Seasonal Variation in Ecological Stoichiometric Characteristics of Carbon, Nitrogen, and Phosphorus in Fine Roots of Karst Forests and Their Influencing Factors: Postprint

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Abstract

The ecological stoichiometric characteristics of plant fine root nutrients represent a strategy for vegetation adaptation to soil environments. To understand the seasonal variation in ecological stoichiometric ratios of carbon (C), nitrogen (N), and phosphorus (P) in fine roots of different forest types in karst regions and their influencing factors, we investigated the C, N, and P contents and ratios in living and dead fine roots of shrublands and woodlands in a karst region and their relationships with environmental factors. The results showed: (1) Overall, the C, N, and P contents in both types of fine roots in woodlands were higher than those in shrublands, indicating that fine roots of tree species have stronger nutrient uptake and storage capacity than those of shrub species. Additionally, for both forest types, the C content in living fine roots was significantly higher than that in dead fine roots ($P < 0.05$), whereas the N and P contents in living fine roots were lower than those in dead fine roots. (2) The C contents in both types of fine roots for both forest types were lower in the rainy season than in the dry season; the N and P contents in living fine roots of shrublands were higher in the rainy season than in the dry season, while the opposite pattern was observed in woodlands. The C:N, C:P, and N:P ratios in living fine roots of shrublands were all lower in the rainy season than in the dry season; in woodlands, the C:N and C:P ratios in both types of fine roots were higher in the rainy season than in the dry season, whereas the N:P ratio was lower in the rainy season than in the dry season. The lower N:P ratios in living fine roots during the rainy season indicated that plants in both shrublands and woodlands experienced a lower degree of P limitation during the rainy season. (3) The C contents in both types of fine roots at upper slopes were higher than those at middle and lower slopes for both forest types, whereas the N and P contents were relatively higher at lower slopes in shrublands and at middle slopes in woodlands; the C:N, C:P, and N:P ratios in both types of fine roots at upper slopes were relatively higher in shrublands, while in woodlands, the C:N ratio at lower slopes was higher than at other slope positions, and the C:P and N:P ratios at upper slopes were higher than at other slope positions, indicating that plants in both forest types were more strongly affected by P limitation at upper slopes and less affected at middle and lower slopes. (4) Redundancy analysis indicated that forest type, available phosphorus, and season were the main factors influencing fine root C, N, and P nutrient contents and ratios, with individual explanatory powers of 18.8%, 6.6%, and 6.5%, respectively. These results suggest that when artificially promoting vegetation restoration, appropriate forest types, seasonal variations, and the effects of slope position differences on N:P ratio changes should be considered in order to accelerate the restoration of karst ecosystems.

Full Text

Seasonal Variation and Influencing Factors of Fine Root C:N:P Ratios in Two Forests of a Karst Ecosystem

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Abstract: The ecological stoichiometry of fine root carbon (C), nitrogen (N), and phosphorus (P) reflects plant adaptation strategies to soil environments. To understand seasonal variations in fine root C:N:P ratios and their influencing factors across different forest types in karst regions, we investigated the C, N, and P contents and ratios of living and dead fine roots in shrubland and arbor forests, along with their relationships with environmental factors. Our results showed: (1) Overall, fine root C, N, and P contents in arbor forests were higher than those in shrublands, indicating stronger nutrient absorption and storage capacity in arbor species. Additionally, living fine roots had significantly higher C content than dead fine roots ($P < 0.05$), while N and P contents were lower in living fine roots. (2) For both forest types, fine root C contents were lower during the rainy season than the dry season. In shrublands, living fine root N and P contents were higher in the rainy season, whereas the opposite pattern occurred in arbor forests. The C:N, C:P, and N:P ratios of living fine roots in shrublands were lower in the rainy season, while in arbor forests, the C:N and C:P ratios of both fine root types were higher in the rainy season but N:P ratios were lower. The lower rainy season N:P ratios suggest reduced P limitation for plants in both forest types during this period. (3) Fine root C contents at upper slopes were higher than at middle and lower slopes for both forest types. Shrublands showed relatively higher N and P contents at lower slopes, while arbor forests had higher N and P contents at middle slopes. Shrublands exhibited relatively higher C:N, C:P, and N:P ratios at upper slopes, whereas arbor forests showed higher C:N ratios at lower slopes and higher C:P and N:P ratios at upper slopes, indicating stronger P limitation effects on upper slopes in both forest types. (4) Redundancy analysis revealed that forest type, available phosphorus, and season were the main factors influencing fine root C, N, and P contents and ratios, with individual explanatory powers of 18.8%, 6.6%, and 6.5%, respectively. These findings suggest that vegetation restoration efforts in karst regions should consider the effects of N:P ratio variations associated with forest type, season, and slope position to accelerate ecosystem recovery.

Keywords: Karst ecosystem, different forest types, fine root nutrients, soil nutrients, ecological stoichiometry

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Plant roots serve as a bridge connecting aboveground vegetation with soil. Extensive root systems not only facilitate soil particle stabilization and effectively control soil erosion, providing important soil and water conservation functions (Su et al., 2018), but also regulate carbon (C), nitrogen (N), and phosphorus (P) nutrient balance and promote nutrient cycling in ecosystems. Fine roots, defined as root portions with diameters less than 2 mm (Chen et al., 2018), undergo rapid turnover cycles of production, death, and decomposition. Through death and decomposition, they return nutrients to soil at rates exceeding aboveground litter inputs (Vogt et al., 1986; Zhang and Wu, 2001; Wei et al., 2013). When soil nutrients become depleted or heterogeneous, fine roots can adapt by regulating their life cycles and ecological stoichiometric ratios, making them the most sensitive component responding to soil environmental changes (Liu et al., 2014).

Ecological stoichiometry provides a comprehensive approach for studying multiple chemical elements and their balance relationships (He and Han, 2010; Pan et al., 2011). Fine root C:N:P ratios can characterize plant nutrient use efficiency (Xiong et al., 2015; Zhang et al., 2021) and indicate growth limitations—N limitation occurs when N:P ratio is below 14, P limitation when above 16, and co-limitation by N and P when between 14 and 16 (Tan et al., 2022; Zheng et al., 2022). Factors such as forest type, season, slope position, and changes in soil nutrient pools and availability can trigger adaptive adjustments in fine root nutrient strategies (Chen et al., 2018; Zhang et al., 2022). Applying ecological stoichiometry theory to analyze fine root C, N, and P contents and ratios helps understand plant adaptation capabilities and strategies to external environments. However, how fine root nutrients in different forest types of karst regions respond to environmental changes remains unclear.

Different forest types exhibit substantial differences in species composition and community structure (Liu et al., 2021), leading to variations in soil nutrient content (Tian et al., 2015). Roots are most directly affected by soil nutrient changes, and nutrient limitations are reflected in fine roots (Tan et al., 2022). Seasonal variations strongly influence root nutrient dynamics through differential soil nutrient availability. For example, Li et al. (2016) found that soil total N, total P, available N, and available P contents in northern Guangxi grasslands were relatively higher in summer and autumn, subsequently affecting fine root C, N, and P contents. Seasonal changes also correlate with fine root growth dynamics, with fine root biomass and growth peaks occurring during rainy seasons (Rufat and Dejong, 2001; Chen et al., 2004; Xu et al., 2018). Thus, seasonal variations affect both aboveground and belowground vegetation characteristics and soil environments. Additionally, topographic factors such as slope position influence soil environmental properties (Yu et al., 2019). While upper slopes typically have poorer soil nutrients in non-karst regions (Yang et al., 2018), karst areas exhibit an inverse pattern with upper slopes having greater nutrient content than lower slopes (Liang et al., 2017), affecting plant nutrient allocation to fine roots and ultimately causing differences in fine root nutrient content across slope positions (Qin et al., 2008; Chen et al., 2018).

Recent studies have examined ecological stoichiometric characteristics of soils, vegetation, and different plant organs. He et al. (2020) suggested that vegetation type is closely linked to soil C:N:P stoichiometry, where changes in soil C:N:P ratios can affect vegetation species composition (Bui et al., 2013), while vegetation can influence soil C, N, and P cycling through litter and root systems, thereby affecting soil nutrient content (Gao et al., 2014). Guo et al. (2021) found that nutrient distribution patterns differ among plant organs, with plants responding to environmental changes by adjusting C:N:P ratios among organs, highlighting the critical importance of roots.

Karst regions in China suffer severe rocky desertification, accelerated soil erosion, and declining land productivity. Many scholars (Lü et al., 2016; Wu et al., 2020) have studied nutrient limitation status and acquisition efficiency through aboveground components like leaf stoichiometry. However, aboveground parts are less sensitive to soil environmental changes than fine roots (Guo et al., 2021). Therefore, understanding fine root nutrient variations across forest types and seasons, along with nutrient allocation patterns and influencing factors, is essential for comprehending plant root adaptation mechanisms in these regions. To investigate how fine root nutrients respond to environmental changes across different forest types, we collected fine root and soil samples from shrubland and arbor forests in karst regions to examine variations in fine root C, N, and P contents and ratios across forest types, seasons, and slope positions. These studies reveal response patterns of fine roots to environmental changes such as season and slope position in different forest types of karst regions, providing scientific basis for ecological restoration and management.

1.1 Study Area

The study area is located in Huanjiang Maonan Autonomous County, Guangxi Zhuang Autonomous Region, China, encompassing the Huanjiang Karst Ecosystem Observation and Research Station of the Chinese Academy of Sciences (108°18'–108°19' E, 24°43'–24°44' N) and the Mulun National Nature Reserve (107°53'–108°05' E, 25°06'–25°12' N). This region features typical karst peak-cluster depressions and a subtropical monsoon climate, with mean annual temperature of 19.9°C, extreme minimum temperature of -5.2°C, extreme maximum temperature of 38.7°C, and mean annual precipitation of 1,389.1 mm. Precipitation is abundant but seasonally uneven, with the rainy season (April–September) accounting for over 70% of annual rainfall (Chen et al., 2012) and the dry season extending from October to March of the following year.

The Huanjiang station experienced frequent burning and grazing from 1958 to the mid-1980s. After all residents relocated in 1985, the degraded system began to recover. The typical landscape unit is peak-cluster depression, with approximately 70% of the study area covered by shrubland (Pan et al., 2020). Dominant plants include *Alchornea trewioides*, *Cipadessa cinerascens*, *Rhus chinensis*, and *Indigofera atropurpurea.*

The Mulun National Nature Reserve covers a total area of 190.2 hm^2 with forest coverage of 94.8%, representing the most intact and largest primary forest preserved in global karst regions (Pan et al., 2011; Zhang et al., 2013). Dominant species include *Cyclobalanopsis glauca*, *Loropetalum chinense*, *Miliusa balansae*, and *Pteroceltis tatarinowii* (Pan et al., 2020).

1.2 Sampling Methods

In May 2014, we established 15 standard plots (10 m $\times$ 10 m, with 5 plots per slope position) in both the Huanjiang Karst Ecosystem Observation and Research Station and the Mulun National Nature Reserve. These plots were distributed across upper, middle, and lower slope positions, with each plot spaced more than 10 m apart.

We studied seasonal fine root dynamics over one year using sequential root coring (Song et al., 2010). From May 2014 to May 2015, fine roots were sampled every two months using root coring (sampling times: May, July, September, and November 2014, and January, March, and May 2015; rainy season: April–September; dry season: October–March). Sampling depth was 10 cm. Each plot was divided into four subplots (5 m $\times$ 5 m), and soil cores were collected using a root corer (10 cm inner diameter $\times$ 10 cm length), then combined into one composite sample. A total of 210 samples were collected (2 forest types $\times$ 15 plots $\times$ 7 sampling times).

Soil core samples were soaked in water for 24 hours before washing to remove soil. Fine roots were selected based on the $ $2 mm diameter criterion (Shi and Tang, 2002). Living and dead fine roots were distinguished by color, shape, elasticity, and ease of separation between cortex and stele (Ostonen et al., 2005). Each fine root sample was dried at 65°C for at least 48 hours, then ground to pass through a 0.154 mm sieve. This yielded a total of 420 samples for analysis (living and dead fine roots).

In May 2014, soil samples were collected from the four subplots of each plot at 10 cm depth, combined into one composite sample, air-dried, and ground to pass through a 2 mm sieve for physicochemical analysis.

1.3 Sample Analysis Methods

Fine root C and N contents were determined using an elemental analyzer (Vario MAX CN, Elementar, Germany). Fine root P content was measured by molybdenum-antimony anti-spectrophotometry after digestion with H_2SO_4+H_2O_2 (Pan et al., 2011).

Soil total nitrogen (TN) was determined by the Kjeldahl method using a flow injection analyzer (FIAstar 5000, FOSS, Hillerød, Denmark). Soil available nitrogen (AN) was measured by the alkali diffusion method. Soil total phosphorus (TP) was digested with NaOH in a muffle furnace, washed with H_2SO_4+HCl, developed with molybdenum blue reagent, and measured spectrophotometrically. Soil available phosphorus (AP) was extracted with $NaHCO_3$ solution, with color development and measurement following the same procedure as TP (Bao, 2000).

1.4 Data Processing and Analysis

Raw data were processed using Excel 2013 and SPSS 26.0. All variables were tested for normal distribution before analysis. One-way ANOVA and least significant difference (LSD) tests were used to analyze differences in soil and fine root nutrient contents between the two forest types. Pearson correlation analysis was performed between fine root C, N, and P contents and stoichiometric characteristics and soil nutrients. Redundancy analysis (RDA) was used to assess the contribution of environmental factors to variations in fine root nutrient contents and stoichiometric ratios.

2.1 Soil Nutrient Characteristics Across Forest Types

Soil TN and AN contents differed significantly between shrubland and arbor forest ($P < 0.05$), while TP and AP contents showed no significant differences. TN:TP and AN:AP ratios also did not differ significantly between forest types. From shrubland to arbor forest, soil TN, AN, and TP contents and TN:TP and AN:AP ratios increased, while AP content decreased.

Note: Different lowercase letters indicate significant differences between shrubland and arbor forest.

[Figure 1: see original paper] Soil total nitrogen, available nitrogen, total phosphorus, available phosphorus, and their stoichiometric ratios

2.2 Effects of Forest Type on Fine Root C, N, P Contents and Stoichiometry

Living fine root C content was significantly higher than dead fine root C content in both forest types ($P < 0.05$, [Figure 2: see original paper]a), while living fine root N and P contents were lower than those in dead fine roots ([Figure 2: see original paper]b, c). Arbor forest fine roots had higher C, N, and P contents and N:P ratios but lower C:N and C:P ratios than shrubland, with significant differences in N content ($P < 0.05$).

2.3 Seasonal Effects on Fine Root C, N, P Contents and Stoichiometry

In shrubland, fine root C contents were lower during the rainy season (448.70 $g \cdot kg^{-1}$ and 412.98 $g \cdot kg^{-1}$) than the dry season (457.99 $g \cdot kg^{-1}$ and 422.22 $g \cdot kg^{-1}$) ([Figure 2: see original paper]a). Living fine root N and P contents were higher in the rainy season (14.24 $g \cdot kg^{-1}$ and 1.22 $g \cdot kg^{-1}$) than the dry season (14.16 $g \cdot kg^{-1}$ and 1.15 $g \cdot kg^{-1}$), while dead fine roots showed the opposite pattern. All stoichiometric ratios of living fine roots were lower in the rainy season, whereas dead fine root C:P and N:P ratios (314.70 and 13.87) were higher than in the dry season (294.20 and 12.61), with significant differences in dead fine root N:P ratio ($P < 0.05$).

In arbor forest, both fine root types had lower nutrient contents in the rainy season ([Figure 2: see original paper]a, b, c), with significant differences in N content between seasons ($P < 0.05$). Rainy season C:N and C:P ratios of living and dead fine roots (21.97, 391.49, 20.36, 304.91) were higher than dry season ratios (21.30, 361.54, 17.72, 274.48) ([Figure 2: see original paper]d, e), while N:P ratios showed the opposite pattern ([Figure 2: see original paper]f).

Note: Different lowercase letters indicate significant differences in fine root nutrients and stoichiometric ratios under the same forest type across seasons; different uppercase letters indicate significant differences between forest types within the same season. SB: Shrubland; AF: Arbor forest; RS: Rainy season; DS: Dry season.

[Figure 2: see original paper] Seasonal dynamics of fine root C, N, P contents and stoichiometric ratios across forest types

2.4 Effects of Slope Position on Fine Root C, N, P Contents and Stoichiometry

In shrubland, living and dead fine root C contents gradually decreased from upper to lower slopes ([Figure 3: see original paper]a). Lower slopes had higher N and P contents than other positions ([Figure 3: see original paper]a, b). C:N and C:P ratios of living and dead fine roots at upper and middle slopes were significantly higher than at lower slopes ($P < 0.05$; [Figure 3: see original paper]d, e). N:P ratios of living and dead fine roots were lowest at middle slopes ([Figure 3: see original paper]f).

In arbor forest, upper slopes had higher C contents than other positions ([Figure 3: see original paper]a). Middle slopes showed higher N and P contents than upper and lower slopes ([Figure 3: see original paper]b, c). C:N ratios of living and dead fine roots were higher at lower slopes ([Figure 3: see original paper]d). C:P and N:P ratios of living and dead fine roots were significantly higher at upper slopes than other positions ($P < 0.05$; [Figure 3: see original paper]e, f).

Note: Different lowercase letters indicate significant differences in fine root nutrients and stoichiometric ratios across slope positions within the same season and forest type. US: Upper slope; MS: Middle slope; LS: Lower slope; SB: Shrubland; AF: Arbor forest.

[Figure 3: see original paper] Variations in fine root C, N, P contents and stoichiometric ratios across slope positions and forest types

2.5 Relationships Between Fine Root C, N, P Contents and Soil Nutrients

Living and dead fine root N contents were significantly positively correlated with soil TN ($P < 0.05$), while living fine root N content was significantly negatively correlated with soil AP ($P < 0.01$). Dead fine root N content was significantly positively correlated with living fine root N and P contents ($P < 0.01$), and living fine root N content was significantly positively correlated with P content ($P < 0.01$). Living and dead fine root N contents and living fine root P content were significantly positively correlated with N:P ratios ($P < 0.01$) and significantly negatively correlated with C:N ratios ($P < 0.01$) ().

RDA analysis indicated that variations in fine root C, N, P contents and ratios were primarily influenced by forest type (individual explanatory power: 18.8%, F=7.9338, P=0.001), AP (6.6%, F=3.2784, $P < 0.05$), and season (6.5%, F=2.5048, $P < 0.05$) ([Figure 4: see original paper]).

Correlation analysis between fine root nutrient contents, stoichiometric ratios, and soil nutrients

Note: * indicates significance at $P < 0.05$ level (two-tailed); ** indicates significance at $P < 0.01$ level (two-tailed).

Note: TN: Total nitrogen; TP: Total phosphorus; AN: Available nitrogen; AP: Available phosphorus; LC: Living fine root carbon; LN: Living fine root nitrogen; LP: Living fine root phosphorus; DC: Dead fine root carbon; DN: Dead fine root nitrogen; DP: Dead fine root phosphorus; LC:LN: C:N ratio of living fine roots; LC:LP: C:P ratio of living fine roots; LN:LP: N:P ratio of living fine roots; DC:DN: C:N ratio of dead fine roots; DC:DP: C:P ratio of dead fine roots; DN:DP: N:P ratio of dead fine roots. Redundancy analysis (a), Variance partitioning analysis (b).

[Figure 4: see original paper] Analysis of soil properties and fine root nutrients and their stoichiometric ratios

3.1 Effects of Forest Type on Fine Root Nutrient Contents and Stoichiometry

Arbor forest fine roots had higher C, N, and P contents and N:P ratios but lower C:N and C:P ratios than shrubland fine roots. Previous studies (Liao and Guo, 2022) have shown that increased soil TN and TP contents promote fine root nutrient absorption. In this study, arbor forest soils had higher TN, TP, and AN contents than shrubland soils ([Figure 1: see original paper]), suggesting greater nutrient absorption capacity in arbor forests. Shrubland fine root N:P ratios were below 14, indicating predominant N limitation, likely due to relatively high P content reducing the N:P ratio. Arbor forest fine roots (N:P > 16) experienced P limitation, possibly because higher soil N content relative to shrubland, combined with asynchronous N and P absorption by fine roots (Guo et al., 2018), resulted in higher N than P content and elevated N:P ratios. Growth-related dilution effects may also have contributed to relatively low P content (Wen et al., 2022).

Correlation analysis showed no significant relationship between fine root C content and soil nutrients, as C is a structural element with strong stability that is not directly involved in metabolic activities (Hu et al., 2018). Living fine root N content was significantly positively correlated with soil TN and significantly negatively correlated with soil AP, indicating that N and P are the main factors influencing fine root C:N:P stoichiometry. Since shrubland fine root N and P contents were lower than those in arbor forest, shrubland exhibited higher C:N and C:P ratios. RDA results confirmed forest type as the primary factor influencing fine root C, N, P contents and stoichiometry, likely because arbor species have stronger nutrient absorption and utilization capabilities. Compared with shrubland, arbor forests have increased species richness (Yang et al., 2009), leading to greater litter input and root biomass, which improves soil quality (Sun et al., 2021). As fine roots are sensitive to soil environmental changes, this ultimately enhances nutrient absorption and storage.

3.2 Seasonal Effects on Fine Root Nutrient Contents and Stoichiometry

During the rainy season, both forest types showed lower C contents in living and dead fine roots, and arbor forests also had lower N and P contents in both root types and dead fine roots in shrubland, while shrubland living fine roots had higher N and P contents. In shrubland, stoichiometric ratios of living fine roots were lower in the rainy season, whereas in arbor forest, C:N and C:P ratios of both root types were higher in the rainy season but N:P ratios were lower. The study area experiences concurrent rainfall and heat during the rainy season, with abundant precipitation and high temperatures promoting vigorous plant growth (Deng et al., 2010). This alters C allocation patterns, with more carbon allocated to aboveground growth and reproduction (Pregitzer, 2003; Li et al., 2021), resulting in lower fine root C content during the rainy season. Shrubland vegetation at this growth stage requires substantial nutrients, and dead fine roots may transfer some nutrients back to the plant before senescence (Zhang and Wu, 2001), leading to lower dead fine root N and P contents in the rainy season.

Carbon content in plants is relatively high and stable, not typically limiting plant growth (Niu et al., 2011), and correlation analysis showed no significant relationship between fine root C content and N, P nutrients or stoichiometric ratios. Therefore, variations in C:N and C:P ratios are primarily driven by N and P elements. In shrubland, higher rainy season N and P contents in living fine roots resulted in lower C:N and C:P ratios. In contrast, arbor forest living fine roots had lower N and P contents during the rainy season, possibly due to: (1) differences in species composition and vegetation growth, with arbor forests having higher species richness and greater fine root biomass (Yang et al., 2009; Du et al., 2010; Wang et al., 2014), and the rainy season being the peak growth period for fine root biomass (Rufat and Dejong, 2001; Chen et al., 2004), which may dilute N and P concentrations; and (2) different water stress response mechanisms between forest types. Shrublands contain more shallow-rooted species primarily utilizing shallow soil water (Huang et al., 2021), while arbor forests include both deep- and shallow-rooted species that can utilize hydraulic lift to supply shallow-rooted plants (Chen et al., 2013; Chen et al., 2022), facilitating nutrient absorption. During the dry season, N and P may be transferred from senescing fine roots to living fine roots, enriching living root nutrients while depleting dead roots (Tripathi et al., 1999), resulting in higher rainy season C:N and C:P ratios in arbor forest.

Studies have shown that fine root C:N and C:P ratios reflect turnover rates, with higher ratios indicating slower turnover (Terzaghi et al., 2013). Therefore, shrubland fine root turnover was faster in the rainy season, while arbor forest turnover was faster in the dry season. N:P ratio serves as an indicator of nutrient limitation on productivity (He et al., 2017), and lower rainy season N:P ratios in both forest types indicate reduced P limitation during this period. In summary, seasonal changes in precipitation and temperature elicit different responses from different vegetation components, and fine roots, as the most sensitive belowground component, actively regulate nutrient cycling in response to environmental changes, thereby affecting their own C, N, P contents and stoichiometric ratios.

3.3 Effects of Slope Position on Fine Root Nutrient Contents and Stoichiometry

Both forest types showed higher fine root C contents at upper slopes than at other positions. Shrubland had higher N and P contents at lower slopes, while arbor forest had higher N and P contents at middle slopes. Shrubland and arbor forest at middle and lower slopes exhibited lower N:P ratios, indicating reduced P limitation at lower slope positions.

Slope position, as an important topographic factor, influences water-heat conditions and soil nutrient distribution, indirectly affecting fine root nutrients (Fan et al., 2019). Upper slopes experience less surface runoff, forcing reduced soil water retention and causing water loss (Zhang et al., 2010). To enhance root water retention capacity, plants increase carbon allocation to roots to maintain normal physiological functions (Luo et al., 2020), resulting in higher fine root C contents at upper slopes in both forest types. Since fine root C content showed no significant correlation with stoichiometric ratios, N and P were the main elements affecting these ratios. Two factors may explain differences in fine root N, P contents and stoichiometric ratios across slope positions: (1) Spatial heterogeneity of environmental factors (soil nutrients). Southwestern karst regions exhibit inverted soil nutrient patterns with upper slopes > lower slopes (Zhang et al., 2006; Liu et al., 2010; Liang et al., 2017). Lower slopes with relatively poor soil nutrients may trigger increased nutrient allocation to fine roots (Cai et al., 2022) and higher nutrient use efficiency (Zeng et al., 2015), resulting in relatively higher N and P contents in shrubland lower slopes. Shrubland upper slopes had lower P content, leading to higher C:P ratios, while higher N content at lower slopes resulted in lower C:N ratios. Middle slope N:P ratios were lower than other positions, indicating weaker P limitation at middle slopes in shrubland. (2) Spatial heterogeneity of biological factors and human activities. Arbor forest middle slopes have high community diversity, intact community structure, and minimal human disturbance (Peng et al., 2012), leading to more developed root systems, greater root biomass, higher vegetation coverage, and increased litter accumulation (Liu et al., 2016), which enhances soil fertility and promotes nutrient absorption and storage, resulting in higher N and P contents at middle slopes. In arbor forest, upper slopes had higher N content than lower slopes, while P content showed the opposite pattern, leading to lower N:P ratios at lower slopes and thus weaker P limitation compared to other positions. These findings demonstrate that environmental and biological factors, combined with human disturbance, directly or indirectly affect nutrient absorption by fine roots across slope positions, causing variations in fine root nutrient contents and stoichiometric ratios.

Conclusions

Arbor forest fine roots had higher nutrient contents but lower C:N and C:P ratios than shrubland fine roots, suggesting stronger N and P absorption and storage capacity in arbor species. Shrubland fine root N:P ratios were lower than arbor forest, indicating greater P limitation in arbor forests.

In shrubland, living fine root N and P contents were higher in the rainy season than the dry season, while arbor forest showed the opposite pattern. Shrubland rainy season living fine root C:N and C:P ratios were lower than in the dry season, whereas arbor forest showed the reverse trend. However, both forest types had lower N:P ratios in the rainy season, indicating reduced P limitation during this period.

Middle and lower slope positions resulted in higher fine root N and P contents but lower N:P ratios, suggesting weaker P limitation at lower slope positions.

References:

Bao SD, 2000. Soil Agricultural Chemistry Analysis[M]. Beijing: China Agriculture Press.

Bui EN, Henderson BL, 2013. C:N:P stoichiometry in Australian soils with respect to vegetation and environmental factors[J]. Plant Soil, 373(1/2): 553-568.

Cai YM, Zhang CF, Li XY, et al., 2022. Effect of litter input on the growth and stoichiometry of fine roots of *Pinus massoniana*[J]. J Northeast For Univ, 50(6): 32-38.

Chen HS, Yang J, Fu W, et al., 2012. Characteristics of slope runoff and sediment yield on karst hill-slope with different land-use types in northwest Guangxi[J]. Trans Chin Soc Agric Eng, 28(16): 121-126.

Chen GS, He ZM, Xie JS, et al., 2004. Comparison on fine root production, distribution and turnover between plantations of *Fokienia hodginsii* and *Cunninghamia lanceolata*[J]. Sci Silv Sin, 40(4): 15-21.

Chen HS, Nie YP, Wang KL, 2013. Spatio-temporal heterogeneity of water and plant adaptation mechanisms in karst regions: a review[J]. Acta Ecol Sin, 33(2): 317-326.

Chen RS, Kang WX, He JN, et al., 2022. Water storage capacities of different vegetation ecosystems[J]. J Cent South Univ For Tech, 42(6): 108-116.

Chen XP, Guo BQ, Zhong QL, et al., 2018. Response of fine root carbon, nitrogen, and phosphorus stoichiometry to soil nutrients in *Pinus taiwanensis* along an elevation gradient in the Wuyi mountains[J]. Acta Ecol Sin, 38(1): 273-281.

Deng PY, Chen HS, Nie YP, et al., 2010. Photosynthetic characteristics of *Radermachera sinica* and *Alchornea trewioides* in karst regions of Northwest Guangxi, China in dry and rainy seasons[J]. Chin J Ecol, 29(8): 1498-1504.

Du YX, Pan GX, Li LQ, et al., 2010. Fine root biomass and its nutrient storage in karst ecosystems under different vegetations in Central Guizhou, China[J]. Chin J Appl Ecol, 21(8): 1926-1932.

Fan Y, Chen ZW, Pan YL, et al., 2019. Effect of stand age and slope position on the stoichiometric characters of *Cunninghamia lanceolata-Aleurites montana* mixed forest[J]. Chin J Appl Environ Biol, 25(2): 246-253.

Gao Y, He NP, Yu GR, et al., 2014. Long-term effects of different land use types on C, N, and P stoichiometry and storage in subtropical ecosystems: A case study in China[J]. Ecol Eng, 67: 171-181.

Guo RQ, Xiong DC, Song TT, et al., 2018. Effects of simulated nitrogen deposition on stoichiometry of fine roots of Chinese fir (*Cunninghamia lanceolata*) seedlings[J]. Acta Ecol Sin, 38(17): 6101-6110.

Guo W, Qi LH, Lei G, et al., 2021. Nutrient distribution patterns and stoichiometry characteristics in *Phyllostachys edulis* and its varieties[J]. J Northeast For Univ, 49(4): 39-44.

He GX, Wang Y, Peng SX, et al., 2020. Soil carbon, nitrogen and phosphorus stocks and ecological stoichiometry characteristics of different vegetation restorations in degraded mountainous area of central Yunnan, China[J]. Acta Ecol Sin, 40(13): 4425-4435.

He HL, Yang XG, Li DD, et al., 2017. Stoichiometric characteristics of carbon, nitrogen and phosphorus of *Sibiraea angustata* shrub on the eastern Qinghai-Xizang plateau[J]. Chin J Plant Ecol, 41(1): 126-135.

He JS, Han XG, 2010. Ecological stoichiometry: Searching for unifying principles from individuals to ecosystems[J]. Chin J Plant Ecol, 34(1): 2-6.

Hu HT, Qiu LJ, Ge LL, et al., 2018. Stoichiometry of fine roots and topsoil of five plantations in coastal sandy[J]. J Sichuan Agric Univ, 36(4): 444-449.

Huang PZ, Li JX, Li DX, et al., 2021. Physiological and ecological adaption of karst woody plants to drought[J]. Guihaia, 41(10): 1644-1653.

Li SP, Wang KL, 2016. Seasonal distribution of soil nutrients and their response to plant diversity of karst mountain grassland[J]. J Soil Water Conserv, 30(4): 199-205.

Li X, Tan ND, Wu T, et al., 2021. Plant growth and C:N:P stoichiometry characteristics in response to experimental warming in four co-occurring subtropical forest tree seedlings[J]. Acta Ecol Sin, 41(15): 6146-6158.

Liang YM, Su YR, He XY, et al., 2017. Various effects on the abundance and composition of arbuscular mycorrhizal fungal communities in soils in karst shrub ecosystems[J]. Chin J Envir Sci, 38(11): 4828-4835.

Liao YN, Guo SJ, 2022. Effects of organic-inorganic fertilizer on the stoichiometric characteristics in chestnut rhizosphere soil and fine roots[J]. J Northeast For Univ, 50(1): 58-63.

Liu C, Xiao WH, Lei PF, et al., 2014. Standing fine root mass and production in four Chinese subtropical forests along a succession and species diversity gradient[J]. Plant Soil, 376(1/2): 445-459.

Liu L, Zeng FP, Song TQ, et al., 2010. Spatial heterogeneity of soil nutrients in Karst area's Mulun National Nature Reserve[J]. Chin J Appl Ecol, 21(7): 1667-1673.

Liu SN, Li G, Yang CJ, et al., 2021. Seasonal variation of soil carbon, nitrogen and phosphorus stoichiometry under different vegetation types in loess hilly region[J]. J Soil Water Conserv, 35(6): 343-349.

Liu X, Huang YX, Yuan H, 2016. Effects of vegetation type and slope position on soil nitrogen transformation rate in Karst regions[J]. Acta Ecol Sin, 36(9): 2578-2587.

Luo HB, Huang CM, Zhu HM, et al., 2020. Effects of drought stress on carbon and nitrogen metabolism of sugarcane roots[J]. J Southern Agric, 51(6): 1332-1338.

Lü WQ, Zhou CY, Yan JH, et al., 2016. Leaf C, N, and P stoichiometry for four typical artificial forests in the karst region of Guizhou Province[J]. J Zhejiang A & F Univ, 33(6): 984-990.

Niu DC, Dong XY, Fu H, 2011. Seasonal dynamics of carbon, nitrogen and phosphorus stoichiometry in *Stipa bungeana*[J]. Pratac Sci, 28(6): 915-920.

Ostonen I, Lohmus K, Pajuste K, 2005. Fine root biomass, production and its proportion of NPP in a fertile middle-aged Norway spruce forest: Comparison of soil core and ingrowth core methods[J]. For Ecol Manag, 212(1-3): 264-277.

Pan FJ, Qian Q, Liang YM, et al., 2022. Spatial variations in fine root turnover, biomass, and necromass of two vegetation types in a karst ecosystem, Southwestern China[J]. Forests, 13(4): 611.

Pan FJ, Wang KL, Zhang W, et al., 2020. Seasonal changes and rhizosphere effects of soil nutrients and enzymatic activities in two vegetation successions of karst ecosystem[J]. J Guilin Univ Tech, 40(1): 209-217.

Pan FJ, Zhang W, Wang KL, et al., 2011. Litter C:N:P ecological stoichiometry character of plant communities in typical Karst Peak-Cluster depression[J]. Acta Ecol Sin, 31(2): 335-343.

Peng WX, Song TQ, Zeng FP, et al., 2012. Relationships between woody plants and forest environmental factors in karst mixed evergreen-deciduous broadleaf forest, southwest China[J]. J Food Agric Environ, 10(1): 890-896.

Pregitzer KS, 2003. Woody plants, carbon allocation and fine roots[J]. New Phytol, 158(3): 421-424.

Qin Y, Wang LH, Zhang GS, et al., 2008. Fine root biomass seasonal dynamics and spatial changes of *Sabina vulgaris* and *Artemisia ordosica* communities in Mu Us Sandland[J]. J Desert Res, 28(3): 455-461.

Rufat J, DeJong TM, 2001. Changes in fine root production and longevity in relation to water and nutrient availability in a Norway spruce stand in northern Sweden[J]. Tree Physiol, 21(15): 1057-1063.

Shi JP, Tang JW, 2002. Fine root dynamics of several types of tropical communities in Xishuangbanna[J]. Guihaia, 22(6): 509-512.

Song RQ, Zhai MY, Jia LM, et al., 2010. Fine root dynamics of different aged triploid *Populus tomentosa* pulp forests during growth period[J]. Chin J Ecol, 29(9): 1696-1702.

Song TQ, Peng WX, Zeng FP, et al., 2009. Spatial heterogeneity of surface soil moisture content in dry season in Mulun National Natural Reserve in karst area[J]. Chin J Appl Ecol, 20(1): 98-104.

Su L, Du H, Wang H, et al., 2018. Root architecture of the dominant species in various vegetation restoration processes in karst peak-cluster depression[J]. Acta Bot Bor-Occid Sin, 38(1): 150-157.

Sun CL, Wang YW, Wang CJ, et al., 2021. Effects of land use conversion on soil extracellular enzyme activity and its stoichiometric characteristics in karst mountainous areas[J]. Acta Ecol Sin, 41(10): 4140-4149.

Tan X, Li LB, Xiang GW, et al., 2022. Ecological stoichiometric characteristics of carbon, nitrogen, and phosphorus in the fine root of *Taxodium* and their correlation with soil nutrients in the riparian zone of the Three Gorges Reservoir Region[J]. J Southwest Univ (Natl Sci Ed), 44(7): 14-23.

Terzaghi M, Montagnoli A, Di Iorio A, et al., 2013. Fine-root carbon and nitrogen concentration of European beech (*Fagus sylvatica* L.) in Italy Prealps: possible implications of coppice conversion to high forest[J]. Front Plant Sci, 4: 192.

Tian NN, Zhang JJ, Ru H, et al., 2015. Soil moisture and nutrient characteristics of soil and water conservation forests in Loess Plateau of western Shanxi Province[J]. Sci Soil Water Conserv, 13(6): 61-67.

Tripathi SK, Singh KP, Singh PK, 1999. Temporal changes in spatial pattern of fine-root mass and nutrient concentrations in Indian bamboo savanna[J]. Appl Veg Sci, 2(2): 229-238.

Vogt KA, Grier CC, Vogt DJ, 1986. Production, turnover, and nutrient dynamics of aboveground and belowground detritus of world forests[J]. Advan Ecol Res, 15: 303-377.

Wang WW, Huang JX, Chen F, et al., 2014. Effects of tree species diversity on fine-root biomass and morphological characteristics in subtropical *Castanopsis carlesii* forests[J]. Chin J Appl Ecol, 25(2): 318-324.

Wei P, Li XW, Fan C, et al., 2013. Fine root biomass and carbon storage in surface soil of *Cinnamomum camphora* plantation in Rainy Area of West China[J]. Chin J Appl Ecol, 24(10): 2755-2762.

Wen YX, Feng KQ, Tong R, et al., 2022. Response of C, N, P stoichiometry of fine and coarse roots of *Metasequoia glyptostroboides* plantation to nitrogen addition[J]. For Res, 35(3): 161-168.

Wu P, Cui YC, Zhao WJ, et al., 2020. Leaf stoichiometric characteristics of 68 typical plant species in Maolan National Nature Reserve, Guizhou, China[J]. Acta Ecol Sin, 40(14): 5063-5080.

Xiong K, Jin ML, Yu T, et al., 2015. Stoichiometric characteristics of C, N, P in typical steppe plant at different grazing gradients[J]. J Green Sci Tech, 17(7): 4-7.

Xu ZY, Zhang QD, Yang L, 2018. Analysis of temporal variation of soil water in a growing season in semi-arid loess hilly area[J]. J Arid Land Resour Environ, 32(3): 145-151.

Yang HB, Wei XL, Dang W, 2009. Species composition and diversity variations at different succession stages of karst vegetation in central Guizhou[J]. J Mount Agric Bio, 28(3): 203-207.

Yang SL, Zhang J, Yang WQ, et al., 2018. A study on the growth characteristics of roots of *Pennisetum sinese* in different slopes of the dry-hot valley[J]. J Yunnan Agric Univ (Nat Sci Ed), 40(3): 577-585.

Yu M, Wang ZM, Xue L, 2019. Effects of different slope positions on leaf nutrient of four broadleaf native tree species[J]. J Cent South Univ For Tech, 39(5): 89-94.

Zeng ZX, Wang KL, Liu XL, et al., 2015. Stoichiometric characteristics of plants, litter and soils in karst plant communities of Northwest Guangxi[J]. Chin J Plant Ecol, 39(7): 682-693.

Zhang C, Chen HS, Nie YP, et al., 2013. Dynamics of soil profile water content in peak-cluster depression areas in karst region[J]. Chin J Eco-Agric, 21(10): 1225-1232.

Zhang JG, Chen HS, Su YR, et al., 2010. Variability of soil moisture and its relationship with environmental factors on Karst hillslope[J]. Trans Chin Soc Agric Eng, 26(9): 87-93.

Zhang XL, Zhou JH, Lai LM, et al., 2021. Carbon, nitrogen and phosphorus stoichiometric characteristics of *Tamarix ramosissima* shrubland and their influencing factors in a desert riparian area of China[J]. Res Environ Sci, 34(3): 698-706.

Zhang W, Chen HS, Wang KL, et al., 2006. The heterogeneity of soil nutrients and their influencing factors in peak-cluster depression areas of karst region[J]. Sci Agric Sin, 39(9): 1828-1835.

Zhang X, Wang DM, Wen WJ, et al., 2022. Seasonal patterns in fine root biomass and nutrient storage of four plantations in the alpine region of Qinghai Province[J]. Sci Silv Sin, 58(6): 13-22.

Zhang XQ, Wu KH, 2001. Fine-root production and turnover for forest ecosystems[J]. Sci Silv Sin, 37(3): 126-138.

Zheng X, Liu Q, Cao MM, et al., 2022. A review of responses of soil nitrous oxide emissions to nitrogen input in forest ecosystems[J]. Acta Pedol Sin, 59(5): 1190-1203.