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How do mycorrhizal suppression and plant functional group loss affect plant communities in Inner Mongolia Steppe?

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Abstract

Questions: Although many studies have investigated how biodiversity loss impacts on ecosystem functioning, we still have little understanding of how it interacts with arbuscular mycorrhizal fungi (AMF) to affect plant communities in natural grasslands. Therefore, here, we conducted a removal experiment to examine how AMF suppression and plant functional group (PFG) removal affect aboveground productivity and community composition in a grassland, and to determine whether AMF alter the compensation ability of the remaining plants.

Location: Inner Mongolian grassland, China.

Methods: We suppressed AMF activities by applying Topsin®-M as a soil drench [Page 2] and selectively removed PFGs to give three treatments (no removal, removal of C_3 grasses, and removal of both C_4 grasses and forbs). We then measured various plant, soil, and AMF parameters for each treatment combination.

Results: We found that the addition of Topsin-M effectively reduced mycorrhizal root colonization across all of the PFG removal treatments. Furthermore, aboveground productivity was significantly impacted by both the presence of AMF and the removal of PFGs. When C_3 grasses were removed, neither C_4 grasses nor forbs compensated for the biomass decline, and the presence of AMF did not affect their compensation ability. Conversely, C_3 grasses could completely compensate for the removal of both C_4 grasses and forbs but the presence of AMF reduced their compensation ability. The removal of both C_4

grasses and forbs dramatically increased the shoot phosphorus (P) content of C_3 grasses but AMF slightly decreased this. By contrast, AMF significantly increased the plant P content of C_4 grasses and forbs across all three PFG removal treatments.

Conclusion: Our results highlight the importance of AMF in structuring natural aboveground productivity under various biodiversity loss scenarios and indicate that AMF will be able to modify vegetation dynamics in response to the future loss of plant diversity.

Keywords: Arbuscular mycorrhizal fungi; Biodiversity; Grassland ecosystem; Plant functional group; Removal experiment; Compensation; Plant productivity; Ecosystem functioning

Nomenclature: Fu & Li (1995) for plants.

[Page 3] Abbreviations: AMF = arbuscular mycorrhizal fungi

Running Title: Mycorrhizal effects on compensation

[Page 4] Introduction

The effect of biodiversity loss on ecosystem functions and services has attracted a large amount of interest among ecologists in recent years (Loreau et al. 2001; Hooper et al. 2005; Tilman et al. 2012). However, although it has been shown that biodiversity loss greatly affects ecosystem structure and functioning in artificial communities (Cardinale et al. 2012), it has been argued that these results and conclusions cannot be generalized to native communities because synthetic communities only contain particular plant species and

assemblages (Duffy 2009; Jiang et al. 2009). Specially, plant diversity lies at the core of the links between biodiversity and ecosystem functions (Isbell et al. 2011). Therefore, knowledge of vegetation dynamics in natural ecosystems responses to plant diversity loss is important to understand the possible effects of future biodiversity loss.

A growing body of research has demonstrated that plant diversity loss affects vegetation properties (e.g. primary productivity and plant species richness), plant-available nutrients in the soil, and net ecosystem carbon exchange (Wardle et al. 1999; McLaren & Turkington 2010; Kong et al. 2011; Maestre et al. 2012; Winfree et al. 2015; Pan et al. 2016). Furthermore, recent experimental studies have shown that plant diversity loss also affects the structure and functioning of the belowground community (Marshall et al. 2011; Cardinale et al. 2012; Chen et al. 2016). For example, Chen et al. (2016) found that the loss of dominant plant functional groups (PFGs) in a temperate ecosystem altered the belowground communities (microbes and nematodes), whereas the loss of sub-dominant PFGs had no effect. Here, PFGs are [Page 5] defined as the groups of plants in community that share similar plant morphological characteristics and show similar growth responses to environmental changes, and therefore have similar influences on main ecosystem processes (Lavorel et al. 1997). However, Marshall (2011) found that the soil microbial community in a northern Canadian grassland ecosystem was relatively insensitive to the loss of PFGs. Therefore, differences in the responses of soil microbes to PFG loss may relate to specific characteristics of different grasslands.

Arbuscular mycorrhizal fungi (AMF) are an important component of the soil microbial community as they play a critical role in altering plant community diversity, composition, and primary productivity (van der Heijden et al. 1998; Hartnett & Wilson 1999). AMF improve the uptake of phosphorus (P) in host plant, which, in return, provides them with photosynthates for their survival (Smith & Read 2008). Experiments with artificial communities have demonstrated that plant community diversity affects AMF properties such as mycorrhizal colonization and the number and species composition of the spores (van der Heijden et al. 1998; Burrows & Pfleger 2002; Chen et al. 2004), whereas field studies found that the PFG removal did not affect AMF colonization or spore density in a mountain shrubland (Urcelay et al. 2009) or a northern grassland (Marshall et al. 2011), indicating AMF community resilience to experimental PFG loss. However, we still lack a comprehensive understanding of how AMF interact with their host plants under different diversity loss scenarios in natural grasslands.

A major feature of native grasslands is that the PFGs have different abundances [Page 6] within a particular community (Ives & Cardinale 2004). Previous studies have shown that loss of dominant PFGs has a greater impact on ecosystem functions, such as primary productivity and soil nitrogen (N) use, than that loss of rare PFGs (Longo et al. 2013; Winfree et al. 2015; Pan et al. 2016). For example, Pan et al. (2016) found that the loss of two dominant PFGs from a C_3 grass-dominated grassland resulted in declines in plant community biomass, even when the sub-dominant PFGs were present. However, McLaren & Turkington (2010, 2011) found that the biomass declines that were induced by the loss of dominant PFGs in a forb-dominated grassland were partly compensated for by sub-dominant PFGs. Thus, it

appears that dominant and sub-dominant PFGs may have different and/or context-dependent effects on primary productivity. Moreover, the mycorrhizal dependence of sub-dominant species or PFGs determines the effects of AMF on plant community composition (Urcelay & Díaz 2003). Whereas, to the best of our knowledge, there is little understanding of how AMF alter the growth responses of dominant and sub-dominant PFGs under PFG loss.

Pioneering experiments have shown that the effects of biodiversity loss on ecosystem properties depend on the identities of not only the lost PFGs but also those that remain (McLaren & Turkington 2010; Cardinale et al. 2012), as these can expand to replace those that were lost through a process known as compensation (Adler & Bradford 2002; Pan et al. 2016). It has previously been found that grasses colonize quickly after the removal of other species, whereas forbs show no increase in biomass (McLaren & Turkington 2011). In addition, PFGs are also considered to be more [Page 7] important than other factors for predicting plant mycorrhizal growth responses, with forbs showing more positive responses to AMF than C_3 grasses (Hoeksema et al. 2010). However, few studies have examined whether AMF alter the degree of biomass compensation after PFG removal.

The aim of this study was to improve our understanding of the effects of AMF on native vegetation dynamics under PFG loss by conducting a removal experiment in the Inner Mongolia grassland, China. Removal experiments are particularly useful in ecosystems where artificial communities are difficult to create, such as Arctic and temperate ecosystems that are dominated by long-lived perennial species (McLaren & Turkington 2011; Wu et al. 2015). Specifically, we addressed the following questions: (1) How do AMF and PFG removal

affect aboveground productivity, community composition and plant nutrient uptake (P)? (2) Do AMF also alter the compensation ability of the remaining plants after PFG removal?

Methods

Study site

This experiment was conducted in a semiarid steppe (42°01′N, 116°17′E; 1324 m above sea level) in Inner Mongolia, China. This region has a monsoon climate, with a long-term (1953–2010) mean annual precipitation of approximately 378 mm and mean annual temperature of 2.1°C. The soils are classified as Calcis-orthic Aridisol and the vegetation type is classified as a temperate steppe, in which the dominant plant species are mainly perennial herbs such as *Artemisia frigida* and *Stipa krylovii* (Bai et al. 2015). More than 80% of the plant species at the study site have been [Page 8] identified as mycorrhizal plants (Bao & Yan 2004; Tian et al. 2009). Previous studies have shown that PFG richness is more strongly related to primary productivity than plant species diversity (Bai et al. 2004; Hooper et al. 2005), and that C_4 grasses and forbs have stronger mycorrhizal dependencies than C_3 grasses (Hoeksema et al. 2010; Lin et al. 2015). Therefore, prior to our study, we categorized the initial plant community into three PFGs: C_3 grasses, C_4 grasses, and forbs, which accounted for approximately 14.77%, 11.16%, and 74.06% of the total aboveground biomass, respectively (Yang et al. 2014).

Experimental design

From May 2015 to September 2017, we carried out a removal experiment using a two-factor random block design, which included factorial combinations of fungicide (F; no fungicide vs.

fungicide treatment) and PFG removal (Removal; no removal, removal of C_3 grasses, and removal of both C_4 grasses and forbs), as well as one treatment with no water or fungicide addition (Appendix S1: Table S1; Appendix S2: Fig. S1). The plots were 2.2 × 2.2 m in size and were spaced 2.0 m apart. The AMF suppression (fungicide treatment) plots received Topsin®-M as a soil drench (1.25 g of the active ingredient in 1.875 L of water per m^2 every 2 weeks), while the control (mycorrhizal treatment) plots only received 1.875 L of water per m^2 every 2 weeks, and both treatments were simultaneously conducted from May to September of each year (2015–2017). Previous field studies have used the fungicide benomyl to suppress AMF (Wilson et al. 2009; Yang et al. 2014; Zhang et al. 2016). However, here, we chose to use Topsin-M because it is a successful alternative for the suppression of [Page 9] AMF that has the same mode of action as benomyl (Wilson & Williamson 2008) and can effectively reduce the mycorrhizal colonization of roots to approximately 60%–80% compared with those in control plots (Wilson & Williamson 2008; McCain et al. 2011).

For the PFG removal treatments, the target PFGs were completely removed by clipping the aboveground parts of the plants and tillering the nodes at 0–5 cm soil depth to minimize physical disturbance to the soil (Chen et al. 2016). To ensure that the growth of the targeted PFGs stopped or at least was significantly suppressed, we clipped the plants in early June of each year.

Sampling procedure

We established one permanent quadrat (1 × 1 m) in each plot to estimate plant species richness in early August of each year. In mid-August of each year, we then clipped all of the

plants to ground level in one sampling quadrat (0.5 × 1 m) within each plot to determine the aboveground biomass of the community and each plant species. All plants were sorted into species and oven-dried at 65°C for 48 h before being weighed. The dry weight of each plant species was recorded separately and expressed as the shoot biomass per m^2. Unlike the determination of shoot biomass, the root biomass of each species / PFG can not be determined precisely due to the roots of plants being tangled in the field. The relatively coarse roots of individual plant species may be collected by washing with water (Bai et al. 2015), but fine roots could not be completely isolated because they were intermingled with roots of other plants (van der Heijden et al. 2006). Therefore, we did not measure the root biomass of each species /PFG in the present study. In addition, we calculated the Shannon–Wiener index (H') for each sampling occasion using the equation:

$$H' = -\sum_{i=1}^{S} pi \ln(pi)$$

where S is the number of plant species in a given plot and pi is the relative biomass of species i in a plot.

In mid-August of each year, we also randomly collected three soil cores (10 cm depth and 7 cm diameter) from the outside area of the permanent quadrat and the previously sampled quadrats in each plot, which were combined *in situ* into one composite sample. Stones and roots were removed from each soil sample (approximately 200 g) by sieving it through a 2 mm mesh, and the roots were then separated from the stones by washing with water. In addition, to assess the impacts of fungicide application and PFG removal on soil

microbes, the soil samples (approximately 15 g) were frozen at −80°C for phospholipid fatty acid (PLFA) analysis in 2016 (see below).

Calculation of the compensation index

To evaluate the compensation capabilities of PFGs and their potential interactions with AMF, we calculated the compensation index (*CI*) for each treatment using the following equation:

$$CI = \frac{\sum_{i}^{n}(O_i - E_i)}{\sum_{i}^{N} E_i - \sum_{i}^{n} E_i}$$

where O_i is the observed shoot biomass of PFG *i* in the depleted community, E_i is the [Page 11] expected shoot biomass of PFG *i* in the depleted community (equal to the yield of PFG *i* in the full community), *n* is the set of PFGs in the depleted community, and *N* is the set of PFGs in the full community. $CI < 0$ indicates no compensation, $0 < CI < 1$ indicates partial compensation, $CI = 1$ indicates complete compensation, and $CI > 1$ indicates overcompensation (Adler & Bradford 2002).

Laboratory analyses

To estimate the effects of field treatments on the main function of AMF in the host plant growth (P acquisition), we analyzed the shoot P concentrations of C_3 grasses, C_4 grasses, and forbs that produced sufficient shoot biomass for measurement. After digesting the shoots with

perchloric and nitric acids (Bélanger & Rees 2007), the shoot P concentration was measured using a spectrophotometer, with ammonium molybdate and ascorbic acid as color reagents.

Soil nutrient availablity can also alter AMF effects on plant growth and P acquisition (Johnson et al. 2010; Johnson et al. 2015). To estimate potential effects of field treatments on soil chemical properties, we measured soil available P and inorganic N. The soil available P was determined using the Olsen method (Bélanger & Rees 2007), the soil inorganic N (NH_4^+-N and NO_3^--N) contents were measured with a flow injection autoanalyzer (Flowsys; Ecotech, Germany), and soil moisture was measured by oven-drying a fresh soil sample from each plot (20 g) at 105°C for 24 h.

To estimate the influences of treatments on the extra-radical and intra-radical abundance of AMF, we analyzed PLFA concentration in soil and mycorrhizal root [Page 12] colonization, respectively. Qualitative and quantitative PLFA analyses were performed using a modification of the Bligh and Dyer method (Frostegard et al. 1991) with an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) and Sherlock software (MIDI Inc., Newark, NJ, USA). The following fatty acids were used as indicators: 16:1ω5c for AMF; 18:2ω6,9 for other fungal PLFAs (Schnoor et al. 2011); a15:0, a17:0, i15:0, i16:0, i17:0, 16:1ω7, 17:0, cy17:0, and cy19:0 for the total bacterial PLFAs (Buyer et al. 1999; Moore-Kucera & Dick 2008).

Mycorrhizal root colonization could not be determined precisely for each species due to the roots of plants being tangled in the field. To estimate AMF root colonization, the tangled roots were cut into 1 cm root segments, which were cleaned in 10% (w/v) potassium

hydroxide (KOH) at 90°C in a water bath for 2 h, acidified with 2% (w/v) hydrochloric acid (HCl) for 5 min, and then washed and stained with 0.05% (w/v) trypan blue. We examined 30 root segments from each root sample microscopically to estimate AMF root colonization (Trouvelot 1986).

Statistical analyses

We found no significant difference between the treatment with no water or Topsin-M addition and the treatment with water and no Topsin-M addition in terms of the AMF properties and shoot biomass of each PFG. Therefore, we removed the former treatment from all subsequent analyses.

To test the effects of year and treatments on each of the plant, soil, and AMF parameters, we used repeated measures analysis of variance (ANOVA), with the AMF and PFG removal treatments included as between-subject factors and year [Page 13] included as a within-subject factor. Because year had a significant effect for nearly all of the parameters tested (shoot biomass, species richness, Shannon–Wiener index, compensation index and shoot P content), a separate two-way ANOVA (generalized linear model; GLM) was performed for each year to test the effects of treatments and their interactions. A two-way ANOVA was used to analyze the effect of treatments on the soil microbial PLFA concentrations because these were estimated only once in 2016. We also used a two-way ANOVA to analyze the treatment effects on AMF root colonization, soil inorganic N, and soil available P because these were

only estimated once in August 2017. An independent-samples *t*-test was used to detect significant differences between the control and Topsin-M treatments for each PFG removal level. The homogeneity and normality of variances were verified for all data using Levene and Kolmogorov–Smirnov tests, respectively. All data were analyzed using the SPSS statistical package (version 17.0; IBM, Armonk, New York, USA) with a significance level of $P < 0.05$.

Results

Effects of treatments on plant shoot biomass and diversity

There was a significant interaction effect between fungicide and PFG removal on the shoot biomass of community over time (F × Removal: $F_{2, 25} = 4.73$, $P < 0.05$; Table 1). The fungicide treatment significantly increased the community shoot biomass in the combined C_4 grasses and forbs removal plots but had less effect in the control and C_3 grasses removal plots (Fig. 1a–c). Across 3 years treatment, PFG removal significantly reduced plant species richness and the Shannon–Wiener index, [Page 13] particularly when both C_4 grasses and forbs were removed (Fig. 2). The fungicide treatment only reduced the Shannon–Wiener index across all removal treatments in 2017 (Fig. 2).

There was also a significant interaction between fungicide and PFG removal in their effects on the shoot biomass of C_3 grasses across the 3 years (F × Removal: $F_{1, 25} = 5.66$, $P < 0.05$; Table 1). The fungicide treatment significantly increased the shoot biomass of C_3 grasses only in the combined C_4 grass and forb removal plots from 2015 to 2017 (Fig. 3a–c)

and significantly reduced the shoot biomass of C_4 grasses only in 2017 (Fig. 3d–f). Neither fungicide nor PFG removal significantly affected the shoot biomass of forbs (all $P > 0.05$; Fig. 3g–i).

Effects of treatments on plant shoot P content

There was a significant interaction effect between fungicide and PFG removal on the shoot P content of the plant community over time (F × Removal: $F_{2, 25} = 7.46$, $P < 0.01$;Table S2). The fungicide treatment increased the shoot P content of the plant community in both the control and the combined C_4 grasses and forbs removal plots but reduced it in the C_3 grasses removal plots from 2015 to 2017 (Table S2; Fig. 4). PFG removal significantly increased the shoot P content of C_3 grasses and this was not affected by AMF across 3 years (Table S2; Fig. 5a–c), whereas the fungicide treatment significantly reduced the shoot P contents of C_4 grasses and forbs which was not affected by PFG removal (Table S2; Fig. 5d–i).

Effects of treatments on compensation index

[Page 15] The *CI*s were much higher for C_3 grasses (close to 1 in 2016 and 2017; Fig. 6a) than for C_4 grasses and forbs (much less than 0; Fig. 6b and c). The fungicide treatment significantly increased the *CI*s of C_3 grasses but did not affect those of C_4 grasses and forbs (independent-samples *t*-test; Fig. 6a–c).

Effects of treatments on AMF and soil properties

There was a significant interaction between fungicide and PFG removal in their effects on mycorrhizal root colonization (F × Removal: $F_{2, 25} = 12.45$, $P < 0.01$), with the addition of

fungicide decreasing mycorrhizal root colonization by 26.92% in the no removal plots and by 63.04% in the C_3 grasses removal plots, but having no effect on mycorrhizal root colonization in the combined C_4 grasses and forbs removal plots (Fig. 7). Fungicide addition significantly suppressed the AMF PLFA concentration across all removal treatments ($F_{1, 25} = 4.93$, $P < 0.05$; Fig. S2) but did not alter the bacterial and other fungal PLFA concentrations in 2016 (all $P > 0.05$; Table S3). Neither fungicide addition nor PFG removal affected soil inorganic N and available P contents (all $P > 0.05$; Fig. S3).

Discussion

Aboveground productivity was significantly decreased when both C_4 grasses and forbs were removed from plots, but it was not affected by the loss of C_3 grasses (Fig. 1; Table 1). It is suggested that the PFG loss could erode aboveground productivity in a temperate steppe community (Fig. 1; Table 1), consistent with the conclusions of other removal experiments (Wardle & Zackrisson 2005; Flombaum & Sala 2008; Pan et al. 2016). Changes in aboveground productivity were derived from not only the [Page 16] identity of the PFGs but also the presence of AMF. Our results showed that AMF strongly suppressed C_3 grass growth when both C_4 grasses and forbs were removed from the plots (Fig. 3a–c; Table 1), it might be reasoned from AMF suppressing the shoot P content of C_3 grasses (Fig. 5a–c; Table S2). Similar to our study, previous studies also found that the application of a fungicide increased graminoid shoot biomass (McLaren & Turkington 2010, 2011). However, belowground productivity of plant community was much higher than aboveground productivity in a

temperate steppe (Yang et al. 2010). Unfortunately, we only measured the aboveground productivity, without belowground one, thus total plant productivity was not assessed and might make a future study.

The removal of both C_4 grasses and forbs dramatically reduced plant species richness and diversity, whereas the removal of C_3 grasses had little effects (Fig. 2). This suggests that forbs are the main contributor to plant richness and diversity in this grassland ecosystem (Fig. 2). The addition of fungicide also had no effect on plant richness over time regardless of the PFG removal (Fig. 2a–c), which is consistent with the findings in a similar grassland ecosystem (Yang et al. 2014).

Wardle et al. (1999) predicted that compensation for biomass loss depended on the traits of the remaining plants than of those removed. However, the degree of biomass compensation depended on both the identity of the remaining PFGs and the presence of AMF in this study. After 3 years of recovery, C_3 grasses could completely compensate for the biomass loss of both C_4 grasses and forbs but AMF decreased this biomass compensation ability (Fig. 6). The present results might be reasoned from the [Page 17] C_3 grasses at our study site contains perennial rhizomatous grasses (*Carex korshinskyi*) and perennial bunch grasses (*Stipa krylovii*), which have been shown to have a strong compensation ability (Fig. S4), due to their highly developed rhizome system (Wang et al. 2004) allowing them to quickly occupy the space caused by species losses (Symstad & Tilman 2001). Alternatively, the C_3 grasses usually have fibrous and highly branched root systems with rapid nutrient uptake rates as a result of the high root surface area (Wilson & Hartnett 1998). Therefore, C_3

grasses are less dependent on AMF–indeed, and often show few positive and sometimes even negative responses to AMF inoculation (Wilson & Hartnett 1998; Lin et al. 2015).

In the C_3 grass removal plots, neither C_4 grasses nor forbs compensated for the biomass loss of C_3 grasses (Fig. 6), and AMF slightly improved the biomass of C_4 grasses (Fig. 3f) and shoot P content of them (Table S2; Fig. 5). Similarly, previous studies have also found that C_4 grasses and forbs do not usually compensate for the PFG loss (Symstad & Tilman 2001; McLaren & Turkington 2011; Pan et al. 2016). C_4 grasses have strong recruitment limitation and did not quickly occupy the space caused by PFG losses (Symstad & Tilman 2001). The responses of forbs to PFG loss have great variability because they range from small, annual, early-season, low mycorrhizal responsive plants such as *Salsola collina* to robust, perennial, deeply rooted, late-season, high mycorrhizal responsive species such as *Artemisia frigida* (Symstad & Tilman 2001; Tian et al. 2009; Yang et al. 2014). In this study, we evaluated the *CI* fairly soon after the initiation of PFG removal, whereas compensatory growth of the remaining PFGs (and particularly C_4 grasses and [Page 18] perennial forbs) may last for several years (Pan et al. 2016), such as forbs had compensated completely for the biomass loss of graminoids after 5 years of recovery (McLaren & Turkington 2011).

The identity of the removal PFGs also affects mycorrhizal root colonization at the community level, which was only increased in the C_3 grass removal plots (Fig. 6). Our results were similar to the findings of Urcelay et al. (2009), that graminoid loss tends to increase mycorrhizal root colonization. Most C_3 grasses, particularly *C. korshinskyi* and *Stipa krylovii*, exhibit a low percentage of mycorrhizal colonization (Bao & Yan 2004). Therefore, the

higher proportion of both C_4 grasses and forbs that were present in the C_3 grass removal plots might explain the higher mycorrhizal root colonization observed in this study (Fig. S5).

The addition of Topsin-M over three growing periods led to a significant decrease in the mycorrhizal root colonization and PLFA concentration of AMF in the control plots, which is consistent with the findings applied Topsin-M to the native grasslands (Wilson & Williamson 2008; McCain et al. 2011). The shoot P content of plant community was lower in the Topsin-M addition plots than the control, which illustrates that Topsin-M can effectively suppress the functions of AMF in the host plant. Several studies have previously shown that fungicide addition may alter plant growth by increasing soil nutrient availability (Chen & Edwards 2001; Allison et al. 2007). However, in the present study, Topsin-M addition did not measurably alter the soil inorganic N and available P contents, consistent with previous work in the field (Yang et al. 2014). Furthermore, Topsin-M addition did not totally suppress [Page 18] mycorrhizal root colonization in this study, suggesting that the *in situ* effects of AMF on the growth and compensation ability of plants might have been underestimated (Kahiluoto et al. 2000; Yang et al. 2014).

Conclusions

AMF could decrease aboveground productivity responses to the PFG loss, furthermore, affect the degree of compensation in the remaining PFGs, that decrease the biomass compensation ability of C_3 grasses to buffer the impacts of the biomass loss of both C_4 grasses and forbs in natural ecosystem (Adler & Bradford 2002; McLaren & Turkington 2011; Pan et al. 2016). Thus, our results highlighted AMF affected plant growth responses at the PFG and

community level and could increase our ability to understand the responses of plant communities to future plant diversity loss.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

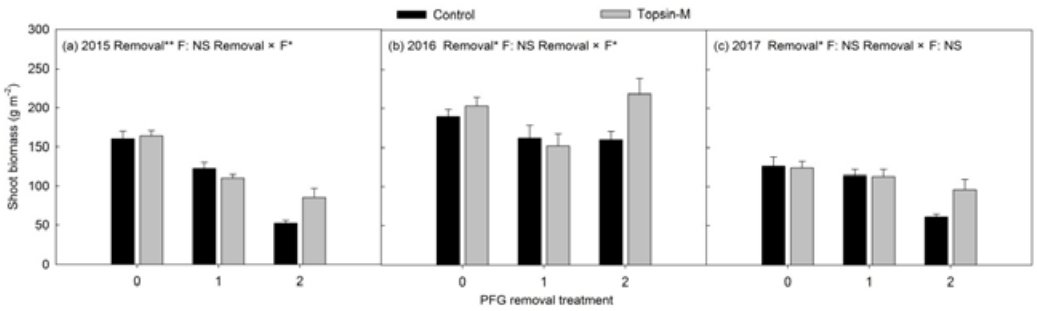
Appendix S1. Effects of fungicide and PFG removal on phospholipid fatty acid analysis and plant shoot P content from 2015 to 2017.

Appendix S2. Effects of fungicide and PFG removal on AMF PLFA concentration, soil available nutrients and plant community composition from 2015 to 2017.

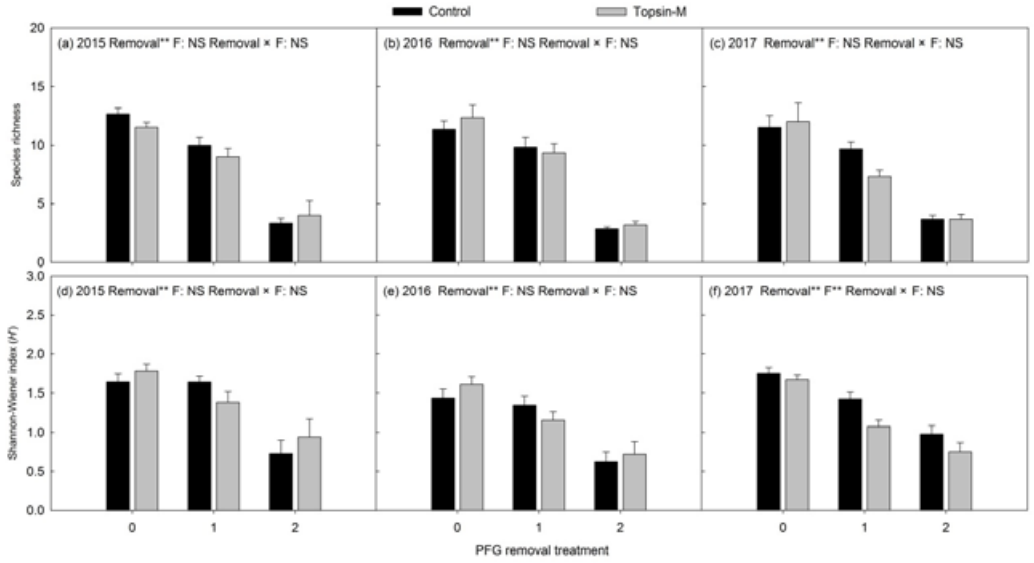
[Page 29] Table 1 *F* ratios resulting from the repeated-measures ANOVA testing the effects of year (Y) and treatments on the plant shoot biomass from 2015 to 2017.

		Community biomass			C_3 grasses biomass		C_4 grasses biomass		Forbs biomass	
Effects	d.f.	*F*	*P*	d.f.	*F*	*P*	*F*	*P*	*F*	*P*
Between-subject										
Block	5, 25	1.56	0.21	5, 15	1.36	0.29	0.53	0.75	2.01	0.14
F	1, 25	3.69	0.07	1, 15	**10.23**	**0.01**	**6.85**	**0.02**	0.03	0.86
Removal	2, 25	**17.49**	**< 0.01**	1, 15	**118.58**	**< 0.01**	0.14	0.71	0.06	0.81
F× Removal	2, 25	**4.73**	**0.02**	1, 15	**5.66**	**0.03**	0.12	0.73	0.16	0.70
Within-subject										
Y	2, 50	**182.95**	**< 0.01**	2, 30	**141.41**	**< 0.01**	1.23	0.31	**41.43**	**< 0.01**
Y× Block	10, 50	**2.70**	**0.01**	10, 30	**2.15**	**0.05**	1.87	0.09	1.29	0.28
Y× F	2, 50	1.24	0.30	2, 30	**2.77**	**0.08**	2.91	0.07	0.74	0.48
Y× Removal	4, 50	**24.99**	**< 0.01**	2, 30	**169.58**	**< 0.01**	0.61	0.55	2.24	0.12
Y×F× Removal	4, 50	0.81	0.52	2, 30	2.08	0.14	0.80	0.46	0.13	0.88

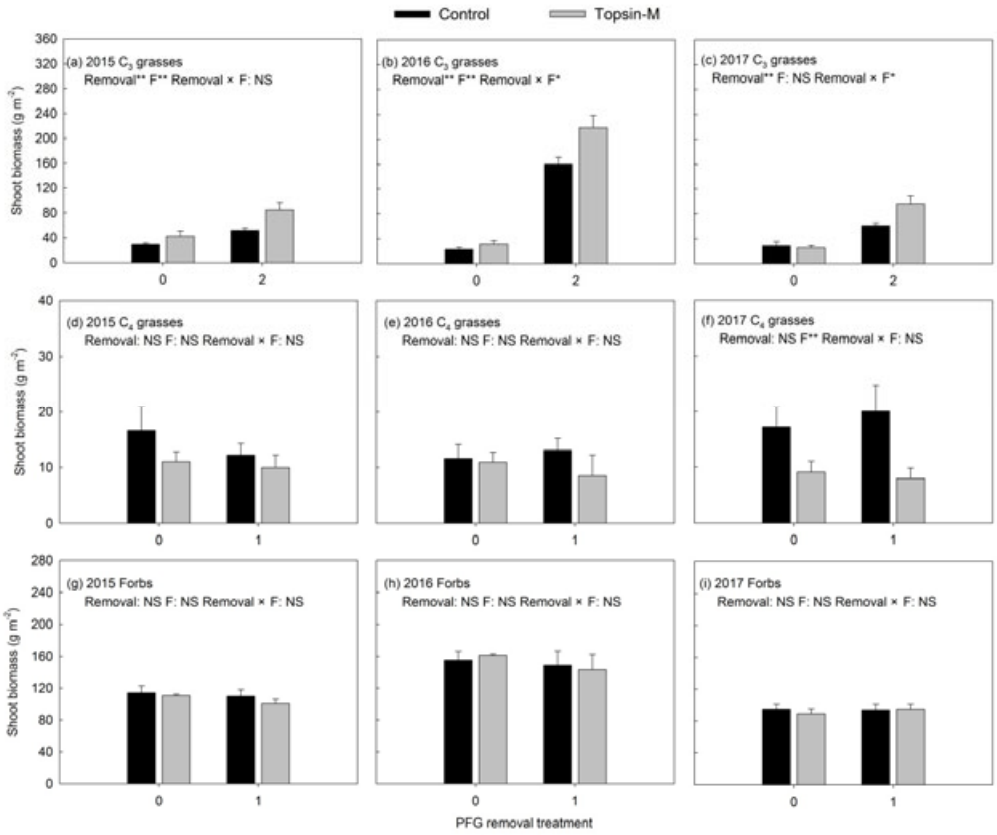
Notes: Treatments were fungicide (F), plant functional group removal (Removal). C_3 grasses, C_4 grasses and forbs all have the same d.f. Bold values are significant at $P < 0.05$.



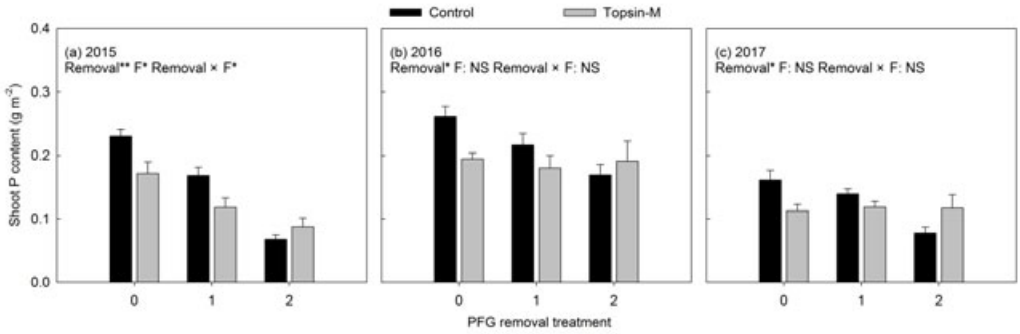
[Page 30] Figure 1 Effects of fungicide (F) and functional group removal (Removal) on the shoot biomass of plant community in 2015 (a), 2016 (b) and 2017(c). 0, 1 and 2 indicate no removal, removal of C_3 grasses, and removal of both C_4 grasses and forbs, respectively. Data are means + SE. $*P < 0.05$; $**P < 0.01$; NS $P > 0.05$.



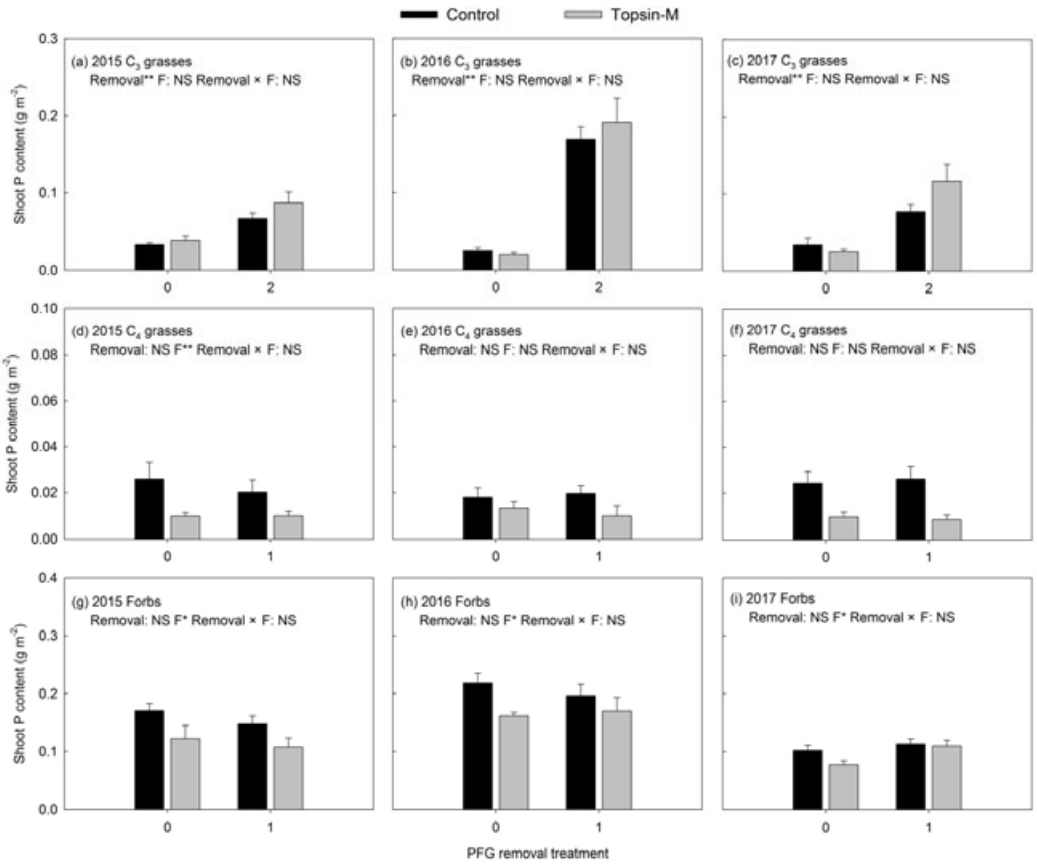
[Page 31] Figure 2 Effects of fungicide (F) and functional group removal (Removal) on the species richness (a, b and c) and Shannon-Wiener index (d, e and f) from 2015 to 2017. See Fig. 1 for treatment abbreviations. Data are means ± SE. $*P < 0.05$; $**P < 0.01$; NS $P > 0.05$



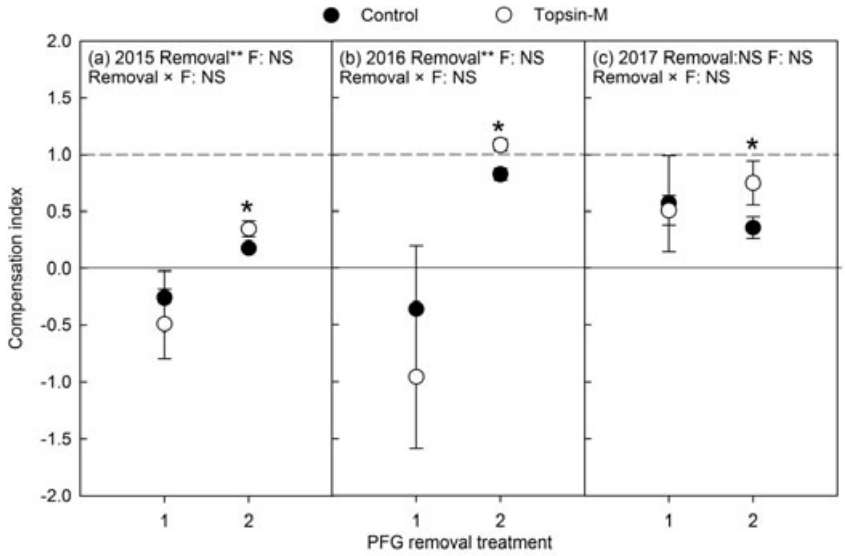
[Page 32] Figure 3 Effects of fungicide (F) and functional group removal (Removal) on the shoot biomass of C_3 grasses (a, b and c), C_4 grasses (d, e and f) and forbs (g, h and i) from 2015 to 2017. See Fig. 1 for treatment abbreviations. Data are means + SE. $*P < 0.05$; $**P < 0.01$; NS $P > 0.05$.



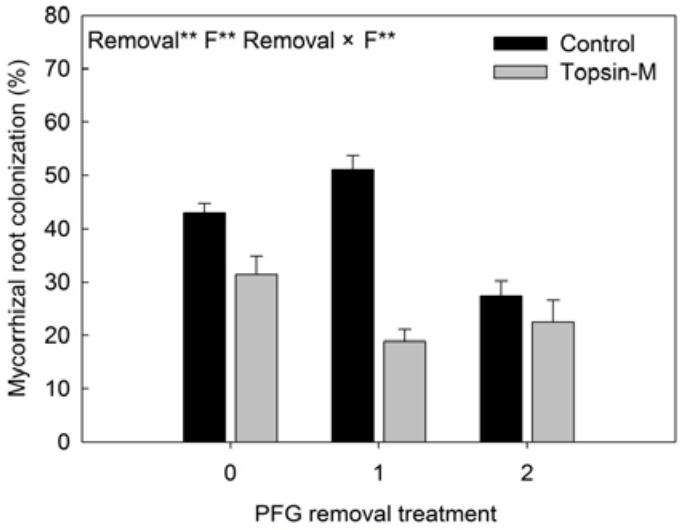
[Page 33] Figure 4 Effects of fungicide (F) and functional group removal (Removal) on the shoot P content of plant community in 2015 (a), 2016 (b) and 2017 (c). See Fig. 1 for treatment abbreviations. Data are means + SE. $*P < 0.05$; $**P < 0.01$; NS $P > 0.05$.



[Page 34] Figure 5 Effects of fungicide (F) and functional group removal (Removal) on the shoot P content of C_3 grasses (a, b and c), C_4 grasses (d, e and f) and forbs (g, h and i) from 2015 to 2017. See Fig. 1 for treatment abbreviations. Data are means + SE. $^{*}P < 0.05$; $^{**}P < 0.01$; NS $P > 0.05$.



[Page 35] Figure 6 Effects of fungicide (F) and functional group removal (Removal) on compensation index in 2015 (a), 2016 (b) and 2017(c). See Fig. 1 for treatment abbreviations. Data are means ± SE. **P* < 0.05; ***P* < 0.01; NS *P* > 0.05.



[Page 36] Figure 7 Effects of fungicide (F) and plant functional group removal (Removal) on mycorrhizal root colonization in 2017. See Fig. 1 for treatment abbreviations. Data are means ± SE. *$P < 0.05$; **$P < 0.01$; NS $P > 0.05$.