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Response of soil microbial communities and nitrogen thresholds of *Bothriochloa ischaemum* to short-term nitrogen addition on the Loess Plateau

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ABSTRACT

China is becoming the world's third largest area of nitrogen (N) deposition, which is attracting increasing attention. Understanding how N affects soil microbial communities and determining the thresholds for the effect of N on microorganisms and ecosystems are critical. We investigated the changes to the characteristics of microbial communities after two years of adding N to soil with the perennial grass *Bothriochloa ischaemum*, simulating N deposition on the Loess Plateau, at four rates of N addition (0 (CK), 2.5 (N1), 5.0 (N2), and 10.0 (N3) g N m^{-2} y^{-1}) and a control BL (bare land without vegetation or N addition). Soil microbial biomass carbon (C) and N contents and microbial activity increased in N1. The lowest rate of N addition (N1) increased soil total, bacterial, fungal, and actinomycetic phospholipid fatty acid (PLFA) contents, but excessive N addition decreased bacterial and actinomycetic PLFA contents. N addition did not alter microbial-community structure. The effect of N addition on soil microbial properties was influenced by soil C content (SOC and DOC), increased the diversity and evenness of the microbial community and decreased the diversity of the bacterial community. Soil microbial biomass and activity increased in N1, which was beneficial to the stability of the soil ecosystem on the plateau and defined the threshold of N addition for microorganisms and the ecosystem. More attention should thus be paid to depositional level represented by N2 (5 g N m^{-2} y^{-1}), which might limit microbial communities. The microbial community was inhibited, the diversity decreased, and the ecological system was affected by the level represented over N2.

1. Introduction

Nitrogen (N) is an important limiting element in most terrestrial ecosystems, and increased N contents can increase food production, plant diversity and plant coverage in degraded areas and improve the function of ecosystems (Isbell et al., 2013). The burning of fossil fuels, the production and use of chemical fertilizers, and the influence of human activities and animal husbandry are increasing atmospheric N deposition (Holland et al., 1999). And soil N content is close to or even beyond the threshold of ecosystems in some areas, which is causing various serious ecological environmental problems such as a decrease in plant diversity (Clark and Tilman, 2008; Stevens et al., 2004) and soil acidification caused by changes to the physical and chemical environment (Phoenix et al., 2012). These issues have drawn the widespread attention of the scientific community and are being actively studied for their contributions to global climate change.

The response of microbial communities to N in terrestrial ecosystems is mainly influenced by the duration and content of N inputs and is weakly affected by N type and mode of application (Treseder, 2008). Changes to a microbial community can be obvious within the first five years of N addition (Treseder, 2008), and long-term N addition can reduce microbial biomass, inhibit respiration, and decrease microbial diversity (Janssens et al., 2010; Liu and Greaver, 2010; Zhong et al., 2015). The results of experimental short-term N addition, however, have not been inconsistent. Zhang et al. (2005) indicated that short-term N addition significantly increased grassland microbial biomass in the dry, hot valleys along the Jinsha River in China, and some studies have shown that short-term N addition can significantly increase soil respiration (Zong et al., 2013). A study in New Zealand indicated that N addition decreased microbial biomass (Sarathchandra et al., 2001), and Ramirez et al. (2012) reported that microbial biomass and respiration intensity decreased in a short-term N-addition incubation experiment. Johnson et al. (2005) reported that N addition did not change microbial biomass in Scotland. The response of microorganisms

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to N deposition in a region thus likely depends on soil N content and the rate and duration of N deposition. N content is relatively low in some areas, and an appropriate amount of N addition can mitigate N limitation in an ecosystem, increase the activity of microorganisms (Yao et al., 2014), and change community structure (Bai et al., 2010). N input beyond saturation will inhibit soil microorganisms. Analyzing the effect of N addition on microbial characteristics and understanding the threshold of the effect of N addition on microbial communities in terrestrial ecosystems are therefore very important.

Thresholds are critical values beyond which ecosystemic functions will change. When the contents of N deposition exceed the tolerance of a system, the function of the system will change in an unpredictable manner (Wei et al., 2013). The threshold of N addition that different ecosystems can sustain in different regions is not consistent. A farmland ecosystem had a threshold of 180 kg ha^{-1} y^{-1} (Zhong et al., 2015), a grassland in Inner Mongolia had a threshold of 56 kg ha^{-1} y^{-1} (Wei et al., 2013), and a forest in the USA had a threshold of 19 kg ha^{-1} y^{-1} (Fenn et al., 2010). The N-saturation concentrations that plants, soil microbes, and soil physical and chemical properties can endure are also inconsistent, even within the same ecosystem. For example, Wei et al. (2013) found that N-deposition rates > 112 kg ha^{-1} y^{-1} significantly decreased microbial biomass, while the two main functional communities of plant have inconsistent threshold (56 kg ha^{-1} y^{-1} for perennial bunch grass; no specific threshold for perennial rhizome grasses), and soil pH significantly changed at 56 kg ha^{-1} y^{-1}. Understanding the thresholds of different components in the ecosystem of the hilly-gully region of the Loess Plateau in China is extremely important, so comprehensively determining the threshold of N deposition for regional environmental protection and for developing policies and regulations that inhibit N deposition is necessary.

The impact of N on microorganisms can be divided into direct and indirect effects by soils and plants. The effects of N on microorganisms are likely associated with the supply of plant carbon (C) and productivity even though the forms of N added to the soil may differ (Mooshammer et al., 2014) and are also likely associated with the production and efficiency of enzymes that decompose organic matter. N can also decrease soil pH and thus has an indirect influence on soil microbial communities (Mooshammer et al., 2014; Vitousek et al., 1997) by inhibiting bacterial diversity (Zhang and Han, 2012), and directly lower the C:N ratio that can increase the relative abundance of fungi, and significantly decrease the bacteria: fungi ratio (Yevdokimov et al., 2008), thus changing the structure of microbial communities.

Soil erosion is a serious problem on the Loess Plateau, which is a typical ecologically fragile area in the country. Low effective N contents in the soil and serious soil and water losses make this area the most representative grassland ecosystem affected by N deposition. The amount of N deposition has recently increased dramatically, so conducting experiments to analyze the impact of N addition on microbial characteristics and to determine the N threshold for the ecosystem is particularly urgent. We therefore used an area of the plateau with native vegetation (*Bothriochloa ischaemum* (L.) Keng, a perennial grass) as experimental materials, testing various levels of N addition to simulate N deposition. Our aim was to determine the effect of short-term N addition on soil microbial activity and community structure, identify the N threshold and mechanism of N deposition on typical grassland soil microorganisms, and estimate the threshold for the ecosystem.

2. Material and methods

2.1. Site description

The experiment was conducted at the State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau in Yangling (34°27′N, 108°7′E; 530 m a.s.l.). This region is characterized by a temperate continental monsoon climate with a mean annual temperature of 13.2 °C and a mean annual precipitation of 674.3 mm. Homemade soil bins with a slope of 15° were used to simulate the natural slope where natural *B. ischaemum* communities live. The bins were 2.0 m long, 1.0 m wide, and 0.5 m deep. Seeds of *B. ischaemum* were acquired in autumn 2012 from the experimental fields at the Ansai Research Station (ARS) of the Chinese Academy of Sciences (36°51′30″N, 109°19′23″E; 1068–1309 m a.s.l.). The loessial soil used in our experiment was obtained from the upper 20 cm of an arable field at ARS. The soil had a bulk density of 1.2 g cm^{-3}, organic-matter content of 1.3 g kg^{-1}, and total N (TN) and phosphorus (TP) contents of 0.19 and 0.27 g kg^{-1}, respectively. Soil was added to the bins in 10-cm layers to a depth of 40 cm, with a bulk density of about 1.2 g m^{-3}. The soil was well watered before sowing to ensure seedling establishment. The seeds were sown at a density of 10 × 10 cm. Excess grass plants and weeds were manually removed during the experiment to restrict plants of the same size to one per hole.

2.2. Experimental design

The experiment had four levels of N addition, based on the global N sedimentation levels (Bobbink et al., 2010) and the amounts of N addition in experiments in China and other countries, and five treatments: bare land (BL) with no vegetation or N addition, CK (0 g N m^{-2} y^{-1}) with vegetation but no N addition, N1 with vegetation and 2.5 g N m^{-2} y^{-1}, N2 with vegetation and 5 g N m^{-2} y^{-1}, and N3 with vegetation and 10 g N m^{-2} y^{-1}. Three replicates of the five treatments received additional N in the form of urea ($CO(NH_2)_2$). The experiment ran for two years. The seed were sown in June 2013. N was applied in August 2013 (the amount of N for one year) and in May, June, July, and August in 2014 as a solution of urea in 1 l of deionized water (equivalent to the amount of N in a year divided into four applications; CK and BL received the same volume of water).

2.3. Soil sampling and analysis

Soil was sampled in September 2014. Soil cores (20 × 20 cm quadrat) were collected to a depth of 20 cm from six randomly selected locations in each bin and combined into one composite sample. This sample was sieved through a 2-mm mesh after the stones and roots were manually removed. The sieved samples were divided into two subsamples. One subsample was air-dried and then divided into two parts. One part was sieved through a 0.25-mm mesh for the determination of soil total organic C (SOC), TN, TP, nitrate N, and ammonium N contents, and the other part was sieved through a 1-mm mesh for the determination of pH and available P (aP) content. The other subsample was also further divided into two parts. One part was stored at 4 °C for measuring soil microbial biomass C (SMBC), soil microbial biomass N (SMBN), basal respiration (BR), and soil-induced respiration (SIR). The other part was stored at − 80 °C for the determination of phospholipid fatty acid (PLFA) contents.

The chemical and physical properties of the soil were determined using standard procedures. SOC was measured with the H_2SO_4-$K_2Cr_2O_7$ method. TN was measured using the Kjeldahl method (Bremner and Mulvaney, 1982). Soil TP was determined colorimetrically after digestion with H_2SO_4 and $HClO_4$ (Schade et al., 2003). Soil NH_4^+-N and NO_3^--N in filtered 2.0 mol l^{-1} extracts of fresh soil sample were measured with a flow injection autoanalyzer. Soil pH was determined in 1:2.5 (w:v) solutions. Soil aP was measured by molybdenum-antimony colorimetry with $Na(HCO_3)_2$ extracts. Dissolved nutrients were extracted with deionized water after shaking 1 h and then filtering through prewashed cellulose acetate filters (0.45 μm pore size). TDN (total dissolved N), DOC, N-NH_4^+, N-NO_3^- were measured. The DOC concentrations were determined using TOC analyzer (liqui TOC II, elementar, Germany). The TDN concentrations were determined using alkaline digestion-UV spectrophotometric method. Dissolved organic nitrogen (DON) was calculated as TDN- ($NH_4^+ + NO_3^-$). Table 1 shows the basic physical and chemical properties of the soil, And C:N is the

Table 1
Soil physicochemical properties in the treatments.

Treatments	TOC ($g\ kg^{-1}$)	TN ($g\ kg^{-1}$)	TP ($g\ kg^{-1}$)	NO_3^--N ($mg\ kg^{-1}$)	NH_4^+-N ($mg\ kg^{-1}$)	pH	aP ($mg\ kg^{-1}$)	DOC ($mg\ kg^{-1}$)	DON ($mg\ kg^{-1}$)	C:N
BL	1.58 ± 0.15a	0.22 ± 0.02a	0.42 ± 0.00a	3.07 ± 0.57a	9.59 ± 0.54b	8.68 ± 0.04a	4.09 ± 0.14c	33.16 ± 1.67a	0.75 ± 0.04ab	7.23 ± 0.79b
CK	1.75 ± 0.06ab	0.23 ± 0.02a	0.44 ± 0.02ab	2.67 ± 0.51a	5.18 ± 0.31a	8.69 ± 0.11a	3.01 ± 0.15b	42.67 ± 4.40b	0.77 ± 0.04b	7.50 ± 0.72b
N1	1.80 ± 0.05b	0.24 ± 0.01bc	0.45 ± 0.01bc	3.10 ± 0.53a	7.91 ± 2.50b	8.59 ± 0.01a	2.07 ± 0.28a	36.46 ± 2.04a	0.79 ± 0.06b	7.42 ± 0.40b
N2	1.75 ± 0.12ab	0.26 ± 0.01bc	0.46 ± 0.01bc	3.17 ± 0.99a	7.22 ± 1.27ab	8.56 ± 0.07a	2.07 ± 0.17a	34.24 ± 1.75a	0.68 ± 0.03a	6.61 ± 0.47ab
N3	1.61 ± 0.06ab	0.28 ± 0.04c	0.46 ± 0.01c	3.10 ± 0.17a	9.09 ± 1.08b	8.54 ± 0.09a	2.12 ± 0.21a	31.77 ± 3.05a	0.77 ± 0.05b	5.84 ± 0.78a

The results are reported as means ± standard deviations. Different letters in table indicate significantly different at P = 0.05.

ratio of SOC: TN of the soil. Soil microbial biomass was measured by chloroform fumigation, SMBC was determined using a liqui TOC analyzer, and SMBN was determined by ultraviolet spectrophotometric colorimetry.

Basal respiration (BR) and the induced respiration (SIR) were measured by an infrared gas analyzer by a method adapted from Hueso et al. (2011). The structures of the soil microbial communities were determined using a modified Blight-Dyer microbial PLFA method (Frostegard et al., 1993). Briefly, fatty acids were extracted from 3.0 g of lyophilized soil by a solution containing citrate buffer, chloroform, and methanol. The PLFAs were separated from neutral and glycolipid fatty acids by solid-phase-extraction chromatography. After mild alkaline methanolysis, the PLFAs were analyzed using a gas chromatograph (GC7890A, Agilent Technologies) equipped with MIDI Sherlock software (Microbial ID, Inc., Newark, USA). An external standard of 19:0 methyl ester was used for quantification, and the amounts were expressed as nmol g^{-1} for dry soil.

Specific PLFA signatures can serve as indicators of specific microbial groups: iso- and anteiso-branched fatty acids for Gram-positive bacteria (Zelles, 1999) and monounsaturated fatty acids for Gram-negative bacteria (Zelles, 1999). 12:1 w4c, 13:0 iso, 14:1 w5c, 15:1 iso w9c, 15:0 iso, 15:0 anteiso, 16:0 iso, 16:1 w7c, 17:1 iso w9c, 17:0 anteiso, 17:0 iso, 17:1 w8c, 17:0 cyclo w7c, 17:1 w5c, 18:1 w9c, 18:1 w7c, 19:anteiso, 20:1 w9c, 22:0 iso, 22:1 w9c, 22:1w6c, and 22:1w3c were used as indicators of bacteria. The lipid 18:2w6c indicated fungal PLFAs, and 10-methyl fatty acids indicated actinomycetic PLFAs (Zelles, 1999). The fungal:bacterial PLFA ratio was used as an index of the fungal:bacterial biomass ratio. Total PLFAs were obtained by summing the contents of all fatty acids detected in each sample.

2.4. Calculations and statistical analyses

Induced respiration (qCO_2), also known as metabolic quotient, relates mineralized C with microbial biomass, reflecting the effect of environmental factors and management measure on microbial C pool (Powlson, 1976):

$$qCO_2 = \text{BR:SMBC}$$

Microbial N-use efficiency (NUE) describes the partitioning of organic N taken up between growth and the release of inorganic N to the environment (that is, N mineralization). C-use efficiency (CUE) is the efficiency of conversion of organic matter to microbial biomass. The microbial-community CUE:NUE ratios were calculated as (Mooshammer et al., 2014):

$$\text{CUE: NUE} = B_{C:N}: R_{C:N}.$$

where $B_{C:N}$ is the C:N ratio of the microbial biomass and $R_{C:N}$ is the C:N ratio of the soil.

All data were analyzed by one-way ANOVAs. Duncan's test at a probability level of $P < 0.05$ was used to perform multiple comparisons. All statistical analyses were performed using SPSS 20.0. Differences were considered statistical significantly at $P < 0.05$. A redundancy analysis (RDA) and principal component analysis (PCA) were conducted using R software to analyze the response of microbial-community composition to the soil characteristics during N addition. Microbial-community compositions (PLFAs) were used as species data, and soil factors were used as environmental variables.

The diversities of the fatty acids were calculated using the Shannon richness index H:

$$H = -\sum_{i=1}^{n} Pi \ln Pi,$$

where Pi is the relative abundance of each fatty acid in the total PLFAs and n is the number of fatty acids detected. The equitability of the fatty acids was calculated with the Shannon evenness index E:

$$E = H/\ln(S),$$

Table 2
Microbial biomass, respiration strength, and qCO_2 of the soils in the treatments.

Treatments	SMBC ($mg\ kg^{-1}$)	SMBN ($mg\ kg^{-1}$)	BR ($mg\ kg^{-1}\ h^{-1}$)	SIR ($mg\ kg^{-1}\ h^{-1}$)	qCO_2 ($10^3\ h^{-1}$)	CUE:NUE
BL	94.22 ± 5.90a	2.23 ± 0.94a	0.30 ± 0.08a	3.22 ± 0.04ab	3.23 ± 0.66a	5.56 ± 0.17b
CK	157.36 ± 14.16c	3.87 ± 0.54b	0.62 ± 0.24b	3.57 ± 0.12ab	3.96 ± 1.46a	5.51 ± 0.51b
N1	206.10 ± 9.11d	5.37 ± 0.33c	0.69 ± 0.08b	3.64 ± 0.25b	3.36 ± 0.49a	5.19 ± 0.17ab
N2	146.85 ± 15.00c	5.02 ± 0.38c	1.00 ± 0.21c	3.52 ± 0.20b	6.80 ± 1.58b	4.43 ± 0.19a
N3	123.25 ± 4.07b	3.78 ± 0.40b	0.87 ± 0.04bc	3.10 ± 0.38a	7.10 ± 0.56b	5.64 ± 0.22b

The results are reported as means ± standard deviations. Different letters in table indicate significantly different at P = 0.05.

where S is the total number of fatty acids tested in the community (Shannon, 1948). Pearson correlation analysis was used to inspect the relationship between the environmental factors and the diversity indices.

3. Results

3.1. Soil microbial biomass and soil respiration

The average soil microbial biomasses and soil respiration for all treatments are summarized in Table 2. SMBC and SMBN were highest at the lowest level of N addition (N1) with the maximum value, but decreased at the higher levels. SMBC and SMBN were significantly higher in N1 than CK. SMBC was significantly lower in N3 than CK, and SMBN was similar in N3 and CK. SMBC and SMBN were significantly higher in the N-addition treatments than in BL.

The rate of BR first increased and then decreased as the amount of added N increased and was highest in N2. BR was significantly higher in N2 than in the low-N treatments (CK and N1) and did not differ significantly between N2 and N3. BR was significantly higher in the N-addition treatments than in BL. The range of SIR was not large. SIR was significantly higher in N1 and N2 than N3, and the rest of the treatments had not reached significant level. qCO_2 did not differ significantly between BL, CK, and N1 but was significantly lower in those treatments than in N2 and N3. CUE:NUE tended to decrease before increasing as the amount of added N increased (Table 2) and was highest in N2. CUE:NUE did not differ significantly between N1 and but was significantly lower in those treatments than in BL, CK, and N3.

3.2. Microbial-community structure and diversity index

Total PLFAs increased with N addition, differing significantly from BL. Total PLFAs increased at the lowest level of N addition (N1) but decreased at the higher levels (N2 and N3) and was highest in N1. Total PLFA content was significantly higher in N1 than CK, N2, and N3 (Fig. 1A). The change of bacterial, fungal, actinomycetic PLFAs had patterns similar to that of total PLFAs, increasing at a low level of N addition and decreasing at higher levels. Bacterial and actinomycetic PLFAs were highest in N1 and differed significantly from those in the other treatments. Fungal PLFA also peaked in N1 but did not differ significantly from CK and N2, and was significantly lower in N3 than N1 and N2 (Fig. 1B, C, D). The bacterial, fungal, and actinomycetic PLFAs were generally significantly higher in CK, N1, and N2 than in BL. Bacterial and fungal PLFAs did not differ significantly between N3 and BL, and actinomycetic PLFA was significantly lower in N3 than BL. Fungal:bacterial PLFAs did not differ significantly among the N-addition treatments and tended to be lower in BL, but the difference was not significant (Fig. 1E).

Microbial-community H_{PLFA} differed significantly among the N-addition treatments, but N addition did not have a significant impact on microbial-community E_{PLFA} (Table 3). Microbial-community H_{PLFA} was highest in CK and N1 and decreased at the higher levels of N addition, with the lowest value in N3. Microbial-community H_{PLFA} in BL did not differ significantly from that in the other treatments. The changes in bacterial-community H_{PLFA} as the amount of added N increased had a similar pattern as the bacterial-community E_{PLFA}, increasing with a maximum in N3.

Microbial-community H_{PLFA} was significantly positively correlated with SOC and C:N (Table 4). Microbial-community E_{PLFA} was significantly positively correlated with SOC. Bacterial-community H_{PLFA} and E_{PLFA} were significantly correlated negatively with SOC, DOC and C:N, and positively with TN. Bacterial-community E_{PLFA} was also significantly negatively correlated with DOC and C:N.

3.3. Factors driving the structure of the soil microbial communities

The results of a principal component analysis (PCA) of the PLFAs of the microbial community in the N-addition treatments are shown in Fig. 2. The first axis of the PCA (PC1) contributed 34.36% of the variation in the PLFA data, PC2 accounted for 23.15% of the variation, and the cumulative contribution rate was 57.51%. PCs 1 and 2 could distinguish between the N-addition treatments. N1 and N2 were to the right of PC1, and CK and N3 were to the left of PC1. The four N-addition treatments were widely distributed, indicating that N addition changed the structure of the microbial communities.

The RDA ordination plot (Fig. 3.) shows the relationships between the environmental factors and microbial communities, including the soil properties SOC, TN, TP, NH_4^+-N, NO_3^--N, pH, aP, and C:N and the above- and below-ground biomasses of *B. ischaemum*. The first and second axes explained 57.33 and 17.75% of the variation, respectively. SOC and DOC (long arrows) were significantly correlated with the microbial community and had a positive role in increasing the PLFA contents and SMBC (P = 0.022 and P = 0.036, respectively). TN was more strongly correlated with the indices of bacterial diversity.

3.4. Threshold of effect on soil, microbial community, and ecosystem

The selection of SOC, TN, TP, NH_4^+-N, NO_3^--N, aP, DOC, DON and pH as soil factors depended on the PCA conducted, another PCA was also conducted based on the microbial indices, including BR, SIR, SMBC, SMBN, bacterial PLFAs, fungal PLFAs, and actinomycetic PLFAs. The third PCA was conducted on the integration of the soil factors and microbial indices. The eigenvalues, contribution rates, and cumulative contribution rates of these PCAs are shown in Table 5. The eigenvalues of PCs 1, 2 and 3 for each analysis were > 1, and the cumulative contribution rates of PCs 1, 2 and 3 for soil, microbial community, and ecosystem were 95.617, 96.290, and 100.00%, respectively, illustrating that most of the information system could be summarized by retaining the first two or three factors.

The score matrix and the contribution rate of each PC, score, and ranking of soil, microbial community, and ecosystem were obtained by counting the standardized original variables (Table 6). The soil ranking was N3 > N2 > N1 > CK; the microbial-community ranking was N1 > N2 > CK > N3; and the ecosystemic ranking was N1 > N2 > CK > N3, with the pattern of change the same as for the microbial community.

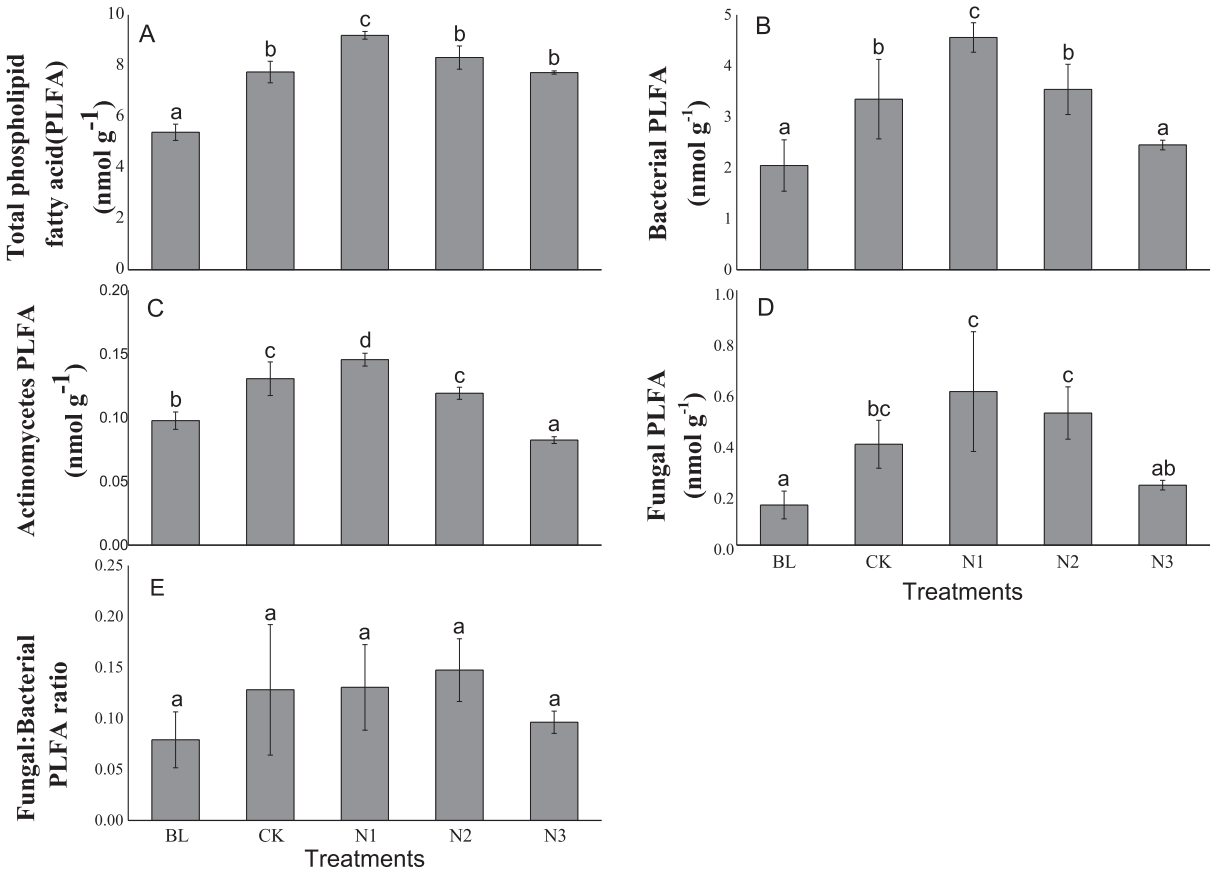

Fig. 1. Phospholipid fatty acid (PLFA) contents in the treatments (BL, CK, N1, N2, N3). Error bars are SE (n = 3). Different letters above bars indicate significantly different at P = 0.05.

Table 3
Changes in Shannon richness (H_{PLFA}) and Shannon evenness (E_{PLFA}) in the treatments.

Treatments	Microbial-community H_{PLFA}	Microbial-community E_{PLFA}	Bacterial-community H_{PLFA}	Bacterial-community E_{PLFA}
BL	2.48 ± 0.13ab	0.82 ± 0.03a	0.64 ± 0.17a	0.26 ± 0.08a
CK	2.62 ± 0.09b	0.86 ± 0.02a	0.63 ± 0.12a	0.27 ± 0.05a
N1	2.62 ± 0.05b	0.84 ± 0.03a	0.64 ± 0.08a	0.27 ± 0.06a
N2	2.35 ± 0.36ab	0.89 ± 0.09a	0.74 ± 0.28a	0.44 ± 0.28a
N3	2.14 ± 0.14a	0.81 ± 0.08a	1.38 ± 0.09b	0.75 ± 0.06b

The results are reported as means ± standard deviations. Different letters in table indicate significantly different at P = 0.05.

4. Discussion

4.1. Effect of N addition on microbial biomass and activity

SMBC and BR first increased and then decreased in this short-term N-addition experiment, with SMBC highest in N1 and BR highest in N2. SMBC and BR were highest at different N-addition levels because BR represents only active microbial biomass C and SMBC represents both active and dormant microbial biomass C. The influence of short-term N addition on microbial biomass and activity were inconsistent. Xue et al. (2005) found that a certain level of N content would increase the quantity of soil microorganisms. Yuan et al. (2012) found that a low level of N addition increased SMBC in a seven-year experiment simulating N deposition, but moderate and high N inputs decreased the quantity of microorganisms, similar to the results of our study. Wallenstein et al. (2006) found that N decreased soil microbial biomass, especially fungal biomass. Tu et al. (2011) found that N contributed significantly to soil respiration in a short-term simulated N-deposition experiment. SMBC and SMBN in our study differed between BL and the treatments containing *B. ischaemum*, illustrating that root secretion and C input had a significant effect on SMBC and SMBN (Eilers et al., 2010; Shen et al., 2010).

This trend of SMBC and SMBN first increasing and then decreasing

Table 4
Correlation coefficients between soil properties and the microbial and bacterial diversity indices.

Treatment	TOC	TN	TP	NH_4^+-N	NO_3^--N	pH	aP	DOC	DON	C/N
Microbial community H_{PLFA}	0.574*	− 0.358	− 0.290	− 0.413	− 0.121	0.476	0.293	0.558	0.082	0.628*
Microbial community E_{PLFA}	0.531*	− 0.150	0.293	− 0.395	0.380	0.207	− 0.187	0.121	0.102	0.375
Bacterial community H_{PLFA}	− 0.522*	0.538*	0.404	0.354	− 0.014	− 0.437	− 0.374	− 0.626*	0.048	− 0.732**
Bacterial community E_{PLFA}	− 0.490	0.593*	0.440	0.339	− 0.040	− 0.548*	− 0.437	− 0.600*	0.064	− 0.764**

* in table indicate significantly different at P = 0.05. ** in table indicate significantly different at P = 0.01.

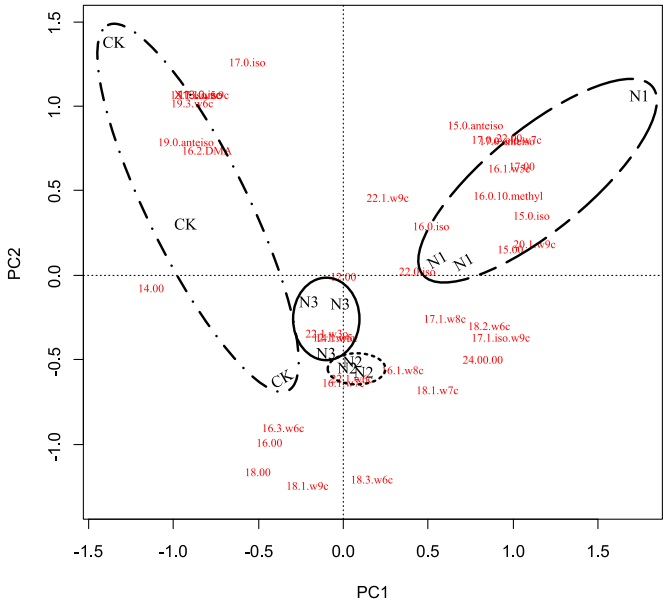

Fig. 2. Principal component analysis of PLFA composition in different N-addition treatments (CK, N1, N2, N3). CKs, N1s, N2s and N3s represent N-addition treatments with 3 replicates.

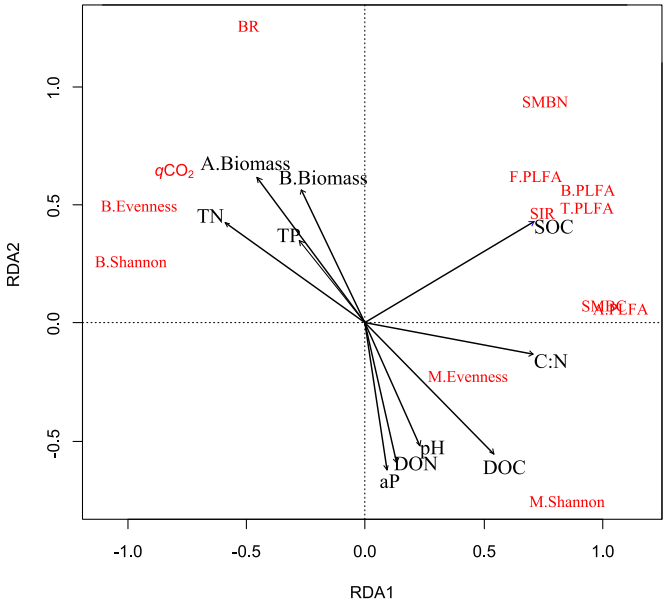

Fig. 3. Redundancy analysis of the soil microbial PLFAs and environmental variables in N-addition treatments (CK, N1, N2, N3). A. Biomass represents the aboveground biomass of *B. ischaemum*. B. Biomass represents the belowground biomass of *B. ischaemum*. T.PLFA represents total PLFA. B.PLFA represents bacterial PLFA. F.PLFA represents fungal PLFA. A.PLFA represents actinomycetic PLFA. M.Shannon represents microbial-community H_{PLFA}. M.Evenness represents microbial-community E_{PLFA}. B.Shannon represents bacterial-community H_{PLFA}. B.Evenness represents bacterial-community E_{PLFA}.

Table 6
Ranking of the principal components of the soil properties and microbial indices.

Treatments	Soil		Microbial community		Ecosystem	
	Score	Ranking	Score	Ranking	Score	Ranking
CK	−0.85	4	−0.27	3	0.62	3
N1	0.20	3	0.88	1	1.09	1
N2	0.27	2	0.35	2	0.01	2
N3	0.78	1	−0.96	4	−1.72	4

may have been due to the C contents of the soil (He et al., 2010; Xue et al., 2005). Soil organic C, TN, and TP contents are relatively low on the Loess Plateau (Liu, 2013). Short-term N addition can alleviate the limitation of soil N and can also increase belowground biomass and root secretion, thus increasing microbial biomass C and N. The increase in N content mitigated the N limitation of the soil. The plants, however, would decrease the allocation of underground resources by decreasing root growth and the release of active material (Liu et al., 2014), thereby inhibiting the growth of microorganisms, so SMBC and SMBN were lower in N2 and N3 than N1. Decreasing SMBC and increasing BR led to a lower CUE:NUE in N2 than the other treatments. These results indicated that the ability of the microbial community to mineralize was stronger in N2, which decreased CUE (Zhong et al., 2015) and which might be associated with the change in content of available N (Mooshammer et al., 2014; Zhong et al., 2015). Odum (1985) argued that microorganisms under environmental stress must divert some of the energy for maintaining growth and reproduction to compensate for the extra energy needed to cope with the environmental stress. The growth of microorganisms was inhibited in our study as the amount of added N increased. The microorganisms in this soil environment had to expend extra energy to maintain their normal lives, so qCO_2 increased in N2 and N3.

4.2. Effect of N addition on microbial-community structure and diversity

Total, bacterial, fungal, and actinomycetic PLFAs had the same patterns of change of SMBC and SMBN. All types of PLFAs firstly increased and then decreased, and contents were highest in N1. Experiments of the effect of N addition on microbial communities have produced inconsistent results. Ramirez et al. (2012) found that short-term N addition mainly increased the quantity of actinomycetes in an incubation experiment in North America where the contents of soil C and N were relatively low. Li et al. (2015) found that N addition in a three-year experiment did not change total PLFAs and had no significant effect on fungi but decreased the quantity of bacteria. Nilsson et al. (2007) also found that N deposition did not have a significant effect on fungi in an oak forest. Habitats on the Loess Plateau are fragile, and the soil is poor and limited by N (Wang et al., 2004), so the short-term N addition in our study alleviated the N limitation. Vegetation biomass and litter increased, leading to an increase in soil organic matter, DOC and an increase in PLFAs. N addition may have lowered the soil pH, which may have inhibited the production of microbial biomass or changed of the structure of the microbial

Table 5
Eigenvalue, contribution rate, and cumulative contribution rate.

Component	Soil			Microbial community			Ecosystem		
	Eigenvalue	Contribution rate (%)	Cumulative contribution rate (%)	Eigenvalue	Contribution rate (%)	Cumulative contribution rate (%)	Eigenvalue	Contribution rate (%)	Cumulative contribution rate (%)
1	6.679	74.208	74.208	5.428	77.538	77.538	10.334	57.413	57.413
2	1.927	21.408	95.617	1.313	18.752	96.290	6.532	36.291	93.703
3	0.394	4.383	100.000	0.260	3.710	100.000	1.133	6.297	100.000

communities (Smolander et al., 1994).

Fungal: bacterial PLFA did not generally differ significantly among the N-addition treatments. Microbial-community H_{PLFA} was significantly lower in these treatments than CK, but bacterial-community H_{PLFA} and E_{PLFA} were higher in N3. Microbial-community H_{PLFA} was significantly lower in N3, due to the decrease in mycorrhizal fungi from the inhibition of microbial growth and lower plant belowground C allocation by the high level of N (Zhao et al., 2015). Bacterial-community H_{PLFA} and E_{PLFA} were negatively correlated with SOC, DOC and C:N, mainly because the C:N ratio was lower for bacteria than fungi. Excessive N addition can increase the effectiveness of soil N, leading to a decrease in C:N ratios, and bacteria are better able to take advantage of organic matter with low C:N ratios (Zhao et al., 2015) and can thus adapt better to soil environments with lower C:N ratios. Frey et al. (2004) found that long-term N addition decreased fungal biomass but did not obviously affect bacterial biomass, thereby significantly decreasing the fungal:bacterial biomass ratio. Allison et al. (2008) reported that N addition did not change the fungal:bacterial biomass ratio, even though the structure of the fungal community changed. Freitag et al. (2005) found that N was not conducive to improving grassland soil microbial diversity. Yang et al. (2015) reported that soil bacterial H_{PLFA} and E_{PLFA} did not change significantly with N gradients. These inconsistent results may have been due to different N contents, soil types, climatic conditions, and sensitivities to N of the different regional ecosystems.

4.3. Implications of ecosystemic N thresholds

N saturation means that the supply of ammonium and nitrate N exceeds the critical concentration of total demand by plants and microorganisms, which is the threshold. A low amount of added N (N1) increased SMBC in this short-term experiment but higher amounts decreased it, with the threshold at 2.5 g N m^{-2} y^{-1}. N addition can change CUE:NUE ratios associated with changes to bacterial and fungal communities due to the different CUEs of fungi and bacteria (Keiblinger et al., 2010). N2 represented the threshold for microbial activity. The threshold of soil properties is inconsistent among studies, and a biomass threshold for *B. ischaemum* of 5 g N m^{-2} y^{-1} has been reported (Ai et al., 2017), which is lower than the plant-productivity threshold of 10.5 g N m^{-2} y^{-1} (Bai et al., 2010), consistent with the study by Wei CZ where plants, microorganisms, and soil had different thresholds.

The PCA of soil physical and chemical properties, plant biomass, and soil microbial indicators (Tables 5 and 6) indicated that N1 (2.5 g N m^{-2} y^{-1}) represented a threshold for soil microorganisms and the thresholds for the entire ecosystem, including soil, plant biomass, and soil microorganisms, which was lower than the previously reported threshold for plants, soil microorganisms, and soil pH of 56 kg ha^{-1} y^{-1} (Wei et al., 2013). The threshold in our study was also lower than the threshold of 180 kg ha^{-1} y^{-1} in a farmland ecosystem (Zhong et al., 2015) but similar to the threshold of 1.9 g N m^{-2} y^{-1} in European and American forests (Fenn et al., 2010).

The amount of N deposition in Shanxi Province has recently increased sharply, from 16.12 kg ha^{-1} in 2010 (Wei et al., 2010) to 28.89 kg ha^{-1} in 2014 (Liang et al., 2014) in the region of Yangling. The current amount of N deposition in this loessial hilly region is similar to or even larger than the threshold in this experiment (2.5 g N m^{-2} y^{-1}), which should be taken seriously by the government and researchers. N deposition > 5 g N m^{-2} y^{-1} will cause problems, such as decreasing the amount of microorganisms and destabilizing ecosystems.

5. Conclusion

N addition had a significant effects on soil microbial biomass, microbial activity and PLFAs reported in this study by soil carbon (SOC, DOC and C:N). The addition of 2.5 g N m^{-2} y^{-1} was the ecosystemic threshold. With the rapid increase in N deposition in Shaanxi Province, we should pay more attention to depositions of 5 g N m^{-2} y^{-1}, at which microbial biomass decreased, microbial activity was inhibited. Therefore, 5 g N m^{-2} y^{-1} in the background of global climate change can provide data for developing environmental policies, which are necessary and urgent. However, there are some disadvantages in this experiment. We should combine some field experiments which were completely close to natural soil conditions to support our results.

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