AGE-RELATED CHANGES IN SOIL AND MICROBIAL C:N:P STOICHIOMETRY IN *ZENIA INSIGNIS* PLANTATIONS IN KARST REGIONS OF SOUTHWEST CHINA

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Abstract. Afforestation is a key strategy for vegetation restoration in the karst rocky desertification regions of southern China. *Zenia insignis* is widely cultivated for this purpose. However, there is a scarcity of data on soil nutrient status and biogeochemical cycles concerning different stand ages in *Z. insignis* plantations within these ecosystems. This study assessed the concentrations of carbon (C), nitrogen (N), and phosphorus (P) and their stoichiometric ratios in soil and microbial biomass across *Z. insignis* plantations of varying ages (3-5, 9-11, 14-16, 19-21, and 24-26 years) in the karst region of Guangxi Province, Southwest China. The results showed that soil C, N, and P concentrations increased with stand age, with significant differences in C content among different ages. Notably, the C:P ratio varied significantly between stands aged 3-5 years and those aged 19-26 years, as did the N:P ratio between stands aged 3-11 years and those aged 24-26 years. The concentrations of soil microbial biomass C (SMBC), microbial biomass N (SMBN), and microbial biomass P (SMBP) also tended to increase with stand age. Differences were observed between stands aged 3-5 years and those aged 24-26 years for SMBC, SMBN, and SMBP but not for the microbial C:N:P ratio. Our study revealed no significant associations between soil and microbial stoichiometric ratios. However, significant correlations were found between SMBC:SMBP and SMBC:SMBN, as well as between SMBN:SMBP. This study underscores the complex responses of soil and microbial stoichiometry to age variations in *Z. insignis* plantations in the karst rocky desertification region of Southwest China.
Keywords: *soil organic carbon, stand age, stoichiometry, karst, Zenia insignis*

Introduction

Carbon (C), nitrogen (N), and phosphorus (P) are three essential elements for biological composition. The biogeochemical cycles of C, N, and P are closely connected, and their respective ratios, known as C:N:P ratio, is an important indicator of ecosystems nutrient status (Liu et al., 2020a). Changes in this ratio can have significant effects on ecosystem processes and functions (Mooshammer et al., 2012; Fan et al., 2015; Smith et al., 2020). C:N:P ratio is particularly associated with both aboveground and belowground ecosystem components and shows complex responses to changes in vegetation (Abbott et al., 2011; Zhou et al., 2018; Zhang et al., 2021a). For example, soil C, N, and P stoichiometry significantly influences vegetation structure and growth, which subsequently affects soil C:N:P ratios through plant litter and root secretions (Zechmeister-Boltenstern et al., 2015; Liu and Wang, 2021).

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Previous research has shown temporal variations in global carbon and nitrogen stoichiometry in forest ecosystems (Yang and Luo, 2011; Liang et al., 2018), and studies have explored how soil C, N, and P stoichiometry affects vegetation patterns (Boyden et al., 2013). The C:N:P ratio in soil is a direct measure of soil fertility and indirectly reflects plant nutritional status (Batjes, 2014). Studies have indicated that the average C:N:P ratio of soil microbial biomass in terrestrial ecosystems tends to reach a dynamic equilibrium of 60:7:1 (Tian et al., 2010). However, studies conducted in diverse ecosystem types have challenged this concept (Chen et al., 2016). Therefore, the association between soil C:N:P stoichiometry and soil microbes is still being investigated, with ongoing debates on whether microbial communities maintain strict equilibrium (Fanin et al., 2013).

Karst landscapes are an indispensable element of the Earth's surface system. In Southwest China, these landscapes cover approximately 340,000 km^2, which is approximately 15% of the world's land area. They extend over approximately 51 million km^2 of carbonate rock areas (Jiang et al., 2014) that are continuously exposed. However, in recent decades, intensive human activities in Southwest China have resulted in severe soil erosion, extensive bedrock exposure, and further ecosystem degradation (Zhou et al., 2020). These activities negatively affect soil fertility and ecosystem services, leading to the widespread occurrence of rocky desertification in the region (Du et al., 2011). This issue is of significant ecological and environmental concern nationwide. To address these problems, the Chinese government has implemented ecological restoration projects such as the Returning Cultivated Land to Forestry Project and the Natural Forest Conservation Project. These initiatives have proven effective in restoring degraded land, enhancing soil fertility, improving ecological conditions, and promoting socioeconomic development (Shidong and Moucheng, 2022). Additionally, the karst region in Southwest China has become an important area for global carbon sequestration (Zhu et al., 2012). Recent studies have emphasized the considerable influence of soil nutrient conditions, especially N and P, on the growth and development of plantations in karst areas (Pan et al., 2015). Ecological stoichiometry provides valuable insights for predicting plant community productivity and vegetation succession in karst ecosystems (Liao et al., 2023). However, limited attention has been given to the stoichiometric characteristics of C, N, and P in soil and soil microbial biomass, as well as their associations with plantation age, in karst ecosystems.

Zenia insignis, a rare and endangered species found mainly in limestone habitats in Southwest China, belongs to the genus Zenia. It is officially protected at the national level as a second-order protected plant on the Red List of Plants of China and is also classified as Vulnerable (VU) on the IUCN Red List (Lai et al., 2019). As a pioneer species used for afforestation to combat rocky desertification in Southwest China, *Z. insignis* has the ability to fix nitrogen. When it decomposes, it contributes to an increase in nutrient elements in the topsoil, thereby improving the physical and chemical properties of the soil. This species holds significant value for widespread planting, comprehensive utilization, and development and plays a crucial role in the ecological restoration and reconstruction of areas affected by rocky desertification (Zhang et al., 2019). Currently, research on *Z. insignis* plantations has expanded beyond seedling cultivation and cultivation techniques to include investigations into its physiological and ecological stress tolerance, wood utilization, ecological stoichiometry, and root traits (Chan et al., 2019). However, there is still a lack of comprehensive understanding

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regarding the ecological stoichiometric characteristics of soil in *Z. insignis* plantations of varying ages (Zhang et al., 2020).

This study examined the correlation between the soil C:N:P ratio and microorganisms at different stages in *Z. insignis* plantations. The primary goal was to comprehensively assess nutrient limitations in the ecosystem processes of *Z. insignis* plantations and develop management strategies to enhance the sustainability of the karst ecosystem. This research analyzed the stoichiometric characteristics of soil and microbial C, N, and P contents throughout the development of *Z. insignis* plantations in karst mountainous areas. Two key questions were addressed: (1) How do soil and microbial C:N:P ratios change with the development of *Z. insignis* plantations? (2) What factors influence the C:N:P stoichiometry of soil microorganisms, assuming that the soil C:N:P ratio of microorganisms is in a nonsteady state?

Materials and methods

Study area

The study area is located in Guohua Town (E107°22’34″, N23°20’19″) of Pingguo City in Guangxi Province, Southwest China (*Fig. 1*). The region has a subtropical monsoon climate characterized by hot and humid summers, dry springs and autumns, and cold and dry winters. The annual temperature ranges is 21.5°C. The frost-free period lasts approximately 300-350 days per year, and the average annual rainfall is 1297 mm, with a relative humidity exceeding 80%. Rainfall is concentrated from April to September, constituting the rainy season, whereas rainfall from October to March is characterized by the dry season (Ou et al., 2020; Zhang et al., 2021b). The area has distinctive karst formations, including valleys and hills (*Fig. 1*). The prevalent soil types are brown calcareous soil with high calcium content. Understory vegetation in the *Z. insignis* plantations includes species such as *Alchornea trewioides*, *Radermachera sinica*, *Litsea glutinosa*, *Cipadessa baccifera*, *Sageretia thea*, *Embelia vestita*, *Flueggea virosa*, *Pteris semipinnata*, *Miscanthus floridulus*, and *Pilea cavaleriei* (Ou et al., 2020).

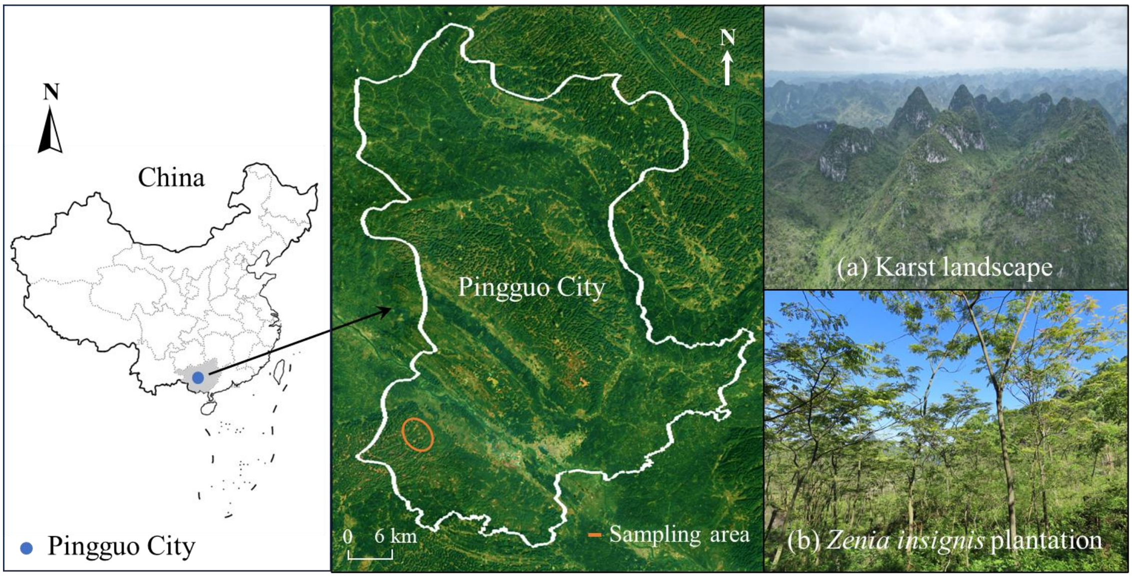

Figure 1. *Map showing the location of the study area, along with images of the karst landscape (a) and the Zenia insignis plantation (b)*

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Soil sampling

For this study, we selected *Z. insignis* plantations with ages ranging from 3-5 years, 9-11 years, 14-16 years, 19-21 years, and 24-26 years in study area. A total of eight 20 m × 20 m plots were established for each age, with varying distances ranging from 200 m to 3 km between them. All the plantation sites were converted from abandoned sloping fields with uniform soil types. Each plot had similar slopes and aspects, and shared the same history and land management practices before afforestation. The stand characteristics in *Z. insignis* plantations are presented in *Table 1*.

Surface soil samples (0-15 cm depth) were collected from each plot using a soil auger (100 cm^3). Six random soil cores were retrieved from each plot and thoroughly mixed to create composite samples. The fresh soil samples were immediately refrigerated until they were transported to the laboratory. In the laboratory, the fresh soil samples were sieved through a 2 mm sieve to remove roots and stones. Subsamples of fresh soil were stored at -4°C for subsequent soil microbial biomass analysis. Another subsample of fresh soil was air-dried, ground, and sieved through a 0.25 mm sieve for the quantification of soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP).

Table 1. *Stand characteristics in Zenia insignis plantations at varying ages.*

Stand age (years)	**Elevation (m)**	**DBH (cm)**	**Tree hight (m)**	**Canopy cover (%)**	**Understory richness**	**Dominant understory species**
3-5	236-265	4.3 ± 0.35	5.3 ± 0.79	43.6 ± 4.51	42.5 ± 3.84	*Pterolobium punctatum, Ticanto crista*
9-11	242-254	8.3 ± 0.43	7.6 ± 0.89	52.7 ± 6.62	50.6 ± 4.76	*Cipadessa baccifera, Flueggea virosa*
14-16	231-260	14.5 ± 0.78	10.5 ± 1.34	64.0 ± 3.33	54.4 ± 3.93	*Alchornea trewioides, Cipadessa baccifera*
19-21	226-267	18.7 ± 0.75	15.8 ± 1.65	74.6 ± 3.81	46.6 ± 2.91	*Alchornea trewioides, Zanthoxylum armatum*
24-26	236-259	23.5 ± 1.19	18.9 ± 1.13	80.9 ± 2.86	42.8 ± 3.78	*Sageretia thea, Litsea glutinosa*

Values are the means ± standard error (SE) (n = 8)

Chemical analysis

The SOC content was determined through wet oxidation with $KCr_2O_7 + H_2SO_4$, followed by titration with $FeSO_4$ (Bao, 2000). TN levels were quantified using an elemental analyzer (EA 3000, Euro Vector, Italy). TP was measured via colorimetry after acid digestion with $H_2SO_4 + HClO_4$ solution (John, 1970). soil microbial biomass C (SMBC), microbial biomass N (SMBN), and microbial biomass P (SMBP) were assessed using the chloroform fumigation-extraction method (Brookes et al., 1982; Wu et al., 1990).

Data analysis

In this study, soil and microbial biomass C:N, C:P, and N:P ratios were calculated on a molar basis. Differential analyses of the stoichiometric ratios of C, N, and P in the soil and microbial biomass across different successional stages were performed using one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test. Pearson correlation analysis was used to examine the associations between the stoichiometric ratios of C:N,

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C:P, and N:P in the soil and the microbial biomass. Statistical analyses were conducted using SPSS v.19.0 (SPSS Inc., Chicago, Illinois, USA). Additionally, redundancy analysis (RDA) was carried out to evaluate the influence of soil environmental factors on microbial C:N:P stoichiometry using the software Canoco 5.3. Unless stated otherwise, all significant differences in this study were considered at $P < 0.05$.

Results

Soil C:N:P stoichiometry

The concentrations of SOC, TN, and TP gradually increased with increasing stand age, with significant trends observed in the SOC and TN concentrations (*Fig. 2*). Significant differences in SOC were found among the different stand ages, whereas TN exhibited significant variations between the 3-5-year-old and 19-26-year-old stands. TP differed significantly between the 3-5-year and 24-26-year stages (*Fig. 2*). There were no significant changes in the soil C:N ratios across the different stand age groups (*Fig. 2*). The patterns of the soil C:P and N:P ratios were similar, with significant differences observed in the C:P ratio between the 3-5-year and 19-26-year stages and in the N:P ratio between the 3-11-year and 24-26-year stages (*Fig. 2*).

Microbial C:N:P stoichiometry

As the *Z. insignis* plantations matured, the concentrations of SMBC, SMBN, and SMBP increased with the development of the forest stand (*Fig. 3*). Additionally, significant differences in the concentrations of SMBC, SMBN, and SMBP were detected between the 3-5-year-old and 24-26-year-old plantations (*Fig. 3*). Although the ratios of SMBC:SMBN, SMBC:SMBP, and SMBN:SMBP in the soil microbial biomass did not significantly differ among the different age groups in the *Z. insignis* plantations, they generally demonstrated a decreasing trend overall (*Fig. 3*). Notably, the microbial C:N:P ratios were greater at the 3-5-year-old stage than at the later stages of the plantation (*Fig. 3*).

Associations among soil, microbial C:N:P stoichiometry and factors

The results demonstrated highly significant correlations between SOC and TN and between the contents of soil microbial biomass C (SMBC), microbial biomass N (SMBN), and microbial biomass P (SMBP)($P < 0.01$, *Table 2; Fig. 4*). Furthermore, a significant negative correlation was found with the ratio of soil microbial biomass carbon to phosphorus (SMBC:SMBP ratio) ($P < 0.05$, *Table 2; Fig. 4*). The TP content showed highly significant positive correlations with SMBC and SMBP and highly significant negative correlations with the SMBN:SMBP ratio ($P < 0.01$, *Table 2; Fig. 4*). The soil N:P ratio was significantly correlated with the SMBC, SMBN, and SMBP contents ($P < 0.05$), with a strong positive correlation observed between the soil N:P ratio and the SMBN:SMBP ratio ($P < 0.01$, *Table 2; Fig. 4*). However, no significant correlation was found between the soil C:N:P ratio and the SMBC:SMBN:SMBP ratio. Nevertheless, the SMBC:SMBP ratio exhibited significant and extremely significant correlations with the SMBC:SMBN ratio and SMBN:SMBP ratio (*Table 2*; *Fig. 4*). No significant differences were detected for other components, such as carbon, nitrogen, phosphorus, or chemical stoichiometry ratios. RDA revealed that SOC accounted for the greatest percentage of the total variation, at 29.6%. Additionally, the percentages of variation explained by the x-axis and y-axis were 32.14% and 6.09%, respectively (*Fig. 4*).

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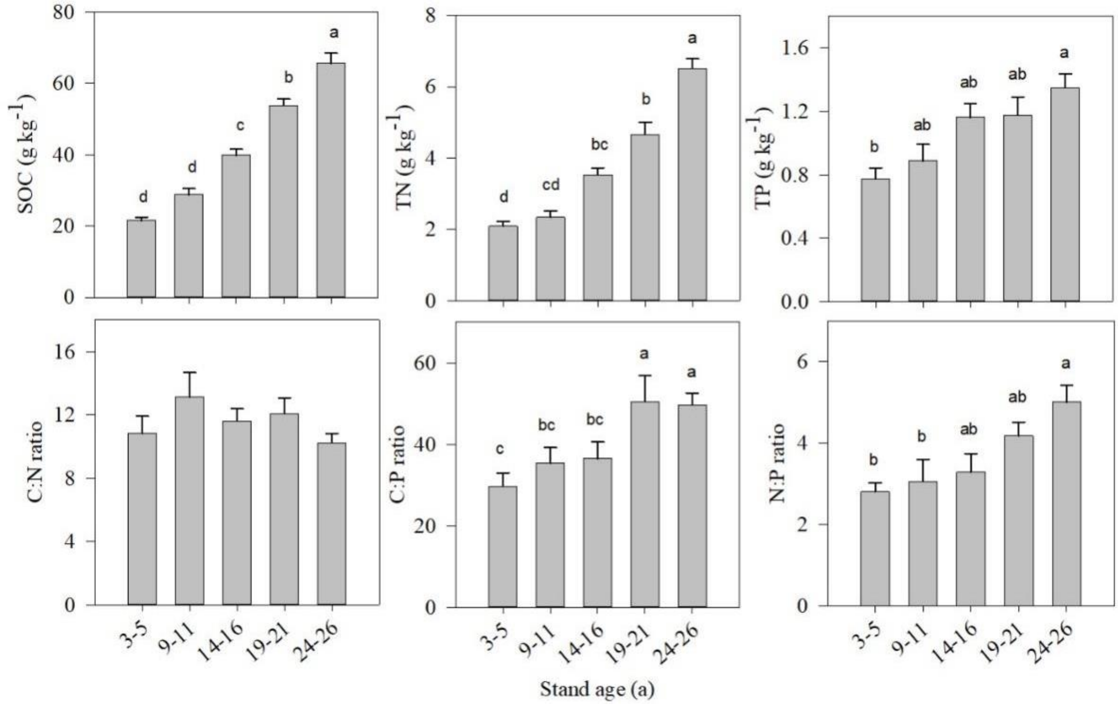

Figure 2. *Changes in SOC, TN, TP, and stoichiometric ratios of soil C:N, C:P and N:P during vegetation succession in a karst region of Southwest China. The values are the means ± SE (n = 8). The different lowercase letters on the columns denote significant differences between successional stages at p < 0.05*

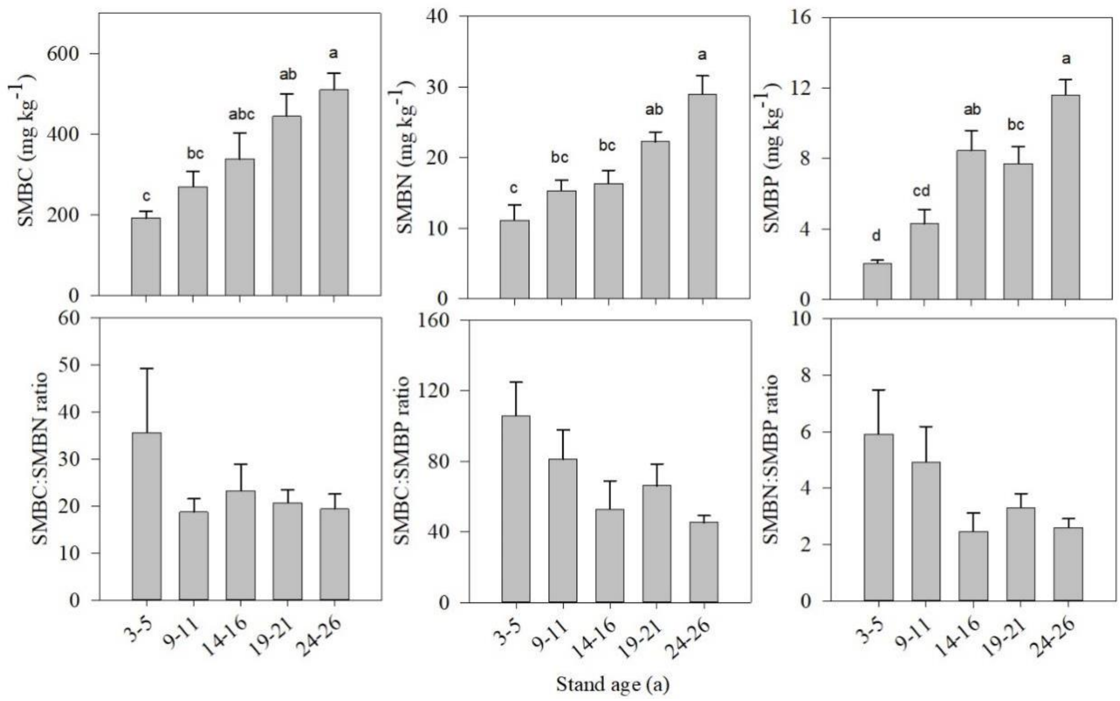

Figure 3. *Changes in SMBC, SMBN, SMBP, and stoichiometric ratios of SMBC:SMBN, SMBC:SMBP and SMBN:SMBP during vegetation succession in a karst region of Southwest China. The values are the means ± SE (n = 8). The different lowercase letters on the columns denote significant differences between successional stages at p < 0.05*

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Table 2. *Pearson's correlation coefficients (r) between the concentrations of C, N, and P and their stoichiometric ratios in the soil and microbial biomass*

	SOC	TN	TP	C:N	C:P	N:P	SMBC	SMBN	SMBP	SMBC:SMBN	SMBC:SMBP
TN	0.86**										
TP	0.59**	0.52**									
C:N	0.05	-0.42**	-0.04								
C:P	0.60**	0.43**	-0.25	0.24							
N:P	0.49**	0.69**	-0.19	-0.45**	0.72**						
SMBC	0.67**	0.57**	0.49**	0.06	0.34*	0.27					
SMBN	0.73**	0.76**	0.27	-0.07	0.55**	0.62**	0.39*				
SMBP	0.74**	0.76**	0.42**	-0.13	0.45**	0.51**	0.44**	0.53**			
SMBC:SMBN	-0.17	-0.17	0.13	-0.02	-0.29	-0.29	0.18	-0.53**	-0.19		
SMBC:SMBP	-0.34*	-0.38*	-0.19	0.14	-0.16	-0.24	0.13	-0.25	-0.68**	0.36*	
SMBN:SMBP	-0.32*	-0.30	-0.43**	0.08	0.04	0.04	-0.22	0.12	-0.58**	-0.31	0.64**

***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$

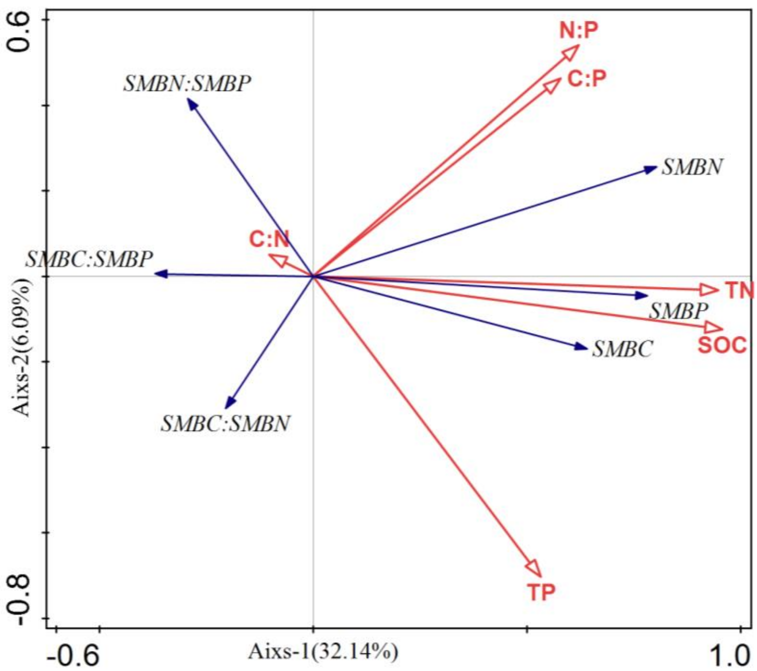

Figure 4. *Redundancy analysis (RDA) was used to identify the associations between microbial biomass stoichiometry and soil properties*

Discussion

Effects of stand development on soil C:N:P stoichiometry

Soil ecological stoichiometry is a crucial indicator for assessing soil quality and plays a fundamental role in determining the equilibrium characteristics of soil C, N, and P (Batjes, 2014). These nutrients have a profound influence on plant growth (Fan et al., 2015). In our study, we followed the nutrient grading standards outlined in the second national soil survey (Bao, 2000). The SOC content in our study progressively increased and reached the first-level threshold in the 19- to 26-year-old stands, whereas the TN content surpassed the first-level standard. Although the TP content met the first-level

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standard in the 14–26-year-old stands, it remained notably lower than the global average of 2.8. This finding is consistent with prior research that has indicated comparatively low phosphorus levels in Chinese soils compared to the global norm (Chen et al., 2006). Consequently, the overall nutrient content within the plantations is relatively high, which fosters biological reproduction and growth. This, in turn, results in pronounced biological “self-fertilization” and expedites processes such as rock weathering, soil formation, and development (Wu et al., 2020).

Throughout our investigation, the SOC, TN, and TP contents exhibited synchronous increases across the different forest age stages. This corresponding increase in SOC and TN contents may be attributed to inputs from litter decomposition, root residues, and their exudates. Furthermore, the increase in soil nitrogen content could be attributed to nitrogen fixation by leguminous plants. However, Cao et al. (2020) argued that as stand age progresses, the C, N, and P contents within the ecosystem do not consistently increase with stand development. Additionally, they proposed that the controversy surrounding the impact of stand age on soil C, N, and P levels may be influenced by other factors, such as previous land use, climate, and tree species, all of which have the potential to obscure the influence of stand age.

The soil C:N ratio serves as an indicator of soil organic matter decomposition (Fan et al., 2015). In our experiment, despite variations in forest age, the soil C:N ratio remained relatively narrow, ranging from 7.2 to 17.5. This finding aligns with observations by Li et al. (2012), who noted a constrained soil C:N ratio ranging from 6.9 to 14.6 in subtropical ecosystems in China. Comparatively, our soil C:N ratio is lower than the global average of 14.3 (Yue et al., 2017). Moreover, we observed no significant changes in the C:N ratios across different stand age stages, indicating sufficient nitrogen availability for microbial growth and organic matter transformation, with excess nitrogen being released into the soil. Additionally, both the SOC and TN contents increased, resulting in minimal variation in the C:N ratio. Correlation analysis (*Table 2*) demonstrated that the soil C:N ratio was not significantly correlated with soil organic C but was significantly correlated with total soil N. These findings suggest that changes in nitrogen content primarily drive variations in the soil C:N ratio across different forest ages.

The soil C:P ratio serves as an indicator of the ability of soil microorganisms to absorb and release P (Tian et al., 2010). In our study, the soil C:P ratio fell below the Chinese average of 136 (Yue et al., 2017), indicating that soil microorganisms act as a reserve for supplying phosphorus to the soil through organic carbon mineralization (Li et al., 2022). Additionally, the low C:P ratio observed in our study promoted the mineralization of soil microorganisms and the release of phosphorus from soil organic matter (Tian et al., 2010). Analysis revealed a strong and significant correlation between the soil C:P ratio, soil organic carbon, and total nitrogen, emphasizing that changes in soil carbon and nitrogen are the primary factors influencing variations in the C:P ratio.

The soil N:P ratio serves as an indicator of whether nitrogen or phosphorus is deficient in a habitat and can be used to evaluate the soil nutrient supply during plant growth (Wang and Yu, 2008). In our study, the soil N:P ratio in *Z. insignis* plantations at different ages ranged from 1.33 to 7.09, which is lower than the Chinese average of 9.3 (Tian et al., 2010). We observed rapid increases in soil organic carbon and TN contents with forest age, whereas the total phosphorus content increased moderately, resulting in elevated soil C:P and N:P ratios. Importantly, changes in the N:P ratio are primarily influenced by nitrogen levels. A lower N:P ratio suggests insufficient nitrogen in the soil, but it may not necessarily indicate nitrogen limitation for plant growth

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(Zhang et al., 2021c). Additionally, both the soil C:P and N:P ratios exhibited an upward trend, peaking at ages 14-26 (*Fig. 2*), indicating an increase in phosphorus limitation with forest development. Moreover, previous studies have highlighted the potential for phosphorus deficiency in karst ecosystem plantations (Du et al., 2011). Chen et al. (2020) demonstrated that phosphorus deficiency in karst areas limits vegetation restoration, whereas rapid growth in plantation ecosystems depletes phosphorus, hampering tree growth and restricting phosphorus nutrition in the soil, ultimately leading to seedling mortality. Therefore, it is crucial to pay attention to the phosphorus supply to ensure the sustainability of *Z. insignis* plantations in later forest age stages (Yu et al., 2022). Overall, our findings show that the soil C:N:P ratios in *Z. insignis* plantations change with increasing forest age, significantly impacting soil nutrient status and biogeochemical cycles.

Effects of stand development on microbial C:N:P stoichiometry

The stoichiometry of carbon, nitrogen, and phosphorus in soil microbial biomass is widely recognized as a critical and sensitive indicator of soil health and fertility. Concurrently, stand age has emerged as a significant factor that influences soil microbial biomass in forest ecosystems during vegetation development (Liu et al., 2020b; Heuck et al., 2015). This study demonstrated that SMBC and SMBN in *Z. insignis* plantations gradually increased with stand development, accompanied by a rising trend in SMBP, which aligns with the findings of Bauhus and Pare (1998). This finding suggests that reforestation has a positive influence on soil quality from a microbial perspective over a certain period (Chen et al., 2020).

Despite the challenging conditions posed by karst's unique calcareous rocks and thin, rugged soil, we initially expected a low microbial biomass in the soil. However, our research shows that the global average levels of SMBC and SMBN in karst forests are relatively high (Xu et al., 2013), with some forest age stages even surpassing those of nonkarst forest soils in the same subtropical climate (Wang and Wang, 2011). This indicates a certain tolerance of karst microorganisms to unfavorable soil habitats. The SMBC:SMBN and SMBC:SMBP ratios in *Z. insignis* plantations exceeded those in Chinese soils, generally at 7.6, and declined in the late stage of 14-26 years post-afforestation (70.2), whereas the SMBN:SMBP ratio was lower than that in Chinese soils (6) (Wang and Yu, 2008). This suggests reduced soil nitrogen bioavailability, with phosphorus released by soil microorganisms supplementing the P pool (Zhang et al., 2011).

In this study, we observed no significant correlation ($P > 0.05$) between the soil C:N:P ratio and the MBC:MBN:MBP ratio in the *Z. insignis* plantations. This may be attributed to adequate soil nutrients being preserved in the original field to meet microbial growth requirements. This observation aligns with Li's research on natural vegetation restoration on fallow land, where the changes in SMBC:SMBN:SMBP during the initial 18 years of vegetation succession were unnoticeable (Hu et al., 2016). Li et al. (2019) suggested that the elemental chemical stoichiometry of soil microbial biomass in subtropical terrestrial ecosystems in China may be nonhomeostatic, as both microbial and soil environments influence C:N:P stoichiometry and flexibly respond to environmental changes. Furthermore, under continuously changing environmental conditions, microbial biomass C:N:P stoichiometry may shift due to variations in microbial community structure as the C:N:P ratios of species in microorganisms fluctuate (Ren et al., 2016). Additionally, microorganisms can absorb excess resources

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and store them as glycogen or polyphosphate, thereby altering the microbial biomass C:N:P ratio (Wilson et al., 2010; Achbergerova and Nahalka, 2011).

RDA revealed that variations in soil C:N:P stoichiometry are attributed to changes in soil properties (*Fig. 4*). In this study, there was no significant correlation between soil C:N:P and SMBC:SMBN:SMBP. However, significant and highly significant correlations were observed between SMBC:SMBP and SMBC:SMBN, as well as between SMBN:SMBP. These findings are consistent with previous studies by Tian et al. (2010) and Li et al. (2019), suggesting that soil microorganisms can adjust their composition, activity, and population dynamics to maintain a consistent C:N:P ratio irrespective of soil elements (Hu et al., 2016; Yu et al., 2018).

A significant positive correlation was observed between soil nitrogen and SMBN, consistent with the findings of Qiu et al. (2020). This indicates a significant positive association between SMBN and soil N content. Additionally, there was a significant positive correlation between the soil and microbial biomass C, N, and P contents. However, no significant correlation was found between their C:N ratios (*Table 2*; *Fig. 4*). This suggests that in karst regions, the soil microbial C:N:P ratio may not adhere to a homeostatic system, as there is a positive correlation between the C:P ratio in the soil environment and the SMBC: SMBP ratio when sufficient carbon is available. These findings also imply that phosphorus acts as a limiting factor for soil biological productivity in this region, supporting the hypothesis that the stoichiometricities of soil microbial C:N:P ratios are nonhomeostatic.

Conclusions

Our study revealed that the establishment of *Z. insignis* plantations in karst regions has led to variations in both soil and microbial C:N:P stoichiometry with increasing forest age. This highlights the significant influence of forest age on soil nutrient status and microbial dynamics. Specifically, as the forest age increased, there was a simultaneous increase in the soil C and N levels, resulting in minimal changes in the soil C:N ratio. However, the response of soil TP to forest age was less pronounced, leading to an increase in the soil C:P and N:P ratios during vegetation succession. These increases suggest a worsening of phosphorus limitation during forest development. Alongside the soil C, N, and P contents, the concentrations of SMBC, SMBN, and SMBP also increased with forest age. Furthermore, the prolonged growth of plantations does not increase the SMBC: SMBN, SMBC: SMBP, and SMBN: SMBP ratios. Instead, these ratios show a declining trend. However, unlike soil C:N:P stoichiometry, there was no correlation observed between the SMBC:SMBN:SMBP ratios at different stand age stages. This study outlines the contrasting responses of soil and microbial stoichiometry to forest age in subtropical karst regions in China, emphasizing the advantages of *Z. insignis* plantation development for the accumulation of soil and microbial C, N, and P. Phosphorus appears to be a limiting factor for growth of *Z. insignis* plantation in karst regions of Southwest China.

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Conflict of interests. The authors declare that they have no competing interests.

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