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ORIGINAL PAPER

Effects of Short-term N Addition on Fine Root Morphological Features and Nutrient Stoichiometric Characteristics of *Zanthoxylum bungeanum* and *Medicago sativa* Seedlings in Southwest China Karst Area

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Abstract
The aim is to explore the response law and adaptation mechanism of karst plants to N deposition and provide scientific bases for the vegetation restoration and social-economic sustainable development of the southwest China karst area. *Zanthoxylum bungeanum* and *Medicago sativa* seedlings were selected as study materials. Responses of the fine root morphological features and stoichiometric characteristics of C, N, P, Ca, and Mg to short-term (1 year) N addition were studied by pot experiments. There were significant differences in specific root length (SRL) and specific surface area (SSA) of 0–0.5 mm diameter fine root of *M. sativa* seedlings between different N additions, but no remarkable effect of N additions on those of *Z. bungeanum* seedlings. Short-term N additions increased N content, decreased C content and C:N, but did not significantly affect Mg and Ca content of the both two plant fine roots. Short-term N additions significantly affected rhizosphere soil N, NO_3^--N, and NH_4^+-N content, C:P and N:P of the two plants tested. One-year short-term N addition significantly affected the fine root morphology of *M. sativa* and the nutrient stoichiometric characteristics of *Z. bungeanum* and *M. sativa* fine roots and rhizosphere soils. Different N addition levels caused different effects on the fine root morphology and nutrient stoichiometric characteristics. Impacts of N additions on nutrient stoichiometric characteristics of *M. sativa* (N fixing plant) fine roots and rhizosphere soils were more remarkable than those in *Z. bungeanum* (non-N fixing plant). Changing fine root SRL and SSA to optimize nutrient absorption ability was the important response strategy of these two plants tested under the N deposition context.

Keywords N deposition · Fine root · Rhizosphere soil · Stoichiometry · Response

1 Introduction

Since the Industrial Revolution, the amount of atmospheric nitrogen (N) content in the twentieth century had increased 3–5 times by the combustion of massive fossil fuel and increasement of agricultural fertilization, and the increase rate of global N deposition was expected to increase 1–2 times of 2000s by the 2050s (Davidson 2009; Janssens et al. 2010; Jia et al. 2016). At present, atmospheric N deposition in southwest China has exceeded 22.2 kg N $hm^{-2}\cdot a^{-1}$. Southwest China is not only one of the three major N deposition areas in China, but also one of the regions with high N deposition in the whole world. In this area, the N deposition continues to rise (Liu et al. 2013). Appropriate amounts of N deposition can promote plant growth and increase ecosystem productivity, but excessive N deposition suppresses plant growth and causes the negative and potential effects of reduced biodiversity and soil acidification (Verma and Sagar 2020; Camarero and Carrer 2017). N deposition causes the changes in soil nutrient environment by affecting the soil available N content, and plants adapt to the changes in soil nutrient environment firstly through root self-regulations,

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such as changing root biomass, form, nutrient content and ratio, and physiological activity. This is the important adaptation strategy of plants to address changes in the soil environment (Yan et al. 2017; Dong et al. 2020). Fine root, the part with diameter ≤ 2 mm in root system, is a key organ for nutrition and water circulation in plants and the most active and sensitive part of the plant root system (Knute 2000; Carfora et al. 2021). It plays extremely important roles in plant adaptation to soil environmental changes. Therefore, studying the effects of N deposition on plant fine roots will help to elucidate plant growth strategies for adapting the environmental changes of N deposition. In particular, in fragile karst ecosystems, this study has great scientific and practical significances for the protection and restoration of regional vegetation under the N deposition context of global climate change.

Effects of N deposition on plant fine root were very complicated and jointly driven by many factors such as climate, soil, and plant interaction. There still are many scientific problems need to be clarified and there are obvious conflicts in the research results obtained (Freschet et al. 2017; Drescher et al. 2020; Li et al. 2021a). Some studies showed that N deposition could significantly increase the specific root length and specific surface area of plant fine roots (Freschet et al. 2017; Chen et al. 2018). However, some other studies obtained the different conclusion that N deposition had not significant effects on fine root morphology (Li et al. 2015). This inconsistency may be related to the differences of N addition quantity and duration time, and also can be attributed to the physiological demand differences of plant itself for N nutrient. N deposition also significantly affects nutrient stoichiometric characteristics of fine roots and rhizosphere soils. Most studies showed that N deposition could improve the available N content and reduce the concentrations of C and P both in fine roots and rhizosphere soils, resulting in the lower C:N and aggravating the P limitation in plant root systems and rhizosphere soils (Chang et al. 2018; Li et al. 2021a). Recent results showed that N deposition could affect the physiological and ecological function of plant roots by affecting K, Ca, and Mg content in the roots and rhizosphere soils (Chen et al. 2017; Liu et al. 2017). And there were obvious differences in effects of N deposition on the nutrient stoichiometric characteristics between the karst ecosystem and other ecosystems because of the special soil and geology context in southern China karst area. Nevertheless, it can be seen that the responses of plant fine roots to N deposition were still unclear, and it was very urgent to conduct systematic researches in this study field. In particular, in the fragile karst ecosystems of southwest China, insufficient research of this study field has obviously hindered the regional vegetation restoration and social-economic development under the remarkable N deposition context.

Southwest China Karst with area of over 550,000 km^2 is the largest karst contiguous distribution area and one of the extremely fragile ecosystems in the whole world (Wang et al. 2021). In Southwest China Karst, the fragile ecological environment combined with the long-term unreasonable social-economic activities of human beings have caused serious vegetation damage, soil erosion, and rock exposure, leading to the severe ecosystem degradation and forming the karst rocky desertification (KRD) (Sheng et al. 2018). The KRD control and rehabilitation has become an important task in the regional economic construction and social development of Chinese government. In the KRD vegetation restoration, plants of *Zanthoxylum bungeanum* and *Medicago sativa* are the typical control species and have played important roles because these two plants can adapt the habitats and have good effects in water and soil conservation (Guo et al. 2020). In addition, these two plants have great economic value and have been cultivated widely in Southwest China, obviously promoting the regional social-economic development. However, so far, there is almost zero in studies on the growth response of *Z. bungeanum* and *M. sativa* to N deposition although N deposition is serious and continues to rise in Southwest China Karst. The lack of these studies seriously limited the sustainable utilization of *Z. bungeanum* and *M. sativa* in the KRD control and regional economic development.

Under the N deposition increase in Southwest China Karst, the follows can be hypothesized: (1) As the most active and sensitive part of root system, the fine root morphology and nutrient stoichiometric characteristics of *Z. bungeanum* and *M. sativa* can be significantly affected by N deposition; (2) different N deposition levels can cause obviously opposing effects of promotion and inhibition on fine root growth of *Z. bungeanum* and *M. sativa*; (3) responses of the both species to N deposition may be totally different because *M. sativ* is a nitrogen fixing plant and its requirement to N supply is different with *Z. bungeanum*. So, in the present study, to verify these hypotheses, the *Z. bungeanum* and *M. sativa* seedlings were selected as study materials, the responses of fine root morphological features and stoichiometric characteristics of C, N, P, Ca, and Mg to short-term (1 year) N addition were studied, and the fine root response strategies of these two plant species tested to the soil environmental changes caused by the N additions were inferred. Results can provide scientific evidences for the further studies on responses of plant growth to N deposition and the scientific cultivation of these two plants tested in Southwest China Karst under the N deposition text.

2 Materials and Methods

2.1 Field Site and Experimental Design

The study site (106° 43′ E and 26° 35′ N, elevation 1078 m a.s.l.) is located in Guiyang city of Guizhou, SW China. The climate belongs to subtropical monsoon humid climate. Long-term (1980–2020) mean annual precipitation, temperature, sunshine hour, and relative humidity is 1178 mm, 15.2 °C, 1214.6 h, and 85%, respectively.

The experiments were conducted by pot experiments. The sizes of pots cultivating *Z. bungeanum* and *M. sativa* were 100 cm × 100 cm × 50 cm and 80 cm × 45 cm × 45 cm (long × wide × high), respectively. Planting matrix was the yellow lime soil coming from the local karst soil with pH of 7.1 ± 0.18, SOC (soil organic carbon) content of 30.8 ± 1.52 g·kg^{-1}, total N content of 1.89 ± 0.13 g·kg^{-1}, and total P content of 0.68 ± 0.02 g·kg^{-1}. The soil depth, water condition, and other factors in the nature also have been simulated in the experiments. The *Z. bungeanum* seedlings and *M. sativa* seeds tested were from a typical KRD area of Guanling county, Guizhou province, Southwest China. The two plant materials were set with different repetitions because *Z. bungeanum* is woody and *M. sativa* is herbaceous. In March 2019, *Z. bungeanum* seedlings of 1 year old were transplanted to the pots, 6 were planted in each pot at horizontal and vertical intervals of 25 cm. *M. sativa* seeds were sown in the pots and 15 seedlings were reserved per pot at horizontal and vertical intervals of 15 cm after sprouted for 30 days. The seedling watering was decided by soil moisture tested by a soil moisture meter. According to the local N deposition level, four gradient treatments of N addition, i.e., control (CK, 0 g N m^{-2}·a^{-1}), low N (LN, 5 g N m^{-2}·a^{-1}), middle N (MN, 10 g N m^{-2}·a^{-1}), and high N (HN, 20 g N m^{-2}·a^{-1}), were set. Each treatment on *Z. bungeanum* and *M. sativa* seedlings was conducted by 3 and 5 repeats, respectively. NH_4NO_3 was used as the N addition substance, and the each month amount of NH_4NO_3 addition was calculated. Since July 2019, in the first day of each month, the month amount of NH_4NO_3 demanded of each treatment was dissolved in 1L water with the concentrations of LN (14.28 g·L^{-1}), MN (28.57 g·L^{-1}) and HN (42.86 g·L^{-1}), respectively, and evenly sprays the seedlings tested. The seedlings of CK were sprayed in the same time by the same amount of pure water. The duration of these treatments was one year.

2.2 Collection of Fine Root and Soil Samples

In August 2020, the plant of *Z. bungeanum* and *M. sativa* seedlings grown well of each N addition treatment were selected randomly. Ensuring the integrity of root systems as far as possible, the root systems were dug carefully by a small shovel and brush. The fine roots (diameter ≤ 2 mm) were cut from the root systems and divided into two types of live and dead according to the appearance, color, and elasticity of fine roots (Guo et al. 2008). The live fine root samples were collected and brought back to the laboratory. In the laboratory, after soaked in water for 6 h and washed by running waters, the fine root samples were divided into three groups with the root diameter of 0–0.5 mm, 0.5–1 mm, and 1–2 mm, respectively. All the live fine root samples were put into the 4 °C refrigerator to conserve or used immediately to determine the morphological features.

The rhizosphere soils (soils within radius 4 mm around the plant root system) of each plant material were sampled separately. The rhizosphere soils were gently shaken off from roots. After removing small stones and other impurities, the rhizosphere soil samples were divided into two groups. One group was sieved by 2-mm sieves and conserved in a refrigerator (−4 °C) for the determination of NO_3^--N and NH_4^+-N content. Another group was air-dried and used to determine pH and nutrient element contents.

2.3 Morphological Measuration of Fine Root

The fine root images of different treatments were obtained by the digital scanner (Expression 10000XL, Epson, Japan) and then the parameters of fine root morphological features, that is, length, diameter, surface area, and volume, were measured by the professional root analysis system (WinRhizo, Regent Inc., Canada). After measuration, the fine root samples were put in the oven of 65 °C to constant weight and then the dry masses were weighed. The special root length (SRL, root length/dry mass), tissue density (TD, dry mass/volume), specific surface area (SSA, surface area/dry mass) were calculated (Table S1 and S2).

2.4 Chemical Determination of Fine Root and Rhizosphere Soil

The dried fine roots and air-dried rhizosphere soils were ground by a ball grinder and sieved by 0.15-mm sieves for the chemical determination. The C, N, and P contents of fine root and rhizosphere soil samples were determined by the methods of potassium dichromate oxidation-heating method, Kjeldahl digestion method, and ammonium molybdate method, respectively (Wang et al. 2018). For the determination of Ca and Mg contents, samples of fine root and rhizosphere soil were firstly dissolved by nitric acid-perchloric acid method and tetraic acid method, respectively, and then determined by atomic absorption spectrophotometry (Hu et al. 2018). Contents of soil NO_3^--N and NH_4^+-N were determined by a continuous flow analyzer (Skalar san, Skalar, Holland).

2.5 Statistical Analysis

All the statistical analyses were conducted by the Excel 2017 and SPSS 20.0 software. One-way ANOVA was used to analyze the effect differences of N addition treatments on fine root morphological features and rhizosphere soil stoichiometric characteristics. Before the variance analysis conducted, the normality and homogeneity of variances were tested. Datasets that did not meet the normality and homogeneity were transformed by the natural logarithm method. Multiple comparisons were conducted by the LSD method. Relationships between fine root morphological features, fine root, and rhizosphere soil stoichiometric characteristics were studied by the Pearson correlation analysis. Canoco 5.0 software was used to conduct the RDA analysis and Origin 7.5 software was used to draw the figures. All data in the figures and tables were the mean ± standard error.

3 Results

3.1 Effects of Short-term N Addition on Fine Root Morphological Features

The short-term (1 year) N addition significantly affected the fine root morphological features of *M. sativa* seedlings (herbaceous plant) (Fig. 1b, d, f, and h), but did not significantly affect the fine root morphological features of *Z. bungeanum* seedlings (woody plant) (Fig. 1a, c, e, and g). There were significant differences in SRL and SSA of 0–0.5 mm diameter fine root of *M. sativa* seedlings between different N addition treatments and the averages of SRL and SSA reached the maximum in the LN treatment (Fig. 1d and f). The short-term N addition had no significant effect on the morphological features of 0.5–1 mm and 1–2 mm diameter fine roots in *M. sativa* seedlings.

3.2 Effects of Short-term N Addition on Fine Root Stoichiometric Characteristics

The short-term N addition significantly affected the contents and ratios of C, N, and P of fine root both in *Z. bungeanum* and *M. sativa* seedlings. Compared with the larger diameter (0.5–1 mm and 1–2 mm) fine roots, the C, N, and P stoichiometric characteristics of 0–0.5 mm diameter fine root were more easily affected significantly by the short-term N addition in the both two plants. In the 0–0.5 mm diameter fine roots of *Z. bungeanum* seedlings, the MN treatment decreased significantly C content (Fig. 2a), P content (Fig. 2c), and C:N (Fig. 2d), but increased significantly N content (Fig. 2b). In the 0–0.5 mm diameter fine roots of *M. sativa* seedlings, treatments of HN, MN, and LN decreased significantly P content (Fig. 3c), Mg content (Fig. 3g), and C:N (Fig. 3d), and treatments of MN and HN increased significantly N content (Fig. 3b), C:P (Fig. 3e), and N:P (Fig. 3f). In the 0.5–1 mm diameter fine roots of *M. sativa* seedlings, MN treatment significantly increased N content (Fig. 3b), C:P (Fig. 3e), and N:P (Fig. 3f).

3.3 Effects of Short-term N Addition on Rhizosphere Soil Stoichiometric Characteristics

The short-term N addition significantly affected rhizosphere soil N (Fig. 4b), NO_3^--N (Fig. 4e) and NH_4^+-N (Fig. 4f) content, C:P (Fig. 4h), and N:P (Fig. 4i) of *Z. bungeanum* and *M. sativa* seedlings, but did not significantly affect the pH (Fig. 4d), Mg (Fig. 4j), Ca (Fig. 4k), and P (Fig. 4c) content. Concretely, the short-term N addition significantly increased the N, NO_3^--N and NH_4^+-N content in the both two plants, and these content averages reached the maximum in the HN treatment. With the concentrations increase of N addition treatment, N:P of the both two plants increased accordingly and reached the maximum in HN treatment. The responses of C:P and C:N of the two plants tested were not concordant. With the concentrations increase of N addition, C:P and C:N of *Z. bungeanum* rhizosphere soils decreased. However, in the rhizosphere soils of *M. sativa* seedlings, C:P increased significantly but C:N showed no significant change with the concentrations increase of N addition.

3.4 Correlations Between Fine Root Morphological Features, Fine Root, and Rhizosphere Soil Nutrient Stoichiometric Characteristics

There were significant correlations between fine root morphological features and nutrient stoichiometric characteristics both in *Z. bungeanum* and *M. sativa* seedlings (Tables 1 and 2). In *Z. bungeanum* seedling fine roots, MD significantly positively related with C content, C:P and N:P; SSA significantly positively related with C and N content, and significantly negatively related with C:N; TD significantly positively related with Mg content. In *M. sativa* seedling fine roots, SRL extremely significantly positively related with N content, significantly positively related with P content, and significantly negatively related with C:N; SSA significantly positively related with C, N, and P content; and TD significantly negatively related with N and P content.

There were also significant correlations between fine root morphological features and rhizosphere soil stoichiometric characteristics in the two plants (Tables 1 and 2). In *Z. bungeanum* seedlings, fine root MD significantly positively related with soil NH_4^+-N content; SRL significantly positively related with soil N, NO_3^-N, and NH_4^+-N content and

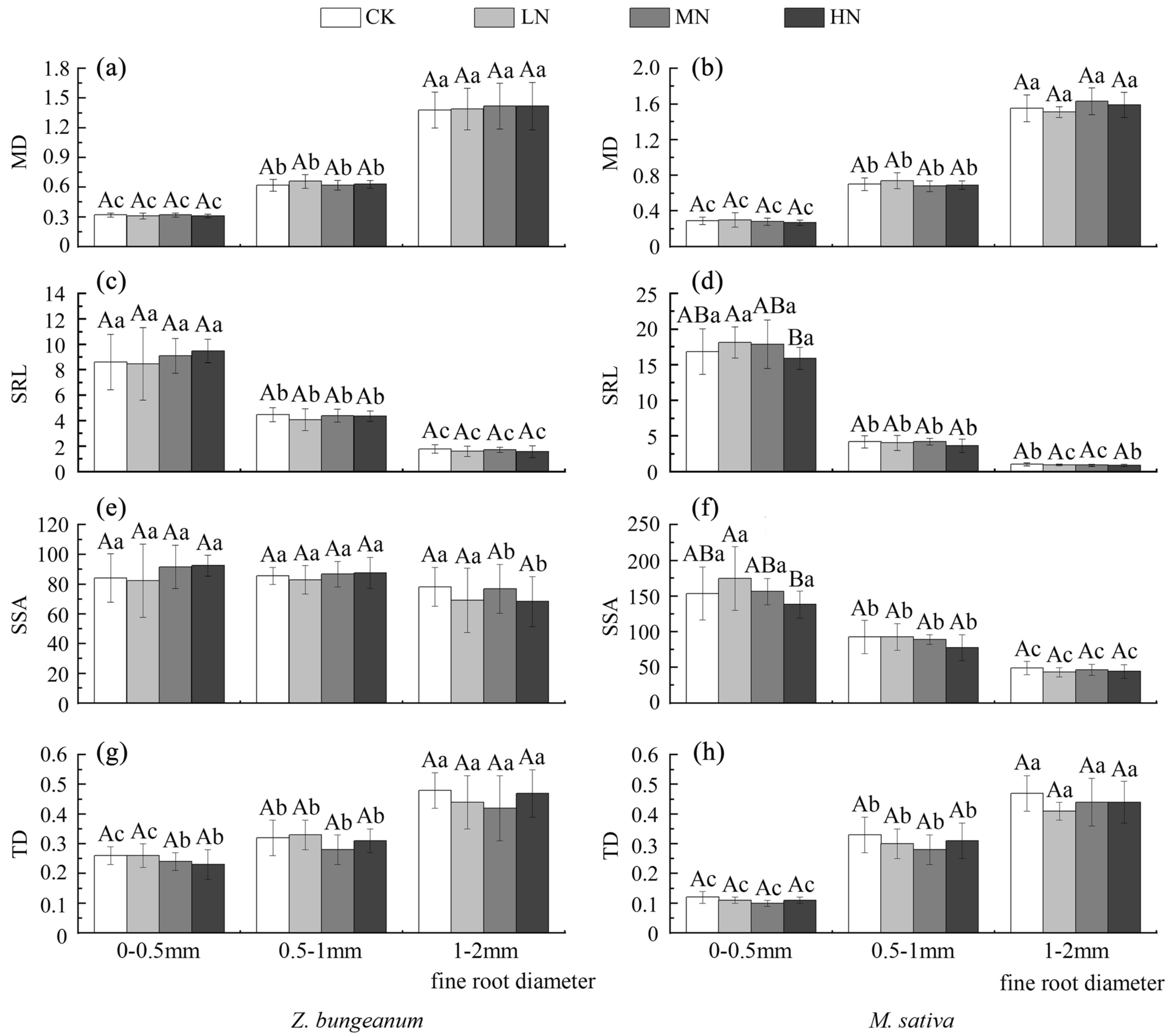

Fig. 1 Effects of short-term (1 year) N addition on fine root morphological features of *Z. bungeanum* and *M. sativa* seedlings. MD, mean diameter; SRL, specific root length; SSA, specific surface area; TD, tissue density; CK, control; LN, low N treatment; MN, middle N treatment; HN, high N treatment. Different capital letters represent the significant difference between different treatments of same diameter fine root and different small letters represent the significant difference between different diameter fine roots of same treatment

N:P; SSA significantly positively related with soil C, N, and NO_3^--N content, C:P and N:P, and extremely significantly positively related with soil NH_4^+-N content; TD significantly positively related with soil P content. In *M. sativa* seedlings, SRL and SSA significantly positively related with soil N, P, and NH_4^+-N content and C:P, and extremely significantly positively related with soil C content; and TD significantly negatively related with soil N content.

The correlations were also significant between the nutrient stoichiometric characteristics of fine roots and rhizosphere soil both in the two plants (Tables 1 and 2). As a whole, there were significantly positively correlations in the stoichiometric characteristics of C, N, P, and Mg, but significantly negatively correlation in Ca content between fine roots and rhizosphere soils of the both two plants. In *Z. bungeanum* seedlings, correlations of soil NO_3^--N and NH_4^+-N content with fine root stoichiometric characteristics all were obvious. However, in *M. sativa* seedlings, correlations of soil NO_3^--N content with fine root stoichiometric characteristics were more remarkable than those of soil NH_4^+-N content.

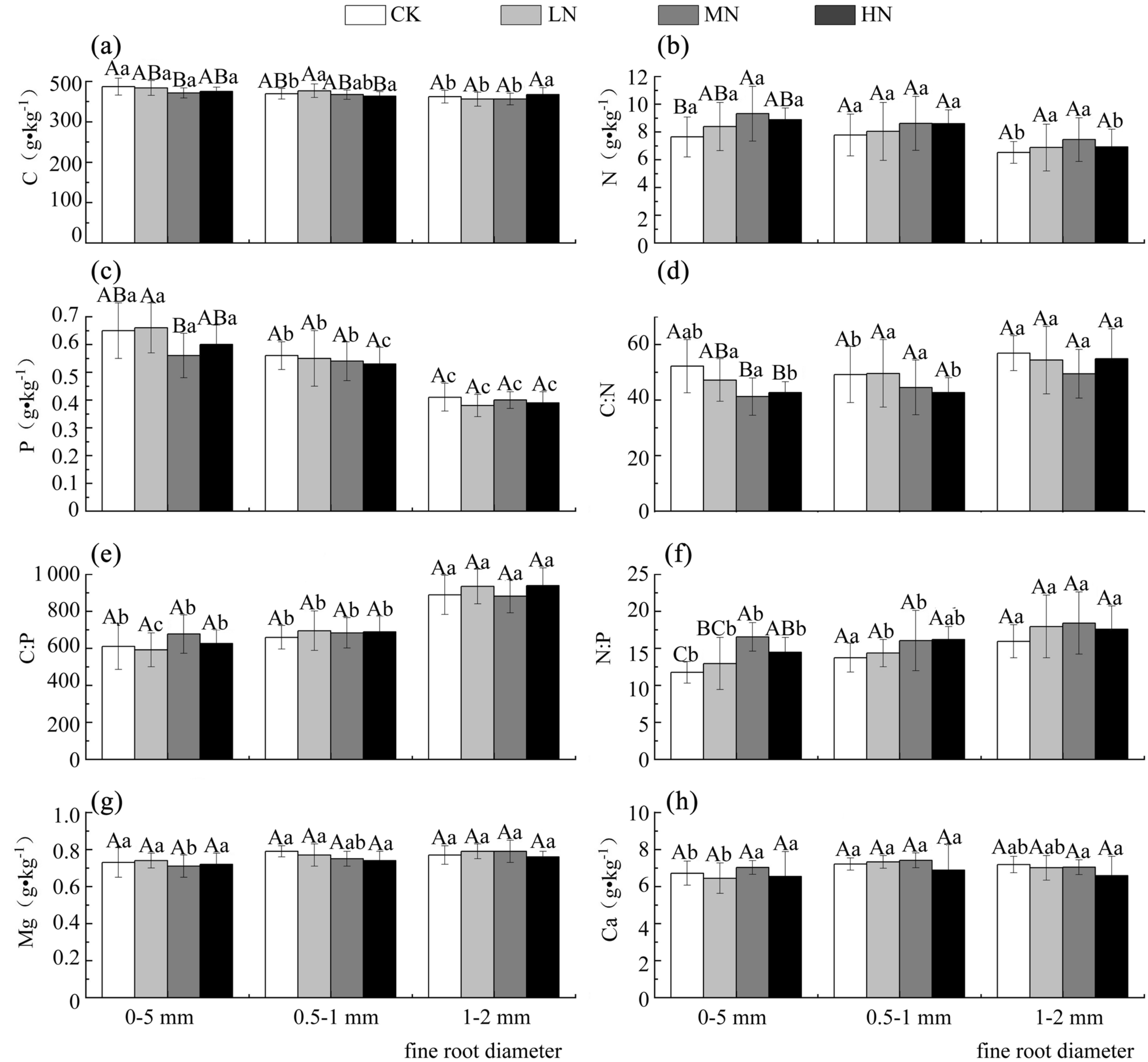

Fig. 2 Effects of short-term (1 year) N addition on nutrition stoichiometric characteristics of fine root in *Z. bungeanum* seedlings. CK, control; LN, low N treatment; MN, middle N treatment; HN, high N treatment. Different capital letters represent the significant difference between different treatments of same diameter fine root and different small letters represent the significant difference between different diameter fine roots of same treatment

3.5 RDA Analysis

The cumulative percent of the first and second axis in the RDA analyses of *Z. bungeanum* (Fig. 5a) and *M. sativa* (Fig. 5b) was 47.44% and 41.35%, respectively. RDA analysis of *Z. bungeanum* showed rhizosphere soil NO_3^--N, NH_4^+-N, N, and P content positively related with fine root SRL, SSA, and MD and were the main influencing factors of rhizosphere soils on fine root morphology. RDA analysis of *M. sativa* showed that rhizosphere soil C, N, and NO_3^--N content and C:P positively related with fine root SRL and SSA, negatively related with fine root TD, and were the main influencing factors of rhizosphere soils on fine root morphology. In addition, results also showed, both in the two plants, the correlations of C, N, P, and Mg contents and ratios between fine root and rhizosphere soil were positive and the correlation of Ca content between fine root and rhizosphere soil were negative.

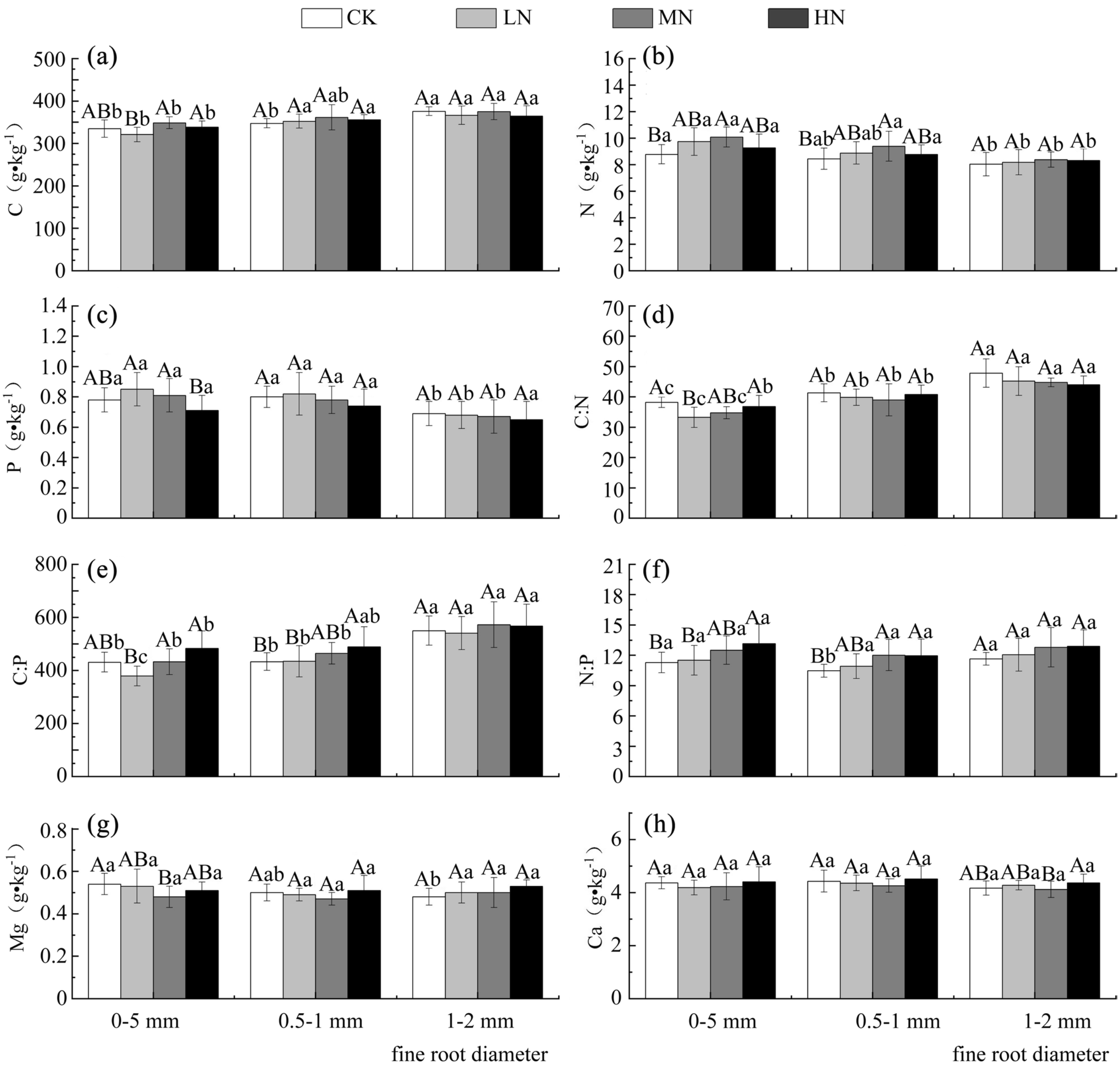

Fig. 3 Effect of short-term (1 year) N addition on nutrition stoichiometric characteristics of fine root in *M. sativa* seedlings. CK, control; LN, low N treatment; MN, middle N treatment; HN, high N treatment. Different capital letters represent the significant difference between different treatments of same diameter fine root and different small letters represent the significant difference between different diameter fine roots of same treatment

4 Discussion

The short-term (1 year) N addition significantly affected the fine root SRL and SSA of the herbaceous plant (*M. sativa*) seedlings, consistent with most of previous research reports on the effect of N addition on plant fine root morphology (Liu et al. 2017; Jia et al. 2019; Zheng et al. 2019). However, the short-term N addition had no significant effect on fine root morphological characteristics of the woody plant (*Z. bungeanum*) seedlings. Meanwhile, results of the present study showed that with the diameter increase of fine root, the fine root SRL, and SSA decrease while the TD increase in the both two plants, according with the previous results of Yan et al. (2017) and Chen et al. (2017). Large diameter fine roots were mainly responsible for the transportation function of nutrients due to their longer average life, greater root diameter, and tissue density (Wang et al. 2019). And smaller diameter fine roots were more sensitive to environmental changes and could quickly adapt and utilize the soil effective nutrient changes caused by N additions through changing

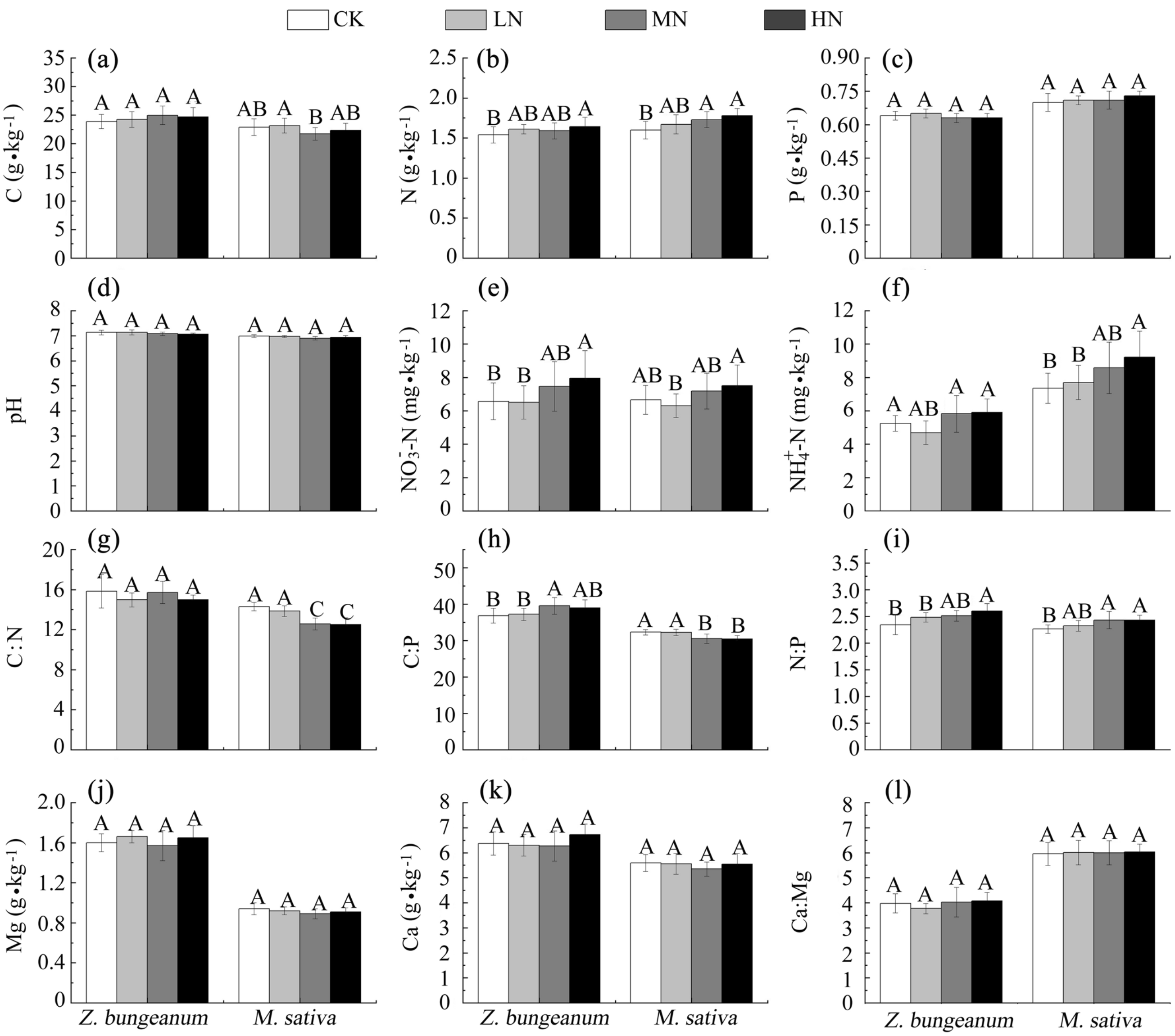

Fig. 4 Effect of short-term (1 year) N addition on the nutrition stoichiometric characteristics of rhizosphere soil in the *Z. bungeanum* and *M. sativa* seedlings. CK, control; LN, low N treatment; MN, middle N treatment; HN, high N treatment. Different capital letters represent the significant difference between different treatments

their SRL and SSA. The present study results confirmed that, compared with woody plants, fine roots of herbaceous plants are smaller and respond more sensitively to the soil environmental nutrient changes caused by N additions (Li et al. 2015).

In the present study, the short-term N addition increased N content and decreased C content of the two plant fine roots, consistent with the previous results (Yang et al. 2018; Chen et al. 2018). So it can be inferred that N addition can increase soil available N, promote N nutrition absorption of fine root and fine root growth, leading to the significant increase of N content, decrease of C content and C:N ration in fine roots (Pressler et al. 2020). And N addition resulted in the changes of plant biomass and C concentration reflecting the C allocation and leading to the reduction of fine root C content. It is also possible that N deposition increases the fine root growth, thus diluting the C concentration. However, some studies had also drawn different conclusion that N addition did not decrease fine root C content (Li et al. 2021b), which should be attributed to the differences in physiological characteristics and C allocation strategies adapted to environments between different plants. In addition, it was found that HN and MN treatment significantly reduced fine root P content, consistent with results of Sardans et al. (2017), supporting the conclusion that N input could significantly change

Table 1 Correlations between fine root morphological features, fine root and rhizosphere soil nutrient stoichiometric characteristics in *Z. bungeanum* seedlings

Subject and parameters		Rhizosphere soil nutrient stoichiometric characteristics											Fine root nutrient stoichiometric characteristics							
		C	N	P	pH	NO_3^--N	NH_4^+-N	C:N	C:P	N:P	Mg	Ca	C	N	P	C:N	C:P	N:P	Mg	Ca
Fine root morphological features	MD	0.247	−0.027	0.055	−0.243	0.272	0.334*	0.177	0.212	−0.019	0.189	0.066	0.360*	0.323	−0.275	−0.280	0.407**	0.495**	0.181	0.117
	SRL	0.310	0.385*	0.010	−0.253	0.329*	0.409*	−0.095	0.309	0.399*	0.068	0.134	0.087	0.207	0.084	−0.243	−0.050	0.115	−0.162	−0.109
	SSA	0.400*	0.398*	0.036	−0.297	0.416*	0.481**	−0.011	0.379*	0.375*	0.064	0.312	0.348*	0.409*	0.118	−0.410*	0.017	0.273	−0.186	−0.072
	TD	0.093	0.147	0.382*	−0.015	−0.018	−0.174	−0.086	−0.159	−0.062	0.094	−0.176	0.322	0.086	−0.060	−0.032	0.089	0.044	0.405*	−0.107
Fine root nutrient stoichiometric characteristics	C	0.508**	0.321	0.548**	−0.157	0.425**	0.450**	0.027	0.152	0.090	−0.132	0.309								
	N	0.568**	0.609**	0.512**	−0.246	0.602**	0.492**	−0.177	0.231	0.386*	0.228	0.282								
	P	0.024	0.129	0.356*	0.195	−0.168	−0.062	0.008	−0.208	−0.169	−0.022	0.142								
	C:N	−0.493**	−0.594**	−0.435**	0.320	−0.603**	−0.423*	0.244	−0.206	−0.434**	−0.206	−0.267								
	C:P	0.088	−0.041	−0.245	−0.208	0.269	0.207	−0.011	0.246	0.204	−0.007	−0.014								
	N:P	0.398*	0.345*	0.102	−0.281	0.571**	0.454*	−0.109	0.321	0.387*	0.128	0.157								
	Mg	0.154	0.258	0.511**	−0.034	0.134	−0.099	−0.169	−0.182	−0.005	0.304	−0.114								
	Ca	−0.471**	−0.377*	−0.141	−0.141	−0.266	−0.280	−0.165	−0.374*	−0.192	−0.387*	−0.179								

MD, mean diameter; *SRL*, special root length; *SSA*, specific surface area; *TD*, tissue density. * and ** represents the significant ($\alpha = 0.05$) and extremely significant ($\alpha = 0.01$) correlation, respectively

Table 2 Correlations between fine root morphological features, fine root and rhizosphere soil nutrient stoichiometric characteristics in *M. sativa* seedlings

Subject and parameters		Rhizosphere soil nutrient stoichiometric characteristics											Fine root nutrient stoichiometric characteristics							
		C	N	P	pH	NO_3^--N	NH_4^+-N	C:N	C:P	N:P	Mg	Ca	C	N	P	C:N	C:P	N:P	Mg	Ca
Fine root morphological features	MD	−0.003	−0.082	0.035	0.092	−0.024	−0.244	0.074	−0.053	−0.141	0.005	0.054	0.104	−0.110	0.034	0.214	−0.003	−0.134	0.113	−0.005
	SRL	0.533**	0.376*	0.322*	−0.187	0.360*	0.140	0.049	0.385*	0.213	0.021	−0.328	0.274	0.436**	0.395*	−0.343*	−0.300	−0.076	0.141	0.188
	SSA	0.547**	0.347*	0.371*	−0.264	0.354*	0.062	0.086	0.344*	0.126	−0.035	−0.274	0.351*	0.380*	0.396*	−0.209	−0.250	−0.109	0.137	0.124
	TD	−0.285	−0.358*	−0.258	0.067	−0.247	−0.248	0.151	−0.106	−0.249	−0.130	0.052	−0.177	−0.371*	−0.331*	0.333*	0.263	0.051	0.172	−0.128
Fine root nutrient stoichiometric characteristics	C	0.504**	0.707**	0.622**	−0.401*	0.630**	0.295	−0.295	0.001	0.375*	0.273	−0.271								
	N	0.431*	0.580**	0.530**	−0.504**	0.483**	0.409*	−0.234	0.004	0.295	0.137	−0.345*								
	P	0.732**	0.479**	0.540**	−0.134	0.280	0.005	0.117	0.414*	0.160	0.451**	−0.208								
	C:N	−0.153	−0.183	−0.188	0.316	−0.130	−0.300	0.066	−0.005	−0.082	0.055	0.242								
	C:P	−0.577**	−0.200	−0.298	−0.053	−0.026	0.163	−0.273	−0.473**	−0.011	−0.381*	0.105								
	N:P	−0.460**	−0.087	−0.179	−0.245	0.055	0.324	−0.294	−0.442**	0.036	−0.398*	−0.023								
	Mg	−0.420*	0.508**	0.327	−0.258	0.355*	0.103	−0.157	0.222	0.363*	0.163	−0.277								
	Ca	0.656**	0.401*	0.570**	−0.026	0.411*	−0.041	0.129	0.279	0.038	0.223	−0.212								

MD, mean diameter; *SRL*, special root length; *SSA*, specific surface area; *TD*, tissue density. * and ** represents the significant ($\alpha = 0.05$) and extremely significant ($\alpha = 0.01$) correlation, respectively

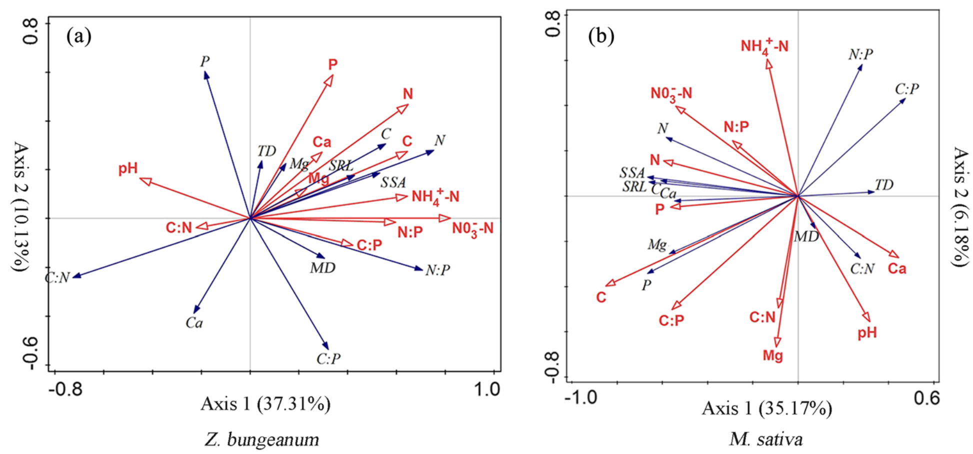

Fig. 5 RDA analyses for the correlations among fine root morphological features, fine root and rhizosphere soil stoichiometric characteristics in *Z. bungeanum* and *M. sativa* seedlings. MD, mean diameter; SRL, specific root length; SSA, specific surface area; TD, tissue density. The blue and red arrows indicate the factors of fine root and rhizosphere soil factors, respectively

plant root microflora and result in the decrease of fine root P element absorption because that the promotion of fine root P absorption by plant root microflora reduced (Cao et al. 2020). And N addition increased fine root N:P and aggravated the risk of P restriction to plants growth. Different from the long-term N addition (Chen et al. 2017), the present results showed the short-term N addition did not significantly affect fine root Mg and Ca content in the both two plants.

Consistent with most of the previous results (Zheng et al. 2019; Chen et al. 2020; Fan et al. 2020), the present N addition significantly increased rhizosphere soil N, NO_3^--N and NH_4^+-N content. But, the impacts of N addition on rhizosphere soil N, NO_3^--N and NH_4^+-N content of the N fixation plant (*M. sativa*) were more remarkable than those of the non-N fixation plant. Fine roots of *M. sativa*, a Leguminosae plant, have a large number of rhizobia with strong N fixation function, leading to the higher rhizosphere soil N content under the N addition. Karst soil C:N in southwest China was significantly higher than the average of the global and China and the plant growth was obviously restricted by N element (Wang et al. 2018). The present study results showed that N addition increased N content and reduced C:N of the rhizosphere soil of the both two plants, suggesting that moderate N deposition would reduce the N limitation and promote the vegetation growth in southwest China karst area. In the present study, N addition did not significantly affect P content but significantly increase N content and N:P of the rhizosphere soil both in *Z. bungeanum* and *M. sativa* seedlings, especially the HN treatment. The N:P increase should be caused by the more N input and no change in P content under the N addition. Studies of Sun et al. (2016) showed that the moderate N addition could improve soil microorganism and enzyme activity. Soil total N content and N:P were positive correlations with soil enzyme activity (Tian et al. 2019). So the present results may be caused by that the N input increased soil available N content, improved soil microorganism and enzyme activity.

Nutrients of plant fine root come mainly from soil, and changes in soil nutrients can significantly affect the fine root morphological features (Bardgett et al. 2014). As expected, the present results showed that fine root morphological features were significantly correlated with nutrient stoichiometry characteristics of rhizosphere soil both in *Z. bungeanum* and *M. sativa* seedlings. The present results also showed that, compared with MD and TD, SRL, and SSA of fine root showed greater correlations with nutrient stoichiometric characteristics of rhizosphere soil, showing that fine root of *Z. bungeanum* and *M. sativa* seedlings adapted to soil nutrient changes mainly by changing SRL and SSA, and further confirming the conclusion that nutrient absorption strategies of fine root, as the main organ for acquiring soil nutrients and water and the most active and sensitive part of root tissues, could be optimized by changing SRL and SSA to achieve the maximize absorption ability (Bardgett et al. 2014; Wang et al. 2016). It was also concluded that changing fine root SRL and SSA to optimize nutrient absorption ability was an important adaptation strategy of these two plants tested to adapt the nutrient changes of karst soil environment. The present study also showed that compared with C, Ca, and Mg content, rhizosphere soil N and P content both of the two plants tested had greater influences on fine root morphological features, which may be related to the N and P limitation in the karst ecosystem (Zhang et al. 2018; Sheng et al. 2018; Jia et al. 2019).

Rhizosphere soil is not only the most intense contact area between plants and soils, but also the main nutrient sources for plant fine root absorption and utilization (Peng et al. 2017; Liu et al. 2021). As expected, the present study results showed there were significant correlations in nutrient stoichiometry characteristics between rhizosphere soil and fine root both of *Z. bungeanum* and *M. sativa* seedlings. Some studies have shown that if the nutrient element content in the plant was proportional to the supply capacity in soil, this plant growth would be limited with a high probability by this nutrient element (Güseell 2004;

Drescher et al. 2020; Zhang et al. 2018). The present study showed between fine roots and rhizosphere soil of the two plants tested, there were positive correlations in N, P and Mg content, and a negative correlation in Ca contents, indicating that the growths of *Z. bungeanum* and *M. sativa* seedlings in the karst area were susceptible by N, P, and Mg limitation and not susceptible by Ca limitation. Moreover, this present study showed that the rhizosphere soil Ca and Mg content both of *Z. bungeanum* and *M. sativa* seedlings were remarkably higher than those of non-karst area plants (Li et al. 2020; Hu et al. 2020a, b), indicating that the adaptation mechanisms of these two plant fine root to the high-Ca and rich-Mg karst soil environment were contributing. But the adaptation mechanisms of these two plants tested to these two elements in the karst soil environment were different because there was a positive correlation in Mg contents and a negative correlation in Ca contents between the fine roots and rhizosphere soil.

5 Conclusions

One-year short-term N addition significantly affected the fine root morphology of *M. sativa* by changing the specific root length and specific surface area. The fine root morphological features and nutrient stoichiometry characteristics were significantly correlated with rhizosphere soil nutrient stoichiometry characteristics in the both two plants tested under the N additions. Changing the specific root length and specific surface area of fine root to optimize nutrient absorption ability was an important response strategy of the plants tested under the N additions. Different N addition levels can cause obviously opposing effects of promotion and inhibition on fine root growth in the both plants tested. Responses of fine root morphology and nutrient stoichiometric characteristics of *M. sativa* to N addition was different with those of *Z. bungeanum* can been attributed to that *M. sativa* is an N fixing plant. Impacts of N additions on nutrient stoichiometric characteristics of *M. sativa* (N fixing plant) fine roots and rhizosphere soils were more remarkable than those of *Z. bungeanum* (non-N fixing plant). The response mechanisms of plant fine roots to the high calcium and rich magnesium karst soil environment were existent and different. Results of the present study had important significances for the further studies on the response laws and adaptation mechanisms of plant growth to N deposition.

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Declarations

Conflict of Interest The authors declare no competing interests.

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