RESEARCH ARTICLE

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Seasonal precipitation and soil microbial community influence plant growth response to warming and N addition in a desert steppe

Yi Zhu · Kailiang Yu · Qian Wu · Xu Cheng · Zhiguo Li · Zhongwu Wang · Mengli Zhao · Andreas Wilkes · Ton Bisselling · Guodong Han · Haiyan Ren

Abstract

Aims Global environmental changes are known to affect terrestrial ecosystems functions (i.e., plant growth and carbon storage). However, how plants and soil microorganisms respond to warming and nitrogen deposition in dry ecosystems with strong seasonal precipitation remains largely unexplored.

Methods Based on a 13-year manipulative field experiment, we investigated the effects of warming and nitrogen addition on soil microbial communities and plant net photosynthetic rates during dry and wet months in a desert steppe in Inner Mongolia.

Results We found that in the wet month, warming and nitrogen addition significantly increased soil microbial biomass. Yet, warming and nitrogen addition significantly increased soil inorganic nitrogen and leaf nitrogen concentration, thus promoting an increase in the net photosynthetic rate of *Stipa breviflora*. Moreover, warming and nitrogen addition significantly shifted soil microbial composition with an increase in soil bacterial phospholipid fatty acids (PLFAs) but a reduction in fungal PLFAs. The increased soil inorganic nitrogen indirectly enhanced leaf nitrogen and plant photosynthesis by changing soil microbial community structure. These changes were not significant in the dry month.

Conclusions Our study indicates that warming and nitrogen addition can promote plant photosynthesis by increasing soil nitrogen availability and changing soil microbial community structure. These changes only occurred when there was sufficient precipitation. These results highlight the crucial role of the soil microbial community and precipitation availability in

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influencing plant growth responses to global change drivers such as warming and nitrogen deposition. These findings suggest the importance of manipulating multiple factors rather than single factors in global change experiments and the role of plant and soil microbial interactions in ecosystem functions (i.e., plant growth and carbon storage) under global change.

Introduction

The Earth's surface temperature has risen by 1.1 ℃ between 1850 and 2020. If temperature continues to rise at its current rate, the magnitude of global warming will exceed 1.5 ℃ and 2 ℃ in the mid-twenty-first century (IPCC 2021). Such unprecedented changes in temperature are predicted to affect soil microbial communities and plant growth with potential feedbacks to climate change (Lin et al. 2010; Nottingham et al. 2019). Since the Industrial Revolution, human beings have increased nitrogen (N) deposition from the atmosphere by applying chemical fertilizers and burning fossil fuels (Galloway et al. 2008), and N deposition is likely to at least double from their current values (10 kg N ha^{-1} yr^{-1}) by 2050 (Galloway et al. 2008; Phoenix et al. 2012). As N is one of the key limiting nutrients for terrestrial plant growth (LeBauer and Treseder 2008), increased N deposition has widespread ecological consequences, including effects on species composition, biodiversity and ecosystem functions (Stevens et al. 2004; Clark and Tilman 2008; Isbell et al. 2013).

Soil microbial communities, as the catalyst of soil nutrient cycling and transformation, are an important component of terrestrial ecosystems (Lucas et al. 2007; Stroud et al. 2007; Liang et al. 2011). Soil microorganisms not only play an important driving role in soil processes, but also are sensitive to biological indicators of climate change (Schindlbacher et al. 2011; Delgado-Baquerizo et al. 2017). Although many studies have shown that warming and N supply may have impacts on soil microbial communities, existing reports on the effects of warming and N deposition on soil microorganisms are not consistent, with reported increase (Gong et al. 2019) or decrease

(Li et al. 2010; Fu et al. 2012) in soil microbial biomass. Moreover, it has been reported that warming changes the structure of soil microbial communities (Sheik et al. 2011; Chen et al. 2021). A few studies have shown that warming reduced the fungi to bacteria (F/B) ratio because of carbon substrate depletion and increase in soil nutrients with a lower soil C/N ratio (Zhang et al. 2005; Melillo et al. 2017; Deng et al. 2018). However, it remains largely unclear how changes in soil nutrients and soil microbial composition under warming affect plant growth, and thus our capability of predicting plant growth response to global warming still remain highly uncertain.

The effects of N addition on increased soil nutrient availability have been demonstrated (Lu et al. 2011; Ren et al. 2018), and reports indicate that the responses of soil microbial communities are ecosystem specific (Lo Cascio et al. 2021). For example, studies have reported increase of soil microbial biomass in desert (Liu and Greaver 2010) and meadow steppe (Gong et al. 2019) and decrease in forests (Liu and Greaver 2010) and semi-arid grassland (Li et al. 2010). Most studies suggest that N addition would also significantly change the structure of the soil microbial community (Clegg et al. 2003; Griepentrog et al. 2015), with either an increase in the F/B ratio (Chen et al. 2019) or non-significant change (Huang et al. 2015). Moreover, it has been reported that warming and N addition have interactive effects on the soil F/B ratio (Gutknecht et al. 2012), or have additive effects on the taxonomic composition and diversity of the soil microbial community (Adair et al. 2019). The increase in soil nutrients due to N addition and warming could directly increase plant growth by increasing nitrogen mineralization and nutrient uptake by roots, but soil microorganisms also compete for soil nutrients (Sorensen et al. 2008; Ma et al. 2011). Indeed, the positive effects of increase in soil nutrient availability on plant growth could be offset or even outweighed by the increased nutrient demand and competition from soil microbial community (Dunn et al. 2006; Dijkstra et al. 2015). Thus, whether the impacts of warming and N addition on plant growth are regulated by soil microbial community structure (such as the soil F/B ratio) and how it contributes to a net effect on plant growth response remain to be understood.

Water is a limiting factor in the desert steppe of northern China (Liu et al. 2009). Differences in

hydrological conditions affect the response of plants to global change drivers. For instance, Ren et al. (2018) found that plant nutrient resorption efficiency had different responses to warming and N addition under different water availability conditions. Yang et al. (2011a) reported that N addition and increased precipitation changed the structure and composition of the plant community. Warming has been found to interact with fluctuation in water availability and to affect the soil microbial community in arid and semi-arid ecosystems (Allison and Treseder 2008; Liu et al. 2009; Nielsen and Ball 2015). Water stress usually causes adverse growth conditions for the soil microbial community and thus inhibits the activities of most microorganisms, which may lead to a lack of response of microbial communities to warming in dry conditions (Sheik et al. 2011). In addition, it has been demonstrated that the effect of N on soil microbial composition may also strongly depend on water status (Herman et al. 1993; Bi et al. 2012; Huang et al. 2015). However, the effects of global change, such as warming, increasing N deposition and precipitation, on plants and soil microorganisms and their potential interactions in the desert steppe are still unclear. Since global change involves multiple factors covarying or interacting with each other, global change experiments manipulating multiple factors would be needed to shed light on the overall response of ecosystem functions under global change (Song et al. 2019).

To investigate how soil microorganisms and plant growth change in the context of global change (i.e., warming and N deposition), and whether and how these changes vary with variation in water availability, we conducted an experiment using a 13-year warming and N addition platform in a *Stipa breviflora* desert steppe in northern China. In 2018, we sampled in two hydrologically contrasting months (i.e., dry in June and wet in July) to examine the characteristics of soil microorganisms and the dominant plant (*S. breviflora*) in response to warming and N deposition. Our experiment aimed to address three main questions. First, do warming and N addition increase soil microbial biomass and change its community structure? Second, are the effects of warming and N addition on plant and soil microorganism additive and regulated by seasonal precipitation? Third, what are the pathways by which warming and N addition affect plant photosynthesis? We hypothesized that 1)

warming and N addition could influence soil microbial biomass, the F/B ratio and nutrient availability, thus leading to higher plant photosynthetic rate; 2) these effects (or plant growth responses) depend on seasonal precipitation; and 3) the effects on plant growth of increased soil nutrients under warming and N addition are regulated by soil microbial community structure.

Material and methods

Study site and experimental design

The study was conducted at the site of a long-term warming and N addition experiment in a desert steppe at Siziwang Banner, Inner Mongolia, northern China (41°46′43.6″N, 111°53′41.7″E, at 1,456 m a.s.l.). Long-term mean annual precipitation in this area is 229 mm (2006−2018), 78% of which falls in June to September. Mean annual temperature was 3.9 ℃ in 2018, with monthly mean temperature of -18.3 ℃ in January, 19.7 ℃ in June and 21.3 ℃ in July. The soil in the study site has a sandy loam texture and is classified as Kastanozem based on the FAO (Food and Agriculture Organization of the United Nations) classification system. The perennial grass, *S. breviflora*, was the dominant species in our study site, and made up approximately 40% of aboveground plant biomass. Other species, such as *Cleistogenes songorica*, *Convolvulus ammannii* and *Artemisia Frigida,* account for about 60% of aboveground plant biomass. The growing season in this desert grassland runs from early April to late September.

In May 2006, the experiment was set up in a homogeneous and flat field using a split-plot design with warming as the main plot and N addition as the subplot. Six pairs of 3 m×4 m main plots were established and the distance between two main plots in each pair was 3 m. One main plot in each pair was assigned to the ambient treatment (W0, no warming) and the other to the warming treatment (W1). Each main plot was divided into two 3 m×2 m subplots, with one of the subplots randomly assigned to no N addition (N0, ambient N addition rate) and the other to N addition (N1). Thus, there were four treatments: no warming and no N addition as the control (C), warming without N addition (W), N addition without warming (N) and combined warming and N addition

(WN). Each treatment had six replicates, so there were 24 subplots in total (Figure S1). The warming plots were heated continuously for 24 h a day using MSR-2420 infrared radiators (Kalglo Electronics Inc. Bethlehem, PA, USA). The infrared radiators were positioned 2.25 m above the ground in each warming plot and set to a radiation output of about 2000 W. In each no-warming plot, a dummy radiator of the same shape and size was positioned at the same height to simulate the shading and other effects of the radiators. The surface soil temperature in the warming plots was 1.3 ℃ higher than in the control (Wang et al. 2015). Before a rainfall event in late June every year, NH_4NO_3 was dissolved in water and evenly sprayed into the N addition plot. At the same time, the same amount of water was sprayed into the no-N addition plot. The N addition rate was 10 g N m^{-2} yr^{-1}, which was chosen based on our goal to alleviate potential N limitation. This N addition rate is most commonly used amount in field experiment in temperate steppe in northern China (Bai et al. 2010).

We examined the responses of plant net photosynthesis and soil microbial community structure to warming and N addition during two (dry and wet) months in 2018. Total precipitation in June was only 6.3 mm, and total precipitation in July was 106.1 mm. Mean monthly temperature was 19.7 ℃ in June and 21.3 ℃ in July (Figure S2). This provided a unique opportunity to investigate whether and how the responses of plant productivity and soil microorganisms to warming and N addition are influenced by seasonal rainfall.

Field sampling and biogeochemistry measurements

We collected 0–10 cm mineral soil samples by taking three 3 cm diameter cores in each subplot on June 15, June 30, July 14 and July 30 in 2018. We then mixed the three soil samples from each subplot into one sample. The mixed soil sample was sieved (2 mm) to remove impurities such as stones and roots. These soil samples were brought back to the laboratory for inorganic N and microbiological analysis. Soil inorganic N (including NH_4^+ and NO_3^-) was measured using a continuous flow spectrophotometer (FIAstar 5000 Analyzer; Foss Tecator, Hillerod, Denmark). Soil pH was measured by PHS-3G digital pH meter (Precision and Scientific Crop., Shanghai, China). Soil temperature and moisture in the 0–10 cm layer

were continuously monitored by soil temperature and water content sensors (ECH2O-TE/TM, Decagon Devices Inc., USA) throughout the year (i.e., 2018).

Plant leaf N content and photosynthesis

On a sunny morning from 9:00 to 11:00 at the end of June and July 2018, three *S. breviflora* plants in each subplot were randomly selected to measure photosynthetic rate using a Li-6400 Portable Photosynthesis System (Li-Cor, Inc., Lincoln, NE, USA). The Li-6400 used red and blue LED as a light source. The light intensity was set to 1500 μ mol m^{-2} s^{-1}. The temperature of the leaf chamber was set to 25 ℃, and other parameters were consistent. In addition, we randomly collected some leaves of these plants, dried them at 65℃ for 48 h, and weighed and ground them to determine N concentration using the Auto-Kjeldahl method (Kjektec System 1026 distilling unit; Kjeltec Systems, Sweden).

Soil microbial biomass and community structure

Soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined using the chloroform-fumigation-extraction (CFE) method (Vance et al. 1987). We first weighed two portions of 12.5 g fresh soil samples, one of which was fumigated with dealcoholized chloroform at 25 ℃ in the dark for 24 h, and the other was cultured without dealcoholized chloroform at 25 ℃ in the dark for 24 h. Then, both soil samples were digested with 50 mL of 0.5 mol L^{-1} K_2SO_4 solution for 30 min, respectively. Finally, we used a multi N/C 3100 TOC total organic C / total N analyzer (Analytik Jena GmbH, Jena, Germany) to determine the C and N content of these two digestions, respectively. Soil MBC and MBN were calculated according to the formula: $MBC(MBN) = E_{C(N)} / K_{EC(N)}$, where $E_{C(N)}$ is equal to the C (or N) content of digested soil samples minus the C (or N) content of non-digested soil samples, $K_{EC(N)}$ is the digestion efficiency, where K_{EC} is 0.38 and K_{EN} is 0.45. Soil microbial community structure was assessed using phospholipid fatty acids (PLFA) analysis (Bossio et al. 1998). The main processes include: extraction, separation, hydrolysis and esterification of phospholipids. The obtained fatty acid methyl esters were separated by Agilent 6890 gas chromatograph (Agilent Technologies, Inc., Santa

Clara, CA, USA), and PLFA composition was analyzed by MIDI microbial identification system (MIDI Inc., Newark, DE, USA). Bacterial biomass was predicted from the sum abundance of the following FAs: i14:0, i15:0, i16:0, i17:0, a15:0, a17:0, 16:1ω7c, 17:1ω8c, 18:1ω9c, 18:1ω7c, cy17:0 and cy19:0, and fungal biomass was predicted from the abundance of the fungi-specific FA 18:2ω6,9 (Frostegard et al. 2011). The fungi to bacteria (F/B) ratio was calculated using fungi FAs and bacteria FAs.

Statistical analyses

We used linear mixed-effects models to examine the main and interactive effects of warming, N addition and sampling month on soil physical and chemical properties, soil microorganisms, plant photosynthetic rate and leaf N concentration. All statistical analyses were performed in the *nlme* package of R (Version 4.0.3, R Core Team 2021; Pinheiro et al. 2021). The code used in R was "*lme(Y ~ W*N*M, random = ~ 1|Rep/subplot, correlation = corAR1(form = ~ 1|Rep/subplot))*", where Y is the response variable (such as soil MBC, MBN), W is binary (0 = no warming, 1 = warming), N is binary (0 = ambient N, 1 = N addition), M denotes the sampling month (i.e. June or July), and Rep is an integer (1 to 6 for replicates from north to south). The denominator degrees of freedom for main effects and interactions in these models reflect sample sizes for the subplots to which warming and N addition were randomly allocated. In addition, in order to study the responses of each factor to warming and N addition in each month, we used linear mixed-effects models to analyze each factor in each month (Table S1). We also used the *psych* package to analyze the Pearson's correlation between plant leaf N concentration and photosynthetic rate with biotic and abiotic factors (Revelle 2020).

We established a structural equation model (SEM) to investigate the direct and indirect effects of warming, N addition and seasonal precipitation on soil microbial structure (F/B ratio) and the net photosynthetic rate of *S. breviflora*. The SEM analysis was carried out using the *lavaan* package in R (Oberski 2014). We first considered a model including all possible pathways and sequentially eliminated non-significant pathways until we obtained the final model. The path coefficients were obtained using maximum likelihood estimation. We used the chi-square (χ^2), P value, comparative fit index (CFI) and root mean square error of approximation (RMSEA) to evaluate the quality of fit of model. When $0 \leq \chi^2 \leq 2df$, $0.05 < P \leq 1$, $0 \leq RMSEA \leq 0.05$ and $0.97 \leq CFI \leq 1.00$, the model has a good fit (Schermelleh-Engel et al. 2003). We note that SEM is typically applied on large datasets, but test statistics have been developed that allow for estimation of the models with less data (Bentler and Yuan 1999; Ullman and Bentler 2012).

Results

Soil biogeochemistry

Soil temperature in the 0 ~ 10 cm soil layer was increased significantly by warming in 2018. On average, soil temperature in the warming plots was increased by 1.41 ℃ (W0: 5.56 ± 1.24 ℃ [mean ± SE, same below], W1: 6.96 ± 1.17℃; $P < 0.001$; Figure S3; Table S1). Neither warming nor N addition had a significant effect on soil moisture (Figure S3, Table S1). Soil pH did not change under warming, but N addition affected soil pH significantly, and there was a significant negative interaction between warming and N addition ($P < 0.01$; Table S1; Figure S4).

The results showed that soil inorganic N was affected by warming, N addition and month ($P < 0.01$ for W, $P < 0.001$ for N and M; Table 1). In the dry month (i.e., June), neither warming nor N addition had significant effect on soil inorganic N. However, in the wet month (i.e., July), compared to the control, warming and N addition significantly increased soil inorganic N by 40.6% and 89.1%, respectively ($P < 0.01$ for W, $P < 0.001$ for N; Figure S5a; Table S2). There was no significant interaction between warming and N addition. Similar to the results for inorganic N, warming significantly increased NO_3^- by 45.0% in the wet month, but it had no significant effect on NH_4^+ ($P < 0.05$; Figure S5b, c; Table S3). N addition significantly increased NO_3^- by 94.2%, and significantly increased NH_4^+ by 67.8% in the wet month ($P < 0.001$; Figure S5b, c; Table S3).

Table 1 Results from linear mixed-effect models on the effects of warming and N addition on soil moisture, inorganic N, microbial biomass and microbial PLFAs

	df_1	df_2	Inorganic N	MBC	MBN	MBC/MBN	Total FAs	FAs(B)	FAs(F)	F/B
Warming (W)	1	15	9.68**	1.97	16.53**	8.84**	22.78***	56.34***	22.69***	43.75***
Nitrogen (N)	1	15	62.42***	3.15	20.50***	3.35	40.85***	110.06***	23.95***	60.85***
Month (M)	3	68	1012.26***	146.48***	220.71***	6.20*	6296.69***	5224.17***	53.29***	322.24***
W×N	1	15	1.01	1.14	0.14	0.20	28.71***	77.87***	17.33***	49.02***
W×M	3	68	23.81***	4.58*	15.00***	0.79	84.63***	111.28***	16.22***	45.65***
N×M	3	68	158.31***	4.33*	24.43***	5.93*	80.66***	138.28***	19.61***	51.32***
W×N×M	3	68	2.01	0.13	10.75**	9.96**	28.99***	68.90***	19.76***	40.06***

Note. The F ratios are presented together with their levels of significance. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. df_1 and df_2 denote the numerator and denominator degrees of freedom, respectively. MBC, MBN and MBC/MBN represent soil microbial biomass carbon, soil microbial biomass nitrogen and the MBC to MBN ratio, respectively. Total FAs, FAs(B), FAs(F) and F/B represent soil total FAs, bacterial FAs, fungal FAs and the ratio fungi to bacteria ratio, respectively

Table 2 Results from linear mixed-effect models on the effects of warming and N addition on leaf N concentration (Leaf N) and net photosynthetic rate (P_{net}) of *Stipa breviflora* in each month

		df_1	df_2	Leaf N	P_{net}
Warming (W)		1	15	7.10*	4.11
Nitrogen (N)		1	15	10.36**	7.68*
Month (M)		1	20	2478.70***	646.03***
W×N		1	15	4.32	5.44*
W×M		1	20	8.64**	9.51**
N×M		1	20	16.06***	8.58**
W×N×M		1	20	10.86**	5.21*
Jun	W	1	15	3.04	0.37
	N	1	15	3.96	0.77
	W×N	1	15	1.21	0.96
Jul	W	1	15	11.85**	7.63*
	N	1	15	18.34***	9.95**
	W×N	1	15	8.75**	6.59*

Note. The F ratios are presented together with their levels of significance. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. df_1 and df_2 denote the numerator and denominator degrees of freedom, respectively

Leaf N content and photosynthesis of *S. breviflora*

Results from linear mixed-effects model showed that warming, N addition and month had significant interactions on plant leaf N concentration and photosynthesis ($P < 0.05$; Table 2). In the relatively wet month, compared to the leaf N content of plants in the control plots (C: 2.36% ± 0.06%), leaf N concentration of *S. breviflora* was significantly increased by warming (W:

2.69% ± 0.07%) and N addition (N: 2.74% ± 0.04%), which represent increases of 14.0% and 15.9%, respectively ($P < 0.01$ for W, $P < 0.001$ for N; Fig. 1a; Table 2). However, the significant increase in leaf N concentration did not occur in the dry month. Results from the linear mixed-effect model showed that there was a significant interaction between warming and N addition on leaf N concentration ($P < 0.01$; Table 2).

Similar to the effects of warming and N addition on leaf N concentration, in the wet month, warming and N addition significantly increased the net photosynthetic rate of *S. breviflora* by 46.1% and 49.5%, respectively ($P < 0.05$ for W, $P < 0.01$ for N; Fig. 1b; Table 2). There was a significant positive interaction between warming and N addition on plant photosynthesis ($P < 0.05$; Table 2). However, these effects were not detected in the dry month. The results from Pearson's correlation analysis showed that there was a significant negative correlation between photosynthetic rate and soil temperature in June. However, in July, both leaf N concentration and photosynthetic rate were significantly positively correlated with soil MBC, MBN and inorganic N, which is in contrast to the significantly negative correlation with the soil F/B ratio (Table S4).

Soil microbial biomass and community structure

Warming and N addition had no significant effects on soil MBC (Table 1, MBC), but they both had significant effects on soil MBN, ($P < 0.01$ for W, $P < 0.001$ for N; Table 1, MBN). The soil MBC/MBN ratio was significantly affected by warming

Fig. 1 Effects of warming and nitrogen addition on leaf N concentration and net photosynthetic rate of *Stipa breviflora* (June and July). Error bars show one standard error of the mean. Different letters in each treatment indicate significant difference among treatments (one-way ANOVA, $P < 0.05$)
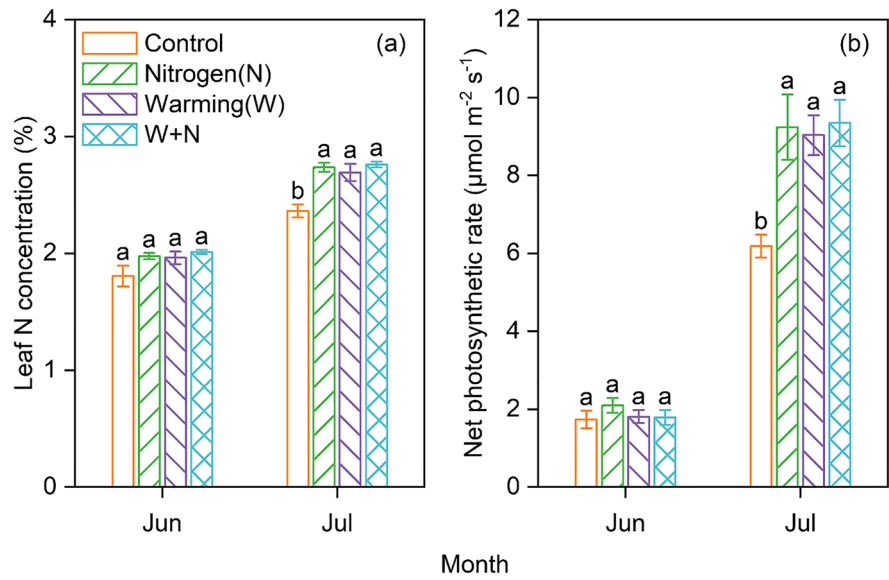
but not by N addition ($P < 0.05$ for W; Table 1, MBC/MBN). There was a significant interaction between warming, N addition and month (Table 1; $W \times M$, $N \times M$). Therefore, we analyzed the effects of warming and N addition on soil MBC and MBN in each month. In the relatively dry month, neither warming nor N addition significantly affected soil MBC, MBN or MBC/MBN (Table S2). By contrast, in the wet month, warming and N addition significantly enhanced soil MBC by 16.6% and 18.0%,

respectively ($P < 0.001$; Fig. 2a; Table S2). Similar to MBC, in the wet month, soil MBN was significantly increased by 48.1% under warming and 54.9% under N addition, respectively ($P < 0.001$; Fig. 2b; Table S2). Due to a greater increase in MBN than MBC, in the wet month warming and N addition significantly decreased the soil MBC/MBN ratio by 22.7% and 23.6%, respectively ($P < 0.001$; Fig. 2c; Table S2). Warming and N addition had significant interactions on soil MBC, MBN and
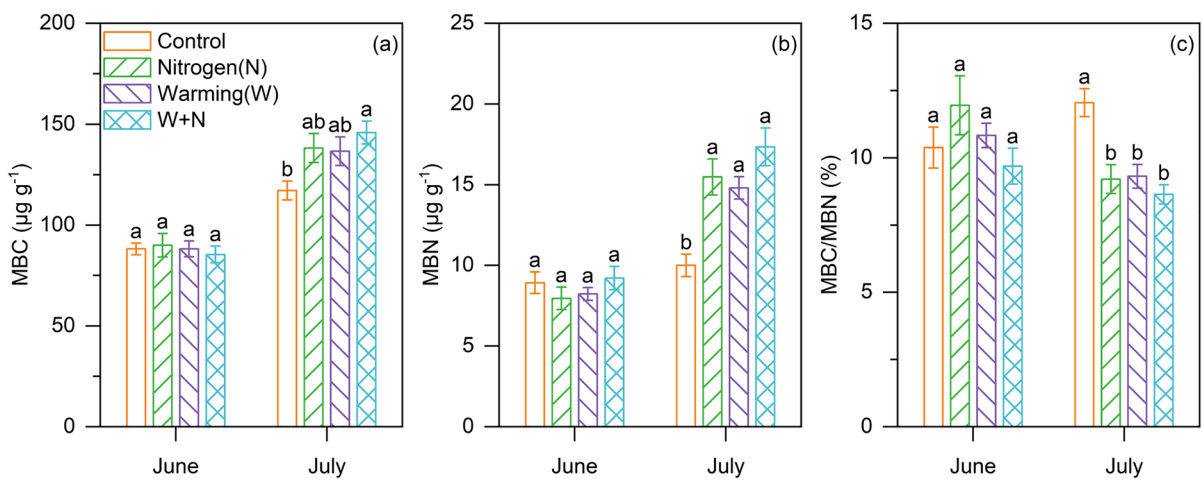
Fig. 2 Effects of warming and N addition on (**a**) soil microbial biomass carbon (MBC), (**b**) microbial biomass nitrogen (MBN) and (**c**) the ratio of MBC/MBN (June and July). Error bars show one standard error of the mean. Different letters in each treatment indicate significant difference among treatments (one-way ANOVA, $P < 0.05$)

the MBC/MBN ratio in the wet month ($P < 0.01$ for MBN, $P < 0.001$ for MBC and MBC/MBN; Table S2).

Both warming and N addition significantly affected soil microbial FAs, and there was a significant interaction between warming and N addition. Moreover, the FAs were significantly different in each month ($P < 0.001$; Table 1). Thus, we also analyzed the responses of soil microbial FAs to warming and N addition in each month. In the dry month, warming and N addition had no significant effect on soil microbial FAs (Fig. 3, dotted lines; Table S2). In the wet month, warming and N addition significantly increased bacterial FAs by 23.1% and 26.1%, but decreased fungal FAs by 23.9% and 24.8%, respectively, resulting in decreases in the F/B ratio of 38.0%

and 40.1%, respectively ($P < 0.001$; Fig. 3, solid lines; Table S2).

Pathways of warming and N addition effects on soil microbial community structure and plant photosynthesis

We used a SEM to analyze the direct and indirect effects of warming, N addition and seasonal precipitation on soil microbial community structure (the F/B ratio) and plant photosynthesis (Fig. 4). First, warming and N addition directly affected leaf N, and consequently enhanced net photosynthesis. Second, warming and N addition directly increased soil inorganic N, but the effect of the increased soil inorganic N on leaf N and photosynthesis was indirect via the

Fig. 3 Effects of warming and N addition on soil microbial phospholipid fatty acids (PLFAs) (June and July). Error bars show one standard error of the mean. Different letters in each treatment indicate significant difference among treatments (one-way ANOVA, $P < 0.05$)
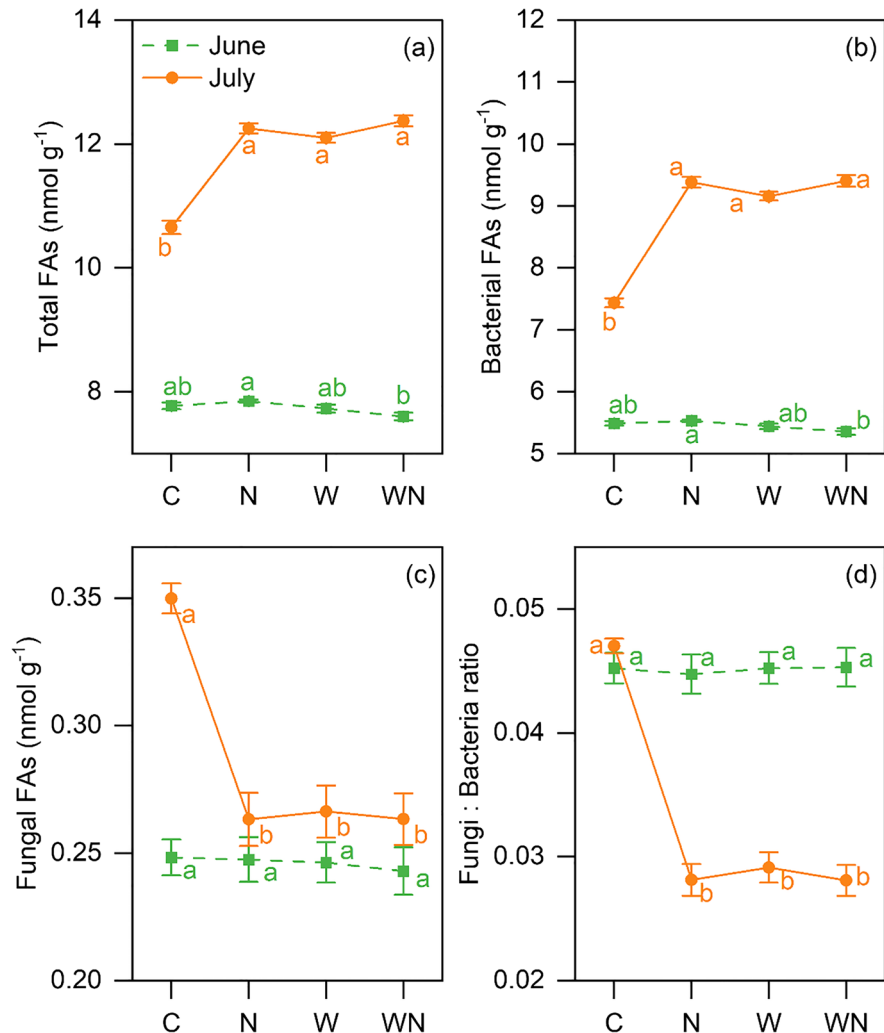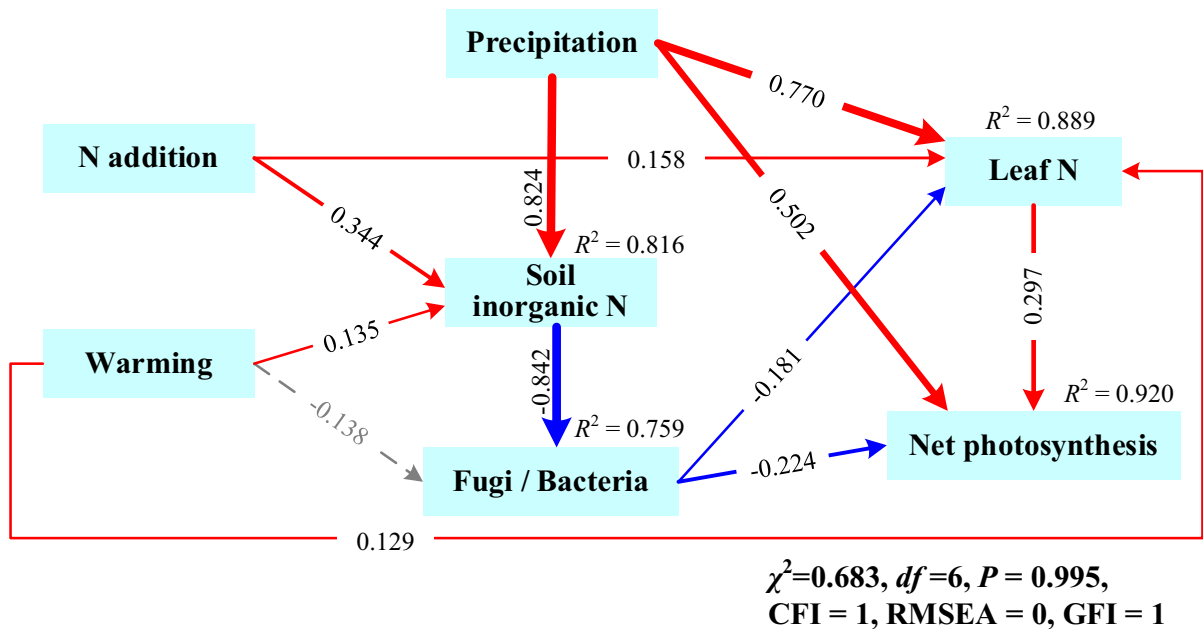
Fig. 4 Structural equation modeling (SEM) analysis showing the direct and indirect effects of warming and N addition on the net photosynthetic rate of *S. breviflora* in July. Results of model fitting are shown in the figure. The R^2 represents the proportion of variance explained. Solid arrows indicate significant positive (red) or negative (blue) pathways, and gray dashed arrows indicate nonsignificant pathways. The values near arrows are the standardized path coefficients. Arrow width is proportional to the strength of the relationship

regulation by soil microbial community composition (the F/B ratio), whereby the F/B ratio had a negative effect on plant photosynthesis. Moreover, the responses of soil N availability, the F/B ratio and plant photosynthesis to warming and N addition were mediated by seasonal precipitation with precipitation favoring soil inorganic N, leaf N and photosynthesis.

Discussion

Our results showed that warming and N addition significantly increased soil microbial biomass (MBC and MBN) in the wet month (Fig. 2), thus supporting our first hypothesis. Previous studies on the effects of warming on soil microbial biomass have been inconsistent, with increase (Zogg et al. 1997), decrease (Allison and Treseder 2008) and non-significant changes (Zhang et al. 2005) all being reported. The differences among these results may be attributed to differences of ecosystems, magnitude of warming or to different warming methods. Contrary to our results showing increased soil microbial biomass under N

addition, it is generally believed that long-term N addition will lead to soil acidification and changes in soil structure, which could have toxic effects on soil microorganisms and lead to a decrease in soil microbial biomass (Lovell et al. 1995; Tietema 1998; DeForest et al. 2004). Our results may be because the N addition rate was lower than the level of inhibition of microbial activities in the present study. For example, Zhang et al. (2008) reported that a relatively low N addition rate (16 g N m^{-2} yr^{-1}) increased soil microbial biomass, but high N addition rates (32 g N m^{-2} yr^{-1} and 63 g N m^{-2} yr^{-1}) significantly reduced soil microbial biomass in a semi-arid grassland in northern China.

In July (i.e., the wet month), both warming and N addition significantly increased bacterial FAs and decreased fungal FAs, resulting in a reduction in the soil F/B ratio (Fig. 3), thus further supporting hypothesis 1. Previous studies suggested that the increased plant growth and decreased soil N availability caused by warming are more beneficial to fungi than bacteria, resulting in an increase of soil F/B ratio (Zhang et al. 2005). Our results showed the opposite, as warming

increased soil available N and decreased the soil F/B ratio. This is presumably because warming decreased soil C substrates or increased soil nutrients with a lower soil C/N ratio, while a lower soil C/N ratio is more conducive for bacteria (Elliott et al. 1983; Fierer et al. 2009), consistent with other studies (Melillo et al. 2017; Deng et al. 2018). Another possible reason is that warming has a stronger inhibitory effect on non-mycorrhizal fungi than on bacteria in temperate grasslands (Zhang et al. 2015). In addition, it is generally believed that long-term N input has a greater negative impact on fungi than on bacteria, leading to a decrease in the F/B ratio (Frey et al. 2004; Leff et al. 2015). Grasslands with higher N input also showed low soil F/B ratios (de Vries et al. 2006). This may be because bacteria need more N to accumulate per unit of biomass C, and a lower C/N ratio may increase the relative abundance of bacteria (Elliott et al. 1983; Fierer et al. 2009). Higher N availability may also induce the accumulation of compounds that are toxic to fungi (Treseder 2008). Moreover, mycorrhizal fungi may be adversely affected by N addition because plants tend to allocate less C to mycorrhizal fungi when N is more available (Treseder 2004). Our results from the SEM also support these suggestions, as the increase of soil available N caused by warming and N addition decreased the soil F/B ratio (Fig. 4).

Consistent with our second hypothesis, the responses of soil nutrient availability, microorganisms and plant photosynthesis were strongly regulated by seasonal precipitation. First, warming and N addition significantly increased soil inorganic N in the wet month of July (Figure S5). The contrasting responses of soil inorganic N in the two months to warming and N addition indicated that the response is regulated by water availability, especially in water-limited ecosystems (e.g. Ren et al. 2018). Compared to June, soil inorganic N increased significantly in the wet month of July, which could be caused by precipitation pulse. A number of studies in arid and semi-arid ecosystems have indicated that water pulses can immediately enhance soil N mineralization and N availability after an extended drought (Austin et al. 2004). Second, warming and N addition effects on plant leaf N concentration and photosynthesis occurred only in the wet month of July (Fig. 1). In the semi-arid desert steppe, water availability, as the most important factor affecting plant function (Weltzin et al. 2003), will inevitably have an important impact on plant growth.

A large number of studies have shown that precipitation has combined effects on plant growth with warming and N addition (Wu et al. 2011; Yang et al. 2011b; Kong et al. 2013; Zhao et al. 2019). Third, warming and N addition significantly increased soil microbial biomass in the wet month of July (Fig. 2). Most microbial activities are usually inhibited under conditions of water stress, which may lead to a lack of response of microbial communities to warming. When water is abundant, the microbial population is more active than under drought conditions (Sheik et al. 2011; Wang et al. 2018). The effects of N addition on microorganisms also depends on variation in precipitation (Zhang et al. 2015). Water is an important condition for nutrient diffusion and replenishment in soil. Abundant water availability could help to replenish N into soil solution and therefore increase available N for microbial use (Park et al. 2002). Consequently, N supplementation can promote the growth of microorganisms by reducing N restriction when water is sufficient. Finally, in the wet month of July, warming and N addition significantly increased bacterial FAs and decreased fungal FAs, resulting in a reduction in the soil F/B ratio (Fig. 3). As bacteria are more sensitive to water than fungi (Kwon et al. 2013), soil inorganic N supplementation caused by warming and N addition stimulated the bacteria more than the fungi under high water availability, which also explains the decrease in the soil F/B ratio (Zhang et al. 2015).

Our study showed significant interactions between warming and N addition on soil microbial biomass, PLFAs, plant leaf N content and photosynthetic rate in the wet month (Table 2, S2). This indicates that the impacts of warming or N addition on soil microorganisms and plants are influenced by another treatment. Many reports have shown that soil microbial community and plants are affected by multiple global change drivers and their interactions (Castro et al. 2010; Komatsu et al. 2019; Du et al. 2020), thus highlighting the importance of manipulating multiple factors rather than single factors in global change experiments (Song et al. 2019). On the one hand, warming might change soil available C and N, or change microbial C and N utilization efficiency (Li et al. 2018; Sun et al. 2022), and affect the responses of microorganisms to N addition. On the other hand, warming might also change the ability of plants to absorb N (Chapin et al. 1986; Clarkson et al. 1988;

Bassirirad et al. 1993), and affect the responses of plants to N addition. Further research is needed to reveal the specific mechanisms of the interaction between warming and N addition.

Our study showed that warming and N addition significantly increased plant net photosynthetic rate in the wet month of July (Fig. 1). Previous studies have shown that warming can change the morphological structure, stomatal conductance and photosynthetic pigment of plant leaves and the activities of enzymes required by plant for photosynthesis, consequently affecting plant photosynthetic rate (Ruiz-Vera et al. 2013; Yang et al. 2013). As N is an important element in the synthesis of chlorophyll and photosynthesis protein (Ordonez et al. 2009), and leaf N content is closely related to the photosynthesis of plants (Wright et al. 2004), N addition can increase plant leaf N concentration, and consequently promote the synthesis of chlorophyll and enhance the net photosynthetic rate of plants (Zhang et al. 2022). Interestingly, results from the SEM showed that the increase in soil inorganic N did not directly increase leaf N concentration and photosynthesis. Instead, its effect was indirect, whereby soil inorganic N increased the abundance of some bacteria related to nutrient cycling and plant N absorption (Tao et al. 2019), consequently leading to increased leaf N concentration and photosynthesis, thus supporting our third hypothesis. The non-significant direct effect of increased soil inorganic N on leaf N and photosynthesis suggested a potential role of competition between plants and soil microorganisms for soil nutrient uptake. In many ecosystems, N is often the most limiting nutrient for plant growth, but microorganisms also need N for growth. Previous studies have shown that plant–microbe competition could be intense when the demand for N by both plant and microorganisms was high (Dunn et al. 2006; Inselsbacher et al. 2010; Xu et al. 2011).

Despite this competition, our results showed the (net) positive response of plant photosynthesis indirectly regulated by the soil microbial community. Our results could be in contrast to the scenarios whereby the positive effects of increased soil nutrient availability on plant growth could be outweighed by the high demand for N and the competitive advantage for N by soil microbes (Dunn et al. 2006; Dijkstra et al. 2015). Overall, plant and soil microbial competition for nutrients is expected to largely influence biogeochemical cycling with implications for plant

diversity and productivity and plant-soil interactions and feedbacks across spatial resource gradients and under global change (Zak et al. 1990; van der Heijden et al. 2008; Averill et al. 2014). Further studies are needed to clarify the mechanisms affecting plant leaf N concentration, with a focus on plant vs. soil microbial competition for soil nutrients and the role of the soil microbial community in nutrient cycling. A quantitative understanding or estimation of plant vs soil microbe demand or uptake for N would be also needed across different spatial and temporal scales.

Conclusions

Overall, our results showed that soil microorganisms and plant responses to warming and N addition were strongly regulated by seasonal precipitation in the water-limited desert steppe. Warming and N addition stimulated plant photosynthesis and soil microorganisms with significant interactions only under high water availability conditions, while warming and N addition had no effects on plant photosynthesis or soil microorganisms under water stress. The interactions thus highlight the importance of manipulating multiple factors rather than single factors to shed light on plant responses to global change. Moreover, warming and N addition directly increased soil inorganic N, but the effects of increased soil inorganic N on leaf N and plant photosynthesis were indirect and occurred via their effects on soil microbial community structure. These results suggest the crucial role of the soil microbial community in regulating the response of the plant community to global change drivers, such as warming and N deposition. These findings highlight the importance as well as crucial knowledge gaps in plant and soil microbe competition for N in ecosystem functions (i.e., carbon cycling) under global change.

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