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Decreased plant productivity resulting from plant group removal experiment constrains soil microbial functional diversity

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Abstract

Anthropogenic environmental changes are accelerating the rate of biodiversity loss on Earth. Plant diversity loss is predicted to reduce soil microbial diversity primarily due to the decreased variety of carbon/energy resources. However, this intuitive hypothesis is supported by sparse empirical evidence, and most underlying mechanisms remain underexplored or obscure altogether. We constructed four diversity gradients (0–3) in a five-year plant functional group removal experiment in a steppe ecosystem in Inner Mongolia, China, and quantified microbial taxonomic and functional diversity with shotgun metagenome sequencing. The treatments had little effect on microbial taxonomic diversity, but were found to decrease functional gene diversity. However, the observed decrease in functional gene diversity was more attributable to a loss in plant productivity, rather than to the loss of any individual plant functional group per se. Reduced productivity limited fresh plant resources supplied to microorganisms, and thus, intensified the pressure of ecological filtering, favoring genes responsible for energy production/conversion, material transport/metabolism and amino acid recycling, and accordingly disfavored many genes with other functions. Furthermore, microbial respiration was correlated with the variation in functional composition but not taxonomic composition. Overall, the amount of carbon/energy resources driving microbial gene diversity was identified to be the critical linkage between above- and belowground communities, contrary to the traditional framework of linking plant clade/taxonomic diversity to microbial taxonomic diversity.

KEYWORDS
biodiversity, biodiversity loss, functional diversity, grassland, inner Mongolia, metagenome, microbial diversity

1 | INTRODUCTION

Anthropogenic environmental changes, such as increased land allocation for agricultural use, nitrogen deposition, and the rising global mean temperature, are accelerating the rate of biodiversity loss on Earth (Bálint, Domisch, Paul, & Nowak, 2011; Dirzo & Raven, 2003). It is a central goal in ecology research to investigate the effect of plant diversity loss on soil microbial communities, for soil habitats

possess the highest biodiversity on Earth and play critical roles in driving multiple ecosystem functions such as carbon and nutrient cycling (Fierer & Jackson, 2006; Fierer et al., 2012; Garbeva, van Veen, & van Elsas, 2004; Jing et al., 2015; Lange et al., 2015). However, the relationships between plant and belowground microbial communities remain elusive, owing to a high degree of heterogeneity in situ and in response to environmental stimuli/change, making consistent, reproducible patterns difficult to ascertain (Culman et al., 2010; Eisenhauer et al., 2011; Goberna, Navarro-Cano, & Verdú, 2016; Liu, Liu, Fu, & Zheng, 2007; Milcu et al., 2013; Prober et al., 2015; Stephan, Meyer, & Schmid, 2000; Wardle et al., 1999; Zak, Holmes, White, Peacock, & Tilman, 2003). Thus, predicting how plant diversity loss will affect soil microbial diversity remains challenging, despite numerous recent innovations that have expanded our capacity to characterize complex microbial communities. Plant diversity loss is likely to both reduce plant productivity and community structure (diversity and composition), and soil microbial communities may respond at both the taxonomic and functional levels. It is not well understood which plant community attributes most crucially influence belowground communities, nor which microbial features are most responsive to aboveground alterations.

On one hand, plant productivity determines the amount of carbon/energy resources supplied to soil microbial communities. In fact, net primary productivity has been demonstrated to play a fundamental role in driving both plant and animal diversity (Hutchinson, 1959; Rosenzweig, 1995). For microorganisms in particular, the decrease in productivity reduces the amount of plant-derived organic material entering the soil, thus potentially stimulating microorganisms with strong energy-acquisition abilities. On the other hand, plant community structure largely governs the diversity of carbon/energy resources (exudates and litter), syntrophic niche space (plant-microbe interactions) and the diversity and composition of soil microhabitats supplied to soil microorganisms (Hiiesalu et al., 2014; Kardol & Wardle, 2010). Thus, the loss of aboveground plant diversity is traditionally predicted to reduce belowground microbial diversity. However, this intuitive hypothesis is supported by scarce in-situ empirical evidence (Goberna et al., 2016; Milcu et al., 2013; Stephan et al., 2000), and most studies did not observe a strong relationship between above- and belowground diversity (Carney, Matson, & Bohannan, 2004; Culman et al., 2010; Jing et al., 2015; McElroy, Papadopoulos, & Adl, 2012; Millard & Singh, 2010; Porazinska et al., 2003; Prober et al., 2015; Wardle, 2006; Wardle, Yeates, Williamson, & Bonner, 2003; Wardle et al., 1999). This phenomenon implies that the relationship between above- and belowground community diversity may not be the most critical linkage between both communities, or that the relationships are obscured by their complexity and/or a limitation in currently applied/established community analysis methods.

Until recent advances in metagenomic sequencing, which have broadened and advanced our ability to analyze complex microbial communities, most studies focused primarily on the taxonomic diversity of soil microbial communities, such as by investigating rRNA gene-based taxonomy. However, many recent studies have demonstrated a greater association between microbial communities and environmental factors (abiotic and biotic) through functional gene assessment (Burke, Steinberg, Rusch, Kjelleberg, & Thomas, 2011; Louca, Parfrey, & Doebeli, 2016; Nemergut et al., 2013; Zhang, Johnston, Li, Konstantinidis, Han, 2017). One explanation is that microbial species can share their genetic material easily through horizontal gene transfer, and thus taxonomic diversity could be mismatched from functional gene diversity (Konstantinidis et al., 2009; Thomas & Nielsen, 2005). A recent population-level investigation of soil microbiota revealed that many coexisting community members within 97% 16S rRNA gene similarity possessed distinct (i.e., not shared) functional traits related to SOM utilization/degradation, and demonstrated how recent disturbances can result in the loss (or acquisition) of several functional traits in just a few years (Johnston et al., 2016). Also, many functions, particularly those for the degradation of plant litter, are widespread across phylum boundaries. Meanwhile, the ecosystem functioning of soil microbial communities, such as soil respiration/organic matter decomposition activity, has shown a significant response to plant diversity in several studies (Eisenhauer et al., 2010; Lange et al., 2015; Meier & Bowman, 2008; Steinauer et al., 2015; Stephan et al., 2000). These results imply that plant diversity loss may influence the microbial functional gene repertoire; thus, microbial functional gene diversity may be more sensitive to plant diversity loss than taxonomic diversity (Barberán, Fernández-Guerra, Bohannan, & Casamayor, 2012).

Studies investigating above- and belowground biodiversity relationships often adopted manipulative experiments (Carney et al., 2004; Lange et al., 2014) or field investigations of natural communities (Barberán et al., 2015; Garbeva et al., 2004). The manipulative experiments often constructed artificial communities with a biodiversity gradient by seeding a different number of plant species into soils, in which the original vegetation had been removed and the original soil physicochemical condition had been heavily disturbed. The investigations of natural communities often compared communities at different spatial sites, which should have different species components and physicochemical environments, besides the different biodiversity per se, and thus, have limited utility for studying the direct effects of plant community diversity on belowground biota in the context of anthropogenic disturbance. Actual ecological communities are formed in a long-term process and the biotic/abiotic components are closely linked, so the rapid biodiversity loss caused by human activities might lead to a cascade of consequences. Therefore, an experimental design manipulating biodiversity gradient in a natural community, such as in situ removal of plant species (Chen et al., 2016; Diaz, Symstad, Chapin, Wardle, & Huenneke, 2003; Wardle et al., 1999; Wu et al., 2015), should be conducted to reveal the impact of biodiversity loss.

Here, we conducted a five-year plant functional group (PFG) removal experiment in a steppe ecosystem of Northern China, which is representative of much of the Eurasian steppe region floristically and ecologically (Li, Yong, & Li, 1988). We previously analyzed the taxonomic diversity of soil bacterial communities through 454 pyrosequencing of 16*S rRNA* gene amplicons, and found that it was

not changed by the PFG treatment (Zhang, Barberán, Zhu, Zhang, & Han, 2014). In this study, we employed shotgun metagenomic sequencing to measure both the taxonomic diversity and the functional gene diversity of soil microbial communities in order to test the following hypotheses: (i) plant productivity is more important than plant community structure in driving soil microbial diversity, at least over short to moderate timescales, (ii) microbial functional gene diversity is more sensitive to PFG removal than microbial taxonomic diversity, (iii) the decrease in plant productivity and diversity will promote the contribution of deterministic relative to stochastic processes in driving soil microbial diversity and composition, and (iv) the change in microbial diversity will lead to a shift in microbial respiration.

2 | MATERIALS AND METHODS

2.1 | Study site, experimental design, and sampling

This study is part of the Inner Mongolia Grassland Removal Experiment (IMGRE) from the Chinese Academy of Sciences. The detailed experimental design has been described previously (Zhang et al., 2014), and we provide only a brief summary here. The experiment was conducted in a typical steppe semi-arid ecosystem (43°38′N, 116°42′E). The mean annual temperature is ~0.3°C and the mean annual precipitation is 346 mm. The vegetation is dominated by *Achnatherum sibiricum*, *Agropyron michnoi*, *Cleistogenes squarrosa*, *Leymus chinensis* and *Stipa grandis*. All plant species were classified into five plant functional groups (PFGs) based on their life forms (Kong et al., 2011; Mclaren & Turkington, 2010). Perennial bunchgrasses (PB), perennial rhizome grass (PR) and perennial forbs (PF) comprised about 49%, 36%, and 14% of the total aboveground biomass, respectively, and they combined to comprise >99% of the total biomass (Zhang et al., 2014). A full combinatorial design was employed with the three PFGs (a total of eight PFG combinations) and five replicates (in five random blocks) for each combination. In other words, there were four PFG diversity gradients: removing 0, 1, 2, and 3 (all) PFGs. PFG diversity gradient was established in early July every year from 2005 to 2009 by manual removal of the aboveground biomass of non-target plants in each plot (6 m × 6 m). Stems and leaves were removed by clipping at the surface while taking great care to reduce disturbance to soil and other plants, and the clipped plant material was removed from the plots.

In late August of 2009 (period with highest plant biomass), aboveground vegetation was sampled by clipping all plants at the soil surface using a 1 m^2 quadrat randomly placed in the plot. All living vascular plants were sorted to species, and were oven-dried at 65°C for 48 hr and weighed (Data S1). The dry mass of all living plants approximated the aboveground net primary productivity in this temperate grassland (Bai et al., 2007; Sala & Austin, 2000). On 22 June 2010, four soil cores (10 cm deep, 3.5 cm diameter) were collected from each plot at random and thoroughly mixed. After removing roots and stones using a 2-mm sieve, part of the soil samples was used to measure soil physicochemical indices and microbial respiration and the rest was frozen for DNA extraction. Soil characteristics (pH and the contents of total carbon, total N (nitrogen), NH_4^+-N, NO_3^--N and water) have been described before (Zhang et al., 2013). Microbial respiration was measured with the alkali absorption method (Hu & Bruggen, 1997). Briefly, the fresh soil (20 g dry weight equivalent) was incubated in a 500-ml glass flask at 25°C in the dark. The glass flask was connected to a glass tube (6 cm in diameter), in which 5 mL of 50 mM NaOH solution was injected to capture CO_2 evolved by the soil. After four days of incubation, the respired CO_2 was determined by titrating the residual OH^- with a standardized HCl solution.

2.2 | Metagenomic sequencing, microbial taxonomic/functional composition analysis

Soil DNA was extracted with the MoBio PowerLyzer PowerSoil DNA isolation kit according to manufacturer's instructions. To obtain sufficient DNA for shotgun metagenomic sequencing and to overcome the experimental constraints of soil habitat heterogeneity, 4–5 replicates were conducted for each sample (0.25 g soil per replicate). In order to prepare DNA libraries for sequencing, DNA extracts were processed according to the description of the Illumina Paired-End Prep kit protocol. DNA was sheared mechanically, size-selected to ~180 bp and gel purified. Sequencing was performed on an Illumina Hiseq 2000 platform located at Shanghai Majorbio Bio-pharm Technology Co., Ltd. 2.21 ± 0.06 (mean ± *SE*) Giga base pairs of DNA sequences were generated for each sample (Table S1). While the sequencing depth may appear relatively low, the large number of replicated samples per treatment (five replicates) and the fact that all datasets were similar in size, which makes comparisons of functional diversity among datasets robust (Fierer et al., 2012; Rodriguez-R & Konstantinidis, 2014; Zhang et al., 2017), offset the low coverage and provided for meaningful comparisons.

To improve the reliability and quality of subsequent analysis, the raw sequence data was processed with the following two steps. First, the Seqprep software (https://github.com/jstjohn/SeqPrep) was used to remove the adapter sequences. Second, the library sickle (https://github.com/najoshi/sickle) was used to trim the reads from 5′ end to 3′ end using a sliding window (size 50 bp, step by 1 bp). If the mean quality of bases inside a window drops below 20, the remainder of the read below the quality threshold will be trimmed. We also discarded quality-trimmer reads that were shorter than 50 bp or containing N (ambiguous bases). Taxonomic profiling of clean reads is proceeded by BLASTn (blast+ version 2.2.31, cut-off e-value: 1e-5) analysis against the SSUrRNA database of silva (Release 119 http://www.arb-silva.de) (Quast et al., 2013). According to the results of taxonomic assignment, the taxon abundances are assigned for the two taxonomic levels of kingdom and phylum. We further calculated the relative abundances of bacteria, archaea, and fungi, and also the relative abundance of each phylum.

Bacteria was dominant among the three groups, and we further calculated its richness. 16S rRNA gene encoding metagenomic reads were assigned against the 16S rDNA gene full-length sequences in the Greengenes database (May 2013 release), which were

subsequently clustered into OTUs (operational taxonomic units) at the 97% threshold, using UCLUST closed-reference OTU picking in Qiime (Caporaso et al., 2010). To exclude the influence of unequal sampling, the relatively rarer OTUs with <1/892 relative abundance (there were 892 reads assigned to OTUs in the smallest sample) in each sample were removed for the calculation of OTU richness and Shannon diversity.

Paired reads of shotgun metagenomic sequences were merged with FLASH using default parameters (Table S1) (Magoc & Salzberg, 2011). Using MBLASTX, merged reads were mapped against the protein sequences from the STRING database (E-value cutoff $1e^{-6}$) (Davis et al., 2013; von Mering et al., 2003). The abundance of each COG (Clusters of Orthologous Groups) gene was counted as the sum of reads mapping to it (Tatusov, Galperin, Natale, & Koonin, 2000), which was then normalized by the size of the dataset. To exclude the influence of unequal sampling, the relatively rarer COG genes with $<10^{-6}$ relative abundance in each sample (there were $> 10^6$ reads assigned to COG genes for each sample) were removed for the calculation of COG richness (Fierer et al., 2012), which was used to represent the functional richness of soil microbial communities (5,580 ± 29 functional genes per sample on average). These COGs have been clustered into dozens of categories (there are a total of 25 COG categories); for each COG category, we also calculated its relative abundance and functional gene richness.

Using MBLASTX, merged reads were also mapped against the protein sequence of the KEGG database (Table S1) (*E*-value cutoff $1e^{-6}$; Kanehisa & Goto, 2000), and the relative abundance of each KO gene was also calculated. To estimate the soil organic matter (SOM) degradation potential of soil microbial communities, we focused on the KO genes responsible for the degradation of plant sugar materials (e.g., cellulose, chitin, poly-, oligo-, di-, and monosaccharides), amino acids and aromatic compounds. A total of 277 SOM-degradation genes were identified in this study (Data S2).

2.3 | The relative contribution of deterministic and stochastic processes

Two different methods were adopted to identify the relative contribution of deterministic and stochastic processes in driving soil microbial taxonomic/functional diversity (Chase, 2010; Zhang, Liu, Bai, Zhang, & Han, 2011). The null model method compared the observed communities to the stochastically assembled communities, and thus assessed the deterministic effect caused by both current experimental treatment and past historical factors (such as the environmental changes occurred before the experimental treatment). In contrast, the direct-calculation method compared the treatment communities with the control communities and thus separated the deterministic and stochastic components of only the treatment effect (Zhang, Pu, Li, Han, 2016).

β diversity, which represents the compositional variation between communities, is often used to infer the possible mechanisms of community assembly (such as the relative importance of deterministic vs. stochastic processes). However, the difference in the β diversity indexes may be caused by differences in the ecological processes, as well as α and γ diversity. To exclude the influence of the other two diversity components, Chase (2010) developed a null model method, which compares the observed β diversity to the theoretical β diversity from stochastically assembled communities. Because this method depends on the presence or absence of OTUs/genes and is sensitive to the noise from rare OTUs/genes, the OTUs/genes with low abundance (bacteria OTU with <1/892 relative abundance and COG genes with $<10^{-6}$ relative abundance) were removed in each sample (Ferrenberg et al., 2013). To identify the relative contribution of deterministic and stochastic processes in driving soil microbial assembly, we analyzed the community data of each treatment, following the steps of Chase (2010) and Zhou et al. (2014). First, for any given pair of plots within the treatment, we calculated the observed OTU/gene richness (e.g., α_1 and α_2 for plot 1 and 2, respectively) and the number of shared OTUs/genes (SS_{obs}). Second, the total number of OTUs/genes detected in the "OTU/gene pool" (γ diversity) from all plots of the treatment, and the proportion of the plots occupied by each OTU/gene was measured. Third, we calculated the distribution of the expected shared OTUs/genes from the null model (SS_{exp}) by randomly drawing α_1 and α_2 OTUs/genes from the OTU/gene pool with the probability of an OTU/gene to be drawn proportional to its among-plot occupancy. The SS_{exp} and the expected Jaccard's similarity (J_{exp}) are obtained for each drawing, and the average Jaccard's similarity ($\bar{J}_{exp}$) and its SD are estimated based on 10,000 drawings (σ_{exp}). For each treatment, permutational analysis of multivariate dispersions (PERMDISP) was adopted to test the difference between the observed community similarity (J_{obs}) and the average of the expected community similarity ($\bar{J}_{exp}$) (Anderson, 2004). The non-significant difference ($p > .05$) meant that stochastic processes were the primary driver of microbial diversity, while the significant larger (or smaller) J_{obs} relative to $\bar{J}_{exp}$ suggested that the deterministic process of ecological filtering (or competitive conclusion) was the primary driver.

To investigate whether the treatments affected the relative contribution of deterministic and stochastic processes in driving microbial diversity, the community data of all plots of all treatments was analyzed together to calculate J_{obs} and $\bar{J}_{exp}$ (and its σ_{exp}), following the same steps as stated above. In other words, here the OTUs/genes of all treatments were taken as the OTU/gene pool. The magnitude of deterministic processes on community structure was further quantified with the index of SES (standard effect size) (Kraft et al., 2011; Zhou et al., 2014): $SES = (J_{obs} - \bar{J}_{exp})/\sigma_{exp}$. For each of the eight treatments, the mean of SES value between every pair of replicate plots was calculated. Linear regression was further used to construct the relationship between the mean SES value of each treatment and aboveground plant productivity.

The deterministic and stochastic changes in microbial taxonomic/functional structure caused by each treatment were calculated with a recently developed method (Zhang et al., 2016; Zhang et al., 2011). Because there was no significant influence of spatial distance on soil microbial structural variation among all plots (Mantel tests: taxonomic structure, $r = .036$, $p = .403$; functional structure, $r = .025$, $p = .358$),

we assumed that there was no systematic variation in the spatial heterogeneity of these communities before the treatments were applied. In other words, these plots were within a local homogeneous area (the distance between any two plots was <300 m) with same environmental conditions (e.g., climate and soil). Therefore, the compositional variation between plots is caused by the deterministic processes of experimental treatments and the stochastic processes of birth/death, dispersal/colonization, etc. The compositional variation between the control plots is not caused by experimental treatments, and the mean compositional variation between each pair of control plots can be taken as *the reference point*. Although the compositional variation between the control and treatment plots is caused by both deterministic and stochastic processes, the changes of stochastic processes are expected to be non-directional and those of deterministic processes to be directional. Thus, for each treatment, *the deterministic change* caused by the treatment can be approximated by: D = ([mean compositional variation between control and treatment]−[reference point]). For each treatment in every experiment, we used Mantel test (Bonnet & Peer, 2002) to check the influence of the difference in soil physiochemical indices among replicate plots on microbial structural variation and found no significant effects for almost all treatments ($p > .05$; Table S2), suggesting that deterministic processes were not mainly responsible for the structural variation among replicate plots and thus stochastic processes were the primary driver. Therefore, for each treatment, *the stochastic change* induced by the experimental treatment can be approximated by: S = ([mean compositional variation within treatment]−[reference point]). Then, for each treatment, we could calculate *the relative importance of the deterministic change* = $\frac{|D|}{|D|+|S|}$.

2.4 | Statistical analyses

Three-way analysis of variance (ANOVA) was used to assess the effect of PFG composition on aboveground plant productivity and the relative abundance of bacteria, archaea and fungi. Bray-Curtis distance based on the relative abundance of bacterial phyla or COG categories was calculated to represent taxonomic/functional compositional variation among these samples (Bray & Curtis, 1957), and principal coordinate analysis (PCoA) was used to visualize the relative differences among these samples (Anderson, 2003). One-way ANOVA was performed to assess the effect of the number of removed PFG on aboveground plant productivity, the taxonomic/functional richness and composition (represented by PCoA axis 1), and microbial respiration. For multiple comparisons, the Least-Significant Difference method and Tamhane's T2 method was used for equal and unequal variance, respectively. Pearson's correlations were used to analyze the effect of aboveground plant productivity on the taxonomic/functional richness and compositional PCoA axis 1. In addition, permutational multivariate analysis of variance (PERMANOVA) was used to reveal the effect of PFG composition on the taxonomic/functional structure based on Bray-Curtis distance matrices (Anderson, 2005). Mantel test or partial Mantel test was adopted to assess the relationships among the taxonomic structure, functional structure, and the plant/soil indices (Bonnet & Peer, 2002). Stepwise regression analysis was used to identify the factors that could effectively explain the changes in soil microbial gene richness (and also the functional PCoA axis 1) from 13 potential plant indices, including the presence/absence of each of the three PFGs, removed PFG number, aboveground plant productivity, plant richness, the biomass of seven dominant plant species (they taken up >98% of the community biomass) and five potential soil physicochemical indices, including soil pH, and the contents of total carbon, total *N*, available *N* and water. Before regressions, all the data were tested for normal distribution. Collinearity was detected by calculating the condition index for each explanatory variable and it was less than 50 for each variable, suggesting autocorrelation did not occur.

We further adopted structural equation modeling (SEM) to gain a mechanistic understanding of how PFG removal affected soil microbial taxonomic/functional structure and microbial respiration. SEM is based on a simultaneous solution procedure, where the residual effects of predictors are estimated (partial regressions) once common causes from inter-correlations have been statistically controlled for (Grace, 2006). The first PCoA axes were used in SEM analysis to represent plant/PFG community composition and microbial taxonomic/functional composition. We started SEM analysis with the specification of a conceptual model of hypothetical relationships, based on a priori and theoretical knowledge (Fig. S1). We assumed that the variation in plant community structure is represented by the changes in plant/PFG richness and composition. The variation in plant community structure will change plant productivity, and both the structure and productivity will affect soil NO_3^--N content, which has been found to be the only responsive soil physicochemical variable to PFG removal (Zhang et al., 2014). The changes in these plant and soil variables will affect soil microbial taxonomic/functional richness and composition, and all these plant, soil and microbial variables will affect microbial respiration (Fig. S1). In the SEM analysis, we compared the model-implied variance–covariance matrix against the observed variance–covariance matrix, and the data were fitted to the models using the maximum likelihood estimation method. Adequacy of the models was determined using χ^2 tests, and adequate model fits are indicated by a non-significant χ^2 test ($p > .05$) (Grace, 2006; Wei et al., 2013). SEM analyses were performed using AMOS 18.0 (Amos Development, Spring House, PA, USA).

3 | RESULTS

3.1 | Response in plant productivity

Plant productivity decreased significantly only when both PR and PB were removed (Figure 1a). When one of the two PFGs was removed, the other would overgrow and thus the total productivity changed little; in other words, there was a compensatory effect (Wardle et al., 1999). This resulted in the phenomenon that as the removed PFG number increased from 0–2 to 3, aboveground

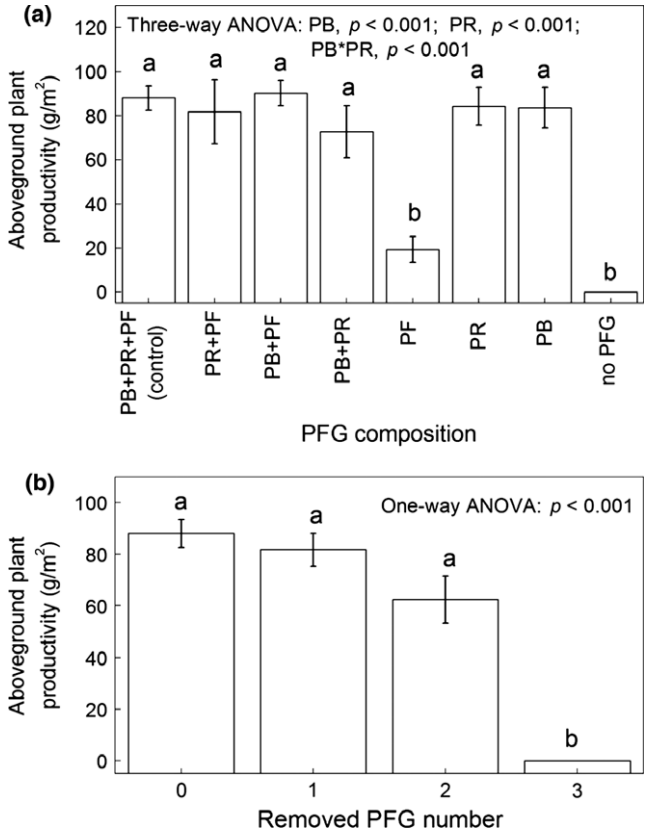

FIGURE 1 Effect of PFG composition and removed PFG number on aboveground plant productivity. Error bars represent one standard error. In the x axis of Figure 1a, PB, PR and PF represent perennial bunchgrass, rhizome, and forbs, respectively, and "+" means their combination

plant productivity first changed little and then decreased sharply (Figure 1b).

Among the measured soil physicochemical indices, the treatments only significantly changed soil NO_3^--N content, as reported previously (Zhang et al., 2014). Soil NO_3^--N content was negatively correlated with aboveground plant productivity (Pearson Correlation: $r = -.598$, $p < .001$), meaning that the reduced productivity maintained less N in the plant biomass and thus there was excess retained in the soil.

3.2 | Response in bacteria OTU diversity and composition

Neither removed PFG number nor aboveground plant productivity had significant effects on bacterial OTU richness ($p > .05$; Figure 2a, b). The abundance-based index of Shannon diversity also showed non-significant response (Fig. S2). These results were consistent with the previous results from 454 pyrosequencing, which targeted only at 16S rRNA gene (Zhang et al., 2014), further confirming these findings.

Different from the non-significant effect on OTU richness, removed PFG number and plant productivity changed soil bacterial community composition (Figure 2c, d). PERMANOVA also revealed that PB and PR removal had significant interactive effect on bacterial community composition ($p = .033$). Mantel test also revealed that bacterial community compositional variation showed a marginally significant correlation with plant productivity ($p < .10$).

Taken together, the treatment changed the taxonomic composition but not the OTU diversity of the soil bacterial community. In other words, the treatment stimulated some OTUs and depressed others, but the total OTU richness remained unchanged. Actually, aboveground plant productivity showed a marginally significant ($p < .10$) positive correlation with the relative abundance of Acidobacteria phylum, and significant negative correlations with those of Actinobacteria, Armatimonadetes, Gemmatimonadetes and Nitrospirae phyla (Table S3).

3.3 | Response in functional gene diversity and composition

Different from the non-significant effect on OTU richness, removed PFG number and aboveground plant productivity had significant effects on functional gene richness ($p < .05$; Figure 3a, b). Specifically, functional richness first showed a weak increase and then a sharp decrease with increasing number of removed PFG (Figure 3a), and showed a linear increase with increasing plant productivity (Figure 3b). The Shannon diversity of functional genes also showed a similar response to removed PFG number and plant productivity as the gene richness (Fig. S3). Stepwise regression analysis further revealed that aboveground plant productivity was the primary explanatory variable for the changes in the functional gene richness ($y = 4912 + 2.56$*(plant productivity), $p < .001$). Actually, in 17 out of all the 19 COG-categories, there was a positive linear relationship between functional gene richness and plant productivity (Table 1).

Both removed PFG number and aboveground plant productivity significantly changed the soil microbial community functional structure (Figure 3c, d). PERMANOVA also revealed that PFG composition had significant effects on the functional structure; specifically, PR had a significant effect ($p < .05$) and PR and PB had a marginally significant interactive effect ($p < 0.10$). Stepwise regression analysis revealed that aboveground plant productivity was the primary explanatory variable for the changes in the functional PCoA axis 1 ($y = 0.005-0.000073$*(plant productivity), $p = .001$).

Consistent with these results of stepwise regression analysis, Mantel test revealed that functional gene richness was significantly correlated with plant community productivity ($p = .001$; Table 2), while the correlations with plant/PFG richness and composition were non-significant ($p > .05$). Although microbial functional composition was significantly correlated with both plant productivity and PFG richness ($p < .05$; Table 2), partial Mantel test revealed that the correlation with plant productivity was still significant when the effect of PFG richness was controlled, but the correlation with PFG richness was non-significant when the effect of plant productivity was controlled. Taken together, the treatment conditions significantly altered both the functional diversity and

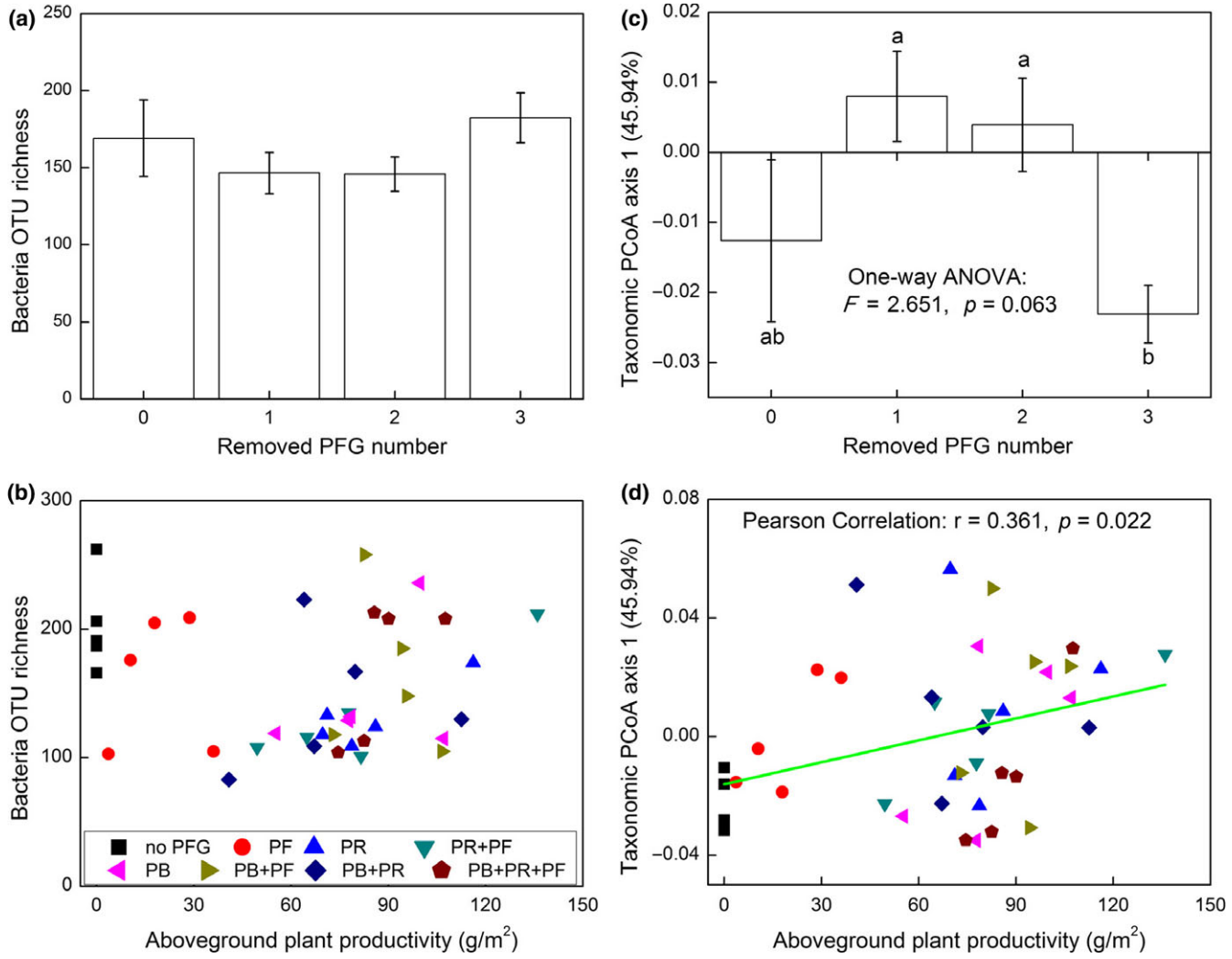

FIGURE 2 Effect of removed PFG number and aboveground plant productivity on the taxonomic diversity (a), (b) and composition (c), (d) of soil bacterial communities. Error bars represent one standard error. The value in the bracket of the y axis of Figure 2c and d represents the percentage of community compositional variation explained by axis 1 of the PCoA [Colour figure can be viewed at wileyonlinelibrary.com]

composition of soil microbial communities, and the corresponding change in plant productivity was identified as the primary driver of these changes.

3.4 | Association between functional and taxonomic diversity

The relative abundance of bacteria, archaea and fungi in the community was about 93%, 6% and 1%, respectively. Among the three groups, only the relative abundance of fungi showed a significant positive correlation with aboveground plant productivity (Pearson Correlation: $r = .361$, $p = .022$; Table S4). The result suggested that the decline in functional gene diversity is partly attributable to the decrease in fungi relative abundance.

Because fungi comprised only 1% of the relative abundance, the decline in gene diversity observed here should not be caused by the decrease in its relative abundance alone. The functional compositional variation was significantly correlated with bacterial community variation (Mantel test: $P = .020$), meaning that the variation in bacterial community composition should also be responsible for part of the decline.

3.5 | Difference between functional and taxonomic diversity

Although the functional and taxonomic composition of soil microbial community were correlated, the taxonomic and functional diversity demonstrated somewhat unlinked responses to our experimental manipulations. First, functional gene diversity decreased significantly with reduced plant productivity (Figure 3b) but taxonomic OTU diversity did not (Figure 2b). Second, functional β diversity (compositional variation) showed a very significant correlation with plant productivity (Mantel test: $p = .001$) but taxonomic β diversity showed only a marginally significant correlations with plant productivity ($p < .10$). Overall, the functional diversity was more closely associated with environmental factors than the taxonomic diversity.

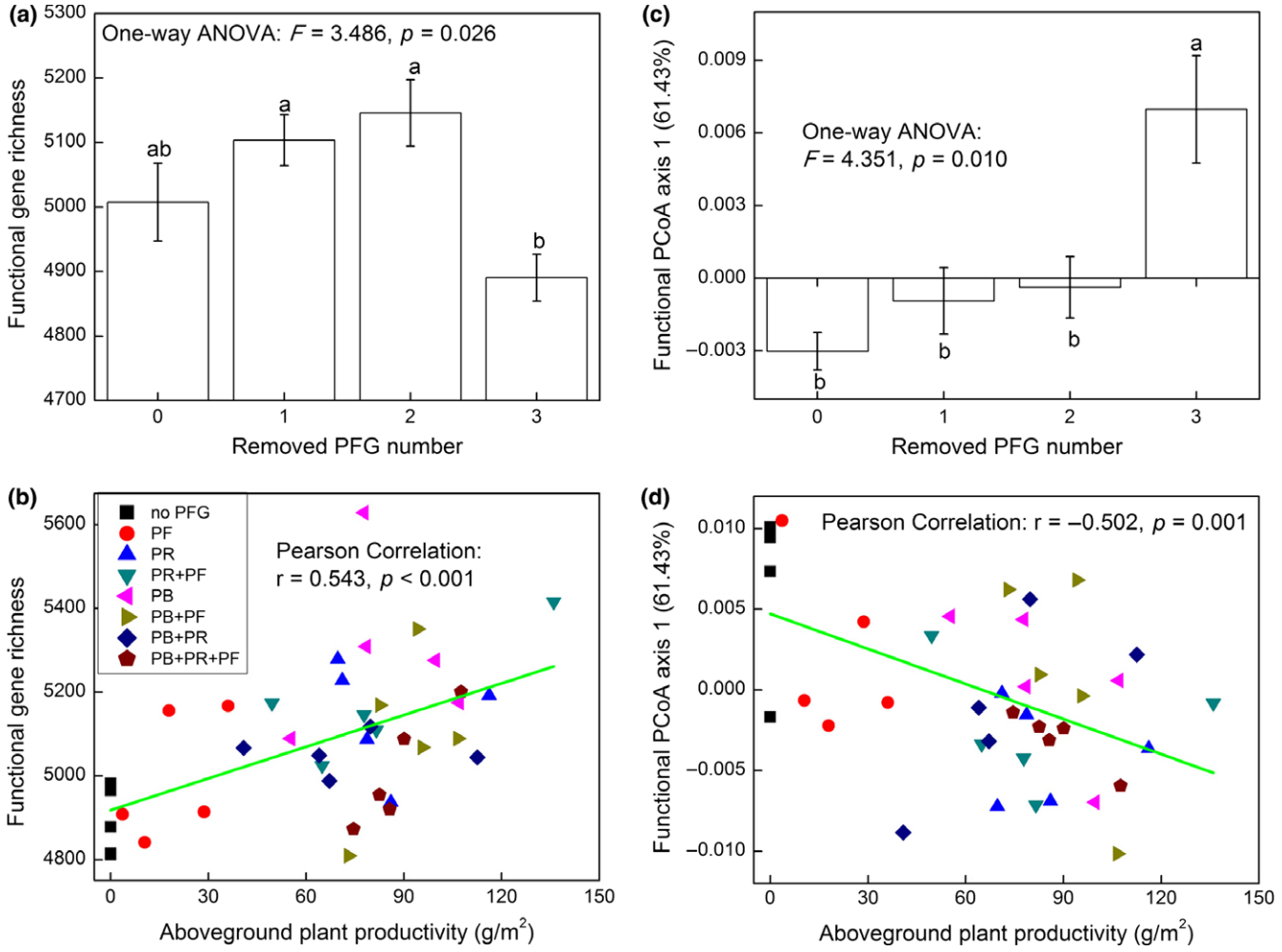

FIGURE 3 Effect of removed PFG number and aboveground plant productivity on the functional diversity (a), (b) and composition (c), (d) of soil microbial communities. Error bars represent one standard error. The value in the bracket of the y axis of Figure 2c and d represents the percentage of community compositional variation explained by axis 1 of the PCoA [Colour figure can be viewed at wileyonlinelibrary.com]

3.6 | The contribution of deterministic vs. stochastic processes and the role of plant productivity in driving microbial diversity

For the taxonomic OTU data, there were non-significant differences between the observed and expected community similarity in seven out of the eight treatments ($p > .05$; Table S5), suggesting that stochastic processes were the primary driver for the changes in taxonomic diversity. In contrast, the observed similarity was significantly larger than the expected similarity for the functional gene data in all the eight treatments ($p < .05$; Table S5), suggesting that the deterministic process of ecological filtering was the primary driver. Taken together, deterministic processes played a more important role in driving the functional than taxonomic diversity. Consistently, both the SES values and the relative importance of deterministic changes were smaller for the taxonomic structure than the functional structure (Figure 4), further confirming this conclusion.

There was a non-significant linear relationship between aboveground plant productivity and the taxonomic SES value, but there was significant negative linear relationship between plant productivity and the functional SES value ($p < .05$; Figure 4a). Also, a similar relationship was observed between plant productivity and the relative importance of the deterministic change (Figure 4b). This suggests that reduced productivity caused by PFG removal promoted the contribution of deterministic, relative to stochastic, processes in driving microbial functional diversity.

3.7 | Linkage between plant community indices, microbial composition and respiration

As removed PFG number increased from 0–2 to 3, microbial respiration first changed little and then showed a sharp decrease (Fig. S4). More precisely, the change in microbial respiration was significantly correlated with the variation in both plant productivity and microbial functional composition, even when the effect of one of the two factors was controlled ($p < .05$; Table 2). Meanwhile, the correlation coefficient was larger for aboveground plant productivity than for microbial functional composition (0.407 vs 0.147; Table 2). These

TABLE 1 Linear regression results between the gene richness or relative abundance of each COG category and aboveground plant productivity (only the results with $p < .05$ are shown)

	Gene richness				Relative abundance			
COG category abbreviation and functional description	Slope	p	P_{FDR}[a]	R^2	Slope	p	P_{FDR}	R^2
A: RNA processing and modification	0.150	0.003	0.005	0.217	5.54E-07	<0.001	<0.001	0.439
B: Chromatin structure and dynamics	0.103	<0.001	<0.001	0.380	4.16E-07	0.002	0.003	0.239
C: Energy production and conversion	0.052	0.042	0.042	0.107	-1.46E-05	0.005	0.005	0.195
D: Cell cycle control, cell division, chromosome partitioning	0.140	0.002	0.003	0.231				
E: Amino acid transport and metabolism	0.052	0.005	0.007	0.193	-2.08E-05	0.001	0.002	0.261
G: Carbohydrate transport and metabolism	0.054	0.015	0.018	0.150	-1.04E-05	0.005	0.005	0.191
H: Coenzyme transport and metabolism	0.028	0.033	0.037	0.117	-6.72E-06	0.002	0.003	0.238
I: Lipid transport and metabolism	0.120	<0.001	<0.001	0.423	-1.14E-05	0.001	0.002	0.246
J: Translation, ribosomal structure and biogenesis	0.156	0.005	0.007	0.193				
K: Transcription	0.218	<0.001	<0.001	0.305				
L: Replication, recombination and repair	0.173	0.001	0.002	0.281				
M: Cell wall/membrane/envelope biogenesis					7.41E-06	0.001	0.002	0.245
N: Cell motility	0.020	0.036	0.038	0.114	2.68E-06	0.001	0.002	0.241
O: Posttranslational modification, protein turnover, chaperones	0.327	<0.001	<0.001	0.356				
P: Inorganic ion transport and metabolism	0.082	0.001	0.002	0.264				
T: Signal transduction mechanisms	0.311	<0.001	<0.001	0.323	2.67E-05	0.002	0.003	0.224
U: Intracellular trafficking, secretion, and vesicular transport	0.239	<0.001	<0.001	0.318	3.90E-06	0.001	0.002	0.249
V: Defense mechanisms					5.87E-06	0.003	0.004	0.214
Z: Cytoskeleton	0.124	<0.001	<0.001	0.353	9.19E-07	<0.001	<0.001	0.584

[a]P_{FDR} refer to corrected p-values using the false discovery rate (FDR) method (Benjamini & Hochberg, 1995).

results meant that reduced plant productivity was the major driver of changes in microbial respiration, and microbial communities have likely adapted to productivity reduction by alteration of their functional structure. Different from the functional structure, the variation in microbial taxonomic composition did not show a significant correlation with microbial respiration ($p > .05$; Table 2), consistent with the different sensitivities of taxonomic and functional diversity to the PFG treatments.

3.8 | Integrated response of the plant-soil-microbe system to PFG removal

The final SEM model adequately fit the data describing interaction pathways among plant, soil, and microbial variables in response to PFG removal ($\chi^2 = 27.959$, $p = .141$; standardized path coefficients are given in Figure 5). As shown in the final model, plant community structure was well represented by the four variables of species/PFG richness and composition ($p < .05$). The model explained 60% and 97% of the variation in plant species richness and composition, respectively, and 10% and 100% of the variation in PFG richness and composition, respectively. The model also explained 6% of the variation in plant productivity, 37% of the variation in soil NO_3^--N content, and 82% of the variation in microbial respiration. The model explained only 6% and 15% of the variation in microbial taxonomic richness and composition, respectively, but explained 29% and 37% of the variation in microbial functional richness and composition, respectively (Figure 5).

Plant community structure had a non-significant effect on microbial taxonomic/functional richness and composition. The decrease in plant productivity did not change microbial taxonomic richness and composition, but it significantly reduced microbial gene richness and altered microbial functional composition (Figure 5). The decrease in plant productivity had a direct negative effect on microbial respiration. In addition, the decrease in plant productivity also had an indirect effect on microbial respiration through altering microbial functional gene composition, consistent with the result from Mantel test (Table 2). The relationships between the remaining variables were non-significant, but improved the model fit (Table S6).

4 | DISCUSSION

The importance of aboveground plant diversity to belowground microbial diversity has always been assumed, but with little empirical evidence to date (Fierer & Jackson, 2006; Lange et al., 2015; Zak et al., 2003). In this study, we manipulated plant diversity in situ by removing the aboveground biomass of different PFGs in the temperate steppe ecosystem over a five year period and quantified both the taxonomic and functional diversity of soil microbial communities. The reduction in plant productivity caused by PFG removal was

TABLE 2 The relationships among plant and microbial community indices revealed by Mantel test or partial Mantel test

			Microbial gene richness		Microbial functional composition		Microbial respiration	
Method	**Effective factor**	**Controlled factor**	***r***	***p***	***r***	***p***	***r***	***p***
Mantel test	Plant productivity		0.191	**0.011**	0.268	**0.001**	0.449	**<0.001**
	PFG composition		−0.009	0.501	0.028	0.296	0.073	0.086
	PFG richness		0.026	0.334	0.162	**0.018**	0.091	0.180
	Plant species composition		−0.005	0.503	0.049	0.209	0.050	0.178
	Plant species richness		−0.004	0.517	0.083	0.057	0.070	0.109
Partial mantel test	Plant productivity	PFG richness			0.229	**0.002**		
	PFG richness	Plant productivity			0.074	0.146		
Mantel test	Microbial taxonomic composition						0.086	0.090
	Microbial functional composition						0.238	**0.001**
Partial mantel test	Plant productivity	Microbial functional composition					0.407	**<0.001**
	Microbial functional composition	Plant productivity					0.147	**0.019**

p values < .05 in bold.

found to decrease the functional diversity, rather than the taxonomic diversity, of soil microbial communities. This finding was confirmed by Mantel test as well as SEM (Table 2; Figure 5), and the microbial respiration data further supported our metagenome result by providing evidence of recent community activity that was affected by the experimental treatment. Genetic and ecological mechanisms work in tandem to drive the difference in the sensitivities of taxonomic and functional diversity. Genetically, microorganisms can gain (via horizontal gene transfer) and lose genes (Thomas & Nielsen, 2005), thus obscuring taxonomic-functional relationships. There also exist many microbial functions that are widespread across taxonomic boundaries without being universally held within a clade; for instance, representatives capable of nitrogen-fixation have been identified from several different microbial phyla, but this function is not ubiquitous in any particular phylum (Dos Santos, Fang, Mason, Setubal, & Dixon, 2012). Thus, microbial species of close taxonomic relation could be less functionally similar than to more distantly related species (Konstantinidis et al., 2009). As a consequence, taxonomic and functional diversity might be governed by different ecological drivers and relate differently to biotic and abiotic environmental characteristics (Barberán, Casamayor, & Fierer, 2014).

Ecologically, functional attributes should be more closely associated with environmental factors than the taxonomic attributes (Burke et al., 2011). Consistent with this, the null model method revealed that deterministic processes played a more important role in driving functional gene diversity than taxonomic/OTU diversity (Table S5), and that the deterministic factors of plant productivity were comparatively less associated to taxonomic diversity (Figure 4). Reduced plant productivity decreased the supply of fresh carbon/energy resources to soil microbial communities, which was overwhelmingly dominated by heterotrophic microorganisms in the soil of this steppe ecosystem (Liu, Zhang, & Wan, 2009). Thus, reduced productivity promoted the deterministic process of ecological filtering and likely favored OTUs with a pronounced ability for efficient energy usage under limiting conditions, which was supported by shifts in the relative abundance of functional mechanisms.

There were two pieces of functional evidence. First, reduced productivity increased the relative abundances of five COG-categories associated with energy generation/conversion and material transport/metabolism (Table 1). The increase in these categories' relative abundances with reduced plant productivity also led to a decrease in the relative abundances of eight other functional categories, such as those for RNA processing and modification (see details in Table 1). Second, reduced plant productivity also stimulated the relative abundance of nearly every gene for the degradation of amino acids (Table S7), while it did not consistently increase/decrease the relative abundances of all genes responsible for the degradation of plant-derived sugar materials and aromatic compounds. Actually, plant root exudates and litter are often decomposed and transformed or assimilated by soil microbes into other SOM components, such as amino acids and other non-sugar polymer bacterial cell components (Kallenbach, Frey, & Grandy, 2016; Liang & Balser, 2011). Due to the lack of fresh sugar materials as plant productivity decreased, soil microbial community members might have become more reliant on the scavenging of starved/dead microbial cells (Table S7), which is expected to be more recalcitrant than fresh plant-derived carbohydrates. Reduced plant productivity favored OTUs with these functions and accordingly disfavored many other OTUs without these functions (but likely with many other specific functional genes due to the tradeoff in genomic content Konstantinidis & Tiedje, 2004), and thus the relative abundance of the other OTUs would decrease in the ecological filtering process, leading to the decline of the total gene richness. In other words, greater energy availability/input is likely needed to maintain such a high functional gene repertoire in this soil environment.

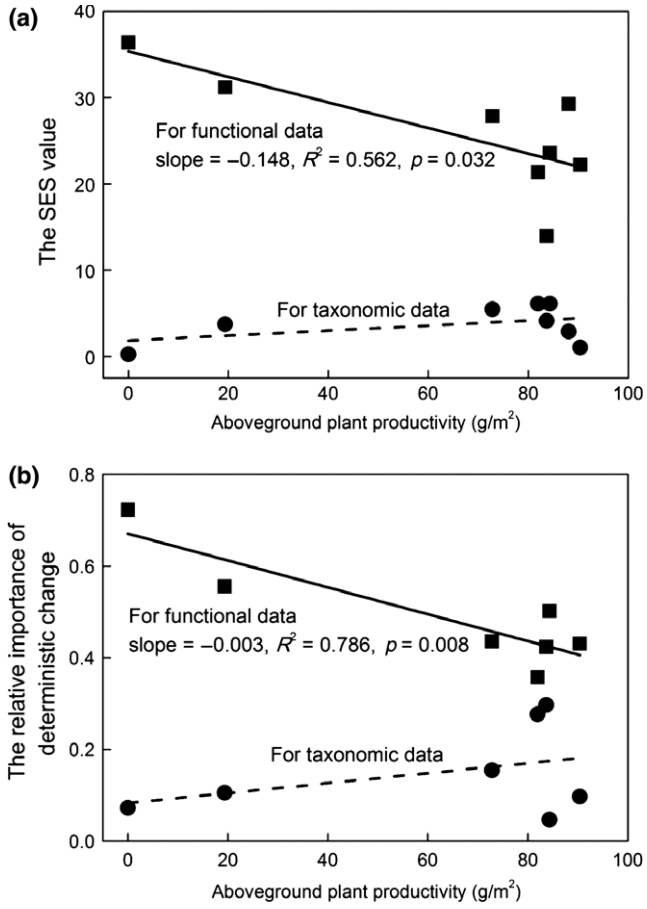

FIGURE 4 Effect of aboveground plant productivity on the SES value (a) and the relative importance of the deterministic change (b) for both the taxonomic and functional structure of soil microbial communities

In contrast to the diversity-diversity hypothesis, here we identified the key ecological linkage between above- and belowground communities in this temperate steppe ecosystem; that was, reduced plant productivity (rather than PFG diversity per se) intensified the pressure of ecological filtering due to the lack of carbon/energy resources and thus, decreased microbial functional gene diversity. These results suggest that an increase in plant productivity might relax the pressure of ecological filtering and promote the contribution of stochastic processes (e.g., dispersal) in community assembly (Hubbell, 2001), and thus un-constrain microbial functional diversity. Because it has been well demonstrated that primary productivity plays a fundamental role in driving plant/animal diversity through promoting stochastic processes (Chase, 2010; Hutchinson, 1959; Rosenzweig, 1995), the key role of plant productivity in driving biodiversity seems to be general across both macro- and microbial communities. However, here the effect of plant productivity on microbial diversity was exhibited within a local ecosystem and over only five years' treatments, while plant productivity had a positive effect on plant/animal diversity only over larger spatiotemporal scales (e.g., across different ecosystems) (Hutchinson, 1959; Rosenzweig, 1995). The discrepancy at spatiotemporal scales was possibly because microorganisms have shorter life history (greater turnover) than higher organisms (Jessup et al., 2004).

It is also plausible that a larger quantity of belowground plant biomass (e.g., roots), regardless of plant species, promotes the diversity of chemical and physical micro-gradients in the soil, resulting in a greater breadth of "niche space" (i.e., the density of unique lifestyles supported) represented in the soil subsample used for DNA extraction and community analysis. This interpretation could also provide a mechanistic explanation for the increased importance of deterministic processes coinciding with reduced plant productivity; specifically, microbes associated with these plant-induced micro-gradients would be disfavored.

Another explanation for the comparatively stronger relationship between plant productivity and microbial functional diversity is that productivity could largely govern the number of effective intra-bacterial trophic levels supported, an idea that coincides with the "bottom-up" community theory of trophic structuring (Fretwell & Barach, 1977). Previously, the abundance of soil microbiota was shown to be almost entirely bottom-up regulated (Mikola & Setälä, 1988). And while studies have begun to reveal the existence of several intra-bacterial predator-prey relationships (Lueders, Kindler, Miltner, Friedrich, & Kaestner, 2006), the number of expected trophic chains, or even the linearity of these linkages, is far from understood. It is plausible that the height of the trophic pyramid (i.e., number of trophic levels supported) constrains microbial functional diversity more so than the breadth of unique detritivore strategies. This fundamental ecological principle may also be relatable to the observed decline in fungal relative abundance in treatments with low productivity and the positive correlation between productivity and genes belonging to the defense mechanisms COG category (Table 1), but its verification requires future testing and an improved framework of soil food web dynamics.

In this experiment, the treatment without all the three PFGs was comparatively more distinguishable from all the other treatments, because there was negligible aboveground plant biomass in this treatment. While it seems illogical to attribute the effect of this treatment to plant productivity alone, aboveground plant productivity was still identified to be the primary explainable variable for the change in functional gene richness when the five samples of this treatment were excluded from the stepwise regression analysis (p = .019). Meanwhile, when this treatment was excluded, the treatment of PR+PB removal had the smallest plant productivity (Figure 1a), which still caused larger functional SES value and larger relative importance of deterministic change than the other six treatments (Figure 4). Taken together, plant productivity was the most important factor driving microbial functional gene diversity.

We did not observe an effect of PFG richness/composition on microbial OTU diversity, which might be caused by several different reasons. First, plant diversity is traditionally expected to drive microbial diversity through providing more diverse types of carbon resources (Garbeva et al., 2004); however, similar carbon resources can be provided by different plant species (Klimeš & Klimešová, 2002; Pan, Han, Bai, & Yang, 2002). Therefore, here we hypothesize

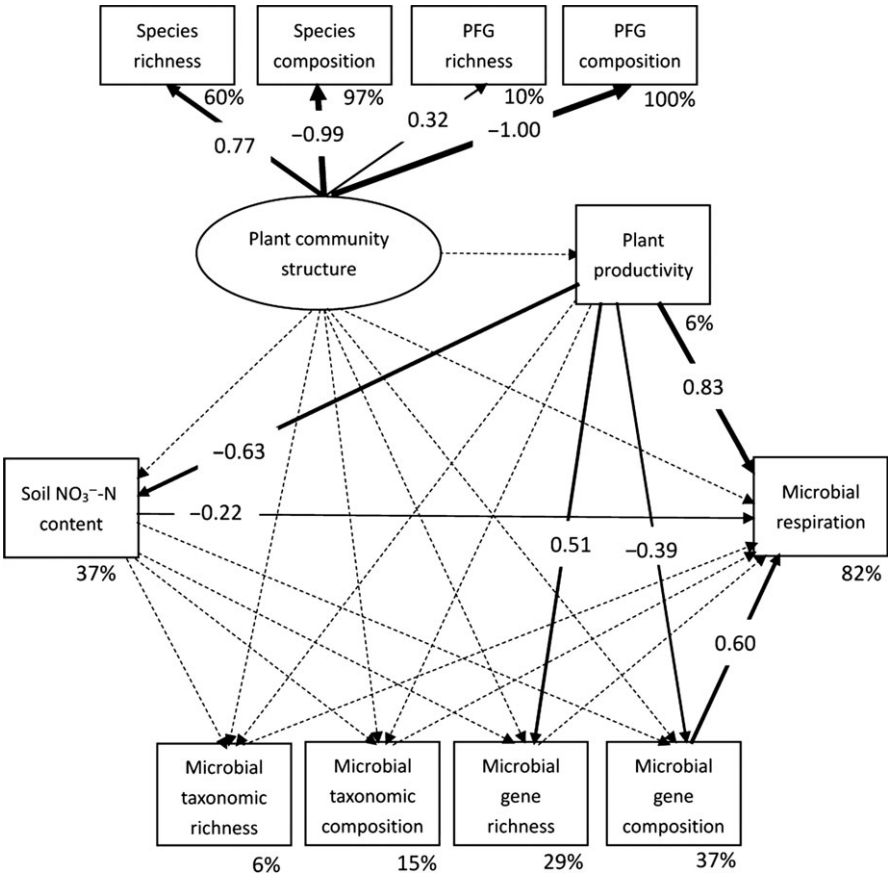

FIGURE 5 Structural equation model analysis of the effect of PFG removal on plant-soil-microbe system. The final model fit the data well: $\chi^2 = 27.959$, $p = .141$, $df = 21$, $n = 40$. Numbers at solid arrows ($p < .05$) are standardized path coefficients (equivalent to correlation coefficients), and width of the arrows indicates the strength of the relationships. The dashed arrows indicate non-significant relationships ($p > .05$). Percentages close to variables indicate the variance explained by the model (R^2)

that PFG richness/composition may have a comparatively small effect on the diversity of carbon resources, resulting in only a small stimulation effect on soil microbial taxonomic diversity, at least in grassland ecosystems like the one studied here (forested areas characterized by more numerous and diverse PFGs may offer a greater diversity of carbon and nitrogen substrates to soil heterotrophs). This hypothesis needs to be further confirmed in future studies. Second, it should be noted that the key role of plant productivity and the sensitive response of microbial functional diversity was found after only a five-year treatment. Thus, a greater importance of PFG diversity/composition and the response of microbial taxonomic diversity may also be exhibited over larger temporal scales (Wardle, 2006; Gao et al., 2013; Prober et al., 2015), particularly for less responsive or slower growing microbial groups. In other words, microbial taxonomic diversity may not have had sufficient time to respond to changes in plant diversity/composition. Third, the definition method of OTU (>97% similarity of 16S rRNA sequences) might not have enough resolution to reveal the sensitive response to the PFG treatments as functional gene diversity, as many coexisting members within 97% similarity of the 16S rRNA sequences have been found to possess many distinguishing (e.g., not shared) functional traits (Johnston et al., 2016). Finally, it should be noted that here we quantified the diversity of the bulk soil microbial communities, which should be less associated with particular plant functional groups than the rhizosphere communities (e.g., high specificity syntrophic relationships). In other words, a closer association between PFG diversity and the taxonomic diversity of rhizosphere microbial communities may have been found if included in the experimental design.

In summary, reduced plant productivity (rather than the decrease in PFG richness *per se*) decreased soil microbial functional gene diversity, but not taxonomic diversity, through promoting the deterministic process of ecological filtering. Thus, the maintenance of plant productivity may be more paramount to conserve belowground microbial functional gene diversity. The two dominant PFGs, PB, and PR (especially PR, which has only one species of *Leymus chinensis* in this ecosystem Zhang et al., 2014), have a crucial and compensatory effect in maintaining plant productivity in this semi-arid steppe ecosystem. Policies on biodiversity conservation should pay more attention on perennial rhizomatous grasses and perennial bunchgrasses for the sustainable management of this ecologically, culturally and economically important grassland ecosystem. However, whether the key role of plant productivity still holds when plant diversity loss is caused by other environmental change factors

(e.g., climate warming) rather than PFG removal, requires further investigation (Steinauer et al., 2015; Thakur et al., 2015).

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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