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Responses of soil microbial functional genes to global changes are indirectly influenced by aboveground plant biomass variation

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ABSTRACT

Global nitrogen (N) deposition and precipitation change are two important factors influencing the diversity and function of terrestrial ecosystems. While considerable efforts have been devoted to investigate the responses of aboveground plant communities to altered precipitation regimes and N enrichment, the variations of belowground soil microbial communities are not well understood, particularly at the functional gene structure level. Based on a 9-year field experiment established in a typical steppe in Inner Mongolia, China, we examined the impacts of projected N deposition and precipitation increment on soil microbial functional gene composition, and assessed the soil/plant factors associated with the observed impacts. The overall functional gene composition significantly shifted in response to precipitation increment, N deposition and their combinations (all ADONIS $P < 0.05$), and such changes were primarily correlated with soil pH, microbial biomass, and microbial respiration. Water supply increased the abundances of both carbon (C) and N cycling genes, suggesting that the projected precipitation increment could accelerate nutrient cycling in this semi-arid region. N effects were mainly observed on the genes involved in vanillin/lignin degradations, implying that the recalcitrant C would not accumulate in soil under future scenarios of higher N deposition. Structural equation modeling (SEM) analysis revealed that soil dissolved organic carbon (DOC) was a key factor directly determining the abundance of C degradation and N cycling genes, and aboveground plant biomass indirectly influenced gene abundance through enhancing DOC. The present work provides important insights on the microbial functional feedbacks to projected global change in this semi-arid grassland ecosystem, and the mechanisms governing C and N cycles at the regional scale.

1. Introduction

The diversity and function of terrestrial ecosystems are greatly influenced by anthropogenic disturbance and global climate change worldwide. Increased atmospheric nitrogen (N) deposition, caused by fossil fuel combustion and application of N fertilizers, is an important aspect of anthropogenic global change (Galloway et al., 2008; Schlesinger, 2009; Canfield et al., 2010). In addition, precipitation regimes are predicted to change at both global and regional scales (IPCC, 2013). Predicting ecosystem responses to global change is a fundamental issue in ecology. It has been well documented that the aboveground plant community is sensitive to precipitation change (Cleland et al., 2013; Eskelinen and Harrison, 2015) and N deposition (Stevens et al., 2004; Clark and Tilman, 2008), particularly in arid and semi-arid grassland ecosystems, where water and N are generally considered to be limiting resources (Harpole et al., 2007; Yang et al., 2011; Xu et al., 2012b). A growing body of evidence suggests that higher precipitation increases plant species richness (Bai et al., 2008; Xu et al., 2015b),

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whereas N fertilization is reported as the main driver of the loss of species richness (Gough et al., 2000; Stevens et al., 2004; Yang et al., 2011). Plant primary productivity and litter quality are generally enhanced by both precipitation increment (Harpole et al., 2007; Bai et al., 2008; Wu et al., 2011; Allison et al., 2013) and N inputs (Suding et al., 2005; LeBauer and Treseder, 2008; Phoenix et al., 2012; Xu et al., 2015b) in N-limited arid and semi-arid terrestrial ecosystems.

Although considerable effort has been devoted to investigating aboveground plant communities in response to precipitation change and N deposition in the past decade, the impacts of projected global change on belowground microbial communities and soil carbon (C) and N cycling processes they mediate are not well understood. By sequencing taxonomic gene markers (e.g., 16S rRNA), a number of recent studies indicated that N addition (Campbell et al., 2010; Ramirez et al., 2010; Zhang et al., 2014a) and precipitation changes (Clark et al., 2009; Evans et al., 2014; Barnard et al., 2015) could alter the diversity and composition of soil microbial communities. In general, increases in N input would increase the relative abundance of copiotrophic taxa (those taxa that have higher N demands and adept at catabolizing more labile C pools), such as Proteobacteria; and reduce that of oligotrophic taxa (those taxa that are adept at catabolizing more recalcitrant C pools), typically represented by Acidobacteria (Fierer et al., 2007). As a consequent, N enrichment is proposed to result in a shift towards labile C decomposition (Fierer et al., 2007; Ramirez et al., 2010). The responses of microbial communities to precipitation regimes are inconsistent, though it was generally hypothesized that increased precipitation would increase soil nutrients level, and subsequently the relative abundance of copiotrophic taxa. In a shortgrass steppe ecosystem, it was reported that Actinobacteria tended to be less abundant under drought (Evans et al., 2014). However, an experiment conducted in a California annual grassland showed that the relative abundance of Acidobacteria increased, and that of Actinobacteria decreased, with wet-up treatment (Barnard et al., 2015). The ecological categories of Actinobacteria are not very clear, and previous publications documented the mixed results, with their relative abundance increasing (Ramirez et al., 2010, 2012; Zeng et al., 2016), or remaining unchanged (Fierer et al., 2007; Zhang et al., 2014a; Li et al., 2016) in response to nutrients addition. Another field experiment demonstrated that the larger, but less frequent, rainfall events enhanced the relative abundance of Verrucomicrobia and Alphaproteobacteria (Evans and Wallenstein, 2012). Verrucomicrobia typically showed an oligotrophic tendency (Ramirez et al., 2012), however, the irresponsiveness of this phylum to elevated nutrients level has also been documented (Li et al., 2016). Although increasing information on the effects of N deposition and precipitation regimes on microbial taxonomic genes has been collected, the responses of soil microbial functional gene diversity and composition to global change, and their potential linkages to soil processes, still remain unclear. Soil microbial communities play important roles in litter decomposition and are key drivers of nutrient cycling in terrestrial ecosystems. Evaluating the effects of global change on soil microbial functional genes is critical for predicting functional feedbacks to the climate system (Zhou et al., 2012; Xue et al., 2016) and deepening our understanding of C and N cycles at a regional scale.

The semiarid temperate grassland in northern China is of a typical vegetation type in the Eurasian continent, covering 78% of the grasslands area in China (Kang et al., 2007). These grasslands support diverse species of plants and are of great importance to ecosystem services, functions, and socio-economics of the region. It has been predicted that the summer precipitation in Northern China will increase by 50% (Sun and Ding, 2010) and the atmospheric N deposition in this area is also expected to increase in the coming decades (Bai et al., 2008, 2010). To predict the ecosystem response to the projected global change, a field experiment manipulating water and multi-level N addition was established in 2005 in a natural steppe in Duolun, Inner Mongolia, China (Xu et al., 2010). After 9 years of treatment, our previous results showed that the plant communities are sensitive to N enrichment and increased precipitation in terms of species richness, community composition and stability (Xu et al., 2010, 2012a, b; 2014, 2015a, b). We recently analyzed the response of soil bacterial diversity and community composition to the increased precipitation and N deposition by sequencing of 16S rRNA gene amplicons, and found that the N enrichment induced co-variation between bacterial and plant communities, and increased precipitation dampened the N effects (Li et al., 2016). However, microbial functional feedback to global change has been rarely studied and requires more direct observed evidence. The specific aims of this study were to examine the impacts of long-term N amendments and precipitation increment on soil microbial functional genes, and to determine what mechanisms might be responsible for the observed responses.

We hypothesized that the abundance of functional genes involved in C and N cycling would be stimulated by precipitation increment and N enrichment in this semi-arid grassland ecosystem, directly associated with increased water availability and nutrient resources. We also expected that the increase in plant productivity induced by water supply (Bai et al., 2008) and N deposition (Gough et al., 2000) would indirectly stimulate C degradation genes through enhancing plant C inputs to the soil. In particular, because increased precipitation and N amendments can reduce the C:N ratio and improve the chemical quality of the litter inputs (Lü et al., 2012; Allison et al., 2013), we further predicted that the abundance of labile C degradation genes will respond more strongly to projected global change, whereas the genes involved in recalcitrant C degradation will show weaker responses. We tested these hypotheses using the newest generation of the functional gene array GeoChip (version 5.0_60K) to detect microbial functional gene abundance and composition in soil samples treated with 9-year N fertilization and water addition.

2. Materials and methods

2.1. Site description and experimental design

The study sites were located at the Restoration Ecological Research Station (E 116°17′20″, N 42°2′29″), Duolun, Inner Mongolia, Northern China. Mean annual precipitation (MAP) is ~380 mm, with >80% of the precipitation occurring between June and September. Mean annual temperature is 2.1 °C, with mean monthly temperature ranging from −17.5 °C in January to 18.9 °C in July. The soil is classified as Calcis-orthic Aridisol according to the US Soil Taxonomy classification. Soil texture of the experimental site is sandy loam with 62.75% sand, 20.30% silt, and 16.95% clay (Chen et al., 2009). The experiment was established in a natural steppe ecosystem that has been fenced since 2000 to prevent grazing by large vertebrate herbivores (Xu et al., 2010).

A field experiment with a constant increase in precipitation and N supply was established in 2005 to examine the potential effects of N deposition and changes in precipitation regime on ecological processes. A split-plot experimental design was employed, 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. The estimated mean nitrogen deposition rate in northern China is about 8.3 g N m^{-2} $year^{-1}$ (He et al., 2007). Consequently, the total amounts of N inputs varied from approximately 8.3 to 23.3 g N m^{-2} $year^{-1}$, simulating the continuous increasing of N deposition in this region. Each

subplot was 8 m × 8 m, and was separated by 1 m wide corridors. This design was replicated in seven experimental blocks. In the middle of the growing season, from June to August, the water addition plots received 15 mm of precipitation weekly by sprinkling irrigation. A total of 180 mm precipitation, approximately 50% of mean annual rainfall, was added yearly during the growing season from 2005 to 2013. The N amendment is granular urea (applied twice, half in early May and the other half in late June), a widely used nitrogenous fertilizer to simulate atmospheric nitrogen deposition in various grassland ecosystems (Zhang et al., 2008; Shen et al., 2011). Moreover, urea is a commonly applied N fertilizer in China, because it is a convenient source of nitrogen and has the highest nitrogen content of all solid nitrogenous fertilizers in use.

2.2. Soil collection and analysis

Our previous studies revealed that the species richness, community composition and stability of aboveground plant community had been significantly changed at the N level of 10 g N m^{-2} $year^{-1}$ (Xu et al., 2012a, b; 2014, 2015a, b). The soil physical and chemical properties and bacterial communities were also significantly altered by N amendments at the level of 10 g N m^{-2} $year^{-1}$ (Wang et al., 2014; Li et al., 2016). Consequently, four treatments, including CK (control), N (10 g N m^{-2} yr^{-1}), W (increased precipitation), WN (interaction of increased precipitation and 10 g N m^{-2} yr^{-1}), were selected for microbial functional gene analysis in this study. Soil samples were collected from each subplot after the harvest of plant biomass by the end of August 2013. For each subplot, five soil cores were randomly taken from the topsoil (0–15 cm) and then mixed to form one composite sample. All samples were placed on ice and transported to the laboratory within two days. Soil samples were passed through a 2.0 mm sieve and stored at 4 °C for soil chemical analysis, and another set of samples were stored at −80 °C for soil genomic DNA extraction.

The following soil and site characteristics were determined for each sample and used in the subsequent statistical analyses: 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, pH, microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and respiration-related parameters, including basal respiration (BR), substrate induced respiration (SIR), and carbon availability index (CAI). TC, TN and TS were determined using an elemental analyzer (2400II CHN elemental analyzer; Perkin-Elmer, USA). Soil C/N ratio was calculated using TC and TN datasets. Soil TP was measured by a Mo-Sb Anti spectrophotometric method. Nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N) was extracted with 2 M KCl, and then analyzed on a continuous-flow ion auto-analyzer (Scalar SANplus segmented flow analyzer, The Netherlands). Soil DOC was extracted with 0.5 M K_2SO_4, and was analyzed using a TOC analyzer (Analytikjena HT1300, Germany). Soil pH was determined in a soil slurry at 2.5:1 water: soil (w/v) ratio using a glass electrode. Soil moisture was measured by drying 10 g of fresh soil for 24 h at 105 °C. Soil MBC and MBN were determined using the fumigation-extraction method (Vance et al., 1987) within 7 days after soil sampling. Soil basal respiration was measured using Li-COR 8200 Infrared Gas Analyzer (IRGA) (Li-COR Biosciences, Lincoln, NB, USA) as previously described (Gershenson et al., 2009). After measuring basal respiration, 8 mg glucose g^{-1} soil was added to determine SIR. The CAI index was calculated by dividing the basal respiration rate with the SIR rate (Gershenson et al., 2009).

2.3. Plant biomass measurement

The aboveground plant biomass was measured by randomly clipping a 15 cm × 2.0 m strip within the 8 m × 8 m subplot. The clipped strip was changed within the subplot every year. The harvested plant biomass was sorted by species, dried for 48 h at 65 °C and weighed. Plant species were categorized into two functional groups: grasses (GR) and non-gramineous forbs (NF). We used the average of the previous two years' (2011 and 2012) aboveground biomass to determine correlations between plant biomass and functional gene abundance.

2.4. Microbial community DNA isolation and GeoChip analysis

The DNA was extracted from approximately 0.35 g of moist soil using a MoBio Power Soil DNA extraction kit following the manufacturer's instructions. The eluted DNA was stored at −80 °C until used. DNA quality was assessed using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE), and final soil DNA concentrations were quantified with a Quant-iT PicoGreen kit (Invitrogen, Carlsbad, CA, USA) using a FLUOstar Optima plate reader (BMG Labtech, Jena, Germany).

The newest generation of the functional gene array GeoChip 5.0 (60K) was used in this study to detect soil microbial functional gene diversity and composition. The GeoChip 5.0_60K (manufactured by Agilent) contains more than 57,000 oligonucleotide probes covering over 144,000 gene sequences in more than 373 gene families for different microbial functional and biogeochemical processes, including carbon, nitrogen, phosphorus, sulfur cycling, metal homeostasis, organic remediation, secondary metabolism, and virulence. Samples were prepared and hybridized as described previously (Yan et al., 2015; Van Nostrand et al., 2016). For each sample, 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 purified, dried, and rehydrated with 13 μL DNase/RNase-free distilled water. A total of 42 μL buffer including 1 × HI-RPM hybridization buffer, 1 × Acgh blocking agent, 0.05 μg/μL Cot-1 DNA, 10 pM universal standard, and 10% formamide (final concentrations) was added to each sample, incubated at 95 °C for 3 min, and then maintained at 37 °C until loaded onto microarray slides. The prepared samples were then hybridized to the array at 67 °C for 24 h in hybridization oven. After washing and drying, the microarrays were scanned by using a NimbleGen MS200 Microarray Scanner (Roche NimbleGen, Inc., Madison, WI, USA) at 633 nm using a laser power of 100% and a photomultiplier tube gain of 75%. The scanned images of hybridized GeoChips were converted and extracted by the Agilent Feature Extraction 11.5 software (Agilent Technologies, Inc.).

2.5. GeoChip data processing

Raw data from the Agilent Feature Extraction were submitted to the laboratory's Microarray Data Manager System (http://ieg.ou.edu/microarray/) and analyzed by the following major steps as described previously (He et al., 2010). (i) Adjusting signal-to-noise ratio (SNR) so that Thermophile probes account for 3.5% of all positive probes with a minimum SNR = 2.0, and then removing spots with a signal intensity less than 1.3 times the background. (ii) After the poor-quality spots were removed, a two-step normalization method was used. First, the universal standard spots were used for spatial normalization within a slide. Second, the Cy3 intensity (sample signal) was then normalized by the mean intensity of the universal standards of all slides. (iii) 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. The relative abundance was then multiplied by the

mean value for the sums of the original signal intensity in all samples. A logarithm transformation was performed for the amplified relative abundance plus 1.

2.6. Statistic analysis

Normality tests (Shapiro-Wilk) were performed with data that were used for analysis of variance (ANOVA). If the normality test revealed a non-Gaussian distribution, data were log-transformed. Effects of N, water addition and their interaction on soil physico-chemical properties, plant biomass, and functional gene abundance were analyzed by split-plot two-way ANOVAs using SPSS 13.0 (SPSS Inc, v 17.0, Chicago, Illinois). Stepwise regression analysis was used to examine the soil and plant factors that could effectively explain the variation in the abundance of key functional genes involved in C and N cycling. Because of the collinearity among environmental factors, we pre-selected the parameters by removing the environmental factors that were highly correlated with other factors ($r > 0.6$). A subset of soil parameters were retained for stepwise regression analysis, including TN, C/N ratio, TP, NO_3^--N, NH_4^+-N, DOC, soil moisture, pH, MBC, BR, CAI, and average aboveground plant biomass of the previous two years, in terms of both total plant community, and the sorted grass (GR) and non-gramineous forbs (NF).

Detrended correspondence analysis (DCA) was used to illustrate the overall changes in functional gene structures in response to N and water addition. To determine whether N amendment and precipitation increment significantly influenced functional gene composition, permutational multivariate analysis of variance was performed using the Adonis function implemented in the R statistical environment (v 3.1.0), based on Bray-Curtis dissimilarity matrix of functional genes. To link the soil/plant variables and the functional gene composition, Mantel test were performed to examine the correlation between the functional gene Bray-Curtis distances with the corresponding differences in soil and plant variables.

Structural equation modeling (SEM) (Grace, 2006) was used to estimate the direct and indirect effects of soil and plant variables on microbial functional gene abundance under environmental changes. The bivariate relationships between all variables with simple linear regressions were checked before SEM analysis to ensure the appropriateness of the linear models. A conceptual model of hypothetical relationships was constructed (Fig. S1), assuming nitrogen enrichment and precipitation increment would impact soil C and N cycling genes, directly by increased nutrients and water availability, or indirectly through altering plant productivity and the available C inputs to soils (as illustrated in the Introduction). We compared the model-derived variance-covariance matrix against the observed variance-covariance matrix, and data were fitted to the models using the maximum likelihood estimation method. We used the χ^2-test ($0.05 \leq P \leq 1.00$) and the root mean square error of approximation (RMSEA; $0 \leq RMSEA \leq 0.05$) to evaluate the fit of the model. The final model was improved by removing relationships between observed variables from prior models based on these indices (Table S1). SEM analyses were performed using AMOS 18.0 (Amos Development Co., Greene, Maine, USA).

3. Results

3.1. Effects of long-term water and nitrogen addition on plant community and soil properties

Soil TC and TN exhibited a significant increase under the precipitation increment treatment ($P < 0.05$ for the water effect), whereas N addition, alone or in combination with water, had no significant effect (Table 1). N addition significantly enhanced DOC and NO_3^--N ($P < 0.05$); however, NH_4^+-N concentration was not influenced by N addition ($P > 0.05$). Soil pH significantly ($P < 0.05$) decreased under N addition, but was not altered by precipitation increment and water by N interaction. N addition tended to suppress soil basal respiration, substrate induced respiration (SIR) and microbial biomass ($P < 0.05$ for all cases). Under the normal precipitation treatment, N addition reduced MBC by 36.9% and MBN by 47.7%, as generally observed in unmanaged natural ecosystem (Treseder, 2008; Geisseler and Scow, 2014). Nevertheless, the simulated precipitation increment alleviated the inhibition by N addition. Compared with ambient water treatment, water addition significantly ($P < 0.05$) increased microbial biomass and respiration (Table 1). The CAI index, estimated by dividing basal respiration with SIR, showed no response to precipitation increment, but increased significantly with N addition and N plus water addition (Table 1).

The average aboveground plant biomass of the total community

Table 1
Effects of nitrogen and precipitation increment on soil properties and aboveground plant biomass. Shown are the mean values and the standard deviations (n = 7).

Soil properties	CK	N	W	WN	*P* value of two-way ANOVA		
					W	*N*	*W* × *N*
TC (g kg^{-1})	17.96 ± 1.60	17.82 ± 1.07	19.71 ± 1.85	19.30 ± 2.96	**0.049**	0.725	0.564
TN (g kg^{-1})	1.85 ± 0.15	1.83 ± 0.13	2.03 ± 0.16	1.99 ± 0.26	**0.039**	0.659	0.888
C/N	9.70 ± 0.20	9.73 ± 0.16	9.70 ± 0.22	9.67 ± 0.23	0.434	0.984	0.747
TS (g kg^{-1})	0.517 ± 0.068	0.513 ± 0.052	0.754 ± 0.244	0.565 ± 0.098	**0.049**	**0.013**	**0.016**
TP (g kg^{-1})	0.316 ± 0.019	0.287 ± 0.025	0.307 ± 0.021	0.311 ± 0.033	0.448	0.250	0.116
DOC (mg kg^{-1})	239.3 ± 8.39	254.7 ± 11.8	251.0 ± 15.7	261.8 ± 14.7	0.138	**0.004**	0.547
NH_4^+-N (mg kg^{-1})	14.44 ± 3.02 a	15.90 ± 2.60	15.03 ± 2.79	15.57 ± 3.43	0.916	0.373	0.677
NO_3^--N (mg kg^{-1})	6.55 ± 1.12	13.46 ± 4.66	6.04 ± 1.71	11.39 ± 3.45	0.339	**0.000**	0.457
Moisture (%)	12.83 ± 1.19	12.43 ± 0.61	15.16 ± 3.01	13.81 ± 2.11	0.076	0.059	0.279
pH	6.38 ± 0.17	6.00 ± 0.17	6.41 ± 0.17	6.14 ± 0.12	0.231	**0.000**	0.275
Respiration	0.565 ± 0.052	0.417 ± 0.046	0.676 ± 0.041	0.530 ± 0.060	**0.000**	**0.000**	0.953
SIR	6.596 ± 0.229	4.021 ± 0.289	8.424 ± 1.406	5.645 ± 0.903	**0.001**	**0.000**	0.709
CAI	0.081 ± 0.006	0.117 ± 0.012	0.091 ± 0.009	0.097 ± 0.017	0.193	**0.001**	**0.006**
MBC (mg kg^{-1})	210.5 ± 44.2	132.8 ± 28.6	285.2 ± 26.8	177.7 ± 34.9	**0.003**	**0.000**	0.116
MBN (mg kg^{-1})	26.52 ± 8.19	13.86 ± 2.58	37.69 ± 4.42	17.48 ± 4.08	**0.001**	**0.000**	0.130
Total plant biomass	195.2 ± 23.0	281.0 ± 62.3	216.1 ± 33.2	364.3 ± 72.7	**0.019**	**0.000**	0.147
GR biomass	84.5 ± 23.4	156.2 ± 62.6	100.8 ± 40.0	229.6 ± 62.1	**0.042**	**0.000**	0.137
NF biomass	110.7 ± 30.0	124.8 ± 41.1	115.24 ± 31.9	134.75 ± 56.5	0.657	0.289	0.864

Results from two-way ANOVAs with split-plot design were shown in the right panels of the table, and significant *P* values were shown in bold.
The unit of plant biomass is g m^{-2}.
Abbreviation: CK, control; N, N addition; W, increased precipitation; WN, N addition plus increased precipitation; GR, grasses; NF, non-gramineous forbs.

Table 2
Effects of water and nitrogen addition on functional gene richness and diversity indices (mean ± SE, $n = 7$). Results from two-way ANOVA with split-plot design were shown in the lower panels of the table, and significant P values were shown in bold.

	Gene richness[a] (No. of probes detected)	Shannon-Wiener index[b] (H′)	Simpson's reciprocal index[c] (1/D)
CK	18,541 ± 127	9.817 ± 0.007	18,144 ± 118
N	18,620 ± 125	9.821 ± 0.007	18,226 ± 123
W	19,058 ± 94	9.844 ± 0.005	18,641 ± 82
WN	19,228 ± 76	9.853 ± 0.004	18,812 ± 80
Two-way ANOVA analysis with a split-plot design			
W	***F* = 33.823, *P* < 0.001**	***F* = 32.720, *P* < 0.001**	***F* = 34.426, *P* < 0.001**
N	$F = 1.155$, $P = 0.304$	$F = 1.112$, $P = 0.312$	$F = 1.279$, $P = 0.280$
W × *N*	$F = 0.138$, $P = 0.717$	$F = 0.144$, $P = 0.710$	$F = 0.160$, $P = 0.696$

[a] Gene probes were treated as "species," and their abundances were represented by the normalized signal intensities.
[b] Shannon-Wiener index is defined as H' = -$\sum Pi \times \ln Pi$, where pi is the proportional abundance of species i.
[c] Simpson's index is based on D = $\sum Pi^2$ and invsimpson returns (1/D).

and GR group was significantly enhanced by both precipitation increment ($F = 7.401$ and 5.195 for total biomass and GR biomass, respectively, $P < 0.05$ in both cases) and N addition ($F = 33.842$ and 31.225 for total biomass and GR biomass, respectively, $P < 0.001$ in both cases). Nevertheless, the NF biomass showed no responses to any of the factors or their interactions (Table 1). We found a significant positive correlation between previously returned plant biomass and soil DOC (spearman correlation $r = 0.640$, $P < 0.001$) and CAI ($r = 0.521$, $P = 0.005$), suggesting the great contribution of plant litter to the top soil available carbon pool.

3.2. Overall responses of functional genes diversity and composition to long-term increased precipitation and added nitrogen

A total of 21,161 gene probes were detected across all treatments, including 8931, 2763, 897, and 1768 gene probes involved in C, N, S, and P cycling, respectively (Table S2). The numbers of gene probes detected in different treatments are shown in Table 2. Two-way ANOVA analysis revealed that precipitation increment caused significant ($P < 0.05$) increases in functional gene richness, Shannon diversity (H'), and Simpson's reciprocal index (1/D). Nevertheless, N addition and N plus water addition showed no significant effects on these diversity indices (Table 2). Similarly, significant watering effects were observed on the number of detected probes of genes involved in C, N, S, and P cycling (Table S2), whereas N addition, alone or in combination with water, elicited no significant responses.

DCA of all detected functional genes showed that samples from all three treatments (nitrogen, water, and water plus nitrogen) harbored gene assemblages distinct from the ambient control (CK) (Fig. 1). It was shown in the DCA profile that the functional gene composition of the increased precipitation treatments (triangles) were separated from those under normal precipitation conditions (circles) along Axis 2, while Axis 1 mainly differentiated the microbial communities by N addition treatment. Adonis analysis consistently showed that the overall functional gene composition of microbial communities significantly changed by N addition ($F = 4.485$, $P = 0.001$), precipitation increment ($F = 4.356$, $P = 0.001$) and combinations of these ($F = 2.221$, $P = 0.006$). No replicate (subplots) effects were detected ($F = 1.582$, $P = 0.063$). A total of 64% of the variation was explained by this model, with precipitation treatment as the main factor (12.8%), followed by N addition (12.4%), and precipitation plus N (6.3%). The replication only contributed 4.4% of the total variation. Adonis analysis of subsets of genes involved in C, N, S and P cycling showed similar patterns, being significantly influenced by N addition, precipitation increment and interaction of these two factors (Table S3).

3.3. Relationships between functional gene composition and environmental variables

Mantel test was performed to determine the relationships between functional gene composition and all measured soil properties and plant variables. The overall functional gene composition was significantly correlated with soil pH, microbial biomass (MBC and MBN), and microbial activity-related indices, such as basal respiration (BR), SIR, and CAI (Table 3). The overall carbon cycling gene composition was significantly ($P < 0.05$) correlated with soil pH, BR and CAI (Table 3). However, the carbon degradation gene composition only showed significant ($P < 0.05$) correlation with BR, implying a close linkage between microbial respiration and carbon degradation processes. Soil NO_3^--N was an additional factor influencing genes involved in N cycling, in particular, the nitrification and denitrification processes ($P < 0.05$ for both gene subsets, Table 3). The composition of nitrogen fixation genes and ammonification genes were observed to have significant correlations with respiration-based indices (Table 3). As with plant variables, the aboveground biomass of the total plant community showed significant correlation with microbial functional gene composition, based on all detected genes and subsets of the genes involved in sulfur (S) cycling (Table 3). Deep insights into specific plant functional groups revealed that GR biomass significantly influenced S cycling gene composition, whereas the biomass of NF group exhibited no correlations with any functional gene subgroup.

3.4. Effects of increased precipitation and nitrogen addition on signal intensities of key functional genes involved in C and N cycling processes

To further understand the effect of nitrogen addition and precipitation increment on specific biogeochemical processes, the key functional genes involved in C and N cycling were further studied below.

3.4.1. Carbon cycling genes

GeoChip 5.0 contains seven subcategories for the genes encoding CO_2 fixation enzymes: 3-hydroxypropionate bicycle, 3-hydroxypropionate/4-hydroxybutyrate cycle, Calvin cycle, dicarboxylate/4-hydroxybutyrate cycle, reductive acetyl-CoA pathway, reductive tricarboxylic acid cycle, and bacterial microcompartments. All of these processes were detected, indicating that diverse microbial C fixation processes exist in this temperate grassland ecosystem. The abundance of C fixation genes, estimated by the signal intensities standardized by the probe numbers, were significantly affected by precipitation increment ($P < 0.05$ for watering effects; Table 4), implying that the increased precipitation in

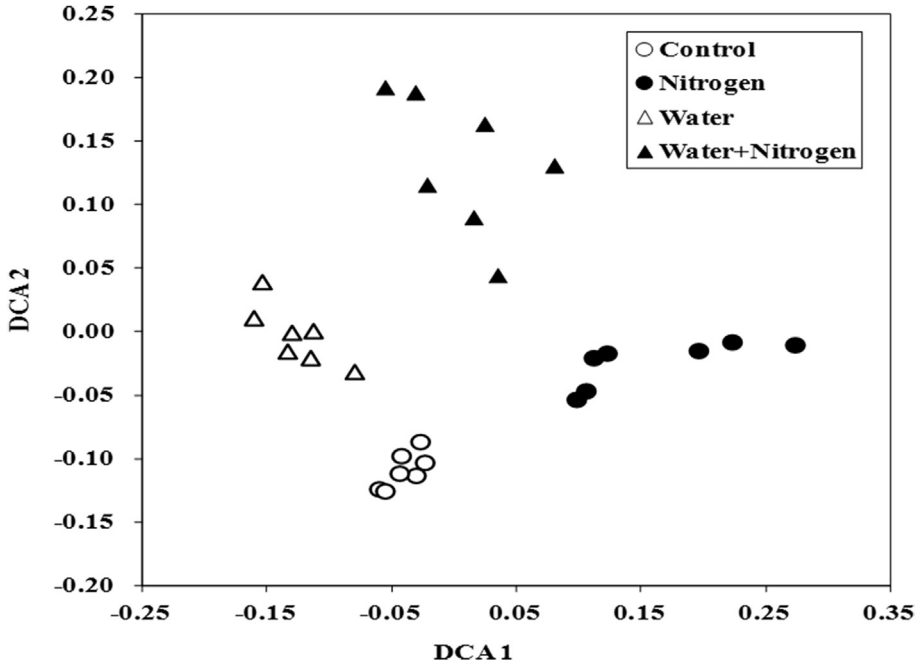

Fig. 1. Detrended correspondence analysis (DCA) of all detected genes showing that long-term nitrogen and water treatments had substantial influences on functional gene compositions of soil microbial communities.

future scenarios may potentially lead to more C fixation in soil. The specific processes, 3-hydroxypropionate bicycle, bacterial microcompartments, Calvin cycle, reductive tricarboxylic acid cycle, and reductive acetyl-CoA pathway, were significantly affected by increased precipitation ($P < 0.05$; Table 4). N addition only showed a significant positive effect on genes involved in the dicarboxylate/4-hydroxybutyrate cycle.

Effects of N addition and precipitation increment on functional genes/enzymes involved in degradation of a variety of C substrates are shown in Fig. 2 and Table 4. Overall, increased precipitation showed significant effects on the degradation of all the covered C sources, including starch, hemicellulose, cellulose, chitin, simple aromatic compounds (terpenes and vanillin/lignin), and lignin ($P < 0.05$ for all cases; Table 4). However, a significant N effect was only observed on the vanillin/lignin decomposition process ($P < 0.05$; Table 4). With respect to the specific C degradation genes/enzymes, it was found that most of the C degradation genes/enzymes were significantly enhanced by water addition (Fig. 2), whereas N enrichment only stimulated genes involved in recalcitrant C decomposition, such as *vanA* for vanillin decomposition, and genes coding for lignin phenol oxidase and chitinase ($P < 0.05$; Fig. 2). Though several genes/enzymes responsible for the degradation of starch and hemicelluloses (the relative labile C source), such as *cda*, *xylA*, arabinofuranosidase, and mannanase, were slightly inhibited by N addition under regular precipitation, significant negative N effects were only observed on *pulA* gene ($P < 0.05$; Fig. 2), which was associated with starch decomposition. Thus, in general, long-term N fertilization had less impact on labile C degradation.

3.4.2. Nitrogen cycling genes

The detected *nif*H genes are phylogenetically related to a variety of organisms, such as Archaeal phylum of Euryarchaeota (Methanobacteria and Methanococci), and bacterial phyla of Acidobacteria, Proteobacteria, Firmicutes, Cyanobacteria, Chloroflexi and some unknown bacteria, implying there is a diverse N_2 fixation process in this grassland ecosystem. Of 18 detected *amo*A genes, eight genes were derived from the bacterial phylum, and the

Table 3
Correlations between microbial functional gene composition (based on Bray-Curtis distances) and soil and/or plant variables. Shown are the significant *P*-values of Mantel test. Because a subset of soil properties (TC, TN, C/N ratio, TS, TP, DOC, moisture, and NH_4^+-N) and NF (non-gramineous forbs) biomass showed no significant correlations with any selected gene datasets, these variables were not presented here.

	pH	NO_3^-	MBC	MBN	BR	SIR	CAI	Plant biomass	GR biomass
All genes	0.018	NS	0.023	0.045	0.001	0.015	0.003	0.042	NS
Carbon cycling	0.041	NS	NS	NS	0.003	NS	0.002	NS	NS
Carbon fixation	0.035	NS	NS	NS	0.005	0.048	0.002	NS	NS
Carbon degradation	NS	NS	NS	NS	0.001	NS	NS	NS	NS
Nitrogen cycling	0.019	0.046	0.032	NS	0.001	0.024	0.009	NS	NS
Nitrogen fixation	NS	NS	NS	NS	0.029	NS	0.046	NS	NS
Ammonification	NS	NS	NS	NS	0.002	0.002	0.018	NS	NS
Nitrification	NS	0.018	0.009	NS	0.019	NS	0.010	NS	NS
Denitrification	0.008	0.020	0.005	0.021	0.001	0.001	0.003	NS	NS
Assimilatory N reduction	0.039	NS	NS	NS	0.033	NS	0.032	NS	NS
Dissimilatory N reduction	0.034	NS	NS	NS	0.001	NS	NS	NS	NS
Phosphorus cycling	0.015	NS	0.026	NS	0.002	0.025	0.008	NS	NS
Sulfur cycling	NS	NS	NS	NS	0.027	NS	NS	0.021	0.008

Abbreviation: GR, grasses; NS, non significant.

Table 4
Variables responsible for the changes in the abundance of genes involved in the key C and N cycling processes. Plant biomass is the average value of 2011–2012. Effective factors represent the factors having significant effects on the abundance of functional genes accessed by two-way ANOVA. N, W and W × N represent N addition, watering, and the interaction of watering and N addition, respectively.

Gene Category	Model	R^2	F	P	Effective factors
Carbon cycling	y = 7025.986 + 1.064(MBC)-22.204(NH_4^+)+3.726 (DOC)	0.448	6.482	0.002	W
Carbon fixation	y = 1.783 + 0.112 (TN)	0.181	5.748	0.024	W
3-hydroxypropionate bicycle	y = −7.840 + 2.409(C/N)	0.200	6.502	0.017	W, W × N
3-hydroxypropionate/4-hydroxybutyrate	y = 6.434 + 0.052(NO_3^-)	0.170	5.314	0.029	–
Calvin cycle	–				W
Dicarboxylate/4-hydroxybutyrate cycle	y = 66.581−6.52(Respiration)	0.231	7.805	0.010	N
Reductive acetyl-CoA pathway	y = 273.766 + 0.054 (plant biomass)	0.185	5.919	0.022	W
Reductive tricarboxylic acid cycle	y = 57.973 + 35.738 (moisture)+0.017(NF biomass)	0.283	4.928	0.016	W
Bacterial microcompartments	y = 314.093−1.209 (NH_4^+) +16.555 (TN)	0.358	6.982	0.004	W
Carbon degradation	y = 2.704 + 0.002(DOC)-0.008(NH_4^+)	0.342	6.488	0.005	W
Starch	–				W
Hemicellulose	–				W
Cellulose	y = 402.523 + 0.070 (GG biomass)	0.228	7.684	0.010	W
Chitin	y = 806.429 + 0.090 (plant biomass)	0.162	5.024	0.034	W
Terpenes	y = 101.741 + 5.995 (TN)	0.244	8.403	0.008	W
Vanillin/Lignin	y = 147.721 + 0.021 (plant biomass)	0.343	13.574	0.001	W, N
Lingin	y = 190.226 + 0.215 (DOC)	0.310	11.680	0.002	W
Nitrogen cycling	y = 5.072 + 0.283 (TN)	0.190	6.091	0.020	W
Nitrogen fixation	y = 0.162 + 2.231*10^{-4} (DOC)	0.197	6.387	0.018	W
Ammonification	y = 0.599 + 0.001(DOC)-0.003(NH_4^+)	0.390	7.998	0.002	W
Nitrification	y = 0.338 + 0.058 (TN)	0.204	6.650	0.016	W
Denitrification	y = 1.981 + 0.101 (TN)	0.204	6.652	0.016	W
Assimilatory N reduction	y = 1.444 + 0.088(TN)-0.005(NH_4^+)	0.346	6.624	0.005	W
Dissimilatory N reduction	y = 0.518 + 1.114*10^{-4}(plant biomass)-0.002(NH_4^+)	0.404	8.486	0.002	W, N

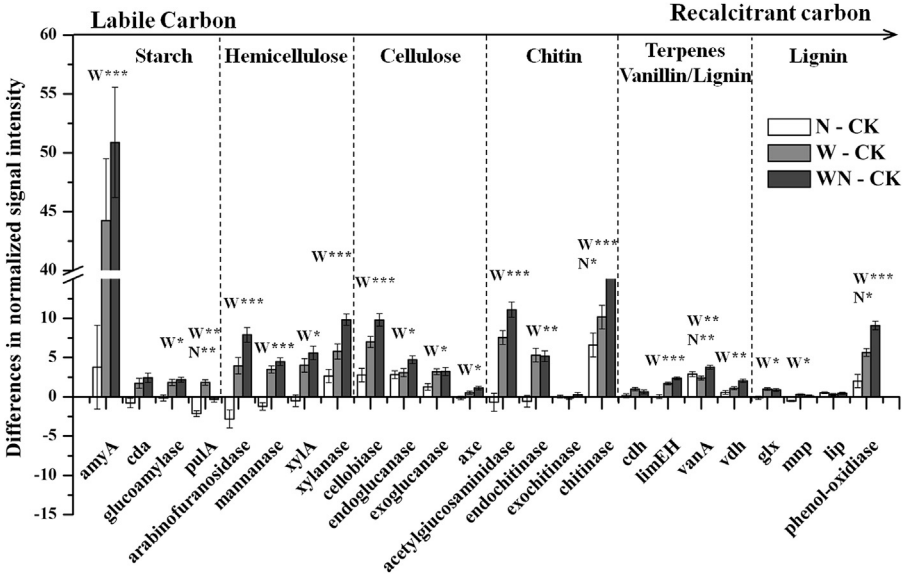

Fig. 2. Effects of projected N deposition and precipitation increment on the abundance of key C degradation genes. Shown are the signal differences between three treatments (N amendments, water addition, and N plus water addition) and control, designated as N-CK, W-CK, and WN-CK, respectively. The complexity of carbon is presented in order from labile to recalcitrant. All data are presented as mean ± SE. The effects of watering (W), N addition (N), and their combinations (W × N) on specific genes were analyzed by two-way ANOVA with a split-plot design. *0.01 < P < 0.05, **0.001 < P < 0.01, ***P < 0.001. No labels representing there was no treatment effect.

remaining ten genes were phylogenetically associated with archaea, suggesting that the ammonia oxidation process in this grassland ecosystem may be performed by both bacterial and archaeal communities.

Precipitation increment significantly (P < 0.05) enhanced the abundance of genes involved in specific N cycling processes (Fig. 3 and Table 4), including N fixation, ammonification, nitrification, denitrification, assimilatory and dissimilatory N reduction, suggesting a close linkage between precipitation regimes and N cycling processes. In contrast, N addition only showed significant effects on the dissimilatory N reduction (P < 0.05; Table 4), as estimated by the signal intensities standardized by the probe numbers. The *nas*A and *nrf*A genes associated with N reduction showed no responses to either watering or N addition (Fig. 3).

3.5. Relationships between functional gene abundances and soil/plant variables

To understand the key environmental factors responsible for the changes in gene abundance, stepwise regression analysis was performed on specific processes or key functional genes involved in C degradation and N cycling (Table 4 and Table S4). In addition, SEM analysis was conducted on the C degradation genes and all N cycling genes (Fig. 4) to discriminate the direct and indirect effects

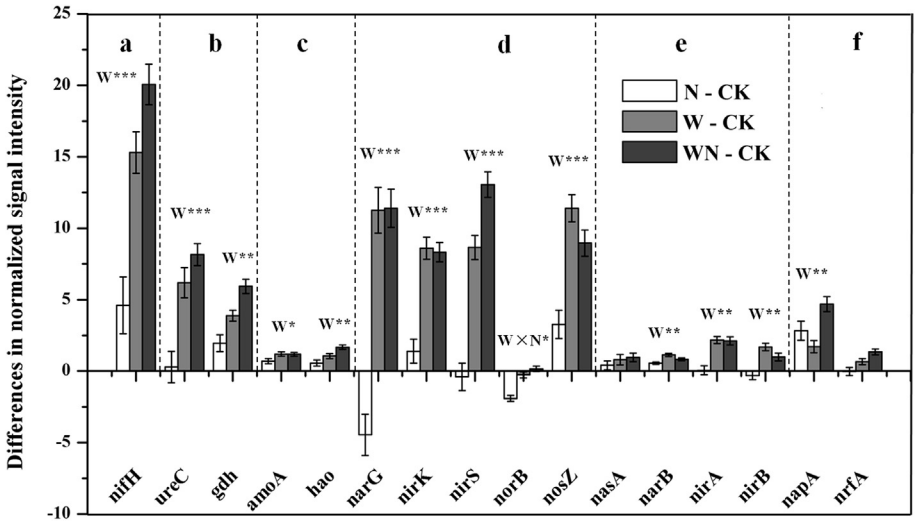

Fig. 3. Effects of projected N deposition and precipitation increment on the abundance of key N cycling genes. Shown are the signal differences between three treatments (N amendments, water addition, and N plus water addition) and control, designated as N-CK, W-CK, and WN-CK, respectively. The processes involved in N cycles include (a) N_2 fixation; (b) Ammonification; (c) Nitrification; (d) Denitrification; (e) Assimilatory N reduction; (f) Dissimilatory N reduction. All data are presented as mean ± SE. The effects of watering (W), N addition (N), and their combinations (W × N) on specific genes were analyzed by two-way ANOVA with a split-plot design. *0.01 < P < 0.05, **0.001 < P < 0.01, ***P < 0.001. No labels representing there was no treatment effect.

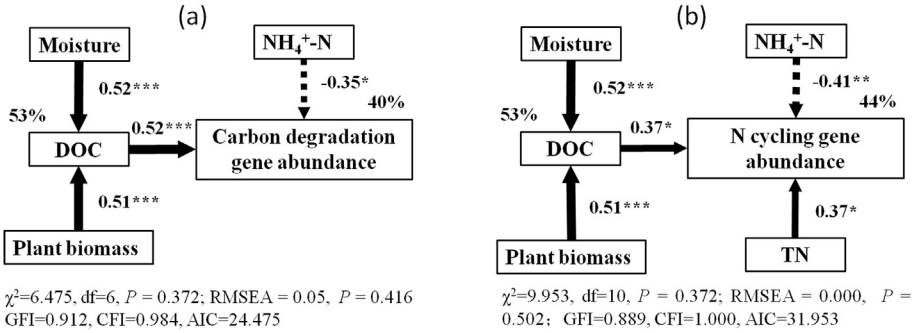

Fig. 4. Structural equation modeling (SEM) showing the relationships between soil/plant variables and the abundance of carbon degradation genes (a) and N cycling genes (b). The gene abundances were estimated by the signal intensities standardized by the probe numbers. Solid arrow indicates a significant positive correlation, and the dashed arrow represents a negative relationship. The width of the arrows is proportional to the strength of the relationship, and the numbers adjacent to arrows are standardized path coefficients. Percentages close to variables refer to the variance accounted for by the model (R^2). Goodness-of-fit statistics for each model are shown below the model. *0.01 < P < 0.05, **0.001 < P < 0.01, ***P < 0.001.

of soil and plant variables on microbial functional gene abundance. Stepwise regression revealed that the abundance of C fixation genes showed a positive correlation with soil TN, indicating that the microbial carbon fixation processes depended on N resources, due to microbial stoichiometric homeostasis. Nevertheless, the abundance of genes involved in different carbon fixation pathways were each correlated with different subsets of soil and plant variables (Table 4). The total abundance of C degradation genes was positively correlated with soil DOC, and negatively correlated with soil NH_4^+-N concentration. Still, the abundance of genes involved in the degradation of relatively recalcitrant carbon, including cellulose, chitin, and vanillin/lignin, were positively correlated with the plant biomass returned to the soil. However, the abundance of genes involved in labile C (starch and hemicelluloses) degradation showed no association with any selected variables. Consistent with stepwise regression analysis, SEM indicated that the total abundance of C degradation genes was directly stimulated by soil DOC, but suppressed by NH_4^+-N concentration. The abundance of C degradation genes was indirectly influenced by soil moisture and plant biomass through enhancing soil DOC. Soil TC, which was positively correlated with the abundance of C degradation genes, was eliminated from the model.

As expected, the total signal intensity of N cycling genes was positively correlated with soil TN, as revealed by stepwise regression and SEM analysis. Most of the specific N cycling processes were related to soil TN, NH_4^+-N or DOC, rather than plant resources returned to the soil (Table 4). In detail, the abundance of *nif*H genes was positively correlated with soil DOC, indicating that microbial nitrogen fixation was closely associated with soil available carbon resource, which could be also explained by the intrinsic C:N organismal stoichiometry. Nitrification and denitrification processes were strongly related to soil TN, as revealed by stepwise regression (Table 4). The abundance of specific denitrification genes, *narG*, *nirK/S*, *norB*, and *nosZ*, were each correlated with different soil variables (Table S4). The assimilatory and dissimilatory N reduction, and ammonification were negatively correlated with soil NH_4^+-N, a metabolic product of these processes. SEM analysis showed similar trends. Soil DOC and TN had direct positive effects on the abundance of total N cycling genes, whereas NH_4^+-N exhibited negative effects. Soil moisture and plant biomass indirectly elevated the abundance of N cycling genes through increasing soil DOC (Fig. 4).

4. Discussion

4.1. Microbial functional gene abundances were significantly elevated by precipitation increment in semi-arid grassland ecosystems

Consistent with our initial hypothesis, the abundance of functional genes involved in C and N cycling were significantly elevated by the long-term precipitation increment treatment. It suggests that water availability is a key driver in accelerating soil nutrient cycling processes in this semi-arid region. Because soil water availability is crucial for the growth and survival of microbes in arid and semi-arid regions, it could alleviate the physiological stress and constraints on enzymatic activities caused by drought (Tiemann and Billings, 2011). In this study, we did find significant positive watering effects on microbial biomass and the respiration rate (Table 1), which could attribute to the positive effects of precipitation increment on nutrients cycling processes. Precipitation events can also increase the availability of nutrients by directly releasing drought accumulated SOC, inorganic N and plant residues (Austin et al., 2004; Manzoni et al., 2012), which would further promote microbial activity. An increase in soil TC, TN and TS were observed under the precipitation increment treatment, which could be partially caused by this releasing process. The altered precipitation regimes may indirectly improve soil nutrients through enhancing plant biomass. Consistent with previous studies (Harpole et al., 2007; Bai et al., 2008; Wu et al., 2011), we observed a significant positive response of plant productivity to water supply (Table 1), which would subsequently increase the amount of nutrients returned to the soils, and subsequently stimulate the C and N cycling genes.

As for the specific C degradation genes, we initially expected that the abundance of labile C degradation genes would show a stronger response to the water supply compared with recalcitrant C degradation genes, because increased water availability usually reduce the C:N ratios of plant litter (Lü et al., 2012; Allison et al., 2013). However, significant positive effects of precipitation increment were observed on the abundance of both labile and recalcitrant C degradation genes. The turnover of soil C pool is a complicated process. The new plant C incorporated into the soils and the minor amounts of old soil organic matter were likely first decomposed to labile or relatively labile C, which is associated with the promotion of recalcitrant C degradation genes. Thereafter, the generated labile C resources would be further decomposed, corresponding to the flourish of labile C degradation genes. The positive effects of water supply on both labile and recalcitrant C degradation genes implies that the projected precipitation increment would increase regional C turnover rates and accelerate the replacement of soil organic matter by fresh plant C inputs.

4.2. Long-term N enrichment stimulated genes involved in recalcitrant C decomposition but showed no impacts on N cycling genes

Taxanomic gene sequencing analyses have indicated that N addition selects a more copiotrophic bacterial community and results in a shift towards labile C decomposition (Fierer et al., 2012; Ramirez et al., 2012). Consequently, we initially assume that C degradation genes involved in labile C degradation would be enhanced, and recalcitrant C decomposition genes would be reduced under N enrichment. However, our findings are not in line with such hypothesis. Most of the labile C degradation genes showed no significant response to N enrichment, which may attribute to the unchanged labile C fraction with added N, as revealed by our previous study (Wang et al., 2014). The increased abundance of functional genes involved in degrading relatively recalcitrant C implies that under long-term N addition treatment, the soil recalcitrant C pool could be activated by microbial communities, and would not accumulate in soils. This finding has great implications for predicting the response of soil C stocks to N deposition and the underlying mechanisms. In grassland ecosystems, impacts of N enrichment on soil C storage are inconsistent. A number of previous studies documented the positive effects of N deposition on soil C stocks (Liu and Greaver, 2010; Fornara et al., 2013), which could be considered somewhat intuitive given that plant productivity generally increases with N enrichment (Gough et al., 2000), which would likely increase C inputs to soils. However, increasing evidences suggest the lack of response of soil total C stock to N enrichment, illustrated by this field experiment (Table 1; Wang et al., 2014), and previous studies conducted in a tall-grass prairie in the USA (Ajwa et al., 1999) and similar grasslands in northern China (Lü et al., 2011; Song et al., 2014). These works postulated that the unchanged soil C pool under N deposition is likely caused by the balance between plant C inputs and microbe-mediated decomposition; our results provide solid evidence supporting this hypothesis. The increase in recalcitrant C decomposition genes and the unchanged SOC content suggest that future scenarios of N deposition would not impact soil C sequestration in this semi-arid temperate steppe.

In contrast to general hypothesis, most of the genes involved in N cycling processes, including N fixation, ammonification, nitrification, and denitrification, showed no responses to N addition. This may be attributed to the sampling time. The N fertilizer was added before and in the middle of the growing season, and the peak stage of ammonification, nitrification and denitrification processes are proposed to be essentially completed within a few weeks of fertilization (Schimel and Parton, 1986). We collected the soil samples by the end of August, and thus, the dynamic changes in mineral N and N cycling genes may have been missed. Indeed, we found no significant N effects on soil TN and NH_4^+-N at the sampling time (Table 1), which may at least partially explain the irresponsiveness of N cycling genes to N amendments. Despite all that, our results preliminarily imply that N enrichment would not show accumulative effects on microbial N cycling gene abundances, based on the long-term perspective of this 9-year field experiment. The interactive effects of N and watering were only observed on *nor*B gene involved in the denitrification process, which could be explained by the fact that soil water availability has large consequences for the inorganic N mobility and for the denitrifier physiology in semi-arid grassland ecosystem (Saetre and Stark, 2005; Dijkstra et al., 2012). The effects of watering plus N on the other C and N cycling genes were not statistically significant, implying that precipitation increment and N deposition might not impact each other on microbial functional feedbacks.

Here, it should be noted that the actual N input level might be lower than that we try to simulate, because of the potential for ammonia (NH_3) volatilization from the surface application of granular urea. A laboratory-based experiment has estimated that approximately 10%–40% of the added urea could be lost in the form of NH_3 in Aridic Calciustolls soils, depending on initial soil moisture content, rainfall pattern, wind speed, and air-humidity (Bouwmeester et al., 1985). However, a previous study performed in a semiarid region in northern China revealed small urea-derived N losses caused by ammonia volatilization (less than 0.1% at the application rate of 160 kg N ha^{-1}) due to the low soil moisture content (Fu et al., 2010). Unfortunately, the atmospheric losses of N were not determined in this experiment. Moreover, the frequency of N addition may influence the N effects on plant-soil systems. A field experiment conducted in another region in Inner Mongolia grassland revealed that soil NH_4^+-N and NH_3 emissions were much

higher at a higher frequency of NH_4NO_3 application (monthly), than at a lower frequency (twice per year) (Zhang et al., 2014b). However, plant species richness decreased more quickly at high rates and at low frequency of NH_4NO_3 addition (Zhang et al., 2014c). In the present study, the urea fertilizer was applied in two doses, and thus, we postulated that the plant species richness could be reduced relatively fast in this field experiment, compared to natural deposition. Further study of frequent N additions is recommended to simulate future scenarios of chronic N deposition.

4.3. Soil microbial functional genes are indirectly stimulated by aboveground plant biomass through increasing soil DOC

We initially hypothesized that N enrichments and increased precipitation would impact soil nutrient cycling processes, either directly through increased N and water availability, or indirectly through increasing plant C inputs to soils. Our results basically support this hypothesis. The SEM analysis revealed that soil DOC was directly correlated with the abundance of C degradation and N cycling genes, whereas soil moisture and aboveground plant biomass indirectly stimulated the gene abundance through enhancing soil DOC. The results suggest that soil DOC is a key factor determining the abundance of genes involved in C degradation and N cycling. Soil DOC is both a substrate and a product of microbial metabolic processes, and is of great importance to microbial assimilation and dissimilation. Previous studies also reported strong links between microbial activity and DOC concentrations (Van Hees et al., 2005; Bolan et al., 2011). Unexpectedly, soil moisture showed no direct effects on gene abundance, but instead indirectly, by influencing C and N cycling via soil DOC. Soil moisture may change from time to time, and we only examined one time point (late growing season) in this study. The high variability of soil moisture may partially explain the lack of direct linkage between soil moisture and available nutrients and/or microbial activities.

Consistent with our hypothesis, plant biomass previously returned to soils would indirectly related to the abundance of genes involved in C degradation and N cycling, through enhancing soil DOC. A previous study in an agro-ecosystem also demonstrated the positive correlation between crop yield and the abundance of genes involved in C degradation (He et al., 2014), supporting the close relationship between microbial C utilization potential and the returned plant resources. With regard to the specific C components, increased aboveground plant biomass tended to stimulate the genes responsible for recalcitrant C degradation, as illustrated by stepwise regression, whereas the genes/enzymes involved in labile C degradation showed no response to plant biomass and selected soil variables (Table 4). We collected soil samples by the end of the growing season, and there is not much fresh plant litter at that time. Instead, the proportion of labile C compounds within plant litter that returned to soil in previous years has been consumed, with recalcitrant C being left in the soil (Wang et al., 2014). That is a possible explanation on the decoupling of labile carbon degradation genes with plant biomass.

Both stepwise regression and SEM analysis indicate that the N cycling processes are positively correlated with soil TN, indicating the important roles soil TN plays in driving N cycling in this N-limited ecosystem, in particular nitrification and denitrification (Table 4). The assimilatory and dissimilatory N reduction processes, and ammonification were negatively correlated with soil NH_4^+-N, which may be caused by the inhibition by metabolic products. In contrast to our general hypothesis, no correlation was found between NO_3^--N and specific N cycling genes, in particular those associated with denitrification. The conventional denitrification process, reducing NO_3^- to NO_2^-, then to NO and N_2O, and finally to N_2, is typically performed by denitrifiers under anaerobic conditions. Given the low water filled pore space (WFPS), this classical denitrifier-denitrification process has been proposed to be less dominant in this semi-arid grassland (Zhang and Han, 2008), which may explain the accumulation of NO_3^--N at sampling (Table 1) and decoupling of NO_3^--N concentration and denitrification genes. Instead, the nitrifier-denitrification process (Kool et al., 2011), a pathway in which ammonia (NH_3) is oxidized to NO_2^- followed by the reduction of NO_2^- to NO and N_2O, may contribute more to N_2O emission in this region, as previously postulated (Zhang and Han, 2008).

5. Conclusions

In this field experiment, we predicted that the projected increased precipitation in future climate change scenarios would accelerate C and N cycling processes in this semi-arid region, by increasing microbial functional gene abundances. The promotion of recalcitrant C degradation genes induced by N enrichment implied that the soil organic C pool would not change, at least not greatly, under future scenarios of N deposition. We still observed that the abundance of C and N cycling genes were directly influenced by soil DOC and ammonium, and indirectly associated with aboveground plant biomass through enhancing soil available C resources. The present work is a first step towards understanding how climate change will alter regional C and N cycles at the functional gene level. In future work, the temporal dynamics of soil microbial functional genes in response to projected global change should be addressed. It would be also interesting to expand the observations in this typical steppe to other types of grasslands, such as meadow steppe, desert steppe, and alpine meadow, in order to test the generality of the observations. In addition, it is important that microbial functional parameters should be incorporated into the current global C, N and climate models for more accurate and reliable predictions.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.10.009.

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