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Yating He[1,2]
Yuchun Qi[1]
Yunshe Dong[1]
Shengsheng Xiao[3]
Qin Peng[1]
Xinchao Liu[1,2]
Liangjie Sun[1,2]

[1]Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Science, Beijing, P. R. China
[2]Graduate University of Chinese Academy of Science, Beijing, P. R. China
[3]Jiangxi Provincial Research Institute for Soil and Water Conservation, Jiangxi, P. R. China

Research Article

Effects of Nitrogen Fertilization on Soil Microbial Biomass and Community Functional Diversity in Temperate Grassland in Inner Mongolia, China

Nitrogen (N) fertilization may profoundly affect soil microbial communities. In this study, a field fertilization experiment was conducted in temperate grassland in Inner Mongolia, China to examine the effect of N fertilization on soil microbial properties and the main factors related to the characteristics of soil microbial community. Soil microbial biomass carbon (MBC) and microbial functional diversity along an N gradient were measured over three months (June to August). The result showed that N fertilization significantly decreased MBC under high N treatment (N200, 200 kg N $ha^{-1} y^{-1}$) compared with the control (N0, 0 kg N $ha^{-1} y^{-1}$) in the three months. Microbial functional diversity in July and August were significantly increased by low N treatment (N50, 50 kg N $ha^{-1} y^{-1}$). Among the three fertilization treatments, microbial functional diversity under N200 in the three months was significantly lower than that of N50. The decrease of MBC and functional diversity under N200 were mainly due to the significant decline of plant belowground biomass under high N treatment. The increase of functional diversity under N50 treatment was due to the higher plant aboveground biomass as a result of the higher soil moisture availability. This finding highlighted that the higher N fertilization (N200) was not suitable for the growth and improvement of functional diversity of the soil microbial community, and that site and plant community play an important role in regulating the characteristics of soil microbial community.

Keywords: Biolog; Grassland; Nitrogen fertilization; Soil microbial biomass

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1 Introduction

Nitrogen (N) limitation is widespread in terrestrial ecosystems and atmospheric N deposition is a primary component of global change [1, 2]. External N addition and atmospheric N deposition has been found to affect the aboveground biology processes and the underground biochemistry of soil, both directly and indirectly [3, 4]. As an important component in regulating belowground ecological processes, the soil microbial populations are facilitators of nutrient availability, particularly soil N availability [5]. Thus, any changes in the availability of soil N may in turn, affect the soil microbial community, and hence obviates their role in the turnover of soil organic matter. Additionally, changes in soil microbial function and community composition may trigger a series of responses, such as impacting litter and organic matter decomposition rates [6], humus formation [7], nutrient transformation and cycling [8], and then alter the interaction between soil microbes and plant communities.

The soil microbial biomass (SMB) is regarded as one of the most sensitive indicators of ecosystem function although it is only a small part of soil organic carbon. Studies regarding the effects of N fertilization on SMB remain equivocal. For instance, Zhang et al. [9] have observed significant increase of SMB to two years N fertilization in deteriorated grassland in China, however, Sarathchandra et al. [10] have reported significant decrease of SMB in a perennial pasture of New Zealand due to two years N fertilization. Meanwhile, Johnson et al. [11] have found that two years application of N did not affect SMB in an upland grassland of Scotland. The mechanisms behind the variations may depend on other soil features, such as soil moisture, soil organic matter, total N, pH, the rate of N addition etc. [12–14], but the specific drivers are still not completely identified [15, 16].

Another key indicator of the health of the soil microbial community is the catabolic diversity as measured by Biolog analysis, which has been used to reflect the response of microbial communities to N fertilization [17]. Similar to findings of fertilization effects upon SMB, the response of microbial carbon utilization to fertilization also varied in different studies. Grayston et al. [18] have found N fertilization significantly improved the diversity of carbon substrate utilization of soil microbial communities in an upland grassland in England, while Johnson et al. [17] and Williams et al. [19] have reported a significant reduction in carbon utilization as a response to the N fertilization in the grasslands of Northern Wales and Scotland, respectively. These equivocal effects of N fertilization upon soil microbial carbon utilization indicated the necessity to

Correspondence: Y. Dong, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, 11A Datun road, Anwai, Beijing 100101, P. R. China
E-mail: dongys@igsnrr.ac.cn

Abbreviations: AWCD, average well color development; **CCA,** canonical correspondence analysis; **D,** Simpson index; **H,** Shannon–Weaver index; **MBC,** microbial biomass carbon; **SMB,** soil microbial biomass; **TN,** total nitrogen; **TOC,** total organic carbon

determine the influences behind soil microbial community response.

Thus far, a considerable amount of effort regarding N fertilization impacts on soil microbial communities have been conducted in North America and Europe [20–22], while there is little available information about China, and this is particularly the case in grassland ecosystems. In China, grasslands are the most widespread ecosystem type and occupy about 40% of the nation's total land area [23]. About 78% of China's grassland is located in the northern temperate arid and semiarid regions and its vegetation type is representative of Eurasian grasslands [24–26]. In addition, the temperate steppe in Inner Mongolia is the major base of livestock production in China. In past years, the steppe was exposed to overgrazing and autumn clipping, which has resulted in degradation of vegetation and soil N deficiencies [27, 28]. Coupled with changes in atmospheric N deposition, anthropogenic N inputs lend uncertainty to the future of this important ecosystem.

In order to better understand how N fertilization affects soil microbial communities and their functional diversity, an *in situ* N fertilization experiment in a typical semiarid temperate steppe in Inner Mongolia, China has been conducted since 2008. The main objectives of this study included: (1) to test if the soil microbial community was affected by two years of N fertilization; (2) to investigate the edaphic characteristics that influence the soil microbial community under the influence of additional nitrogen.

2 Materials and methods

2.1 Site description

The study site was located in the Inner Mongolia Grassland Ecosystem Research Station (IMGERS; Chinese Academy of Sciences) in the Xilin River Basin (43°33′N, 116°40′E, and 1265 m altitude), Inner Mongolia, China. The Xilin River Basin site is a member of the Chinese Terrestrial Ecosystem Flux Observational Network (ChinaFlux). The site has a typical moderate temperate monsoon climate with a mean annual temperature of 0.6°C and an annual precipitation of 341 mm, most (70%) occurring from July to September [29]. The soil is classified as dark chestnut soil (Chinese classification) and calcic-orthic Aridisol in the US soil taxonomy classification with a texture comprised of 60% sand, 21% clay, and 19% silt, respectively. The depth of the soil profiles range from 100 to 150 cm with a 20 to 30 cm humus layer. The grass community is dominated by *Leymus chinensis*, *Stipa grandis*, and *Agropyron michnoi.* The area was used as clipping pasture and, to our knowledge, has never received any anthropogenic N input prior to this experiment.

2.2 Experimental design and soil sampling

A 100 m × 100 m enclosure was established in 2008 in a flat and homogeneous area and a core area (76 m × 65 m) was selected in it for intensive sampling. Forty-two (10 m × 10 m) plots were arranged into seven rows and six columns following a completely randomized block design. All of the plots were separated by a 1 m-wide buffer zone. Except for the two southeastern corner plots, each plot was randomly assigned to any one of the following NH_4NO_3 treatment: (1) N0, 0 kg N ha^{-1} y^{-1}; (2) N50, 50 kg N ha^{-1} y^{-1}; (3) N100, 100 kg N ha^{-1} y^{-1}; (4) N200, 200 kg N ha^{-1} y^{-1}. The NH_4NO_3 solution was sprayed twice a year during the growing season, half in the late June or early July and the other half in the early August when the rainfall was most abundant in this area (8th July and 1st August in 2008, 29th June and 2nd August in 2009). The N0 plots received the same water treatments with other plots to minimize the effects of additional water on plant growth and microbial response. Among the forty fertilized plots, twelve plots with three replicates for each treatment (4 N treatment × three replicates) were randomly selected for the microbiological studies, and the other 12 plots (4 N treatment × three replicates) for the plant biomass studies.

Six soil samples (5 cm in diameter and 10 cm in depth) were randomly taken in each plot on 18 June, 11 July, and 16 August of 2009 and their positions were at least 1 m apart from the edge of the plot to avoid edge effects. Then the six soil samples were uniformly mixed to get a composite fresh sample for each plot. The three composite soil samples from the same N input level made up the three replicates of each treatment. After removing plant roots and large stones by sieving (2 mm), all the composite samples were stored in an icebox and transferred to laboratory. Each composite sample was then divided into three subsamples. One of them was stored at 4°C prior to the analysis for microbial community carbon sources utilization and microbial biomass, another one was air-dried and ground prior to the determination of total organic C (TOC), total N (TN) and soil pH, and the last one was stored at −20°C for mineral N concentration analysis and the soil moisture determination. Results are presented on per unit weight of oven-dry soil (105°C).

2.3 Microbial biomass

Microbial biomass was determined by the chloroform fumigation extraction method [30]. A 25-g fresh soil sample was fumigated with ethanol-free $CHCl_3$ for 24 h at 25°C. Then the fumigated and non-fumigated soil samples were extracted by shaking with 100 mL of 0.5 M K_2SO_4 for 30 min, and the extracts were vacuum filtered through 0.45-μm polycarbonate filter membrane. The amount of total organic C in the extracts was measured using an automatic total organic carbon analyzer (Elementar High TOCII, Germany). Microbial biomass carbon (MBC) was calculated from the differences between the amount of organic C extracted from fumigated and non-fumigated samples using conversion factor (K_{EC}) of 0.45.

2.4 Soil microbial community carbon sources utilization pattern

Soil microbial community carbon sources utilization were constructed using Biolog EcoPlates (Biolog, Hayward, CA, USA). Briefly, 10 g soil were suspended in 100 mL 0.145 M NaCl and then the soil suspensions were shaken at 200 rpm for 20 min on a reciprocal shaker. After setting for 30 min, the soil suspensions were diluted to 10^{-3} and a 150 μL aliquot of the soil solution was dispensed into each well of the Biolog EcoPlate using an 8-channel repeating pipette. The plates were then incubated at 25°C in the dark for 10 days. The color development was measured as absorbance at 590 nm every 24 h using the Microlog Rel 4.20 software. Microbial community carbon utilization activity was expressed as average well-color development (AWCD), calculated using the 120 h incubated data as follows [31]:

$$\mathrm{AWCD} = \sum_{i=1}^{n} \frac{(x_i - c)}{31} \quad (1)$$

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where x_i is the optical density value measured at 590 nm in substrate i in Ecoplates. c is the value measured in the control well, and 31 is the number of carbon sources.

The functional diversity as measured by the Shannon index (H) and the Simpson index (D), defining the species richness and the most common species of community, was calculated as follows [32]:

$$H = -\sum_{i=1}^{n} p_i(\ln p_i) \quad (2)$$

$$D = 1 - \sum_{i=1}^{n} (p_i)^2 \quad (3)$$

where p_i is the ratio of the corrected absorbance value of each well to the sum of absorbance value of all wells, n is the total number of carbon sources.

2.5 Soil properties analysis and root biomass

Soil pH was measured by glass electrode using a soil to water ratio of 1:2.5. The soil samples were oven dried for 24 h at 105°C to determine soil moisture. Soil total organic C and total N contents were determined by wet oxidation with $K_2Cr_2O_7$ and the semi-micro Kjeldahl methods using air-dried soil samples [33]. NO_3^-−N and NH_4^+−N content were measured by a continuous-flow ion auto-analyzer (AA3 Bran + Luebbe, Germany). Plant belowground biomass at 0–10 cm depth was measured by soil auger, and aboveground biomass was measured by clipping method.

2.6 Statistical analyses

The treatment effects on soil and microbial properties were tested by one-way ANOVA and the significances of differences between means of each treatment were judged by LSD test with spss17.0. To determine the key factors affecting microbial carbon utilization pattern, canonical correspondence analysis (CCA) was performed by Canoco version 4.5. Statistical significant differences were set up at $p < 0.05$.

3 Results

3.1 Soil nutrient contents and root biomass

During the three sampling months, N fertilization had no significant effects on soil TOC and TN concentrations, while it significantly increased soil NO_3^-−N and NH_4^+−N concentrations under N200 treatment compared with the control (N0) ($p < 0.05$; Table 1). Contrary to soil available N, a significantly decreased soil pH under N200 treatment was observed ($p < 0.05$). Meanwhile, N fertilization increased plant aboveground biomass, but significantly decreased plant belowground biomass under N200 treatment compared with the control ($p < 0.05$; Table 2).

3.2 Microbial biomass

Two years N fertilization led to considerable variation of SMB among treatments during the three sampling months (Table 1). MBC significantly decreased under N200 treatment in June and August and significantly decreased under both N100 and N200 treatments in July compared with the control ($p < 0.05$). Across the 3 months, MBC was

Table 1. SMB and soil physicochemical characteristics during the three sampling months (means ± SD, $n = 3$)

	18 June				11 July				16 August			
Soil factor	N0	N50	N100	N200	N0	N50	N100	N200	N0	N50	N100	N200
MBC (mg/kg)	$279.69^a \pm 74.73$	$147.33^{ab} \pm 36.10$	$150.44^{ab} \pm 36.57$	$118.43^b \pm 15.78$	$267.74^a \pm 13.16$	$233.26^{ab} \pm 20.16$	$171.37^b \pm 26.21$	$193.59^b \pm 13.25$	$288.70^a \pm 7.45$	$285.76^a \pm 25.19$	$317.73^a \pm 17.42$	$220.74^b \pm 11.49$
TOC (%)	$1.69^a \pm 0.31$	$1.49^a \pm 0.09$	$1.53^a \pm 0.14$	$1.64^a \pm 0.27$)	$1.90^a \pm 0.31$	$1.64^a \pm 0.16$	$1.48^a \pm 0.08$	$1.50^a \pm 0.11$	$1.56^a \pm 0.08$	$1.71^a \pm 0.17$	$1.67^a \pm 0.21$	$1.78^a \pm 0.23$
TN (%)	$0.19^a \pm 0.02$	$0.20^a \pm 0.01$	$0.19^a \pm 0.02$	$0.20^a \pm 0.01$)	$0.18^a \pm 0.01$	$0.19^a \pm 0.01$	$0.20^a \pm 0.03$	$0.18^a \pm 0.01$	$0.19^a \pm 0.01$	$0.20^a \pm 0.02$	$0.19^a \pm 0.02$	$0.24^a \pm 0.13$
NO_3^- − N (mg/kg)	$1.92^a \pm 0.19$	$4.77^b \pm 1.25$	$4.11^{ab} \pm 1.05$	$4.94^b \pm 1.36$)	$2.22^b \pm 1.08$	$4.38^b \pm 0.27$	$6.40^b \pm 1.91$	$23.40^a \pm 3.50$	$6.16^b \pm 0.47$	$14.89^b \pm 4.06$	$28.49^a \pm 5.07$	$49.44^a \pm 9.07$
NH_4^+ − N (mg/kg)	$2.35^a \pm 0.57$	$3.75^a \pm 1.07$	$2.45^a \pm 0.85$	$5.63^b \pm 0.51$)	$2.00^d \pm 0.39$	$7.78^c \pm 1.88$	$20.21^b \pm 1.33$	$36.51^a \pm 1.24$	$1.70^b \pm 0.96$	$4.45^b \pm 1.35$	$15.57^b \pm 5.83$	$36.28^a \pm 3.97$
pH (H_2O)	$8.39^a \pm 0.04$	$7.75^b \pm 0.05$	$7.63^c \pm 0.06$	$7.60^c \pm 0.03$)	$8.23^a \pm 0.05$	$8.18^{ab} \pm 0.10$	$8.17^{ab} \pm 0.02$	$8.08^b \pm 0.06$	$8.19^a \pm 0.03$	$8.25^a \pm 0.04$	$8.24^a \pm 0.04$	$8.15^b \pm 0.06$

N0, 0 kg N/ha/y; N50, 50 kg N/ha/y; N100, 100 kg N/ha/y; N200, 200 kg N/ha/y.
NO_3^- − N, soil NO_3^- content; NH_4^+ − N, soil NH_4^+ content; pH, soil pH value.
The same letters in each column are not significantly different at $p < 0.05$.

Table 2. Three months average plant biomass in the treatments (kg/ha) (means ± SD, $n = 9$)

	Treatment	Treatment	Treatment	Treatment
Plant biomass	N0	N50	N100	N200
Aboveground biomass	$173.46^{a} \pm 32.25$	$193.28^{a} \pm 37.83$	$189.13^{a} \pm 23.94$	$190.59^{a} \pm 46.97$
Belowground biomass	$1335.07^{a} \pm 121.98$	$1321.81^{ab} \pm 280.36$	$1339.18^{a} \pm 234.70$	$1108.28^{b} \pm 259.71$

See the footnote in Table 1 for the explanations for abbreviations.
The same letters in each column are not significantly different at $p < 0.05$.

positively correlated with TOC ($r = 0.54$, $p < 0.01$) and C/N ratio ($r = 0.47$, $p < 0.01$). Meanwhile, a positive correlated relationship was also found between MBC and plant belowground biomass ($r = 0.31$, $p < 0.05$).

3.3 Microbial community carbon sources utilization and functional diversity

N fertilization had no significant effect on microbial community carbon sources utilization, Shannon index (H) and Simpson index (D) in June, but AWCD in July and August were both significantly increased by N50 treatment compared with the control ($p < 0.05$). Among the three fertilization treatments, AWCD under N200 in the three months were all significantly lower than those of N50 ($p < 0.05$). The two functional diversity indices were significantly decreased by N100 treatment in July and significantly increased by N50 treatment in August ($p < 0.05$), respectively (Table 3). Across the three months, the Shannon index and the Simpson index were positively correlated with TOC ($r = 0.36$, $p < 0.05$; $r = 0.36$, $p < 0.05$) and C/N ratio ($r = 0.33$, $p < 0.05$; $r = 0.39$, $p < 0.05$), respectively.

CCA was performed to test the key soil factors, which might control the microbial carbon sources utilization in the three months (Fig. 1). CCA1 and CCA2 significantly correlated with the soil physico-chemical factors in the three months ($p < 0.001$) and they jointly explained 43.7, 61.1, and 56.8% of the variance in soil microbial carbon utilization patterns in June, July, and August, respectively. The microbial carbon sources utilization were mainly affected by TOC ($p < 0.05$), C/N ratio ($p < 0.05$), soil moisture ($p < 0.05$) and soil pH ($p < 0.001$) in June, and by soil moisture ($p < 0.001$), TN ($p < 0.01$) and C/N ratio ($p < 0.1$) in July, while microbial carbon sources utilization in August were under the influence of TOC ($p < 0.05$) and C/N ratio ($p < 0.05$).

4 Discussion

4.1 The effects of N addition on soil microbial biomass

N addition may result in the decreasing of MBC [10, 34], and may also cause a shift from N limiting to C limiting [17]. In our study, SMB had a negative response to the increased soil N, while a positive correlation between MBC and TOC as well as C/N ratio were observed.

Table 3. Functional diversity of soil microbial communities during the three months (means ± SD, $n = 3$)

	18 June				11 July				16 August			
Functional diversity	N0	N50	N100	N200	N0	N50	N100	N200	N0	N50	N100	N200
AWCD	$0.48^{ab} \pm 0.05$	$0.58^{a} \pm 0.08$	$0.43^{ab} \pm 0.02$	$0.40^{b} \pm 0.14$	$0.31^{b} \pm 0.04$	$0.46^{a} \pm 0.04$	$0.20^{c} \pm 0.03$	$0.21^{c} \pm 0.04$	$0.34^{b} \pm 0.01$	$0.49^{a} \pm 0.02$	$0.37^{b} \pm 0.04$	$0.30^{b} \pm 0.06$
H	$2.97^{a} \pm 0.09$	$3.06^{a} \pm 0.07$	$2.79^{a} \pm 0.12$	$2.86^{a} \pm 0.24$	$2.83^{a} \pm 0.04$	$2.76^{a} \pm 0.12$	$2.15^{b} \pm 0.18$	$2.65^{a} \pm 0.12$	$2.51^{b} \pm 0.20$	$2.93^{a} \pm 0.19$	$2.72^{ab} \pm 0.09$	$2.76^{ab} \pm 0.12$
D	$0.94^{a} \pm 0.01$	$0.95^{a} \pm 0.01$	$0.93^{a} \pm 0.01$	$0.93^{a} \pm 0.02$	$0.93^{a} \pm 0.01$	$0.93^{a} \pm 0.01$	$0.82^{b} \pm 0.04$	$0.91^{a} \pm 0.01$	$0.89^{b} \pm 0.02$	$0.94^{a} \pm 0.01$	$0.92^{ab} \pm 0.01$	$0.92^{ab} \pm 0.02$

The same letters in each column are not significantly different at $p < 0.05$.

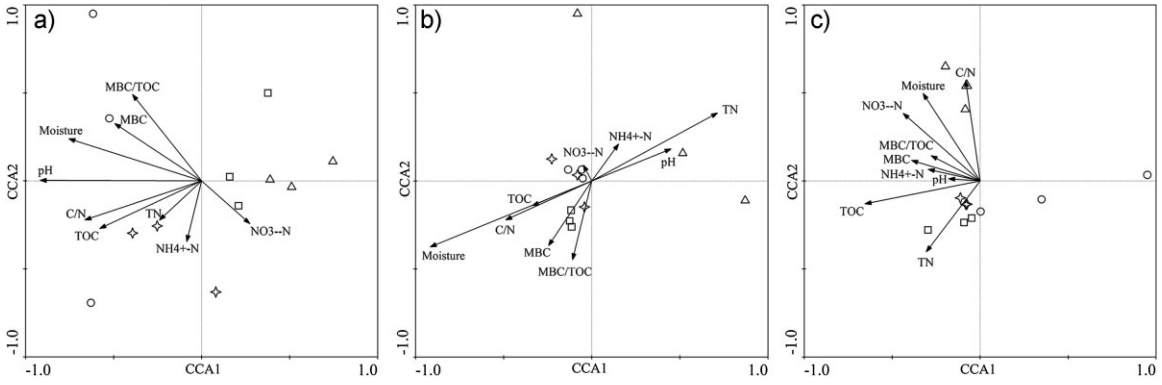

Figure 1. CCA ordination biplot of soil microbial carbon sources utilization with soil chemical variable in June (a), July (b) and August (c). N0 (0 kg N/ha/y, ○), N50 (50 kg N/ha/y, □), N100 (100 kg N/ha/y, △), N200 (200 kg N/ha/y, star). MBC, microbial biomass carbon; C/N, C/N ratio; Moisture, soil water content; pH: soil pH value; $NO_3^- - N$ and $NH_4^+ - N$, concentrations of soil inorganic N.

These results indicated that N alone was not a limiting factor in this study area, but accumulation of N may cause the soil microbial population to become C limited. Previous reports indicated that microorganisms may be restricted by C supply when the C/N ratio of soil is below 30:1 [35, 36]. In the present study, the grassland was used as a hay pasture for years and had never received any anthropogenic fertilizers before this experiment. Soil organic carbon content was rather low (<1.90%) and the C/N ratio varied between 7.1 and 10.3. Therefore, the SMB was evidently limited by C availability. However, one can see that no significant effects of N fertilization on soil organic carbon content over the three months were found under N200 treatment (Table 1), so the significant decrease of MBC under N200 treatment cannot be simply explained by the change of soil organic carbon. In present study, a positive correlated relationship between MBC and plant belowground biomass was found ($r = 0.31$, $p < 0.05$), and the plant belowground biomass was significantly decreased under high N treatment (N200), in conformity with the findings of Haynes and Gower [37] and Li et al. [38]. The lower plant belowground biomass under N200 treatment may reduce the amount of root exudates released to soil, which have important functions in promoting the growth of soil microorganisms [39], and consequently resulted in the decrease of MBC under high N fertilization rate. Our results were in agreement with previous studies in the grassland in the USA that higher N fertilization decreased MBC due to the reduced root exudation [40]. Another nitrogen addition research in a temperate grassland in China found the reductions in MBC under higher N addition (320 and 640 $kg\,N\,ha^{-1}\,y^{-1}$) are partly attributed to the deleterious effects of soil pH [41]. The lower N fertilization rates in our study may in part indicate differing causation for similar results in higher N fertilization studies; namely, the reduction of SMB.

4.2 The effects of N addition on soil microbial community carbon sources utilization pattern

The interactions between plant communities and soil microbial communities is well known, and plant productivity was believed to exert strong selective pressures on functional diversity of soil microbial communities [42, 43]. In the present study, AWCD under N200 treatment in the 3 months were all significantly lower than that of N50, which was partly in agreement with the response of MBC to N fertilization. The significant decline of AWCD under N200 treatment was mainly due to the lower plant belowground biomass under N200 treatment through decreasing root exudates in soil. In addition, soil $NO_3^- - N$ concentrations were significantly increased and the pH values were significantly decreased under N200 treatment in the 3 months. The associated soil chemical changes along with the change of pH may also be important factors controlling soil microbial communities [44].

Meanwhile, AWCD significantly increased by N50 treatment compared with the control (N0) in July and August. The exact reason for the increase has not yet been identified. The possible mechanisms could be as follows: Firstly, the importance of soil moisture in regulating microbial diversity is well known [45], and soil moisture was also an important limiting factor for plant aboveground biomass in the Inner Mongolia steppe [29]. In our study, we observed a significant relationship between soil moisture and AWCD (Fig. 1), but the higher plant aboveground biomass under N50 treatment was also observed (Table 2). Therefore, the increase of AWCD under N50 treatment may partly due to the higher plant aboveground biomass, which may return to the soil and provide as carbon sources for soil microbial communities. Second, in our canonical analysis, the soil microbial communities under N50 treatment were well separated from the control in July and August. These separations might have been associated with increases in utilization of specific type of carbon under N50 treatment.

In contrast to the responses of AWCD to nitrogen fertilization in July and in August, the Shannon index and the Simpson index were both significantly increased under N50 treatment in August and decreased by N100 treatment in July. Meanwhile, the two diversity indices were both positively correlated with TOC and C/N ratio. The Shannon index provides a way to quantify substrate utilization richness of the soil microbial community, while Simpson index provides information about the abundances of the most common species [46]. In our study, the TOC and C/N ratio of N100 treatment were relatively lower in July than the other nitrogen treatments. Besides, the lower belowground biomass under N100 treatment in July and the higher belowground biomass under N50 treatment in August were also observed. These results suggested that the functional diversity and the abundance of a few common species of soil microbial communities were regulated by the availability of soil organic carbon to a certain extent, and the balance between C and N is another important factor which accounting for the potential carbon utilization of soil microbial communities.

5 Conclusions

This study demonstrated that soil microbial community has a close relationship with plant communities. The decreases in MBC and microbial functional diversity under N200 were mainly due to the significant decline of plant belowground biomass under high N treatment. The increase of carbon utilization of soil microbial communities under N50 treatment was partly due to the higher plant aboveground biomass. The discrepancy between of the two functional diversity indices (Shannon index and Simpson index) was regulated by the availability of soil organic carbon to a certain extent and by the balance between C and N. This indicated that higher N level (N200) would not be suitable for the growth and functional diversity of soil microbial community in temperate grassland in Inner Mongolia, China, and moderate fertilization was a much better choice for grassland recovery and for maintaining high level soil microbial community in this N deficient ecosystem in China.

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