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Effects of nitrogen addition on root traits and soil nitrogen in the long-term restored grasslands

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Abstract: Fine root traits are plastic and responsive to increased nitrogen (N) deposition. However, with the restoring of the ecosystem after grain for green, little research has been reported about the response of root traits in a long-term restored ecosystem to increased N deposition. Therefore, a successive N addition experiment was conducted in a long-term restored grassland on the Loess Plateau to analyse the effects of different N addition levels (0, 2.5, 5, 10, 20 g N/m²/year) on root morphological traits, soil carbon (C) and N. Our results showed that root morphological traits (except for root diameter) firstly increased and then declined, with the maximum in the N level of 5 g/m²/year. N addition significantly increased soil organic carbon, total nitrogen, ammonium nitrogen (NH₄⁺-N) and nitrate-nitrogen (NO₃⁻-N) with the increasing N addition level, especially in the soil surface layer. Specific root length and specific root area had remarkable negative correlations with NO₃⁻-N, while root diameter and root length density had positive correlations with soil available N and soil microbial biomass carbon. This study indicated that plants could have the threshold response to adapt to the N addition and prefer to slowly grow rather than quickly invest and return in order to adapt to the environmental stress.

Keywords: macronutrient; nitrogen cycle; growth strategy; nitrification; available nitrogen

With extensive combustion of fossil fuels, heavy application of nitrogen (N) fertiliser, N fixing plant cultivation, and expansion of animal husbandry, global atmospheric N deposition has increased by approximately three- to five-fold over the past century (IPCC 2013) and is predicted to considerably increase in the coming years. A large amount of active N in the atmosphere subsequently increases available N across various ecosystems. Terrestrial ecosystems' underground processes play critical roles in terrestrial carbon (C) and N cycles (Ma et al. 2018, López-Angulo et al. 2020). Especially, plant fine roots and soil nutrient availability are sensitive to the

increase of N deposition (Wu et al. 2021). Therefore, it is essential to study the effects of atmospheric N deposition on root trait, soil C and N to understand ecosystem nutrient cycles under future scenarios of increasing N deposition.

Over the past few decades, a number of simulated field studies about the effects of increasing N input on the belowground community have been well-conducted (Bai et al. 2010, Chen et al. 2015a). On one hand, enhanced N deposition through N addition not only changes soil N availability but also induces soil acidification, which may influence N transformation processes (i.e., N mineralisation and nitrification),

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carbon cycle, and microbial activity (Gao et al. 2015, Chen et al. 2015b). On the other hand, enhanced N deposition affects root traits directly by damaging root tissues and mycorrhiza and indirectly by increasing the availability of soil nutrients (Yue et al. 2016, Zheng et al. 2019). However, effects (positive, neutral, and negative) of increased N deposition on belowground biomass were inconsistent because of inherent variability in ecosystem properties and environmental factors. Furthermore, less is known about how the impacts of N deposition on the relationships between soil C, N and root traits, especially in long-term restored grasslands after grain for green.

In this study, we carried out an N addition experiment in the different levels with a randomised complete block in a long-term restored grassland on the Loess Plateau. N addition, significantly decreased soil pH (Bejarano et al. 2014), which indicated that soil acidification had the potential to disrupt root morphology traits (Zheng et al. 2019). Therefore, our hypotheses regarding the effects of five years cumulative N addition were: (1) alteration of root morphology traits; (2) an increase of soil C and N; and (3) a positive relationship between available N and

root morphology traits. The objective of this research was to reveal how root morphology traits respond to N deposition in the long-term restored grassland ecosystem, which could be helpful to understand the growth strategies of plants for the restored ecosystem in response to face the increasing N deposition.

MATERIAL AND METHODS

Establishment of the sampling plots. This study was carried out in the grassland of the Yunwu Mountain National Natural Reserve, protected as a long-term monitoring site since 1982, and was located on the Loess Plateau of China (106°21'–106°27'E, 36°10'–36°17'N). The study area has a semiarid climate within the middle temperate zone. The mean annual temperature is 5 °C, with the daily average maximum and minimum temperatures occurring in July (24 °C) and January (–14 °C), respectively. The mean annual precipitation is 425 mm, 60–75% of which falls from July to September. The vegetation community consists of 297 plant species and is dominated by *Stipa bungeana*, *S. grandis*, *S. przewalskyi*, *Artemisia sacrorum*, and *Thymus mongolicus*. The soil type is montane

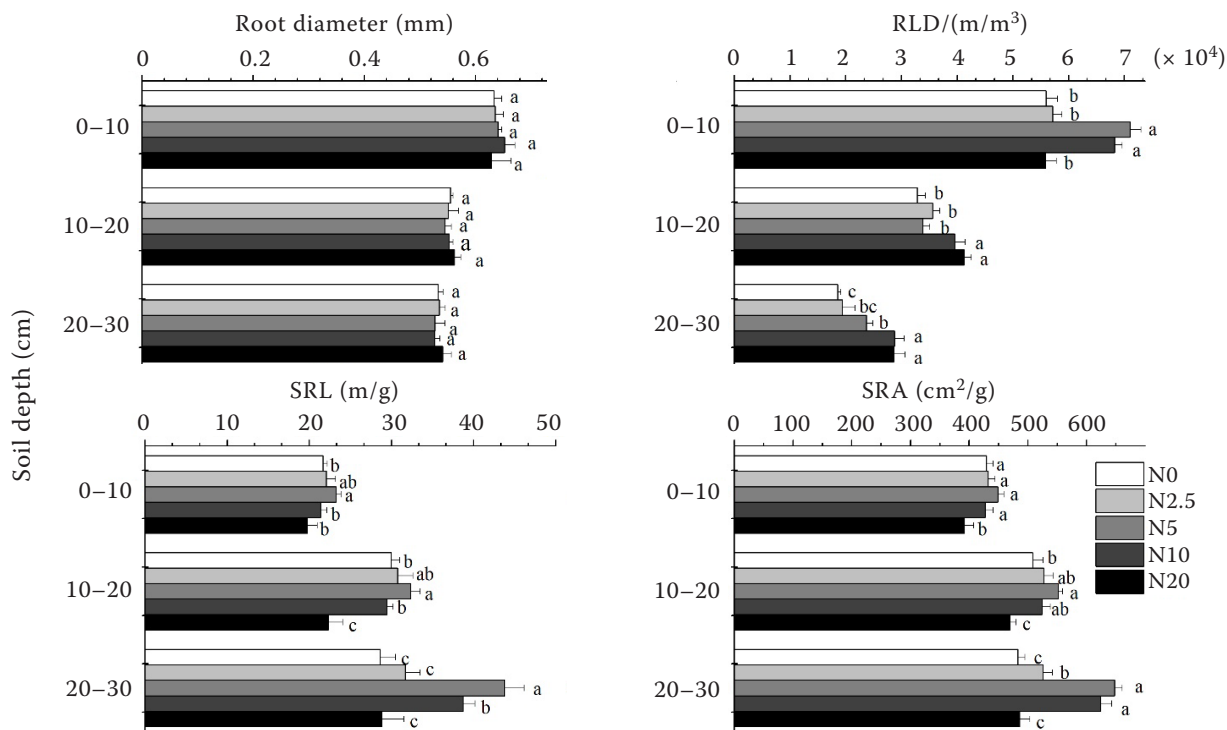


Figure 1. Changes of root morphological traits in each depth between different nitrogen (N) addition levels. Different letters mean a significant difference at 0.05 level in each depth between different N addition. RLD – root length density; SRL – specific root length; SRA – specific root area; N0 – 0, N2.5 – 2.5, N5 – 5, N10 – 10, N20 – 20 g N/m²/year

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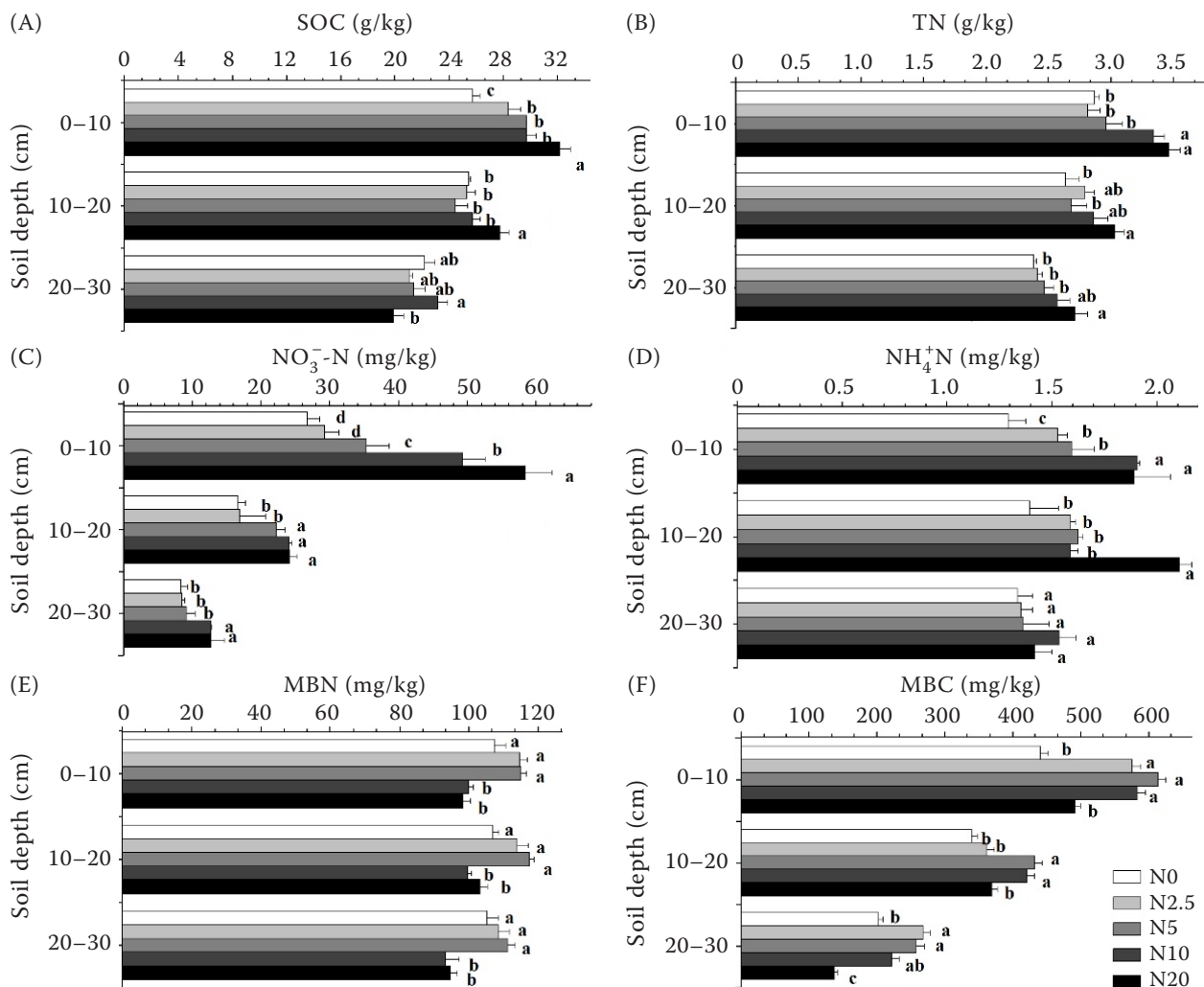


Figure 2. Changes of soil carbon and nitrogen in each depth between different nitrogen (N) addition levels. Different letters mean a significant difference at 0.05 level in each depth between different N addition. SOC – soil organic carbon; TN – total nitrogen; $\text{NH}_4^+ \text{-N}$ – ammonium nitrogen; $\text{NO}_3^- \text{-N}$ – nitrate nitrogen; MBN – microbial biomass nitrogen; MBC – soil microbial biomass carbon; N0 – 0, N2.5 – 2.5, N5 – 5, N10 – 10, N20 – 20 g N/m²/year

grey-cinnamon soil, classified as a CalciOrthic Aridisols according to the Chinese taxonomic system. The mean values of pH, total nitrogen (TN), soil organic carbon (SOC), ammonium nitrogen ($\text{NH}_4^+ \text{-N}$) and nitrate nitrogen ($\text{NO}_3^- \text{-N}$) were 8.65, 1.71 g/kg, 31.19 g/kg, 1.97 mg/kg, 33.37 g/kg, respectively. Our experiments were conducted in the long-term restored grassland, with undisturbed mature grassland community. Urea ($\text{CH}_4\text{N}_2\text{O}$) fertilisers were applied to the experimental plots at the level of N was added at 0, 2.5, 5, 10, and 20 g N/m²/year in the end of May in the early growing period for the successive years from 2014 to 2018 to simulate the change of N deposition, referring to Bai et al. (2010). Weather conditions are shown in Figure 1.

Accordingly, N addition levels were short for N0, N2.5, N5, N10, and N20, respectively. There were eight replicates for each of five treatments which included a control treatment (i.e., no N addition) and four levels of N addition. Forty plots (6 m × 10 m) were randomly laid out in a randomised complete block design. Plots were separated by 2-meter walkways. In brief, five N addition levels were established with each treatment replicated eightfold resulting in 40 plots sampled in total. The experimental design is shown in Figure 2.

Field sampling and measurements. Because root and soil were less insensitive to N addition than aboveground community, we just collected soil samples containing roots in 2018, not each year

after N addition. In each plot, three soil samples were randomly collected at a depth of 0–10, 10–20 and 20–30 cm by using a bucket auger (9.0 cm in diameter) regardless of community composition, then soil samples of the same layer were mixed into one sample. Therefore, 120 soil samples containing roots were collected (detailed sample size shown in Table 1). Roots were picked out from each soil sample with tweezers and then washed with distilled water to remove tightly attached organic matter and mineral soils. It was difficult to identify the species of each root. All the roots in each plot were as one sample and were not measured separately. Roots were spread on a transparent plastic tray and scanned at a resolution of 300 dpi (Epson Scanner, 10000XLPro, Nagano-ken, Japan). Root images were analysed with WinRhizoPro software (V2012b, Régent Instruments, Québec, Canada) to calculate the parameters, including mean root diameter (RD, mm), cumulative root length (m), cumulative root surface area (cm²) (Su et al. 2017, Zheng et al. 2019). After scanned, roots were oven-dried for 48 h at 65 °C and then weighed to gain root mass. Root biomass, root length density, specific root length and specific root area are calculated in equations as follows: root length density (RLD, m/m³) = root length/sampling volume; specific root length (SRL, m/g) = root length/root mass; specific root area (SRA, cm²/g) = root surface area/root mass.

After removing the roots, the remaining soil sample was divided into two parts. One part was air-dried to determine SOC and N. SOC was measured using

the dichromate oxidation method. TN was analysed by an automatic Kjeldahl apparatus (2300, Haganas, Sweden). The second part was kept fresh at 4 °C for measuring soil available nitrogen and soil microbial biomass. NH₄⁺-N and NO₃⁻-N were measured by continuous flow auto-analysis (AutAnalyel, Bran + Luebbe GmbH + Norderstedt, Germany). Soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were measured using the chloroform fumigation extraction method. The experimental determination methods of soil C and N contents were similar to Su et al. (2017).

Statistical analysis. A two-way analysis of variance (ANOVA) followed by Tukey's *HSD* (honestly significant difference) test was conducted to determine the effect of N addition level and soil depth on grassland root traits (RD, RLD, SRL, and SRA) and soil properties (SOC, TN, NH₄⁺-N, NO₃⁻-N, MBC, and MBN). *P*-values of less than 0.05 were considered to indicate statistical significance. The statistical analyses were conducted using IBM SPSS 20.0 (IBM, Chicago, USA). Redundancy analysis (RDA) was used to assess the relationship between soil C and N content and root traits by Canoco 5.0 after logarithmic transformation (ter Braak and Smilauer 2012). Graphs were created with Origin 8.5 (Systat Software, Northampton, USA).

RESULTS AND DISCUSSION

Effects of N addition on root morphology. N addition influences plant root systems directly by injuring tissues and indirectly by changing soil N

Table 1. Analysis of variance results for all the variables (*n* = 120)

		Nitrogen addition			Soil depth			Nitrogen addition × soil depth		
		<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
Root traits	SRL	4	72.039	< 0.0001	2	392.204	< 0.0001	8	22.977	< 0.0001
	SRA	4	30.619	< 0.0001	2	137.41	< 0.0001	8	8.38	< 0.0001
	RLD	4	40.828	< 0.0001	2	1 564.7	< 0.0001	8	18.06	< 0.0001
	RD	4	0.096	0.983	2	76.759	< 0.0001	8	0.323	0.951
Soil C and N	TN	4	21.932	0.001	2	86.405	< 0.0001	8	2.281	0.049
	SOC	4	6.037	< 0.0001	2	178.517	< 0.0001	8	7.479	< 0.0001
	MBC	4	68.27	< 0.0001	2	1 288.369	< 0.0001	8	12.531	< 0.0001
	MBN	4	25.716	< 0.0001	2	6.231	0.005	8	0.389	0.918
	NO ₃ ⁻ -N	4	262.054	< 0.0001	2	2 392.915	< 0.0001	8	86.345	< 0.0001
	NH ₄ ⁺ -N	4	61.681	< 0.0001	2	67.993	< 0.0001	8	15.974	< 0.0001

SRL – specific root length; SRA – specific root area; RLD – root length density; RD – root diameter; TN – total nitrogen; SOC – soil organic carbon; MBC – soil microbial biomass carbon; MBN – microbial biomass nitrogen; NH₄⁺-N – ammonium nitrogen; NO₃⁻-N – nitrate nitrogen

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availability (Galloway et al. 2004, Zheng et al. 2019). For example, N addition significantly increased root length density to improve root activity in the severely degraded grassland (Qin et al. 2014). N addition increased significantly fine root diameter and SRL to improve N absorption at the low level (Yan et al. 2017). In our study, the results supported basically the first hypothesis. Except that root diameter changed slightly with no significant difference, RLD, SRL and SRA were significantly affected by N addition level (Table 1). SRA and SRL firstly increased and then declined, with the maximum at the level of N5 (Figure 1), which showed that plant has the threshold response to adapt to the N addition. The possible reason was that N addition relieved nutrient restriction and increased the contact area between root and soil to enhance SRL and SRA (Leuschner et al. 2013). However, N addition in the high level might cause aluminum (Al) toxicity due to the decreasing pH (Vanguelova et al. 2007), which limited root absorption capacity. So root morphological traits did not continue to rise with the increase of N addition, which were also found in the Inner Mongolia steppe (Zheng et al. 2019).

Effects of N addition on soil C and N. N addition has diverse effects on soil C content by altering C allocation and turnover, varying N addition levels. For example, N addition in the low level had no significant effects on SOC (Fornara et al. 2013), while N addition in the high level declined the SOC (Liu et al. 2016). In our study, N addition in the high level significantly increased SOC in the depth of 0–10 cm by 20.8% (Figure 2A) because N addition promoted plant growth and increased the input of organic matter by plant residues. However, soil organic carbon was relatively stable and had an insensitive response to short-term N addition (Zhang et al. 2017), so N addition had no significant influence on SOC in the depths of 10–20 cm and 20–30 cm. The results indicated that the effects of N addition on SOC mainly concentrated on the soil surface layer.

N addition directly increases mineral nitrogen content in the soil and litter layers, which promotes soil N mineralisation and nitrification and increases soil available N (Bai et al. 2010). Soil TN, $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ contents were significantly affected by N addition and soil depth (Table 1). The response of available soil N to N addition in the surface layer was more sensitive to N addition than that in other depths. For example, compared to the control treatment, N20 increased soil $\text{NO}_3^-\text{-N}$ in the depths of

0–10, 10–20 and 20–30 cm by 118.2, 44.4 and 52.1%, respectively (Figure 2), supporting the second hypothesis. Furthermore, the effects of N addition on soil $\text{NO}_3^-\text{-N}$ was relatively stronger than on soil $\text{NH}_4^+\text{-N}$, similar to the study of Su et al. (2016). One possible reason was that redundant $\text{NH}_4^+\text{-N}$ could be converted to $\text{NO}_3^-\text{-N}$ when $\text{NH}_4^+\text{-N}$ produced by soil ammoniation exceeded the amount of plant absorption and microbial fixation. The other possible reason was that N addition might improve the ratio of soil nitrification to soil mineralisation, which led to the $\text{NO}_3^-\text{-N}$ accumulation (Bejarano et al. 2014).

Although soil microbial biomass only accounts by 3% of the soil matter, soil microbial biomass was the most active component in soil and sensitive to human activities. Short-term N addition might have various impacts on soil microbial biomass (i.e., increase, decrease or no effect) (Dijkstra et al. 2005, Liu et al. 2007, Shen et al. 2014). In our study, soil MBC and soil MBN increased after N addition with the maximum in N5 level (Figure 2E–F). The increase of soil MBC and soil MBN in the low level of N addition was because that N addition improved the release of plant C and N into the soil. However, excess nitrogen in the high level of N addition decreased the soil pH, which increased the biological toxicity of exchangeable aluminum ions (Vanguelova et al. 2007, Tu et al. 2015). The efficiency of conversion of plant carbon and nitrogen into microbial biomass carbon and nitrogen reduced under the high level of N addition (Craig et al. 2021). So soil MBC and soil MBN declined with the increase of N addition.

Relationships between root morphological traits and soil C and N. Generally, fine roots can improve their ability to absorb soil nutrients and water by increasing root length density or changing root morphological traits when soil resources availability increased (Meinen et al. 2009, Zheng et al. 2019). The change of root morphology, in turn, affected soil nutrients through altering the root turnover to the soil. For example, increased soil N availability resulted in a reduction in fine root biomass, SRL, and SRA (Wang et al. 2013, Chen et al. 2016). Our study showed that increased soil N availability altered significantly root morphological traits. The possible reason was that root morphology was more plastic and responsive to changes in the soil environment than root production (Chen et al. 2016). Different root morphological traits have diverse impacts on root physiological functions, like increased water and nutrient transport, to reveal resource acquisi-

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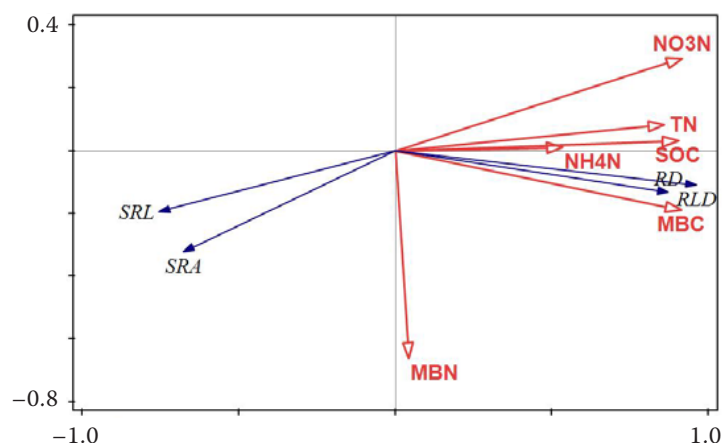


Figure 3. Redundancy analysis (RDA) between root morphology, soil carbon and nitrogen ($n = 120$). SRL – specific root length; SRA – specific root area; RLD – root length density; RD – root diameter; TN – total nitrogen; SOC – soil organic carbon; MBC – soil microbial biomass carbon; MBN – microbial biomass nitrogen; $\text{NH}_4^+\text{-N}$ – ammonium nitrogen; $\text{NO}_3^-\text{-N}$ – nitrate nitrogen

tion mechanisms (Wang et al. 2018). Therefore, the responses of different root morphological traits to availability N had significant heterogeneity, contrary to the third hypothesis. SRL and SRA had remarkable negative correlations with $\text{NO}_3^-\text{-N}$, while RD and RLD had positive correlations with soil availability N and soil C, especially with MBC (Figure 3). This research indicated that N addition in the long-term restored grassland degraded the root functions (i.e., respiration rate), especially in the high rate of N addition. Plants preferred to face environmental stress through extending root lifespans with wide RD and long RLD. For example, plants tended to invest more C in long-lived roots (Tu et al. 2015) for increasing C use efficiency when soil N availability increases because of N addition.

In conclusion, N addition had diverse effects on root morphological traits, soil C and N contents (i.e., increased soil available N, altered root morphological traits) in the long-term restored grassland. This could demonstrate that plants had the threshold response to adapt to the N deposition. SRL and SRA degraded with the increasing N deposition in the long-term restored grasslands, and plants tended to extend root lifespans with wide RD and long RLD to face N deposition. These comparisons revealed that plants preferred to slowly grow rather than quickly invest and return to adapt to the increasing N deposition. Thus, this study could enrich the research about the effects of N deposition on the belowground community and provide a reference to understand the growth strategies of plants for the restored ecosystem in response to face the environmental stress.

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