Science of the Total Environment 515–516 (2015) 215–224

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Effects of nitrogen addition on soil microbes and their implications for soil C emission in the Gurbantunggut Desert, center of the Eurasian Continent

Gang Huang, Yan Feng Cao, Bin Wang, Yan Li *

State Key Lab of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, 818 South Beijing Road, Urumqi, Xinjiang 830011, China

HIGHLIGHTS

- Effects of N addition on soil microbes were studied in a temperate desert in 2011–2013.
- N addition decreased soil respiration beneath shrubs of *H. ammodendron* in 2012.
- N addition inhibited microbial biomass and respiration beneath *H. ammodendron* in 2012.
- N addition had no effects on microbial community composition.
- N addition decreased soil respiration with no alteration of soil carbon stability.

ARTICLE INFO

Article history:
Received 8 July 2014
Received in revised form 18 January 2015
Accepted 18 January 2015
Available online 14 February 2015

Editor: E. Capri

Keywords:
Carbon utilization
Desert ecosystem
Nitrogen deposition
Microbial biomass
Microbial community composition
Microbial respiration
Soil respiration

ABSTRACT

Nitrogen (N) deposition can influence carbon cycling of terrestrial ecosystems. However, a general recognition of how soil microorganisms respond to increasing N deposition is not yet reached. We explored soil microbial responses to two levels of N addition (2.5 and 5 gN m^{-2} yr^{-1}) in interplant soil and beneath shrubs of *Haloxylon ammodendron* and their consequences to soil respiration in the Gurbantunggut Desert, northwestern China from 2011 to 2013. Microbial biomass and respiration were significantly higher beneath *H. ammodendron* than in interplant soil. The responses of microbial biomass carbon (MBC) and microbial respiration (MR) showed opposite responses to N addition in interplant and beneath *H. ammodendron*. N addition slightly increased MBC and MR in interplant soil and decreased them beneath *H. ammodendron*, with a significant inhibition only in 2012. N addition had no impacts on the total microbial physiological activity, but N addition decreased the labile carbon substrate utilization beneath *H. ammodendron* when N addition level was high. Phospholipid fatty acid (PLFA) analysis showed that N addition did not alter the soil microbial community structure as evidenced by the similar ratios of fungal to bacterial PLFAs and gram-negative to gram-positive bacterial PLFAs. Microbial biomass and respiration showed close correlations with soil water content and dissolved carbon, and they were independent of soil inorganic nitrogen across three years. Our study suggests that N addition effects on soil microorganisms and carbon emission are dependent on the respiratory substrates and water availability in the desert ecosystem.

1. Introduction

Anthropogenic nitrogen (N) deposition resulting from increasing fertilizer application and fossil fuel combustion is 30–50% greater than that from natural sources (Galloway et al., 2008), and it has become one of the most important topics in global climate change (de Vries, 2009). Soil microorganisms play a key role in modulating the biosphere–atmosphere carbon exchange (Allison et al., 2010; Bardgett et al., 2008; Singh et al., 2010) through decomposition and heterotrophic respiration (Singh et al., 2010), and consequently change the carbon release rate into the atmosphere from the soil. Therefore, understanding how soil microbial organisms respond to N deposition has significances to elucidate the mechanisms of soil carbon emission under increasing N deposition (Galloway et al., 2004; Perry et al., 1991).

Empirical studies have shown that increasing N availability has complex influences on soil microbial communities (LeBauer and Treseder, 2008; Lu et al., 2011; Nohrstedt et al., 1989; Sinsabaugh and Linkins, 1989; Treseder, 2008). Several reasons are responsible for the inconsistent microbial responses to N addition. First, soil microbial activity responds nonlinearly to a gradient of N addition (Bai et al., 2010; Mo et al., 2008), suggesting that microbial responses to N addition are dependent on the N addition dose and experimental site. Second, the background of soil N content and microorganisms differ among studies;

* Corresponding author at: 818 South Beijing Road, Urumqi, Xinjiang 830011, China.
E-mail address: liyan@ms.xjb.ac.cn (Y. Li).

http://dx.doi.org/10.1016/j.scitotenv.2015.01.054
0048-9697/

the degree of N saturation might influence the direction and magnitude of soil microbial responses (Mo et al., 2008; Treseder, 2008). Third, the associated variations in soil properties generated by N addition can affect microbial responses to N addition. For instance, soil acidification by N addition (Lu et al., 2011; Wei et al., 2013; Zhang et al., 2013b) can cause toxicity to microbes and thus decrease microbial activities (Edwards et al., 2011). Moreover, plant root growth decreases while aboveground biomass increases with N addition, which can alter the respiratory substrates to microbes and subsequently affect microbial responses to N addition (Aerts and Chapin, 2000; LeBauer and Treseder, 2008; Treseder, 2004). Fourth, N addition can alter a series of critical ratios of soil organic matter, including C:N ratio, ligin:N ratio, N:P ratio. These ratios are nutrient limiting indicators of soil microbial decomposition (Parton et al., 2007; Schimel et al., 2007; Sterner and Elser, 2002; Vivanco and Austin, 2006).

Some geographical studies have demonstrated that the effects of N addition on soil microbes are dependent on soil nutrients (Collins et al., 2008). Especially, soil carbon availability has important influences on soil microbial activity in nutrient limited areas (Gallardo and Schlesinger, 1992). For example, the addition of labile organic matter can increase microbial activity and alter the microbial community structure by supporting more competitive populations (Collins et al., 2008). 'Fertile islands' is a sign of shrub inhabitation in arid lands. Soil nutrients and moisture beneath shrubs are much better than in interplant soils (Herman et al., 1995). Compared with forest and grassland ecosystems, nutrient heterogeneity in deserts is more significant to ecosystem responses to climate change (Austin, 2011; Austin et al., 2004). However, little is known on the interactive effects of 'fertile islands' and N addition on soil microbial communities in temperate deserts.

The desert accounts for one fifth of the total terrestrial area, and represents the most human affected and sensitive areas to climate change. However, few studies are available in desert ecosystems as compared with other terrestrial ecosystems (Vishnevetsky and Steinberger, 1996; Zhang and Zak, 1998). Because of the relatively high vegetation cover (30–50%) and potential carbon resorption ways, the temperate desert in northwestern China is a potential C reservoir (Ma et al., 2013; Su et al., 2013; Zhang et al., 2013a). To examine the responses of soil microbes to increasing N deposition and its consequences to soil carbon emission, we conducted a manipulative experiment of 2.5 and 5 gN m^{-2} yr^{-1} addition in the Gurbantungute Desert, northwestern China. Soil microbial biomass and respiration, microbial carbon utilization profiles, microbial community composition and soil respiration were investigated in two contrasting microsites of interplant soil and beneath the constructive shrub of *Haloxylon ammodendron*. First, we hypothesized that N addition would affect microbial biomass and activity, or it would alter microbial community composition via different influences on different microbial groups; Second, we hypothesized that N addition effects on soil microbial communities would depend on substrate availability in the microsite; Third, we hypothesized that the altered soil communities under N addition would change soil carbon emission. Moreover, this study was conducted across three years with contrasting precipitations, we also investigated the impacts of water availability on the responses of soil microbial communities to N addition.

2. Materials and methods

2.1. Study site description

The field site was in the vicinity of the Fukang Station of Desert Ecology, Chinese Academy of Sciences, on the southern edge of the Gurbantunggut Desert (44°17′N, 87°56′E and 475 m a.s.l.), Xinjiang, northwestern China. This region has a continental arid, temperate climate, with a hot, dry summer and cold winter. The annual mean temperature is 6.6 °C. The yearly average temperature ranged from 6.4 °C to 7.7 °C in 2011–2013 (Fig. S1). The annual mean precipitation is 160 mm. The annual precipitation was 167.4 mm, 102 mm and 133.7 mm in 2011, 2012 and 2013, respectively (Fig. S1). Soils are desert solonetz in 0–100 cm, with aeolian sandy soil at the top. Shrubs and semi-shrubs are mainly *Tamarix ramosissima*, *H. ammodendron* and *H. persicum*, with coverage of ca. 30%. The herbaceous layer is composed of *Alyssum linifolium*, *Leptaleum filifolium*, *Erodium oxyrrhynchum*, *Myosotis scorpioides*, *Eremurus inderiensis*, *Salicornia brachiata* and *Ceratocarpus arenarius*, with a cover of 40% in wet years (Fan et al., 2012, 2014), and the herbaceous community composiiton is different in interplant soil and beneath shrubs due to the different soil properties (Huang et al., 2015; Su et al., 2007).

Table 1
The effects of N1 (2.5 g N m^{-2} yr^{-1}) and N2 (5 g N m^{-2} yr^{-1}) addition on soil volumetric water content (SVWC), inorganic nitrogen content (In-N), dissolved carbon (DC) and soil pH (mean ± S.E., n = 6). Results from three-way ANOVA were shown in the lower panels of the table, and significant *P* values were shown in bold. Different small letters represent significant differences within year based on post hoc two-way ANOVA testing at $P < 0.05$. *IP* interplant soil, *BS* beneath shrubs of *H. ammodendron*, *Y* year, *C* control, *N* nitrogen addition, *S* microsite.

	SVWC (%)		In-N (mg kg^{-1})		DC (mg kg^{-1})		pH	
	IP	BS	IP	BS	IP	BS	IP	BS
2011								
N0	2.6 ± 0.3a	2.7 ± 0.2a	26.0 ± 2.5 a	28.2 ± 1.9 a	382.7 ± 24.9a	687.9 ± 24.7a	9.5 ± 0.1 a	9.7 ± 0.1a
N1	1.9 ± 0.3a	2.7 ± 0.2a	30.5 ± 3.4ab	30.6 ± 2.1a	455.6 ± 6.8b	469.2 ± 18.7b	9.4 ± 0.1a	9.6 ± 0.1a
N2	1.9 ± 0.4a	2.2 ± 0.2 a	34.8 ± 2.2 b	45.5 ± 3.9 b	476.7 ± 5.2b	451.9 ± 38.8b	9.4 ± 0.1 a	9.5 ± 0.1a
2012								
N0	5.4 ± 0.6a	5.7 ± 1.6a	27.4 ± 1.9a	30.2 ± 4.5a	362.5 ± 54.8a	639.2 ± 19.1a	9.5 ± 0.1a	9.6 ± 0.1a
N1	4.9 ± 0.8a	5.3 ± 0.5a	35.1 ± 5a	42.1 ± 3.6a	460.5 ± 25.6b	489.1 ± 14.3b	9.4 ± 0.1a	9.6 ± 0.1a
N2	4.9 ± 0.5a	4.7 ± 0.2b	55.5 ± 4b	65.3 ± 6.5 b	472.4 ± 120.6b	468.9 ± 10.7b	9.3 ± 0.1a	9.5 ± 0.1a
2013								
N0	3.2 ± 0.1a	3.4 ± 0.5a	15.4 ± 2.4a	26.7 ± 10.8 a	304.8 ± 17.4a	594.7 ± 23.4a	9.5 ± 0.1a	9.7 ± 0.1a
N1	2.8 ± 0.2a	3.4 ± 0.4a	18.3 ± 5.1a	31.1 ± 5.4a	426.1 ± 10.4b	441.4 ± 21.2b	9.4 ± 0.1a	9.4 ± 0.1a
N2	2.6 ± 0.2a	3.5 ± 0.2a	46.5 ± 6.1b	41.3 ± 2.8 b	432.3 ± 19.1b	432.5 ± 15.9b	9.3 ± 0.1a	9.4 ± 0.1a
Significance of repeated-measures ANOVA								
Y			<**0.001**	**0.023**	<**0.001**		0.271	
N			0.057	<**0.001**	<**0.001**		0.067	
S			<**0.001**	**0.001**	<**0.001**		0.254	
Y × N			**0.001**	**0.011**	0.274		**0.001**	
Y × S			0.354	0.442	0.381		0.351	
N × S			0.571	**0.042**	<**0.001**		0.218	
Y × N × S			**0.024**	0.054	0.144		0.376	

2.2. Plot layout, soil sample collection and related soil parameter measurements

The experiment used a completely randomized block design. There were six blocks and each block had three plots. Six blocks stretched in six interlands between sandy dune belts (Fig. S2). The CK, N1 and N2 treatments were randomly arranged into plots in each block, totally, each treatment had six replications. Each plot was 10 × 10 m, with 10 m wide buffer zone between adjacent plots. In addition, subplots (interplant and beneath shrubs of *H. ammodendron*) were created in each plot. Based on the real atmospheric nitrogen deposition in the Gurbantunggut Desert (2–2.5 g N m^{-2} yr^{-1}, Zhang et al., 2012), three levels of N addition were applied in our experiment: 0 gN m^{-2} (CK), 2.5 gN m^{-2} (N1) and 5 gN m^{-2} (N2) addition per year. N was treated in the form of NH_4NO_3, which was diluted in 15 L of water (equal to 0.15 mm rainfall) and evenly sprayed into the N1 and N2 addition plots in each block in April (for spring annuals) and July (for summer annuals). The same amount of distilled water was added in the control plots. All experimental design and instrument arrangements were conducted in 2010. Before N treatment, vegetation and soil nutrient characteristics (soil organic matter, soil total and inorganic nitrogen, soil total phosphorus and soil potassium) all showed no statistically significant difference between treatments using block as a covariance in ANOVA analysis. Five soil cores (5 cm in diameter, 5 cm in depth) were used to collect a mixed soil sample in interplant and beneath shrubs of *H. ammodendron* on August 10th in 2011, 2012 and 2013. After removing plant roots and large stones using a 2-mm sieve, soil samples were packed into a portable refrigerated box and transported to the laboratory for soil inorganic nitrogen, dissolved carbon, pH, microbial biomass, microbial respiration, microbial community level physiological profiles

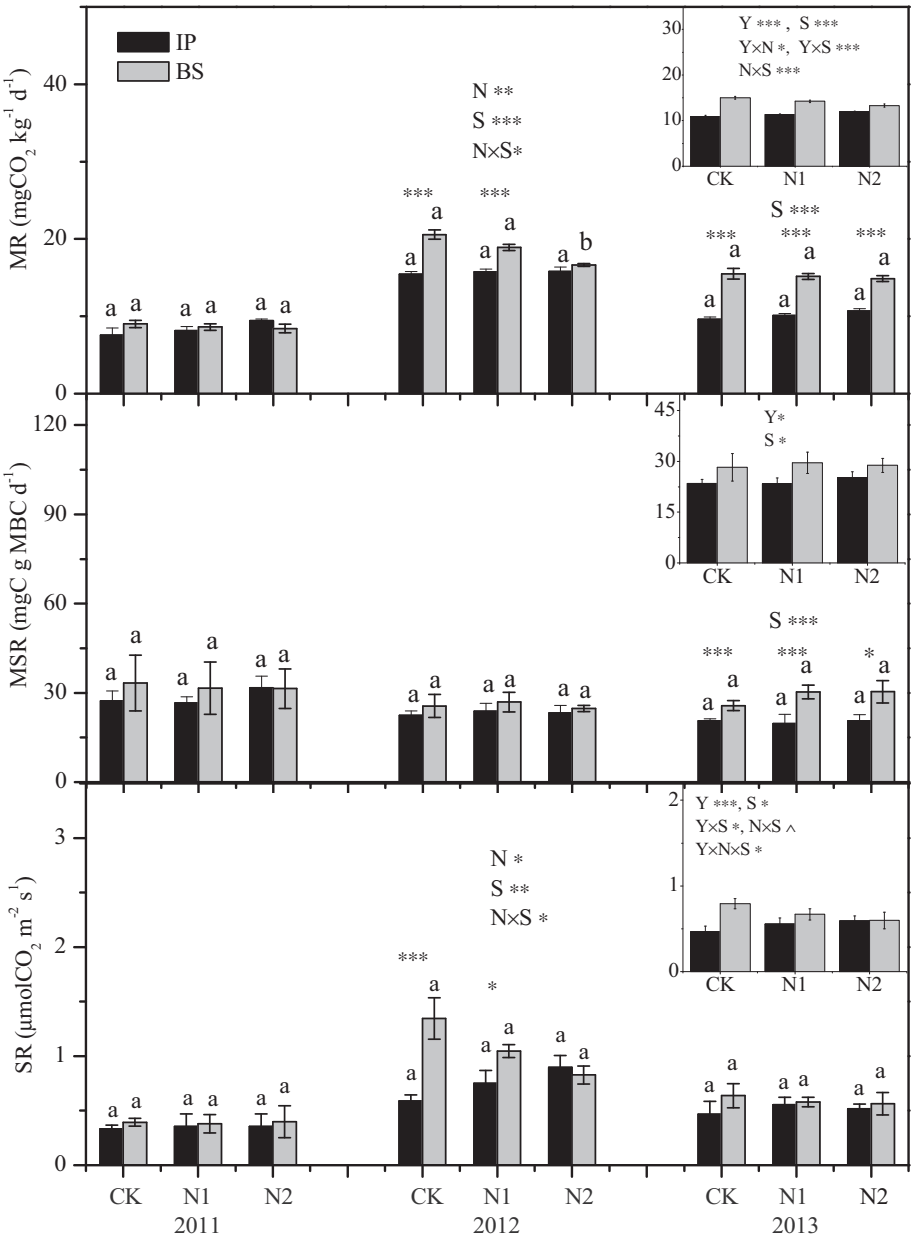

Fig. 1. Inter-annual dynamics (mean ± S.E., n = 6, main panels) and means (mean ± S.E., n = 18, top right insets) of microbial respiration (MR), mass specific respiration (MSR) and soil respiration (SR) averaged over three years. Significant results of the repeated-measures ANOVA on the effects of year (Y), nitrogen addition (N), microsite (S), and their interactions on MR, MSR and SR are shown in insets. Significant results of two-way ANOVA on the effects of nitrogen, site and their interactions are also shown within years, and bar with different small letters indicating significant differences between nitrogen treatments within microsites, and asterisks indicating significant differences between microsites. Significance: ***$P < 0.01$, **$P < 0.01$, *$P < 0.05$ and ∧$P < 0.1$. *IP* interplant, *BS* beneath shrubs of *H. ammodendron*, *CK* control, *N1* 2.5 g N m^{-2} yr^{-1} addition treatment, *N2* 5 g N m^{-2} yr^{-1} addition treatment.

(CLPPs) and microbial phospholipid fatty acid (PLFA) analysis as soon as possible. In addition, soil volumetric water content was also measured at sampling.

Nitrate–N (NO_3^-–N) and ammonium–N (NH_4^+–N) were extracted with 2 M KCl and measured with Auto Analyzer 3 (AA3, BRAN-LUEBBE Ltd., Hamburger, Germany). Dissolved carbon (DC) was extracted by adding 50 ml of 0.5 M K_2SO_4 to subsamples of 12.5 g homogenized soil, and agitated it on an orbital shaker at 120 rpm for 1 h. The filtrate was analyzed using a TOC analyzer (multi N/C 3100, Jena, Germany). Soil volumetric water content (SVWC) at 0–5 cm soil layer was measured using a portable TDR (HH_2-Delta T Device moisture meter, UK). Soil pH was determined with a glass electrode in a 1:5 soil:water solution (w/v).

Soil respiration (SR) was measured on the 10th of August in 2011, 2012 and 2013 using a portable infrared gas analyzer (Li-6400; LI-COR Inc., Lincoln, NE), which was incorporated into a photosynthesis system and attached to a closed dynamic soil respiration chamber designed for the Li-6400 system. In each plot, three polyvinyl chloride collars (inner diameter: 10.4 cm; height: 5.8 cm) were randomly placed beneath shrubs of *H. ammodendron* and in interplant soil, and then permanently installed 2 cm into soil. For each measurement, the soil respiration chamber was placed on each polyvinyl chloride collar. The measurement was repeated three times for each collar, and the chamber was displaced and repositioned on the collar with each repetition.

2.3. Microbial biomass and respiration measurements

Soil microbial biomass carbon (MBC) and nitrogen (MBN) was estimated using the chloroform fumigation extraction method (Brookes et al., 1985). Paired 20-g fresh soil samples that were either unfumigated or fumigated with alcohol-free $CHCl_3$ for 24 h were extracted with 0.5 M K_2SO_4 (1:2.5 w/v). Total C and N in fumigated and non-fumigated extracts were analyzed using a TOC analyzer (multi N/C 3100, Jena, Germany). The efficiency factors for MBC ($K_c = 0.38$, Vance et al., 1987) and MBN ($K_n = 0.54$, Brookes et al., 1985) were used to calculate the respective biomass.

Microbial respiration was measured as CO_2 evolution of fresh soil samples at 60% of water-holding capacity and incubated in sealed containers for 72 h at 25 °C. The CO_2 efflux from the soil was determined by the alkali absorption method (Page et al., 1982). Before incubation, 5 ml of 50 mM NaOH solution was injected into the connecting tube to absorb CO_2 released from the soil, and the respired carbon was determined by titrating the residual OH^- with a standardized HCl solution. Besides, mass specific respiration was calculated as microbial respiration dividing MBC.

2.4. Microbial carbon utilization profiles measurement

Microbial community level physiological profiles (CLPPs) were conducted using BIOLOG EcoPlates (Haywood, CA, USA) to identify patterns in C-substrate use. The EcoPlates are 96-well microtiter plates containing triplicate wells for 31 C substrates (plus a negative control with no C substrate; n = 3 each) representing common root exudates and soil compounds (Campbell et al., 1997). Analyses were conducted on soils within two days of collection. 10-gram fresh soil was added to 1000 ml of deionized water, and shaken at 200 rpm/min for 30 min. After shaking, soil suspensions were stirred continuously while 150 μL aliquots per well were transferred to each plate. Plates were incubated in the dark at 30 °C, and well-color development measured as optical density (OD) at 590 nm every 24 h for 240 h. Plate measurements at 96 h are used in this study (Classen et al., 2003). The net for each substrate was calculated by subtracting the substrate OD from the control well OD. If the result was less than 0.06, the net OD was set to zero due to the detection limits of the system (San Miguel et al., 2007). The net OD matrixes were analyzed to estimate the community-level changes in microbial C utilization. Microbial metabolic potential expressed as the average well-color development (AWCD) was determined as follows (Garland and Mills, 1991):

$$AWCD = \sum_{i=1}^{n}(x_i - c)/n$$

where x_i is the OD value of the sample substrate well, c is the OD value of the control well, and n is the number of sole C source types on each microtiter plate (n = 31).

Patterns of substrate use were characterized by grouping individual well substrates by chemical guild (Insam, 1997): amines, amino acids, carbohydrates, carboxylic acids, phenolic compounds, and polymers. Microbial metabolism of each guild (Gx) was determined by equally weighting all guilds to the largest one (carbohydrates, 10 substrates) using the following equation (Leflaive et al., 2005):

$$Gx = \frac{10}{n} \times \sum_{i}^{n} OD_i$$

where n is the number of substrates in the guild, and OD_i is the optical density of substrate i within the guild.

2.5. Microbial community composition measurement

Phospholipid fatty acid (PLFA) analysis was used to evaluate microbial community composition. PLFA of ca. 10 g dry soil was extracted and then fractionated and quantified following protocols described by Bossio and Scow (1998). Polar lipids in initial soil extracts were separated from neutral and glycolipids by elution with 5-ml chloroform and 10-ml acetone followed by 5-ml methanol. Mild alkaline methanolysis was then performed on the polar lipid fraction. A 2-μl sample of each fatty acid methyl ester extract was injected and analyzed by an Agilent 6850N gas chromatograph with a flame ionization detector and a HP-1 Utra 2 capillary column (Agilent Technologies, Santa Clara, CA, USA). Gas chromatography was performed as recommended by the MIDI standard protocol (Microbial ID, Newark, DE, USA). The peaks were identified by chromatographic retention time and a standard qualitative mix that ranged from C9 to C30 using a microbial identification system (Microbial ID). The relative concentration of each PLFA in each soil extract was represented by the mole percentage (nmol %) of total PLFAs. Although more than 60 PLFAs were identified, the analysis of data from the four treatments was restricted to 21 PLFAs that were consistently present in each sample. We chose i15:0, a15:0, i16:0, 16:1w7c, 16:1w9c, i17:0, a17:0, cy17:0, cy19:0, 18:1w7c as the bacterial markers

Table 2
Correlations of microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), total microbial PLFAs (T-PLFAs), bacterial PLFAs (B-PLFAs), fungal PLFAs (F-PLFAs), microbial respiration (MR) and soil respiration (SR) with soil volumetric water content (SVWC), dissolved carbon (DC), pH (pH), inorganic nitrogen (In-N) in interplant soil and beneath shrubs of *H. ammodendron*. Significant correlations are highlighted in bold.

	SVWC	DC	pH	In-N
Interplant				
MR	**0.884 (0.002)**	**0.803 (0.009)**	−0.171 (0.669)	0.178 (0.649)
SR	**0.828 (0.006)**	**0.771 (0.015)**	−0.221 (0.569)	−0.270 (0.482)
MBC	**0.801 (0.01)**	0.620 (0.075)	−0.294 (0.442)	0.054 (0.890)
MBN	**0.886 (0.001)**	**0.869 (0.002)**	−0.124 (0.750)	−0.319 (0.403)
T-PLFA	**0.871 (0.002)**	**0.884 (0.002)**	0.126 (0.746)	−0.455 (0.218)
B-PLFAs	**0.812 (0.008)**	**0.863 (0.003)**	−0.120 (0.758)	−0.290 (0.449)
F-PLFAs	**0.784 (0.012)**	**0.829 (0.006)**	−0.158 (0.684)	−0.272 (0.478)
Beneath shrubs of H. ammodendron				
MR	**0.750 (0.020)**	0.651 (0.058)	−0.027 (0.946)	0.276 (0.472)
SR	**0.883 (0.002)**	**0.878 (0.002)**	0.034 (0.931)	0.280 (0.466)
MBC	**0.851 (0.004)**	**0.705 (0.034)**	0.110 (0.778)	0.063 (0.872)
MBN	**0.783 (0.012)**	**0.915 (0.001)**	−0.048 (0.902)	−0.326 (0.391)
T-PLFA	**0.835 (0.005)**	**0.800 (0.010)**	0.138 (0.724)	0.269 (0.485)
B-PLFAs	**0.768 (0.016)**	0.610 (0.081)	0.244 (0.526)	−0.071 (0.857)
F-PLFAs	**0.828 (0.006)**	0.663 (0.052)	0.153 (0.695)	0.052 (0.895)

(Ringelberg et al., 1997; White et al., 1996; Zelles, 1997; Zogg et al., 1997). Terminally branched PLFAs, i.e., i15:0, a15:0, i16:0, i17:0 and a17:0, were used as indicators of gram-positive (GP) bacteria and cyclopropyl fatty acids cy17:0, cy19:0, 16:1w7c, 16:1w9c and 18:1w7c were used as indicators of gram-negative (GN) bacteria (Frostegård and Bååth, 1996; Ringelberg et al., 1997; Zak et al., 1996; Zelles, 1997; Zogg et al., 1997). Unsaturated PLFAs, 18:1w9c and 18:2w6,9c were used as the fungal markers (Zak et al., 1996). The ratio of fungal to total bacterial PLFAs was used to estimate the ratio of fungal to bacterial biomass (F:B) in soils (Frostegård and Bååth, 1996; White et al., 1996). The ratio of gram-negative bacteria to gram-positive bacteria PLFAs was used to estimate the ratio of gram-negative and gram-positive bacterial biomass (GN:GP). In addition, other PLFAs such as 14:0, i14:0, 16:0, 17:0, 18:0, 20:0, 14:1w5c, 17:1w8c and 20:1w9c, were also used to analyze the composition of microbial communities.

2.6. Statistical analysis

All statistical analyses were performed using R software version 3.0.2 (http://www.r-project.org). The repeated-measures ANOVA (aov function) was used to evaluate variations in soil inorganic nitrogen, soil pH, soil volumetric water content, soil dissolved carbon, soil respiration, microbial biomass, microbial respiration, mass specific respiration, and averaged well color development (AWCD), mole percentage of major groups of PLFA biomarkers, including gram-negative bacteria

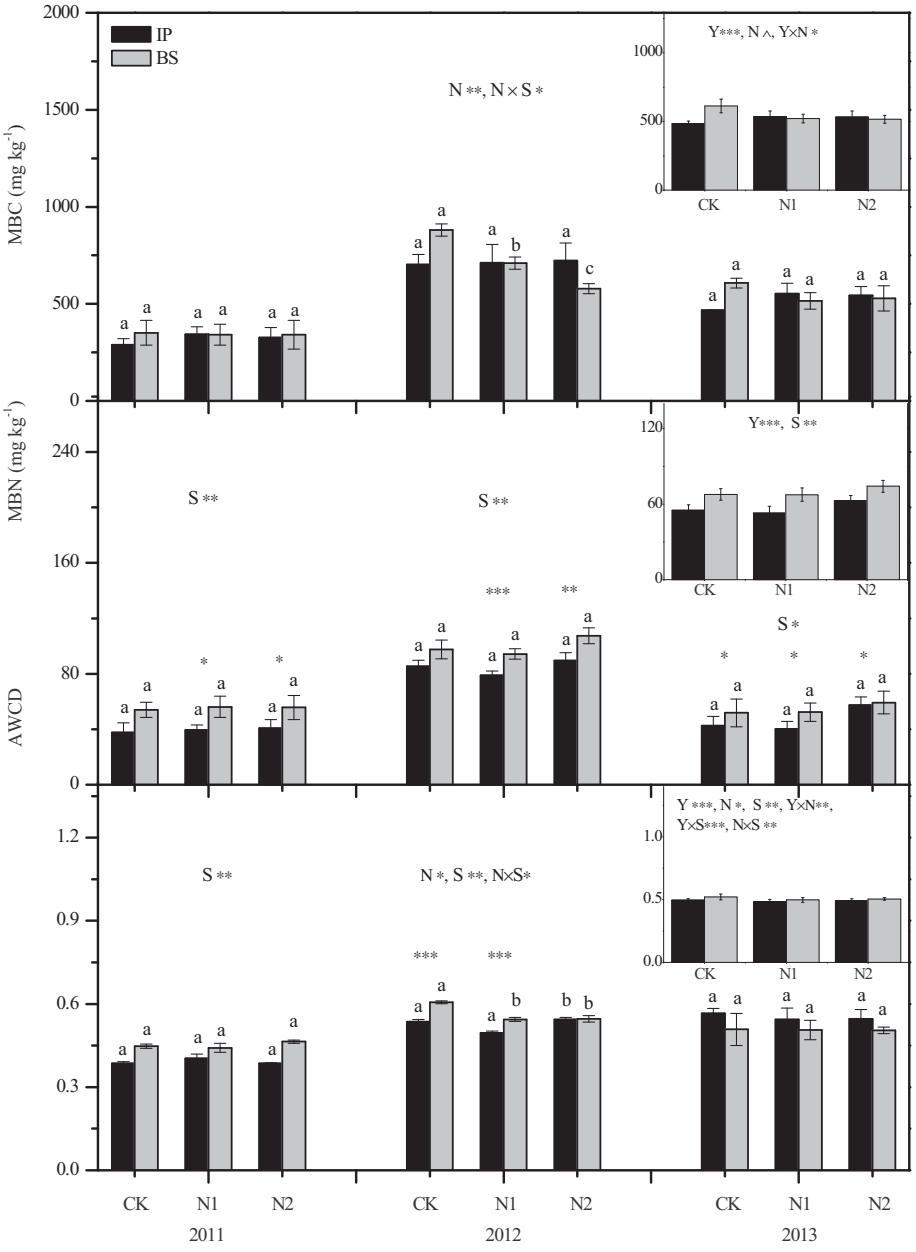

Fig. 2. Inter-annual dynamics (mean ± S.E., n = 6, main panels) and means (mean ± S.E., n = 18, top right insets) of microbial biomass carbon (MBC), nitrogen (MBN) and averaged well color development (AWCD) averaged over three years. Significant results of the repeated-measures ANOVA on the effects of year (Y), nitrogen addition (N), microsite (S), and their interactions on MBC, MBN and AWCD are shown in insets. Significant results of two-way ANOVA of the effects of nitrogen, microsite and their interactions are also shown within years, and bar with different small letters indicating significant differences between nitrogen treatments within microsites and asterisks indicating significant differences between microsites. Significance: $^{***}P < 0.01$, $^{**}P < 0.01$, $^{*}P < 0.05$ and $\wedge P < 0.1$. *IP* interplant, *BS* beneath shrubs of *H. ammodendron*, *CK* control, *N1* 2.5 g N m^{-2} yr^{-1} addition treatment, *N2* 5 g N m^{-2} yr^{-1} addition treatment.

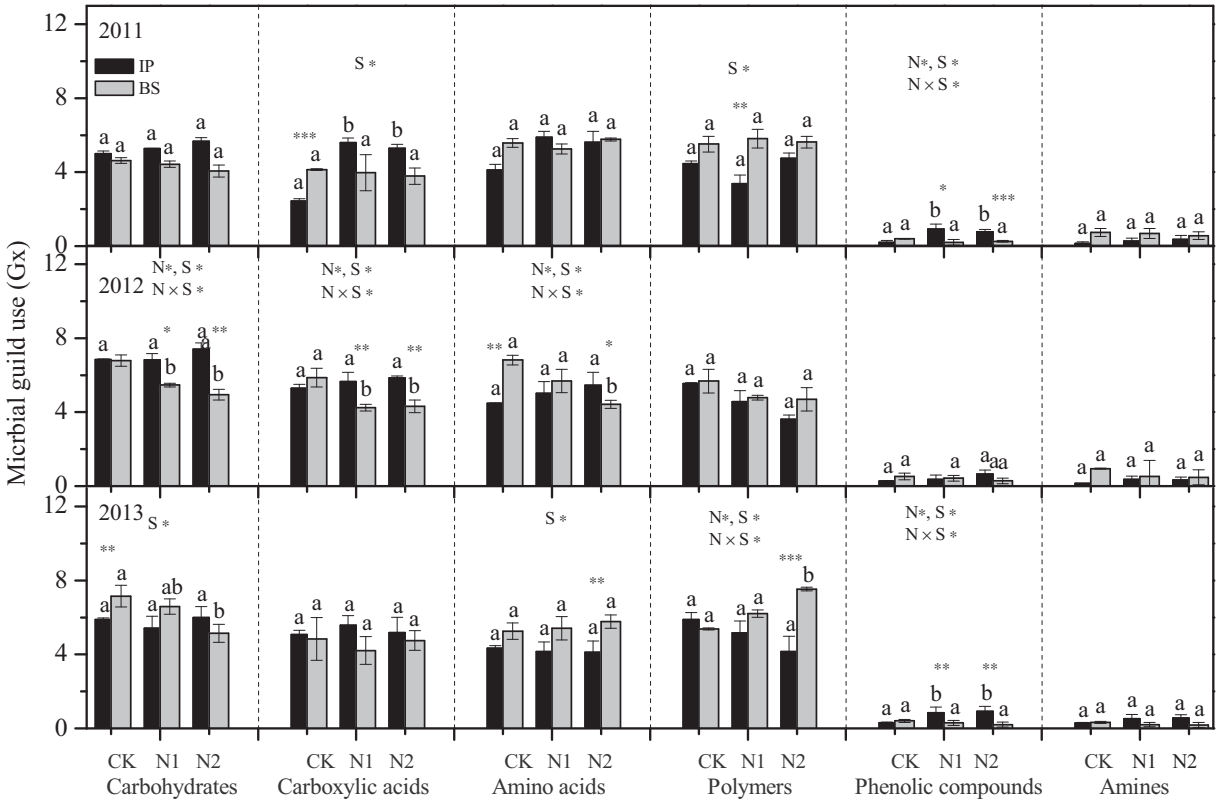

Fig. 3. Effects of increased nitrogen (N), microsite (S), and their interactions on microbial metabolism of each guild (Gx) in 2011, 2012 and 2013 (means ± S.E., n = 6). The significant results of two-way ANOVA on the effects of nitrogen and microsite, and their interactions on microbial metabolism of each guild are shown in each year. Significance: ***$P < 0.001$, **$P < 0.01$ and *$P < 0.05$. *IP* interplant, *BS* beneath shrubs of *H. ammodendron*, *CK* control, *N1* 2.5 g N m^{-2} yr^{-1} addition treatment, *N2* 5 g N m^{-2} yr^{-1} addition treatment.

(GN), gram-positive bacteria (GP), fungi and bacteria, and ratios of gram-negative bacteria to gram-positive bacteria (GN:GP) and fungi:bacteria (F:B) induced by microsite and N addition from 2011 to 2013. When the effects of treatments depended upon year (based on the repeated measures ANOVA), a two-way ANOVA was performed to analyze the yearly impacts of N addition and microsite on the parameters. The default 'cor.test' function (Pearson correlation) was used to test the significance of correlations among microbial biomass, microbial respiration, mole percentage of major groups of PLFA biomarkers, soil respiration and soil parameters. The multiple response permutation procedure (MRPP) in the vegan package was used to test the effects of year, microsite and N addition on microbial community composition. Nonmetric multidimensional scaling (NMDS, 'metaMDS' function of vegan package) analysis was used to analyze multivariate changes in microbial community composition.

3. Results

3.1. N addition effects on soil water content, inorganic nitrogen, dissolved carbon and pH

N addition and microsite had no significant effects on soil water content (Table 1). Soil water content showed a large interannual variation, with the highest value in 2012, followed by 2013 and 2011 (Table 1, $P < 0.001$). N addition significantly increased soil inorganic nitrogen (Table 1, $P < 0.001$). Soil dissolved carbon increased in interplant and decreased beneath shrubs with the addition of nitrogen, indicating the significant interactive effects of N addition and microsite (Table 1, $P < 0.001$). N addition decreased soil pH at the marginal significant level (Table 1, $P = 0.067$). Soil dissolved carbon was significantly higher beneath shrubs across three years, moreover, it showed a large interannual variation, with the highest value in 2012 (Table 1).

3.2. N addition effects on microbial respiration and soil respiration

N addition effects on microbial respiration were dependent on year (Fig. 1, F = 3.5, $P = 0.012$) and microsite (Fig. 1, F = 14.8, $P < 0.001$).

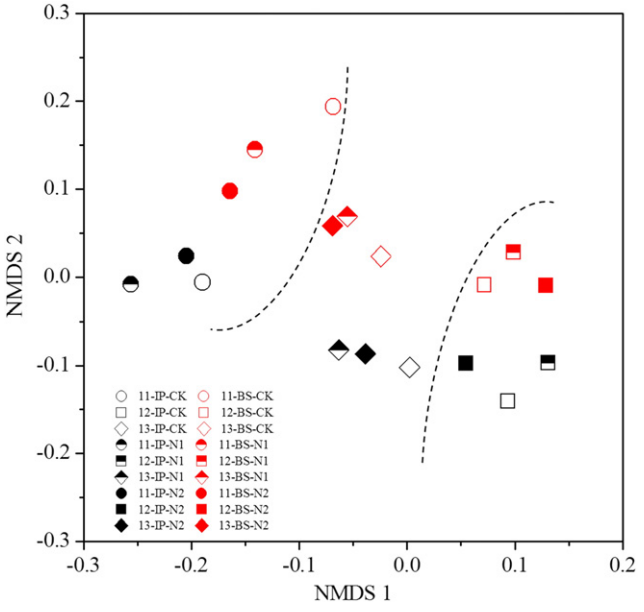

Fig. 4. Non-metric multidimensional scaling (NMDS) analysis on microbial community composition. Manually drawn dashed lines were used for easily visualizing the NMDS scores between years. The label represents Year-Site-Treatment. 11, 12 and 13 represents measurements conducted in 2011, 2012 and 2013, *IP* interplant soil, *BS* beneath shrubs of *H. ammodendron*, *CK* control, *N1* 2.5 gN m^{-2} yr^{-1} nitrogen addition treatment, *N2* 5 gN m^{-2} yr^{-1} nitrogen addition treatment.

N addition only significantly declined microbial respiration beneath shrubs in 2012 (Fig. 1, F = 11.9, $P < 0.001$), while exerting no impacts in 2011 (Fig. 1, F = 0.7, $P = 0.5$) and 2013 (Fig. 1, F = 0.12, $P = 0.8$). N addition exerted no impacts on mass specific respiration (Fig. 1, F = 0.1, $P = 0.9$). Similar to microbial respiration, N addition had negative impacts on soil respiration beneath shrubs in 2012 (F = 6.05, $P = 0.012$), N1 and N2 treatments decreased soil respiration by 22.3% and 38.5%, respectively (Fig. 1), while N addition had no influences on soil respiration in 2011 and 2013. Microbial respiration (F = 456.3, $P < 0.001$) and soil respiration (F = 58.3, $P < 0.001$) differed profoundly among years (Fig. 1). In the control plots, microbial respiration and soil respiration were highest in 2012, followed by 2013 and 2011. Microbial respiration (F = 72.8, $P < 0.001$) and soil respiration (F = 10.4, $P = 0.003$) were significantly higher beneath shrubs than in interplant soil under control in 2012, while having no difference between microsites in 2011 (Fig. 1).

Microbial respiration was independent of soil inorganic nitrogen (Table 2, $P > 0.05$) and soil pH (Table 2, $P > 0.05$). Microbial respiration was positively related to soil water content (IP: r = 0.884, $P = 0.002$; BS: r = 0.750, $P = 0.020$) and dissolved carbon (IP: r = 0.803, $P = 0.009$; BS: r = 0.651, $P = 0.058$). Similarly, soil respiration was also independent of soil pH and inorganic nitrogen across three years (Table 2, $P > 0.05$), and it was positively related with soil water content (IP: r = 0.828, $P = 0.006$; BS: r = 0.883, $P = 0.002$) and dissolved carbon (IP: r = 0.771, $P = 0.015$; BS: r = 0.878, $P = 0.002$) across three years (Table 2).

3.3. N addition effects on microbial biomass and carbon utilization profiles

N addition effects on microbial biomass carbon (MBC) were dependent on year (Fig. 2, F = 4.24, $P = 0.018$). N1 and N2 treatments significantly declined MBC by 19.3% and 34.2% beneath shrubs in 2012 (Fig. 2), while having no impacts on MBC in 2011 and 2013 (Fig. 2, $P > 0.05$). N addition had no significant impacts on microbial biomass nitrogen (Fig. 2, MBN, F = 1.65, $P = 0.21$). Similarly to MBC, N addition effects on the average well color development were dependent on year (Fig. 2, AWCD, F = 2.4, $P = 0.009$), N addition also significantly decreased AWCD beneath shrubs in 2012 (Fig. 2, F = 3.8, $P < 0.05$), while having no impacts in 2011 and 2013. N addition significantly increased microbial utilizations of carboxylic acids in interplant in 2011 (Fig. 3, $P = 0.006$) and phenolic compounds in interplant in 2011 (Fig. 3, $P = 0.021$) and 2013 (Fig. 3, $P = 0.035$). In contrast to the interplant soil, N addition significantly decreased microbial utilizations of carbohydrates (Fig. 3, $P = 0.035$), carboxylic acids (Fig. 3, $P = 0.027$) and amino acids (Fig. 3, $P = 0.041$) beneath shrubs in 2012 and carbohydrates beneath shrubs in 2013 (Fig. 3, $P = 0.044$).

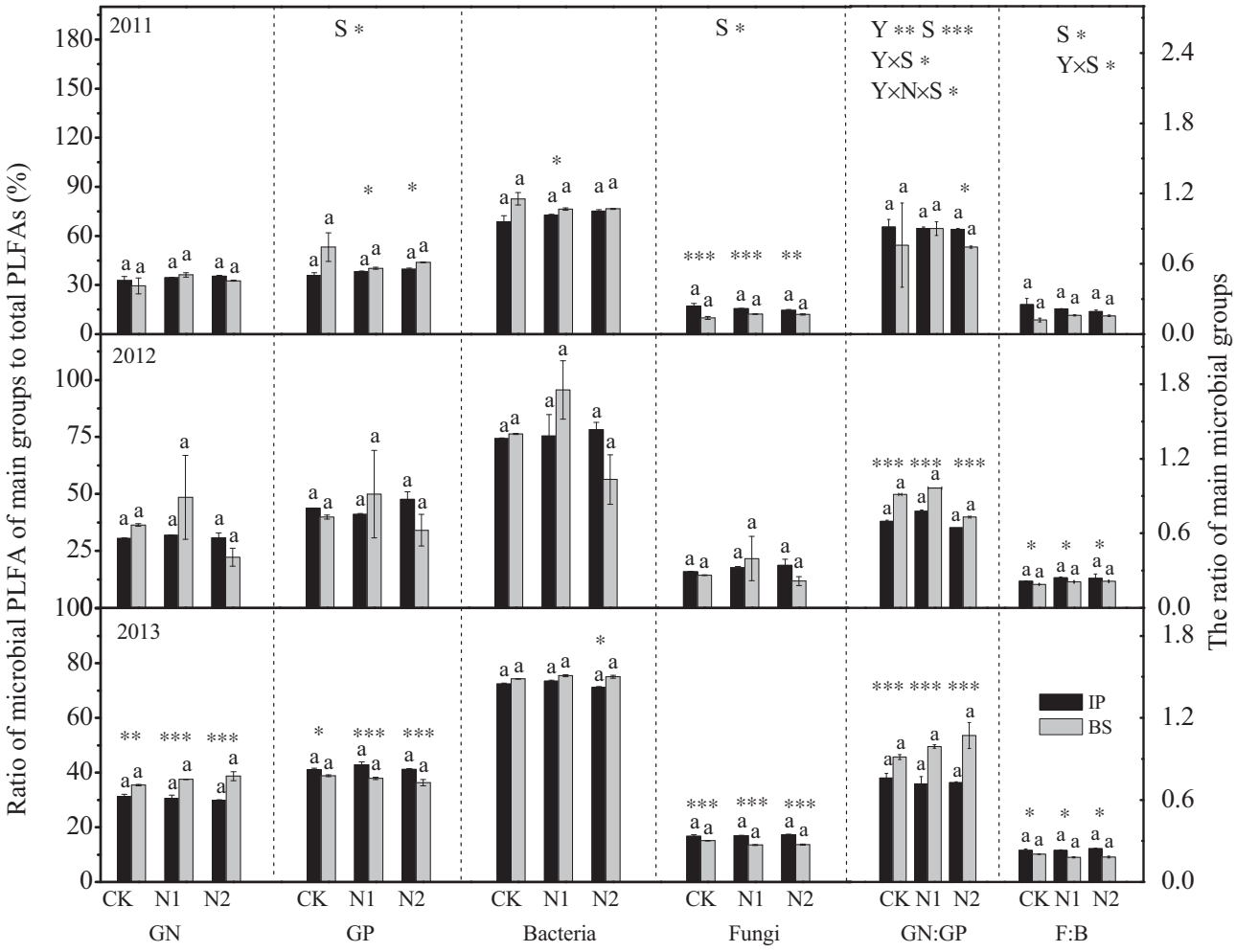

Fig. 5. Effects of year, nitrogen addition and microsite on microbial PLFAs of main groups and the ratio of different microbial groups (means ± SE, n = 6). Significant results of the repeated-measures ANOVA on the effects of year (Y), nitrogen addition (N), microsite (S), and their interactions on each parameter are shown on top of panel. Significant results of two-way ANOVA of the effects of nitrogen, site and their interactions are shown within years, and bar with different small letters indicating significant differences between nitrogen treatments and asterisks indicating significant difference between sites. Significance: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ and ∧$P < 0.1$. *GN* gram-negative bacteria PLFAs, *GP* gram-positive bacteria PLFAs, *GN:GP* the ratio of gram-negative to gram-positive bacteria PLFAs, *Fungi* fungal PLFAs, *Bacteria* bacterial PLFAs, *F:B* the ratio of fungi to bacteria PLFAs, *IP* interplant, *BS* beneath *H. ammodendron*, *CK* control, *N1* 2.5 gN m^{-2} yr^{-1} nitrogen addition treatment, *N2* 5 gN m^{-2} yr^{-1} nitrogen addition treatment.

MBC (F = 66.0, $P < 0.001$), MBN (F = 22.9, $P < 0.001$) and AWCD (F = 51.7, $P < 0.001$) all differed significantly among years (Fig. 2). MBC, MBN and AWCD were the highest in 2012, followed by 2013 and 2011. MBC was slightly higher beneath shrubs than interplant soil. MBN ($P = 0.006$) and AWCD ($P = 0.004$) were significantly higher beneath shrubs than in interplant soil (Fig. 2).

MBC was independent of soil inorganic nitrogen (Table 2, $P > 0.05$) and soil pH (Table 2, $P > 0.05$), and it was positively related to soil water content (IP: r = 0.801, $P = 0.010$; BS: r = 0.851, $P = 0.004$) and dissolved carbon at a marginal significant level in interplant soil (r = 0.620, $P = 0.075$) and at a significant level beneath shrubs (r = 0.705, $P = 0.034$). MBN was significantly correlated with soil water content (IP: r = 0.886, $P = 0.001$; BS: r = 0.783, $P = 0.012$) and dissolved carbon (IP: r = 0.869, $P = 0.002$; BS: r = 0.915, $P < 0.001$).

3.4. N addition effects on microbial community composition

N addition exerted no significant impacts on microbial community composition (Fig. 4, $P = 0.991$, MRPP). Similarly, N addition had no impacts on the mole percentage of gram-negative bacteria (Fig. 5, GN, F = 2.037, $P = 0.173$), gram-positive bacteria (Fig. 5, GP, F = 0.149, $P = 0.863$), total bacteria (Fig. 5, F = 0.803, $P = 0.471$), fungi (Fig. 5, F = 0.725, $P = 0.504$), and the ratio of GN to GP (Fig. 5, F = 0.991, $P = 0.400$) and F to B (Fig. 5, F = 0.154, $P = 0.859$).

The sampling year had significant impacts on microbial community composition (Fig. 4, $P = 0.002$, MRPP), and the ratio of gram-negative bacteria to gram-positive bacteria differed significantly among years (Fig. 5, GN:GP, F = 12.9, $P = 0.002$). Microsite also significantly affected microbial community composition (Fig. 4, $P = 0.014$, MRPP). PLFA percentage of gram-positive bacteria (GP, F = 5.8, $P = 0.03$) and fungi (F = 5, $P = 0.03$), the ratio of fungi:bacteria (F:B, F = 5.8, $P = 0.032$) and gram-negative bacteria to gram-positive bacteria (GN:GP, F = 50.5, $P < 0.001$) all differed significantly between microsites (Fig. 5).

Microbial, bacterial and fungal PLFAs were all positively related with soil water content across three years (Table 2, $P < 0.05$), and they were positively related with dissolved carbon in interplant (Table 2, $P < 0.005$). Only microbial PLFAs were significantly related with dissolved carbon beneath shrubs (Table 2, $P < 0.05$). Microbial, bacterial and fungal PLFAs were independent of soil pH and inorganic nitrogen across three years (Table 2, $P > 0.05$).

3.5. Correlations of microbial and soil respiration with microbial properties

Microbial respiration was strongly related to MBC (IP: r = 0.943, $P < 0.001$; BS: r = 0.972, $P < 0.001$), average well color development (IP: r = 0.918, $P < 0.001$; BS: r = 0.964, $P < 0.001$), total microbial (IP: r = 0.933, $P < 0.001$; BS: r = 0.839, $P < 0.05$), bacterial (IP: r = 0.956, $P < 0.001$; BS: r = 0.932, $P < 0.001$) and fungal (IP: r = 0.948, $P < 0.001$; BS: r = 0.976, $P < 0.001$) PLFAs across three years (Fig. 6). Soil respiration was positively related to MBC (IP: r = 0.908, $P < 0.001$; BS: r = 0.948, $P < 0.001$), average well color development (IP: r = 0.929, $P < 0.001$; BS: r = 0.935, $P < 0.001$), total microbial (IP: r = 0.956, $P < 0.001$; BS: r = 0.878, $P < 0.05$), bacterial (IP: r = 0.962, $P < 0.001$; BS: r = 0.856, $P < 0.05$) and fungal (IP: r = 0.972, $P < 0.001$; BS: r = 0.909, $P < 0.001$) PLFAs across three years (Fig. 6).

4. Discussion

Anthropogenic N deposition resulting from increasing fertilizer application and fossil fuel combustion is a global issue, and ecosystem-

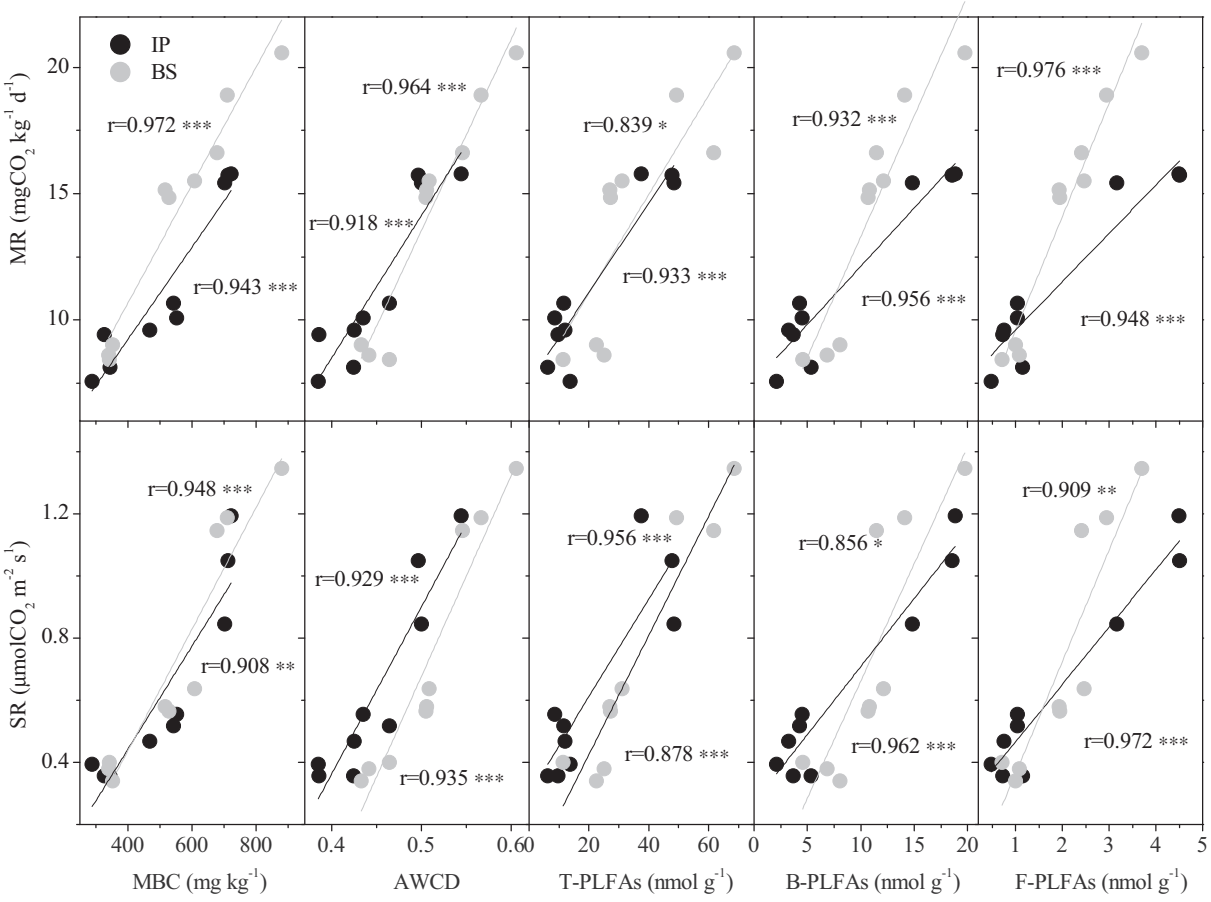

Fig. 6. Correlations of microbial respiration (MR) and soil respiration (SR) with microbial biomass carbon (MBC), average well color development (AWCD), total microbial PLFAs (T-PLFAS), bacterial PLFAs (B-PLFAs), and fungal PLFAs (F-PLFAs). Correlation coefficients and associated *P* values were based on Person correlation tests.

level responses to N deposition have been widely studied. Positive and neutral responses of plant growth have been observed (LeBauer and Treseder, 2008). However, soil microbial organisms respond differently from plants to N deposition, with positive, negative and no responses to N addition (Lu et al., 2011; Treseder, 2008). Many avaliable studies on N addition effects on soil microbial organisms are confined to a short-term period, and results showed that although small amounts of N addition can exert positive influences on soil microbes, the amount of N addition over the threshold value can trigger inhibition of microbial growth (Ball and Virginia, 2014).

Our study showed that N addition effects on microbial biomass and respiration differed with regard to the soil water content and microsite in this desert ecosystem. This result suggests that environmental cues can affect the N addition effects on soil microbes. Significant N addition effects only occurred in 2012. Among the three investigation years, soil temperature at 0–5 cm showed no difference among the three years, ranging from 10.8 to 11.1 °C. However, soil water content at 0–5 cm differed significantly, being the lowest in 2011 (1.92%) and the highest in 2012 (5.71%). The different microbial responses to N addition among the three investigation years are likely due to different soil water contents. The interactive effects of N and soil water content on microbial growth and respiration have also been observed in a grassland steppe (Bi et al., 2012) and desert ecosystems (Ball and Virginia, 2014). For instance, 5 gN m^{-2} yr^{-1} addition significantly increased microbial biomass when soil water content exceeded 10%, while N addition had no impacts under soil water content less than 10% irrespective of the N addition dose (2.5, 5 and 10 gN m^{-2} yr^{-1}) in the Negev Desert (Vishnevetsky and Steinberger, 1996). Soil microbes in deserts always experience drought stress as precipitation is sporadic and low (Loik et al., 2004; Sala and Lauenroth, 1982). In this study, no responses of soil microbes to N addition may be caused by the low microbial growth under drought, which led to the negligible effects of N addition. Thus, the contrasting responses of soil microbial biomass to N addition are closely associated with water availability in desert ecosystems.

N addition exerted opposite effects on microbial biomass and respiration between two microsites in 2012, with slight stimulation in interplant soil and significant inhibition beneath shrubs. The contrasting microbial responses to N addition have also been observed in some experiments with a gradient of N addition, where N loading thresholds for microbial biomass may occur (Mo et al., 2008). The amount of N addition which exceeds the N threshold value can trigger negative microbial responses to N addition. However, in our study, MBN in both microsites was slightly stimulated by N addition, so N limitation on soil microbes might generally occur in interplant soil and beneath shrubs. Although some studies attributed soil acidification to the reduction of microbial biomass under high N addition (Lu et al., 2011; Wei et al., 2013), this deleterious effect of declined soil pH on microbial communities might be impossible in the high alkaline soil in our study site (averaged pH > 10). Therefore, other possible causes, like C limitation, may have taken effect in our study, as suggested by the significant correlations of microbial biomass and respiration with soil dissolved carbon. The C limitation on microbes contradicts the high soil organic matter beneath shrubs. Because soil organic matter beneath shrubs is more resistant to decompose than that in interplant soil, the high N addition can inhibit the oxidative enzyme activity when C availability is relatively low, generating an unfavorable condition for microbes (He et al., 2007). Further studies using C isotope to trace the microbial carbon utilization can explore the causes of the inhibited microbial growth beneath shrubs under N addition.

Although microbial community level physiological profiles (CLPPs) can only reflect the changes of cultivable microbes, it can be used for analyzing microbial community physiological activity and their relations with microbial biomass. In our study, N addition only significantly inhibited microbial community physiological activity beneath shrubs in 2012, as evidenced by the decreased AWCD under N addition. Combining this result with the positive relation of microbial respiration with microbial biomass and AWCD, we can deduce that N addition effects on heterotrophic respiration results from altering microbial biomass and changing physiological activity. Moreover, the decreased utilization of carbohydrates and carboxylic acids under high N addition beneath shrubs in 2012 indicates that microbes can alter the type of C utilization under N addition. Considering that soil dissolved carbon increased in interplant and decreased beneath shrubs with the addition of nitrogen, the substrate utilization under high N level might have been different between two microsites. The close correlations of MBC and microbial respiration with soil organic matter suggest that the labile carbon substrates (carbohydrates, carboxylic acids and amino acids) determines soil carbon emission and the stability of soil organic matter in this desert ecosystem (Manzoni et al., 2012; Schimel et al., 2007).

As suggested by soil PLFA profiles, N addition did not alter the proportion of soil fungi and bacteria, and the ratio of gram-negative to gram-positive bacteria PLFAs. Similarly, N addition also had no impacts on microbial community composition. This is consistent with the consequences of N addition in a hardwood forest (DeForest et al., 2004) and temperate steppe (Zhang et al., 2013b). However, recent studies using molecular methods showed that although the ratio of soil fungi to bacteria was not altered, it's possible that N-using taxa of bacteria have changed under N addition. For example, N addition increased the abundance of ammonia-oxidizing bacteria (AOB) rather than archaeo (AOA), especially, when C availability was low, high N addition inhibited the oxidative enzyme activity, facilitating heterotrophic bacterial growth, which could outcompete the AOB (He et al., 2007). Therefore, N addition may not trigger a shift of F:B at the community level, but could induce a shift in N-transformers, which needs to be further analyzed.

5. Conclusion

Soil microbial communities in response to N addition and their implication for soil carbon emission were studied in two microsites (interplant and beneath shrubs of *H. ammodendron*) in a temperate desert across three years. N addition slightly increased microbial biomass carbon and respiration in interplant soil and decreased them beneath shrubs. The increased N stimulated vegetation growth beneath shrubs (Huang et al., 2015), thus, water and carbon availability decreased for microbes, which largely contributed to the decline in microbial biomass and respiration beneath shrubs under high N addition. In contrast to our hypothesis, there was no shift in microbial community structure under N addition. In particular, microbial respiration was positively related with microbial biomass and AWCD, suggesting that microbial biomass and community physiological activity were the primary determinants of carbon emission. Our study suggests that N addition effects on microbial organisms and carbon emission are mediated by nutrient and water availability in this temperate desert.

Acknowledgments

We are very grateful to the three anonymous reviewers for their valuable and constructive comments on this manuscript. We greatly appreciate Dr. Hong-mei Yang for the BIOLOG assay, and Dr. Yan-feng Cao for the soil PLFA extraction and GC analysis of the PLFA extracts. This work was sponsored by the Chinese National Natural Scientific Foundation (Nos: 31370010 & 41371004) and the West Light Foundation of the Chinese Academy of Sciences (RCPY201101).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.01.054.

References

Aerts, R., Chapin, F.S., 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Adv. Ecol. Res. 30, 1–67.

Allison, D.B., Stramski, D., Mitchell, B.G., 2010. Seasonal and interannual variability of particulate organic carbon within the Southern Ocean from satellite ocean color observations. J. Geophys. Res. Oceans 115 (C06002).

Austin, A.T., 2011. Has water limited our imagination for arid land biogeochemistry? Trends Ecol. Evol. 26, 229–235.

Austin, A.T., Yahdjian, L., Stark, J.M., Belnap, J., Porporato, A., Norton, U., et al., 2004. Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia 141, 221–235.

Bai, Y.F., Wu, J.G., Clark, C.M., Naeem, S., Pan, Q.M., Huang, J.H., et al., 2010. Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: evidence from inner Mongolia Grasslands. Glob. Chang. Biol. 16, 358–372.

Ball, B.A., Virginia, R.A., 2014. Microbial biomass and respiration responses to nitrogen fertilization in a polar desert. Polar Biol. 37, 573–585.

Bardgett, R.D., Freeman, C., Ostle, N.J., 2008. Microbial contributions to climate change through carbon cycle feedbacks. ISME J. 2, 805–814.

Bi, J., Zhang, N.L., Liang, Y., Yang, H.J., Ma, K.P., 2012. Interactive effects of water and nitrogen addition on soil microbial communities in a semiarid steppe. J. Plant Ecol. 5, 320–329.

Bossio, D.A., Scow, K.M., 1998. Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. Microb. Ecol. 35, 265–278.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil-nitrogen — a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17, 837–842.

Campbell, C.D., Grayston, S.J., Hirst, D.J., 1997. Use of rhizosphere carbon sources in sole carbon source tests to discriminate soil microbial communities. J. Microbiol. Methods 30, 33–41.

Classen, A.T., Boyle, S.I., Haskins, K.E., Overby, S.T., Hart, S.C., 2003. Community-level physiological profiles of bacteria and fungi: plate type and incubation temperature influences on contrasting soils. FEMS Microbiol. Ecol. 44, 319–328.

Collins, S.L., Sinsabaugh, R.L., Crenshaw, C., Green, L., Porras-Alfaro, A., Stursova, M., et al., 2008. Pulse dynamics and microbial processes in arid land ecosystems. J. Ecol. 96, 413–420.

de Vries, W., 2009. Assessment of the relative importance of nitrogen deposition and climate change on the sequestration of carbon by forests in Europe: an overview introduction. For. Ecol. Manag. 258.

DeForest, J.L., Zak, D.R., Pregitzer, K.S., Burton, A.J., 2004. Atmospheric nitrate deposition, microbial community composition, and enzyme activity in northern hardwood forests. Soil Sci. Soc. Am. J. 68, 132–138.

Edwards, I.P., Zak, D.R., Kellner, H., Eisenlord, S.D., Pregitzer, K.S., 2011. Simulated atmospheric N deposition alters fungal community composition and suppresses ligninolytic gene expression in a northern hardwood forest. PLoS One 6, e20421.

Fan, L.L., Ma, J., Wu, L.F., Xu, G.Q., Li, Y., Tang, L.S., 2012. Response of the herbaceous layer to snow variability at the south margin of the Gurbantonggut Desert of China. Chin. J. Plant Ecol. 126–135.

Fan, L.L., Tang, L.S., Wu, L.F., Ma, J., Li, Y., 2014. The limited role of snow water in the growth and development of ephemeral plants in a cold desert. J. Veg. Sci. 25, 681–690.

Frostegård, Å., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol. Fertil. Soils 22, 59–65.

Gallardo, A., Schlesinger, W.H., 1992. Carbon and nitrogen limitations of soil microbial biomass in desert ecosystems. Biogeochemistry 18, 1–17.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., et al., 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70, 153–226.

Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z.C., Freney, J.R., et al., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320, 889–892.

Garland, J.L., Mills, A.L., 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl. Environ. Microbiol. 57, 2351–2359.

He, J.Z., Shen, J.P., Zhang, L.M., Zhu, Y.G., Zheng, Y.M., Xu, M.G., et al., 2007. Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. Environ. Microbiol. 9, 2364–2374.

Herman, R.P., Provencio, K.R., Herreramatos, J., Torrez, R.J., 1995. Resource islands predict the distribution of heterotrophic bacteria in Chihuahuan Desert soils. Appl. Environ. Microbiol. 61, 1816–1821.

Huang, G., Li, Y., Padilla, F.M., 2015. Ephemeral plants mediate responses of ecosystem carbon exchange to increased precipitation in a temperate desert. Agric. For. Meteorol. 201, 141–152.

Insam, H., 1997. Substrate utilization tests in microbial ecology. J. Microbiol. Methods 30, 1–2.

LeBauer, D.S., Treseder, K.K., 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89, 371–379.

Leflaive, J., Cereghino, R., Danger, M., Lacroix, G., Ten-Hage, L., 2005. Assessment of self-organizing maps to analyze sole-carbon source utilization profiles. J. Microbiol. Methods 62, 89–102.

Loik, M.E., Still, C.J., Huxman, T.E., Harte, J., 2004. In situ photosynthetic freezing tolerance for plants exposed to a global warming manipulation in the Rocky Mountains, Colorado, USA. New Phytol. 162, 331–341.

Lu, M., Zhou, X.H., Luo, Y.Q., Yang, Y.H., Fang, C.M., Chen, J.K., et al., 2011. Minor stimulation of soil carbon storage by nitrogen addition: a meta-analysis. Agric. Ecosyst. Environ. 140, 234–244.

Ma, J., Wang, Z.Y., Stevenson, B.A., Zheng, X.J., Li, Y., 2013. An inorganic CO_2 diffusion and dissolution process explains negative CO_2 fluxes in saline/alkaline soils. Scientific Reports 3, p. 2025.

Manzoni, S., Schimel, J.P., Porporato, A., 2012. Responses of soil microbial communities to water stress: results from a meta-analysis. Ecology 93, 930–938.

Mo, J., Zhang, W., Zhu, W., Gundersen, P., Fang, Y., Li, D., et al., 2008. Nitrogen addition reduces soil respiration in a mature tropical forest in southern China. Glob. Chang. Biol. 14, 403–412.

Nohrstedt, H.O., Arnebrant, K., Baath, E., Soderstrom, B., 1989. Changes in carbon content, respiration rate, ATP content, and microbial biomass in nitrogen-fertilized pine forest soils in Sweden. Can. J. For. Res. 19, 323–328.

Page, A., Miller, R., Keeney, D., 1982. Soil respiration. Methods Soil Anal. 2, 143–156.

Parton, W., Silver, W.L., Burke, I.C., Grassens, L., Harmon, M.E., Currie, W.S., et al., 2007. Global-scale similarities in nitrogen release patterns during long-term decomposition. Science 315, 361–364.

Perry, D.A., Borchers, J.G., Turner, D.P., Gregory, S.V., Perry, C.R., Dixon, R.K., et al., 1991. Biological feedbacks to climate change — terrestrial ecosystems as sinks and sources of carbon and nitrogen. Northwest Environ. J. 7, 203–232.

Ringelberg, D.B., Stair, J.O., Almeida, J., Norby, R.J., ONeill, E.G., White, D.C., 1997. Consequences of rising atmospheric carbon dioxide levels for the belowground microbiota associated with white oak. J. Environ. Qual. 26, 495–503.

Sala, O.E.S., Lauenroth, W.K., 1982. Small rainfall events: an ecological role in semiarid regions. Oecologica 53, 301–304.

San Miguel, C., Dulinski, M., Tate, R.L., 2007. Direct comparison of individual substrate utilization f\rom a CLPP study: a new analysis for metabolic diversity data. Soil Biol. Biochem. 39, 1870–1877.

Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress–response physiology and its implications for ecosystem function. Ecology 88, 1386–1394.

Singh, B.K., Bardgett, R.D., Smith, P., Reay, D.S., 2010. Microorganisms and climate change: terrestrial feedbacks and mitigation options. Nat. Rev. Microbiol. 8, 779–790.

Sinsabaugh, R.L., Linkins, A.E., 1989. Cellulase mobility in decomposing leaf litter. Soil Biol. Biochem. 21, 205–209.

Sterner, R.W., Elser, J.J., 2002. Ecological Stoichiometry: The Biology of Elements From Molecules to the Biosphere. Princeton University Press, Princeton, New Jersey, USA.

Su, Y.G., Li, X.R., Cheng, Y.W., Tan, H.J., Jia, R.L., 2007. Effects of biological soil crusts on emergence of desert vascular plants in North China. Plant Ecol. 191, 11–19.

Su, Y.G., Wu, L., Zhou, Z.B., Liu, Y.B., Zhang, Y.M., 2013. Carbonflux in deserts depends on soil cover type: A case study in the Gurbantunggute desert. North China. Soil Biol. Biochem. 58, 332–340.

Treseder, K.K., 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. New Phytol. 164, 347–355.

Treseder, K.K., 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecol. Lett. 11, 1111–1120.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707.

Vishnevetsky, S., Steinberger, Y., 1996. Desert system microbial biomass determined by phospholipid phosphate and muramic acid measurement. Land Degrad. Dev. 7, 257–265.

Vivanco, L., Austin, A.T., 2006. Intrinsic effects of species on leaf litter and root decomposition: a comparison of temperate grasses from North and South America. Oecologia 150, 97–107.

Wei, C.Z., Yu, Q., Bai, E., Lu, X.T., Li, Q., Xia, J.Y., et al., 2013. Nitrogen deposition weakens plant-microbe interactions in grassland ecosystems. Glob. Chang. Biol. 19, 3688–3697.

White, D.C., Stair, J.O., Ringelberg, D.B., 1996. Quantitative comparisons of in situ microbial biodiversity by signature biomarker analysis. J. Ind. Microbiol. 17, 185–196.

Zak, D.R., Ringelberg, D.B., Pregitzer, K.S., Randlett, D.L., White, D.C., Curtis, P.S., 1996. Soil microbial communities beneath *Populus grandidentata* grown under elevated atmospheric CO_2. Ecol. Appl. 257–262.

Zelles, L., 1997. Phospholipid fatty acid profiles in selected members of soil microbial communities. Chemosphere 35, 275–294.

Zhang, Q.H., Zak, J.C., 1998. Effects of water and nitrogen amendment on soil microbial biomass and fine root production in a semi-arid environment in West Texas. Soil Biol. Biochem. 30, 39–45.

Zhang, Y., Song, L., Liu, X.J., Li, W.Q., Lu, S.H., Zheng, L.X., et al., 2012. Atmospheric organic nitrogen deposition in China. Atmos. Environ. 46, 195–204.

Zhang, C., Li, C.F., Luo, G.P., Chen, X., 2013a. Modeling plant structure and its impacts on carbon and water cycles of the Central Asian arid ecosystem in the context of climate change. Ecol. Model. 267, 158–179.

Zhang, N.L., Liu, W.X., Yang, H.J., Yu, X.J., Gutknecht, J.L.M., Zhang, Z., et al., 2013b. Soil microbial responses to warming and increased precipitation and their implications for ecosystem C cycling. Oecologia 173, 1125–1142.

Zogg, G.P., Zak, D.R., Ringelberg, D.B., MacDonald, N.W., Pregitzer, K.S., White, D.C., 1997. Compositional and functional shifts in microbial communities due to soil warming. Soil Sci. Soc. Am. J. 61, 475–481.