Does the Mixture of Species change the C:N:P Ecological Stoichiometric Characteristics of fine Roots? A Case Study of Cupressus Funebri Mixed Forest

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Abstract

The growth of fine roots of trees is affected by environmental changes and biological factors. At present, there have been many researches on the physiological plasticity of fine roots caused by environmental changes, but there are still few studies on the influence of biological factors on fine roots. This paper focused on the contents of carbon (C), nitrogen (N), and phosphorus (P), and their ecological stoichiometric ratios in different root orders of *Cupressus funebris* fine roots in 11 mixed stands with *Koelreuteria paniculate* or *Toona sinensis* at different ratios, and the effects of soil physical and chemical properties on the root chemical properties. It aimed to provide new insights into the fine-root nutrient distribution pattern and the transformation or reconstruction of low-efficiency pure forests from the standpoint of forest types. The results showed that: soil pH, and the content of available nitrogen (SAN), available phosphorus (SAP) and available potassium (SAK) differed significantly in the tested mixed forest stands. No significant differences in carbon content of fine roots were observed in different mixed stands. The content of nitrogen and phosphorus in fine roots in mixed forests showed heterogeneity. Species mixing changed the C/N, C/P and N/P of the *C. funebris* compared the pure stands. The "*T. sinensis* + *C. funebris*" forest alleviated the limitation of the lack of phosphorus on fine roots of *C. funebris* on. The principal component analysis showed that mixed stands of "*T. sinensis* + *C. funebris*" had the highest comprehensive score at ratio of "3:1". Thus, our results recommended the adoption of *T. sinensis*, especially at 75%, to reconstruct the low-efficiency pure *C. funebris* forest.

1. Introduction

As many problems, such as low productivity, poor ecological functions, and serious pests and diseases, has been observed for the large area of concentrated and contiguous pure *C. funebris* forests in the hilly area of central Sichuan, (Marino and Onda, 2011), native tree species have been adopted as the companion tree species to build mixed forests to enhance the efficiency of forest land, maintain and protect the biodiversity and the stability of the ecosystem in this region (Zhou et al. 2019). Mixed forest is a plant community composed of different species, and the coexistence of species on a local scale is an important aspect for the mechanism research of community construction and biodiversity maintenance (Henning et al., 2016; Thomas et al., 2018). There are two hypotheses for the species coexistence theory which insists that there are differences among species, Average Fitness Difference (AFD) (HilleRisLambers et al., 2012) and Niche Difference (ND) (Andrew et al., 2017). The former emphasizes the regional equality of average fitness among coexisting species. The latter refers to factors that avoid or buffer the elimination of competition, and achieve stable coexistence through niche differentiation and trade-offs. Previous studies on species coexistence tended to qualitatively describe differences in relative fitness and niche differentiation through theoretical models. It is not only difficult to quantify the differences in species' ecological strategies and ecological functions, but also lacks other relevant information for explaining the mechanisms of biodiversity maintenance .

As plant functional traits are easy to determine, can characterize qualitative and quantitative indicators such as species' adaptation strategies for the environmental and physiological processes, and reflect the advantages of plants' adaptability to the surrounding environment and resource utilization efficiency (Martina et al., 2020), they have been introduced into the study of species coexistence. The C, N and P content of organisms and their ecological stoichiometric characteristics are one of the plant functional traits that are easy to measure, and are closely related to community structure, ecosystem stability, and biogeochemical cycles (James et al., 2009). Some scholars have investigated the soil C, N and P, and ecological stoichiometric ratios of different tree species in different regions (such as subalpine, tropical, cold temperate, etc.) (Wang et al., 2018; Michael et al., 2017; Yang et al. 2014; Feng et al., 2017). However, there are few reports on the relationship between ecological stoichiometric ratios and soil C, N or P of different stands under the same main tree species. In addition, can mixed forests change their stability and adopt new survival strategies and adaptability? For lake ecosystems and herbaceous plants, the stoichiometry of bacteria and multicellular animals is considered to have absolute homeostasis, but there are few studies on higher plants and no consensus has been reached (Sabine, 2005; Virginia and Peter, 2009). In addition, ecological stoichiometry is closely related to ecological processes, and most of the reported researches focused on the above-ground leaves (Liu et al., 2020; Gong et al., 2018). However, due to the differences in the content and ratio of C, N, and P among organs (Yang and Luo, 2011; Yuan and Chen, 2010), it is not feasible to replace the root system with the content of carbon, nitrogen and phosphorus in leaves.

The root system is the main body of the underground functional traits of plants. In particular, fine roots (diameter $\leq 2\text{mm}$), as the main organs for plants to absorb, transport and store nutrients and carbohydrates, play a very important role in the nutrient cycle of plants and forest ecosystems (Kyotaro et al., 2017). When the external environment changes, the fine roots are more sensitive than the above-ground organs, and their functional properties are a good indicator for understanding the subtle changes in the environment (Zhou et al., 2017). The root system has a branching structure similar to branches, and different root orders have great differences in morphology and physiological functions (Amandine et al., 2018; Kyotaro et al., 2017; Zhou et al., 2017). Therefore, it is worthy of in-depth discussion to study the differences of the functional properties of fine roots in different root orders.

Present studies mainly paid more attention to the effects of multi-species ecosystems on fine roots (Amandine et al., 2020; Marie et al., 2019), and how mixing affects the content and ratio of C, N, and P in fine roots. The mechanism still remains under debate. Therefore,

understanding the mechanism of mixed regulation of fine root changes will help scientifically managing low-efficiency plantations. As the most direct living space for roots, soil provides water and mineral elements for plant growth (Cao et al., 2020). At the same time, the growth, death, and turnover of fine roots will also be affected by soil factors (Sawsan et al., 2019). In addition, during plant growth and development, litter and root exudates can also improve certain physical and chemical properties of the soil, thereby changing its growth state, morphology, and physiological plasticity (E. Oleghe et al., 2017). Some studies also showed that there is a strong relationship between fine root nutrients and soil nutrient availability, pH, soil water content, soil bulk density and other soil physical and chemical properties (Dina et al., 2020; Su et al., 2019). Therefore, it is very important to study the relationship between the content and ratio of fine root C, N, P in different *C. funebris* forests, and the physical and chemical properties of soil.

Based on the above questions and that *C. funebris* is one of the most important protection forest tree species selected and bred in this area the "*Koelreuteria paniculata* + *C. funebris*", "*Toona sinensis* + *C. funebris*" and the pure *C. funebris* forest in the early stage of construction were employed in this research, with the research methods of plant functional traits and ecological stoichiometry. Therefore, this study explored the response characteristics of *C. funebris* fine roots in the mixed model through the determination of the carbon, nitrogen and phosphorus of *C. funebris* fine roots and the physical and chemical properties of rhizosphere soil, clarified its adaptation strategies to different forest stand environments, which provided a certain theoretical basis and reference value for further understand the underground growth process of different *C. funebris* forest stands, and the construction or reconstruction of *C. funebris* forest stands.

2. Materials And Methods

2.1 Study sites

The experimental site was located in Chongzhou Forest Farm, Chongzhou, Chengdu City, Sichuan Province, China (103°38'50" ~ 103°38'50" E, 30°33'30" ~ 30°33'31" N). The region has a subtropical humid monsoon climate, with four distinct seasons, short spring and autumn, long summer and winter, abundant rainfall, less sunshine, and long frost-free period. The annual average temperature is 16.8°C, average rainfall 1011.3 mm, annual average sunshine duration 994.9 h, and annual average frost-free period 282 d.

2.2 Plant materials

The adopted tree species were *C. funebris* (sapling height 77.0 ± 1.2 cm, ground diameter 7.76 ± 0.15 mm), *K. paniculata* (seedling height 94.1 ± 1.4 cm, ground diameter 7.40 ± 0.13 mm) and *T. sinensis* (seedling height 68.0 ± 1.1 cm, ground diameter 6.97 ± 0.08 mm), which were 2-year-old nutrient-cup seedlings. Ground diameter and height of seedlings for a given species were basically similar, and were growing well.

2.3 Experimental design

The 11 stands of "*K. paniculata* + *C. funebris*", "*T. sinensis* + *C. funebris*", and *C. funebris* pure forest built at the end of October 2015 were selected as the research objects. The mixture of "*K. paniculata* + *C. funebris*" at ratios of "1:3", "1:2", "1:1", "2:1" and "3:1" were referred to as T₁ to T₅, while "*C. funebris* + *T. sinensis*" as T₆ to T₁₀. *C. funebris* pure forest was recorded as the control (CK). A total of 33 sample plots (11 treatments × 3 replicates) were set up in this experiment, consisting of 756 *C. funebris* seedlings, 540 *K. paniculata* seedlings, 540 *T. sinensis* seedlings, i.e., 1386 seedlings in total (72 seedlings × 33 plots). The area of each plot was 5 m × 5 m, and in order to avoid mutual interference between treatments, a buffer distance of 4 ± 0.1 m was set between each plot. The mixed pattern adopted inter-plant mixing to ensure that the roots of the tree species overlap each other when planting, and the soil is solidified during the planting process. The seedlings were pretreated with mechanical soil preparation, manual weeding and watering 3 days before transplantation, and no management treatment was required after transplantation.

2.4 Sampling and index determination

The root and soil samples were collected on October 30, 2017. Three *C. funebris* trees were randomly collected from each plot. The complete root system and the associated rhizosphere soil were dug out, and the root system with fine roots scattered in the soil were sealed in a plastic bag. Some rhizosphere soil were sampled and labeled. The samples are brought back to the laboratory in 12 h, and the roots are rinsed with running water. The dead and alive root were distinguished according to the shape, color and elasticity of the roots, and they were placed in another sealed bags and stored at 4°C. The cryopreserved roots were washed with deionized water at room temperature to remove the surface residues, were graded using the root order grading method (Amandine et al., 2018). In specific, the roots that are farthest from the main root axis of the root system, having no branches, but having root tips, are defined as the first-level root; the mother root of the first-level root is defined as the second-level root; the second-level root is originated from the third-level root, and so on, until the fifth-level root. The born roots without branches on the high-level roots are also classified as first-level roots. For the scattered roots, the root sequence is divided according to its color, shape and degree of lignification. The roots at different root order were put into corresponding vessels, dried in a 65°C oven to constant weight, ground and sieved for the determination of C, N and P content. Soil pH, soil organic carbon (SC), soil total nitrogen (SN), soil

total phosphorus (SP), soil available nitrogen (SAN), soil available phosphorus (SAP) and soil available potassium (SAK) were also measured at the same time.

The pH of the soil is determined by the glass electrode method (the water-to-soil ratio is 1:2.5), the SC was determined by the potassium dichromate oxidation-external heating method, the SN was determined by the Kjeldahl method, and the SP was determined by the Kjeldahl method. Sodium hydroxide fusion-molybdenum-antimony colorimetric method was used for determination, the SAN is determined by alkaline hydrolysis diffusion method, the SAP is determined by sodium bicarbonate extraction-molybdenum-antimony colorimetric method, and the SAK is leached with neutral ammonium acetate Extraction-atomic absorption spectrophotometry determination.

2.5 Statistical analysis

Microsoft Excel 2007 was used to manage, sort and calculate the data; SPSS 20.0 was used for data statistics. The effect of tree species, mixing ratio, orders and their interactions on the *C. funebris* fine root C, N, P concentration and ecological stoichiometric ratio were analyzed with Three-way ANOVA. Furthermore, the data of C, N, P concentration and stoichiometric ratio of the fine roots of *C. funebris* of 1 ~ 5-order under different tree species or different ratios were analyzed by One-way ANOVA, followed by multiple comparison of Duncan ($P \leq 0.05$). Considering the growth of seedlings, the root system is concentrated on the surface of the soil, the physical and chemical properties of the surface rhizosphere soil and the content of C, N, P and their ratios in fine roots were selected for correlation analysis. Principal component analysis was used to obtain the comprehensive index. Sigmaplot 12.5 was used for figure drawing.

3. Results

3.1 Soil physical and chemical properties of different forest stands

The introduction of *K. paniculata* or *T. sinensis* into the *C. funebris* forests significantly reduced the pH of the rhizosphere soil ($P < 0.05$), but the differences between mixed forests were not significant ($P > 0.05$) (Table 1). There was no significant differences in SC or SN in different forest stands ($P > 0.05$). In addition, there was no significant differences for SP or SK between mixed forests ($P > 0.05$), but they were significantly higher than those in pure *C. funebris* forests ($P < 0.05$). Mixing significantly increased the available components such as SAN, SAP and SAK ($P < 0.05$). In mixed forests, as the proportion of *C. funebris* decreased, SAN, SAP and SAK increased in a high extend. Finally, under the same ratio, *T. sinensis* was more conducive to increasing SAN, SAP and SAK in the mixed forest.

3.2 C, N and P content and stoichiometric ratio in fine roots of *C. funebris*

The Two-way ANOVA showed that the fine roots chemical content and stoichiometric ratio of *C. funebris* were significantly influenced under mixing species, mixing ratios, root order and their interaction effects ($P < 0.05$), except that the fine root N was not affected under the interaction of root order and tree species ($P > 0.05$) (Table 2). That is to say, mixed tree species could affect the content and ratio of C, N, P in fine roots of *C. funebris*.

The fine root C content of *C. funebris* did not differ among the mixed treatments ($P > 0.05$), but they were all significantly lower than that of pure *C. funebris* forests ($P < 0.05$) (Figure 1A). The fine root N content of *C. funebris* showed a significant difference among different forest stands ($P < 0.05$) (Figure 1B). The N content of 1 ~ 4-order fine roots under T_1 treatment showed the highest ($P \leq 0.05$), and the N content of T_8 treatment was lower than that of pure forest, but the difference was not significant ($P > 0.05$) (Figure 1B). The change of fine root P content in mixed *C. funebris* forest showed a heterogeneous trend and was not always higher than that of pure forest (Figure 1C). However, there is a significant difference in fine root P content between "*K. paniculata* + *C. funebris*" and "*T. sinensis* + *C. funebris*" (Figure 1C). In general, "*T. sinensis* + *C. funebris*" was higher than "*K. paniculata* + *C. funebris*", and the P content of 1 ~ 5-order fine roots under T_{10} showed the highest (Figure 1C).

Fine root C/N, C/P and N/P were all affected by the mixing forest models (Figure 2A, 2B, 2C). The C/N of 1 ~ 5-order fine roots of the mixed forests were higher than those of pure *C. funebris* forests except for the T_1 treatment (Figure 2A). The fine root C/P in T_2 , T_4 , and T_5 treatments was higher than that of pure forest, while the other treatments were lower than those of pure forest (Figure 2B). Overall, T_4 treatment had the highest C/P for fine roots (Figure 2B). The fine root N/P of "*K. paniculata* + *C. funebris*" were between 13.95 and 24.51, and "*T. sinensis* + *C. funebris*" between 6.60 and 11.51 (Figure 2C).

3.3 Correlation analysis between fine root C, N, P content and stoichiometric ratio of soil physical and chemical properties

There were varying degrees of correlation between the content of C, N, and P in fine roots of *C. funebris* and their stoichiometric ratios, and the physical and chemical properties of rhizosphere soil (Table 3). Fine root C was significantly positively correlated with soil pH ($P < 0.01$), but significantly negatively correlated with SAN, SAP and SAK ($P < 0.01$) (Table 3). Fine root N was significantly negatively correlated with SAN, SAP and SAK ($P \leq 0.05$) (Table 3). Fine root C/P was significantly negatively correlated with soil pH, SAP and SAK ($P < 0.05$) (Table 3).

3.4 Principal component analysis of the concentration of fine root C, N, P content and its stoichiometric ratio and soil physical and chemical properties

The characteristic indexes of *C. funebris* fine roots (fine root C, N, P, C/N, C/P and N/P) and soil physical and chemical properties (pH, SC, SN, SP, SK, SAN, SAP, SAK), 14 indicators in total, were adopted for principal component analysis. Four principal component variables were extracted, and the cumulative contribution rate reached 79.681%, which contained sufficient information for most of the original data to meet the requirements of principal component analysis (Table 4). These 4 principal components can be used to analyze the fine root characteristics and soil physical and chemical properties of *C. funebris* under 11 different forests.

Maximum Variance Method was used to rotate the load of each factor, so that the coefficients in the matrix are differentiated from 0-1, which makes it easier to analyze the connotation of the principal components, i.e., the rotated principal component matrix (Table 5). In component 1, fine root N and fine root C/N contributed the largest load, and fine root N contribution was negative. In component 2, pH and SAP showed the largest and consistent load. In component 3, fine root C and fine root C/P had the largest load. SC contributed the largest load to Component 4.

The comprehensive evaluation model adopted:

$$F = \frac{\lambda_1}{\lambda_1 + \lambda_2 + \lambda_3 + \lambda_4} \times F_1 + \frac{\lambda_2}{\lambda_1 + \lambda_2 + \lambda_3 + \lambda_4} \times F_2 + \frac{\lambda_3}{\lambda_1 + \lambda_2 + \lambda_3 + \lambda_4} \times F_3 + \frac{\lambda_4}{\lambda_1 + \lambda_2 + \lambda_3 + \lambda_4} \times F_4$$

In the formula: F is the comprehensive evaluation value; λ_1 , λ_2 , λ_3 and λ_4 are the characteristic root indexes, and F_1 , F_2 , F_3 and F_4 are the main component values.

Comprehensive index based on principal component analysis of the rhizosphere soil environment and the characteristic factors of *C. funebris* fine roots under different forest stands was obtained through Table 6 (Table 7). The changes of soil physical and chemical properties and characteristic factors of *C. funebris* fine roots under different forest stands tended to be consistent in the 1~5-order fine roots, which generally increased with the increase of root order. *K. paniculata* or *T. sinensis* was selected as the associated tree species for *C. funebris* transformation, and the comprehensive index of "*T. sinensis* + *C. funebris*" was higher under the same mixing ration. The comprehensive index of "*T. sinensis* + *C. funebris*" increased with increasing proportion of *T. sinensis*, and the score was the highest under the T₁₀ treatment.

4. Discuss

4.1 Effect of mixing on soil physical and chemical properties

In this study, the physical and chemical properties of soil under different forest stands were measured. The introduction of *K. paniculata* or *T. sinensis* in the *C. funebris* forest significantly reduced the rhizosphere pH, while the difference between the mixed forest stands was not significant (Table 1). This may be resulted from the coexistence of species that secreted organic acids such as formic acid, acetic acid, and oxalic acid into the soil through roots, dissociating more H⁺, and lowering the soil pH to a certain extent (Ma et al., 2020). Indexes such as soil organic carbon and soil total nitrogen were not significantly differed among mixed stands (Table 1), which is similar as the results of study on different stands of beech, fir and spruce by Maïke et al. (Maïke et al., 2016). But significant differences were observed in the study of forests in North China (Wang et al., 2016), which showed that although fine root turnover is one of the important pathways for soil carbon input, it is unlikely to lead a significant change in a short term due to the huge soil organic carbon and soil total nitrogen storage capacity (Xu et al., 2018). Even in some mature forests, the changes are not obvious (Wang et al., 2017). The total phosphorus and total potassium of the soil in the mixed forest were increased compared to pure *C. funebris* forest (Table 1), indicating that the introduction of mixed tree species has changed the type and quantity of microbes in the rhizosphere soil, and transformed them into forms of inorganic phosphorus compounds and K⁺ that are easily absorbed by plants through ammonification and mineralization (Xi et al., 2019). The increased intensities of soil total phosphorus and soil total potassium in the "*K. paniculata* + *C. funebris*" and "*T. sinensis* + *C. funebris*" forest stands differed from each other (Table 1), which may be due to the different interaction modes between species and the different sensitivity of receptors (Ding et al., 2018), thus showing that *T. sinensis* is more suitable for the mixed forests of *C. funebris*. In the mixed forest, the SAN, SAP and SAK had all been improved (Table 1). Three possible reasons might be used to explain this: firstly, the organic acids and protons secreted from the roots of the mixed forest increase the solubility of insoluble nutrients in the soil through acidification or complexation (Li et al., 2007). Secondly, the mixed forest secretes more root exudates into the soil, which in turn provides nutrients and energy substances for the soil microorganisms, so that the

soil microorganisms multiply, combined with the catalysis of soil enzymes, accelerating the decomposition of insoluble nutrients in the soil (Andres et al., 2018). Thirdly, both *T. sinensis* and *K. paniculata* are deciduous tree species, and more litter can be returned to the soil, which accelerates the circulation of available nutrients in the soil (Wang et al., 2017). In addition, mixed litter can also accelerate its return efficiency (Lou et al., 2017; Michael et al., 2019).

4.2 Effect of mixing on the C, N, P content and stoichiometric ratio in fine roots

C, N, and P are the most basic constituent elements of organisms. The essence of biological growth and development is actually the process of regulating the accumulation and relative proportion of these basic elements (James et al., 2009; Michael et al., 2017). Plant roots, especially fine roots, are the main organs for plants to absorb water and nutrients. At the same time, their functional traits also reflect plant adaptation strategies for soil nutrient availability and intraspecific/interspecific competition (Kyotaro et al., 2017; Amandine et al., 2018). Therefore, the study of nutrient elements in fine roots is of great significance to comprehensively understand the recycling of nutrients in the entire forest ecosystem, especially the underground forest ecosystem.

Stand types have significant effects on the N, P and C/N, C/P and N/P of *C. funebris* fine roots (Figure 1A, 1B, 1C, Figure 2A, 2B, 2C), reflecting the profound significance of species mixing for nutrient balance in plants. In this study, it was found that the C content in 1~5-order fine roots was different between the mixed treatments, but it was not significant (Figure 1A). This may be because the C, as a structural substance, mainly acts as a skeleton in the plant, and its content is affected by the environment, being less influenced by environment and more stable (Alexandra et al., 2016). But the fine root C of *C. funebris* in mixed stands was significantly higher than that in the pure forest ($P < 0.05$), which is similar to the results of Leena et al. (Leena et al., 2019) on mixed forests in northern Europe, which suggested that some of the photosynthetic products were used for identification or defense between plants, resulting in a decrease in the C content of fine roots. The results of this study on the N content of fine roots in different *C. funebris* forests were heterogeneous (Figure 1B), indicating that only an appropriate mixing ratio can make the fine roots have higher nutrient accumulation and turnover capabilities than pure forests. In addition, the N element, as a functional substance, is more susceptible to environmental and inter-species changes (Justin et al., 2016). The mixing of *C. funebris* and *K. paniculata* has a certain promotion effect on the N content at different root orders of *C. funebris*, and the mixing of "*K. paniculata* + *C. funebris*" at ratio of 1:3 can maximize the use of N in the soil by the mixed forest. It can significantly increase the total N content of the 1~4-order fine roots of *C. funebris*. There might be two possible explanations: one is that there is a certain difference in nutrient requirements between species, showing the dislocation of time or nutrient type (Josie and Stephen, 2020); another is that the space utilized by different species have certain differentiation (Andrew et al., 2017). The changes of P content in fine roots of *C. funebris* mixed forest were heterogeneous (Figure 1C). In general, P content of "*T. sinensis* + *C. funebris*" is higher than that of "*K. paniculata* + *C. funebris*", and the P content of 1~5-order fine roots is the highest under T_{10} treatment. This may be due to the interactive effect between mixed tree species, which makes the content and distribution of P element different in pure forest from that in mixed forest (Xavier et al., 2020). At the same time, it may also attribute to the difference of tree species that different tree species have different competitive positions in the mixed forest, and this kind of competition has changed the growth of fine roots of different tree species, resulting in changes in the P content that can be obtained by the root system, and thus affecting the absorption of P element by fine roots (Wang et al., 2017). In addition, from the point of the classification of fine root orders, the lower roots are attached to the higher roots, which may also be the reason for the heterogeneity of P content between root orders (Chen et al., 2017).

Plant functional traits do not work separately, but can adapt to environmental changes through a series of combinations and trade-offs between traits. Yuan suggested that there were differences in morphology, chemistry and metabolism of fine roots, so there are differences in stoichiometric ratios (Yuan and Han, 2011). Generally speaking, C/N and C/P can usually reflect the absorption and utilization efficiency of N and P elements by plants. Higher C/N and C/P have higher N and P utilization efficiency, that is, plants have a higher growth rate (Yuan and Han, 2011; Alexandra et al., 2016; Leena et al., 2019). Species mixing does not change the stoichiometry of *C. funebris* fine roots. Contrary to our results, the introduction of mixed tree species changed the early identification of species and interaction effects between species, thereby influencing the growth and development rate. This study found that the C/N and C/P of mixed forests are not always higher than those of *C. funebris* cypress forests (Figure 2A, 2B), and the interaction between species would be beneficial, neutral, or inhibited (Michaela et al., 2016). At the same time, due to differences in plant root biomass, fine root volume, etc., an excessively high mixing ratio will not only lead to a high root exudates accumulation, but also resulted in more serious compression of living space (Vicente et al., 2019). In addition, the interaction between species and the sensitivity of receptors are different, which could also cause significant differences between different forest stands (Xavier et al., 2020). N and P are the most vulnerable nutrient elements for plants, and their ratio reflects the most direct signal of plant growth restricted by nutrients (Yuan and Han, 2011). It is generally believed that, during the process of plant growth, $N/P < 14$ can be considered as plant growth restricted by N, $14 < N/P < 16$ means that plants are both restricted by N and P, and $N/P > 16$ means plant growth is restricted by P (Yuan and Han, 2011; Michaela et al., 2016). Wang Zhiqiang et al. (Wang et al., 2020) found that the contents of C, N, and P in fine roots of Chinese plants were $448.81 \text{ mg} \cdot \text{g}^{-1}$, $10.73 \text{ mg} \cdot \text{g}^{-1}$, and $0.9 \text{ mg} \cdot \text{g}^{-1}$, respectively, while C/N, C/P and N/P were 41.84, 508.32 and 11.73, respectively. Yuan et al. (Yuan and Han, 2011) also concluded that the global fine root N/P ranged between 13~18. In this study, both C/N and

C/P were lower than these data (Figure 2A, 2B), indicating that the element utilization efficiency of the *C. funebris* root system in this area was low. At the same time, the introduction of *T. sinensis* or *K. paniculata* into the *C. funebris* forest can adjust its restriction on the use of chemical elements (Figure 2C). The fine root N/P of *C. funebris* in "*K. paniculata* + *C. funebris*" were 13.95~24.51, which is more restricted by the P element. However, the fine root N/P of *C. funebris* in "*T. sinensis* + *C. funebris*" was 6.60~11.51, which was more restricted by the N element. As the hilly area of central Sichuan is extremely deficient in P element (Zhang et al., 2020), choosing *T. sinensis* as the companion tree species of *C. funebris* can significantly alleviate the P deficiency limit of *C. funebris* forest. On the other hand, as this area also belongs to the area of high N deposition (Peng et al., 2020), the growth limitation of *C. funebris* by N restriction will be alleviated to some extent in the future.

4.3 the relationship between the C, N, P content and stoichiometric ratio in fine roots and soil physical and chemical properties

The content and ratio of C, N, P in fine roots not only depend on the dynamic balance of soil supply and its own morphological construction, but also the mutual adaptation and coordination of species. Many previous studies on subtropical and tropical regions have also shown that there is a certain correlation for physical and chemical properties between plant fine roots and soil (Cao et al., 2020; E. Oleghe et al., 2017; Dina et al., 2020). Our research showed that the C content of fine roots was significantly positively correlated with soil pH (Table 3). The C content and N content of fine roots were significantly negatively correlated with SAN, SAP and SAK (Table 3). Fine root C/P has a significant negative correlation with soil pH, SAP and SAK (Table 3). That is to say, the distribution of different physiological and functional traits in the growth process of *C. funebris* fine roots under different forest stands was different, and the changes in soil environment also led to differences in adaptation strategies. In addition, principal component analysis was carried out on the soil physical and chemical properties, and fine root characteristic factors in the 11 different *C. funebris* stands. The factors with higher loading in principal component 1 were all fine root characteristic factors (Table 5). The high loading in component 2 was the soil characteristic factor (Table 5). It showed that *C. funebris* fine root nutrient reacted very quick to the introduction of tree species. From the comprehensive analysis index, as the proportion of *C. funebris* decreased (Table 7), the comprehensive index increases. Therefore, it can be concluded the development of *C. funebris* fine roots and the change of rhizosphere soil environment were more significant with the introduction of *T. sinensis* or *K. paniculata*.

Conclusion

Mixing changed the C, N, P content and its ecological stoichiometric ratio in *C. funebris* fine roots. Mixing changed the pH, SAN, SAP and SAK, etc., of the rhizosphere soil, thereby changing the nutrient distribution pattern of *C. funebris* fine roots and affecting its growth and development. Different forest stands adopted different survival strategies among root sequences when responding to changes in the soil environment. In comparison, the first three levels of fine roots were more sensitive. Choosing *T. sinensis* as a mixed species of *C. funebris* can alleviate the influence of *C. funebris* fine roots by the P restriction. In general, the "*T. sinensis* + *C. funebris*" model with a mixing ratio of "3:1" had the best effect.

Declarations

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Tables

Table1 the soil physical and chemical properties under different forest stands

Treatment	pH	SC(mg·g ⁻¹)	SN(mg·g ⁻¹)	SP(mg·g ⁻¹)	SK(mg·g ⁻¹)	SAN(mg·g ⁻¹)	SAP(mg·g ⁻¹)	SAK(mg·g ⁻¹)
T ₁	7.66±0.09 ^b	16.68±1.04 ^a	1.43±0.04 ^a	0.84±0.03 ^a	16.41±0.26 ^{ab}	125.27±0.99 ^e	43.89±0.77 ^{gh}	66.56±1.54 ^e
T ₂	7.64±0.05 ^b	16.72±0.64 ^a	1.43±0.02 ^a	0.84±0.03 ^a	16.43±0.20 ^{ab}	126.08±0.75 ^{de}	44.58±1.62 ^{fg}	66.86±1.89 ^e
T ₃	7.58±0.04 ^b	16.73±0.40 ^a	1.43±0.03 ^a	0.84±0.01 ^a	16.46±0.05 ^{ab}	128.12±1.81 ^d	45.86±0.33 ^{efg}	67.13±1.69 ^e
T ₄	7.66±0.04 ^b	16.79±0.43 ^a	1.43±0.03 ^a	0.85±0.01 ^a	16.56±0.06 ^a	134.23±2.17 ^c	47.40±1.99 ^{cde}	69.37±0.96 ^c
T ₅	7.62±0.05 ^b	16.96±0.66 ^a	1.45±0.01 ^a	0.85±0.02 ^a	16.61±0.15 ^a	143.83±1.83 ^a	49.19±1.62 ^{bc}	70.68±1.24 ^{bc}
T ₆	7.60±0.06 ^b	16.48±0.75 ^a	1.43±0.01 ^a	0.84±0.02 ^a	16.37±0.15 ^{ab}	131.76±1.34 ^c	46.60±0.92 ^{def}	69.58±1.25 ^c
T ₇	7.57±0.06 ^b	16.49±0.88 ^a	1.43±0.01 ^a	0.84±0.01 ^a	16.37±0.10 ^{ab}	131.91±1.43 ^c	47.36±1.37 ^{cde}	70.10±1.47 ^c
T ₈	7.63±0.06 ^b	16.56±0.71 ^a	1.44±0.02 ^a	0.84±0.01 ^a	16.44±0.17 ^{ab}	133.41±0.70 ^c	48.55±1.68 ^{bc}	71.09±0.52 ^{bc}
T ₉	7.62±0.03 ^b	16.62±0.84 ^a	1.44±0.01 ^a	0.85±0.00 ^a	16.51±0.11 ^{ab}	139.2±1.09 ^b	50.17±1.21 ^b	72.42±0.77 ^{ab}
T ₁₀	7.66±0.02 ^b	16.74±0.44 ^a	1.45±0.03 ^a	0.86±0.01 ^a	16.58±0.07 ^a	143.68±0.64 ^a	53.91±1.35 ^a	73.72±0.86 ^{ab}
CK	7.78±0.00 ^a	16.27±0.00 ^a	1.42±0.00 ^a	0.83±0.00 ^a	16.26±0.00 ^b	126.9±2.28 ^{de}	42.11±0.36 ^h	62.09±0.44 ^f

NOTE: pH, SC, SN, SP, SK, SAN, SAP, SAK means soil pH, soil organic carbon, soil total nitrogen, soil total phosphorus, soil total potassium, soil alkaline nitrogen, soil available phosphorus, and soil available potassium, respectively. Different lowercase letters in the same column indicate that the relevant indicators between different treatments are significantly different at the 0.05 level.

Table.2 the results of multi-factor analysis of variance of C, N, P content and stoichiometric ratio of fine roots under different stands

source	Type III sum of squares	df	Mean square	F	Sig.	
Fine root carbon	Root sequence	368160.867	4	92040.217	4841.861	.000
	Mixing ratio	581.573	4	145.393	7.649	.000
	species	102.838	1	102.838	5.410	.022
	Root sequence × Mixing ratio	1206.048	16	75.378	3.965	.000
	Root sequence ×species	449.768	4	112.442	5.915	.000
	Mixing ratio×species	1077.215	4	269.304	14.167	.000
	Root sequence × Mixing ratio×species	1319.380	16	82.461	4.338	.000
Fine root nitrogen	Root sequence	889.845	4	222.461	112.820	.000
	Mixing ratio	52.035	4	13.009	6.597	.000
	species	226.035	1	226.035	114.632	.000
	Root sequence × Mixing ratio	78.465	16	4.904	2.487	.003
	Root sequence ×species	5.878	4	1.469	.745	.563
	Mixing ratio×species	271.945	4	67.986	34.479	.000
	Root sequence × Mixing ratio×species	165.576	16	10.348	5.248	.000
Fine root phosphorus	Root sequence	7.836	4	1.959	69.547	.000
	Mixing ratio	3.224	4	.806	28.610	.000
	species	10.968	1	10.968	389.351	.000
	Root sequence × Mixing ratio	3.635	16	.227	8.064	.000
	Root sequence ×species	1.518	4	.379	13.471	.000
	Mixing ratio×species	2.058	4	.514	18.264	.000
	Root sequence × Mixing ratio×species	3.097	16	.194	6.871	.000
Fine root C/N	Root sequence	13833.136	4	3458.284	488.808	.000
	Mixing ratio	221.920	4	55.480	7.842	.000
	species	880.065	1	880.065	124.392	.000
	Root sequence × Mixing ratio	530.665	16	33.167	4.688	.000
	Root sequence ×species	120.760	4	30.190	4.267	.003
	Mixing ratio×species	622.815	4	155.704	22.008	.000
	Root sequence × Mixing ratio×species	581.602	16	36.350	5.138	.000
Fine root C/P	Root sequence	2566506.906	4	641626.727	257.674	.000
	Mixing ratio	306055.126	4	76513.782	30.728	.000
	species	1253795.637	1	1253795.637	503.518	.000
	Root sequence × Mixing ratio	173868.718	16	10866.795	4.364	.000
	Root sequence ×species	201485.670	4	50371.417	20.229	.000
	Mixing ratio×species	342802.391	4	85700.598	34.417	.000
	Root sequence × Mixing ratio×species	174471.029	16	10904.439	4.379	.000
Fine root N/P	Root sequence	122.582	4	30.645	13.367	.000
	Mixing ratio	274.092	4	68.523	29.888	.000
	species	2336.080	1	2336.080	1018.926	.000

Root sequence × Mixing ratio	293.370	16	18.336	7.997	.000
Root sequence ×species	94.765	4	23.691	10.333	.000
Mixing ratio×species	266.671	4	66.668	29.078	.000
Root sequence × Mixing ratio×species	215.453	16	13.466	5.873	.000

Table.3 Correlation coefficients between the concentration and stoichiometric ratio of C, N, P in fine roots of *C. funebris* and the physical and chemical properties of soil

	pH	SC	SN	SP	SK	SAN	SAP	SAK
F _C	0.019	0.012	-0.005	-0.026	0.011	0.007	-0.008	-0.034
F _N	0.146	0.065	-0.065	-0.137	0.036	-0.176*	-0.179*	-0.233**
F _P	-0.191*	0.020	0.072	0.013	0.013	0.211**	0.362**	0.357**
F _{C/N}	-0.085	-0.050	0.039	0.062	-0.044	0.085	0.101	0.132
F _{C/P}	0.182*	0.014	-0.033	0.035	0.047	-0.082	-0.249**	-0.266**
F _{N/P}	0.298**	0.044	-0.090	-0.028	0.082	-0.199*	-0.397**	-0.445**

NOTE: *and ** represent significant at P≤0.05 and 0.01, respectively.

Table.4 Interpretation of fine root characteristic factors and total variance of soil physical and chemical properties under different forest stands

component	Initial eigenvalue			Extract the sum of squares and load
	total	Variance %	accumulation %	total
1	4.063	29.022	29.022	4.063
2	3.396	24.256	53.278	3.396
3	2.026	14.473	67.751	2.026
4	1.670	11.930	79.681	1.670
5	0.836	5.969	85.650	
6	0.561	4.007	89.656	
7	0.424	3.028	92.684	
8	0.337	2.406	95.090	
9	0.248	1.770	96.860	
10	0.182	1.303	98.163	
11	0.124	0.884	99.047	
12	0.080	0.570	99.617	
13	0.037	0.266	99.883	
14	0.016	0.117	100.000	

Table.5 Rotated component matrix

	component			
	1	2	3	4
Fine root carbon	-0.188	-0.152	0.925	0.045
Fine root nitrogen	-0.915	-0.154	0.107	0.037
Fine root C/N	0.978	0.060	0.011	-0.020
Fine root C/P	0.567	-0.066	0.785	0.033
Soil pH	0.125	0.772	-0.411	0.231
Soil carbon	-0.029	-0.127	-0.014	0.837
Soil available phosphorus	0.077	0.815	-0.335	0.268

Extraction method: principal component analysis method.

Rotation method: Orthogonal rotation method with Kaiser standardization.

a.the rotation converges after 6 iterations.

Table.6 the coefficient matrix of fine root characteristic factors and soil physical and chemical properties score function under different forest stands

	component			
	1	2	3	4
Fine root carbon	-0.132	0.047	0.375	0.058
Fine root nitrogen	-0.313	-0.006	0.102	0.040
Fine root C/N	0.330	-0.023	-0.069	-0.019
Fine root C/P	0.133	0.028	0.266	0.038
Soil pH	0.036	0.225	-0.087	0.015
Soil carbon	-0.004	-0.148	-0.001	0.426
Soil available phosphorus	0.012	0.248	-0.047	0.030

Table.7 Principal component analysis comprehensive index of rhizosphere soil environment and *C. funebris* fine root characteristic factors under different forest stands

Treatment	Root sequence	F1	F2	F3	F4	F
T ₁	1	0.33	-2.24	0.34	0.02	-0.50
	2	0.12	-1.92	0.48	0.09	-0.44
	3	-0.10	-1.33	0.47	0.09	-0.34
	4	-0.31	-1.15	0.68	0.19	-0.31
	5	-0.95	1.03	0.31	0.02	0.03
T ₂	1	0.23	-1.41	0.01	-0.15	-0.37
	2	0.05	-0.99	0.03	-0.14	-0.30
	3	-0.27	0.06	-0.13	-0.21	-0.13
	4	-0.81	1.00	0.14	-0.09	0.02
	5	-1.34	1.74	0.52	0.08	0.15
T ₃	1	0.05	-1.31	-0.11	-0.27	-0.44
	2	-0.23	-0.77	0.00	-0.22	-0.35
	3	-0.44	-0.49	0.18	-0.14	-0.30
	4	-0.88	0.45	0.27	-0.10	-0.15
	5	-1.47	1.68	0.44	-0.04	0.05
T ₄	1	0.63	-0.82	-0.07	-0.07	-0.04
	2	0.44	-0.31	-0.07	-0.07	0.04
	3	0.14	-0.09	0.27	0.08	0.08
	4	-0.53	0.64	0.91	0.37	0.22
	5	-1.07	1.71	1.09	0.45	0.39
T ₅	1	0.75	-0.89	-0.03	-0.16	-0.03
	2	0.43	-0.47	0.21	-0.04	0.05
	3	0.21	-0.36	0.50	0.09	0.07
	4	-0.51	0.64	1.04	0.33	0.25
	5	-1.02	2.47	0.66	0.14	0.52
T ₆	1	0.17	-0.29	-0.81	-0.37	-0.23
	2	0.33	-0.39	-1.02	-0.46	-0.25
	3	0.04	-0.10	-0.74	-0.34	-0.20
	4	-0.38	0.53	-0.46	-0.22	-0.09
	5	-1.05	1.56	-0.05	-0.04	0.08
T ₇	1	0.13	-0.64	-0.66	-0.34	-0.32
	2	0.07	-0.61	-0.58	-0.30	-0.31
	3	-0.19	-0.45	-0.26	-0.15	-0.28
	4	-0.39	-0.15	-0.13	-0.09	-0.23
	5	-1.15	2.25	-0.49	-0.30	0.13
T ₈	1	0.82	-1.90	0.06	0.12	-0.25
	2	0.60	-0.71	-0.35	-0.06	-0.07
	3	0.41	-0.23	-0.35	-0.06	0.01

	T ₉	4	0.10	0.45	-0.28	-0.03	0.12
		5	-0.59	2.09	-0.25	-0.04	0.37
		1	0.86	-1.16	-0.16	0.03	-0.06
		2	0.70	-0.39	-0.4	-0.08	0.05
		3	0.47	-0.20	-0.15	0.04	0.09
	T ₁₀	4	0.16	0.20	0.10	0.16	0.16
		5	-0.37	1.38	0.18	0.18	0.35
		1	1.70	-0.60	-0.42	0.07	0.37
		2	1.68	-0.76	-0.28	0.14	0.35
		3	1.41	0.17	-0.44	0.07	0.49
	CK	4	0.87	0.56	0.17	0.35	0.57
		5	0.39	2.18	-0.11	0.20	0.81
		1	0.54	-1.72	0.13	0.33	-0.25
		2	0.49	-0.76	-0.39	0.10	-0.11
		3	0.17	-0.09	-0.29	0.15	0.00
		4	-0.45	1.19	-0.12	0.22	0.21
		5	-0.97	1.71	0.39	0.45	0.31

Figures

Figure 1

A. The fine root N content of *C. funebris* showed a significant difference among different forest stands ($P < 0.05$). B. The N content of 1 ~ 4-order fine roots under T₁ treatment showed the highest ($P \leq 0.05$), and the N content of T₈ treatment was lower than that of pure forest, but

the difference was not significant ($P > 0.05$). C. However, there is a significant difference in fine root P content between "K. paniculata + C. funebris" and "T. sinensis + C. funebris"

Figure 2

A. The fine root C/P in T₂, T₄, and T₅ treatments was higher than that of pure forest, while the other treatments were lower than those of pure forest. B. Overall, T₄ treatment had the highest C/P for fine roots. C. The fine root N/P of C. funebris in "K. paniculata + C. funebris" were 13.95~24.51, which is more restricted by the P element.

Supplementary Files

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- [Heetal.Appendix.docx](#)