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No synergistic effects of water and nitrogen addition on soil microbial communities and soil respiration in a temperate desert

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ABSTRACT

Soil microbial communities play an important role in regulating land–atmosphere CO_2 exchange in terrestrial ecosystems. However, their responses to climate change are unclear. We explored the effects of water and nitrogen addition and their interaction on soil microbes and the resulting impacts on soil carbon emissions in the Gurbantunggut Desert, northwestern China. A manipulative 30% increase in precipitation and 5 gN m^{-2} $year^{-1}$ deposition alone and in combination was applied across three years from 2011 to 2013. Water addition significantly increased microbial biomass and respiration, metabolic quotient, and the utilization of carbohydrates, carboxylic acids and amino acids. Water addition did not change the microbial community composition. Nitrogen addition only significantly increased soil bacterial PLFAs, while exerting no significant impacts on soil fungal PLFAs, microbial respiration and soil respiration. Moreover, nitrogen addition had no significant impacts on microbial community composition. Water and nitrogen addition in combination did not generate synergistic effects on microbial communities and soil respiration. Across treatments in three years, soil respiration and microbial respiration were positively correlated with microbial total PLFAs, microbial carbon utilization profiles, while being independent of microbial community structure. This study suggests water addition can increase soil carbon emission through increasing microbial utilization of labile carbon, and water plus nitrogen addition had no synergistic effects on soil microbial communities and soil respiration, demonstrating nitrogen is not a limiting factor for soil microbial communities in the scenario of increasing precipitation in this desert ecosystem.

1. Introduction

Soil microbial communities act as a profound carbon sink in soils, and are also a mediator of soil organic matter decomposition (Schimel et al., 2007). Because of the importance of soil microbial communities in soil carbon emission and nutrient dynamics, they are gaining increasing concern (Manzoni et al., 2012a; Delgado-Baquerizo et al., 2013; Serna-Chavez et al., 2013). However, studies exclusively focusing on desert ecosystems are not abundant, and results are not consistent, which greatly hampers our understanding of soil carbon dynamics in the scenario of climate change (Insam and Rangger, 1997; Collins et al., 2008; Bell et al., 2014).

Desert ecosystems are characterized by low water and substrate availability to microbial organisms, and soil microbial activities are largely inhibited due to the low water availability and carbon supply (Austin et al., 2004). In some desert ecosystems, soil microbial biomass is under detectable at dry period, and can reach 15.3 mg N kg^{-1} soil in the wet duration (Gallardo and Schlesinger, 1992, 1995). The varying microbial biomass with soil moisture may exert profound impacts on soil carbon emission. Under drought, soil microbes must accumulate solutes to reduce the water potential in internal cells, which may lead to a decreased microbial respiration and soil carbon emission (Manzoni et al., 2012a; Taylor et al., 2012b). However, when moisture is available, soil microbes dispose osmolytes rapidly, which usually generates a pulse of CO_2 at the ecosystem level (Su et al., 2013). Besides of the microbial biomass, altered soil microbial community with water availability may also elicit the variation of soil carbon emission (Six et al., 2006; Schimel et al., 2007; Manzoni et al., 2012a) due to the contrasting carbon use efficiencies between different functional groups (Sinsabaugh et al., 2013).

Soil organic matter quantity can determine the degradation ability by soil microbes and subsequent carbon emission from the soil. The two most important elements affecting microbes in deserts are carbon and nitrogen, and studies are controversial in terms of their relative importance across ecosystems (Sinsabaugh et al., 2013). Similarly, manipulative experiments also showed contrasting responses of microbial biomass and respiration to altered soil substrates. For instance, 8 years of N addition of 3 g m^{-2} $year^{-1}$ did not alter the microbial community composition, while significantly reducing microbial biomass by 18% in a

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temperate forest (DeForest et al., 2004). In contrast, N application of 2 mg of NH_4NO_3–N g wet soil^{-1} only exerted transitory negative effects on microbial respiration and biomass (Soderstrom et al., 1983). Despite that N addition can trigger soil acidification, which usually exerts negative effects on soil microbial biomass and activity, these studies suggest that nitrogen availability effects on soil microbial communities deserve further studies.

The Gurbantunggut Desert is a temperate desert and located in the center of the Eurasian Continent. Because of the high vegetation cover of shrub-steppe community, this desert provides many ecological and economic services to local inhabitants. It has been predicted that precipitation will be continuously increasing through 2030 in this region (Liu et al., 2010). Besides, atmospheric nitrogen deposits at a rate of 3.6 gN m^{-2} year^{-1} in this desert due to the intensive agricultural activities adjacent to the desert in the past half century (He et al., 2007). Increasing water and nitrogen availability in this desert ecosystem may alter soil microbial communities and thus change soil carbon emission. However, few studies have been aimed to understand their potential impacts (Zhou et al., 2012). To elucidate the dynamics of microbial responses and their implications for soil carbon emission in the context of increasing precipitation and nitrogen deposition, we established a field experiment with manipulations of a 30% increase in precipitation and 5 gN m^{-2} year^{-1} deposition in the Gurbantunggut Desert in 2011–2013. Our previous observations have shown that water addition can significantly promote soil moisture and plant growth, implying that water addition can alleviate the water limitation and increase respiratory substrates to soil microbes (Huang et al., 2015). Thus, our first hypothesis is water addition can stimulate microbial growth and respiratory activity, and therefore soil respiration will be increased. Nitrogen addition can increase soil inorganic nitrogen content, which can change some important elemental ratios, primarily soil C:N. Considering that the primary functional groups of soil microbes have contrasting stoichiometric ratios and carbon use efficiencies (Keiblinger et al., 2010), our second hypothesis is that N addition can change the microbial community structure and microbial respiratory activities, leading to a different soil respiration as compared to the control. Significant promotion of plant growth can be achieved under nitrogen addition when water availability is high, which is thereafter favorable for soil microbial growth, thus our third hypothesis is that concurrent additions of water and nitrogen have synergistic effects on soil microbial communities and soil respiration.

2. Materials and methods

2.1. Study site description

The experiments were conducted in the vicinity of the southeastern Gurbantunggut Desert, northwestern China (44°17′N, 87°56′E, 475 m a.s.l.). This region has a continental arid, temperate climate, with a hot, dry summer and cold winter. The annual mean temperature is 6.6 °C and the annual mean precipitation is 160 mm, in which 70% to 80% fall in April–September. The annual precipitation and mean air temperature were 167.4 mm and 6.4 °C, 102 mm and 6.8 °C, and 133.7 mm and 7.7 °C in 2011, 2012, and 2013, respectively (Fig. S1). The pan evaporation is 2000 mm. The soil great groups are Torripsamments, under the soil order of Entisols; and Haplocalcids, under the soil order of Aridosols (USDA Soil Taxonomy). The soil is silt loam texture, with 81.7% sand, 16.8% silt and 1.5% clay. Soil organic carbon content is 2.4 g kg^{-1}. Soil pH is 9.5. The plants are *Tamarix ramosissima*, *Haloxylon ammodendron*, *Haloxylon persicum*, *Alyssum linifolium*, *Leptaleum filifolium*, *Erodium oxyrrhynchum*, *Myosotis scorpioides*, *Eremurus inderiensis*, *Salicornia brachiate*, and *Ceratocarpus arenarius*, with a cover of 30%. Soil surface was covered by biological soil crusts, which are an intimate association between soil particles and cyanobacteria, algae, microfungi, lichen and bryophytes in different proportions (Li, 2012). Its cover reaches 40% in the study site (Su et al., 2013).

2.2. Experiment design, sample collection, soil and plant properties measurements

Twenty-four 10 × 10 m plots were established and equally distributed in six blocks. Within each block, control (CK), water addition (W), nitrogen addition (N) and water plus nitrogen addition (WN) treatment were applied following a randomized block design. The distance between two adjacent plots was 10 m. In each W and WN treatments, precipitation increased 30%, according to predictions for northern China over the next 30 years (Liu et al., 2010). The 30% extra precipitation was collected using "rainfall collection pans". The pans were constructed from galvanized iron sheets, with an area of 1.9 × 1 m, totaling 18 pans was installed for each plot, the total area of pans was equivalent to 30% of the area in the plot, corresponding to the added water of 50.2 mm, 30.6 mm, and 40.1 mm in 2011, 2012, and 2013, respectively. Each pan was erected at a slight angle, and the rainfall intercepted was collected in a bucket that was buried in the soil. Immediately after a rainfall event, the collected rain was evenly sprayed onto the plots in the early morning or late afternoon to prevent excessive evaporation. Moreover, given the ecological significance of snowmelt in our site (Fan et al., 2014), snow fallen in the pan was also evenly added in the corresponding plot in early spring. In the N and WN treatments, N was applied in liquid form, 1667-g NH_4NO_3 was diluted in 15-L distilled water and evenly sprayed (equal to 0.15 mm rainfall) onto the corresponding plots (10 × 10 m) in early-April and mid-July, corresponding to the rapid growth duration of spring ephemerals and summer annuals in the study site (Huang and Li, 2015), which aimed to eliminate N limit to plant growth. The same amount of distilled water was added in the CK and W treatments. N (5 gN m^{-2} year^{-1}) application was based upon the real mean airborne N deposition rate (3.6 gN m^{-2} year^{-1}) registered in Xinjiang, northern China over the past 10 years (He et al., 2007). All experimental design and instrument arrangements were applied in 2010. Moreover, before the application of water and nitrogen treatments, vegetation and soil nutrient concentrations (soil organic matter, soil total nitrogen, soil total phosphorus, soil potassium and inorganic nitrogen) all showed no statistically significant difference between treatments using block as a covariance in ANCOVA analysis, suggesting no heterogeneity among the blocks undergone four treatments.

Five soil cores (5 cm diameter, 0–5 cm depth) were collected from each plot to get a composite sample on August 10th in 2011, 2012 and 2013. In desert ecosystems, because soil microbial communities exhibit large variations by water availability, we collected soil samples at the day without rainfall occurred in the prior five consecutive days. 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 and microbial properties measurements.

Soil nitrate-N (NO_3^- N) and ammonium-N (NH_4^+-N) were extracted with 2 M KCl (Sala et al., 2012; Reichmann et al., 2013) and measured with Auto Analyzer 3 (AA3, BRAN-LUEBBE Ltd., Hamburg, Germany). Dissolved organic carbon (DOC) was extracted by adding 50 mL of 0.5 M K_2SO_4 to subsamples of 12.5 g homogenized soil, and by agitating 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 pH was measured using a 1:5 dilution of soil:water (ISSCAS, 1978).

Soil respiration was measured with Li-Cor 840 portable photosynthesis systems (IRGA; LI-840, LiCor Inc., Lincoln, NE, USA) equipped with a chamber. The chamber dimension was 0.5 × 0.5 m at the base and 0.3 m in height. This method is widely used and provides valuable information that cannot be obtained easily in other ways (Risch and Frank, 2007). The chamber walls were constructed of clear polyethylene sheeting, and when measuring soil respiration, the chamber was covered with an opaque cloth. Two electric cooling fans were installed to circulate air in the chamber during measurements. Aluminum collars (ca. 3 cm deep) with a groove for chamber

placement were installed in each plot one month prior to the first measurement (late March 2011) to minimize the disturbance of collar insertion. 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).

2.3. Microbial properties measurements

Microbial respiration was determined by the alkali absorption method (Page et al., 1982). Briefly, fresh soils (20 g dry weight equivalent) were adjusted to 60% of field holding capacity and pre-incubated at 25 °C for 20 days. The incubated soils were then spread on the bottom of 500-mL glass jars for 72 h at 25 °C. Before that, 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. Metabolic quotient (qCO_2) was calculated as microbial respiration dividing by MBC.

Soil microbial carbon utilization pattern was measured using BIOLOG EcoPlates (Haywood, CA, USA, Insam and Rangger, 1997). 10 g of 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 (Preston-Mafham et al., 2002). The net OD for each substrate was calculated by the OD of the control well subtracted from OD of substrate well. If net OD was less than 0.06, it was set to zero (San Miguel et al., 2007). Patterns of substrate use were characterized by grouping individual well substrates into chemical guild (Insam and Rangger, 1997): amines, amino acids, carbohydrates, carboxylic acids, phenolic compounds, and polymers. Microbial utilization of each guild (Gx) was determined 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 the optical density of substrate i within the guild.

Microbial biomass in terms of functional group and microbial community composition were evaluated using phospholipid fatty acid (PLFA) analysis. PLFA was extracted from soil samples and then fractionated and quantified following protocols described by Vestal and White (1989) and Bossio and Scow (1998). The extracted fatty acid methyl esters were identified using a MIDI peak identification system (Microbial ID. Inc., Newark, DE, USA). Peak areas were converted to nmol lipid g dry $soil^{-1}$ using internal standards (19:0 nonadecanoic methylester). Totally 60 PLFAs were identified, the analysis of data from the four treatments was restricted to 26 PLFAs. The fatty acids i16:1, 16:1 w5c, 16:1 w9c, cy17:0, a17:1 w9c, 17:1 w8c, cy19:0 w8c, 20:1 w9c, 14:00, 16:00, 17:00, 18:00, 20:00, 15:0 3OH, a15:0, i15:0, a15:1, i15:1, a16:0, i16:0, a17:0, i17:0, 18:0 2OH, and 18:1 w7c 11-methyl represent total bacteria (White et al., 1996; Ringelberg et al., 1997; Zelles, 1997; Zogg et al., 1997). i16:, 16:1 w5c, 16:1 w9c, cy17:0, a17:1 w9c, 17:1 w8c, cy19:0 w8c, and 20:1 w9c represent Gram-negative bacteria (GN). 14:00, 16:00, 17:00, 18:00, 20:00, 15:0 3OH, a15:0, i15:0, a15:1, i15:1, a16:0, i16:0, a17:0, i17:0, 18:0 2OH and 18:1 w7c 11-methyl represent Gram-positive bacteria (GP) (Frostegård and Bååth, 1996; Zak et al., 1996). Unsaturated PLFAs, 18:1w9c and 18:2w6,9c represent fungi (Zak et al., 1996; Madan et al., 2002).

2.4. Statistical analysis

All statistical analyses were performed using R software version 3.0.3 (http://www.r-project.org). Prior to the analysis, data were tested for normality using the Kolmogorov–Smirnov test. Because soil respiration, microbial respiration, bacteria PLFAs, the ratio of fungal to bacterial PLFAs, and microbial carbon utilization of all six guilds were not normally distributed, ln-transformation was applied to these variables to normalize their distribution. Analysis of Covariance, with annual precipitation as the covariate, year as the random factor and water and nitrogen as fixed factors, was used to evaluate variations in soil moisture, soil inorganic nitrogen, soil respiration, microbial respiration, metabolic quotient, microbial substrate utilization of each guild (Gx), and major groups of PLFA biomarkers induced by water and nitrogen addition during the experimental period from 2011 to 2013. Because of the significant annual variation of dependent variables, a One-way ANOVA was used to examine the statistical difference of the measuring variables among the four treatments within a year.

The multiple response permutation procedure (MRPP) in the vegan package was used to test inter-annual variation in microbial community composition and water and nitrogen treatment effects on microbial community composition. Nonmetric multidimensional scaling (NMDS, 'metaMDS' function of vegan package, using Bray–Curtis distance) analysis was used to analyze multivariate changes in microbial community composition (26 PLFAs). The specific procedure of "metaMDS" was to initially run a default isoMDS, using the first solution as the standard, the 'Procrustes' procedure was then used to find a global, convergent and best fit solution. The function of "envfit" with 999 permutations in the vegan package was further used to identify relationships between microbial carbon utilization profiles, community composition and other parameters.

3. Results

3.1. Soil properties in response to water and nitrogen addition

Water addition significantly increased soil moisture by 2.43% and soil dissolved organic carbon across three years (Fig. 1, both $P < 0.01$). Water addition exerted no significant impacts on soil inorganic nitrogen and soil pH (Fig. 1, $P > 0.05$). Nitrogen addition had no significant impacts on soil moisture, dissolved organic carbon and soil pH (Fig. 1, both $P > 0.05$), it significantly promoted soil inorganic nitrogen content, by 1.13 times across three years (Fig. 1, $P < 0.001$). Both soil moisture (Fig. 1, $P < 0.001$) and inorganic nitrogen (Fig. 1, $P = 0.007$) showed a significant inter-annual variation, and they were higher in 2012 (Fig. 1).

3.2. Soil respiration in response to water and nitrogen addition

Water addition increased soil respiration to different extents across all the measurements (Fig. 2). When values were averaged in a year, water addition significantly increased soil respiration (Table 1, Fig. 2, $P = 0.01$), on average by 36%, 18% and 11% in 2011, 2012 and 2013, respectively. Nitrogen addition had no significant impact on soil respiration (Table 1, Fig. 2, $P = 0.72$). Water plus nitrogen addition had no interactive effects on soil respiration (Table 1, Fig. 2, $P = 0.43$).

3.3. Soil microbial biomass and community structure in response to water and nitrogen addition

Water addition significantly increased the total, bacterial and fungal PLFAs (Tables 1 & 2, all $P < 0.001$). Nitrogen addition significantly increased bacterial PLFAs (Tables 1 & 2, $P = 0.02$), while exerting no impacts on fungal PLFAs across three years (Table 1 & 2, $P = 0.28$). Water plus nitrogen addition exerted no interactive effects on the PLFAs of fungi and bacteria (Tables 1 & 2, $P > 0.05$). Water and nitrogen addition alone and in combination exerted no profound impacts on F:B

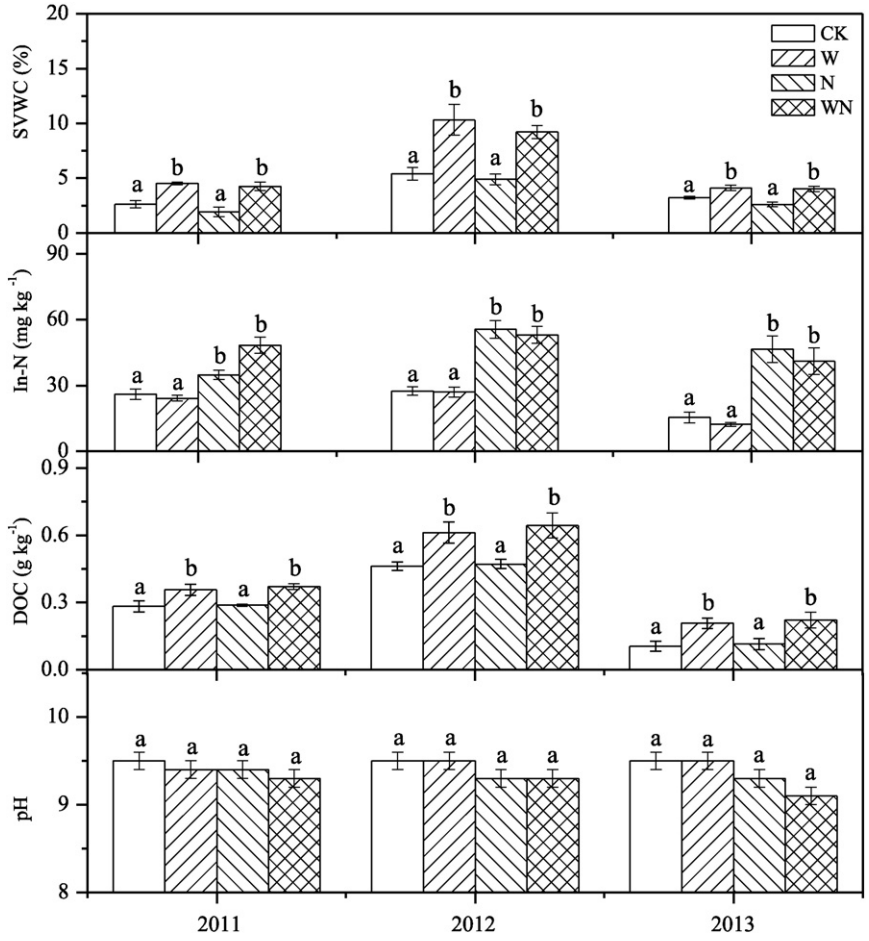

Fig. 1. The effects of water and nitrogen addition on soil volumetric water content (SVWC), inorganic nitrogen content (In-N), soil dissolved organic carbon (DOC) and soil pH (pH) (mean ± S.E., n = 6). Bars with different letters within years represent significant differences between treatments at P < 0.05. *Y* year, *CK* control, *W* water addition, *N* nitrogen addition, *WN* water plus nitrogen addition.

(Tables 1 & 2, P > 0.05), MRPP analysis showed that water and nitrogen addition had no influences on soil microbial community composition (Fig. 3, P > 0.05).

3.4. Microbial respiration in response to water and nitrogen addition

Water addition significantly increased microbial respiration by 95.3% across three years (Tables 1 & 3, P < 0.001). In detail, water addition significantly increased microbial utilization of carbohydrates, carboxylic acids and amino acids in 2012 and 2013 (Tables 1 & 3, P < 0.001). Nitrogen addition slightly increased microbial respiration, but was not significant (Tables 1 & 3, all P > 0.05), nitrogen addition also exerted no significant impacts on all of the six carbon groups (Tables 1 & 3, all P > 0.05) Water and nitrogen addition had no synergistic effects on microbial respiration and carbon utilization of six groups (Tables 1 & 3, all P > 0.05). Besides, water addition significantly increased microbial respiratory quotient by 42.4% across three years (Tables 1 & 3, P < 0.001). Nitrogen addition slightly increased microbial respiratory quotient but not significant (Tables 1 & 3, P > 0.05). Water plus nitrogen addition exert no interactive effects on microbial respiratory quotient.

3.5. Correlation of microbial respiration and soil respiration with microbial biomass, microbial carbon utilization profile and community composition

Microbial respiration was related to microbial carbon utilization profiles (Table 4, $R^2 = 0.49$, P = 0.049) and independent of microbial community composition ($R^2 = 0.31$, P = 0.188). Soil respiration was positively related to microbial biomass (r = 0.94, P < 0.001). Soil respiration was correlated with microbial carbon utilization profiles (Table 4, $R^2 = 0.85$, P = 0.002), while independent of microbial community composition (Table 4, $R^2 = 0.25$, P = 0.274). Moreover, soil respiration was positively related to the microbial respiration (r = 0.71, P = 0.009).

4. Discussion

Soil microbial communities are a soil carbon sink, although it only represents 0.6–1.1% of the total soil organic carbon across different biomes (Fierer et al., 2009). Soil microbial community can significantly affect soil carbon dynamics through microbial decomposition (Schimel et al., 2007). However, elucidating microbial responses to climate change and the subsequent consequences to soil carbon emissions has been challenging, since the literatures often report inconsistent responses (Balser et al., 2010). This study showed that the simulated 30% increase in annual precipitation significantly increased total microbial, fungal and bacterial biomass, microbial respiration and microbial carbon utilization of carbohydrates, carboxylic acids and amino acids, and soil respiration; in contrast, it had no profound effects on the ratio of fungal to bacterial PLFAs and microbial community structure. 5 gN m^{-2} $year^{-1}$ addition significantly increased bacterial PLFAs, and slightly increased fungal PLFAs, microbial respiration and soil respiration. Similar to water addition treatment, N addition also had no significant impacts on the ratio of fungal to bacterial PLFAs and microbial community structure. Water plus nitrogen addition had no interactive effects on microbial communities and soil respiration.

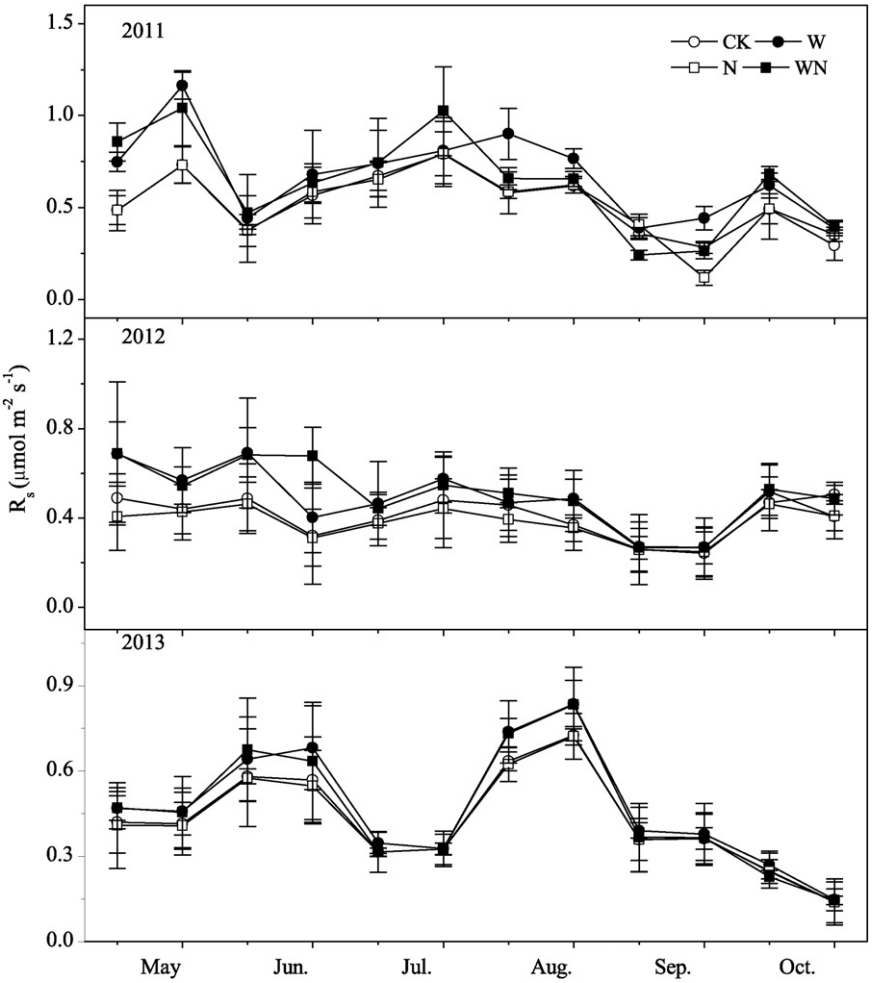

Fig. 2. Soil respiration (R_s) dynamics in control (CK), water addition (W), nitrogen addition (N) and water plus nitrogen addition (WN) plots in 2011–2013.

4.1. Promoted microbial growth and respiration are responsible for soil C emission under water addition

Desert soils are characterized with intermittent wet occasions from sporadic precipitation inputs. Soil microbial organisms become dormant or even dead under drought, and recover their physiological activity with available water (Schimel et al., 2007). Previous studies on the relationships of soil microbial organisms with soil moisture usually focused on the dry–wet cycles, few have investigated the effects of long-term variations of water availability on microbial organisms (Schimel et al., 2007). The 30% increases in annual precipitation significantly increased soil total microbial, fungal and bacterial PLFAs in this study, suggesting a profound stimulation of microbial biomass of both fungi and bacteria. This result is consistent with studies conducted in arid and semiarid regions, showing that increasing water availability could promote microbial growth (Austin et al., 2004; Manzoni et al., 2012a; Zhang et al., 2013). Soil fungi have long been considered to be more drought-tolerant than soil bacteria, and less sensitive to increasing water availability (Sommers et al., 1980; Freckman, 1986). However, our study showed that the biomass of soil fungi and bacteria were both stimulated greatly by increased water availability, suggesting fungi and bacteria are equally water-limited in this desert soil (Manzoni et al., 2012a). Water addition can alleviate diffusive limitation of substrate to soil microbial organism, in the present study, water addition significantly increased soil dissolved organic carbon, which primarily resulted from the increased plant growth (Huang et al., 2015). Soil microbial biomass was positively correlated with

Table 1
Results (P-values) of repeated measure ANOVAs on the effects of year (Y), water addition (W), N addition (N), and their interactions on soil respiration (SR), total microbial PLFAs (total), bacterial PLFAs (bacteria), fungal PLFAs (fungi) and the ratio of fungal to bacterial PLFAs (F:B), microbial respiration (MR), microbial quotient (qCO_2), microbial utilization of carbohydrates (Ch), carboxylic acids (Ca), amino acids (Ac), polymers (Po), phenolic compounds (Pc), and amines (Am).

Treatment	SR	Total	Bacteria	Fungi	F:B	MR	qCO_2	Ch	Ca	Ac	Po	Pc	Am
Y	0.002	<0.001	<0.001	<0.001	0.152	0.002	0.001	<0.001	<0.001	<0.001	<0.001	0.301	0.001
W	0.010	<0.001	<0.001	<0.001	0.887	<0.001	0.014	<0.001	<0.001	<0.001	0.428	0.145	0.049
N	0.718	0.043	0.020	0.278	0.379	0.107	0.421	0.254	0.648	0.350	0.571	0.503	0.427
Y × W	0. 35	0.005	0.002	0.009	0.621	0.117	0.101	0.011	<0.001	0.463	0.482	0.472	0.522
Y × N	0.823	0.43	0.51	0.36	0.289	0.348	0.574	0.063	0.284	0.815	0.823	0.234	0.638
W× N	0.428	0.278	0.267	0.547	0.902	0.672	0.084	0.510	0.327	0.639	0.318	0.113	0.969
Y × W × N	0.384	<0.001	<0.001	0.001	0.087	0.589	0.197	0.551	0.487	0.002	0.961	0.294	0.523

Table 2
Effects of water and nitrogen addition on the PLFAs of the main microbial groups (means ± S.E., n = 6). *Bacteria* bacterial PLFAs, *Fungi* fungal PLFAs, *F:B* the ratio of fungi to bacteria PLFAs. Different letters within years represent significant differences between treatments at $P < 0.05$.

	Bacteria	Fungi	Total	F:B
2011				
CK	2.1 ± 0.7 a	0.6 ± 0.7 a	3.1 ± 1.2 a	0.2 ± 0.0 a
W	5.2 ± 1.2 b	1.5 ± 0.4 b	7.5 ± 1.7 b	0.3 ± 0.0 a
N	3.2 ± 0.5 c	0.8 ± 0.2 a	4.5 ± 0.7 c	0.2 ± 0.0 a
WN	4.8 ± 0.5 bc	1.3 ± 0.2 b	6.9 ± 0.8 bc	0.3 ± 0.0 a
2012				
CK	12.3 ± 1.0 a	5.2 ± 0.4 a	18.7 ± 1.5 a	0.42 ± 0.0 a
W	25.2 ± 1.2 b	10.9 ± 0.8 b	36.8 ± 1.6 b	0.46 ± 0.0 a
N	16.5 ± 0.5 b	7.3 ± 0.1 a	23.8 ± 0.08 c	0.49 ± 0.0 a
WN	14.8 ± 0.5 b	5.8 ± 0.4 a	19.7 ± 1.7 a	0.46 ± 0.0 a
2013				
CK	2.8 ± 0.3 a	1.2 ± 0.7 a	4.4 ± 0.5 a	0.41 ± 0.0 a
W	4.2 ± 0.1 b	1.8 ± 0.2 b	6.5 ± 0.2 b	0.44 ± 0.0 a
N	3.7 ± 0.7 c	1.5 ± 0.3 a	5.9 ± 1.0 c	0.42 ± 0.0 a
WN	2.8 ± 0.5 a	0.9 ± 0.2 a	4.2 ± 0.8 a	0.32 ± 0.0 a

soil dissolved organic carbon, demonstrating increased respiratory substrate is responsible for increased microbial biomass under water addition in the desert.

Water addition had no significant effects on the ratio of soil fungal to bacterial PLFAs. Moreover, NMDS analysis also showed that water addition did not significantly alter microbial community structure. Microbial community structure in response to water variability has been investigated in some previous studies, and results are controversial (Bell et al., 2009; Lamb et al., 2011; Cregger et al., 2012; Sorensen et al., 2013; Bell et al., 2014; Frossard et al., 2015). For instance, Zhang et al. (2013) investigated microbial community structure in the Inner Mongolia steppe under a 30% precipitation addition for 4 years, showing that microbial community structure as indicated by PLFAs was not altered by water addition. Bell et al. (2014) investigated microbial PLFAs under water addition in the Namib Desert, showing that the ratio of fungal to bacterial PLFAs was not altered in the first four years by water addition, however, in the fifth and sixth year, water addition in both summer and winter significantly altered F:B PLFAs, while water addition only in summer or winter did not affect the ratio of F:B PLFAs. Moreover, a 7-year water addition study from a cold desert showed that water addition only significant altered bacterial community structure in summer, while exerting no impacts on bacterial community structure in the winter, in contrast to bacteria, this study also demonstrated that water addition significantly altered fungal community structure in winter, while exerting no influences in the summer (Sorensen et al., 2013). The inconsistent responses of soil microbial community structure to water availability among studies suggest that the microbial response to climate change is highly dependent on background climatic variability and the specific soil and plant traits. Therefore, further studies are needed to elucidate microbial community composition in response to water availability in desert ecosystems.

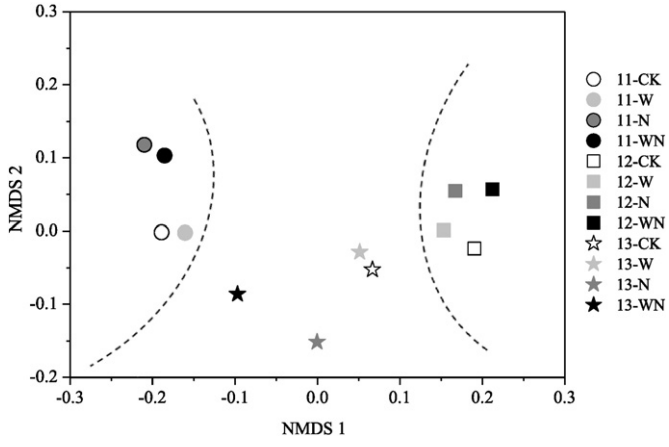

Fig. 3. Non-metric multidimensional scaling (NMDS) analysis on microbial community composition. The label represents Year-Treatment. 11, 12 and 13 represent measurements conducted in 2011, 2012 and 2013. CK, W, N and WN represent control, water addition, nitrogen addition and water plus nitrogen addition.

Soil microbial respiration was also significantly stimulated by water addition in our study. Soil microbial respiration was positively correlated with soil microbial biomass, suggesting increased microbial growth was responsible for the increased microbial respiration. Besides, microbial quotient was also significantly increased under water addition, this result indicates that increased mass specific physiological activity also contributes to increased soil microbial respiration under water addition. In contrast to microbial growth, microbial community as indicated by PLFAs showed no significant variation in response to water addition, this suggests increased microbial respiration was independent of microbial community structure under water addition. Soil respiration was profoundly stimulated by water addition, and it was positively correlated with microbial respiration, suggesting the direct contribution of microbial respiration to soil respiration under water addition. Moreover, water addition significantly increased microbial utilization of carbohydrates, carboxylic acids and amino acids, while having no significant effects on microbial utilization of polymers, phenolic compounds and amines. Carbohydrates, carboxylic acids and amino acids are more labile carbon in soil, the increased utilization of carbohydrates, carboxylic acids and amino acids suggests that water addition can increase labile carbon turnover in desert soil.

4.2. Limited effects of N addition on soil microbial communities and soil C emission

Nitrogen is considered to be the second limiting factor after water in desert ecosystem for plant growth. However, different from plants, soil microbial communities are not always constrained by nitrogen availability (Saito et al., 2007). Empirical studies on nitrogen addition effects on soil microbial communities have shown inconsistent results (Nohrstedt et al., 1989; Sinsabaugh and Linkins, 1989; LeBauer and Treseder, 2008; Lu et al., 2011; He et al., 2013). Nitrogen addition can significantly promote plant growth, which in turn leads to more substrate inputs and stimulate microbial growth. This is a widely accepted mechanism for the positive nitrogen addition effects on soil microbial communities (Lu et al., 2011). On the other hand, soil acidification by N addition can cause toxicity to microbes and thus decrease microbial activities (Edwards et al., 2011). However, among these studies, few have exclusively focused on desert ecosystems (Dennis et al., 2013; Ball and Virginia, 2014; Mueller et al., 2015; Wang et al., 2015) In our study, 5 gN m^{-2} $year^{-1}$ addition significantly increased bacterial PLFAs, while exerting no significant impacts on fungal PLFAs. Soil bacteria and fungi have contrasting stoichiometrical ratio of C:N, with the average ratio of soil bacterial C:N to be 4:1 and soil fungal C:N to be 10:1 across terrestrial ecosystems (Keiblinger et al., 2010), this suggests that soil bacterial community need more available nitrogen than fungal community for normal physiological activity, thus, nitrogen addition in our study only stimulated bacterial PLFAs and had limited stimulation on soil fungal PLFAs. Besides of the different stoichiometrical ratio of C:N between bacteria and fungi, carbon starvation may be another important factor affecting N addition effects on soil microbial communities in our study. Soil organic carbon content is 2.37 g kg^{-1}, and the ratio of soil carbon to nitrogen is 18.2 in our study site. Previous reports indicated that microorganisms may be restricted by carbon supply when the C:N ratio of soil is below 30:1 (Aber, 1992; Kaye and Hart, 1997), therefore, soil microbial biomass might be constrained by carbon availability in our study site.

Microbial community structure can significantly affect microbial respiration, because different microbial functional groups have contrasting

Table 3
Effects of water and nitrogen addition on the microbial respiration (MR) and metabolism of each guild (mean ± S.E., n = 6). Different small letters within a year represent significant differences between treatments at P < 0.05.

	MR	qCO_2	Carbohydrates	Carboxylic acids	Amino acids	Polymers	Phenolic compounds	Amines
2011								
CK	7.6 ± 0.9 a	27.3 ± 3.4 a	4.4 ± 0.1 a	2.4 ± 0.1 a	4.1 ± 0.3 a	4.5 ± 0.1 a	0.2 ± 0.1 a	0.1 ± 0.1 a
W	17.3 ± 0.6 b	37.5 ± 1.2 b	5.4 ± 0.1 b	2.7 ± 0.0 a	4.5 ± 0.0 a	5.4 ± 0.2 a	0.4 ± 0.1 a	0.3 ± 0.1 a
N	9.4 ± 0.3 a	31.7 ± 1.9 a	4.3 ± 0.1 a	2.5 ± 0.0 a	3.7 ± 0.0 a	4.4 ± 0.1 a	0.2 ± 0.1 a	0.2 ± 0.0 a
WN	17.6 ± 0.2 b	38.5 ± 1.5 b	4.9 ± 0.1 b	2.4 ± 0.1 a	4.2 ± 0.0 a	5.4 ± 0.2 a	0.4 ± 0.1 a	0.3 ± 0.0 a
2012								
CK	15.4 ± 0.3 a	22.5 ± 1.4 a	6.8 ± 0.0 a	5.3 ± 0.2 a	4.5 ± 0.0 a	5.5 ± 0.1 a	0.3 ± 0.0 a	0.1 ± 0.0 a
W	26.4 ± 0.9 b	31.7 ± 3.8 b	7.4 ± 0.0 b	6.4 ± 0.0 b	7.3 ± 0.2 b	6.2 ± 0.5 a	0.2 ± 0.0 a	0.2 ± 0.0 a
N	15.8 ± 0.6 a	23.3 ± 2.6 a	6.8 ± 0.0 a	5.2 ± 0.0 a	4.5 ± 0.0 a	5.6 ± 0.2 a	0.3 ± 0.0 a	0.2 ± 0.0 a
WN	28.1 ± 0.3 b	33.1 ± 4.2 b	7.3 ± 0.0 b	6.7 ± 0.1 b	7.0 ± 0.1 b	6.6 ± 0.1 a	0.2 ± 0.1 a	0.2 ± 0.0 a
2013								
CK	9.6 ± 0.3 a	20.6 ± 0.7 a	5.9 ± 0.1 a	5.1 ± 0.2 a	4.3 ± 0.1 a	5.4 ± 0.1 a	0.3 ± 0.0 a	0.3 ± 0.0 a
W	20 ± 0.3 b	30.6 ± 2.2 b	7.1 ± 0.2 b	6.3 ± 0.3 b	6.7 ± 0.3 b	5.8 ± 0.4 a	0.4 ± 0.1 a	0.4 ± 0.1 a
N	10.7 ± 0.3 a	20.5 ± 2.1 a	5.7 ± 0.3 a	5.0 ± 0.0 a	4.6 ± 0.2 a	5.3 ± 0.0 a	0.3 ± 0.1 a	0.3 ± 0.0 a
WN	21.4 ± 1.0 b	38.4 ± 1.9 b	7.0 ± 0.1 b	6.1 ± 0.2 b	6.2 ± 0.1 b	5.9 ± 0.3 a	0.3 ± 0.1 a	0.5 ± 0.1 a

carbon use efficiency and preference for carbon type (Fierer et al., 2009; Keiblinger et al., 2010). For instance, soil bacteria have a lower carbon use efficiency that fungi, and thus more CO_2 is released into atmosphere in bacteria dominated communities (Keiblinger et al., 2010). However, few studies have linked microbial community alteration with soil carbon dynamics under nitrogen addition in desert ecosystems. For instance, Wang et al. (2015) investigated soil microbial community responses to a 7-year nitrogen addition of 3.5, 7 and 14 gN m^{-2} $year^{-1}$ in the Tengger Desert using pyrosequencing data, results showed that bacterial diversity decreased linearly with nitrogen addition, while fungal diversity was highest in the 3.5 gN m^{-2} $year^{-1}$ addition treatment in lichen-dominated crusts. In contrast, Mueller et al. (2015) investigated soil microbial community using high-throughput sequencing of ribosomal RNA genes under N addition of 0.7 and 1.5 gN m^{-2} $year^{-1}$ in the southern Neveda, results showed that nitrogen addition significantly decreased soil bacterial diversity while exerting no impacts on fungal diversity. In our study, 5 gN m^{-2} $year^{-1}$ addition had no significant impacts on soil microbial community structure represented by PLFAs. One reason for the neutral microbial response is the lower resolution of PLFAs as compared with the molecular methodology (Saito et al., 2007). In our study, the ratio of fungal to bacterial PLFAs was 0.23 under control, and nitrogen addition increased bacterial PLFAs, suggesting microbial respiration would be more dominated by the bacterial communities under nitrogen addition. Given the lower carbon use efficiency of soil bacteria, soil carbon emission would be increased under nitrogen addition in this desert ecosystem.

Table 4
Correlations of microbial community composition and microbial carbon source utilization profiles with microbial variables.

Factor	R^2	P value	Factor	R^2	P value
Correlations with microbial community composition			Correlations with microbial carbon source utilization profiles		
SR	0.25	0.274	SR	0.85	0.002
MR	0.31	0.188	MR	0.49	0.049
			Bacteria	0.77	0.002
			Fungi	0.75	0.002
			F:B	0.26	0.241

Microbial community composition was evaluated using phospholipid fatty acid (PLFA) analysis, and 26 PLFAs persistently occurred in all four treatments were used in the analysis. Microbial carbon source utilization profiles was measured by BIOLOG EcoPlates, and 31 carbon sources were used in analysis. *SR* soil respiration, *MR* microbial respiration. *Bacteria* bacterial PLFAs, *Fungi* fungal PLFAs, *F:B* the ratio of fungi to bacteria PLFAs.

4.3. No synergistic effects of water and nitrogen addition on soil microbial communities and C emission

Water and N addition in combination did not generate synergistic effects on soil microbial communities in this study. This may be caused by two reasons. First, water addition can increase N mineralization and N flow in dry soils, which can compensate microbial requirement for N under increasing water availability (Austin et al., 2004). Second, water addition significantly increased soil dissolved organic carbon content, dissolved organic carbon is generally considered to be labile carbon and is more easily to be decomposed, suggesting a low nitrogen demand, thus nitrogen addition may have limited role in dissolved organic carbon decomposition under water addition. The no synergistic effects of water and nitrogen addition on soil microbial communities and soil respiration in the present study suggests that water availability is the most important factor controlling microbial communities and soil carbon emission, nitrogen deposition effects on soil microbial communities would be limited in the scenario of increasing precipitation in this desert ecosystem.

5. Conclusions

This study showed that the 30% increase in precipitation significantly stimulated soil respiration, microbial biomass, microbial respiration, microbial metabolic quotient and microbial utilization of carbohydrates, carboxylic acids and amino acids, while exerting no profound impacts on soil microbial community composition. Nitrogen addition only significantly increased bacterial PLFAs, while had no significant impacts on fungal PLFAs and microbial community structure, and this may be due to the contrasting stoichiometrical ratios of soil bacteria and fungi. Water and nitrogen addition together did not have synergistic effects on soil microbial communities and soil respiration. Our results demonstrated a 30% increase in precipitation can promote soil CO_2 emission primarily from labile carbon decomposition, and the effects of 5 gN m^{-2} $year^{-1}$ addition on soil microbial respiration are limited.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.catena.2016.03.002.

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