

Aridity stimulates responses of root production and turnover to warming but suppresses the responses to nitrogen addition in temperate grasslands of northern China

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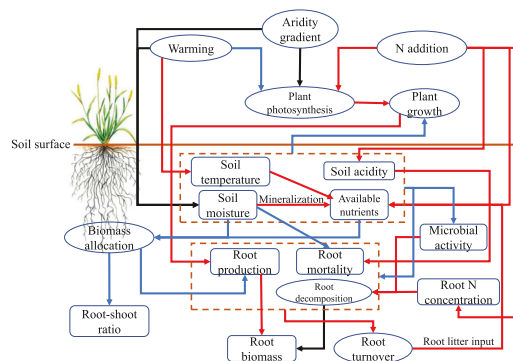
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HIGHLIGHTS

- Warming could reduce root production, but nitrogen (N) addition less affected it.
- Effects of warming or N addition on plant biomass allocation varied with years.
- Warming could suppress root turnover, but N addition may stimulate it.
- Warming and N addition had additive effects on root turnover.
- Aridity promoted root turnover under warming, but suppressed it under N addition.

GRAPHICAL ABSTRACT



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ABSTRACT

Global warming and nitrogen (N) deposition are known to affect root dynamics in grasslands. However, previous studies were based only on a single ecosystem type, so it is unclear how warming and N addition affect root traits (root biomass, root-shoot ratio, root production and turnover) along the aridity gradient. In this study, we conducted an experiment to determine the effects of warming and N addition on root traits in desert, typical, and meadow grasslands in northern China, where the aridity gradually decreases from west to east across the region. Warming increased root-shoot ratio in dry year due to decline in soil water, but had a downward trend in root production and turnover in all three grasslands. N addition decreased root-shoot ratio in humid year due to increase in soil N, whereas did not significantly affect root production in any grasslands and increased root turnover in desert and meadow grasslands rather than typical grassland. Warming combined with N addition had negatively additive effects on root turnover in typical and meadow grasslands rather than desert grassland. N addition-induced changes in root biomass and root-shoot ratio were negatively affected by aridity in dry year. Aridity positively affected responses of root production and turnover to warming but negatively affected those

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Root production
Root turnover

responses to N addition. However, root-shoot ratio, root production and turnover under warming combined with N addition were not affected by aridity. Our results suggest that warming suppresses root carbon (C) input but N addition may exacerbate it in temperate grasslands, and warming combined with N addition suppresses it only in wet grasslands. Aridity promotes root C input under warming but suppresses it under N addition. However, aridity may little affect soil C and nutrient dynamics under global warming combined with N deposition in temperate grasslands in the future.

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1. Introduction

Global temperatures and atmospheric nitrogen (N) deposition have both increased since the Industrial Revolution, especially in temperate regions (IPCC, 2013). These global changes are affecting the terrestrial carbon (C) cycle and attracting the attention of the scientific community. Root litter and exudate contribute C and nutrients to ecosystem in amounts similar to or even more than foliage (Norby et al., 2000). Thus, root production and turnover are important regulators of soil C and nutrient cycles in terrestrial ecosystems and link above- and below-ground C processes (Loya et al., 2004; Norby et al., 2004; Roumet et al., 2016; Ruess et al., 2003; Sullivan et al., 2007). Root turnover is important for sequestering C in soil, but the process is highly sensitive to global changes (Gill and Jackson, 2000). Thus, understanding how global changes influence root dynamics is vital to understanding C distribution and nutrient cycles in an ecosystem (Fig. 1) (Arndal et al., 2018; Trumbore and Gaudinski, 2003).

Previous studies show that multiple drivers of global change have additive effects on terrestrial C storage (Yue et al., 2017) but have interactive effects on gross primary productivity in grassland ecosystems (Niu et al., 2009). However, most researches on how root dynamics respond to global changes are limited to a single factor, and additive or interactive effects between warming and N addition on root turnover have not been investigated in semi-arid grasslands. Further, most relevant studies are small scale, and provide no insight on regional scale relationships.

Warming directly affects soil temperature, soil moisture, and photosynthesis rate (Song et al., 2016; Wertin et al., 2017). Warming also alters plant phenology (Klanderud and Totland, 2005), promotes plant growth (Zhang et al., 2005), and extends the growth phase. Therefore, warming affects production and distribution of plant biomass (Fig. 1) (Bai et al., 2010). In general, warming increases root production in ecosystems where precipitation or water availability is relatively abundant (Sullivan and Welker, 2005; Wan et al., 2004). However, warming can suppress root growth in arid and semi-arid ecosystems by decreasing

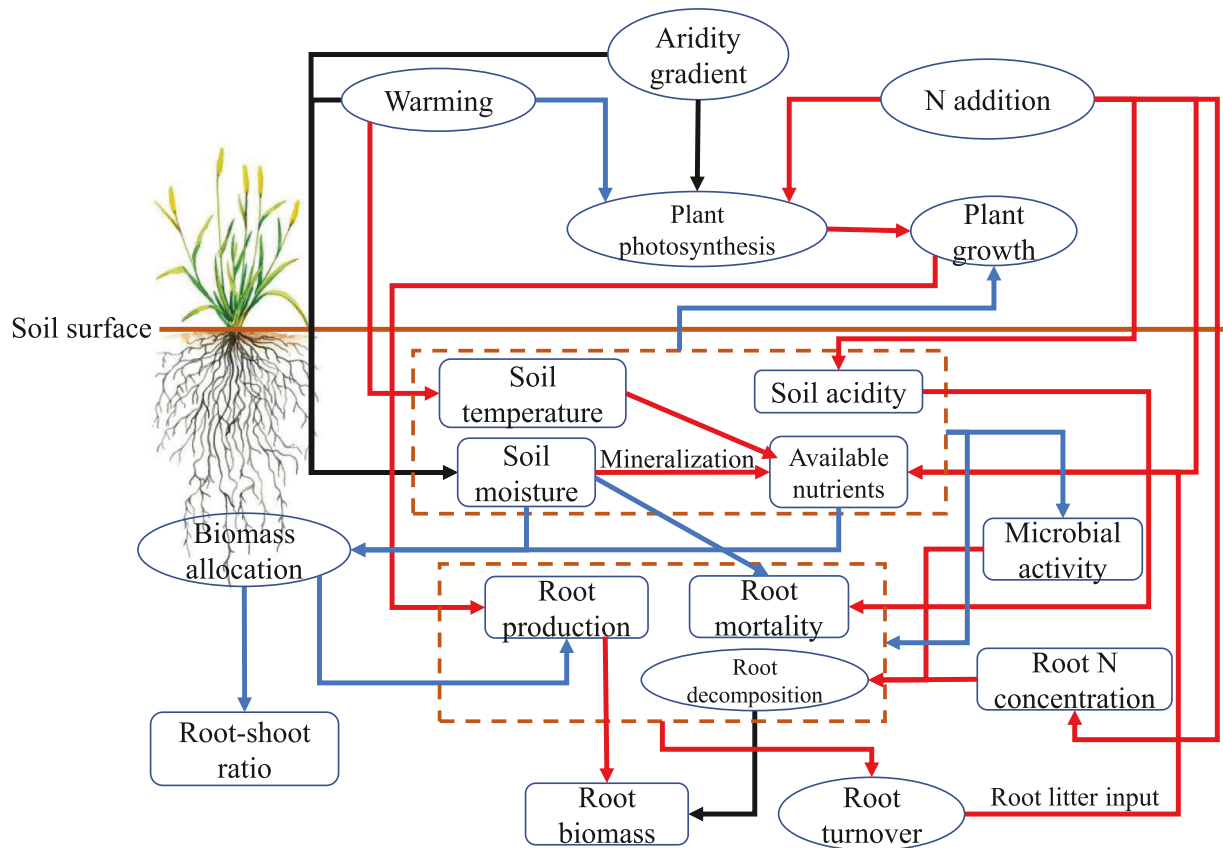


Fig. 1. A conceptual diagram of how warming and N addition influence root dynamics along an aridity gradient. Red, black, and blue arrows indicate positive, negative, and uncertain relationships, respectively. Overall, warming, N addition, and aridity gradient induced changes in microenvironment (temperature, moisture, and nutrients) affect plant photosynthesis. The changed photosynthesis and soil resources (water, nutrients) affect plant biomass allocation and root production. The changes in soil physicochemical properties impact root growth or mortality. Treatments affect soil microbial activity and root nutrient, thus influencing root decomposition. The altered root growth, mortality, and decomposition affect root biomass and turnover. Root turnover influences root litter input, thus in turn altering soil nutrient condition.

water availability (Bai et al., 2010). Therefore, warming may enhance root production in meadow grasslands with high water supplies, but reduce root production in desert grasslands with low water supplies.

As might be expected, previous studies on the effects of warming on root turnover in grasslands yielded inconsistent results. For example, warming was observed to increase (Fitter et al., 1999; Volder et al., 2007), decrease (Wang et al., 2017) or have no effects on (Bai et al., 2010) root turnover in various grasslands. Given these different responses, manipulating temperature conditions along an environmental gradient may explain how warming impacts root dynamics on a regional scale (Fraser et al., 2013; Yuan and Chen, 2010).

N availability in soil is a major limiting factor in temperate grasslands (Chapin et al., 1987; Vitousek and Howarth, 1991) and has vital effects on terrestrial belowground productivity (Fitter et al., 1999; Haynes and Gower, 1995; Li et al., 2015) and C allocation underground in northern China (Fig. 1) (Giese et al., 2013). For example, meta-analyses indicated that N addition significantly decreases root production and turnover, but has minor or no impact on root biomass in grasslands on a global scale (Li et al., 2015; Peng et al., 2016). In field studies, N addition increased (Fitter et al., 1999), decreased (Xu et al., 2017), or had no effect (Gao et al., 2011) on root production or turnover in diverse grassland ecosystems. These differences might be explained by studying the effects of N addition along an environmental gradient.

Water availability influences how warming and N addition affects biomass allocation in plants (Gao et al., 2011; Xu et al., 2015). Meta-analysis found that responses of below ground net primary productivity to warming positively correlated with aridity in grasslands (Wang et al., 2019). So, it can be detected that warming can enhance root production in dry grasslands (such as desert grassland). A field study showed that water didn't interact with N to affect belowground net primary production (Xu et al., 2017). Another study across several grassland ecosystems concluded that alpine grasslands become more and more insensitive to N input with precipitation decrease (Zong et al., 2019). Thus, precipitation may have little effects on responses of root production to N addition and N addition may impact less on root production in dry than that in wet grasslands.

Grasslands accounts for 25% of the total land area of the earth and play an important role in the global C cycle (Hui and Jackson, 2006; Suter et al., 2002). Plant roots and their activities may have a greater influence on ecosystem function in grasslands than in forests, because grassland ecosystems often have a higher proportion of biomass belowground (Gill and Jackson, 2000). Thus, understanding root dynamics is important for adaptive management in grassland ecosystems, especially given that temperate grasslands in northern China are highly sensitive to global changes (Bai et al., 2010; Liu et al., 2009). Across the east to west in this study (meadow to typical to desert grasslands), soil water availability declined and aridity index (AI) decreased (Fig. 2). Moreover, soil nutrient contents often positively correlates with mean annual precipitation (MAP) along the aridity gradient (Yuan et al., 2017). How roots respond to warming and N addition may vary in different grassland ecosystems, because different changes in soil moisture and nutrient contents along aridity gradient. However, no studies have yet addressed this question.

In this study, we investigated how warming and N addition affect root traits (root biomass, root-shoot ratio, root production, and root turnover) in desert, typical, and meadow grasslands along an aridity gradient in northern China. Based on previous studies (Niu et al., 2009; Wang et al., 2019; Xu et al., 2017; Zong et al., 2019), we hypothesized that: 1) warming and N addition affect root traits in an interactive manner; 2) how root traits respond to warming and N addition largely depends on aridity. To test these hypotheses, we conducted a series of manipulative experiments with warming and N addition along an aridity gradient in temperate grasslands of northern China.

2. Materials and methods

2.1. Study sites

We selected nine study sites in Hangjingqi, Damaoqi, Siziwangqi, Sunitezuoqi, Xilinhot, Dongwuqi, Ewenk, and Erguna that ranged from west to east in Inner Mongolia, China (Fig. 2, Table 1). The study area spanned about 1500 km, in which western regions were characterized

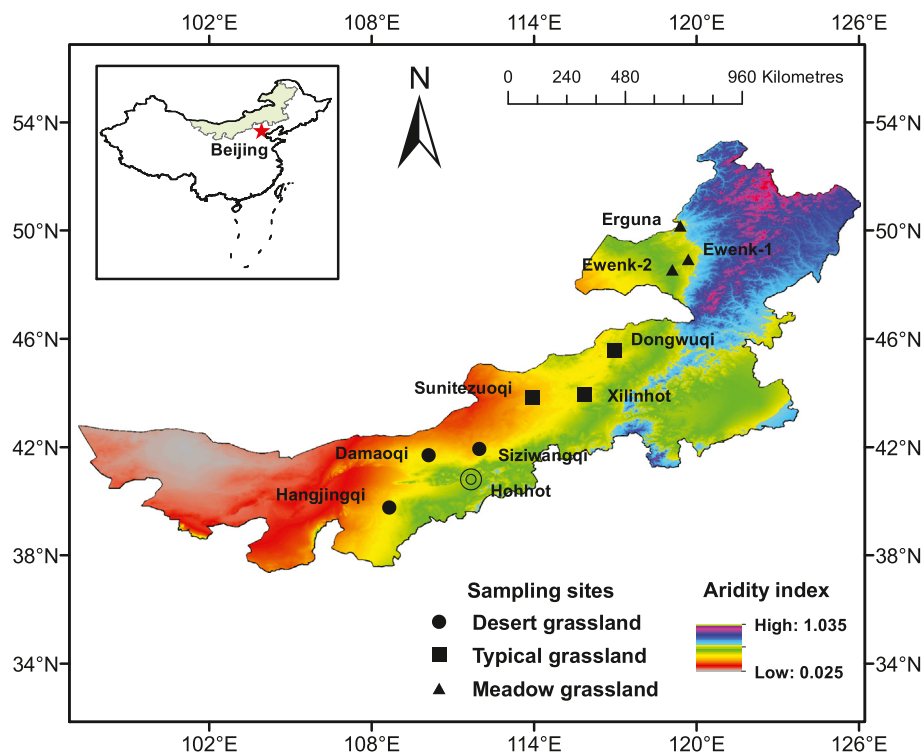


Fig. 2. Map of the experimental sites in this study.

Table 1
Location, grassland type, mean annual temperature (MAT, °C), mean annual precipitation (MAP, mm), aridity, soil type, soil bulk density (SBD, g cm⁻³), total C (TC), N (TN), and P (TP) concentrations (g kg⁻¹) and pH in soil of each study site.

Sites	Latitude	Longitude	Grassland type	MAT	MAP	Aridity	Soil type	SBD	TC	TN	TP	pH
Hangjingqi	39.78°	108.66°	Desert grassland	6.40	305	0.696	Calcaric Arenosols	1.50	6.93	0.70	0.38	8.90
Damaoqi	41.70°	110.10°	Desert grassland	3.79	261	0.703	Calcic Kastanozems	1.32	18.69	1.92	0.42	8.67
Siziwangqi	41.94°	111.97°	Desert grassland	2.94	220	0.736	Haplic Kastanozems	1.28	16.92	1.81	0.40	8.22
Sunitezuoqi	43.83°	113.96°	Typical grassland	2.67	211	0.744	Calcic Kastanozems	1.32	8.03	0.96	0.28	7.23
Xilinhot	43.94°	115.86°	Typical grassland	2.35	263	0.675	Haplic Kastanozems	1.32	19.77	2.09	0.38	8.34
Dongwuqi	45.58°	116.96°	Typical grassland	1.30	241	0.692	Haplic Kastanozems	1.32	23.28	2.32	0.50	6.82
Ewenk-1	48.93°	119.69°	Meadow grassland	-1.39	340	0.531	Calcic Chernozems	1.21	21.79	2.09	0.44	6.60
Ewenk-2	48.55°	119.11°	Meadow grassland	-1.49	320	0.569	Calcic Chernozems	1.21	23.77	2.07	0.45	6.74
Erguna	50.17°	119.40°	Meadow grassland	-2.34	359	0.505	Calcic Chernozems	1.21	28.23	2.30	0.41	6.88

Note. Aridity was calculated as $[1-AI]$, where AI is aridity index; MAT, MAP and AI were average value for 1970–2000 which were obtained from the WorldClim database (<http://www.worldclim.org/bioclimate>); TC, TN, TP, and pH in soil at 0–10 cm soil layer were analyzed.

by a temperate continental climate and eastern regions by a cold temperate continental monsoon climate. The AI of the grasslands gradually increased from west to east, and the grassland types transit from desert (AI: 0.264–0.304), typical (AI: 0.256–0.325), to meadow (AI: 0.431–0.495) grasslands (Fig. 2). Along the west to east gradient, mean annual temperature (MAT) gradually decreased (-2.34 – 6.40 °C) and MAP gradually increased (211–359 mm). Precipitation at each study site was higher in 2018 (humid year) than in 2017 (dry year) (Fig. S1). In the desert grassland, dominant plant species were *Cleistogenes squarrosa*, *Leymus chinensis*, *Stipa krylovii*, *Allium ramosum*, *Heteropappus altaicus*, and *Convolvulus ammannii*. In the typical grassland, dominant plant species were *Salsola collina*, *C. squarrosa*, *A. ramosum*, *S. krylovii*, *C. ammannii*, and *Chenopodium glaucum*. In the meadow grassland, dominant plant species were *L. chinensis*, *Carex duriuscula*, *Poa pratensis*, *Leymus secalinus*, *C. squarrosa*, and *Potentilla bifurca*. From west to east, soil types changed from calcaric arenosols, calcic kastanozems, and haplic kastanozems to calcic chernozems, using the Food and Agriculture Organization of the United Nations (FAO) soil classification system (Table 1).

2.2. Experimental design

In November 2016, we fenced a 50 × 50 m block containing a fairly uniform mixture of plant species at each of the nine study sites. In May 2017, we arranged experimental plots within the fenced areas using a Latin square design. Sixteen 3 m × 3 m plots were arranged into four rows and four columns with a 2 m buffer zone between any two adjacent plots. The treatments were: control (C), warming [W, passive warming with open-top chambers (OTCs)], N addition (N), and warming plus N addition (WN). There were four replicate plots for each treatment. The OTCs in this study were made of polycarbonate screen with over 90% transparency and a 4.39 m² basal area. Each OTC was firmly stabilized and mounted 10 cm above the ground to facilitate air flow and allow rainwater to flow into the OTCs. N was applied with urea at a level of 20 g N m⁻² in May and August 2017. Urea particles were evenly sprinkled on the soil surface of N and WN-treatment plots. Automatic meteorological towers (FY-QBX) were installed within the experiment block at each study site to monitor air temperature and precipitation.

2.3. Air temperature and soil microenvironment

Beginning July 2017, sensor probes connected to meteorological towers in each experimental block automatically recorded temperature and humidity at 30-min intervals. The probes measured air temperature 10 cm above the ground, and soil temperature (ST) and volumetric water content (% V/V) (soil moisture, SM) 10 cm below ground in each plot.

2.4. Soil and plant sampling

In August 2017 and 2018, we selected a subplot (0.75 × 0.75 m²) for sampling within each experimental plot at the nine study sites. To avoid

the disturbance of reduced precipitation by OTCs, we selected our subplots just below the opening of OTCs. All plants in subplots were clipped at the soil surface. Clipped plants were weighed after oven-drying to constant weight as aboveground biomass. After clipping, soil cores were extracted with a soil auger (7-cm inner diameter) at 0–30 cm depth interval. Soils and roots in the soil cores were separated using a 2-mm sieve. Soils were taken to the laboratory and air dried for soil nutrient analysis. After carefully collecting and washing, we oven-dried root samples to constant weight and weighed roots to measure root biomass. Root-shoot ratio was calculated as dividing root biomass by aboveground biomass.

We determined root production using ingrowth cores. Two root ingrowth cores were established in August 2017 in each plot. Root ingrowth bags (nylon net bags with diameter 7 cm and length 35 cm) were positioned after soil columns (30 cm depth) were removed. Ingrowth bags were gradually filled with root-free soils of local origin. All ingrowth cores were removed after one year by carefully removing the soil around the cylinder and collecting roots that had grown into the cores. Soils and roots in ingrowth cores were separated in the same manner as soil cores. After carefully collecting and washing, we oven-dried root samples to a constant mass and weighed them to measure root production. Root turnover was calculated as dividing root production by root biomass (Dahlman and Kucera, 1965).

2.5. Soil chemical analysis

We extracted the available nitrogen (SAN) in soil using 1 M KCl solution and measured it using flow analyzer (Auto Analyzer 3-AA3). The available phosphorus (SAP) in soil was extracted using activated carbon-CH₃COONH₄ solution and analyzed it using UV-visible spectrophotometer (UV2300) with molybdenum-antimony anti-spectrophotometric method.

2.6. Data analyses

We used three-way ANOVA to test main and interactive effects of warming, N addition, and sampling site or grassland type on root-shoot ratio, root biomass, root production, and root turnover. Our analysis was guided by the following: 1) If the combined effect of warming and N addition was greater than the sum of the individual effects, then warming plus N addition was synergistic; 2) If the combined effect of warming and N addition was weaker than the sum of the individual effects, then warming plus N addition was antagonistic; 3) If the combined effect of warming and N addition was equal to or not significantly different from the sum of the individual effects, then warming plus N addition had a simple additive effect (Yuan and Chen, 2015).

Aridity index (AI, the ratio of precipitation to potential evapotranspiration) of each sampling site were obtained from the WorldClim database (<http://www.worldclim.org/bioclimate>). To interpret our results, we substituted the AI with aridity, estimated as $[1-AI]$, which increased as

MAP decreased from east to west in our study region. Aridity might be a more appropriate measure than precipitation in our studied gradient, in which temperature also varies. We used regression analysis to identify correlations between aridity and changes in root traits induced by warming or N addition. Redundancy analysis (RDA) was performed to study the contributions of soil microenvironments and nutrients on changes in root traits caused by warming or N addition. All analyses were performed using R version 3.6.2 (The R Project for Statistical Computing, <https://www.r-project.org/>).

3. Results

3.1. Effects of warming and N addition on microenvironment and soil available nutrients

Compared to control plots, mean air temperatures in warming plots increased by 1.8 °C in desert, 2.9 °C in typical, and 1.2 °C in meadow grasslands (all $P < 0.001$) (Fig. 3a–c and j) during the growing season (May to October in 2018). Mean soil temperatures at 10 cm soil depth in warming plots increased by 1.1 °C in desert ($P < 0.1$), 0.95 °C in typical ($P < 0.01$), and 0.8 °C in meadow grasslands ($P < 0.01$) (Fig. 3d–f and k) during the growing season. Mean soil moisture at 10 cm soil depth in warming plots decreased by 2.3 (% V/V, absolute change) in typical ($P < 0.001$) and 1.5 in meadow ($P < 0.05$) grasslands during the growing season. Mean soil moisture at 10 cm soil depth in warming plots in desert grassland was not significantly different from controls (Fig. 3g–i and l), except for at the Hangjingqi (desert grassland) study site where warming significantly reduced mean soil moisture ($P < 0.01$). We did not detect significant changes in mean air or soil temperature from N addition to all grasslands (Fig. 3j and k). However, N addition significantly decreased mean soil moisture in typical grassland by 0.9 and meadow grassland by 3.7 (both $P < 0.01$) (Fig. 3l).

Warming increased the availability of soil N and P (SAN and SAP) in the 0–30 cm soil layer of all grasslands in 2017 (all $P < 0.05$). However, warming reduced SAN in desert grassland and increased it in typical and meadow grasslands in 2018 (all $P < 0.05$). Warming increased SAP in desert ($P < 0.01$) and meadow ($P < 0.05$) grasslands in 2018 (Fig. 4a and b). N addition increased SAN in all grasslands in both sampling years (all $P < 0.001$), but had no effects on SAP (Fig. 4a and b).

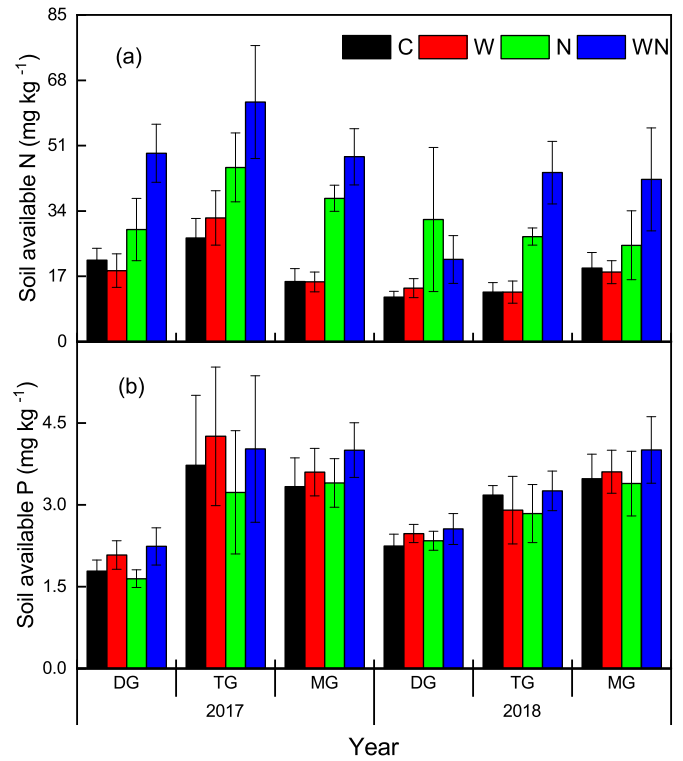


Fig. 4. Effects of warming and N addition on soil available N (a) and P (b) contents in desert (DG), typical (TG), and meadow (MG) grasslands in 2017 and 2018. Error bar represented the standard error of the mean. C: control, W: warming, N: N addition, WN: warming plus N addition.

3.2. Effects of warming and N addition on root biomass, aboveground biomass and root-shoot ratio

Warming increased root biomass only in typical grassland in 2017 ($P < 0.05$), but did not significantly change it in all grasslands in 2018 (Fig. 5a). Aboveground biomass was decreased by warming in all

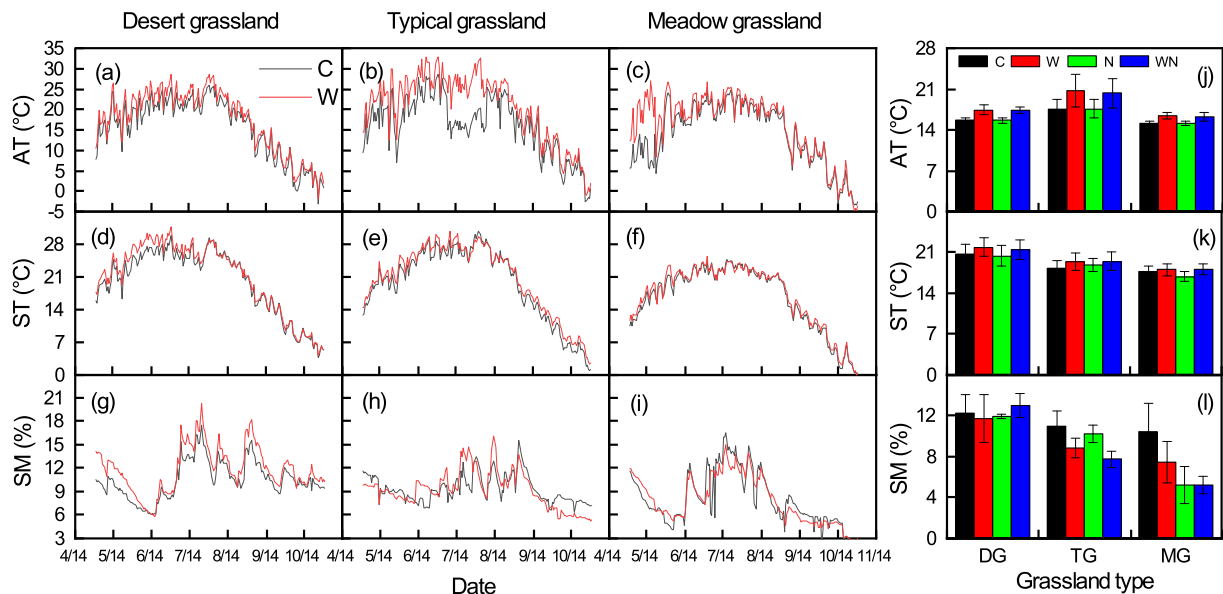


Fig. 3. Daily mean air temperature (AT) (a–c), mean soil temperature (ST) (d–f), mean soil moisture (SM) (g–i) under warming and mean air temperature (j), mean soil temperature (k), mean soil moisture (l) under warming and N addition in desert (DG), typical (TG), and meadow (MG) grasslands from May to October in 2018. Air temperature was measured 10 cm above ground; soil temperature and moisture were measured at 10 cm soil depth. Error bar represented the standard error of the mean. C: control, W: warming, N: N addition, WN: warming plus N addition.

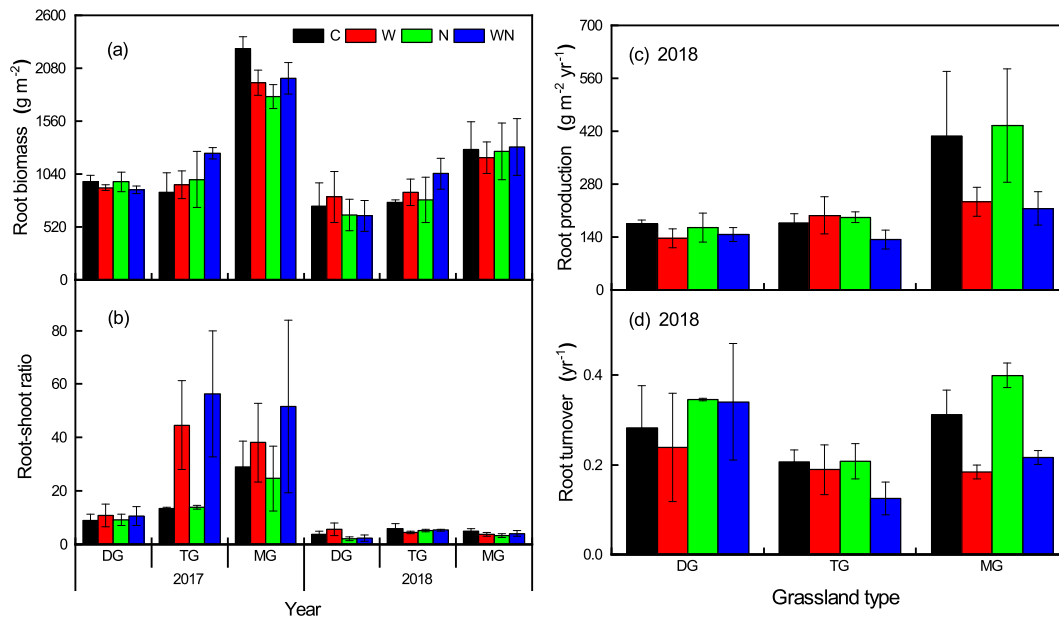


Fig. 5. Effects of warming and N addition on root biomass (a), root-shoot ratio (b), root production (c), and root turnover (d) in desert (DG), typical (TG), and meadow (MG) grasslands. Error bar represented the standard error of the mean. C: control, W: warming, N: N addition, WN: warming plus N addition.

grasslands in 2017 (all $P < 0.01$), but warming increased it in desert grassland and decreased it in typical and meadow grasslands in 2018 (all $P < 0.01$) (Fig. S2). Root-shoot ratio was increased by warming in all grasslands in 2017 (all $P < 0.05$), but it was significantly increased only in desert grassland in 2018 ($P < 0.01$) (Fig. 5b). N addition increased root biomass only in typical grassland in 2017 ($P < 0.01$), but decreased it only in desert grassland in 2018 ($P < 0.05$) (Fig. 5a). Above-ground biomass was marginally increased by N addition in meadow grassland in 2017 ($P < 0.1$), but it was significantly increased by N addition in desert and meadow grasslands in 2018 (both $P < 0.001$) (Fig. S2). N addition increased root-shoot ratio only in typical grassland in 2017 ($P < 0.05$), but it was significantly decreased by N addition in desert and meadow grasslands in 2018 (both $P < 0.05$) (Fig. 5b). Interactive effects of warming and N addition on root-shoot ratio in typical and meadow grasslands in 2017 or desert and meadow grasslands in 2018 were found (all $P < 0.05$).

3.3. Effects of warming and N addition on root production and turnover

In 2018, warming reduced root production in desert and meadow grasslands (both $P < 0.05$), but did not significantly change it in typical grassland (Fig. 5c). Warming decreased root turnover in typical and meadow grasslands (both $P < 0.05$), but had no significant effects on it in desert grassland (Fig. 5d). N addition did not significantly affect root production in all grasslands (Fig. 5c). However, it significantly increased root turnover in desert and meadow grasslands (both $P < 0.05$), but did not significantly change root turnover in typical grassland (Fig. 5d). Interactive effects of warming and N addition on root production only in typical grassland were detected ($P < 0.05$).

3.4. Warming and N addition-induced changes in root traits along the aridity gradient

When pooling all the grassland ecosystems together, aridity had no significant relationships with warming-induced changes in root biomass and root-shoot ratio. However, N addition-induced changes in root biomass and root-shoot ratio (both $P < 0.05$) positively correlated with aridity only in 2017 (Fig. 6a–d). Increased aridity promotes warming-induced changes in root production and turnover (both

$P < 0.05$) in 2018. N addition-induced changes in root production ($P < 0.05$) and turnover ($P < 0.01$) negatively correlated with aridity in 2018 (Fig. 6e and f). However, aridity did not affect changes in root production and turnover under both warming and N addition in 2018 (Fig. 6e and f).

4. Discussion

Our results showed that warming and N addition had interactive effects on root-shoot ratio in different grassland ecosystems in various sampling years. The interactive effects on root production in typical grassland were also detected. These results partially support our hypothesis that warming and N addition affect root traits in an interactive manner. Warming and N addition had negatively additive effects on root turnover in typical and meadow grasslands rather than desert grassland, indicating C input from roots decreased, thus negatively impacting the soil C cycle in wet grasslands. Aridity greatly affected the responses of root production and turnover to warming or N addition, thus largely impacts root C input and soil C cycle in temperate grasslands. However, aridity had little effects on root production and turnover under warming combined with N addition. These results partially support our hypothesis that how root traits respond to warming or N addition largely depends on aridity in temperate grasslands.

4.1. Effects of experimental warming

We found that experimental warming significantly increased root biomass in typical grassland and root-shoot ratios in all grasslands in a dry year (Fig. 5a and b, Table 2), indicating that plants allocate more biomass to roots for obtaining water due to exacerbated dry condition under warming in dry years (Bai et al., 2010; Chapin et al., 1987; Li et al., 2019). Additionally, warming-induced water deficit suppressed microbial activity and decomposition of dead roots (Davidson and Janssens, 2006). The accumulation of dead roots contributed to increase in root biomass (Fig. 1). In desert grassland, the unchanged root biomass under warming may be due to the negative effects of increase in SAP and positive effects of increase in ST on root biomass (Fig. 7a). In meadow grassland, the effects of decrease in SM may offset the effects of increased SAN on root biomass under warming (Fig. 7e). These results

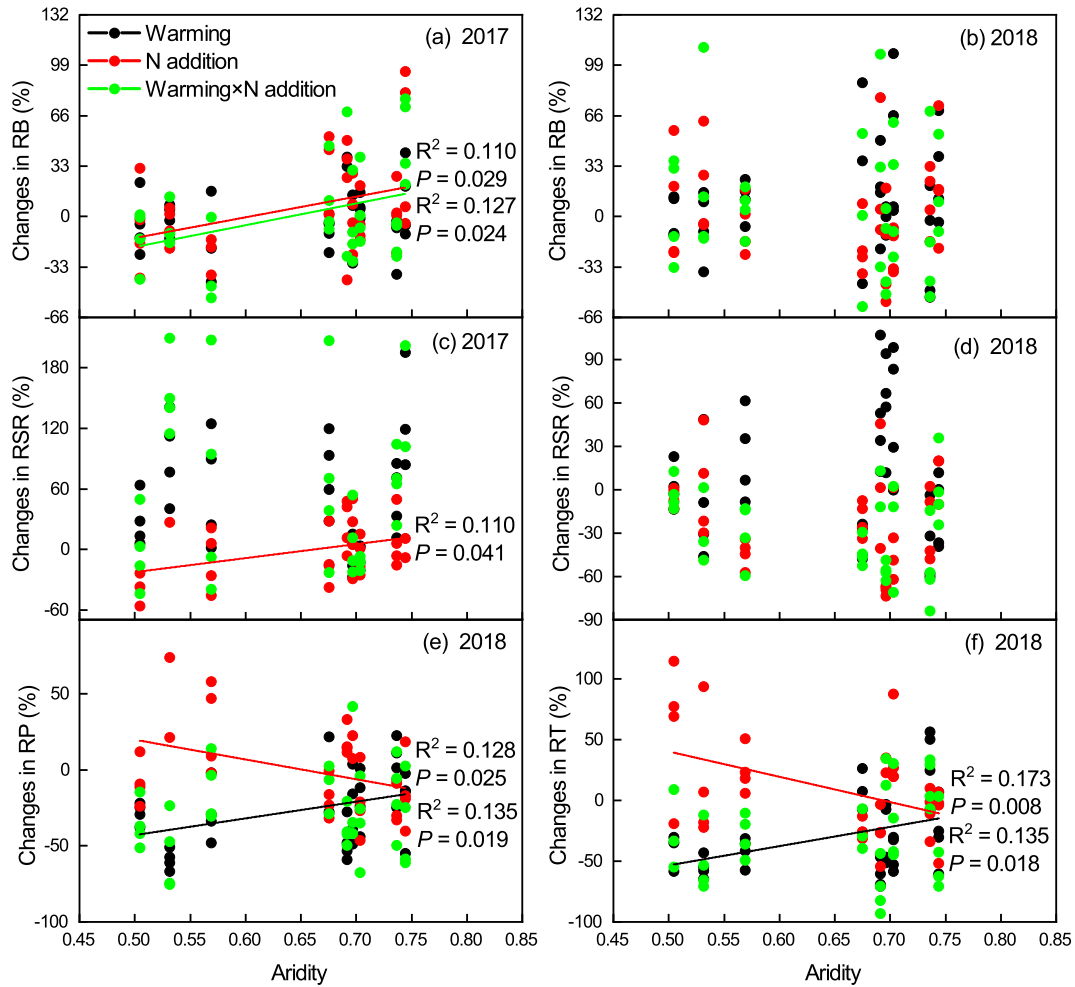


Fig. 6. Regression analysis for warming and N addition-induced changes in root biomass (RB) (a, b), root-shoot ratio (RSR) (c, d), root production (e) and turnover (f) in relation to aridity. Details of the regression analyses are given in each panel.

suggest that warming-induced changes in root biomass in temperate grasslands are jointly affected by soil properties.

Warming can directly suppress photosynthesis due to high temperature (Wertin et al., 2017). Warming-induced decrease in water availability also reduces the absolute amount of carbohydrate supply to roots for growth by reducing plant photosynthesis (Niu et al., 2008).

Table 2

Statistical analysis of main effects and interactive effects of warming (W), N addition (N), and grassland type (G) on root biomass (RB), root-shoot ratio (RSR), root production (RP), and root turnover (RT) in desert (DG), typical (TG), and meadow (MG) grasslands.

Treatment	Grassland type	2017		2018			
		RB	RSR	RB	RSR	RP	RT
W	DG	0.289	0.032	0.531	0.001	0.020	0.442
	TG	0.042	0.000	0.104	0.946	0.309	0.016
	MG	0.626	0.000	0.874	0.573	0.000	0.000
N	DG	0.838	0.717	0.037	0.000	0.975	0.013
	TG	0.009	0.027	0.331	0.324	0.200	0.126
	MG	0.183	0.157	0.679	0.039	0.862	0.042
W × N	DG	0.891	0.838	0.419	0.033	0.391	0.544
	TG	0.254	0.040	0.479	0.134	0.039	0.105
	MG	0.113	0.009	0.568	0.019	0.455	0.336
W × G		0.347	0.001	0.491	0.141	0.001	0.024
N × G		0.036	0.779	0.337	0.016	0.886	0.057
W × N × G		0.222	0.601	0.702	0.068	0.544	0.559

Note: Data represent P-values. Bold font indicates a significant result ($\alpha = 0.05$).

Therefore, warming can inhibited root production in semi-arid grasslands (Fig. 5c, Table 2) (Bai et al., 2010) where the water availability is a key limiting factor (Harpole et al., 2007). However, we found that increased ST had positive effects on root production under warming in typical and meadow grasslands (Fig. 7c and e). Fitter et al. (1999) found that root growth and production are strongly driven by the availability of photosynthate. Our results showed that warming significantly decreased aboveground biomass in typical and meadow grasslands (Fig. S2, Table S2), indicating the plant photosynthesis was largely reduced by warming. Therefore, in semi-arid grasslands, the negative effects of warming-induced decrease in photosynthesis on root production could be stronger than the positive effects of warming-induced increase in ST, thus leading to reduced root production.

Decreased root growth due to warming-induced decrease in photosynthesis (Niu et al., 2008; Wertin et al., 2017) contributed to reduced root turnover. Reduced SM caused by warming suppresses root respiration (Davidson and Janssens, 2006) and mortality (Bai et al., 2010), and decreases soil microbial activity, thus inhibiting microbial decomposition (Kong et al., 2018). Slow root growth, mortality, and decomposition resulted in decreased root turnover under warming (Fig. 5d, Table 2). Decreased root turnover in response to warming indicates reduced C input from roots to soil (McNally et al., 2017; Xiong et al., 2018) in semi-arid grassland ecosystems. This consequence may directly affect soil C and nutrient cycles because most C input to soil is root-derived in grasslands (Poeplau, 2016).

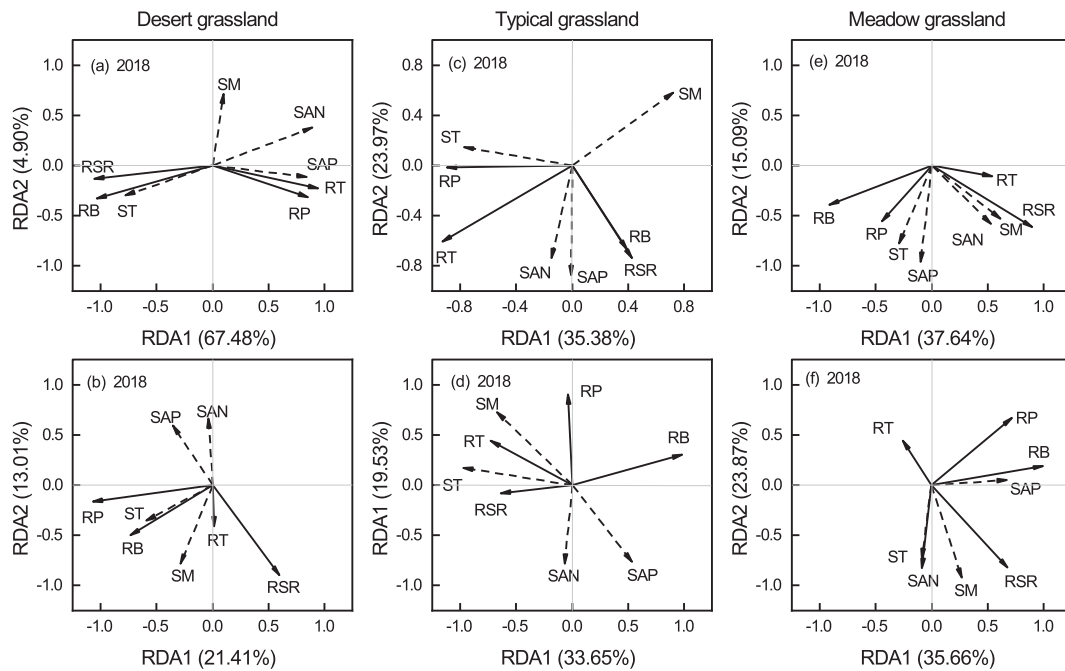


Fig. 7. Redundancy analysis (RDA) of root traits (solid lines) in relation to soil properties (dotted lines) under warming (a, c, e) and N addition (b, d, f). The angle and length of the arrows indicate the direction and strength of the relationships of root traits with soil properties. RB, root biomass; RSR, root-shoot ratio; RP, root production; RT, root turnover; ST, soil temperature; SM, soil moisture; SAN, soil available N; SAP, soil available P.

4.2. Effects of N enrichment

N addition directly increases SAN, allowing plants to allocate less biomass into roots for nutrient acquisition (Li et al., 2019; Poepflau, 2016; Yuan and Chen, 2012). Our results support this view, showing that N addition had a downward trend in root biomass and root-shoot ratio in desert and meadow grasslands and slightly decreased root-shoot ratio in typical grassland in humid years (Fig. 5a and b, Table 2). Our results are consistent with previous studies that reported decreased root-shoot ratios under N addition (Poepflau, 2016; Wang et al., 2018; Yuan and Chen, 2012). However, plants can extract large soil water and decreased soil moisture due to stimulated plant growth by N addition (Fig. S2, Table S2). We found that N addition increased root biomass and root-shoot ratio in typical grassland in a dry year, likely reflecting the fact that plants allocate more biomass to roots for water acquirement (Chapin et al., 1987) due to N addition-induced decline in SM (Fig. 3l, Table S1). Moreover, N addition suppressed root decomposition and increased accumulation of root biomass because N addition-induced water deficit decreased soil microbial activity (Kong et al., 2018) in typical grassland.

In our study, N addition did not significantly affect root production at 0–30 cm soil depth in all grassland ecosystems, consistent with previous studies (Gao et al., 2011; Swindon et al., 2019). Plant production of semi-arid grasslands in Inner Mongolia is co-limited by water and N (Li et al., 2011), and the response of root production to N addition depended on water supply (Gao et al., 2011). Therefore, N addition without enough water supply may little affect root production in semi-arid grasslands.

N addition leads to base cation leaching and soil acidification (Tian and Niu, 2015). Increased soil acidity also causes physical and chemical changes in soils that may accelerate the death of roots (Smithwick et al., 2013). High N concentration in roots due to N addition (Fig. S3, Table S2) may accelerate root respiration and deplete carbohydrates in roots, leading to shortened root life span and a rapid root turnover (Burton et al., 2000; Eissenstat et al., 2000). Additionally, high N concentration in root biomass caused by N addition contributes to microbial decomposition of dead roots (Fig. 1) (Enriquez et al., 1993). Therefore,

N addition promoted root mortality and decomposition, thus enhanced root turnover in desert and meadow grasslands (Fig. 5d, Table 2). Moreover, N addition-induced decrease in SM shortened life span (Bai et al., 2008), contributing to the significant increase in root turnover in meadow grassland (Fig. 7f). Our results in desert and meadow grasslands are consistent with a meta-analysis which indicating that N addition increased root turnover in grasslands (Peng et al., 2016). However, in typical grassland, N addition-induced low SM suppressed microbial decomposition (Kong et al., 2018; Reed et al., 2009) of roots (Fig. 7d), and N addition had little effects on N concentration in root biomass (Fig. S3, Table S2). These results contributed to slight decrease of root turnover under N addition in typical grassland. The increased root turnover in response to N addition in our study implies that N addition can stimulate root C input and soil C cycle (McNally et al., 2017) in desert and meadow grasslands.

4.3. Effect of warming combined with N addition on root traits

Our analysis showed that warming and N addition had synergistic effects on root-shoot ratio in typical and meadow grasslands in 2017 (Fig. 5b, Table 2). The results may be due to N addition exacerbating warming-induced water deficit (Fig. 3l). However, warming and N addition had antagonistic effects on root-shoot ratio in desert grassland but synergistic effects on it in meadow grassland in 2018 (Fig. 5b, Table 2). These results may be ascribed to significant increase in SAN in desert grassland and significant decline in SM in meadow grassland caused by N addition (Figs. 3l and 4a, Table S1). These results indicated that soil water and nutrient play an important role in biomass allocation of plants under both warming and N addition in semi-arid grasslands (Kang et al., 2013; Li et al., 2019; Luo et al., 2013).

We also found antagonistic effects of warming and N addition on root production in the 0–30 cm soil layer in typical grassland (Fig. 5c, Table 2). That is, in typical grassland, warming had no significant effects on root production under ambient N addition, but decreased it under N addition. The result may be due to N addition-induced increase in soil N (Fig. 7d) (Li et al., 2019). Our results also showed that warming combined with N addition had negatively additive effects on root production

in meadow grassland and root turnover in typical and meadow grasslands (Fig. 5c and d, Table 2). These results indicate that global warming combined with N deposition can reduce C input from roots and may negatively affect soil C and nutrient cycles (Aerts et al., 1992; McNally et al., 2017; Xiong et al., 2018) in wet grasslands in the future. However, the additive effects of warming combined with N addition did not significantly change root turnover in desert grassland, which indicating root C input will stay stability under both global warming and N deposition in desert grassland.

4.4. Responses of root production and turnover to warming and N addition depend on water availability along the aridity gradient

In dry years, plant growth largely relies on soil water availability in semi-arid grasslands (Li et al., 2011). Aridity plays a major role in C allocation between aboveground parts and roots under N addition in dry years. Compare to humid areas, low water availability in dry grasslands impedes soil microbes to use input nutrients more effectively and suppresses microbial activity (Hungate et al., 2007; Liu et al., 2009). Decreased microbial activity suppresses root decomposition and promotes accumulation of root biomass (Fig. 1) (Kong et al., 2018; Ngao et al., 2007). Therefore, when we pooled grassland ecosystems together, we found that aridity gradient promoted the effects of N addition on root biomass and root-shoot ratio only in dry years (Fig. 6a–d). However, aridity gradient did not influence how warming affects root biomass and root-shoot ratio in semi-arid grasslands, reflecting the long-term adaptation of biomass allocation to aridity under warming (Yuan et al., 2017). Under warming, though plants could allocate more biomass to roots due to short-term reduction in soil water in manipulative experiment (Fig. 5b), plant biomass allocation will be remained unchanged with long-term aridity based on gradient observation (Fig. 6c and d).

Low water availability in soil in dry areas exacerbates warming-induced drought, promoting plants to allocate more biomass to roots for water foraging (Bai et al., 2010; Chapin et al., 1987; Li et al., 2019) and decreasing root life span (Chen and Brassard, 2013). Thus, increased aridity positively affected warming-induced changes in root production and turnover (Fig. 6e and f), which is consistent with a previous meta-analysis (Wang et al., 2019). Our results suggest that warming results in higher C input to soil from roots in dry grasslands than in wet grasslands. However, aridity suppressed N addition-induced changes in root production and turnover (Fig. 6e and f). Compare to wet grasslands, lower water availability in soil in dry grasslands suppressed urease activity (Sardans and Penuelas, 2005) and the transformation of added urea, decreased microbial activity and root decomposition (Kong et al., 2018), and inhibited root growth (Bai et al., 2010), thus decreasing root production and turnover. Our results are consistent with a previous meta-analysis which reported that N addition decreased root production and turnover in dry areas (Peng et al., 2016).

However, changes in root production and turnover induced by warming combined with N addition were not associated with aridity, suggesting effects of warming and N addition were offset by each other along aridity gradient. The result implies that aridity may have little effects on root C input under warming combined with N addition.

5. Conclusion

In this study, we investigated how warming and N addition affected root traits in three temperate grassland ecosystems along a natural aridity gradient. We found: 1) Warming had minor effects on root biomass, but increased root-shoot ratio in dry year due to decline in soil water. Root production and turnover were trend to be decreased by warming, which likely reduces root C input in semi-arid temperate grasslands. Effects of N addition on root biomass varied with grassland type, but N addition decreased root-shoot ratio in humid year due to increase in soil N. However, N addition had little effects on root production. In contrast, N

addition increased root turnover in desert and meadow grasslands rather than typical grassland and it may promote root C input in these regions. 2) Warming and N addition had antagonistic effects on root-shoot ratio in wet grasslands in dry year, but antagonistic effects on root-shoot ratio in dry grasslands and synergistic effects in wet grasslands were detected in humid year. Warming and N addition had antagonistic effects on root production in typical grassland but negatively additive effects on root turnover in typical and meadow grasslands rather than desert grassland. These results indicate that C input from roots decreased, thus negatively impacting the soil C cycle in wet grasslands. 3) Increasing aridity promoted the effects of warming on root production and turnover but suppressed the effects of N addition on them. However, aridity did not impact root production and turnover under both warming and N addition, suggesting root C and nutrients input may be little affected by aridity under both global warming and N deposition in temperate grasslands. Our study provides a more comprehensive perspective for understanding how warming and N addition affect root traits on a regional scale in temperate grasslands.

CRediT authorship contribution statement

Chuang Yan: Investigation, Data curation, Formal analysis, Writing - original draft, Visualization. **Zhiyou Yuan:** Funding acquisition, Project administration, Conceptualization, Methodology, Writing - review & editing. **Zunchi Liu:** Investigation, Resources. **Jingjing Zhang:** Investigation, Resources. **Kai Liu:** Investigation, Resources. **Xinrong Shi:** Writing - review & editing. **T. Ryan Lock:** Writing - review & editing. **Robert L. Kallenbach:** Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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