicates × 2 duplicates, for a total of 144 pots. The pots were placed in a greenhouse with a completely randomized design at 60 % relative humidity, a day/night cycle of 16/8 h, and temperatures of 21 °C/16 °C, respectively. 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 immediately. The pots were watered every other day by adding sufficient water to bring the soil moisture to 25 % (*w*/w) by weighing. The pots were re-positioned randomly once a week to minimize the effects of greenhouse microclimate variation.

After 12 weeks, aboveground parts of each pot were clipped at the soil surface. Two equal numbers of pots were obtained randomly for each treatment. The first set of pots was used to measure belowground biomass after washing roots with tap water. The roots along with the shoots were then oven-dried at 70 °C for five days and weighed. The second set of pots was used to measure the colonization percentage of soil fungi and to isolate *Fusarium* species (see below).

Measurement of soil fungi in plant roots

For each treatment, 10 root fragments of 5 cm in length were randomly selected from each of the three replicates from the second set of pots. They were immediately transported to the laboratory, where fine roots from each treatment were washed repeatedly with distilled water to remove any soil and stored in 50 % ethanol (Maron et al. 2011). The cleaned root samples were then digested for 40 min at 90 °C in 10 % KOH, stained with Chorazol Black E (Brundrett et al. 1984), and assessed at 250× magnification to determine the percent of internal tissues infected by AMF vs. non-AMF (pathogenic/saprophytic fungi)(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 counted for each replicate, and the colonization percentage was calculated as the number of intersections in which AMF or non-AMF were observed divided by total intersections.

Isolation of pathogenic Fusarium spp. from roots

Members of the genus *Fusarium* are common plant pathogens worldwide (Booth 1971). The isolation frequency of *Fusarium* spp. from the roots of the three plants was determined after the greenhouse experiment was completed, as a preliminary study indicated that members of this genus are the most pathogenic of the isolated fungi (unpublished data). For each treatment, 5 root fragments of 5 cm in length were randomly selected from each of the three replicates from the second set of pots. Tap roots (fibrous roots for *S. bungeana*) were trimmed, washed free of soil with tap water, and used to isolate fungi. The cleaned roots were surface-sterilized with 1 % sodium hypochlorite for 2 min, rinsed three times in sterile water, air-dried on sterile filter paper, and cut into 1–2-mm-long segments, as described by Skipp et al. (1986). Ten random root pieces were then placed on malachite green agar media (containing 200 mg/L penicillin and streptomycin) at 22 °C for 1 week (Castellá et al. 1997). Fungal hyphae from the margin of each colony were picked off under a stereoscopic microscope and transferred to potato dextrose agar (PDA) for purification and identification (Dynowska et al. 2011). *Fusarium* isolates were identified based on the colony and conidial characteristics from single spores on both PDA and carnation leaf agar (Nelson et al. 1983). The isolation frequency of *Fusarium* spp. was calculated as the number of root segments that had *Fusarium* spp. divided by the total root segments.

Statistical analysis

All data analyses were performed using GenStat version 17.1 (VSN International Ltd., UK). For IV and the concentration of soil nutrient, values of subsamples within the same replicate were averaged prior to statistical analysis. Field data were analyzed using one-way analysis of variance (ANOVA) with Tukey's tests to evaluate the effect of grazing on the IVs of the three species. The concentrations of soil nutrients were analyzed using two-way ANOVA, with grazing intensity and soil treatment as factors. Plant biomass, root biomass/total biomass ratio (RWR), the percentage of fungal colonization, and the isolation frequency of *Fusarium tricinctum* were analyzed using generalized linear mixed models (GLMMs) with plant species, grazing intensity, and soil treatment as fixed effects, and replication as a random effect (randomized position within the greenhouse). In a preliminary test, soil organic C was used as a covariate in the GLMMs as the concentration of soil organic C increased by soil sterilization, but later it was omitted from the models because it did not actually affect the outcome of the analyses. Individual comparisons of plant total biomass and the RWR between unsterilized and sterilized soil within each grazing intensity were performed using independent sample *t*-tests at $p < 0.05$. The differences in fungal colonization and *F. tricinctum* frequency among grazing intensities were tested using Tukey's tests at $p < 0.05$. Prior to the ANOVA, data were checked for normality and homogeneity, and were transformed whenever necessary to meet the assumptions. Means are reported with standard errors.

Results

Field observations

The IVs of *A. capillaris* and *L. davurica* decreased significantly with increased grazing intensity (Table 1), indicating that the abundance of the two species decreased as the grazing intensity increased. In contrast, the IV of the grass *S. bungeana* tended to increase with increased grazing intensity (Table 1), indicating that sheep grazing increased the abundance of the grass to some extent.

Table 1 Importance value of *Artemisia capillaris*, *Lespedeza davurica* and *Stipa bungeana* at different grazing intensities (0, 2.7, 5.3 and 8.7 sheep/ha) in Tian Shui Grassland Research Station, Huan County, Gansu Province, in 2014

Grazing intensity (sheep/ha)	*A. capillaris*	*L. davurica*	*S. bungeana*
0	0.426 ± 0.015 a	0.106 ± 0.022 a	0.133 ± 0.012
2.7	0.335 ± 0.021 b	0.081 ± 0.017 ab	0.148 ± 0.074
5.3	0.280 ± 0.040 bc	0.070 ± 0.010 ab	0.159 ± 0.021
8.7	0.246 ± 0.017 c	0.049 ± 0.022 b	0.213 ± 0.056

Data are represented as the mean of three replicates with standard errors. Within columns, different lower-case letters indicate significant difference using Tukey's tests at $p < 0.05$

Soil nutrient concentration

The concentration of soil NH_4^+-N, NO_3^--N, total N, and organic C was significantly affected by grazing intensity (Table 2). Soil sterilization did not affect the concentration of soil NH_4^+-N, NO_3^--N, total N, and total P, but did affect soil organic C, which was significantly higher in sterilized than unsterilized soil (Table 2, Table 3). The concentration of soil NH_4^+-N increased significantly with increased grazing intensity and was highest in the 8.7 sheep/ha treatment (Table 3). The concentration of soil NO_3^--N tended to increase with increased grazing intensity (Table 3). Soil total N tended to decrease with increased grazing intensity (Table 3). Soil organic C decreased significantly as the grazing intensity increased and was lowest in the 8.7 sheep/ha treatment (Table 3). Total P did not vary significantly with grazing intensity and was highest in the 2.7 sheep/ha treatment (Table 3).

Plant biomass and biomass allocation

Plant total biomass was significantly affected by plant species, grazing intensity and soil treatment (Table 4). Total biomass tended to increase with grazing intensity for the three plant species (Fig. 1). There was a three-way interaction effect of soil treatment, plant species, and grazing intensity on plant total biomass (Table 4), indicating that plant growth of the three species responded differently to sterilization among grazing intensities. For *A. capillaris*, plant biomass was significantly higher in

Table 2 Results from two-way ANOVA for the effects of grazing intensity (G) (0, 2.7, 5.3 and 8.7 sheep/ha) and soil treatment (S) (sterilized or unsterilized) on soil total nitrogen (TN), inorganic N (NH_4^+-N and NO_3^--N), total phosphorus (TP) and organic carbon (SOC). F-values (*F*) and *p*-values (*p*) were given. Significant *p*-values ($p < 0.05$) in bold

Source	df	NH_4^+-N		NO3$^-$-N		TN		TP		SOC	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
G	3	13.31	**< 0.001**	4.20	**0.023**	7.80	**0.002**	0.52	0.673	10.66	**< 0.001**
S	1	0.89	0.359	0.08	0.776	0.01	0.940	1.66	0.216	4.81	**0.043**
G × S	3	0.15	0.929	0.10	0.961	0.10	0.959	0.55	0.653	0.57	0.644

the soil from the control that was sterilized than in the unsterilized soil (Fig. 1a). We observed that *A. capillaris* plants grown in the unsterilized soil from the control had smaller and blacker root systems than those grown in the sterilized soil (Fig. 2). For *S. bungeana*, plant biomass in the sterilized soil from the 8.7 sheep/ha treatment was significantly higher than that in the unsterilized soil (Fig. 1c). However, we found that the plant biomass of *L. davurica* in sterilized soil was significantly lower than that in unsterilized soil for all grazing intensities (Fig. 1b).

The RWR was significantly affected by plant species and soil treatment (Table 4). There was an interactive effect of plant species and grazing intensity on RWR (Table 4), indicating that the RWR of the three species differed among grazing intensities. The RWR tended to increase as the grazing intensity increased for *A. capillaris* (Fig. 3a). By contrast, the RWR tended to decrease as the grazing intensity increased for *S. bungeana* (Fig. 3c). The RWR in sterilized soil was significantly higher than that in unsterilized soil from the control and the 2.7 sheep/ha treatment for *A. capillaris* (Fig. 3a).

Fungal colonization on plant roots

Soil sterilization significantly reduced the colonization percentage of AMF and non-AMF (Table 5, Fig. 4). The mean percentages of root colonization by AMF and non-AMF differed among species (Table 5, Fig. 4). Roots of *L. davurica* in the unsterilized soil had high AMF colonization percentages for all grazing intensities (*c*.40 %) (Fig. 4c). There was an interactive effect between plant species and grazing intensity on non-AMF (Table 5), indicating that the colonization of non-AMF responded differently depending on grazing intensity for the three plant species. For *A. capillaris*, the percentage of non-AMF in the unsterilized soil from the control was significantly higher than that in the other three grazing intensities (Fig. 4b). In contrast, the percentage of non-AMF in the unsterilized soil tended to increase as the grazing intensity increased for *S. bungeana* (Fig. 4f).

Table 3 The concentration of soil total nitrogen (TN), inorganic N (NH_4^+-N and NO_3^--N), total phosphorus (TP) and organic carbon (SOC) of the unsterilized and sterilized soil sampled from the four grazing intensities (0, 2.7, 5.3 and 8.7 sheep/ha)

Soil treatment	Grazing intensity (sheep/ha)	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	TN (g/kg)	TP (g/kg)	SOC (g/kg)
Unsterilized	0	14.50 ± 1.07 b	2.45 ± 0.15	0.95 ± 0.12 a	0.38 ± 0.06	6.37 ± 0.78 a
	2.7	15.95 ± 1.09 ab	3.05 ± 0.69	0.99 ± 0.07 a	0.40 ± 0.03	5.25 ± 1.02 ab
	5.3	17.45 ± 1.37 ab	3.19 ± 0.21	0.81 ± 0.18 ab	0.36 ± 0.04	5.03 ± 0.72 ab
	8.7	19.21 ± 2.41 a	3.30 ± 0.49	0.60 ± 0.08 b	0.39 ± 0.01	3.54 ± 1.10 b
Sterilized	0	13.35 ± 0.97 B	2.62 ± 0.41	0.99 ± 0.08	0.41 ± 0.05	7.69 ± 1.09 A
	2.7	15.73 ± 0.79 AB	3.14 ± 0.37	0.94 ± 0.27	0.42 ± 0.05	6.80 ± 1.96 AB
	5.3	16.73 ± 1.75 AB	3.14 ± 0.38	0.83 ± 0.07	0.41 ± 0.01	6.07 ± 0.91 AB
	8.7	19.04 ± 1.57 A	3.28 ± 0.20	0.61 ± 0.18	0.38 ± 0.05	3.56 ± 0.64 B

Data are represented as the mean of three replicates with standard errors. Within columns, different lower-case and capital letters indicate significant differences for the unsterilized and sterilized soil among grazing intensities using Tukey's tests at $p < 0.05$

Table 4 Results from generalized linear mixed models for the effects of plant species (*Artemisia capillaris*, *Lespedeza davurica* and *Stipa bungeana*), grazing intensity (0, 2.7, 5.3 and 8.7 sheep/ha), and soil treatment (sterilized or unsterilized) on plant total biomass (TB) and root biomass/total biomass ratio (RWR) in the greenhouse experiment. F-values (*F*) and *p*-values (*p*) were given. Significant *p*-values ($p < 0.05$) in bold

Factor	df	TB†		RWR	
		F	*p*	*F*	*p*
Plant species (P)	2	148.50	**< 0.001**	142.91	**< 0.001**
Grazing intensity (G)	3	61.50	**< 0.001**	0.63	0.599
Soil treatment (S)	1	11.50	**0.001**	15.57	**< 0.001**
P × G	6	13.19	**< 0.001**	4.95	**0.001**
P × S	2	15.26	**< 0.001**	2.92	**0.064**
G × S	3	3.96	**0.014**	0.74	0.532
P × G × S	6	4.20	**0.002**	0.54	0.775

† Data $\sqrt{x}$ transformed before analysis

Isolation of Fusarium species

In total, four *Fusarium* species were isolated from the three plant species. *Fusarium tricinctum* was the most common *Fusarium* species and had the highest isolation frequency for the three plant species. *Fusarium oxysporum* was also isolated from all three plant species. *Fusarium redolens* was isolated from *L. davurica* and *S. bungeana*. *Fusarium equiseti* was only isolated from *S. bungeana*. Isolates from black stunted roots that were characteristic of *A. capillaris* plants grown in the unsterilized soil from the control were *F. tricinctum* (Fig. 2). Conidia that formed in colonies growing on PDA were consistent in shape and size with those of this *Fusarium* species (Fig. 5).

The isolation frequency of *F. tricinctum* was significantly affected by plant species, grazing intensity and soil treatment (Table 5). Soil sterilization significantly reduced the isolation frequency of *F. tricinctum* (Table 5, Fig. 6). There was an interactive effect between plant species and grazing intensity (Table 5), indicating that the isolation frequency of *F. tricinctum* differed among grazing intensities for the three species. For *A. capillaris*, the isolation frequency of *F. tricinctum* in the unsterilized soil from the control was significantly higher than that in the other three grazing intensities (Fig. 6a). The isolation frequency of *F. tricinctum* in the unsterilized soil from the control was significantly lower than that in the 8.7 sheep/ha treatment for *S. bungeana* (Fig. 6c).

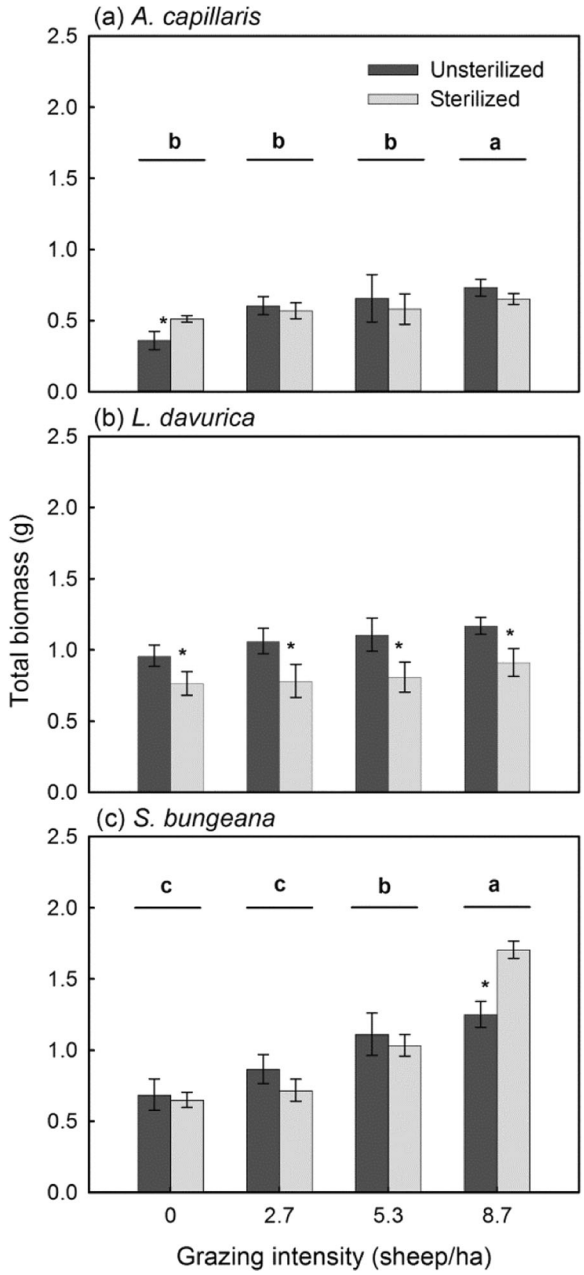

Fig. 1 Plant total biomass of *Artemisia capillaris* (**a**), *Lespedeza davurica* (**b**) and *Stipa bungeana* (**c**) grown in unsterilized and sterilized soil from different grazing intensities (0, 2.7, 5.3 and 8.7 sheep/ha). Data are represented as the mean of three replicates and the bars indicate standard errors. Within each panel, different lowercase letters indicate significant differences among grazing intensities using Tukey's tests at $p < 0.05$. Within each grazing intensity, the asterisk indicates significant differences between the sterilized and unsterilized soil using independent samples *t*-tests at $p < 0.05$

Fig. 2 Root systems of *Artemisia capillaris* grown in the sterilized (left) and unsterilized (right) soil from the control (0 sheep/ha) in the greenhouse

Discussion

In this study, we explored how the intensity of long-term grazing affects plant performance via grazing-induced changes in soil properties. We utilized field-conditioned soil that was either heat-sterilized or not sterilized, to determine the roles of soil fungi and nutrient availability in the observed effects. We found that grazing-induced changes in soil created consistent positive effects with increased grazing intensity, as well as species-specific feedback effects within each grazing intensity for the three species. Our findings strongly indicated that in a grazed ecosystem, plant growth can be modified by the intensity of grazing, and soil fungi and nutrient availability are important drivers of the observed feedback effects.

In support of our first hypothesis, the results showed that plant total biomass tended to increase with increased grazing intensity for all three species, regardless of sterilization, demonstrating that grazing-induced changes in soil have consistent positive effects on plant growth. These results were in agreement with those of previous studies in which higher plant biomass was observed in soils from grazed plots than in soils from un-grazed plots (Medina-Roldán et al. 2012; Veen et al. 2014). We found that the concentration of soil inorganic N tended to increase as the grazing intensity increased. Additionally, in a previous study in the area, the rate of net mineralization in the soil was highest in the 8.7 sheep/ha treatment (Liu et al. 2011). Taken together, we inferred that soil nutrient availability was a key driver of the consistent positive effects with the increased grazing intensity, as previously reported by Olofsson (2009).

Contrary to our second hypothesis, total biomass of the three species was not consistently greater when plants were grown in sterilized soil. Plant biomass in sterilized soil was only higher than that in unsterilized

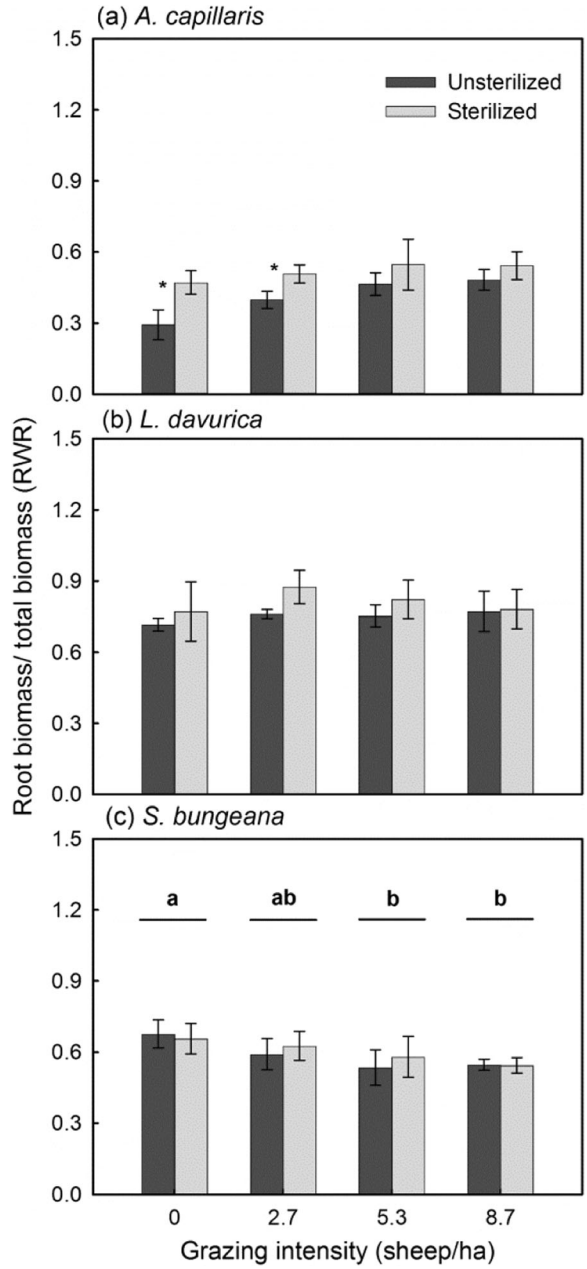

Fig. 3 Root biomass/total biomass ratio (RWR) of *Artemisia capillaris* (**a**), *Lespedeza davurica* (**b**) and *Stipa bungeana* (**c**) grown in unsterilized and sterilized soil from different grazing intensities (0, 2.7, 5.3 and 8.7 sheep/ha). Data are represented as the mean of three replicates and the bars indicate standard errors. Within each panel, different lower-case letters indicate significant differences among grazing intensities using Tukey's tests at $p < 0.05$. Within each grazing intensity, the asterisk indicates significant differences between the sterilized and unsterilized soil using independent *t*-tests at $p < 0.05$

Table 5 Results from generalized linear mixed models for the effects of plant species (*Artemisia capillaris*, *Lespedeza davurica* and *Stipa bungeana*), grazing intensity (0, 2.7, 5.3 and 8.7 sheep/ha), and soil treatment (sterilized or unsterilized) on arbuscular mycorrhiza fungi (AMF) and non-AMF (pathogenic/saprophytic fungi) and *Fusarium tricinctum* in the greenhouse experiment. F-values (*F*) and *p*-values (*p*) were given. Significant *p*-values ($p < 0.05$) in bold

Factor	df	AMF		Non-AMF		*F. tricinctum*	
		F	*p*	*F*	*p*	*F*	*p*
Plant species (P)	2	29.85	**< 0.001**	4.63	**0.014**	9.43	**< 0.001**
Grazing intensity (G)	3	1.54	0.217	6.38	**0.001**	2.87	**0.046**
Soil treatment (S)	1	228.35	**< 0.001**	199.30	**< 0.001**	148.68	**< 0.001**
P × G	6	1.43	0.222	4.52	**0.001**	5.65	**< 0.001**
P × S	2	18.30	**< 0.001**	4.49	**0.016**	0.18	0.833
G × S	3	0.27	0.846	2.44	0.076	0.38	0.766
P × G × S	6	0.44	0.850	1.36	0.251	0.32	0.923

soil with the control for *A. capillaris* and in the treatment of 8.7 sheep/ha for *S. bungeana*. Indeed, the plant biomass of *L. davurica* in sterilized soil from all grazing intensities was lower than that in unsterilized soil. The variation in the growth of the three species in sterilized and unsterilized soil with respect to grazing intensity indicated that grazing-induced changes in soil could also generate species-specific feedback effects within each grazing intensity. It is known that pathogenic fungi are key drivers of species-specific feedback effects (Kardol et al. 2007; Bezemer et al. 2013). In our study, *A. capillaris* plants grown in the unsterilized soil from the control plots had smaller and blacker root systems and lower biomass allocation to roots than those grown in the sterilized soil. In contrast, *A. capillaris* plants grown in unsterilized soil from other grazing intensities did not have conspicuous root blackening. Subsequent isolation of fungi from the roots of *A. capillaris* showed that the occurrence of the pathogenic fungus *F. tricinctum* was highest in the unsterilized soil from the control plots. The comparison of *A. capillaris* growth and *F. tricinctum* colonization in sterilized and unsterilized soil from the four different grazing intensities provided support for the viewpoint that the build-up of fungal pathogens is a key driver of negative feedback effects (Kardol et al. 2007; Bezemer et al. 2013). In addition, beneficial fungi, such as AMF, can build up positive feedback effects by promoting plant uptake of soil nutrients (Reynolds et al. 2003). In our study, the colonization percentage of AMF was high for all grazing intensities for *L. davurica* (*c*. 40 %), providing evidence for the viewpoint that AMF play an important role in positive species-specific feedback effects (Klironomos 2002).

Although we expected to observe consistent negative feedback effects within each grazing intensity for the three species (Kulmatiski et al. 2008; Reinhart 2012), positive effects were detected more commonly across grazing intensities for the three species. For example, *L. davurica* experienced strong positive feedback effects for all grazing intensities. Negative soil feedbacks for dominant plant species are predicted to prevent the competitive exclusion of subordinate species and to maintain species co-existence (Bonanomi et al. 2005; Petermann et al. 2008). In this study, we found that the abundance of *A. capillaris* was highest in the control plots, and the abundance of *S. bungeana* was highest in the plots of 8.7 sheep/ha. Hence, the negative feedback effects detected for the two species in the two contrasting grazing intensities (control for *A. capillaris* and 8.7 sheep/ha for *S. bungeana*) could contribute to maintain species co-existence via reducing plant abundance. *Lespedeza davurica* is a highly preferred legume species for grazing livestock in the semiarid grassland of northwest China and its abundance has decreased greatly due to many years of intense grazing by sheep (Cheng et al. 2011). As a result, the positive feedback for this species is expected to increase the abundance of this species in the grassland (Callaway 2000; Inderjit and der putten WH 2010). It is inferred that in a grazed ecosystem, long-term grazing by livestock strongly consumes aboveground parts of the preferred species, leading to different community compositions in regard to the intensity of grazing. However, grazing-induced changes in soil have the potential to promote species co-existence via altering the species-specific interactions of plant and soil conditions.

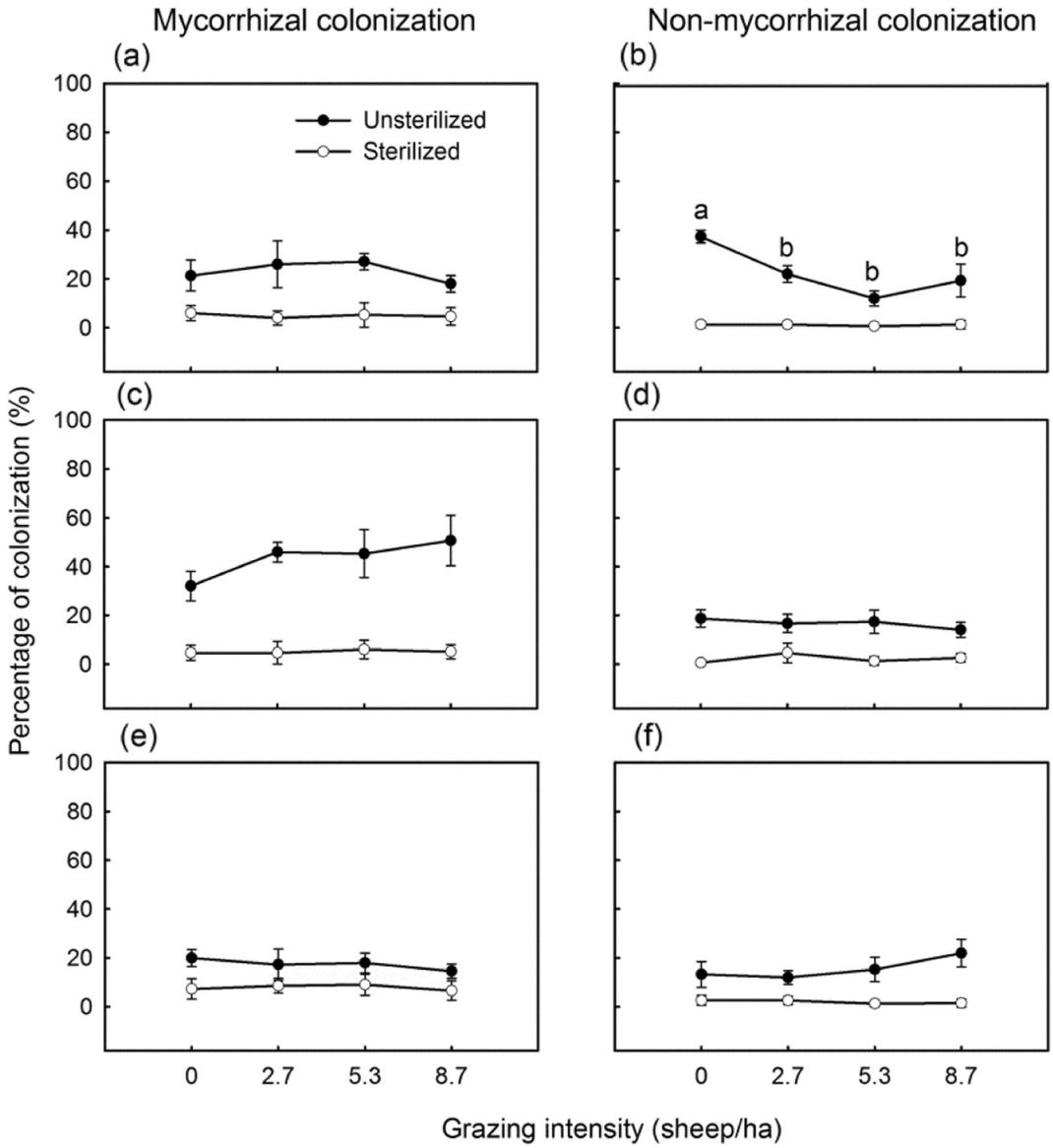

Fig. 4 The mean percentages of *Artemisia capillaris* (**a** and **b**), *Lespedeza davurica* (**c** and **d**) and *Stipa bungeana* (**e** and **f**) root colonized by arbuscular mycorrhizal fungi (AMF) and non-AMF (pathogenic/saprophytic fungi) when they were grown in unsterilized and sterilized soil from different grazing intensities (0, 2.7, 5.3 and 8.7 sheep/ha). Data are represented as the mean of three replicates and the bars indicate standard errors. Different lowercase letters indicate significant differences among grazing intensities using Tukey's tests at $p < 0.05$. Note that percentages of AMF and non-AMF in sterilized soil are not zero but have very small values

While this study indicated the significance of soil fungi in modifying species-specific feedback effects in a grazed ecosystem, there are several concerns that have to be acknowledged. One major concern relates to the use of autoclaving for sterilization. Autoclaving was used to sterilize the soil because the preferred method, gamma irradiation (McNamara et al. 2003), was not available. A major weakness of sterilization is that the concentration of soil nutrients could increase due to decomposition of the killed soil organisms (Berns et al. 2008). In our study, the concentration of soil organic C increased by autoclaving, which can affect plant performance directly or indirectly (Liu et al. 2015), probably resulting in a bias in the results. However, the species-specific responses to sterilization within same grazing intensities support the validity of the procedure.

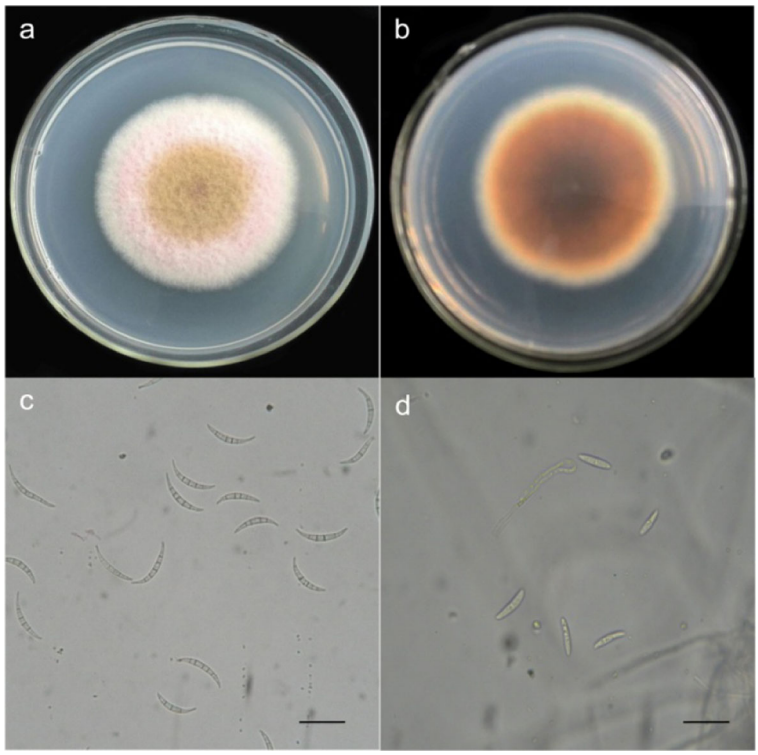

Fig. 5 Morphological characteristics of *Fusarium tricinctum* isolates from roots of *Artemisia capillaris* when grown in unsterilized soil from the control (0 sheep/ha). **a** Colony cultured on a PDA plate for 7 days at 25 °C; **b** undersurface of the colony; **c** sickle-shaped macroconidia, 22.5 ~ 27.5 μm × 2.5 ~ 3.6 μm; **d** spindle-shaped microconidia, 5.0 ~ 9.4 μm × 1.2 ~ 2.0 μm. Scale bar: **c** = 20 μm; **d** = 10 μm

For example, in the treatment of 8.7 sheep/ha, soil sterilization increased the growth of *S. bungeana*, but decreased the growth of *L. davurica*. The variation in the growth of the two species in the sterilized and unsterilized soil is unlikely to result from the increased organic C from autoclaving sterilization as abiotic changes appear to consistently affect plant growth (Aerts and Chapin 2000). Additionally, although we sterilized the soil twice to eliminate all soil fungal communities, the findings that fungal colonization in the sterilized soil was not zero indicated that fungi were still present in the sterilized soil, which may also bias our results. We cannot determine the origin of the fungi (probably resulting from subsequent contamination and/or incomplete sterilization), as the efficacy of sterilization was not tested in culture plates. In general, the side effects of sterilization can be largely avoided by the addition of small amounts of living and sterilized soil to the sterilized background soil (De Long et al. 2014; Gundale et al. 2014; Kardol et al. 2007). However, we did not use the approach considering that the density of soil fungal communities would be diluted by the addition of a soil inoculum and hence may not necessarily reflect the communities in the field soil (Brinkman et al. 2010).

Another important concern regarding sterilization is that other soil organisms that function in the plant–soil interactions, including bacteria (Weidner et al. 2015), root-feeding nematodes (Olff et al. 2000), and other invertebrates (De Deyn et al. 2003), would also be eliminated by sterilization. Different taxonomic groups of soil organisms may have similar or additive effects on plants (Kardol et al. 2007). For example, a study of the dune grass *Ammophila arenaria* showed additive effects of mixtures of soil fungal pathogens and a nematode, compared to the effects of pathogen monocultures, as the presence of nematodes may create access for pathogenic fungi to roots by penetrating the roots with their feeding stylet (De Rooij-Van der Goes 1995). However, interactions such as the competition between different groups of soil organisms may alter the observed feedback effects (van der Putten et al. 2013).

In addition, the origin of seeds is an important concern that could bias our results. Genetically differentiated populations of the same plant species could respond differently to plant–soil interactions (Felker-Quinn et al. 2011). Seeds used in our greenhouse trial were obtained from roadsides near the experimental station, where plants might have experienced a certain extent of grazing and adapted to that level of grazing (Linhart and

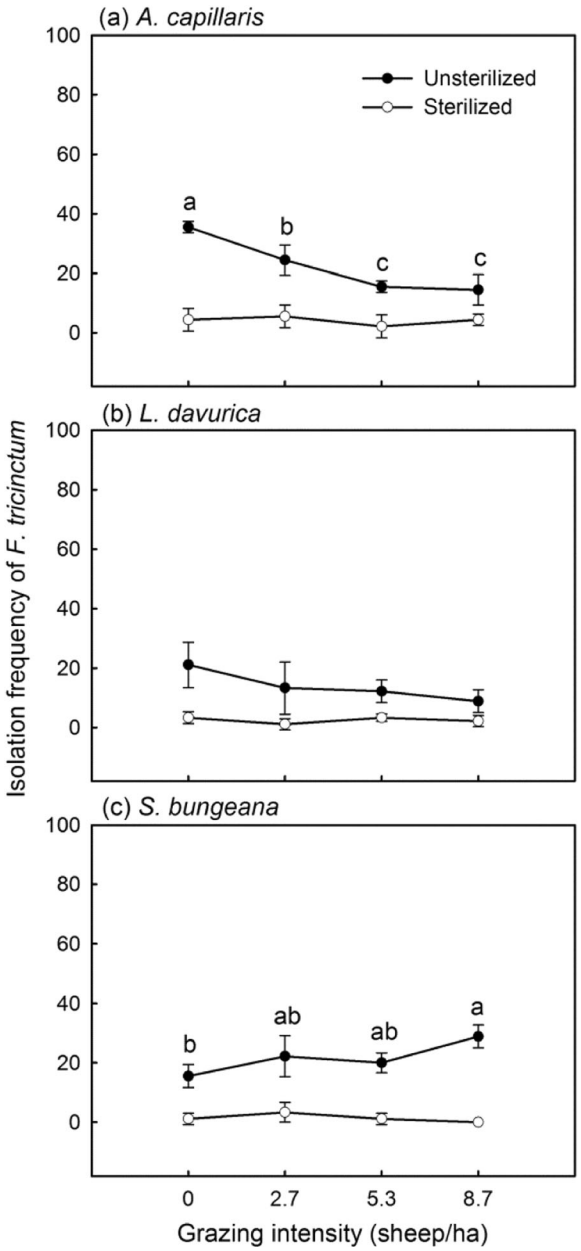

Fig. 6 Isolation frequencies of *Fusarium tricinctum* from roots of *Artemisia capillaris* (**a**), *Lespedeza davurica* (**b**) and *Stipa bungeana* (**c**) when they were grown in unsterilized and sterilized soil from different grazing intensities (0, 2.7, 5.3 and 8.7 sheep/ha). Data are represented as the mean of three replicates and the bars indicate standard errors. Different lower-case letters indicate significant differences in the unsterilized soil among grazing intensities using Tukey's tests at $p < 0.05$. Note that the frequencies of *F. tricinctum* in sterilized soil are not zero but have very small values, except for *S. bungeana* in the treatment of 8.7 sheep/ha, where the value is zero

Grant 1996). Hence, seeds grown in soils from the experimental plots in which the intensity of grazing is similar to that of the roadside may exhibit stronger feedback effects than those grown in soils from other experimental plots.

Furthermore, in this study, we found that fungal colonization was related to species abundance in the field. For example, *L. davurica* had a low plant abundance compared to a high AMF colonization in all grazing intensities. However, variation in root traits is also thought to influence plant responses to fungal colonization (Newsham et al. 1995). It is frequently hypothesized that plant species with a coarse root architecture, characterized by relatively larger diameter roots, lower root hair density, and shorter root hairs, tend to exhibit higher colonization of AMF (Brundrett 2002; Fitter 2004). *Lespedeza davurica* has a large tap root system with very few root hairs, and thus leads to a high colonization of AMF on roots. Therefore, taking plant traits, such as seed origin, fungal colonization and reproduction rate into consideration is necessary for future PSF experiments (Ke et al. 2014).

In fields, a plant species typically grows in mixed communities and hence also interacts with individuals of other species (van de Voorde et al. 2011; van der Putten 2009). A plant species can facilitate or inhibit the performance of other species through their effects on soil biotic or abiotic properties (Bonanomi et al. 2005; van der Putten 2009). It is suggested that individual plant species may generate a mild negative effect in mixtures, opposite to the effects in monoculture, as the density of specialized soil pathogens is diluted (Hendriks et al. 2013). In a grazed ecosystem, the interaction of plants and soil could be more complicated as grazing involves several factors that simultaneously affect soil conditions (Mikola et al. 2009). In this study, only three dominant species were grown in monoculture to investigate how grazing-induced changes in soil affect growth for four grazing intensities. In view of the complexity of plant–soil interactions in a grazed ecosystem, a PSF experiment involving an individual species grown in monoculture and in a mixture of plants for different grazing intensities will be performed in the future to further explore how grazing-induced changes in soil influence plant performance.

In conclusion, we found that long-term grazing-induced changes in soil create consistent positive effects with increased grazing intensity, as well as species-specific feedback effects within each grazing intensity for the three species in the semiarid grassland of northwest China. Although the direction of PSFs is consistent

for the three species with increased grazing intensity, the species-specific effects within grazing intensity suggest that grazing can modify PSFs depending on plant abundance. The direction of PSFs, for example, can shift from negative in control to positive in the presence of grazing as for *A. capillaris*, or from positive in control and/or low-grazing conditions to negative under high-grazing conditions as for *S. bungeana*. Nutrient availability is the key driver of the consistent positive feedback effects with increased grazing intensity, while soil fungi play an important role in the species-specific feedback effects within each grazing intensity. These findings further the understanding that grazing not only indirectly affects plant growth through altering abiotic and biotic soil properties but that the intensity of long-term grazing can modify the direction and strength of PSFs with regard to plant abundance in the field.

Acknowledgments We wish to thank Dr. Chunping Zhang, Dr. Zulfi Jahufer, Dr. Dongwen Luo and Dr. Saman Bowatte for the help in data analysis and the revision of the manuscript. We are grateful to Xiang Yao and Yong Liu for the help of field vegetation survey, and Juan Wang for the assistance of greenhouse experiment. We also acknowledge the excellent informed comments from the two referees. This research was financially supported by National Basic Research Program of China (2014CB138702) and National Public Welfare Industry of Agricultural Science and Technology Special Projects (201303057).

References

Aerts R, Chapin IFS (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Adv Ecol Res 30:1–67

Bakker ES, Ritchie ME, Olff H, Milchunas DG, Knops JM (2006) Herbivore impact on grassland plant diversity depends on habitat productivity and herbivore size. Ecol Lett 9:780–788

Bardgett RD, Wardle DA (2003) Herbivore-mediated linkages between aboveground and belowground communities. Ecology 84:2258–2268

Bardgett RD, Jones AC, Jones DL, Kemmitt SJ, Cook R, Hobbs PJ (2001) Soil microbial community patterns related to the history and intensity of grazing in sub-montane ecosystems. Soil Biol Biochem 33:1653–1664

Berns AE, Philipp H, Narres HD, Burauel P, Vereecken H, Tappe W (2008) Effect of gamma-sterilization and autoclaving on soil organic matter structure as studied by solid state NMR, UV and fluorescence spectroscopy. Eur J Soil Sci 59:540–550

Bever JD (1994) Feeback between plants and their soil communities in an old field community. Ecology 75:1965–1977

Bever JD (2003) Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. New Phytol 157:465–473

Bezemer TM, van der Putten WH, Martens H, van der Voorde TF, Mulder PP, Kostenko O (2013) Above-and below-ground herbivory effects on below-ground plant-fungus interactions and plant-soil feedback responses. J Ecol 101:325–333

Bonanomi G, Giannino F, Mazzoleni S (2005) Negative plant-soil feedback and species coexistence. Oikos 111:311–321

Booth C (1971) The genus Fusarium. Commonwealth Mycological Institute, Kew, England

Brinkman PE, Van der Putten WH, Bakker EJ, Verhoeven KJ (2010) Plant–soil feedback: experimental approaches, statistical analyses and ecological interpretations. J Ecol 98:1063–1073

Brundrett MC (2002) Coevolution of roots and mycorrhizas of land plants. New Phytol 154:275–304

Brundrett M, Piche Y, Peterson R (1984) A new method for observing the morphology of vesicular-arbuscular mycorrhizae. Can J Bot 62:2128–2134

Callaway RM (2000) Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. Science 290: 521–523

Castellá G, Bragulat MR, Rubiales MV, Cabañes FJ (1997) Malachite green agar, a new selective medium for *Fusarium* spp. Mycopathologia 137:173–178

Cheng J, Cheng JM, Hu TM (2011) Distribution responses of *Lespedeza davurica* community on loess plateau to climate change. Chin J Appl Ecol 22:35–40

Christensen L, Coughenour MB, Ellis JE, Chen ZZ (2004) Vulnerability of the Asian typical steppe to grazing and climate change. Clim Chang 63:351–368

Collins SL, Knapp AK, Jmb R, Blair JM, Steinauer EM (1998) Modulation of diversity by grazing and mowing in native tallgrass prairie. Science 280:745–747

De Deyn GB, Raaijmakers CE, Zoomer HR, Berg MP, de Ruiter PC, Verhoef HA, Bezemer TM, van der Putten WH (2003) Soil invertebrate fauna enhances grassland succession and diversity. Nature 422:711–713

De Long JR, Kardol P, Sundqvist MK, Veen GF, Wardle DA (2014) Plant growth response to direct and indirect temperature effects varies by vegetation type and elevation in a subarctic tundra. Oikos 124:772–783

De Rooij-Van der Goes PCEM (1995) The role of plant-parasitic nematodes and soil-borne fungi in the decline of *Ammophila arenaria* (L.) link. New Phytol 129:661–669

Dynowska M, Góralska K, Barańska G, Troska P (2011) Importance of potato-dextrose agar medium in isolation and identification of fungi of the genus *Fusarium* obtained from clinical materials. Med Mycol 2005:291–299

Ehrenfeld JG, Ravit B, Elgersma K (2005) Feedback in the plant-soil system. Annu Rev Environ Resour 30:75–115

Felker-Quinn E, Bailey JK, Schweitzer JA (2011) Soil biota drive expression of genetic variation and development of population-specific feedbacks in an invasive plant. Ecology 92:1208–1214

Fitter AH (2004) Magnolioid roots-hairs, architecture and mycorrhizal dependency. New Phytol 164:15–16

Gilland KE, Mccarthy BC (2014) Microtopography influences early successional plant communities on experimental coal surface mine land reclamation. Restor Ecol 22:232–239

Gundale MJ, Kardol P, Nilsson MC, Nilsson U, Lucas RW, Wardle DA (2014) Interactions with soil biota shift from negative to positive when a tree species is moved outside its native range. New Phytol 202:415–421

Gustafson DJ, Casper BB (2004) Nutrient addition affects AM fungal performance and expression of plant/fungal feedback in three serpentine grasses. Plant Soil 259:9–17

Hamilton EW, Frank DA (2001) Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. Ecology 82:2397–2402

Hendriks M, Mommer L, Caluwe H, Smit-Tiekstra AE, Putten WH, Kroon H (2013) Independent variations of plant and soil mixtures reveal soil feedback effects on plant community overyielding. J Ecol 101:287–297

Hou FJ, Xiao JY, Nan ZB (2002) Eco-restoration of abandoned farmland in the loess plateau. Chin J Appl Ecol 13:923–929

Inderjit, der putten WH V (2010) Impacts of soil microbial communities on exotic plant invasions. Trends Ecol Evol 25:512–519

Kardol P, Cornips NJ, van Kempen MML, Bakx-Schotman JMT, van der Putten WH (2007) Microbe-mediated plant-soil feedback causes historical contingency effects in plant community assembly. Ecol Monogr 77:147–162

Kardol P, Deyn GB, Laliberte E, Mariotte P, Hawkes CV (2013) Biotic plant-soil feedbacks across temporal scales. J Ecol 101:309–315

Ke PJ, Miki T, Ding TS (2014) The soil microbial community predicts the importance of plant traits in plant–soil feedback. New Phytol 206:329–341

Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417: 67–70

Klironomos JN, Rillig MC, Allen MF (1996) Below-ground microbial and microfaunal responses to *Artemisia tridentata* grown under elevated atmospheric CO_2. Funct Ecol 10: 527–534

Kos M, Tuijl MAB, de Roo J, Mulder PPJ, Bezemer TM (2015) Plant-soil feedback effects on plant quality and performance of an aboveground herbivore interact with fertilisation. Oikos 124:658–667

Kulmatiski A, Beard KH, Stevens JR, Cobbold SM (2008) Plant-soil feedbacks: a meta-analytical review. Ecol Lett 11:980–992

Linhart YB, Grant MC (1996) Evolutionary significance of local genetic differentiation in plants. Annu Rev Ecol Syst 27:237–277

Liu TZ, Nan ZB, Hou FJ (2011) Grazing intensity effects on soil nitrogen mineralization in semi-arid grassland on the loess plateau of northern China. Nutr Cycl Agroecosyst 91:67–75

Liu N, Kan HM, Yang GW, Zhang YJ (2015) Changes in plant, soil and microbes in typical steppe from simulated grazing: explaining potential change in soil carbon. Ecol Monogr 85: 269–286

Manning P, Morrison SA, Bonkowski M, Bardgett RD (2008) Nitrogen enrichment modifies plant community structure via changes to plant-soil feedback. Oecologia 157:661–673

Maron JL, Marler M, Klironomos JN, Cleveland CC (2011) Soil fungal pathogens and the relationship between plant diversity and productivity. Ecol Lett 14:36–41

McGonigle T, Miller M, Evans D, Fairchild G, Swan J (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol 115:495–501

McNamara NP, Black HIJ, Beresford NA, Parekh NR (2003) Effects of acute gamma irradiation on chemical, physical and biological properties of soils. Appl Soil Ecol 24:117–132

Medina-Roldán E, Paz-Ferreiro J, Bardgett RD (2012) Grazing-induced effects on soil properties modify plant competitive interactions in semi-natural mountain grasslands. Oecologia 170:159–169

Mikola J, Setälä H, Virkajärvi P, Saarijärvi K, Ilmarinen K, Voigt W, Vestberg M (2009) Defoliation and patchy nutrient return drive grazing effects on plant and soil properties in a dairy cow pasture. Ecol Monogr 79:221–244

Nelson DW, Sommers LE (1982) Total carbon, organic carbon, and organic matter. In: Miller RH, Keeney DR (eds) page, AL. Methods of Soil Analysis American Society of Agronomy and Soil Science Society of American, Madison, WI, pp. 1–129

Nelson P, Toussoun T, Mar W (1983) *Fusarium* species: an illustrated manual for identification. Pennsylvania State University, University Park, PA

Newsham KK, Fitter AH, Watkinson AR (1995) Multi-functionality and biodiversity in arbuscular mycorrhizas. Trends Ecol Evol 10:407–411

Olff H, Ritchie ME (1998) Effects of herbivores on grassland plant diversity. Trends Ecol Evol 13:261–265

Olff H, Hoorens B, De Goede R, Van der Putten W, Gleichman J (2000) Small-scale shifting mosaics of two dominant grassland species: the possible role of soil-borne pathogens. Oecologia 125:45–54

Olofsson J (2009) Effects of simulated reindeer grazing, trampling, and waste products on nitrogen mineralization and primary production. Arct Antarct Alp Res 41:330–338

Petermann JS, Fergus AJ, Turnbull LA, Schmid B (2008) Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. Ecology 89:2399–2406

Reinhart KO (2012) The organization of plant communities: negative plant-soil feedbacks and semiarid grasslands. Ecology 93:2377–2385

Ren JZ (1998) Grassland research methods. China Agriculture Press, Beijing

Reynolds HL, Packer A, Bever JD, Clay K (2003) Grassroots ecology: plant-microbe-soil interactions as drivers of plant community structure and dynamics. Ecology 84:2281–2291

Skipp RA, Christensen C, Nan ZB (1986) Invasion of red clover (*Trifolium pratense*) roots by soilborne fungi. N Z J Agric Res 29:305–313

Smith LM, Reynolds HL (2015) Plant-soil feedbacks shift from negative to positive with decreasing light in forest understory species. Ecology 96:2523–2532

Stark S, Kytöviita MM (2006) Simulated grazer effects on microbial respiration in a subarctic meadow: implications for nutrient competition between plants and soil microorganisms. Appl Soil Ecol 31:20–31

van de Voorde TF, van der Putten WH, Bezemer TM (2011) Intra- and interspecific plant-soil interactions, soil legacies and priority effects during old-field succession. J Ecol 99:945–953

van de Voorde TF, van der Putten WH, Bezemer TM (2012) The importance of plant–soil interactions, soil nutrients, and plant life history traits for the temporal dynamics of *Jacobaea*

vulgaris in a chronosequence of old-fields. Oikos 121:1251–1262

van der Putten WH (2009) A multitrophic perspective on functioning and evolution of facilitation in plant communities. J Ecol 97:1131–1138

Veen G, de Vries S, Bakker ES, van der Putten WH, Olff H (2014) Grazing-induced changes in plant-soil feedback alter plant biomass allocation. Oikos 123:800–806

van der Putten WH, Bardgett RD, Bever JD, Bezemer TM, Casper BB, Fukami T, Kardol P, Klironomos JN, Kulmatiski A, Schweitzer JA (2013) Plant-soil feedbacks: the past, the present and future challenges. J Ecol 101:265–276

van der Putten WH, Vandijk C, Peters BAM (1993) Plant-specific soil-borne diseases contribute to succession in foredune vegetation. Nature 362:53–56

Weidner S, Koller R, Latz E, Kowalchuk G, Bonkowski M, Scheu S, Jousset A (2015) Bacterial diversity amplifies nutrient-based plant-soil feedbacks. Funct Ecol 29:1341–1349