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Soil microbial beta-diversity is linked with compositional variation in aboveground plant biomass in a semi-arid grassland

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Abstract

Background and aims: Exploring biodiversity linkages between aboveground and belowground biota is a core topic in ecology, and can have implications on our understanding of ecosystem process stability. Yet, this topic still remains underexplored. Here, we explored diversity linkages, in terms of both alpha- and betadiversity, between plant and top soil microbial communities in a semi-arid grassland ecosystem. *Methods.* Soil microbial community structure was assessed based on both 16S rRNA and functional genes, and plant community composition was evaluated by traditional “species composition” and a newly-defined “biomass composition”, which includes the information on the biomass of each species. *Results*: The bacterial alpha-diversity, expressed as the richness and Shannon diversity of 16S rRNA genes, was significantly correlated with plant species richness and Shannon diversity, whereas the alpha-diversity of microbial functional genes showed marginal association with total plant biomass. Microbial beta-diversity, evaluated by 16S rRNA genes, showed close relationship with plant beta-diversity estimated by both “species composition” and “biomass composition”, while the microbial beta-diversity based on functional genes was only associated with the compositional variation in aboveground plant biomass. *Conclusions*: These results showed that the differences in metabolic potential of soil microbial communities, which is closely related with ecosystem functions, can be better predicted by the variation of plant-derived resources returned to soil, than merely by the species composition of the macro-organism communities.

Keywords: Aboveground-belowground diversity linkages, 16S rRNA, Soil bacterial diversity, Microbial functional gene diversity, GeoChip functional gene array

INTRODUCTION

Understanding linkages between the diversity of organisms aboveground and belowground is functionally important at the ecosystem scale in terms of the maintenance and stability of ecosystem processes (Wall and Moore 1999; Hooper et al. 2000). Furthering this understanding may provide information critical to the conservation of species, ecosystem maintenance, and the ecosystem services provided to humanity. The way aboveground and belowground communities are linked has received unprecedented attention over the last decade (Hooper et al. 2000; Wardle et al. 2004; De Deyn and Van der Putten 2005; van der Heijden et al. 2008; Prober et al. 2015). Nevertheless, a comprehensive comparison of the aboveground plants and the soil microbes is still lacking, due to the knowledge gap between microbiologist and macro-ecologists.

A few studies have explored relationships between plant and microbial alpha-diversity across a range of plant taxa and with a focus on various root-associated microbial groups, such as arbuscular mycorrhizal fungi (AMF) (Van der Heijden et al. 1998; Thoms et al. 2010; Antoninka et al. 2011; Gao et al. 2013), nitrogen-fixing rhizobia (Van der Heijden et al. 2006), and ammoniaoxidizing bacteria (AOB) (Kowalchuk et al. 2000), and reported positive, negative, or non-significant relationships between aboveground and belowground diversity. In comparison with alpha-diversity linkages, direct comparisons of plant-microbial beta-diversity at the community level are still limited (Prober et al. 2015). This limitation might be caused by the fact that most studies did not survey the corresponding plant and microbial communities within strictly dependent sampling sites (De Deyn and Van der Putten 2005), and plant diversity effects on soil microbial diversity could be context dependent (Tedersoo et al. 2016). Only a couple of publications have documented the aboveground-belowground beta-diversity linkages. By collecting completely paired species information, a strong coupling of plant and soil fungal beta-diversity was reported in a tropical forest ecosystem at a regional scale (Peay et al. 2013). Thereafter, a recent study conducted in grassland ecosystems across a global scale concluded that plant diversity predicts beta- but not alpha- diversity of soil microbes (Prober et al. 2015). In these two studies, the plant community composition was assessed as the number of species and/or the number of individuals observed in each plant species. Given the great influence of plant litter quality and quantity on soil microorganisms (Wardle et al. 2004), the impacts of aboveground plant biomass on the soil microbial community may outweigh that of the plant species composition. Consequently, it was proposed in our previous study that when addressing the aboveground-belowground diversity associations, the composition of a plant community evaluated merely by the number of species and individuals might not be representative enough. Instead, the

patterns of species-specific plant biomass (productivity) should also be taken into account (Li et al. 2015).

Here, we assayed a 9-year old field experiment established in a typical steppe in Inner Mongolia, China, that was designed to explore the ecosystem responses to two projected environmental changes, increased precipitation and nitrogen (N) deposition (Xu et al. 2015). Our previous studies documented that increased precipitation enhanced plant species richness and the percent cover of forbs, whereas N fertilization reduced species richness and enhanced the coverage of grasses (Xu et al. 2012; Xu et al. 2015). A gradient of plant species richness (from 7 to 22) and differences in community composition generated by these long-term treatments (Xu et al. 2012; Xu et al. 2015) provide an ideal experimental site for comparison of aboveground-belowground biodiversity linkages. Although these differences in diversity and community composition were generated artificially, they still could represent, at least partially, the natural gradients caused by environmental stress. We hypothesized that the diversity of soil microbial community would show positive correlations with that of the plant community, in terms of both alpha- and beta-diversity, because of the close associations between the producers (plants) and the decomposers (soil microbes) (Wardle et al. 2004). We also hypothesized that plant community composition estimated by species-specific biomass would be more closely linked with soil microbial community composition than the traditional plant species composition, due to a greater influence of plant litter resources on microbial communities.

MATERIALS AND METHODS

Site description and experimental design

The study sites were located at a temperate steppe in Duolun, Inner Mongolia, northern China. This area falls within the typical temperate zone and is characterized by a semiarid continental monsoon climate. Mean annual precipitation (MAP) is ~380 mm, and mean annual temperature is 2.1 °C. Soil is chestnut according to the Chinese classification and Calcis-orthic Aridisol in the US Soil Taxonomy classification. This grassland is dominated by herbaceous species, e.g. Stipakrylovii spp., Artemisia frigida, and Agropyroncristattum spp.. Plants begin to turn green from early April each year and reach the peak aboveground biomass in early September, and almost all the aboveground plant tissues die during the winter (beginning from November). The naturally assembled plant communities consist of about 15 species per m^2, and cover about 64% of the ground. Because of increased fossil fuel combustion and application of artificial fertilizers, the atmospheric nitrogen (N) deposition is expected to increase in northern China (Zhang et al. 2008; Liu et al. 2011). The summer precipitation is also projected to increase in this region in the coming decades (Sun and Ding 2010).

The field experiment was established in 2005 to examine the ecosystem response to projected N deposition and precipitation change (Xu et al. 2012). A splitplot experimental design was employed in this field experiment, which involves two levels of precipitation (ambient or added) applied at the plot scale and four levels of N (0, 5, 10, 15 g N m^{-2} $yr.^{-1}$) applied to subplots within precipitation treatments. We choose four treatments in this study, including Control (no water and no nitrogen), N addition (no water, 10 g N m^{-2} $yr.^{-1}$), increased precipitation (50% of the mean annual rainfall, no nitrogen), and the combination of N addition and increased precipitation (50% of the mean annual rainfall, and 10 g N m^{-2} $yr.^{-1}$). Each treatment was replicated in seven experimental blocks. For a total of 28 blocks, each block was 8 m × 8 m, and was separated by 1 m wide corridors. In the middle of the growing season, from June to August, the water-added blocks received 15 mm of precipitation weekly by sprinkling irrigation. A total of 180 mm precipitation, approximately 50% of mean annual rainfall, was added yearly. The N amendment was urea (applied twice, half in early May and the other half in late June).

Because the specific aim of this study was to compare aboveground and belowground community diversity across an environmental gradient, we did not attempt to replicate our treatments but instead relied on correlation-based statistical analyses.

Plant investigation and diversity estimates

In mid-July 2013, each plant species presented within a pre-established 1 m × 1 m permanent quadrat was recorded in each subplot to determine plant species richness. In early September, aboveground living plant tissues within a randomly selected 0.15 m × 2.0 m strip in each subplot were harvested by species, and the harvest was dried for 48 h at 65 °C and weighted to determine plant aboveground biomass.

We used two “species by site” matrices to determine plant community beta-diversity: a “species composition” matrix, including the number of individuals observed in each species; and a “biomass composition” matrix, containing the biomass of each species. Bray-Curtis distances were computed using the “Vegan” R package, based on both matrices to examine the dissimilarity of plant community composition among individual blocks.

Soil collection and analysis

Soil samples were collected from each experimental block after the harvest of plant biomass by the end of August 2013. For each soil sample, five soil cores (5 cm diameter) were randomly collected from the topsoil (0– 15 cm) and then mixed to form one composite sample. Soil samples were passed through a 2.0 mm sieve and stored at 4 °C before soil property analysis, and −80 °C before soil genomic DNA extraction. Soil physicochemical properties, including total carbon (TC), total nitrogen (TN), C/N ratio, total phosphorous (TP), total sulfur (TS), nitrate-N (NO_3^- -N) and ammonium-N (NH_4^+ -N),

dissolved organic carbon (DOC), moisture, and pH were determined by regular methods as described previously (Li et al. 2016; Li et al. 2017). Soil microbial biomass carbon (MBC) and nitrogen (MBN) were determined using the fumigation-extraction method (Brookes et al. 1985). Soil basal respiration (BR) and substrate induced respiration (SIR) was measured using Li-COR 8200 Infrared Gas Analyzer (IRGA) (Li-COR Biosciences, Lincoln, NB, USA). The carbon availability index (CAI) was calculated by dividing the basal respiration rate with the SIR rate (Gershenson et al. 2009).

Microbial community DNA isolation and molecular analysis

The DNA was extracted from approximately 0.35 g of fresh soil by using the MoBio Power Soil DNA extraction kit (MoBio Laboratories Inc. Carlsbad, California, USA). The V_1-V_3 region of the 16S rRNA gene was amplified by using primers 27F and 533R (Sun et al. 2014), and sequenced on 300PE MiSeq (Illumina, San Diego, CA, USA) as previously described (Li et al. 2016). The paired-end 300 bp reads were demultiplexed, trimmed of reads containing ambiguous bases and long homopolymers, and merged using QIIME v 1.7.0 (Caporaso et al. 2012). Unique sequences were sorted by abundance, and singletons in the data set were discarded. Chimeras were filtered using the Ribosomal Database Project (RDP) (Wang et al. 2007) and UCHIME (Edgar et al. 2011), and all quality-filtered sequences were clustered into OTUs (Operational Taxonomic Unit) at 97% similarity cutoff using UPARSE (version 7.1, http://drive5.com/uparse/). Since diversity is unavoidably influenced by the number of sequences collected, a subset of 9409 sequences were randomly selected from bacterial 16S rRNA datasets. Representative sequences were classified taxonomically using the RDP classifier against Greengenes (McDonald et al. 2012) based on a 50% confidence threshold. Census at each taxonomical level was processed with Perl scripts. The sequence data have been submitted to the GenBank database under accession number SRP044829 (Biosample numbers SAMN 02919274~277, 282~285, 290~293, 298~301, and SAMN 05200518~529).

The newest generation of the functional gene array GeoChip (5.0_60K) (Aligent), containing 57,000 oligonucleotide probes covering different biogeochemical processes, was used in this study to detect soil microbial functional gene diversity and composition. Samples were prepared and hybridized as previously described (Yan et al. 2015). In brief, 700 ng of soil microbial community genomic DNA was labeled with the fluorescent dye Cy-3 (GE Healthcare) by random priming. The labeled DNA was mixed with 42 μL of buffer (1 × HI-RPM hybridization buffer, 1 × Acgh blocking agent, 0.05 μg/μL Cot-1 DNA, 10 pM universal standard, and 10% formamide), and incubated at 95 °C for 3 min. The prepared samples were then hybridized to the array at 67 °C for 24 h in hybridization oven. The hybridized microarrays were scanned by using a NimbleGen MS200 Microarray Scanner (Roche NimbleGen, Inc., Madison, WI, USA) at 633 nm. The scanned images were converted and extracted by the Agilent Feature Extraction 11.5 software (Agilent Technologies, Inc.). Raw data were analyzed by the following major

steps as described previously (He et al. 2010; Li et al. 2017). First, adjusting signal-to-noise ratio (SNR), and then removing spots with a signal intensity less than 1.3 times the background. Second, the universal standard spots were used for spatial normalization within a slide, and the Cy3 intensity (sample signal) was then normalized by the mean intensity of the universal standards of all slides. Third, the probes that appeared in only one of seven replicates in each treatment were removed as noise. Afterwards, the relative abundance in each sample was calculated by dividing the individual signal intensity of each probe by the sum of the original signal intensity for all detected probes in that sample. Raw data were submitted to the Microarray Data Manager System (http://ieg.ou.edu/microarray/).

The microbial species richness was represented by the number of unique OTUs (16S rRNA gene) or the number of gene probes detected by the Geochip (functional gene). The non-parametric Shannon's diversity index (Shannon 1948) was also calculated based on 16S rRNA gene and functional gene datasets for comparison of alpha-diversity. For microbial beta-diversity, Bray-Curtis distances were computed using the "Vegan" R package, based on either 16S rRNA gene matrix or functional gene matrix. To evaluate whether the relationship with plant communities differed across bacterial taxonomic lineages, we also calculated the alpha- and beta- diversity of two of the most abundant phyla, Proteobacteria and Acidobacteria, which have relatively distinct trophic strategies (Fierer et al. 2007) and are usually reported to be influenced by N and water addition (Ramirez et al. 2010; Evans and Wallenstein 2012; Li et al. 2016). In a broad ecological meaning, the Proteobacteria is usually grouped as the copiotrophic bacteria, and Acidobactria is typically classified as the oligotrophic microbes (Fierer et al. 2007). Moreover, to elucidate which specific functional gene categories are more strongly correlated with plant communities, the diversity of genes involved in carbon (C) degradation and N cycling were also included in the plant-microbe correlation analysis, given the important roles these two gene subsets played in plant litter decomposition and soil nutrient cycling.

Statistical analysis

Two-way ANOVAs with a split-plot design were used to determine the effects of N, increased precipitation and their interactive effects on microbial alpha-diversity data. All these statistic analysis were conducted by using SPSS16.0 (SPSS, Inc., Chicago, IL, U.S.A). To determine whether N amendment and increased precipitation significantly influenced microbial community composition, permutational multivariate analysis of variance (PERMANOVA) was performed using the Adonis function implemented in the R statistical environment (v 3.1.0) (R Development Core Team 2011), based on BrayCurtis dissimilarity matrix of 16S rRNA genes and functional genes.

To correlate microbial gene richness with plant species richness and biomass, we firstly performed a simple linear regression. To correct the N and watering effects on the diversity relationship, a linear mixed-effects

model (lme) with the four treatments as random-effect factor was further performed with the package nlme in R. A multiple regression analysis was used to examine the soil and plant factors that could effectively explain the variation in the microbial taxonomic and functional gene richness. Because of the collinearity among environmental parameters, we pre-selected the soil and plant parameters by removing the parameters that were highly correlated with other parameters ($r > 0.6$). A subset of soil parameters of TC, TN, DOC, NO_3^--N, NH_4^+-N, moisture, pH, MBC, BR, and plant biomass and species richness were retained for further stepwise regression analysis. The variation inflation factor (VIF) was used as the criterion to further identify collinearity among explanatory variables. Since the VIF values will change after each variable is removed, a stepwise selection of variables using VIF was performed. In detail, starting with the full set of explanatory variables, a VIF for each variable was calculated. The variable with the single highest value was removed and the process repeated until all values are below the threshold (here we used $VIF < 5$ as the threshold). The stepwise regression analysis was performed using SPSS (SPSS Inc., v 16.0, Chicago, Illinois).

To link the beta-diversity of the aboveground and belowground community, Bray-Curtis distances of microbial community were correlated with plant community dissimilarities using Mantel test implemented in the Vegan package in R (Oksanen et al. 2015). To remove the influences of soil variables on plant communities, partial Mantel test were further performed to evaluate the beta-diversity relationship between aboveground and belowground communities by controlling soil property distance matrix as constant. Because most soil variables are correlated, the dissimilarity in soil properties was calculated as the Euclidean distance generated from the scaled principal component analysis scores for each blocks based on the 15 soil variables detected. To further identify the relative importance of soil property and plant community dissimilarity contributing to the variation of soil microbial community composition, a multiple regression on matrices (MRM) was performed using “ecodist” R package (Goslee and Urban 2007). The partial regression coefficients of an MRM model gave a measure of rate of change in microbial community similarity for variables of interest when other variables were held constant (Martiny et al. 2011).

RESULTS

Effects of nitrogen and water addition on soil microbial alpha-diversity and beta-diversity

N addition significantly decreased the overall number of bacterial OTUs ($P < 0.05$; Fig. 1a) and the Shannon diversity ($P < 0.05$; Table S1), whereas increased precipitation showed no effects on bacterial alpha diversity index ($P > 0.05$ for both observed OTUs and Shannon diversity). N addition and increased precipitation showed significant ($P < 0.05$) interactive effects on the observed OTUs and Shannon diversity (Fig. 1a and Table S1). Proteobacteria and Acidobacteria were detected as two of the most

abundant phyla in soil bacterial communities, comprising 28.8% and 11.3% of all sequences, respectively (Fig. S1). The richness of Proteobacteria was significantly enhanced by increased precipitation ($P < 0.05$; Fig. 1b). The richness and Shannon diversity of acidobacterial community was only influenced by N addition ($P < 0.05$; Fig. 1c and Table S1), with a sharp decrease under N amendment.

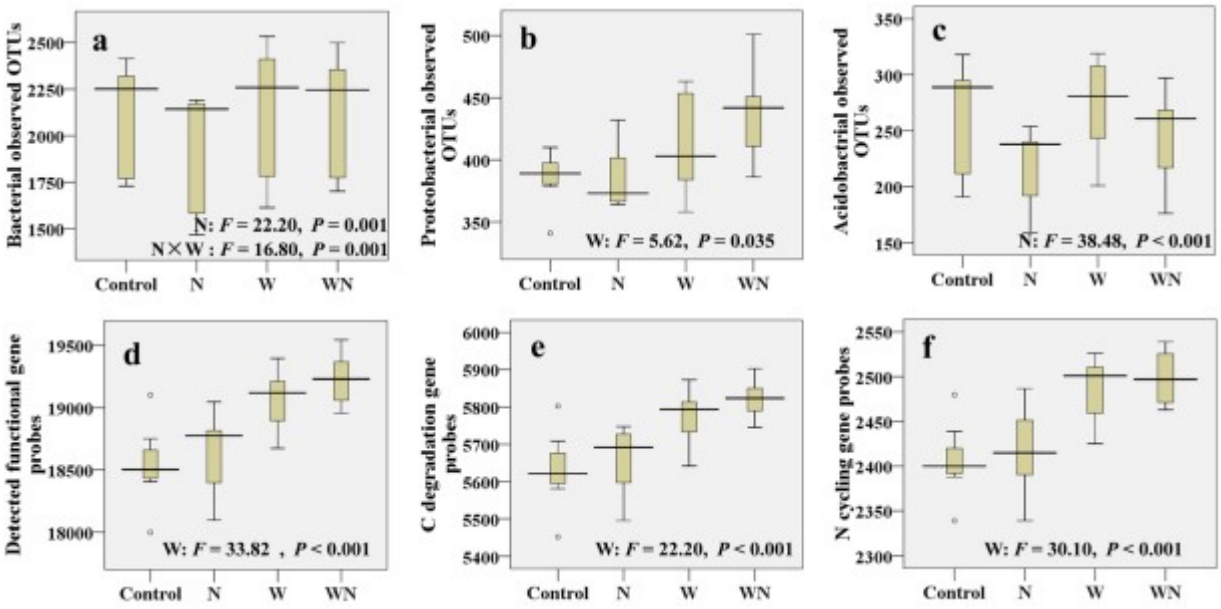

Fig. 1. Responses of soil microbial taxonomic gene (**a**, **b**, **c**) and functional gene (**d**, **e**, **f**) richness to nitrogen addition and increased precipitation. Effects of nitrogen addition and increased precipitation on microbial richness were estimated by two-way ANOVAs with a split-plot design. N: nitrogen addition, W: increased precipitation, N × W: interaction between water and nitrogen. Only significant effects with $P < 0.05$ were shown on the panel. The raw data of functional gene richness (**d**, **e**, **f**) had been previously reported in a closely related research (Li et al. 2017).

Increased precipitation significantly increased the functional gene richness and Shannon diversity ($P < 0.05$ for both, Fig. 1d and Table S1). Nevertheless, N addition and N plus water addition showed no significant effects on these diversity indices. The responses of the alpha-diversity of carbon (C) degradation genes and N cycling genes to increased N and precipitation are in line with the total functional genes. The richness and Shannon diversity of these two gene categories were consistently elevated by water supply ($P < 0.05$; Fig. 1e, Fig. 1f and Table S1), whereas N addition, alone or in combination with water, elicited no significant effects.

Beta-diversity of microbial communities demonstrated significant variations in the community composition under water and N addition. In the NMDS plot based on the Bray-Curtis distance of 16S rRNA genes (Fig. 2a), the horizontal axis clearly separated bacterial communities by N addition treatment. PERMANOVA (Table S2) consistently showed that the overall bacterial community composition was significantly changed by N addition ($F = 5.754$,

P = 0.001). The water supply also had significant effects on the overall bacterial community composition (F = 2.414, P = 0.013), but no water and N interactive effects were detected (P > 0.05). Similarly, the community composition of proteobacterial group was also significantly influenced by N addition (F = 6.446, P = 0.001) and increased precipitation (F = 2.436, P = 0.039). Acidobacterial community composition was significantly changed by N addition (F = 7.140, P = 0.001), increased precipitation (F = 2.386, P = 0.008) and their interactions (F = 2.006, P = 0.023).

NMDS ordination of all detected functional genes showed that the vertical axis mainly differentiated the functional gene structure by water supply treatments (labeled by triangle) from those under the normal precipitation conditions (labeled by circle), whereas the horizontal axis mainly differentiated the functional gene structure by N addition treatment (Fig. 2b). PERMANOVA (Table S2) showed that the overall functional gene structure was significantly altered by N addition (F = 4.485, P = 0.001), increased precipitation (F = 4.356, P = 0.001) and interaction of N and precipitation (F = 2.221, P = 0.006). The results from the subsets of genes involved in C degradation and N cycling process showed similar patterns to the overall functional genes (Table S2).

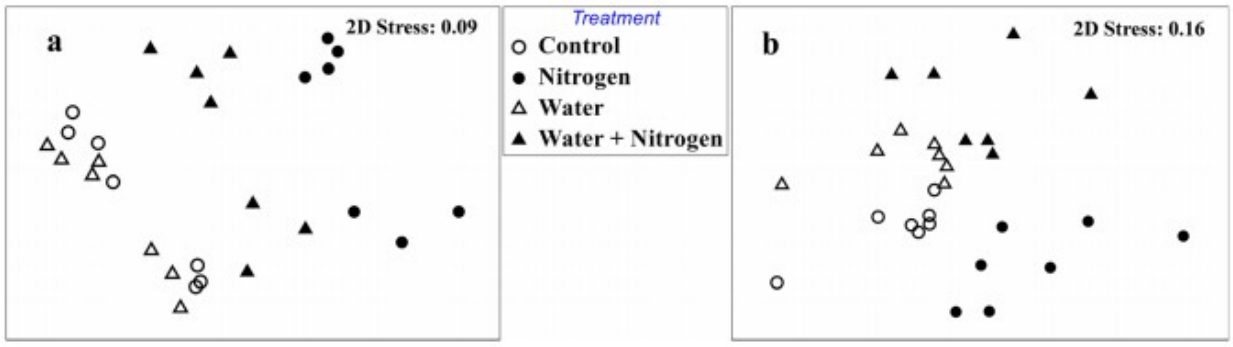

Fig. 2. Non-metric multi-dimensional scaling (NMDS) plots of the microbial communities based on Bray-Curtis distances of 16S rRNA gene matrix (**a**) and functional gene matrix (**b**).

Alpha-diversity linkage between aboveground and belowground communities

To compare the aboveground and belowground biodiversity across the entire environmental gradient studied in this field experiment, the plant-microbe correlation analysis was performed with each replicate representing an individual data point, rather than relying on isolated differences among treatments. With the sequencing depth of 9409 sequences per sample, the whole sequencing dataset comprised a total of 6969 OTUs at a cutoff of 97% sequence similarity. The observed bacterial OTU numbers across all soil samples varied between 1469 and 2531 (mean = 2049, s.d. = 334), and the detected functional gene number (the number of genes detected by Geochip probes) ranged from 17,998 to 19,546 (mean = 18,862, s.d. = 400). The plant species richness in this field experiment varied from 7 to 22 species (mean = 13.79, s.d. = 3.86). The aboveground plant biomass showed great

variation, with the lowest value of 161.4 g/m^2, and highest value of 542.8 g/m^2. The mean aboveground plant biomass was 290.7 (s.d. = 96.9).

Across all soil samples, the observed bacterial OTU numbers was positively correlated with plant species richness (r^2 = 0.338, slope = 50.23, t = 3.641, P = 0.001; Fig. 3a), as estimated by simple linear regression. However, there was no significant correlation between bacterial OTU numbers and total aboveground plant biomass (r^2 = 0.052, slope = −0.784, t = −1.191, P = 0.244; Fig. 3b). In contrast, the functional gene richness showed a positive correlation with total aboveground biomass (r^2 = 0.193, slope = 1.814, t = 2.493, P = 0.019; Fig. 3d), but exhibited no relationship with plant species richness (r^2 = 0.053, slope = 23.75, t = 1.200, P = 0.241; Fig. 3c). To correct the treatment effects on the aboveground-belowground diversity relationship, we further assessed the plant-microbe diversity relationship by using a linear mixed-effect (lme) model, regarding N and watering treatments as random effect factors. In line with the result of simple linear regression, the lme model consistently showed a positive correlation between observed bacterial OTUs and plant species richness (slope = 50.23, t = 3.641, P = 0.001; Table 1), and no significant correlations between bacterial OTU numbers and plant biomass, or between functional gene richness and plant species richness (Table 1). However, the relationship between the functional gene richness and the plant biomass has been slightly changed with lme model. The functional gene richness only showed a marginal positive correlation with the plant biomass in the lme model (slope = 0.846, t = 1.403, P = 0.174; Table 1).

The aboveground-belowground Shannon index correlation showed a similar pattern to that of the richness. Specifically, the bacterial Shannon-index was positively correlated with plant community Shannon-index (slope = 0.275, t = 2.517, P = 0.019; Table S3), but showed no association with plant biomass (slope = -1.1×10^{-3}, t= −1.498, P = 0.148), as evaluated by lme model. Although the regular linear regression illustrated a significant positive correlation between functional gene Shannon diversity and plant biomass (r^2 = 0.198, slope = 9.77×10^{-5}, t = 2.529, P = 0.018), the lme model showed that the functional gene Shannon index only showed a marginal positive correlation with plant biomass (slope = 4.8×10^{-5}, t = 1.471, P = 0.155), and showed a weak negative correlation with plant Shannon diversity (slope = -6.2×10^{-3}, t= −1.142, P = 0.265). Thus, in general, the richness and Shannon diversity of bacterial 16S rRNA genes was significantly correlated with plant species richness and Shannon diversity, whereas the alpha-diversity of microbial functional genes showed marginal association with total plant biomass.

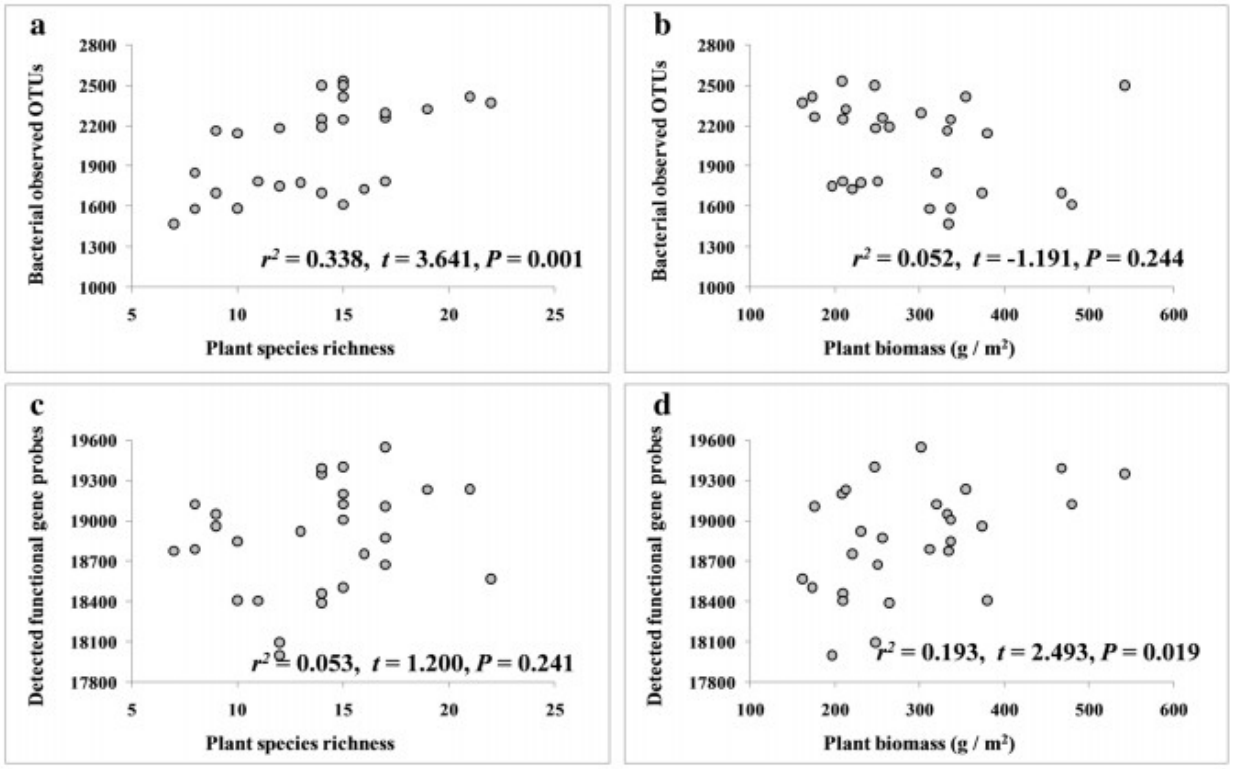

Fig. 3. Comparison of microbial taxonomic/functional gene richness and aboveground plant species richness and biomass (n = 28). We regressed observed bacterial OTUs with (**a**) plant species richness and (**b**) aboveground plant biomass, and detected functional gene probes with (**c**) plant species richness and (**d**) aboveground plant biomass.

To evaluate whether the relationship with plant communities differed across bacterial trophic strategies and functional gene categories, we also assessed the diversity of specific bacterial lineages (Proteobacteria and Acidobacteria) and functional gene groups (carbon degradation genes and N cycling genes). Consistent with the patterns of overall bacterial richness, the observed proteobacterial OTU numbers was positively correlated with plant species richness (slope = 5.770, t = 3.571, P = 0.002; Table 1), whereas the richness of Acidobacteria had no significant correlation with plant species richness (slope = 2.145, t = 1.283, P = 0.212). There was no relationship between the proteobacterial richness and the plant aboveground biomass (slope = −0.031, t = −0.415, P = 0.682); the acidobacterial richness even showed a negative correlation with plant biomass (slope = −0.151, t = −2.325, P = 0.029). A simple regular regression showed that the richness of both carbon degradation genes (r^2 = 0.186, slope = 0.508, t = 2.437, P = 0.022) and N cycling genes (r^2 = 0.200, slope = 0.261, t = 2.551, P = 0.017) were positively correlated with aboveground plant biomass. However, the lme models showed that the richness of these functional genes only exhibited a marginal positive correlation with plant aboveground biomass (Table 1), in line with the total functional genes.

Stepwise regression further supported that microbial taxonomic richness (represented as the richness of 16S rRNA genes) was most regulated by plant species richness (standard coefficient r = 0.642; Table 2), whereas microbial functional gene richness was more governed by the amount of plant-originated resources returned to soil (standard coefficient r = 0.599 for plant biomass; Table 2). In addition to the plant species richness, the overall bacterial richness also showed positive correlation with soil NO_3^--N (standard coefficient r = 0.321, P = 0.046). The functional gene richness was also positively correlated with microbial biomass carbon (MBC) (r = 0.427, P = 0.012) and negatively correlated with NH_4^+-N (r = −0.356, P = 0.026). With respect to the specific bacterial lineages, acidobacterial richness also showed a positive correlation with plant species richness (standard coefficient r = 0.607, P = 0.001), whereas the proteobacterial richness was only linked to soil total nitrogen (TN) (r = 0.549, P = 0.002). The richness of carbon degradation genes and N cycling genes are in line with that of the total functional genes, being positively correlated with aboveground plant biomass and MBC, and negatively correlated with NH_4^+-N (Table 2).

Table 1 Plant-microbe alpha-diversity relationship as estimated by linear mixed-effect models using nitrogen and water treatments as the random effect factor

	AIC	Intercept	Slope	*t*	*P*
Observed bacterial OTUs ~ Plant species richness	385.54	1356.37	50.23	3.641	**0.001**
Proteobacterial OTUs ~ plant species richness	269.68	325.92	5.770	3.571	**0.002**
Acidobacterial OTUs ~ plant species richness	274.36	488.25	2.145	1.283	0.212
Observed bacterial OTUs ~ Plant biomass	400.86	2344.69	−1.018	−1.513	0.144
Proteobacterial OTUs ~ plant biomass	284.49	414.59	−0.031	−0.415	0.682
Acidobacterial OTUs ~ plant biomass	277.29	561.59	−0.151	−2.325	**0.029**
Functional gene richness ~ Plant species richness	391.22	18,827.42	2.488	0.164	0.871
C degradation gene richness ~ Plant species richness	327.84	5709.85	0.744	0.166	0.870
N cycling gene richness ~ Plant species richness	289.31	2449.22	0.194	0.091	0.929
Funcrional gene richness ~ Plant biomass	395.75	18,615.94	0.846	1.403	0.174
C degradation gene richness ~ Plant biomass	332.43	5648.31	0.247	1.381	0.181
N cycling gene richness ~ Plant biomass	293.64	2415.77	0.124	1.469	0.155

The significant *P* values were shown in bold

Beta-diversity linkages between aboveground and belowground communities

The average Bray-Curtis distance of the 16S rRNA gene matrix was 0.433 (s.d. = 0.069, range = 0.265~0.623), and the average functional gene beta-diversity was only 0.052 (s.d. = 0.009, range = 0.028~0.080). This suggested that the similarity of microbial functional gene structure among plots is higher than the similarity of the bacterial taxonomic composition. Plant beta-diversity calculated based on the Bray-Curtis distance of species abundance was 0.616 (s.d. = 0.104, range = 0.301~0.888), and the plant community beta-diversity measured using species-specific biomass matrix was 0.683 (s.d. = 0.131, range = 0~0.958).

Simple Mantel tests revealed that microbial taxonomic beta-diversity calculated using the 16S rRNA gene dataset showed a positive correlation with plant community beta-diversity, estimated by both species composition (Mantel r = 0.252, P = 0.002; Fig. 4a) and a species-specific biomass matrix (Mantel r = 0.245, P = 0.001; Fig. 4b). Unlike the patterns of taxonomic betadiversity, the Bray-Curtis distance of the microbial community generated from the functional gene abundances was not correlated with the plant beta-diversity calculated by the number of individuals observed in each species (Mantel r = 0.143, P > 0.05; Fig. 4c), but was significantly correlated with plant beta-diversity estimated by the species-specific “biomass composition” (Mantel r = 0.275, P = 0.001; Fig. 4d).

Partial Mantel test revealed similar results to that of the simple Mantel test (Table 3). When the effects of soil property distance were held constant, the soil microbial taxonomic beta-diversity was positively correlated with the variation of plant communities (partial Mantel r = 0.214 for species composition distance, and r = 0.222 for biomass composition distance, P < 0.01 for both cases). However, when we removed the effects of the soil dissimilarities, microbial functional gene betadiversity showed no significant correlation with plant species composition distance (partial Mantel r = 0.109, P = 0.147), but was positively correlated with the compositional variation in aboveground plant biomass (partial Mantel r = 0.258, P = 0.004).

Comparisons of beta-diversity between specific bacterial groups/functional gene categories and plant communities consistently revealed that the microbial taxonomic beta-diversity was positively correlated with the variations of both plant species composition and biomass composition, whereas the functional gene beta-diversity was only correlated with the variation in biomass composition (Table 3). Specifically, the beta-diversity of Proteobacteria and Acidobacteria both showed positive correlations with plant species composition distance (partial Mantel r = 0.137 for Proteobacteria, and r = 0.240 for Acidobactia, respectively, P < 0.01 for both cases), and with biomass composition distance (partial Mantel r = 0.123 for Proteobacteria, and r = 0.241 for Acidobactia, respectively, P < 0.01 for both). The variations in carbon degradation genes and N cycling genes structure were only correlated with plant beta-diversity estimated by species-specific biomass matrix (partial Mantel r = 0.277 for C degradation gene, and r = 0.280 for N cycling gene, respectively, P < 0.01 for both), but showed no significant correlation with the variations of plant species composition (P > 0.05 for both).

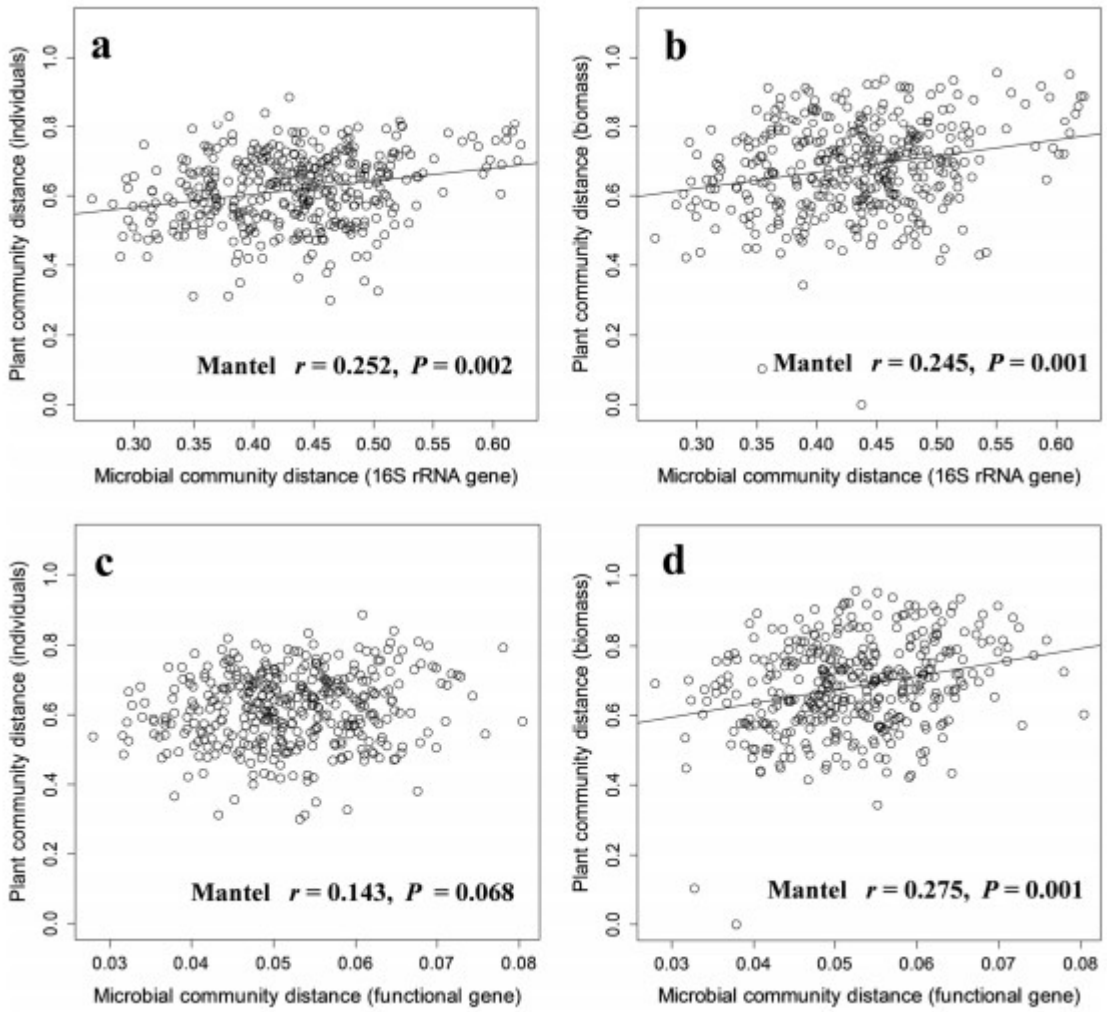

Fig. 4. Comparison of beta-diversity between plant and microbial communities. Bray-Curtis distances of microbial communities computed based on 16S rRNA gene data (**a**, **b**) and functional gene data (**c**, **d**) were regressed against plant community dissimilarity calculated based on the number of individuals in each species (**a**, **c**) and species-specific biomass (**b**, **d**), respectively.

Table 2 Soil and plant variables that explain the variation in the microbial taxonomic and functional genes richness, as revealed by stepwise regression

Variables	Model	R^2	F	P	Standardized coefficient of individual variables
Bacterial OTUs	y = 1051.53 + 55.51 (plant richness) + 24.79 (NO_3^--N)	0.437	9.714	0.001	Plant richness: $\beta = 0.642$, $P < 0.001$, VIF[a] = 1.037 NO_3^-: $\beta = 0.321$, P = 0.046, VIF = 1.037
Proteobacterial OTUs	y = 198.02 + 107.66 (TN)	0.301	11.204	0.002	TN: $\beta = 0.549$, $P = 0.002$, VIF = 1.000
Acidobacterial OTUs	y = 147.01 + 7.30 (plant richness)	0.368	15.140	0.001	Plant richness: $\beta = 0.607$, $P = 0.001$, VIF = 1.000
Functional gene richness	y = 18,374 + 2.47 (plant biomass) + 2.62 (MBC) − 49.83 (NH_4^+-N)	0.466	6.980	0.002	Plant biomass: $\beta = 0.599$, $P = 0.001$, VIF = 1.116 MBC[b]: $\beta = 0.427$, $P = 0.012$, VIF = 1.111 NH_4^+: $\beta = -0.356$, $P = 0.026$, VIF = 1.010
C degradation gene richness	y = 5578.1 + 0.694 (plant biomass) + 0.741 (MBC) − 13.71 (NH_4^+-N)	0.448	6.499	0.002	Plant biomass: $\beta = 0.599$, $P = 0.001$, VIF = 1.116 MBC: $\beta = 0.427$, $P = 0.012$, VIF = 1.111 NH_4^+: $\beta = -0.356$, $P = 0.026$, VIF = 1.010
N cycling gene richness	y = 2367.5 + 0.361 (plant biomass) + 0.406 (MBC) − 6.715 (NH_4^+-N)	0.494	7.806	0.001	Plant biomass: $\beta = 0.599$, $P = 0.001$, VIF = 1.116 MBC: $\beta = 0.427$, $P = 0.012$, VIF = 1.111 NH_4^+: $\beta = -0.356$, $P = 0.026$, VIF = 1.010

The standardized regression coefficient measures the impact of a unit change in the standardized value of variables on the standardized value of microbial richness. The larger standardized coefficient represents more contributions

[a] VIF: variation inflation factor

[b] MBC: microbial biomass carbon

Multiple regression on distance matrices (MRM) analysis (Table 4) further confirmed that the dissimilarity of the plant species composition made the largest contribution to the variation of the overall bacterial taxonomic betadiversity with partial regression coefficients of 0.1110 ($P = 0.018$), but was not strongly correlated with the microbial functional gene beta-diversity ($P = 0.643$). Instead, the compositional variation in plant biomass was a major driver of the microbial functional gene beta-diversity ($\beta = 0.017$, $P = 0.008$). Soil property distance was significantly correlated with microbial beta-diversity of both taxonomic composition ($\beta = 0.0107$, $P = 0.002$) and functional gene structure ($\beta = 0.0011$, $P = 0.043$). Regarding the specific bacterial groups, the acidobacterial beta-diversity showed a significant correlation with the variation of both plant species composition ($\beta = 0.1363$, $P = 0.035$) and plant biomass composition ($\beta = 0.1078$, $P = 0.020$), whereas the proteobacterial beta-diversity only showed significant association with plant species composition dissimilarity ($\beta = 0.1233$, $P = 0.048$). Consistent with the pattern of the overall functional genes, the variations in C degradation genes and N cycling genes structure showed no relationship with plant species composition dissimilarity ($P > 0.05$ for both). The beta-diversity of C degradation genes was only correlated with the compositional variation in plant biomass ($\beta = 0.0270$, $P = 0.001$), and that of N cycling genes was significantly driven by both plant biomass composition dissimilarities ($\beta = 0.0308$, $P = 0.001$) and soil property distance ($\beta = 0.0018$, $P = 0.013$).

DISCUSSION

In this study, we observed that the richness of both overall bacterial communities and specific taxa (Proteobacteria and Acidobacteria) was positively correlated with plant species richness (Fig. 3a, Table 1, and Table 2), in agreement with the general hypothesis that diverse plant species would sustain diverse litter types and root exudates, and thus, a species-rich decomposer community (Kowalchuk et al. 2002; Steinauer et al. 2016). However, the bacterial richness and Shannonindex showed no significant positive correlation with plant biomass. High plant biomass usually generates a relative nutrient-rich ecological niche, which may serve as a selection stress that could lower the microbial diversities (Campbell et al. 2010). We even observed a negative correlation between the richness of Acidobacteria and plant aboveground biomass. Acidobacteria are known as typical oligotrophs, which do not prefer the rich labile carbon resources derived from the fresh plant biomass (Fierer et al. 2007; Li et al. 2016).

Unexpectedly, the microbial functional gene richness, including the total functional gene and genes involved in C degradation and N cycling, showed no significant correlations with plant species richness in this field experiment (Fig. 3c, Table 1 and Table 2). Instead, the functional gene richness was positively correlated with plant aboveground biomass, as estimated by regular linear regression and stepwise regression analysis (Fig. 1d and Table 2) (though the lme model only revealed a marginal positive correlation, $P <$

0.2; Table 1). These results suggest that the microbial taxonomic and functional gene richness were correlated with different plant parameters. A recent publication found marginal positive effects of plant diversity, but no significant effects of root exudates treatment, on soil microbial taxonomic richness (Steinauer et al. 2016). In contrast, the microbial extracellular enzyme activities showed weak responses to plant diversity, but increased significantly with elevated root exudates diversity (Steinauer et al. 2016). These results support, at least partially, our findings that soil microbial taxonomic diversity was more regulated by plant species diversity, whereas the microbial function (i.e. the microbial extracellular activities or the functional genes) was more correlated with the plant-derived resources (i.e. the root exudates or the plant biomass).

Table 3. The correlations between microbial community composition (estimated by 16S rRNA gene and functional gene datasets) and the plant community composition (calculated based on species composition and biomass composition) determined by partial mantel test, with the soil property distance matrix partialed out.

	Plant species composition		Plant biomass composition	
	r	*P*	*r*	*P*
Overall Microbial community composition	0.214	**0.004**	0.222	**0.003**
Proteobacterial community composition	0.137	**0.017**	0.123	**0.021**
Acidobacterial community composition	0.240	**0.008**	0.241	**0.002**
Overall functional gene structure	0.109	0.147	0.258	**0.004**
C degradation gene structure	0.010	0.494	0.277	**0.002**
N cycling gene structure	−0.030	0.611	0.280	**0.001**

The significant *P* values were shown in bold

Table 4. Relative contributions of the dissimilarity of plant community composition and soil property to the variation of microbial community composition

Distance matrix	Model summary			Plant species composition dissimilarity		Plant biomass composition dissimilarity		Soil property distance	
	R^2	*F*	*P*	*β*	*P*	*β*	*P*	*β*	*P*
Overall Bacterial beta-diversity	0.437	9.714	0.001	0.1101	0.018	0.0917	0.005	0.0107	0.002
Proteobacterial beta-diversity	0.301	11.204	0.002	0.1233	0.048	0.0816	0.094	0.0105	0.012
Acidobacterial beta-diversity	0.368	15.140	0.001	0.1363	0.035	0.1078	0.020	0.0109	0.005
Overall functional gene beta-diversity	0.466	6.980	0.002	0.0045	0.643	0.0169	0.008	0.0011	0.043
C degradation gene beta-diversity	0.448	6.499	0.002	−0.0069	0.540	0.0270	0.001	0.0012	0.083
N cycling gene beta-diversity	0.494	7.806	0.001	−0.0131	0.241	0.0308	0.001	0.0018	0.013

A multiple regression on distance matrices (MRM) analysis was performed, and the partial regression coefficients of an MRM model give a measure of the rate of change in soil microbial beta-diversity per standardized unit of dissimilarity for the matrix of interest.

Consistent with our initial hypothesis, the microbial taxonomic beta-diversity was highly linked to plant community beta-diversity, estimated by both species composition and the “biomass composition”. This conclusion is in

line with our previous finding in a broadleaved-coniferous forest ecosystem (Li et al. 2015). These consistent observations implied that more similar soil bacterial taxonomic compositions are usually associated with more similar aboveground plant communities than expected by chance. Interestingly, the microbial functional gene beta-diversity, including beta-diversity of C degradation and N cycling genes, was only correlated with compositional variation in aboveground plant biomass, but not with plant beta-diversity calculated by the traditional species composition. These results suggest that the soil microbial function (implied by various functional genes) is more influenced by the variations in the plant-derived resources returned to the soil than merely governed by the plant species composition. This might be caused by the fact that the composition of the aboveground plant biomass influences the quantity and quality of the litter community (Broughton and Gross 2000), and subsequently, the resources available to the soil microbial communities. Thus, sites with a more similar plant biomass composition would be expected to be associated with microbial communities that have more similar microbial metabolic potentials and soil nutrient cycling processes. Moreover, because soil microbial communities with higher catabolic diversity are expected to be more resistant to stress or disturbance (Degens et al. 2001; Griffiths and Philippot 2013), we postulate that the ecosystem harboring a more diverse plant biomass would have higher resilience and functional stability under environmental changes.

In this study, we found that the microbial taxonomic gene beta-diversity was significantly correlated with plant taxonomic beta-diversity, while the functional gene beta-diversity was not. Although several metagenomic studies have revealed significant correlations between microbial phylogenetic diversity and functional gene diversity (Bryant et al. 2012; Fierer et al. 2012), the differences in their response patterns to environmental changes had been documented by a number of recent publications (Ding et al. 2015; Kuang et al. 2016; Zhang et al. 2016). It has been previously proposed that microbial functional traits were more useful in assessing the relationship between microbial communities and environmental factors than taxonomic composition based on phylogenetic markers (Edwards et al. 2013; Louca et al. 2016; Gibbons 2017). Here, we provide additional evidence on their distinct response patterns to aboveground plant communities. Microbial species may not always have a direct relationship with specific functional traits, because specific functional genes could be widely detected across a variety of taxa or phylogenetic groups (Burke et al. 2011; Gibbons 2017). This hypothesis was supported by a lower dissimilarity of functional gene among plots (average value of 0.052 with range of 0.028~0.080) compared to a higher dissimilarity of taxonomic genes (average value of 0.433 with range of 0.265~0.623). The functional redundancy of the microbial community may explain why microbial taxonomic and functional gene diversity was each correlated with different environmental variables.

The present study fills the knowledge gap associated with the aboveground-belowground diversity relationships in natural steppe ecosystems. However, we only worked with the bacterial subset of the belowground community which may not be representative of other belowground taxa. Previous studies have shown that the bacterial, fungal and archaeal communities responded differently to environmental parameters and aboveground influences (Mamet et al. 2017). Thus, future studies on the fungi-plant or archaeaplant diversity relationships would be helpful to further interpret the aboveground-belowground diversity relationship. Additionally, the plant-derived resources are not limited to plant litter, and the plant root exudates could also have great influences on the soil microbial community. The conclusions generated from this study are based on an artificially manipulated field experiment, and one should be cautious to generalize these conclusions to the real natural system. For example, the field experiment was conducted at the local scale, with identical climate, soil texture, and vegetation type. While, the natural steppe usually distributes at a regional or continental scale, with broader gradient in ecological niche factors, such as temperature, precipitation, soil properties and plant community composition. Despite these limitations, our study still provides a framework for designing future studies aiming to address the problems described above.

In conclusion, our results suggested that when predicting biodiversity associations between aboveground and belowground biota, evaluation of microbial communities at both the taxonomic and functional gene levels was required. In addition, given the close associations between plant litter decomposition processes and soil microbial functional traits, the compositional variation in plant biomass might be more strongly predictive of the differences in metabolic potential of the soil microbial community (and thus, the ecosystem functions), than the traditional plant species composition. We further recommended that when exploring ecosystem functional services and community resilience under environmental stresses, the plant-derived resources diversity and composition should be taken into account in addition to the species diversity and composition.

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Conflict of interest

The authors declare that they have no conflict of interest.

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