Effects of plant functional group loss on soil biota and net ecosystem exchange: a plant removal experiment in the Mongolian grassland

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Summary

1. The rapid loss of global biodiversity can greatly affect the functioning of above-ground components of ecosystems. However, how such biodiversity losses affect below-ground communities and linkages to soil carbon (C) sequestration is unclear. Here, we describe how losses in plant functional groups (PFGs) affect soil microbial and nematode communities and net ecosystem exchange (NEE) in a 4-year removal experiment conducted on the Mongolian plateau, the world’s largest remaining natural grassland.

2. Our results demonstrated that the biomasses or abundances of most components of the two below-ground communities (microbes and nematodes) were negatively affected by PFG loss and were positively related to above-ground plant biomass. The removal of dominant PFGs (perennial bunchgrasses and perennial rhizomatous grasses) reduced the biomass or abundance of below-ground community components while removal of less dominant PFGs (perennial forbs and annuals/biennials) did not change or increased the biomass or abundance of below-ground community components.

3. The biomass-based ratio of fungal to bacterial microbes and the number-based ratio of fungal-feeding to bacterial-feeding nematodes decreased with increasing PFG losses. Variation partitioning analyses showed that the identity of PFGs together with above-ground plant biomass explained most of the total variation in soil microbes and that the identity of PFGs and above-ground plant biomass together with nematode food resources explained most of the total variation in soil nematodes. The increase in NEE with PFG loss was mainly explained by decreases in above-ground plant biomass and the ratio of fungi to bacteria.

4. Synthesis. The shift of below-ground communities from a fungal-based to a bacterial-based energy channel as PFG richness decreases indicates that less diverse grassland ecosystems will have lower nutrient retention and hence be more sensitive to land-use or climate change. The dominant effects of above-ground plant biomass and below-ground communities on NEE indicate that PFG loss resulting from land-use or climate change has the potential to reduce C sequestration in semi-arid grassland soils. These findings suggest that predictive models may need to consider the composition of above-ground and below-ground communities in order to accurately simulate the dynamics of CO2 fluxes in terrestrial ecosystems.

Key-words: biodiversity loss, compensatory effect, mass ratio hypothesis, plant functional group, plant–soil (below-ground) interactions, soil C sequestration, soil food web, soil microbes, soil nematodes, trophic levels

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Introduction

Terrestrial ecosystems worldwide are being affected by multiple anthropogenic stressors (e.g. global climate change, land-use change and biotic invasion) (Sala et al. 2000; Tilman 2000). One of the most significant consequences of these anthropogenic stressors is the rapid loss of biodiversity in terrestrial ecosystems (Sala et al. 2000; Loreau, Naeem & Inchausti 2002). This biodiversity loss in natural ecosystems has far-reaching effects on ecosystem functioning and services (Hooper et al. 2005; Balvanera et al. 2006; Tilman, Reich & Isbell 2012). Previous experimental studies have demonstrated that plant diversity loss affects vegetation characteristics (e.g. primary productivity and plant–plant interactions) and that these responses often depend on the identities of the lost plant species or plant functional groups (PFGs) (Wardle et al. 1999; Balvanera et al. 2006; Cardinale et al. 2012). Recent studies have also demonstrated that reductions in plant diversity alter ecosystem processes and the structure and functioning of below-ground communities (e.g. microbes and nematodes) (Naeem, Hahn & Schuurman 2000; Bardgett & Wardle 2010; Cardinale et al. 2012). Furthermore, studies have shown that PFG richness is more tightly related to primary productivity and ecosystem stability than plant species diversity (Bai et al. 2004; Hooper et al. 2005). Differences between PFGs have been suggested to affect both below-ground communities and soil processes (e.g. C and nutrient cycling) (Wardle et al. 1999; De Deyn et al. 2004; Ward et al. 2009). However, we still lack a comprehensive understanding of how a reduction in PFG richness affects below-ground communities and their linkages to C storage in natural ecosystems.

As primary producers and providers of resources to the soil food web, plants greatly affect the composition, structure and functioning of below-ground communities (Bezemer et al. 2010; Eisenhauer et al. 2010). Therefore, a reduction in plant diversity or PFG richness will greatly affect below-ground communities and the ecosystem processes that they govern (Wardle et al. 2004; Bardgett & Wardle 2010; de Vries et al. 2013). In a study across land-use systems, for example, de Vries et al. (2013) showed that soil biota (e.g. soil microbes and nematodes) explained a large amount of the total variation in soil respiration, dissolved organic C leaching, nitrogen (N) mineralization and N leaching. Recent reports have also demonstrated that the effect of plant diversity loss decreases with the trophic distance from the manipulated level (Balvanera et al. 2006; Viketoft et al. 2009; Scherber et al. 2010). Therefore, observed differences in the responses of below-ground communities to plant diversity may have been due to the investigation of differential trophic positions relative to the manipulated level. Most previous studies have considered how plant diversity reduction or PFG loss affects a single trophic level of below-ground communities (Spohn et al. 2000; Hedlund et al. 2003; Zak et al. 2003). Only a few studies have considered a wide range of taxonomic or functional groups of below-ground communities in a single experiment (Gastine, Scherer-Lorenzen & Leadley 2003; Milcu et al. 2006; Scherber et al. 2010). As a consequence, our understanding of responses of the below-ground communities to PFG richness remains incomplete, and this limits our ability to predict the effect of future PFG/species losses on ecosystem functioning and services (Hooper et al. 2005; Scherber et al. 2010).

The results of previous studies on the effects of PFG/species loss on below-ground communities have been largely inconsistent. Some reports have indicated that plant diversity loss increases total microbial biomass (Eisenhauer et al. 2010), abundance and diversity of soil bacteria or fungi (Wardle et al. 1999) and abundance of earthworms (Milcu et al. 2008). In contrast, other reports showed that plant diversity loss had no effects on total microbial biomass (Hedlund et al. 2003; Habekost et al. 2008) and the abundance and diversity of nematodes or earthworms (Gastine, Scherer-Lorenzen & Leadley 2003; Hedlund et al. 2003). Changes in PFG richness may alter the composition and functioning of below-ground communities by at least two pathways. First, the chemical constituents of litter and root exudates (e.g. N, lignin and soluble C) differ among PFGs and these differences will affect the densities/biomasses of soil organisms and the structure of below-ground communities (Bardgett & Shine 1999; Wardle et al. 1999; De Deyn et al. 2004). Secondly, changes in ecosystem productivity when PFGs are lost can induce changes in C and N allocation to soils and therefore affect the growth and activity of below-ground communities (Wardle et al. 1999; Eisenhauer et al. 2010). Much of the data concerning the effects of plant diversity loss on below-ground communities has been collected in experiments with synthetically assembled grassland communities, and it remains unclear whether such findings can be applied to natural systems, which are likely more complex (Tilman, Reich & Isbell 2012). Taken together, the lack of consistency and mechanistic understanding about how PFG richness affects below-ground communities in natural grassland ecosystems might be caused by the failure to separate the effects of plant composition, above-ground plant biomass and soil factors. A key issue in current ecosystem research is to understand the ability of grasslands to sequester and store C in the face of global diversity loss (Cardinale et al. 2012). The Mongolian steppe, part of the largest contiguous grassland in the world (the Eurasian steppe), is one of the most important stocks of terrestrial soil C (White, Murray & Rohwerder 2000). Although abiotic factors (especially soil temperature and moisture) have been identified as the primary determinants of soil C flux (Raich & Schlesinger 1992), our understanding of how PFG losses influence ecosystem-scale C stores and fluxes in natural grassland ecosystems is limited (Ward et al. 2009; Bardgett & Wardle 2010). A plant removal experiment in a peatland in northern England demonstrated that losses of PFGs, notably ericaceous shrubs, can greatly increase total ecosystem CO2 fluxes (Ward et al. 2009). As noted earlier, PFG loss can greatly affect the composition and functioning of below-ground communities and thereby affect soil C dynamics and storage. However, no study has experimentally tested how changes in below-ground
communities resulting from PFG loss affect ecosystem-scale C stores and fluxes in natural grassland communities on the Mongolian steppe.

Using a 4-year, large-scale PFG-removal experiment in the Inner Mongolian grassland, we investigated the effects of PFG losses on soil microbes and soil nematodes and C sequestration, as both soil microbes and soil nematodes can affect ecosystem C and nutrient cycling (Bardgett & Wardle 2010; Chen et al. 2013a). Five levels of PFG diversity were obtained by removing a single PFG or multiple PFGs in all possible combinations of four PFGs (Table S1 in Supporting Information). In this study, we have addressed three specific questions. First, how do PFG diversity losses affect soil microbes, nematodes, net ecosystem exchange (NEE), above-ground plant biomass and soil variables (e.g. soil pH, NO$_3^-$-N, NH$_4^+$-N, soil organic C and total soil N) in the semi-arid Inner Mongolian grassland? Secondly, which factors (PFG richness, above-ground plant biomass or PFG identity) determine the changes in the composition of the below-ground microbial and nematode communities? Finally, how do PFG loss-induced changes in above-ground plant biomass, below-ground communities and soil variables mediate the changes in NEE? Overall, by assessing how PFG losses change below-ground communities and C sequestration, this study aims to improve our understanding of the dynamics of CO$_2$ fluxes with rapid loss of biodiversity in terrestrial ecosystems.

**Materials and methods**

**STUDY SITE**

This study was conducted at the Inner Mongolian Grassland Ecosystem Research Station (IMGERS, 43°38′N, 116°42′E) of the Chinese Academy of Sciences, which is located in the Xilin River Basin of Inner Mongolia, China, at approximately 1200 m a.s.l. This area is characterized by a semi-arid continental climate with a mean annual temperature of 0.9 °C (Bai et al. 2010). Precipitation mainly falls during the growing season (June–August), which coincides with the highest temperatures. The site has a dark chestnut soil (Calcic Chernozem according to the ISSS Working Group RB, 1998), with a loamy-sand texture. Before the experiment began, the plant community was dominated by Leymus chinensis (a perennial rhizome grass) and Stipa grandis (a perennial bunchgrass), which are widely distributed in the Eurasia steppe region (Bai et al. 2004).

**PFG REMOVAL EXPERIMENT IN THE INNER MONGOLIAN GRASSLAND**

The establishment of the Inner Mongolian Grassland Removal Experiment (IMGRE) was described by Wu et al. (2015). The sampling in this study was conducted in a subset of the IMGRE involving the complete removal of 0–4 PFGs. The complete removal of PFGs was initiated in 2005 and involved five blocks, with 16 plots (6 m × 6 m each) per block, resulting in a total of 80 plots. Plots were separated by 1-m walkways. The PFG removal experiment included all combinations of four PFGs (Table S1). We categorized all plant species into the following four functional groups based on plant species C:N ratio and life form (Bai et al. 2004; Wu et al. 2015): perennial rhizomes (PR), perennial bunchgrasses (PB), perennial forbs (PF), and annuals and biennials (AB). By removing zero or various combinations of one, two, three or four functional groups, we had a total of 16 removal treatments (Table S1). To minimize physical disturbance to soil, plants were removed by cutting the above-ground parts and tilling nodes to 3 cm soil depth. To ensure that the removal treatment stopped or at least significantly reduced the growth of targeted plants, we cut the target plants in early June of each year (2006–2009). In late August 2009, above-ground plant biomass was measured by clipping all plants in a 1 m × 1 m quadrat at the soil surface in each plot. All plants were sorted into PFGs and oven-dried at 65 °C for 48 h and weighed.

**SOIL SAMPLING AND ANALYSIS**

In late August of 2009, four soil cores (2 cm in diameter and 15 cm in depth) were randomly collected from each plot and were combined to form one composite soil sample per plot. After the soil was gently mixed and roots were removed, the moist soil was passed through a 2-mm-mesh sieve and separated into two parts. One part was maintained fresh for measurement of soil moisture, NO$_3^-$-N, NH$_4^+$-N, microbes and nematodes. A 20-g subsample of fresh soil was oven-dried at 105 °C for 24 h to determine soil moisture. NH$_4^+$-N and NO$_3^-$-N concentrations were determined by extracting inorganic N at 100 rpm for 2 h from subsamples with 100 mL of 2 M KCl before and after incubation. The extract was subjected to colorimetric determination on a 2300 Kjeltec Analyzer Unit (FOSS, Höganas, Sweden). The other part was air-dried and used to determine soil pH, soil organic C (SOC) and total soil N (TSN). Soil pH was measured in a 1:2.5 (soil: water) suspension. SOC content was determined using the Walkley–Black modified acid-dichromate FeSO$_4$ titration method. TSN content was determined by micro-Kjeldahl digestion, followed by colorimetric determination with a 2300 Kjeltec Analyzer Unit (Sparks et al. 1996). All results are expressed on a dry weight basis.

**SOIL MICROBIAL AND NEMATODE COMMUNITIES**

The microbial community in soil samples was assessed by analysis of phospholipid fatty acids (PLFAs) (Bossio et al. 1998). Qualitative and quantitative PLFA analyses were performed with an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) and the MIDI Sherlock Microbial Identification System (MIDI Inc., Newark, DE, USA). The abundance of each individual PLFA in a given sample was expressed as PLFA nmol g$^{-1}$ dry soil against an internal standard (methyl ester C19:0; Matreya Inc., State College, PA, USA); PLFAs specific to bacteria (i15:0, a15:0, i15:0, h15:0, a17:0, i17:0, h17:0, 16:1ω7c, 17:1ω8, 18:1ω9c, 18:1ω7c, cy17:0 and cy19:0), fungi (18:2ω6,9) and actinobacteria (10Me 16:0, 10Me 17:0, 18:1ω7c, cy17:0 and cy19:0). All results. Based on feeding habits and life-history characteristics, soil nematodes were assigned to four trophic groups (Bongers 1990).
plant feeders, bacterial-feeders fungal feeders and omnivores + carnivores. Carnivorous and omnivorous nematodes were included in one trophic group because carnivorous nematodes were infrequently found (de Vries et al. 2013). The number-based ratio of fungal-feeding to bacterial-feeding nematodes (BF) was calculated. We also calculated the relative abundance (%) of each nematode group, and we used these relative abundances to describe the structure of the nematode community. All results for microbes and nematodes are expressed on a dry weight basis.

MEASUREMENT OF NEE

Net ecosystem exchange is the balance between net uptake of CO2 by photosynthesis and its loss by respiration and indicates whether an ecosystem is a C source (NEE > 0) or C sink (NEE < 0). NEE was measured by the static-chamber method (Chen et al. 2009). Briefly, in August 2009, a square PVC collar (50-cm × 50-cm and 10 cm in height) was placed in each plot of block 1–4 (64 plots in total). The PVC collars were inserted into the soil to a depth of 5 cm 3 days in August 2009, a square PVC collar (50-cm cube), which was attached to a Li-Cor 840 infrared gas analyser (IRGA) (Li-Cor, Inc., Lincoln, Nebraska, USA). The changes in CO2 concentration in the chamber were automatically monitored every second for 90 s, but only the data from the middle 50 s were used to calculate NEE so as to minimize disturbance effects (Chen et al. 2009). On each measurement day, all soil CO2 efflux measurements were completed between 9:00 am and 11:00 am.

Statistical Analysis

All statistical analyses were done with R version 3.1.1 (R Development Core Team 2009). First, mixed linear models with individual PFGs (absent or present, Table S2) or loss of a species based factors and block as a random factor were used to assess the effect of PFG identity or PFGs loss on below-ground communities, and soil properties; these analyses used the function lmer from the ‘lme4’ package. One-way ANOVAs with Duncan’s multiple-range tests were performed across all response variables to compare the loss number of PFG effects. The data of response variables were transformed with the natural log-rhythm before the analysis to improve normality. Secondly, linear or quadratic regression was used to examine the relationships between above-ground plant biomass and soil factors (i.e. soil moisture, pH, NO3−-N, NH4+-N, SOC, TSN and SOC to TSN ratio) and below-ground community groups. The regression with the lowest AIC value was selected as the final model. Thirdly, variation partitioning analyses (Leps et al. 2011) were used to determine the relative importance of the number of PFGs lost, PFG identity, above-ground plant biomass, soil properties and nematode food resources on below-ground community groups. The nematode food resources for each nematode trophic group are described in Table S3 (de Vries et al. 2013). For example, the food resource was bacterial biomass for BF and fungal biomass for fungal-feeding nematodes (FF). The percentage of variance explained by each variable (the number of PFGs lost, PFG identity, above-ground plant biomass, soil properties and potential nematode food resources) was determined using the varPart function in the ‘LMEConvenienceFunctions’ package (Tremblay & Ransijn 2012). Finally, the potential linkages of above-ground plant biomass and below-ground communities to NEE were further examined using linear or quadratic regression. To facilitate our analyses, principal component analysis performed on seven soil properties (i.e. soil moisture, pH, NO3−-N, NH4+-N, SOC, TSN and SOC to TSN ratio); the first two principal components (PCs) were used as indicators of soil properties.

Results

Effects of PFG Removal on Above-Ground Plant Biomass and Soil Properties

Based on above-ground plant biomass, PB and PRs were considered the dominant PFGs, and perennial forbs (PF) and annuals/biennials (AB) were considered less dominant PFGs (Fig. S1, S2 and Table S4). Removal of PB decreased above-ground plant biomass by 42% and soil moisture by 11% but increased soil NH4+-N content by 12% (Fig. S1, S2 and Table S4). Removal of PR decreased above-ground plant biomass by 31% and soil moisture by 11% but increased soil NO3−-N content by 11%. Removal of PF increased soil moisture by 6% and soil NO3−-N content by 11%, and removal of AB increased soil NO3−-N content by 6% (Figs S1, S2 and Table S4). The removal of PFGs did not change soil pH, SOC, TSN or the ratio of SOC to TSN (Figs S1, S2 and Table S4). PFG loss decreased the above-ground plant biomass and soil moisture but increased soil NO3−-N and NH4+-N contents (Fig. S3 and Table S4). Reduction in the number of PFGs did not change the soil pH, SOC, TSN or the ratio of SOC to TSN (Fig. S3). In addition, the first two principal components (PC1 and PC2) explained 32% and 18% of the total variance for the seven soil properties (Table S5), respectively. Removal of PB and PF increased the PC1 of soil properties and removal of PR and PF increased the PC2 (Fig. S2 and Table S4). Reduction in the number of PFGs increased the first two PCs of soil properties (Fig. S3 and Table S4).

Effects of PFG Identity on Below-Ground Communities

Removal of PFGs altered the below-ground communities, and the effect was greater with loss of the dominant PFGs (PB and PR) than with loss of the less dominant PFGs (PF and AB) (Fig. 1, Figs S4–S6 and Table S4). Removal of PB or PR decreased total microbial biomass by 16–19% (due to decreases in all microbial groups) and the biomass-based ratio of fungal to bacterial microbes (F:B ratio) by 9–12% (Fig. 1a–f and Fig. S4). Removal of PB or PR increased the relative bacterial biomass but did not change the relative fungal or actinobacterial biomass (Fig. 1a–f and Fig. S4). Removal of PF or AB increased the relative biomass of bacteria but did not change any other microbial variable (Fig. 1a–f and Fig. S4).

For nematodes, the removal of PB or PR decreased total nematode abundance by 20–30% (due to decreases in all nematode trophic groups) but did not change the relative abundance of any nematode trophic group (Fig. 1g–k, Fig. S4 and Table S4). Removal of PF did not change the abundance or relative abundance of any nematode trophic group.
Plant diversity loss and soil biota

Effects of PFG loss and above-ground plant biomass on below-ground communities

ANOVA indicated that PFG loss (the loss from 0 to 4 PFGs) greatly affected the two below-ground communities (Fig. 2, Fig. S7 and Table S4). For soil microbes, PFG loss decreased the total microbial biomass (due to a decrease in fungal biomass and actinobacterial biomass) and the F:B ratio (Fig. 2a-f and Fig. S7). PFG loss increased relative bacterial biomass but did not change the relative fungal biomass or relative actinobacterial biomass (Fig. 2a-f and Fig. S7). For soil nematodes, PFG loss decreased the abundance of total nematodes (due to a decrease in all nematode trophic groups) and the FF:BF ratio (Fig. 2g-l and Fig. S7). PFG loss decreased the relative abundance of FFs but did not change the relative abundance of the other three nematode trophic groups (Fig. 2g-l and Fig. S7).

Regression analysis indicated that changes in the two below-ground communities were closely associated with changes in above-ground plant biomass (Fig. 3 and Fig. S8). Above-ground plant biomass was positively related to total microbial biomass, the biomass of each microbial group and the F:B ratio \( (r^2 = 0.18-0.29) \) (Fig. 3a-f and Fig. S8). Above-ground plant biomass was negatively related to the relative bacterial biomass but was not related to the relative fungal biomass nor to the relative actinobacterial biomass (Fig. 3a-f and Fig. S8). Similarly, above-ground plant biomass was positively related to the abundances of total nematodes and of all nematode trophic groups \( (r^2 = 0.21-0.36) \) but was not related to the FF:BF ratio nor to the relative abundance of each nematode trophic group (Fig. 3a-f and Fig. S8).

Relative role of PFG loss, above-ground plant biomass and PFG identity on below-ground communities and their linkages to NEE

According to variation partitioning analyses, variation in most microbial community groups was mainly explained by the identity of the lost PFGs (16-60%) and above-ground plant biomass (1-31%). Variation in most nematode community
groups was mainly explained by the identity of the lost PFGs (13–53%), above-ground plant biomass (1–22%) and nematode food resources (3–63%) (Fig. 4 and Table S6). Other factors (number of PFGs lost and soil factors) explained only a small percentage of the variation in all below-ground community groups (Fig. 4 and Table S7). The abundances of

Our experiment in the world’s largest remaining natural grassland provides robust evidence that PFG loss can reduce the biomass or abundance of microbial and nematode groups in soil. This is consistent with other reports that changes in PFG richness or plant diversity did not affect below-ground communities (Gastine, Scherer-Lorenzen & Leadley 2003; Marshall, McLaren & Turkington 2011). Our 4-year study, however, supported the findings that losses in PFG richness negatively affected below-ground functional groups (Viketoft et al. 2009; Scherber et al. 2010; Eisenhauer et al. 2011). Such negative effects could be explained by reductions in the quality and quantity of resources available to the below-ground communities resulting from decreases in above-ground plant biomass and decreases in the probability of including key PFG of PB or PR for the low PFG-richness plots (Gastine, Scherer-Lorenzen & Leadley 2003; Milcu et al. 2008; Viketoft et al. 2009). The negative effects of reduced PFG richness on below-ground communities in this typical steppe indicate that the availability of nutrients to plants, as mediated by the soil biota, could also be reduced (Wardle et al. 2004; Bardgett & Wardle 2010), potentially resulting in a reduction in ecosystem productivity.

We also found that the structures of below-ground communities changed when PFGs were removed. The decreases in the ratios of fungal to bacterial microbial biomass and of fungal-feeding to bacterial-feeding nematode abundance with increasing PFG loss suggest that fungal-based soil food webs are more important in PFG-rich than in PFG-poor assemblages. These findings are consistent with those from other plant diversity experiments, which reported that the biomass-based ratio of fungal to bacterial microbes (Zak et al. 2003; Scherber et al. 2010) and the number-based ratio of fungal-feeding to BF (Viketoft et al. 2009) decreased with decreasing plant diversity. The dominant role of the bacterial-based energy channel in the low PFG diversity communities might be explained by the decreased quantity of plant materials to the below-ground organisms (Wardle et al. 2004). Contrary to our findings, however, several papers concerning nematode communities reported that decreases in PFG number failed to enhance the bacterial-based energy channel (De Deyn et al. 2004; Viketoft et al. 2009; Bezemer et al. 2010). This inconsistency could be due to the fact that, besides being affected by nematode food resources (bottom-up effects), nematode communities may also be affected by their predators (top-down effects) (Ingham et al. 1985). The shift from fungal-based to bacterial-based soil food webs resulting from PFG loss could result in increases in nutrient losses and CO2 release, which in turn could cause the semi-arid grassland to be more sensitive to changes in land use or climate (de Vries et al. 2013).
ground communities mainly through decreased plant productivity (Spehn et al. 2000; Eisenhauer et al. 2010). This finding is also consistent with the mass ratio hypothesis, which predicts that the relative effect of individual PFGs or plant species on ecosystem properties should be proportional to their relative contribution to community productivity (Grime 1998). Most below-ground organisms are heterotrophs that rely on the plant-derived residues entering the soil via leaf and root litter and rhizodeposition (Spehn et al. 2000; Zak et al. 2003; Wardle et al. 2004). Thus, the decline in the biomass or abundance of microbes and nematodes in response to PFG loss likely results from declines in the quality and quantity of plant materials entering the soil. Some researchers, however, reported that neither above-ground nor root biomass explained the negative effects of plant diversity loss on below-ground communities (Spehn et al. 2000; Gastine, Scherer-Lorenzen & Leadley 2003; Eisenhauer et al. 2010). This lack of consistency with the mass ratio hypothesis might be explained by that the measurement of above-ground plant resource quality may override the effect of above-ground plant biomass can increase the performance of below-ground communities by increasing the quantity of plant-functional groups (PFGs) (Marshall, McLaren & Turkington 2011). Overall, our findings indicate that the loss of dominant PFGs may greatly affect below-ground communities and may lead to a new stable state for ecosystem productivity (NEE). Regressions are based on linear or quadratic models. Statistics ($r^2$ and P values) for regression are indicated (**P < 0.01; ***P < 0.001).

RELATIVE EFFECTS OF PFG LOSS, ABOVE-GROUND PLANT BIOMASS AND PLANT COMPOSITION ON THE BELOW-GROUND COMMUNITIES

In our study, the variation in the below-ground communities induced by PFG removal was mainly explained by PFG identity and PFG biomass rather than by PFG number per se, which is consistent with previous studies (Wardle et al. 1999; Milcu et al. 2008). Because the composition of plant litter and root exudates differs among plant species and PFGs, changes in plant diversity and in PFGs are likely to alter the quality of resources supporting the below-ground communities (Zak et al. 2003; Milcu et al. 2006). Our findings are inconsistent with those from a removal experiment in a northern Canadian grassland that showed that removal of PFGs (graminoids, legumes and forbs) had almost no effect on the soil microbes (Marshall, McLaren & Turkington 2011). In the Canadian study, however, the plant material was only slowly incorporated into the soil. In this experiment, the dominant PFGs (PB and PR) have greater root biomass and deeper roots than the less dominant PFGs and thereby produce larger amounts of fine roots (Wu et al. 2015). In addition, the dominant PFGs (PB and PR) have higher C:N ratios than PF and AB (Wu et al. 2015), and the removal of dominant PFGs may lead to a decline in the soil F:B ratio (Bardgett & Shine 1999; De Deyn, Cornelissen & Bardgett 2008). We therefore suspect that the below-ground communities in our research benefitted more from the dominant PFGs than from the less dominant PFGs because the dominant PFGs provided a greater quantity of resources to the below-ground communities (Scherber et al. 2010; Eisenhauer et al. 2011). The higher C:N ratios of the dominant PFGs produced litter with a higher C:N ratio (Wu et al. 2015), which may have enhanced the biomass or abundance of below-ground community groups (Bardgett & Shine 1999; De Deyn et al. 2004). In addition, the dominant PFGs may have provided physical protection for below-ground communities by producing more plant litter (Eisenhauer et al. 2010).

Although above-ground plant biomass explained less of the variation in below-ground community variables than PFG identity, the effect of above-ground plant biomass on the below-ground communities was significant. As noted earlier, increases in above-ground plant biomass can increase the performance of below-ground communities by increasing the quality of plant-derived inputs into the soil (Stephan, Meyer & Schmid 2000; Milcu et al. 2008). In addition to PFG properties (i.e. identity, number and biomass), nematode food resources were also strongly linked to changes in the soil nematode community. The relatively low percentage of the variation in below-ground community variables explained by soil factors in our PFG removal experiment may be due to the relatively minor effects of PFG removal on SOC, TSN, pH and other soil chemical properties (Marshall, McLaren & Turkington 2011). Overall, our findings indicate that the loss of dominant PFGs may greatly affect below-ground communities and may lead to a new stable state in the grassland ecosystem. The latter possibility is supported by an observational study, which found that in heavily grazed sites where the dominant PFGs (PB and PR) were almost completely replaced by PF and AB, ecosystem productivity did not recover to the original level even 10 years after grazing had been stopped (Chen et al. 2013b).

PFG LOSS-INDUCED DECLINE IN SOIL C STORAGE VIA PLANT BIOMASS AND BELOW-GROUND COMMUNITIES

Our short-term NEE measurements indicate that the ability of the semi-arid grassland to function as a C sink may decline...
with PFG loss in the long term and that this decline was mainly associated with the decrease in above-ground plant biomass and changes in below-ground communities (as indicated by F:B ratio). Generally, the amount of C stored in an ecosystem is determined by the balance between C assimilation (via photosynthesis) and respiration (including both heterotrophic and autotrophic respiration) (De Deyn, Cornelissen & Bardgett 2008). Researchers have previously suggested that differences in PFG traits (e.g. leaf area index) influence photosynthetic rate and community productivity, which in turn may affect ecosystem C sequestration (Ward et al. 2009). In our PFG removal experiment, the decline in plant community productivity with PFG loss likely contributed to reduced C assimilation and this was especially the case when the dominant PFGs, such as PB or PR were removed.

In addition to directly affecting C storage by reducing plant productivity, PFG loss can reduce C storage by inducing a shift from a fungal-based to a bacterial-based soil food web. Relative to fungal-based soil food webs, bacterial-based soil food webs have higher amounts of available nutrients and lower amounts of nutrient-rich organic matter (De Deyn, Cornelissen & Bardgett 2008; de Vries et al. 2013). The high amounts of available nutrients could lead to higher rates of decomposition of soil organic matter and thus to increased losses of C to the atmosphere. Although the lack of measurements of specific components of NEE limits our understanding of which components dominate the effects of PFG loss on soil C storage, our findings indicate that loss of specific PFGs and the associated declines in plant biomass can greatly affect below-ground communities and change C flux and storage in grassland soils. As a consequence, such grassland soils may change from C sinks to C sources that can increase greenhouse gas concentrations and contribute to future global change.

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Data accessibility

Data deposited in the Dryad repository: http://datadryad.org/resource/doi:10.5061/dryad.6sk0g (Chen et al. 2016).

References


