

Arbuscular mycorrhizal fungi alter plant community composition along a grazing gradient in Inner Mongolia Steppe



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Abstract

Arbuscular mycorrhizal fungi (AMF) have a significant influence on plant productivity and diversity in non-grazing grassland. However, the interactive effects between grazing intensity and AMF on plant community composition in natural grassland communities are not well known. We conducted a field experiment that manipulated AMF colonization and grazing intensity to study the impact of AMF suppression on plant community composition and nutrient status over 2 years (2015–2016) with contrasting rainfall levels. We found that AMF root colonization was significantly reduced by the application of the fungicide benomyl as a soil drench. Grazing intensity regulated plant community composition and aboveground biomass mainly by reducing the growth of *Leymus chinensis* over 2 years. AMF suppression increased the growth of *Chenopodium glaucum*, but it did not alter other plant species across all grazing intensities. The effects of AMF suppression on plant community composition changed along a grazing gradient considerably between years: AMF suppression increased the biomass of *C. glaucum* across all grazing intensities in 2015, but slightly increased it in 2016. Interactions between AMF suppression and grazing intensity altered the phosphorus concentration of *Stipa grandis* and *Cleistogenes squarrosa* in 2015 but not in 2016. AMF suppression decreased the shoot phosphorus content of *L. chinensis* but increased that of *C. glaucum* across all grazing intensities. Our results indicate that grazing intensity substantially alters aboveground community biomass and affects growth of dominant species; AMF by itself have limited effects on plant communities along a grazing gradient in typical steppe.

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Keywords: Benomyl; Grassland; Grazing gradient; Grazing intensity; *Chenopodium glaucum*; *Leymus chinensis*

Introduction

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Grazing by ungulate herbivores is a vital biotic process affecting grassland ecosystem structure and function (Eldridge, Poore, Ruiz-Colmenero, Letnic, & Soliveres

2016), and it is the main land use type of semiarid rangelands worldwide (Gossner et al. 2016). In semiarid grassland, the spatial distribution of livestock is dependent on the distance from watering points (James, Landsberg, & Morton 1999), such that the relative grazing intensity decreases with distance from water (Fernandez-Gimenez & Allen-Diaz 2001). This grazing intensity gradient alters plant community productivity (Fernandez-Gimenez & Allen-Diaz 2001; Todd 2006), diversity (Chillo, Ojeda, Capmourteres, & Anand 2017) and physical properties of the soil (Ba et al. 2012; Shahriary et al. 2012). Moreover, grazing intensity can shape plant community composition through shifts in relative abundance among different plant functional groups (Campanella, Bisigato, & Rostagno 2016) and abiotic environment, consequently leading to the alteration of arbuscular mycorrhizal fungi (AMF) root colonization and AMF community composition in the field (Ba et al. 2012).

AMF have symbiotic relationships with plants and thus play a critical role in plant community structure, diversity and productivity in the field (O'Connor, Smith, & Smith 2002; Dostalek, Pankova, Munzbergova, & Rydlova 2013; Yang et al. 2014; Knappova, Pankova, & Munzbergova 2016) and in the laboratory (van der Heijden et al. 1998). AMF improve host plant nutrient status and growth, and in return, host plants provide photosynthate to their fungal partners (Smith & Read 2008). Although livestock–plant and AMF–plant interactions have been studied, relatively few studies have explored the community consequences of multiple biotic interactions among AMF, host plants and livestock.

Grazing can alter plant and soil abiotic properties, which leads to changes in the diversity of AMF in the soil (Eom, Wilson, & Hartnett 2001; Ba et al. 2012). One explanation for this pattern is the carbon limitation hypothesis, which suggests that grazing tends to reduce photosynthetic allocation to roots, leading to reduced AMF growth (Bardgett, Wardle, & Yeates 1998; Barto & Rillig 2010). Previous studies have shown that grazing or defoliation can reduce root colonization, density of external hyphae and AMF diversity in the field (Dhillon & Gardsjord 2004; Liu, Kan, Yang, & Zhang 2015) and in the laboratory (Allsopp 1998; Garcia & Mendoza 2012). Other studies have failed to support the carbon limitation hypothesis, and in some cases, grazing tended to have a neutral or positive effect on mycorrhizal colonization in native grasslands (Tian, Gai, Zhang, Christie, & Li 2009b; van der Heyde, Bennett, Pither, & Hart 2017) and in the greenhouse (Chen, Christensen, Nan, & Hou 2017). Indeed, a meta-analysis suggested that herbivores reduced mycorrhizal colonization by only about 3% (Barto & Rillig 2010). Light to moderate grazing can enhance the photosynthetic capability of plants (Zhao, Chen, Han, & Lin 2009) and, therefore, the plants might not suffer strong carbon limitation (Ba et al. 2012). These conflicting results indicate that the interaction among AMF, host plants and livestock is likely to be context-dependent (Klironomos et al. 2011; Horn, Hempel, Verbruggen, Rillig, & Caruso 2017), varying with grazing intensity, herbivore species and vegetation

type (Barto & Rillig 2010; Mendoza, Cabello, Anchorena, García, & Marbán 2011; Ruotsalainen & Eskelinen 2011). Even though some of the properties that influence AMF distribution and diversity are well understood, the impacts of a grazing intensity gradient on the response and feedback of AMF for host plants are still unclear.

AMF can enhance plant tolerance to herbivores for some plant functional groups in the greenhouse experiments (Walling & Zabinski 2006; Torres et al. 2011). After defoliation, mycorrhizal C₄ grasses and non-N₂-fixing forbs showed accelerated growth compared to C₃ grasses in the laboratory (Kula, Hartnett, & Wilson 2005). Meanwhile, AMF improved shoot growth of a perennial grass with herbivore damage but had no effect on annuals (Borowicz 2013). AMF might increase the capacity for plant regrowth following defoliation by increasing nutrient availability (Mcnaughton & Chapin 1985; Kula et al. 2005; Walling & Zabinski 2006). The effects of insect herbivores on plant–AMF interactions in the laboratory are well known (Kula et al. 2005; Babikova et al. 2013). However, livestock can cause more extensive shoot tissue losses than insects (Barto & Rillig 2010), and livestock can damage plants by trampling and altering the soil surface conditions. To our knowledge, how AMF alter plant community composition and plant nutrient uptake along the grazing intensity in the field is still unclear.

Several studies have investigated the role of AMF on plant community structure and diversity in Australian semiarid herblands (O'Connor et al. 2002), North American prairies (Hartnett & Wilson 1999), the European grasslands (van der Heijden et al. 2006) and Eurasian steppe (Yang et al. 2014). However, the role of AMF on plant community structure and diversity in grazed grassland have only been addressed in a few studies (Dhillon & Gardsjord 2004) and remain uncertain for most grassland ecosystems (Klironomos et al. 2011). Here, we conducted a field experiment to test the effects of grazing intensity and AMF suppression on plant community composition and plant nutrition in the grasslands of Inner Mongolia. Thus, two questions of this study were to be answered: (i) Do arbuscular mycorrhizal fungi (AMF) affect plant community composition along a grazing intensity gradient? (ii) How do AMF alter plant nutrient uptake along the grazing intensity gradient?

Materials and methods

Study site

This experiment was conducted in the grassland ecological protection and sustainable utilization research base of the Chinese Academy of Agricultural Science, which is located in the center of the Inner Mongolian steppe (44°15'N, 116°32'E), China. The site has an altitude range of 1111–1121 m above sea level. The long-term mean annual precipitation in this area is approximately 350 mm, with precipitation mainly falling during the growing season from

June to August (211 mm). The mean annual temperature is -0.1°C , ranging from -22°C in January to 18.3°C in July. The soil in the study area is Calcium Chestnut soil (Calcic Chernozems according to the IUSS Working Group R B, 1998), with a loamy-sand texture.

The vegetation is classified as temperate steppe. The dominant species are *Leymus chinensis* and *Stipa grandis* (perennial C₃ grasses), they are associated with arbuscular mycorrhizae and together account for 82.6% of the total aboveground biomass (see Supplementary Appendix A: Table 1). A highly diverse mixture of other less abundant arbuscular mycorrhizal plant species is also present, including *Cleistogenes squarrosa* (C₄ perennial grass), *Chenopodium glaucum* and *Salsola collina* (C₄ annual forbs). *C. glaucum* and *S. collina* are presumed to have limited mycorrhizal colonization (Tian, Gai, Zhang, Christie, & Li 2009a), and generally considered as non-mycotrophic plants (Jirout, Triska, Ruzickova, & Elhottova 2009; Ba et al. 2012).

Experimental design

Three equally-sized and flat paddocks which were set up in June 2014 and randomly distributed, were used in this study. Each paddock covered 1.33 ha and was grazed moderately (6.01 sheep ha⁻¹) with eight sheep of similar age and weight. All paddocks were fenced from the beginning of the experiment to contain sheep migration in the paddocks. Sheep were allowed to graze continuously during the vegetative growth period from 10 June to 1 September. An artificial watering point was set in each paddock and fixed near the edge of the paddock (see Supplementary Appendix A: Fig. 1). Water and salt were supplied during the grazing period.

In 2015, we conducted an experiment with a two-factor randomized block design, which consisted of combinations of two levels of fungicide application (benomyl treatment and the control treatment without benomyl) and three levels of grazing intensity (heavy grazing, HG, moderate grazing, MG, and light grazing, LG). Benomyl is an effective fungicide, reducing mycorrhizal root colonization (O'Connor et al. 2002). It has been used widely to suppress AMF in field experiments (Hartnett & Wilson 1999; O'Connor et al. 2002), has minimal effects on other soil microflora in typical steppe (Yang et al. 2014; Zhang et al. 2016), and has no direct effects on a wide range of plants (Paul, Ayres, & Wyness 1989). In semiarid and arid grasslands, water is usually available at only a few scattered point sources (Shahriary et al. 2012). Livestock have to drink regularly in this area, and consequently the spatial distribution of livestock is typically highest closest to watering points and decrease with increasing distance from watering points (Todd 2006; Sasaki, Okayasu, Jamsran, & Takeuchi 2008). Based on previous studies in the typical steppe ecosystem (Sasaki et al. 2008), we used the distance from the watering point (10, 50 and 100 m) as a proxy of grazing intensity (heavy grazing, HG, moderate grazing, MG, and light grazing, LG, respectively). Location of the artificial

watering point in a paddock influences the distribution of sheep, resulting in a radial gradient of plant cover (Shahriary et al. 2012). Therefore, we established four 100 m transects radiating outwards from the artificial watering point in each paddock. Two plots (2×2 m) were established randomly at each of the three distances (10, 50 and 100 m) along each transect. Each plot was separated from the other by a 1-m gap (see Supplementary Appendix A: Fig. 1). The two plots were randomly assigned to one of the two treatments, i.e. fungicide and control. The fungicide plots received benomyl as a soil drench (6 g of the active ingredient in 10 L of water per m² every 2 weeks). The control plots received 10 L of water per m² every 2 weeks. Benomyl application was applied simultaneously with grazing treatments and the benomyl and control plots were treated in this manner from 10 June to 1 September each year. The precipitation was vastly different between the two years of the present study, with a growing season (April to August) precipitation of 166.97 mm in 2015 and 67.23 mm in 2016 (see Supplementary Appendix A: Fig. 2). Consequently, we took this opportunity to analyze the inter-annual variation of plant community responses along the grazing intensity gradient (Carmona, Mason, Azcarate, & Peco 2015). To eliminate the potential effect of variations among the paddocks, we defined the three paddocks as blocks.

Plant and soil sampling

One permanent quadrat (1×1 m) was established in each plot to estimate species number in the middle of August. In late August, one sampling quadrat (0.5×1 m) within each plot was cut to ground level to determine the aboveground biomass in 2015 and 2016. In each paddock, we grouped the same fungicide-treated species at each distance along the four different transects as one replicate, oven dried at 65 °C for 48 h before being weighed.

In mid-August of each year, three cylindrical soil cores (10 cm in depth and 7 cm in diameter) were collected from each plot. The soil samples were air-dried after removing roots by sieving through 2-mm mesh. For all plots, the same fungicide-treated soil and root samples were pooled together into one sample for each distance from the four different transects. The soil and root samples were taken immediately to the laboratory.

Laboratory analyses

For the four plant species that occurred in each plot and produced sufficient shoot biomass for measurements, *L. chinensis*, *S. grandis*, *C. squarrosa* and *C. glaucum*, we analyzed P concentration in their shoots. Because *C. glaucum* had low biomass (<0.5 g m⁻²) in 2016, we did not analyze its shoot P in that year. Shoot P concentration was measured using a spectrophotometer, with ascorbic acid and ammonium molybdate as color reagents, following digestion of

Table 1. Analysis of variance for the effects of block (BK), benomyl application (B), grazing intensity (G) and year (Y) on soil available nutrients, soil bulk density and mycorrhizal root colonization. Significant effects of treatments are indicated in bold.

Response	BK	B	G	B*G	Y	Y*BK	Y*B	Y*G	B*G*Y
Df. (factor)	2	1	2	2	1	2	1	2	2
Df. (error)	10				10				
Inorganic N	3.33 ^{NS}	0.47 ^{NS}	0.31 ^{NS}	0.76 ^{NS}	7.27**	4.36*	0.02 ^{NS}	0.38 ^{NS}	1.02 ^{NS}
Available P	4.19*	0.65 ^{NS}	4.14 ^{NS}	0.05 ^{NS}	29.86**	10.66**	0.26 ^{NS}	0.26 ^{NS}	1.26 ^{NS}
Soil bulk density	4.48*	0.24 ^{NS}	1.15 ^{NS}	0.75 ^{NS}	3.04 ^{NS}	0.94 ^{NS}	0.01 ^{NS}	0.01 ^{NS}	0.01 ^{NS}
Mycorrhizal colonization	1.20 ^{NS}	59.48**	52.12**	10.90**	7.18*	0.50 ^{NS}	4.25 ^{NS}	0.60 ^{NS}	1.21 ^{NS}

Note: F-values are shown for each variable followed by their respective significance levels.

* $P < 0.05$.

** $P < 0.01$.

NS $P > 0.05$.

the shoots with perchloric and nitric acids (Bélanger & Rees 2007). Soil inorganic N (ammonium $[NH_4^+ - N]$) and nitrate $[NO_3^- - N]$) contents were analyzed with a flow injection autoanalyser (Flowsys; Ecotech, Germany). Soil available P was determined using the Olsen methods (Bélanger & Rees 2007).

Mycorrhizal root colonization of each plant species could not be precisely estimated because of the tangled roots of the plants in the native grassland. The tangled roots were washed and root segments were cut to estimate AMF root colonization. Root segments were cleaned in 10% (w/v) KOH at 90 °C in a water bath for 2 h and then acidified with 2% (w/v) hydrochloric acid for 5 min. Root segments were washed and stained with 0.05% (w/v) Trypan blue. Thirty root segments from each sample were examined microscopically and the percentage of the root colonized by AMF was assessed (Trouvelot 1986).

Data analysis

Repeated measures ANOVAs were performed to test the effects of benomyl application, grazing intensity and year as well as their interactions on soil available nutrients, percentage of roots colonized, aboveground biomass and species richness. Additional two-factor ANOVAs (with fixed effects: grazing intensity and benomyl application) and one random effect (blocks) were performed to analyze variation in the response among treatments each year. Following ANOVA, *post hoc* comparisons of the means were calculated using Tukey's HSD test ($p < 0.05$). Single linear regression was employed to investigate the relationships between mycorrhizal root colonization and aboveground biomass at the functional group and community levels. The homogeneity and normality of variances were verified for all data using Levene and Kolmogorov–Smirnov tests, respectively. All statistical analyzes were performed using the SPSS statistical package (version 17.0; IBM, Armonk, New York, USA).

Results

The effects of AMF suppression and grazing intensity on mycorrhizal root colonization and nutrients available in soil

AMF suppression effectively reduced mycorrhizal root colonization by 43.1% across all distances (Table 1; Fig. 1A and B). Mycorrhizal root colonization significantly increased with a decrease of grazing intensity (Fig. 1A and B). Across the two years, there was a significant interaction between AMF suppression and grazing intensity on AMF root colonization (Fig. 1A and B). AMF suppression reduced mycorrhizal root colonization by 15.9%, 55.7% and 23.9% at the HG, MG and LG plots, respectively. AMF suppression and grazing intensity did not significantly alter soil inorganic N and available P and soil bulk density (Table 1). In our study, soil inorganic N and soil available P were 6.83 (± 0.29) and 5.52 (± 0.14) mg kg $^{-1}$, respectively, and soil bulk density was 1.31 (± 0.01) g cm $^{-2}$.

The effects of grazing intensity and AMF on plant community composition

The aboveground biomass of the plant community was significantly increased with a decrease of grazing intensity from 2015 to 2016 (Table 2; Fig. 2A and B). AMF suppression did not significantly alter the aboveground community biomass along the grazing gradient in 2015 (Fig. 2A). However, AMF suppression significantly decreased the aboveground community biomass across all grazing intensities in 2016 (Fig. 2B). Across all treatments, aboveground community biomass showed substantial inter-annual variation, with higher aboveground biomass in 2015 than in 2016 (Table 2) and higher AMF colonization in 2015 than 2016. AMF suppression and grazing intensity did not significantly alter species richness across the two years (Table 2).

The aboveground biomass of *L. chinensis* increased with an decrease of grazing intensity (Table 2, Fig. 3A and B). The aboveground biomass of *S. grandis* increased with a decrease

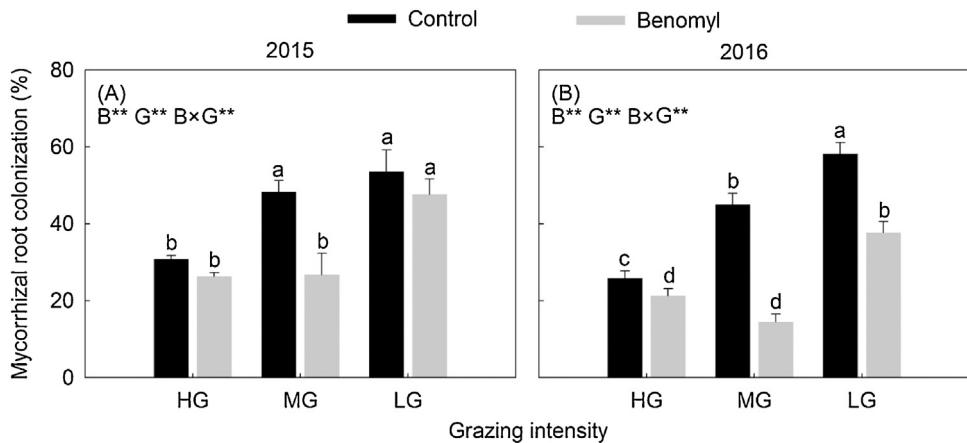


Fig. 1. Effects of benomyl addition (B), grazing intensity (G) and their interaction on mycorrhizal root colonization in 2015 (A) and in 2016 (B). HG, MG and LG indicate heavy grazing, moderate grazing and light grazing, respectively. Data are means \pm SE. ** $P < 0.01$. Bar groups with different capital letters indicate significant difference according to Tukey's HSD test ($P < 0.05$).

Table 2. Analysis of variance for the effects of block (BK), benomyl application (B), grazing intensity (G) and year (Y) on aboveground biomass of the community and individual plant species in 2015–2016.

Response	BK	B	G	B*G	Y	Y*BK	Y*B	Y*G	B*G*Y
Df. (factor)	2	1	2	2	1	2	1	2	2
Df. (error)	10				10				
Community	6.36 [*]	1.69 ^{NS}	49.74^{**}	0.83 ^{NS}	77.10^{**}	4.16[*]	3.61 ^{NS}	6.94[*]	1.20 ^{NS}
Species richness	1.40 ^{NS}	0.05 ^{NS}	0.23 ^{NS}	2.15 ^{NS}	5.39[*]	3.08 ^{NS}	0.08 ^{NS}	6.01[*]	0.07 ^{NS}
<i>L. chinensis</i>	1.07 ^{NS}	1.72 ^{NS}	13.21^{**}	0.26 ^{NS}	22.10^{**}	4.71[*]	0.17 ^{NS}	1.73 ^{NS}	2.53 ^{NS}
<i>S. grandis</i>	0.65 ^{NS}	0.66 ^{NS}	3.77 ^{NS}	0.09 ^{NS}	10.02[*]	0.42 ^{NS}	0.39 ^{NS}	11.31^{**}	0.17 ^{NS}
<i>C. squarrosa</i>	1.47 ^{NS}	0.06 ^{NS}	1.25 ^{NS}	0.13 ^{NS}	16.42^{**}	0.34 ^{NS}	0.37 ^{NS}	4.07 ^{NS}	1.77 ^{NS}
<i>C. glaucum</i>	0.76 ^{NS}	7.42[*]	1.19 ^{NS}	0.90 ^{NS}	64.65^{**}	0.84 ^{NS}	7.01[*]	1.09 ^{NS}	0.91 ^{NS}
<i>S. collina</i>	3.89 ^{NS}	6.27[*]	2.49 ^{NS}	0.19 ^{NS}	37.62^{**}	2.58	5.62[*]	2.04 ^{NS}	0.23 ^{NS}

Note: F-values are shown for each variable followed by their respective significance levels.

* $P < 0.05$.

** $P < 0.01$.

NS $P > 0.05$.

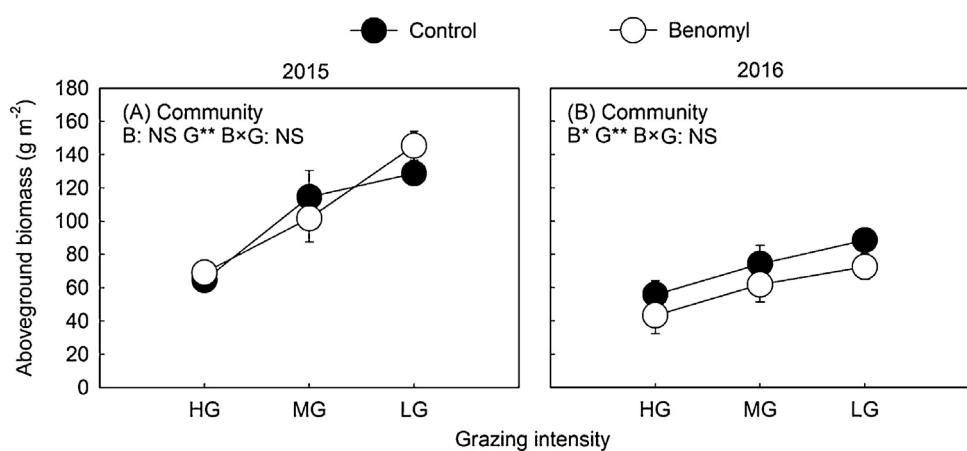


Fig. 2. Effects of benomyl addition (B), grazing intensity (G) and their interaction on aboveground biomass of plant community in 2015 (A) and 2016 (B). HG, MG and LG indicate heavy grazing, moderate grazing and light grazing, respectively. Data are means \pm SE. ** $P < 0.01$; * $P < 0.05$; NS $P > 0.05$.

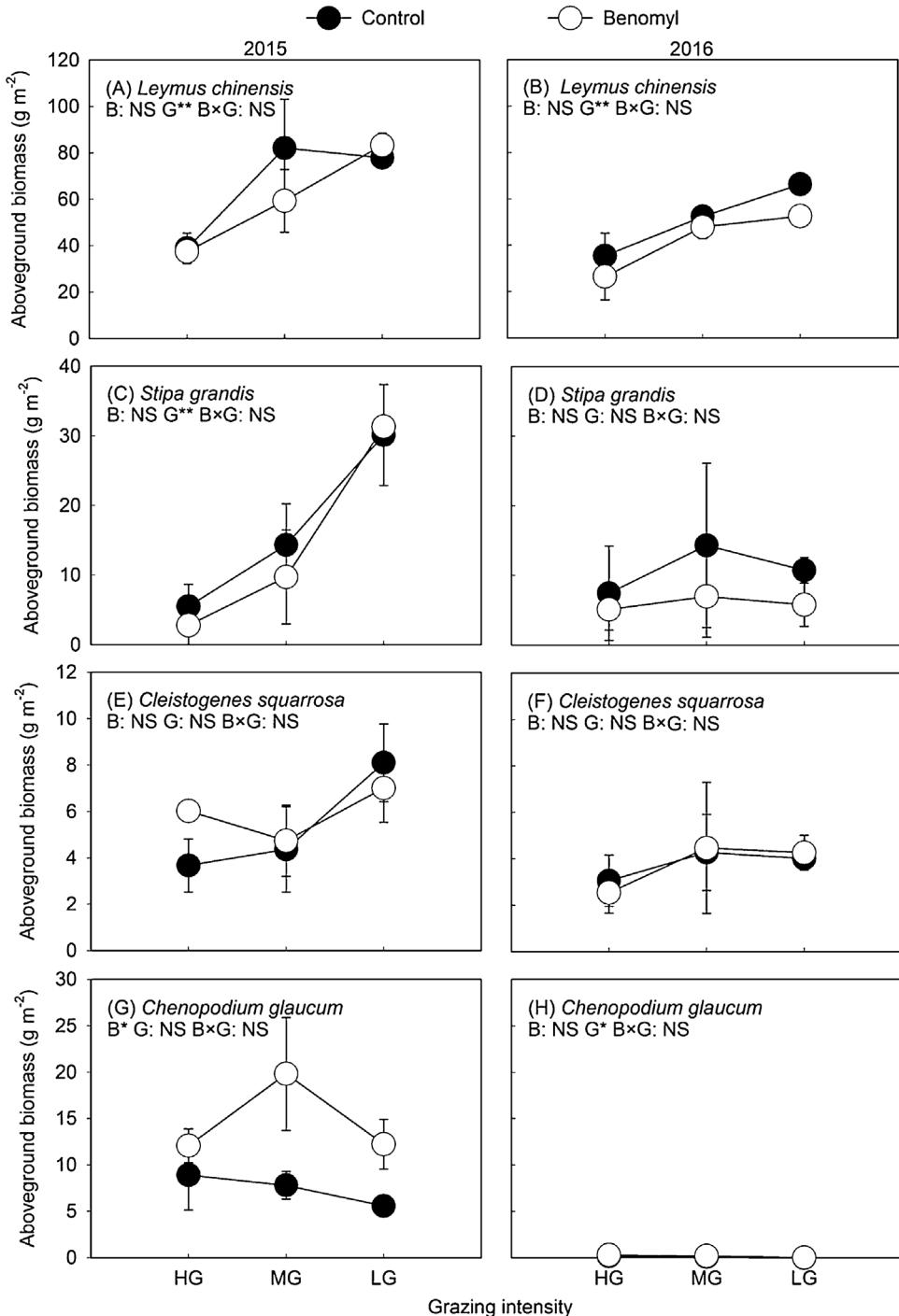


Fig. 3. Effects of benomyl addition (B), grazing intensity (G) and their interaction on aboveground biomass of *L. chinensis* in 2015 (A) and 2016 (B), *S. grandis* in 2015 (C) and 2016 (D), *C. squarrosa* in 2015 (E) and 2016 (F) and *C. glaucum* in 2015 (G) and 2016 (H). HG, MG and LG indicate heavy grazing, moderate grazing and light grazing, respectively. Data are means \pm SE. ** $P < 0.01$; * $P < 0.05$; NS $P > 0.05$.

of grazing intensity only in 2015 (Fig. 3C and D). No significant effects on the aboveground biomass of *C. squarrosa* were detected (Fig. 3E and F). AMF suppression significantly increased the aboveground biomass of *C. glaucum* and *S. collina* in both 2015 and 2016 (Table 2; Fig. 3G and H), but there was no effect on shoot biomass of *L. chinensis*, *S. grandis* and *C. squarrosa* (Table 2).

Mycorrhizal root colonization was positively correlated with the aboveground biomass of the entire community and *L. chinensis* in 2015 and 2016 (Fig. 4A and B), and with the aboveground biomass of *S. grandis* only in 2015 (Fig. 4C). We found no significant relationship between mycorrhizal root colonization and the aboveground biomass of *C. squarrosa* across the 2 years (Fig. 4D). However, mycorrhizal root

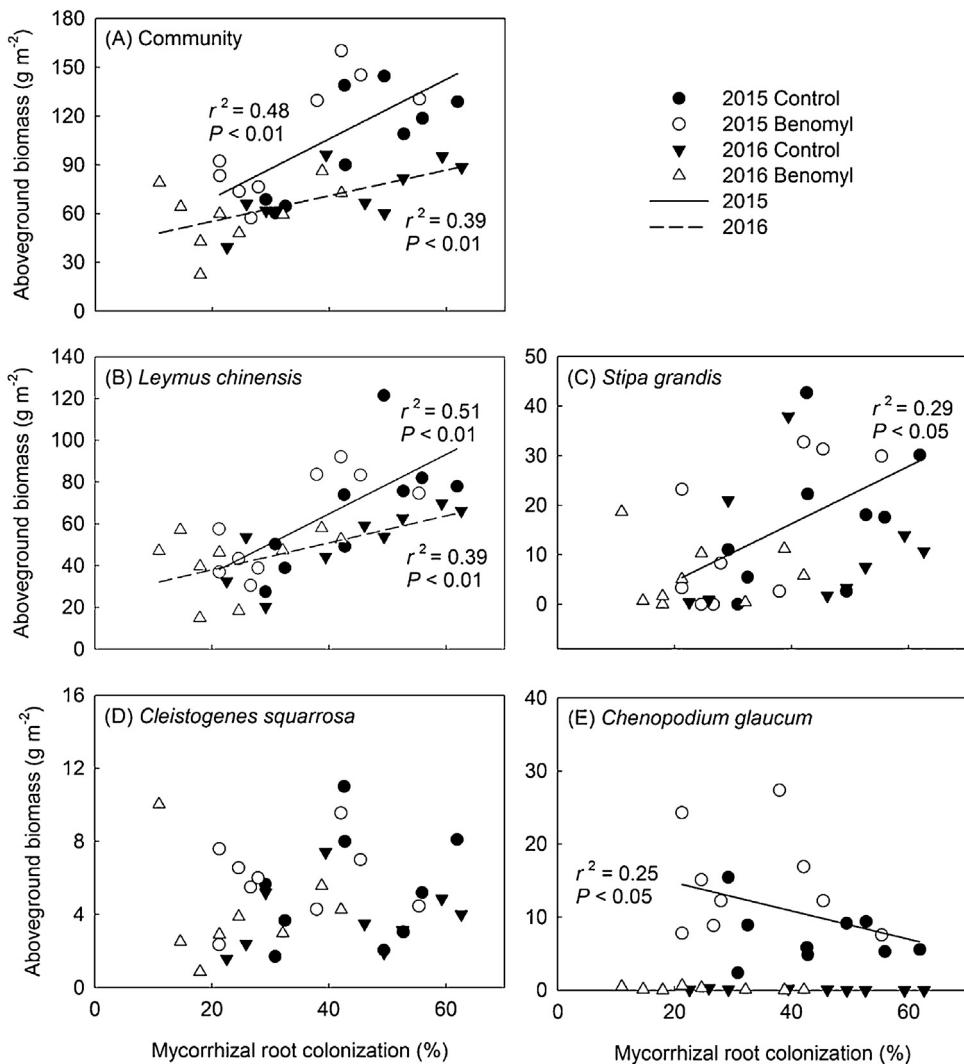


Fig. 4. The mycorrhizal root colonization in relation to community biomass (A), *L. chinensis* biomass (B), *S. grandis* biomass (C), *C. squarrosa* biomass (D), *C. glaucum* biomass (E). Colors indicate the different mycorrhizal treatments: control (black) and benomyl treatment (white). Shapes indicate the different years: 2015 (circle) and 2016 (triangle).

colonization was negatively correlated with the aboveground biomass of *C. glaucum* in 2015 (Fig. 4E).

The effects of grazing intensity and AMF on plant nutrient acquisition

No significant effects on the shoot P concentration of *L. chinensis* and *C. squarrosa* were detected (Fig. 5A, B, E and F). AMF suppression reduced P concentrations in *S. grandis* by 33.15% and 31.13% at the HG and LG plots, respectively, but it did not alter that at the MG plots in 2015 (Fig. 5C and D). Across all grazing intensities, AMF suppression significantly increased P concentration of *C. glaucum* (Fig. 5G).

AMF suppression significantly decreased shoot P content of *L. chinensis* in 2015 and in 2016 (Table 3; Fig. 6A and B), but there was no effect on shoot P content in *S. grandis* and *C. squarrosa* (Fig. 6C–F). The shoot P content of *S. grandis*

increased with decreasing grazing intensity in 2015 (Fig. 6C), and AMF suppression significantly increased shoot P content in *C. glaucum* only in 2015 (Fig. 6G).

Discussion

Grazing intensity changed plant community composition by altering the growth responses of different plant species in the typical steppe ecosystem. Our results showed that heavy grazing around watering points led to selective removal of *L. chinensis*, which tended to promote the growth of forbs. Consistent with previous results (Wan, Bai, Schonbach, Gierus, & Taube 2011; Zhang et al. 2014), aboveground biomass of *L. chinensis* declined and the relative abundance of C₄ forbs increased with increasing grazing intensity. The palatability of C₃ grasses is higher than that of forbs (mainly annual

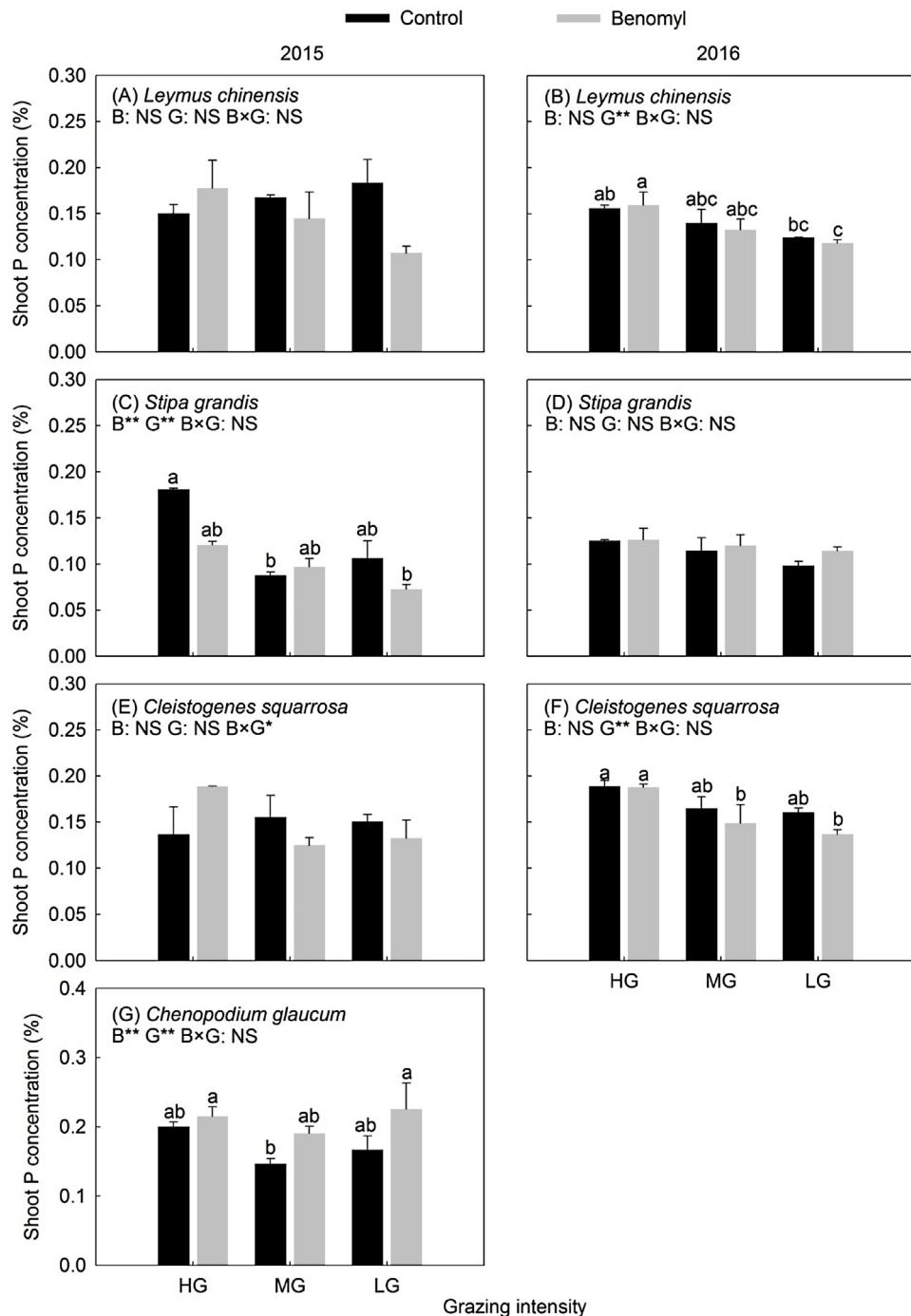


Fig. 5. Effects of benomyl addition (B), grazing intensity (G) and their interaction on aboveground biomass P concentration of *L. chinensis* in 2015 (A) and 2016 (B), *S. grandis* in 2015 (C) and 2016 (D), *C. squarrosa* in 2015 (E) and 2016 (F) and *C. glaucum* in 2015 (G). HG, MG and LG indicate heavy grazing, moderate grazing and light grazing, respectively. Data are means \pm SE. * $P < 0.05$; ** $P > 0.05$. Bar groups with different capital letters indicate significant difference according to Tukey's HSD test ($P < 0.05$).

C_4 forbs *C. glaucum* and *S. collina*; Chen, Hooper, & Lin 2011). Grazing benefits the unpalatable plant species due to preferential removal of the palatable species (Augustine & McNaughton 1998). Our results showed that grazing intensity did not change aboveground biomass of C_4 grasses (*C. squarrosa*). This result can be explained partially by the dif-

ference in height between C_4 and C_3 grasses. C_4 grasses present at the study site have a lower shoot height (<10 cm) than C_3 grasses (20–50 cm), and thus livestock tend to avoid C_4 grasses when grazing (Zhang et al. 2014).

Apart from defoliation, livestock trampling can damage plant foliage and alters the soil physical properties such as soil

Table 3. Analysis of variance for the effects of block (BK), benomyl application (B), grazing intensity (G) and year (Y) on shoot P in four species. Significant effects of treatments are indicated in bold.

Response	BK	B	G	B*G	Y	Y*BK	Y*B	Y*G	B*G*Y	
Df. (factor)	2	1	2	2	1	2	1	2	2	
Df. (error)	10					10				
<i>L. chinensis</i>										
P concentration	0.98 ^{NS}	1.41 ^{NS}	1.93 ^{NS}	2.01 ^{NS}	5.43*	0.01 ^{NS}	2.10 ^{NS}	0.57 ^{NS}	3.58 ^{NS}	
P content	0.52 ^{NS}	7.69*	2.44 ^{NS}	2.48 ^{NS}	13.74**	1.16 ^{NS}	3.98 ^{NS}	5.57*	3.16 ^{NS}	
<i>S. grandis</i>										
P concentration	0.44 ^{NS}	8.78*	17.21**	2.81 ^{NS}	1.78 ^{NS}	3.11 ^{NS}	5.83*	13.27**	6.47**	
P content	0.22 ^{NS}	2.05 ^{NS}	2.37 ^{NS}	0.56 ^{NS}	3.64 ^{NS}	0.59 ^{NS}	0.66 ^{NS}	5.28*	0.80 ^{NS}	
<i>C. squarrosa</i>										
P concentration	2.29 ^{NS}	0.54 ^{NS}	5.39*	3.68 ^{NS}	7.08*	4.57*	1.44 ^{NS}	0.77 ^{NS}	2.65 ^{NS}	
P content	0.99 ^{NS}	0.01 ^{NS}	0.58 ^{NS}	1.09 ^{NS}	5.78*	0.05 ^{NS}	0.08 ^{NS}	2.42 ^{NS}	1.35 ^{NS}	
<i>C. glaucum</i>										
P concentration	2.35 ^{NS}	11.89**	8.08**	0.99 ^{NS}						
P content	0.54 ^{NS}	10.36**	0.54 ^{NS}	1.00 ^{NS}						

Note: F-values are shown for each variable followed by their respective significance levels.

*P<0.05.

**P<0.01.

NS P>0.05.

bulk density (van Klink et al. 2015). In our study, the decrease of shoot biomass of community and dominant species (*L. chinensis*) under heavy grazing may be attributed partly to trampling. Besides, previous studies found that sheep trampling can promote soil–seed contact and seedling emergence (Blumenthal & Ison 1996). In our experiment, heavy grazing promoted growth of annual forbs, which may be due to sheep trampling enhancing seedling establishment. Moreover, our results suggested that there was no evidence for changes in soil bulk density or soil available nutrients due to distance from the watering point. This is consistent with the findings of previous studies, which found that grazing did not alter soil bulk density or soil inorganic N at distances of 10, 50 and 80 m from water or shade (Franzleubbers, Stuedemann, & Schomberg 2000). Moreover, hoof prints and excrements of livestock tend to become concentrated at distances within 1 m of water or shade (Franzleubbers et al. 2000).

Our study showed AMF suppression improved *C. glaucum* growth by increasing its shoot P nutrition along a grazing gradient. *C. glaucum* was known to be less dependent on AM colonization (Tian et al. 2009a). Indeed, recent meta-analyses also indicated that annual species had higher specific root lengths and abilities for rapid nutrient acquisition compared to perennial species (Lin, McCormack, & Guo 2015). Our study also found that AMF suppression decreased the shoot P nutrition of *L. chinensis* especially under moderate and light grazing. Moderate grazing intensity can enhance the photosynthetic capability of *L. chinensis* (Zhao et al. 2009), which can alleviate soil nutrient limitation on plant regrowth (Ba et al. 2012). Moderate grazing induces higher allocation of carbon to AMF (Eom et al. 2001; Hartnett & Wilson 2002), which may provide limited nutrients to *L. chinensis*.

Benomyl addition over two growing periods resulted in substantial decreases in mycorrhizal root colonization along the grazing gradient, particularly in the second year. AMF root colonization decreased by approximately 43.10% relative to the control across all distances during the two growing periods, consistent with previous studies in which benomyl was applied to native grasslands (Hartnett & Wilson 1999; O'Connor et al. 2002; Yang et al. 2014). Several studies have found that benomyl addition may alter plant community composition by increasing soil nutrient availability (Chen & Edwards 2001). However, our study suggested that benomyl addition did not measurably alter soil inorganic N and available P, consistent with previous work that applied benomyl to similar grasslands (Yang et al. 2014). Benomyl addition did not totally suppress mycorrhizal root colonization in our study, consistent with previous findings in steppe ecosystems (Yang et al. 2014; Zhang et al. 2016). Benomyl only inhibits AMF to penetrate through the cell cuticula of plant roots to some extent (Köller, Allan, & Kolattukudy 1982; Kahiluoto, Ketoja, & Vestberg 2000). Furthermore, our results suggested that the effects of AMF on plant community structure and plant nutrition *in situ* may be underestimated (Kahiluoto et al. 2000; Yang et al. 2014).

Inter-annual variation in precipitation is the key factor contributing to variation in plant growth responses in semiarid grassland (Bai, Han, Wu, Chen, & Li 2004; Carmona et al. 2015). The amount of precipitation during the growing season in 2016 was about 31.9% of long-term mean. Our results suggested that plant community biomass and functional groups were dependent on inter-annual variation in precipitation. The variation in plant community biomass resulted mainly from the inter-annual changes in biomass of annual forbs,

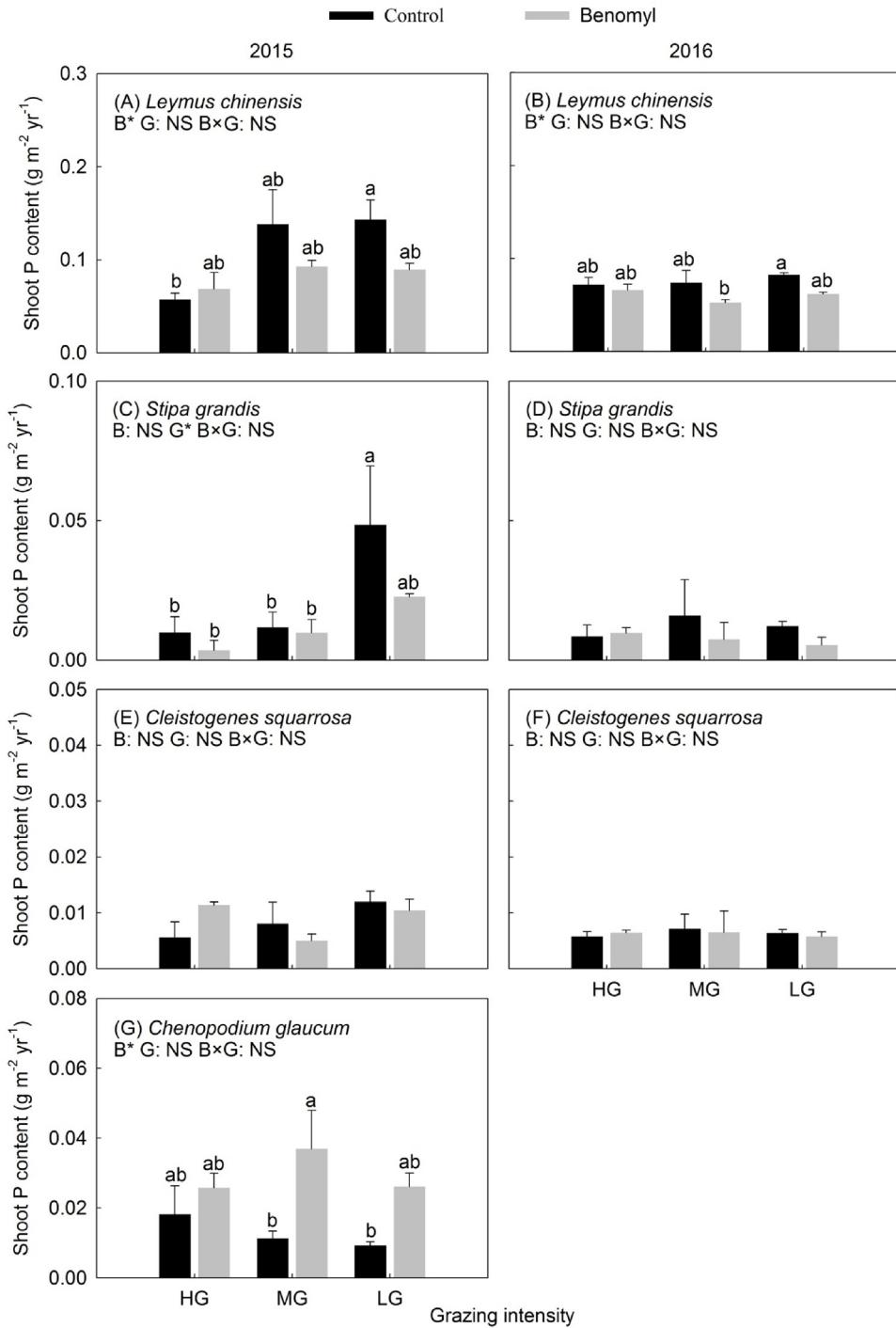


Fig. 6. Effects of benomyl addition (B), grazing intensity (G) and their interaction on aboveground biomass P content of *L. chinensis* in 2015 (A) and 2016 (B), *S. grandis* in 2015 (C) and 2016 (D), *C. squarrosa* in 2015 (E) and 2016 (F) and *C. glaucum* in 2015 (G). HG, MG and LG indicate heavy grazing, moderate grazing and light grazing, respectively. Data are means \pm SE. * $P < 0.05$; $^{NS}P > 0.05$. Bar groups with different capital letters indicate significant difference according to Tukey's HSD test ($P < 0.05$).

which was substantially higher in 2015 than in 2016. Consistent with previous result (Chen et al. 2011), unpalatable annual species became dominant in the relatively wet year and then disappeared in the subsequent dry year. Furthermore, the shoot biomass of *S. collina* contributed most to the total aboveground biomass when soil water availability

was high in this region (Li, Lin, Taube, Pan, & Ditttert 2011). With sharp increases in soil water, fast-growing annuals can obtain a competitive advantage because they have higher water use efficiency than perennials (Ehleringer, Schwinnig, & Gebauer 2000). The experiment was conducted over a relatively short time period, which might have prevented us from

observing substantial growth changes in perennial species and temporal shifts in soil nutrient levels. Thus, we recommend caution in interpreting the effects of short-term grazing and benomyl application as an indicator of their long-term effects.

Conclusions

We found that grazing intensity alone substantially altered plant community shoot biomass and affected growth of dominant species, and AMF by itself had few impacts. AMF only suppressed the dominance of *C. glaucum* independent of grazing intensity, but AMF effects were higher in the wet year than in the dry year. Temporal variability of climatic conditions in terrestrial ecosystems is predicted to increase in the future because of climate change (Knapp et al. 2008). The high variability of the precipitation pattern may lead to a high incidence rate of drought and extreme rainfall events (Dickson & Foster 2011). Therefore, the drastic precipitation fluctuation observed in our study, and the alteration of plant community in response to grazing intensity and AMF, are likely to become even more common in the future.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.baae.2018.07.002>.

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