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Arbuscular Mycorrhizal Fungi Adjusts Root Architecture to Promote Leaf Nitrogen Accumulation and Reduce Leaf Carbon–Nitrogen Ratio of Mulberry Seedlings

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Abstract: In the initial stages of restoring rocky desertification, the proliferation of nutrients strongly influences plant survival. The carbon–nitrogen doctrine in plants argues that a lower leaf carbon–nitrogen (C:N) ratio enhances the growth of plant nutrients. However, the mechanisms by which inoculation with arbuscular mycorrhizal fungi (AMF) can influence plants during the restoration of rocky desertification are not thoroughly understood. This study used mulberry as a suitable example of a mycorrhizal plant in desertification areas to examine changes in growth, leaf carbon, nitrogen accumulation, and the carbon–nitrogen ratio post inoculation using AMF. The correlation between leaf carbon–nitrogen ratio and root morphology following AMF inoculation was also examined. The results demonstrated that inoculating mulberry with the dominant strains *Funneliformis mosseae* (Fm) and *Rhizophagus intraradices* (Ri) not only enhanced above-ground growth and improved carbon and nitrogen nutrient absorption but also had a more pronounced effect on leaf nitrogen accumulation than on carbon accumulation, resulting in a potential decrease in the leaf C:N ratio by 42.13%. It also significantly improved root morphology by exponentially increasing the number of connections and crossings by 120.5% and 109.8%, respectively. Further analysis revealed a negative correlation between leaf C:N ratio and root morphology, as well as between root length and the number of connections. Plants with more developed root systems exhibited greater competitiveness for nitrogen, resulting in a lower leaf C:N ratio. This study suggests that the inoculation of AMF could enhance leaf nitrogen accumulation and reduce the leaf C:N ratio by expanding the spatial absorption range of the root through positive changes in root morphology, thereby promoting plant nutrient growth. This study forms a fundamental scientific basis for the successful management of desertification.

Keywords: rocky desertification; arbuscular mycorrhizal fungi; root morphology; carbon–nitrogen ratio

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1. Introduction

Karst rock desertification is typically characterized by extensive bedrock outcrops accompanied by shallow and discontinuous soil layers. It is also characterized by significant soil erosion and inadequate water and nutrient storage capacity [1]. Several techniques are being developed worldwide to restore rocky desertification. For instance, China has recently utilized vegetation restoration techniques to green vast areas affected by rocky desertification. However, a substantial portion of the area, approximately 7.22 million hectares, remains barren and lacks trees. To effectively combat rocky desertification, it is essential to reforest this land with suitable tree species. It is well recognized that trees primarily undergo nutritional and reproductive growth, which remains integral throughout their developmental stages [2]. Notably, the development of plant nutrients significantly influences their survival during the early stages of recovery. The leaves of plants are the primary organs for photosynthesis, converting light energy into chemical energy and providing carbohydrates essential for plant growth and development [3]. The nitrogen content significantly influences the photosynthesis of plant leaves. A balance between carbon and nitrogen regulates leaf growth and senescence, specifically the C:N ratio, during the nutrient growth phase of plants. The C:N theory posits that the nutritional and reproductive growth of a plant is governed by the ratio of carbohydrates to nitrogen compounds. A lower C:N ratio favors nutritive growth [4]. Truthfully, in unrecovered areas, implementing effective measures to reduce the leaf C:N ratio can promote plant nutrient growth and facilitate early-stage growth and development.

More than 80% of terrestrial plants engage symbiotic relationships with arbuscular mycorrhizal fungi (AMF), forming mycorrhizal-type structures that promote plant growth [5–7]. It is well known that mycorrhizal plants can effectively contribute to the management of unrecovered rocky desertified areas. An extensive mycelial network, formed through symbiotic interactions between plants and AMF, aids in the uptake of nitrogen from microscopic soil pores [8], which are inaccessible to the root system and impassable. This process heightens the plant's competitiveness with soil microorganisms for nitrogen. Concurrently, AMF absorbs carbohydrates from the plants, thereby supporting their autotrophic growth [9]. Meta-analyses have shown that inoculating plants with AMF results in a significant increase in nitrogen accumulation, which is accompanied by either a decrease or a negligible increase in carbon accumulation within plant leaves. A study conducted by Xin et al. [10] revealed a significant reduction in the C:N ratio in the leaf due to AMF, whereas Mei et al. [11] showed that AMF could significantly increase the C:N ratio of ironweed clover leaves under warming conditions. The significance of variations in the exchange of carbon and nitrogen resources between mycorrhizae and plants, especially in diverse nutrient environments, has been examined regarding disparities in AMF for carbon and nitrogen accumulation in plant leaves [12]. Based on different studies, introducing inoculants to nutrient-deficient soils in rocky desertification areas to enhance plant nitrogen accumulation while concurrently reducing the leaf C:N ratio is highly needed to gain a deeper understanding of the successful management of desertification.

The nitrogen in plant leaves predominantly originates from the root system. The morphology of the root plays a pivotal role in determining the capacity of the plant to locate and uptake nutrients from its growth medium [13]. The nitrogen uptake capacity of plants, as distinguished by their varied root morphology, was identified using nitrogen isotope labeling techniques [14]. Nevertheless, these techniques contribute to the expansion of the root absorption area. AMF, which is symbiotic with plants, significantly influences the root morphology of various plants. The effects exhibited by different strains on the root conformation of individual plants can vary considerably under diverse habitats [15]. It was found that seedlings of hedgerow, winged oil tree, sorghum, soil sedum, and strawberry inoculated with AMF under nutrient stress conditions exhibited significantly enhanced root morphological features, including length, surface area, and volume [16–18]. Mulberry cuttings inoculated with *Funneliformis mosseae* exhibited no significant alteration in either total root length or the number of root tips [19]. Conversely, Ren beans inoculated with *Rhizophagus intraradices* showed significantly reduced numbers of root tips, crosses, and a smaller projected area [20]. Typically, plants enhance their nitrogen uptake to influence the C:N ratio, which is achieved through modifications of root conformation and morphology, including increased root length, surface area, and the number and density of root hairs [21]. The length and surface area of plant roots indicate the spatial uptake range and contact with the soil. Plants with a high capacity for nitrogen uptake and those with high nitrogen accumulation tend to have more considerable total root lengths and surface areas [22–24]. Our hypothesis posits that inoculation could enhance full plant root length and surface area while simultaneously reducing the leaf C:N ratio.

The mulberry tree, a quintessential tufted mycorrhizal plant, is extensively utilized in the ecological restoration of rocky desertification due to its inherent characteristics, including stoniness, barrenness, calcium-loving nature, alkali tolerance, and drought resistance. Xing et al. [25] revealed that inoculation enhances the root and above-ground growth of mulberry trees and strengthens their antagonistic capacity in the face of drought stresses. Some hypotheses regarding mulberry trees demonstrated that inoculation leads to leaf nitrogen accumulation and a decrease in the C:N ratio due to positive alterations in the root system, subsequently enhancing their growth. However, these hypotheses were not profoundly investigated. This study aimed to explore (1) the impact of inoculation on the growth of mulberry growth plants, its effects on leaf carbon and nitrogen accumulation, and alterations in the C:N ratio; (2) the influence of inoculation on the root morphology of mulberry trees; and (3) the correlation between leaf C:N ratio and root conformation. In addition, this study explores the root morphological factors that promote early-stage plant nutrition and growth in rocky desertified areas, offering a theoretical basis for managing desertification.

2. Materials and Methods

2.1. Experimental Materials

The strains *Funneliformis mosseae* (Fm) and *Rhizophagus intraradices* (Ri) were taken from the Institute of Microbiology [26], Guangxi Academy of Agricultural Sciences. The tested bacteriological agents were spores, mycelia, infected root segments, and river sand. River sand was used as the substrate, whereas white clover (*Trifolium repens* L.) and corn (*Zea mays* L.) were used as hosts for propagation. Mulberry seeds "Teyou 2" and "Guisang 12" were purchased from Nanning Tianlong Biotechnology Co., Ltd., Guangxi, China. Before the experiment, the seeds were disinfected using a 10% H_2O_2 solution for 10 min. They were rinsed using deionized water several times and placed in a Petri dish on a layer of wet filter paper. The seeds were then placed in a water-isolated constant temperature incubator at 28 °C to promote germination. The tested soil was collected from the topsoil (0–20 cm) of the rocky desertification Mulberry Garden base at the Sericultural Research Institute of the Guizhou Academy of Agricultural Sciences. Subsequently, plant root residues and stones were removed. The soil was then air-dried and thoroughly mixed. The mixture was then passed through a 5 mm nylon mesh screen and sterilized using 10 kGy Cobalt-60 radiation. The physicochemical properties of the soil are presented in Table 1.

Table 1. Physicochemical properties of the soil used. Values are presented as mean (n = 4).

Parameters	Soil
pH	6.91
Organic matter (g/kg)	26.9
Total nitrogen (g/kg)	2.32
Total phosphorus (g/kg)	0.65
Alkali-hydrolyzed nitrogen (mg/kg)	110.11
Available phosphorus (mg/kg)	7.77

2.2. Experimental Methods

2.2.1. Experimental Design

In this study, the installed experiment lasted for 4 months in the greenhouse of the Sericulture Research Institute of the Guizhou Academy of Agricultural Sciences (26°30′ N, 106°39′ E). The experiment was divided into a control group (CK), which was not inoculated, and a combination test of different strains and varieties (3 × 2). Each treatment was replicated four times, resulting in 24 experiments. The plants were exposed to supplemented light daily (light intensity: 30,000 lx) for 14 h using LED plant growth lamps and were maintained at 25 ± 3 °C. Mycorrhizal seedlings were grown in trays with holes containing 600 g of sterilized substrate and 60 g of bactericide in each tray. The control treatment had the same amount of bactericide, which was added to maintain the composition of soil microorganisms except for the target bacteria and other treatments. One seed was planted at each point, and 500 mL of deionized water was added every 3 days during cultivation. Mulberry seedlings with consistent growth were chosen for potting. A circular pot with dimensions of 18.5 cm in height, 13.5 cm in upper diameter, and 10.5 cm in bottom diameter was used. Each pot was planted with 1 seedling (loaded with 3 kg of sterilized soil), and the incubation experiment lasted for 3 months. During the potting period, soil was irregularly supplemented with deionized water.

2.2.2. Sampling and Analysis

At the end of the experiment, the entire mulberry tree was excavated and rinsed using deionized water. The root system was utilized to quantify the mycorrhizal infection. The mycorrhizal seedlings were extracted from 30 root segments, each measuring 1 cm long. They were then cleansed and immersed in a formalin (formaldehyde–acetic acid–ethanol) fixation solution for 12 h. Subsequently, the seedlings were stained using trebly blue staining solution and examined for mycorrhizal infection using an optical microscope [27]. The remaining parts were categorized based on their roots, stems, and foliage and then placed in an oven at 105 °C for 30 min and then dried at 60 °C until a constant weight was achieved. The electronic balance was used to measure the dry weight of each component (biomass), which was subsequently crushed and sealed for future use. The entire root image was scanned using a root scanner (GXY-A, Zhejiang Top Yun non-g). The Win-Rhizo root analysis system software (2021; Regent Instrument Inc., Quebec, QC, Canada) was utilized to analyze the number of root tips, forks, intersections, and projected area. The carbon and nitrogen content in the leaves was determined using the carbon and nitrogen element analyzer Vario micro cube (Elementar, Frankfurt, Germany).

$$\text{Mycorrhizal infection rate (\%)} = \sum (0 \times \text{number of root segments} + 10\% \times \text{number of root segments} + 20\% \times \text{number of root segments} + \ldots + 100\% \times \text{number of root segments})/\text{total number of root segments}. \quad (1)$$

$$\text{Mycorrhizal dependence} = (\text{inoculated plants with dry weight} - \text{uninoculated plants with dry weight})/\text{inoculated plants with dry weight}. \quad (2)$$

$$\text{Leaf nitrogen accumulation} = \text{leaf nitrogen content} \times \text{leaf biomass}. \quad (3)$$

$$\text{Carbon accumulation in leaves} = \text{leaf carbon content} \times \text{leaf biomass}. \quad (4)$$

2.2.3. Statistical Analysis

The data were analyzed using SPSS for single-factor analysis. Fisher's least significant difference (LSD) was performed at a 5% confidence level to investigate significant differences between different treatments. Figures were created using Origin 2021 and Canoco 5, and the Graphical Abstract was generated using BioRender.com.

3. Results

3.1. Effects of Mycorrhization on the Growth and Development of Mulberry Seedlings

After inoculation with Ri and Fm, the root systems of Teyou 2 and Guisang 12 were successfully invaded by the fungal mycelium (Figure 1), where mycelium and vesicles were visible under the microscope. Relative to the uninoculated group, the mycorrhization of seedlings significantly increased their plant height, stem diameter, and root–shoot ratio (Table 2), and the extent of these increases varied depending on the fungal strains and mulberry varieties. The infection effect on Teyou 2 was more pronounced, with an Fm infection rate reaching 80.58%, while no mycelium was observed in the uninoculated group (Table 3). The results demonstrated the disparity between mycorrhized seedlings and uninoculated ones, indicating that the growth of mulberry seedlings was enhanced after mycorrhization.

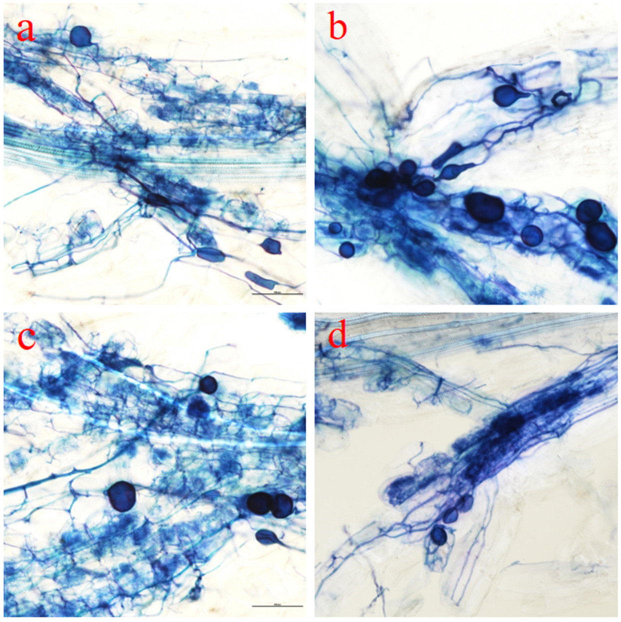

Figure 1. The infection status of Fm and Ri strains on Teyou 2 (**a**,**b**) and Guisang (**c**,**d**), respectively.

Table 2. Physiological characters of arbuscular mycorrhizal mulberry seedlings. Values are given as mean (n = 4) and standard deviation. CK, Ri, and Fm letters represent the control, *Rhizophagus intraradices*, and *Funneliformis mosseae.* The significant difference (LSD test, $p < 0.05$) between the two groups is represented by the superscript letters behind the data.

	Plant Height (mm)		Stem Diameter (mm)		Root Shoot Ratio (mm)	
	Teyou 2	**Guisang 12**	**Teyou 2**	**Guisang 12**	**Teyou 2**	**Guisang 12**
CK	34.44 ± 2.57^{c}	78.60 ± 3.87^{c}	1.34 ± 0.07^{b}	1.83 ± 0.04^{b}	0.09 ± 0.01^{b}	0.16 ± 0.02^{b}
FM	79.31 ± 4.80^{a}	91.11 ± 4.67^{b}	2.21 ± 0.12^{a}	2.16 ± 0.04^{a}	0.12 ± 0.02^{b}	0.27 ± 0.04^{ab}
RI	63.06 ± 1.67^{b}	104.09 ± 0.91^{a}	1.94 ± 0.13^{a}	2.29 ± 0.06^{a}	0.31 ± 0.04^{a}	0.30 ± 0.06^{a}

Table 3. Mycorrhizal infection of arbuscular mycorrhizal mulberry seedlings. Values are given as mean (n = 4) and standard deviation. CK, Ri, and Fm letters represent the control, *Rhizophagus intraradices*, and *Funneliformis mosseae.* The significant difference (LSD test, $p < 0.05$) between the two groups is represented by the superscript letters behind the data.

	Mycorrhizal Infection Rate (%)		Mycorrhizal Dependence (%)	
	Teyou 2	**Guisang 12**	**Teyou 2**	**Guisang 12**
CK	——	——	——	——
FM	42.55 ± 1.78^{b}	70.64 ± 3.46^{b}	80.58 ± 0.02^{b}	48.35 ± 0.10^{b}
RI	63.60 ± 5.18^{a}	75.97 ± 2.77^{a}	78.29 ± 0.03^{b}	44.60 ± 0.13^{b}

3.2. Changes in Carbon and Nitrogen Accumulation and Stoichiometric Ratio of Mycorrhized Mulberry Seedling Leaves

As displayed in Figure 2, the carbon and nitrogen accumulation in the leaves of mulberry seedlings on rocky desertified soil changed after two arbuscular mycorrhizations. The carbon accumulation of Ri and Fm mycorrhizal Teyou 2 increased by 6.4% and 28.9%, respectively (Figure 2a). In contrast, the nitrogen accumulation significantly increased by 79.0% and 17.7%, respectively ($p < 0.05$) (Figure 2b). The carbon and nitrogen accumulation of Guisang 12 increased by 29.5% and 43.9%, respectively. This indicates that arbuscular mycorrhization can enhance the plant's absorption of carbon and nitrogen, with a more substantial effect on nitrogen.

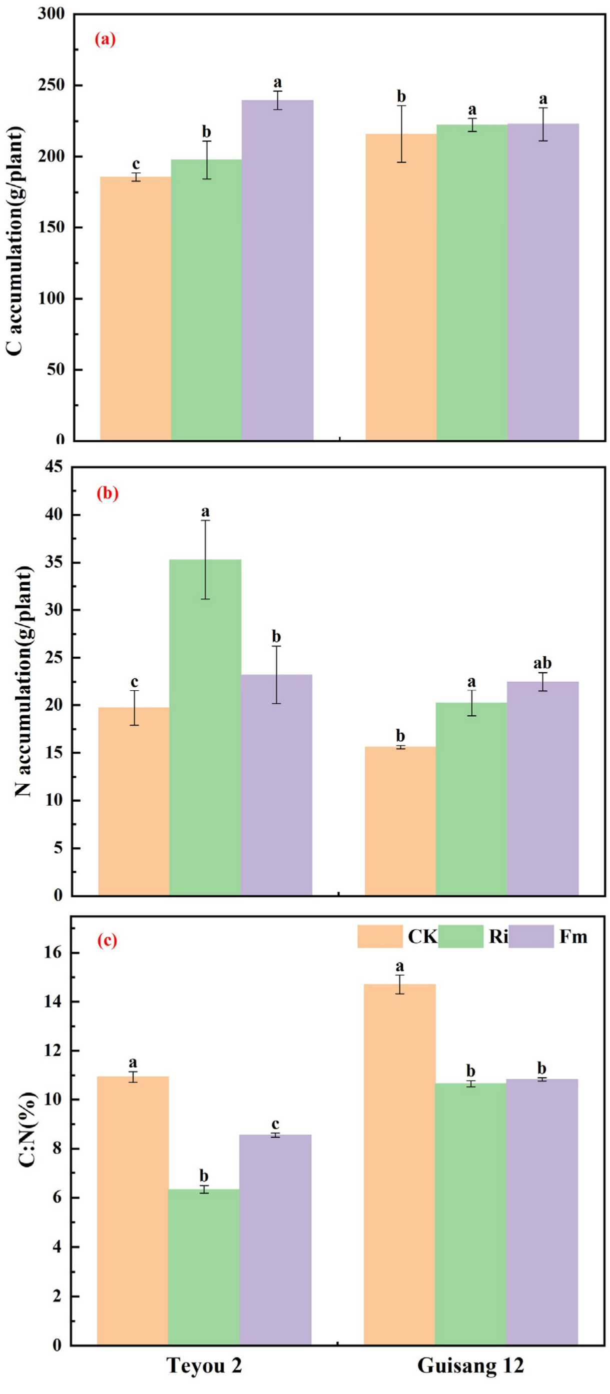

Figure 2. Carbon content (**a**), nitrogen content (**b**), and C:N (**c**) of arbuscular mycorrhizal mulberry leaves in rocky desertification soil. CK, Ri, and Fm letters represent the control, *Rhizophagus intraradices*, and *Funneliformis mosseae.* The significant difference (LSD test, $p < 0.05$) between the two groups is represented by the superscript letters above the error bars.

Compared with the control group, there were significant changes in the C:N ratio in the leaves of both arbuscular mycorrhized mulberry seedlings (Figure 2c) ($p < 0.05$). The C:N ratio of the Ri and Fm mycorrhizal Teyou 2 leaves decreased by 42.1% and 21.7%, respectively. The C:N ratio of the Guisang 12 leaves decreased by 32.5% and 26.3% by Ri and Fm mycorrhization, respectively. These results indicate that arbuscular mycorrhization significantly reduces the C:N ratio of mulberry seedling leaves, with Teyou 2 showing a more significant mycorrhizal effect.

3.3. Morphological Changes in Mycorrhized Mulberry Seedling Root

As depicted in Figure 3, the root length of mulberry seedlings on the rocky desertified soil significantly changed after the two arbuscular mycorrhizations ($p < 0.05$). After being mycorrhized by Fm and Ri, the root length of Teyou 2 increased considerably by 55.9% and 61.7%, respectively, whereas the root length of Guisang 12 increased by 14.2% and 10.9%, respectively. Compared with Guisang 12, the root of TeYou 2 significantly increased after mycorrhization, indicating that Teyou 2 had a higher mycorrhizal dependency on Ri than Fm.

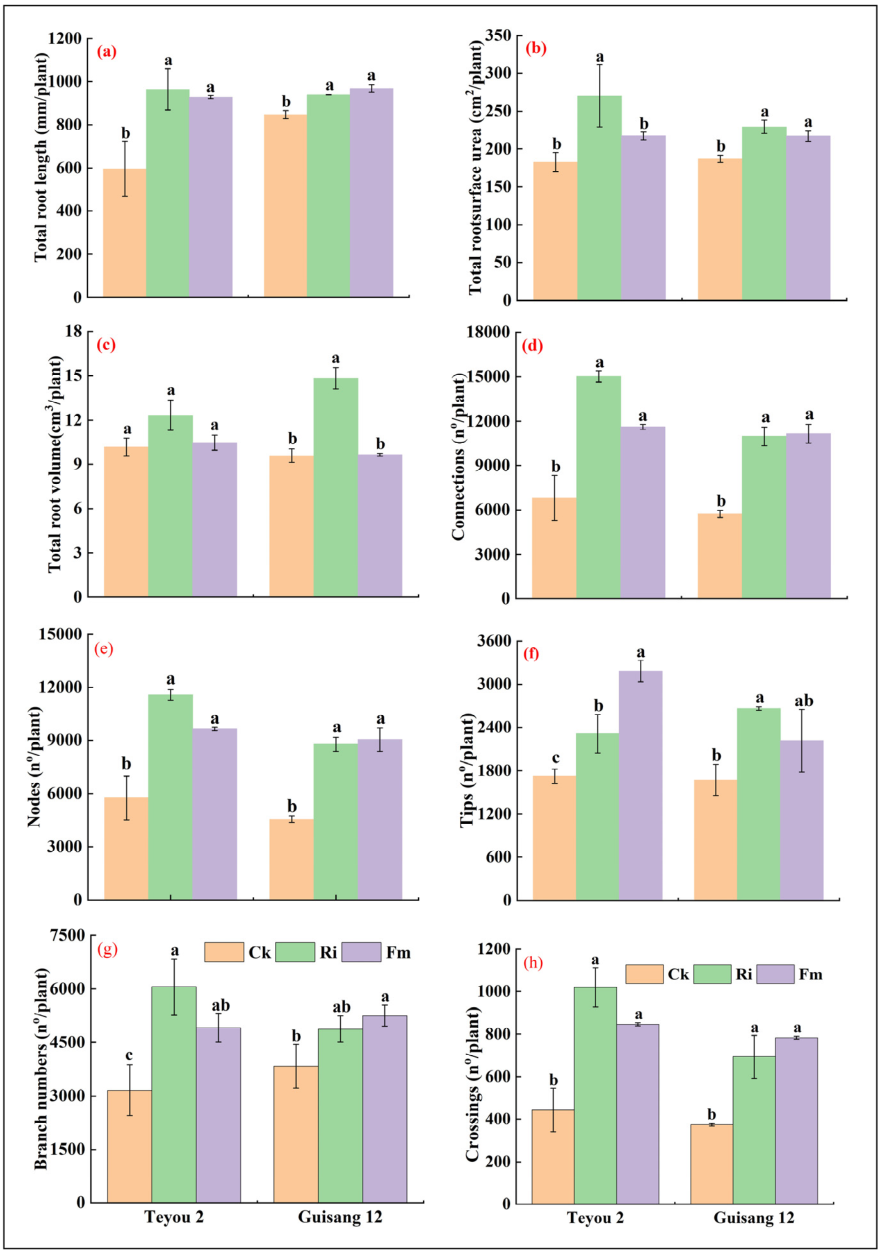

Figure 3. Characteristics of arbuscular mycorrhizal mulberry in total root length (**a**), total root surface area (**b**), total root volume (**c**), connections (**d**), nodes (**e**), tips (**f**), branch numbers (**g**), and crossings (**h**). Ck, Ri, and Fm letters represent the control, *Rhizophagus intraradices*, and *Funneliformis mosseae.* Data are presented as means ± standard deviations ($n = 4$). The significant difference (LSD test, $p < 0.05$) between the two groups is represented by the super-script letters above the error bars.

There was no significant difference in the root surface area of the two arbuscular mycorrhized mulberry seedlings ($p > 0.05$). Compared with the control, the root surface area of the Fm and Ri mycorrhizal Teyou 2 increased by 18.9% and 47.8%, respectively, whereas the root surface area of Guisang 12 increased by 15.9% and 22.5%, respectively. The root volume of the Ri-mycorrhized Teyou 2 and Guisang 12 significantly increased ($p < 0.05$) by 21.2% and 54.7%, respectively, relative to the control. Conversely, the root volume of Fm-mycorrhized mulberry seedlings did not show significant changes; it only increased by 2.8% and 0.8%, respectively, compared with the uninoculated group.

The node number and the connection number of the two arbuscular mycorrhized mulberry seedlings were significantly increased ($p < 0.01$). The connection and node number changes were more significant in the Ri-mycorrhized Teyou 2. Compared with the control, the number of connections increased by 70.7% and 120.5% for the Fm- and Ri-mycorrhized Teyou 2, respectively, and the number of nodes increased by 67.4% and 100.9% for the Fm- and Ri-mycorrhized Teyou 2, respectively. In general, the number of connections increased by 94.5% and 91.2% for Fm- and R-mycorrhized Guisang 12, respectively, and the number of nodes increased by 98.3% and 92.4% for Fm- and Ri-mycorrhized Guisang 12, respectively. The connection and node number changes were more significant for Guisang 12 with Fm mycorrhization.

The number of nodes and links in the roots of two types of arbuscular mycorrhized mulberry seedlings significantly changed ($p < 0.01$). The change in the number of connections and nodes of Ri-mycorrhized TeYou 2 was more impactful. Compared with the control, the relationships between Fm- and Ri-mycorrhized Teyou 2 increased by 70.7% and 120.5%, with the number of nodes increased by 67.4% and 100.9%, respectively. Compared with the Ri group, the change in the number of connections and nodes of Fm-mycorrhized Guisang 12 was more significant. Compared with the control, the number of connections and nodes of Fm and Ri mycorrhizal Guisang 12 increased by 94.5% and 91.2%, and the number of nodes increased by 98.3% and 92.4%, respectively.

After the inoculation of two different types of arbuscular mycorrhizal fungi, the mulberry seedling roots significantly increased in the number of root tips, branches, and crossings ($p < 0.01$). Among them, the increase in crossings was the most significant ($p < 0.001$). Specifically, compared with the control group, the number of branches increased by 84.7% and 34.3% for Fm- and Ri-mycorrhized Teyou 2, respectively. The number of branches increased by 55.5% and 91.7%, and the number of crossings increased by 91.1% and 130.1%, respectively. Compared with the control, in Fm- and Ri-mycorrhized Guisang 12, the number of root tips increased by 32.5% and 59.2%, respectively. The number of branches increased by 37.1% and 27.4% and the number of crossings increased by 109.8% and 85.7%, respectively.

Taken altogether, the two treatments of arbuscular mycorrhization had a positive impact on the physical features of mulberry seedling roots in soil affected by rocky desertification. The inoculation of AMF can potentially enhance root growth and development, although the degree of improvement varies based on the specific strains of AM fungi and the mulberry seedling varieties employed.

3.4. Coupling Relationship between Root Morphological Characteristics and Leaf Carbon to the Nitrogen Ratio of Mulberry Seedling

After being transplanted into rocky desertified soil, the leaf C:N ratio of arbuscular mycorrhized mulberry seedlings showed a significant negative correlation with the indices of root morphology (Figure 4). There were significant correlations between leaf C:N and root length, node number, and surface area, with solid correlations observed with root length and connection number. Among these, root length and connection number had the highest correlation with leaf C:N. In Guisang 12, there was a significant correlation between leaf C:N and root length and branching number. The root morphology and leaf C:N were influenced by mycorrhizal dependency, with higher mycorrhizal dependence resulting in more significant changes in root morphology and leaf C:N.

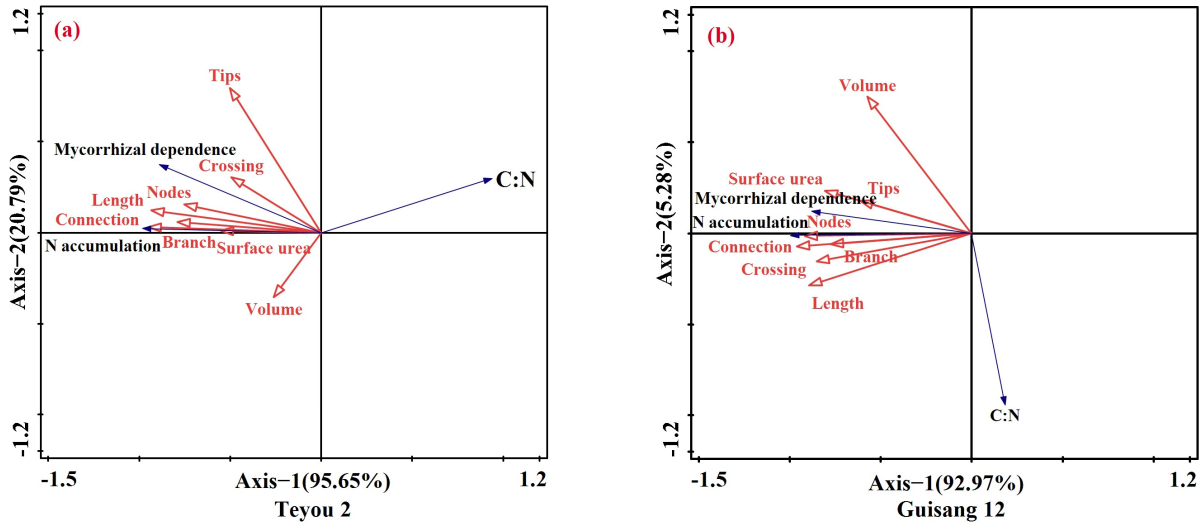

Figure 4. Redundancy analysis of root morphological characteristics and leaf C:N ratio of Teyou 2 (**a**) and Guisang 12 (**b**).

4. Discussion

4.1. Mycorrhization of Mulberry Seedlings Can Change Its Absorption and Utilization of Nitrogen

Human activities have caused the soil in rocky desertification areas to become infertile, significantly damaging productivity [28]. Therefore, arbuscular mycorrhizal fungi enhance plant growth and development by improving the absorption of essential nutrients such as phosphorus, nitrogen, potassium, and sulfate, particularly in nutrient-deficient conditions. Fungi are pivotal in promoting plant growth and development by facilitating the uptake of essential mineral elements [28,29]. Nitrogen availability has a more significant impact on plant growth than other factors. As the central organ of photosynthesis, the leaf can indicate the plant's overall condition by reflecting its stoichiometric ratio [3]. Typically, the C:N ratio is a reliable indicator of plant growth rate and nitrogen utilization, as highlighted by [30]. It has been observed that plants with higher growth rates generally exhibit lower C:N ratios [31].

The results of this study revealed a noteworthy uptick in nitrogen accumulation within mycorrhized mulberry seedlings cultivated in rocky desertification soil. Concurrently, there was a substantial decrease in the C:N ratio. This indicates that the nitrogen utilization of mulberry seedlings was enhanced. This is consistent with previous studies showing that AMF can increase N content and decrease C:N in plants [10,32,33]. AMF can decrease the nitrogen mineralization rate in the soil, thereby promoting better nitrogen absorption by plants [34,35]. Ri exhibited a more pronounced mycorrhizal effect on the C:N ratio of Teyou 2 leaves. The C:N ratio of leaves was regulated by the degree of mycorrhizal dependence, with a higher support resulting in a more significant change. Therefore, the mycorrhization of mulberry on rocky desertified soil could reduce the C:N ratio of leaves, improve plant nitrogen utilization, and subsequently increase the soil's nitrogen content through nutrient exchange between plants and soil.

4.2. Mycorrhization of Mulberry Seedlings Could Promote a Positive Change in Root Morphology

AMF can alter the morphological layout of plants, including root configuration. This study demonstrated that the root morphology of mycorrhized mulberry seedlings significantly improved on rocky desertified soil, which is consistent with previous studies [18,36–38]. This indicates that AMF could promote root growth and alter root morphology, which may occur when AMF infects plant roots, leading to the development of mycelia that invade the internal tissues of the root system. They engage with the root cells, leading to a subsequent transformation in the morphology of all elements within the root system. Additionally, AMF exhibits different effects on the physical characteristics of host plants, which can be attributed to the diverse influences of the strains on the plants [20]. These effects are evident in the root structure of the mycorrhized mulberry. This study showed that Ri notably exerts a substantial mycorrhizal impact on mulberry seedlings, as evidenced by variations in various indicators of root morphology.

4.3. Effects of Root Morphological Changes in Mycorrhized Mulberry Seedlings on Leaf Carbon to Nitrogen Ratio

The morphological characteristics of roots are closely related to plant nutrient uptake efficiency [39,40]. The root structure is a highly adaptable feature that enables plants to adjust to fluctuations in the bioavailability of water and nutrients in the soil, optimizing the efficiency of nutrient uptake, particularly nitrogen. Nitrogen is widely recognized as a critical component in plant proteins, amino acids, essential enzymes, biological enzymes, and other compounds. It is also closely linked to the relationship with root morphology [24,41]. The results of this study are supported by the findings of a previous study, which revealed that enhancing the length of roots, the number of lateral roots, and the surface area is essential for maximizing the efficiency of plant nutrient absorption via AMF symbiosis [42]. When arbuscular mycorrhized mulberry seedlings were transplanted to rocky desertification soil, their root morphology improved, resulting in increased N accumulation in the leaves and decreased leaf C:N ratio. These effects resulted in improved nitrogen utilization and growth rates, especially for Teyou 2, where a more robust symbiotic association with AMF was evident. The leaf C:N ratio of Teyou 2 was mainly influenced by factors such as root length, surface area, and connection number, with root length and surface area having a particularly significant impact.

Root length is a crucial metric for assessing root morphology, indicating the spatial absorption range of roots. It plays a vital role in nutrient and water absorption, serving as an indispensable characteristic for the growth and development of roots [43,44]. The leaf C:N ratio of Guisang 12 was predominantly influenced by root length, the number of root tips, and the number of connections. Notably, root length and the number of tips had the most substantial impact. The number of forks in the root system indicates the distribution space, extension range, and utilization ability of the roots in the soil. The changes in root morphology of arbuscular mycorrhized Teyou 2 were well correlated with leaf C: N. The significant differences between the two mycorrhized seedlings may be attributed to their gene expression, which requires further investigation. The results of this study confirm that mycorrhized mulberry seedlings on rocky desertification soil exhibit an inverse correlation between leaf C:N ratio and root morphology. As the root system developed further, the decrease in leaf C:N ratio became more pronounced, signifying a more favorable nutritional growth for the plant.

5. Conclusions

In summary, a well-developed root system architecture can enhance the interaction between the roots and the soil, facilitating the absorption of water and nutrients by plants. Planting mycorrhized mulberry seedlings in rocky desertified soil could induce significant positive changes in various root indicators, leading to alterations in root morphology. The number of connections and crosses increased exponentially by 120.5% and 109.8%. At the same time, AMF promoted plants to absorb more nitrogen, which reduced the leaf C:N ratio by 42.13%. Longer total root lengths and more connections benefit plants by enabling them to absorb and utilize more nitrogen, resulting in increased nitrogen accumulation in plant leaves and a reduced leaf C:N ratio. A lower C:N facilitates the nutritional growth of vegetation in the early stages of rocky desertification recovery, providing a theoretical basis for rocky desertification management.

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