CASCADING EFFECTS OF LITTER-SOIL NUTRIENT STOICHIOMETRY ON ENZYME ACTIVITIES DURING KARST FOREST SUCCESSION IN SOUTHWEST CHINA

ZHANG, Y.[1] – ZHENG, H. M.[1*] – ZHANG, Z. H.[2] – LI, Z. H.[1]

[1]*Tangshan Key Laboratory of Simulation of Urban Ecosystem, Department of Resource Management, Tangshan Normal University, Tangshan 063000, China*

[2]*School of Karst Science, State Engineering Technology Institute for Karst Desertification Control, Guizhou Normal University, Guiyang 550025, China*

**Corresponding author*
e-mail: hxcandzy@tstc.edu.cn

(Received 16th Aug 2025; accepted 10th Nov 2025)

Abstract. Litter serves as a source of enzymes and substrates, with enzymatic transformations regulating soil nutrient cycling. These three components (litter, enzymes, and soil nutrient) interact synergistically to maintain soil ecological functions and material balance. Currently, a significant knowledge gap exists regarding the cascade effects linking litter decomposition, soil enzyme activity, and soil nutrients during mid-succession in karst forests. The results indicated that: (1) Variations in plant species composition across different successional stages led to significant differences in litter stoichiometry. (2) Changes in litter stoichiometry significantly influenced alterations in soil nutrients and enzyme activity. (3) Soil nutrient factors (SOC, TN, HN, TP, AP, C/N, C/P, N/P) significantly drove changes in soil enzyme activity, with SOC being the most important driving factor. In summary, karst forest succession influences the cascade relationships among litter, soil nutrients, and soil enzyme activity.

Keywords: *nutrient cycling, soil biochemistry, karst landscape, ecological indicators, forest dynamics*

Introduction

Karst forests are forest ecosystems characterized by specific soil and vegetation features, where plants grow on carbonate-dominated substrates under forest climate conditions (Gunn, 2004). Spatial heterogeneity in plant species diversity within karst forests influences soil enzyme activity affecting both litter and soil nutrient content (Zeng et al., 2021). The ecological stoichiometry of carbon (C), nitrogen (N), and phosphorus (P) – fundamental elements of plant composition – reflects the successional stage and growth dynamics of vegetation. Furthermore, it regulates soil nutrient accumulation through the process of litter decomposition (Geekiyanage et al., 2019; Ye et al., 2024). Litter serves as a vector for nutrient transfer between plants and soil. While a portion of nutrients in plant leaves is assimilated by the plant itself, the remainder enters the soil via litterfall (Zhang et al., 2024). This nutrient transfer process directly shapes the composition of the soil nutrient pool, thereby influencing the community structure and successional trajectory of vegetation (Zhu et al., 2023). During vegetation succession, changes occur in plant litter inputs and root exudates, which subsequently affect soil organic carbon (SOC) content (Chari and Taylor, 2022). Soil enzymes are among the most active compounds in the soil ecosystem, participating in biochemical reactions essential for material cycling and energy flow (Zheng et al., 2018). Consequently, soil enzyme activity serves as a sensitive indicator of soil nutrient status and ecosystem functioning (Daunoras et al., 2024). The intensity of soil enzyme activity directly

DOI: http://dx.doi.org/10.15666/aeer/2401_041055

influences the transformation of soil organic matter. Therefore, soil enzyme activity metrics are frequently employed as indicators for assessing soil fertility (Wang et al., 2023). In summary, plant stoichiometric traits regulate soil C, N, and P pools through the release of nutrients from decomposing litter. Concurrently, soil enzymes act as biological catalysts, directly mediating the transformation of organic matter; their activity is, in turn, feedback-regulated by nutrient availability (Manzoni et al., 2010; Sistla and Schimel, 2012). This intricate interplay not only shapes the efficiency of soil nutrient cycling but also drives the trajectory of vegetation succession (Song et al., 2024).

Existing research indicates that topographic factors, elevation, seasonal variations, and litter input can all influence soil enzyme activity characteristics. Liao et al. (2021) revealed that topographic differences indirectly regulate soil enzyme activity by altering plant diversity. Chen et al. (2024) found that soil enzyme activity and its stoichiometric ratios are influenced by the combined effects of forest type and seasonal changes. In a study of the karst desertification region in Southwest China, Chen et al. (2017) demonstrated that soil physicochemical factors significantly affect soil enzyme activity, with soil bulk density and SOC being the primary influencing factors. Gan et al. (2025) found that during vegetation succession, soil P content and C - N content are key drivers of changes in soil enzyme activity ratios. Investigating soil enzyme activity in the Changbai Mountains, Gu et al. (2017) revealed that, apart from soil environmental factors and hydrothermal conditions, soil enzyme activity is also influenced by litter and vegetation community composition. Dao et al. (2024) demonstrated variations in soil enzyme activity under different land use types within the peak-cluster depression karst desertification area.

While numerous studies have investigated the relationships between soil enzymes and soil nutrients, as well as between soil enzymes and litter, across various forest types (Liu et al., 2023; Wu et al., 2024; Xu, et al., 2022), research specifically focused on karst forest ecosystems remains limited. There is a critical need to delve deeper into the unique ecological mechanisms governing these interactions within karst forests. Therefore, this study was conducted in the Shibing karst region of Guizhou Province. We investigated litter and soil C, N, and P concentrations, their stoichiometric ratios, soil enzyme activity, and the interactions among these components across three karst forest successional stages: arbor forest, shrub forest, and shrub-grass mixed forest. We proposed the following hypotheses: Variations in plant species composition across different karst forest successional stages lead to significant differences in litter stoichiometry (H1). Changes in litter stoichiometry mediate alterations in soil nutrients and enzyme activity during karst forest succession (H2). Soil nutrient factors drive changes in soil enzyme activity throughout karst forest succession (H3). The above hypotheses aim to elucidate the cascading effects of litter, soil nutrients, and soil enzyme activity during ecological succession in Karst forests.

Materials and methods

Study area description

The Shibing Karst study area is located in Shibing County, eastern Guizhou Province (108°06’38”E-108°07’50”E, 27°08’22”N-27°09’28”N) (*Fig. 1*). It lies within a montane transitional zone on the eastern edge of the Yunnan-Guizhou Plateau, descending towards the low mountains and hills of western Hunan, marking the boundary between China’s second and third topographic steps. Covering 282.95 km², the study area experiences a subtropical humid monsoon climate with an annual mean temperature of 16°C and average annual precipitation of 1220 mm (Chen et al., 2023). Lithologically dominated by dolomite, the vegetation belongs to the mid-subtropical evergreen broad-leaved forest zone, characterized by diverse forest types with predominant tall arbors and scattered shrublands.

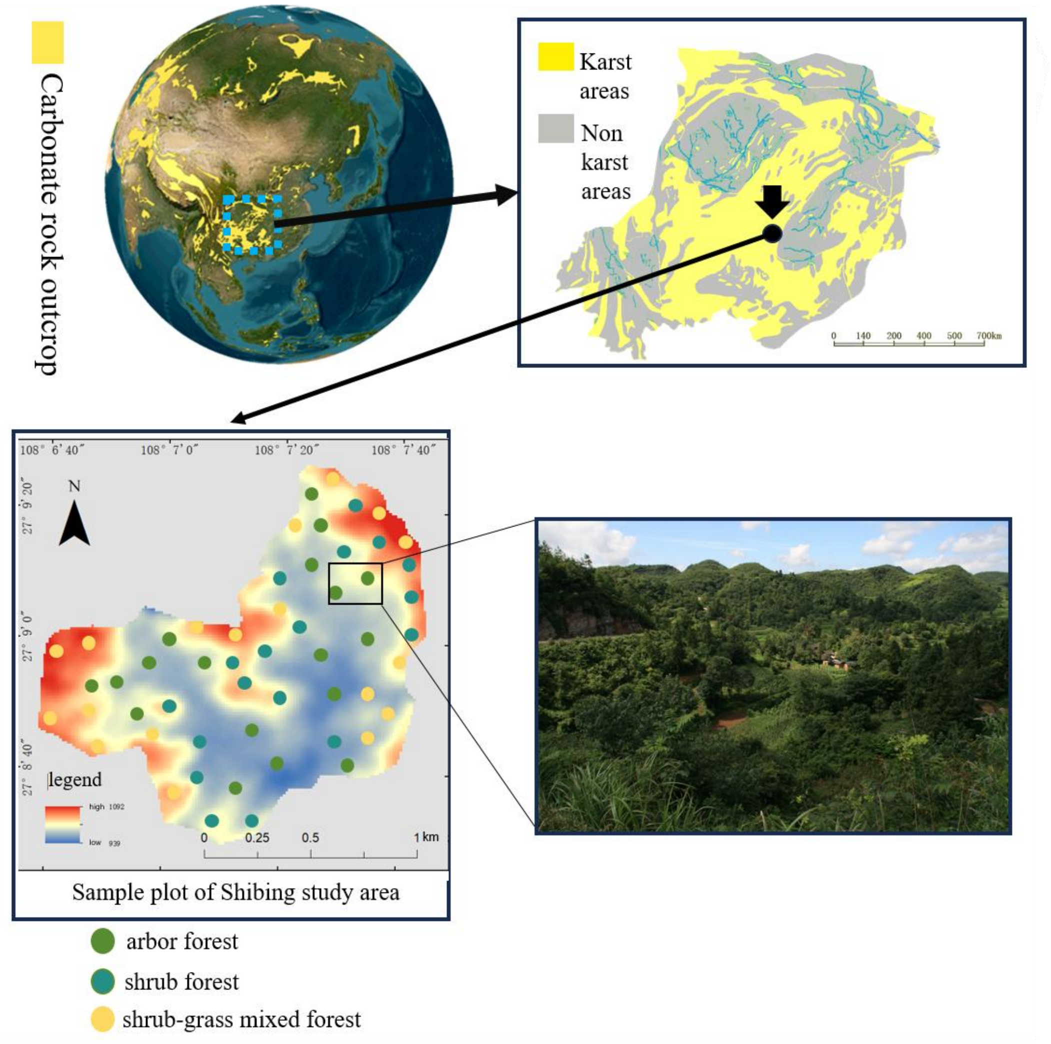

***Figure 1.** The location of the study area and the landscape of the sample plots*

Sample plot selection and setup

Three vegetation types were selected within the Shibing Karst area: arbor forest, shrub forest, and shrub-grass mixed forest. For each type, 18 sample plots (20 m × 20 m) were established to record microenvironmental parameters including altitude, slope aspect, and vegetation composition. Meanwhile, we also collected soil samples and measured soil nutrients and soil enzyme activities.

Litter and soil sampling and processing

This study was conducted during the peak vegetation growth season from August to September 2024. To minimize sampling bias, three 1 m × 1 m subplots were established within each 20 m × 20 m plot. Surface litter was collected, homogenized, sealed in zip-lock bags, and oven-dried at 70°C to constant weight for nutrient analysis. Soil samples (0-10 cm depth) were collected from each plot using a five-point sampling method, combined, sealed, and air-dried. After removing visible roots and debris, samples were ground and sieved: one portion through 0.15 mm mesh for nutrient analysis, and another through 2 mm mesh for enzyme activity assays (Bilen and Turan, 2022).

Laboratory analyses

Litter organic carbon content (OC) and total nitrogen content (TN) were determined using a semi-automatic Kjeldahl apparatus (model K9860, Hanon Instruments, China). Total phosphorus content (TP) in litter was measured via HNO_3-$HClO_4$ digestion followed by molybdenum-antimony colorimetry, and calculate the ratios of C/N, C/P, and N/P (Avramidis et al., 2015). Soil organic carbon content (SOC) and total nitrogen content (TN) were analyzed using identical methods to litter samples. Soil hydrolysable nitrogen (HN) was determined by the alkali hydrolysis diffusion method. Soil total phosphorus content (TP) was determined through NaOH fusion-molybdenum-antimony colorimetry, while soil pH was measured with a pH meter (model PHS-3E, INESA Instrument, China) (Pansu and Gautheyrou, 2006). Soil enzyme activities were quantified as follows: catalase (CAT) via titrimetry, urease (UR) via indophenol blue colorimetry, sucrase (SUC) via DNS colorimetry, and alkaline phosphatase (ALP) via microplate fluorometry.

Statistical analyses

Descriptive statistics (mean ± SD) were calculated using Excel. Differences between groups were assessed using Fisher LSD tests ($p < 0.05$) in R 4.4.2. The relationships among litter, soil nutrients, and soil enzymes were analyzed using Pearson correlation analysis. To identify key drivers of soil enzyme activities, we employed redundancy analysis (RDA), Mantel tests, and random forest modeling. All figures were generated using R 4.4.2 and Origin 2024.

Results

Stoichiometric characteristics of litter and soil

Arbor forest stage showed significantly higher litter total nitrogen content (TN) than shrub stage forest. Soil TN exhibited similar significant differences between these two stages (*Fig. 2b*). Litter total phosphorus content (TP) differed significantly between arbor forest stage and shrub forest stage, as well as between arbor forest stage and shrub-grass forest stage. Soil TP showed significant differences between arbor forest stage and shrub forest stage, as well as between shrub forest stage and shrub-grass forest stage. (*Fig. 2c*). Overall, except for soil organic carbon content (OC) which showed no significant variation, all other elements exhibited stage-specific differences across the three succession stages.

In three forest types, significant differences in litter C/N and soil C/N were observed between the arbor forest stage and shrub forest stage, as well as between the arbor forest stage and shrub-grass mixed forest stage (*Fig. 3a*). Significant differences in soil C/P were detected between the arbor forest stage and shrub forest stage, and between the shrub forest stage and shrub-grass mixed forest stage (*Fig. 3b*). Soil N/P showed significant differences between the shrub forest stage and arbor forest stage, and between the shrub forest stage and shrub-grass mixed forest stage (*Fig. 3c*). Overall, except for litter N/P, which showed no significant differences among the three succession stages, all other stoichiometric ratios exhibited significant variations across the stages.

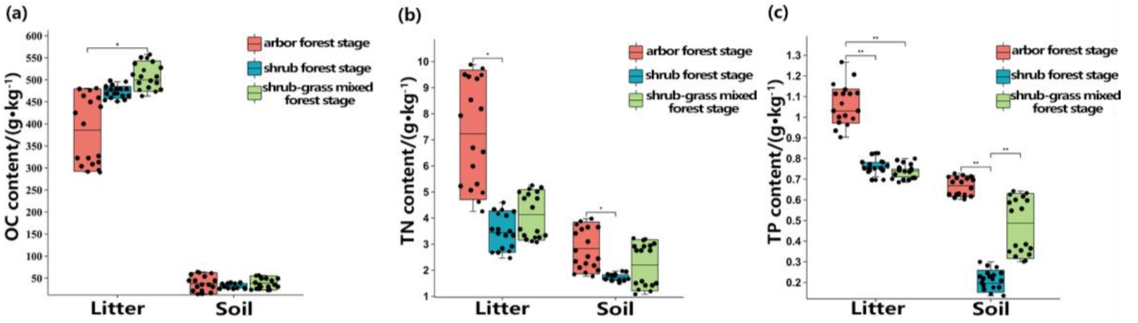

Figure 2. *Litter/soil OC (a), litter/soil TN (b), and litter/soil TP (c) contents across three succession stages (* indicates significant differences between groups (“*”: $p < 0.05$, “*”: $p < 0.01$, Fisher’s LSD test))*

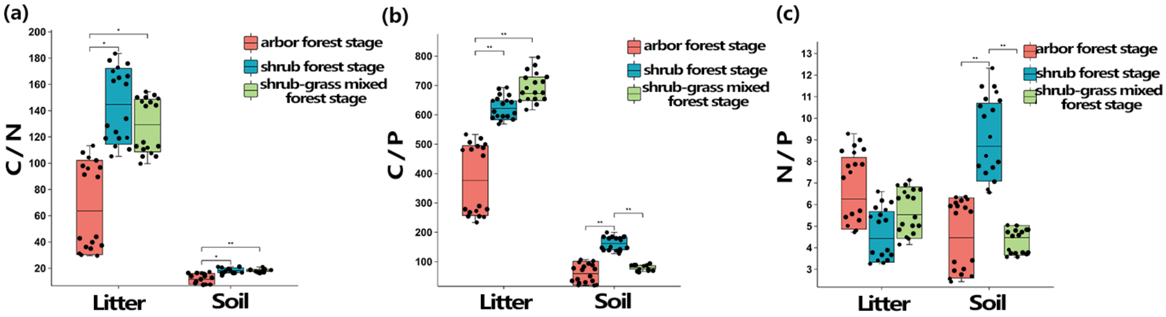

Figure 3. *Stoichiometric ratios (C/N, C/P, N/P) of litter and soil across different forest succession stages. (* indicates significant differences between groups (“*”: $p < 0.05$, “*”: $p < 0.01$, Fisher’s LSD test))*

Soil enzyme activity profiles

Catalase (CAT) activity differed significantly between arbor forest stage and shrub forest stage, as well as arbor forest stage and shrub-grass forest stage (*Fig. 4a*). Both Sucrase (SUC) and alkaline phosphatase (ALP) activities showed significant differences between shrub forest stages and shrub-grass forest stages (*Fig. 4c*, *d*).

Correlations among litter, soil nutrients, and soil enzymes

Correlation analysis revealed significant positive relationships between litter OC and soil OC, C/N, and C/P (*Fig. 5*). Litter TN and TP showed significant negative correlations with soil C/N, C/P, and N/P ratios, but positive correlation with soil TP. Litter C/N and litter C/P were significantly positively correlated with soil C/N and soil C/P, but significantly negatively correlated with TP. Litter N/P was significantly negatively correlated with soil C/N and soil C/P, while significantly positively correlated with soil TP. Correlation analysis revealed: (1) Litter OC was significantly positively correlated with CAT activity, but not significantly correlated with urease (UR) or ALP activity; (2) Litter TN and TP were significantly negatively correlated with CAT activity, with no significant correlations with

SUC or UR activity; (3) Litter C/P and C/N ratios were significantly positively correlated with CAT activity; (4) Litter N/P ratio showed a negative correlation with CAT activity, but no significant correlations with the other three enzymes (SUC, UR, ALP).

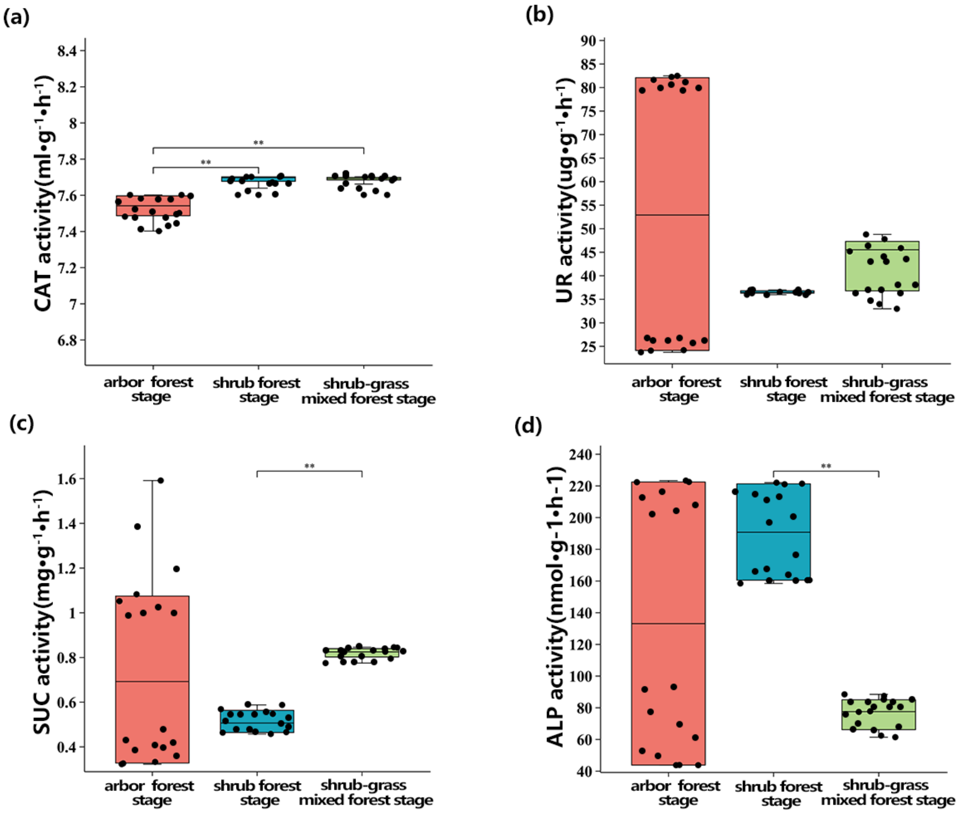

Figure 4. *Soil enzyme activities CAT (a), UR (b), SUC (c), and ALP (d) across succession stages. Bars with different letters indicate significant differences (* indicates significant differences between groups (“*”: $p < 0.05$, “*”: $p < 0.01$, Fisher’s LSD test))*

Drivers of soil enzyme activities

The first two RDA axes explained 0.604 and 0.248 of total variance, with strong correlations (r = 0.995) between enzyme activities and environmental factors (*Table 1*). The first two axes collectively accounted for 96.15% of enzyme variation and 85.15% of enzyme-environment relationships. The RDA ordination effectively captured enzyme-environment relationships, with Axis 1 being the dominant driver.

Table 1. *Eigenvalues and explanation rates of RDA axes for soil enzyme-environment relationships*

Item	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues of soil enzyme activity	0.604	0.248	0.114	0.014
Correlation of soil enzyme activity and soil environment factors	0.995	0.995	0.966	0.874
Cumulative interpretation of soil enzyme activity/%	60.380	85.150	96.530	97.900
Cumulative interpretation of soil enzyme activity-soil environment factors/%	61.680	86.980	98.610	100
Total canonical eigenvalues		0.980		
Total eigenvalues		1		

DOI: http://dx.doi.org/10.15666/aeer/2401_041055

RDA ordination showed that environmental factors (hollow arrows) generally aligned with soil enzymes (solid arrows) except TP, which exhibited opposite direction (*Fig. 6*). Narrow angles between hydrolysable nitrogen (HN) and SUC/UR indicate strong positive correlations, while wider angle with CAT suggests weaker correlation. C/P showed close association with CAT, and ALP was influenced by multiple factors.

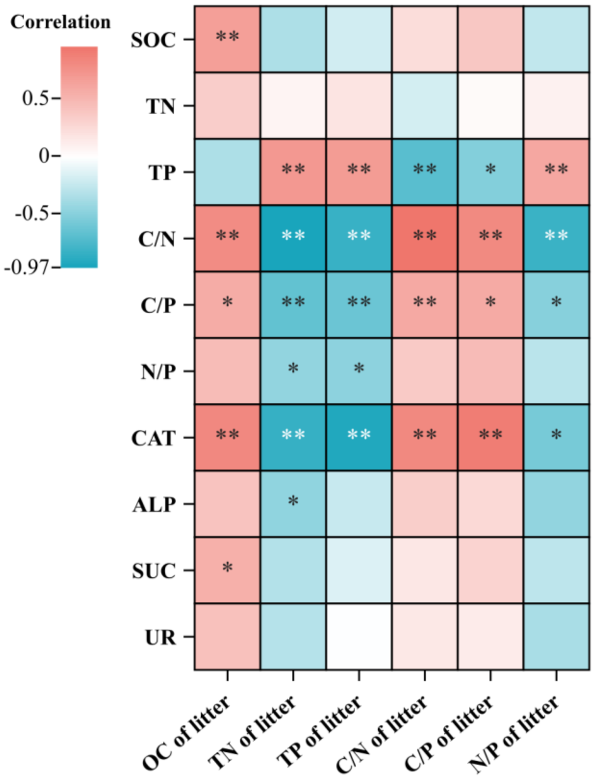

Figure 5. *Pearson correlation matrix among litter properties, soil nutrients, and soil enzyme activities. (“*”: p < 0.05, “**”: p < 0.01)*

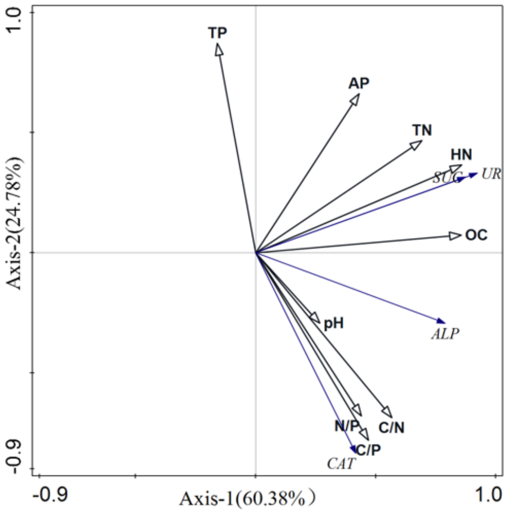

Figure 6. *RDA ordination diagram showing relationships between soil enzymes (solid arrows) and environmental factors (hollow arrows). Arrow length reflects contribution rate to the model*

Mantel test revealed: TN correlated positively with HN, AP, and SOC; HN showed sole significant correlation with SOC; TP negatively correlated with C/N, C/P, N/P; C/N and C/P were positively related; C/P and N/P exhibited strongest correlation. SUC strongly correlated with TN, HN, SOC ($p < 0.001$) and AP, C/N ($p < 0.05$); UR showed strongest ties with C/P, N/P; CAT linked primarily to C/N; ALP associated most with HN and SOC (*Fig. 7*).

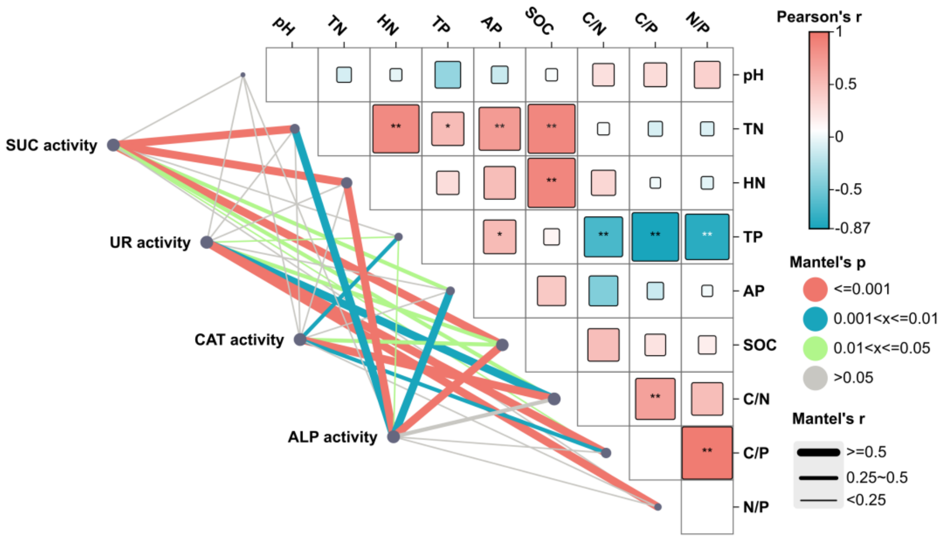

Figure 7. *Mantel test results showing correlations between soil enzymes and environmental factors (Edge width reflects Mantel's r statistic, color gradient indicates p-value significance)*

Random forest analysis revealed: (1) SOC was the strongest predictor for all four enzymes, particularly SUC; (2) TP influenced UR, CAT, and ALP; (3) C/N and C/P affected CAT and SUC; (4) TN, HN, and AP primarily impacted SUC and ALP (with HN being most influential); (5) N/P only affected UR (*Fig. 8*).

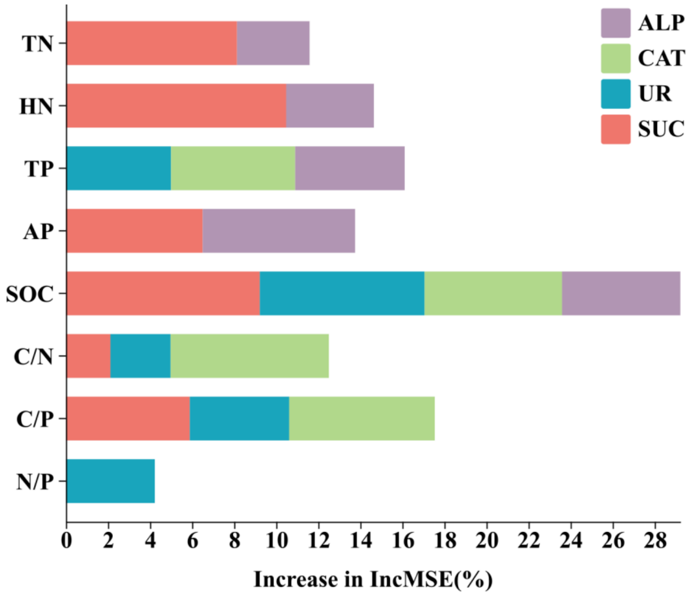

Figure 8. *Random forest model showing drivers of soil enzyme activities. IncMSE values indicate relative importance of environmental factors*

Discussion

Litter stoichiometric characteristics during karst forest succession

Litter in the Shibing karst forest exhibited high OC (454.62 ± 80.23 g/kg) and low TN (4.92 ± 2.37 g/kg) contents, characterized by a high C/N ratio (Wang et al., 2023). This high-C, low-N characteristic is attributed to the high soil pH derived from limestone parent material in the region, which promotes organic matter stabilization. Meanwhile, strong P adsorption in soils leads to N limitation for plants, resulting in greater C retention and enhanced N resorption in litter (Han et al., 2024; Ma et al., 2023). In the Shibing study area, litter C/N ratios (>40) and N/P ratios (<14) across three forest types indicate slow litter decomposition rates and N-limited plant growth. During decomposition, microorganisms require more N to metabolize abundant C, but N scarcity limits microbial growth and metabolic activity, ultimately slowing decomposition processes (Craine et al., 2007; Li et al., 2021). Notably, litter TN and TP contents in the arbor forest stage were significantly higher than those in other succession stages. This is because the arbor forest stage hosts more complex plant communities with higher species diversity. Different tree species and understory vegetation can exploit soil nutrients at varying depths and layers, enhancing N and P acquisition efficiency (Ma et al., 2020; Wu et al., 2022). Arbor species generally exhibit stronger P capacity and biosynthetic activity compared to shrub-grass plants, enabling higher N and P use efficiency during growth. Consequently, more N and P are allocated to constructing plant tissues and cell structures, leading to elevated TN and TP contents in litter (Wang et al., 2018, 2023). Litter C/N and C/P ratios in the arbor forest stage were significantly lower than those in other succession stages. This is attributed to relatively low soil N and P availability in the shrub forest and shrub-grass mixed forest stages, which limits plant nutrient uptake. Consequently, plants accumulate higher C relative to N and P, resulting in elevated litter C/N and C/P ratios (Yang et al., 2022). In contrast, the arbor forest stage employs efficient resource use strategies, enabling plants to accumulate more N and P during growth, which is ultimately reflected in litter stoichiometric ratios (Yang et al., 2021). Overall, differences in plant species composition across karst forest succession stages drive variations in litter stoichiometric characteristics, supporting hypothesis H1.

Correlations among soil nutrients, enzyme activities, and litter nutrients during karst forest succession

Soil nutrients in the Shibing karst study area exhibited average values of SOC (36.08 ± 17.83 g/kg), TN (2.25 ± 0.97 g/kg), and TP (0.449 ± 0.22 g/kg), characterized by high SOC but low TP. Soil C/P (100.14 ± 54.05) and N/P (5.93 ± 2.85) ratios indicated significant P limitation in the Shibing study area, consistent with previous findings (Chen et al., 2019; Cui et al., 2019). We observed that soils in the shrub forest stage exhibited lower TP contents but higher C/P and N/P ratios. This could be attributed to shrub litter potentially containing lower N and P contents and decomposing more rapidly, leading to faster nutrient cycling that limits nutrient accumulation in soils (Berg and Laskowski, 2005). Soil enzymes mediate biochemical reactions involved in material cycling and energy flow. Variations in litter quantity/composition and root exudate quantity/quality across forest types influence soil properties, thereby affecting soil enzyme activities (Wang et al., 2020). CAT activity in the arbor forest stage was significantly lower than in the other two stages. This was because arbor litter contained less OC, failing to provide sufficient carbon sources for soil microorganisms, thereby limiting microbial growth/metabolism and reducing CAT activity (Li et al., 2023). Compared to the shrub-grass mixed forest stage, soils in the shrub forest stage exhibited lower SUC activity but higher ALP activity. This was due to lower SOC in shrub forests limiting SUC activity, coupled with more severe P limitation driving plants and microorganisms to enhance ALP activity for P acquisition (Cui et al., 2019; Xu et al., 2022).

Litter serves as a critical carrier for nutrient transfer between plants and soils, playing a vital role in maintaining soil fertility and promoting material cycling in forest ecosystems (Krishna and Mohan, 2017). Litter OC content was significantly positively correlated with soil SOC, C/N, and C/P ratios, indicating that litter OC serves as a critical source of SOC. Litter TN and TP contents were significantly negatively correlated with soil C/N, C/P, and N/P ratios, but significantly positively correlated with soil TP, suggesting that litter N and P contents significantly influence soil stoichiometric ratios (Yu et al., 2025). This positive correlation also indicates that litter is an important source of soil TP. Litter C/N and C/P ratios were significantly positively correlated with soil C/N and C/P ratios, but significantly negatively correlated with soil TP content. This suggests that litter C/N and C/P ratios directly influence corresponding soil ratios. Higher litter C/N and C/P ratios may reduce soil P availability, as increased carbon input could immobilize soil P (Huang et al., 2021). Litter N/P ratio was significantly negatively correlated with soil C/N and C/P ratios, but significantly positively correlated with soil TP content. This indicates that lower litter N/P ratios reduce soil C/N and C/P ratios, as relatively higher N availability enhances soil N availability, thereby lowering C-to-N and C-to-P ratios (Xu et al., 2022). Litter OC content was significantly positively correlated with CAT activity, indicating that OC input from litter enhances soil CAT activity (Curci and Edwards, 1992). Litter TN and TP were significantly negatively correlated with CAT activity, suggesting that higher litter N and P contents inhibit CAT activity (Morales-Urrea et al., 2023). Litter C/P and C/N ratios were significantly positively correlated with CAT activity, indicating that higher C/P and C/N ratios provide more carbon sources, stimulating microbial growth/metabolism and thereby enhancing CAT activity (Shen et al., 2019). Litter N/P ratio showed a negative correlation with CAT activity, suggesting that higher litter N/P ratios may inhibit CAT activity. This could be due to relatively excessive N increasing microbial carbon demand, thereby affecting CAT synthesis and activity (Huang et al., 2020). Overall, variations in litter stoichiometric characteristics during karst forest succession influence soil nutrient dynamics and enzyme activities, supporting hypothesis H2.

Relationships between soil enzyme activities and soil nutrient characteristics

Soil enzymes, primarily derived from soil microorganisms, are closely linked to soil physicochemical properties. They facilitate the decomposition of organic matter and transformation of inorganic substances in soils, while changes in soil physicochemical properties also influence enzyme activities (Idris et al., 2024). RDA and Mantel tests revealed that soil enzyme activities were significantly influenced by environmental factors, including SOC, TN, HN, TP, AP, and stoichiometric ratios (C/N, C/P, N/P). Random forest analysis demonstrated that SOC significantly affected all four enzymes, with the strongest impact on SUC activity. This indicates that SOC is a foundational factor influencing soil enzyme activities, as it provides energy and c sources for soil microorganisms, thereby promoting enzyme synthesis and secretion (Qiao et al., 2025). TP significantly influenced UR, CAT, and ALP activities, highlighting its multifaceted role in regulating soil enzyme activities. Although TP often showed negative correlations with enzyme activities, its impact remained non-negligible. The negative correlation between TP and soil enzyme activities could be attributed to excessive soil P accumulation, which elevates soil solution P concentrations and subsequently inhibits enzyme activities (Fatemi et al., 2016). C/N and C/P ratios significantly affected CAT, SUC, and UR activities, while TN, HN, and AP primarily influenced SUC and ALP activities. Notably, N/P ratios only showed a significant effect on UR activity. Higher C/P ratios may indicate relatively abundant soil carbon and deficient phosphorus. In such conditions, microorganisms might produce more CAT to decompose organic matter and release phosphorus, meeting their growth demands (Peng et al., 2022). Increases in TN and HN generally correspond to enhanced soil enzyme activities, as these factors directly provide available nitrogen sources for soil microorganisms, stimulating their growth/metabolism and thereby increasing SUC and UR activities (Wu et al., 2024). Overall, SOC emerged as the most critical soil nutrient factor driving variations in soil enzyme activities during karst forest succession, strongly supporting hypothesis H3.

Conclusion

This study investigated litter and soil stoichiometric characteristics, as well as soil enzyme activities, across three succession stages (arbor forest, shrub forest, shrub-grass mixed forest) in the Shibing karst region. The objectives were to clarify variation patterns of litter/soil nutrients and enzyme activities during succession; reveal cascading relationships among litter-soil nutrients-enzyme activities. Key findings include: (1) Plant species composition differences across succession stages drive variations in litter stoichiometric characteristics; (2) Changes in litter stoichiometry during succession influence soil nutrient dynamics and enzyme activities; (3) Soil nutrients, particularly SOC, are the primary drivers of soil enzyme activity variations. Overall, variations in plant community structure and species composition across succession stages lead to differences in litter chemical composition, which alter soil nutrient concentrations/ratios and ultimately drive variations in soil enzyme activities.

Soil microbial community structure and function vary across vegetation types, with microorganisms playing critical roles in litter decomposition, soil nutrient cycling, and enzyme activity regulation. However, our study did not explicitly incorporate microbial mechanisms into the litter-soil nutrient-enzyme activity cascading relationships. Future research should integrate high-throughput sequencing to develop quantitative models linking microbial community structure to soil enzyme activities and nutrient availability. Particular attention should be paid to how plant-microbe interactions shape belowground niches through root exudates.

Acknowledgements. This study was supported by the Hebei Natural Science Foundation (No. D2025105003); Scientific Research Project of Tangshan Normal University (No. 20266130055) and the Project of Geographical Society of Guizhou Province (2020HX05).

Conflict of interests. The authors of this work declare that they have no conflicts of interest.

REFERENCES

[1] Avramidis, P., Nikolaou, K., Bekiari, V. (2015): Total organic carbon and total nitrogen in sediments and soils: a comparison of the wet oxidation—titration method with the combustion-infrared method. – Agriculture and Agricultural Science Procedia 4: 425-430. https://doi.org/10.1016/j.aaspro.2015.03.048.

[2] Berg, B., Laskowski, R. (2005): Decomposers: soil microorganisms and animals. – Advances in Ecological Research 38: 73-100. https://doi.org/10.1016/S0065-2504(05)38003-2.

[3] Bilen, S., Turan, V. (2022): Enzymatic Analyses in Soils. – In: Amaresan, N., Patel, P., Amin, D. (eds.) Practical Handbook on Agricultural Microbiology. Springer, New York, pp. 377-385. https://doi.org/10.1007/978-1-0716-1724-3_50.

[4] Chari, N. R., Taylor, B. N. (2022): Soil organic matter formation and loss are mediated by root exudates in a temperate forest. – Nature Geoscience 15(12): 1011-1016. https://doi.org/10.1038/s41561-022-01079-x.

[5] Chen, H., Luo, P., Wen, L., Yang, L., Wang, K., Li, D. (2017): Determinants of soil extracellular enzyme activity in a karst region, Southwest China. – European Journal of Soil Biology 80: 69-76. https://doi.org/10.1016/j.ejsobi.2017.05.001.

[6] Chen, H., Li, D., Mao, Q., Xiao, K., Wang, K. (2019): Resource limitation of soil microbes in karst ecosystems. – Science of the Total Environment 650: 241-248. https://doi.org/10.1016/j.scitotenv.2018.09.036.

[7] Chen, H., Gou, M., Hu, J., Lei, L., Zhu, S., Hu, R., Zhao, H., Xiao, W., Liu, C. (2024): Seasonal variations in soil enzyme activity and nutrient limitations of differently aged Pinus massoniana plantation. – Microorganisms 12(11): Article 11. https://doi.org/10.3390/microorganisms12112314.

[8] Chen, J., Jiang, J., Zhang, N., Song, Y. (2023): Effects of habitats in typical karst areas of Guizhou on ultrastructural morphology of Typhlocybinae. – Ecology and Evolution 13: e10680. https://doi.org/10.1002/ece3.10680.

[9] Craine, J. M., Morrow, C., Fierer, N. (2007): Microbial nitrogen limitation increases decomposition. – Ecology 88(8): 2105-2113. https://doi.org/10.1890/06-1847.1.

[10] Cui, Y., Fang, L., Deng, L., Guo, X., Han, F., Ju, W., Wang, X., Chen, H., Tan, W., Zhang, X. (2019): Patterns of soil microbial nutrient limitations and their roles in the variation of soil organic carbon across a precipitation gradient in an arid and semi-arid region. – Science of The Total Environment 658: 1440-1451. https://doi.org/10.1016/j.scitotenv.2018.12.289.

[11] Curci, R., Edwards, J. O. (1992): Activation of Hydrogen Peroxide by Organic Compounds. – In: Strukul, G. (ed.) Catalytic Oxidations with Hydrogen Peroxide as Oxidant. Springer, Dordrecht, pp. 45-95. https://doi.org/10.1007/978-94-017-0984-2_3.

[12] Dao, M., Liu, Y., Wang, Y., Yao, P., Shao, H., Zhang, S., Ma, L., Zhang, L. (2024): Effects of land use patterns on soil enzyme activity and fertility in rocky desertification area of peak-cluster depression. – Journal of Southwest Forestry University: Natural Sciences 44(5): 93-100.

[13] Daunoras, J., Kačergius, A., Gudiukaitė, R. (2024): Role of soil microbiota enzymes in soil health and activity changes depending on climate change and the type of soil ecosystem. – Biology 13(2): Article 2. https://doi.org/10.3390/biology13020085.

[14] Fatemi, F. R., Fernandez, I. J., Simon, K. S., Dail, D. B. (2016): Nitrogen and phosphorus regulation of soil enzyme activities in acid forest soils. – Soil Biology and Biochemistry 98: 171-179. https://doi.org/10.1016/j.soilbio.2016.02.017.

[15] Gan, F., Shi, H., Tan, X., Jiang, L., Li, W., Xia, Y., Pu, J., Dai, Q., Yan, Y., Fan, Y. (2025): Responses of soil enzyme activity and microbial nutrient limitations to vegetation types in a degraded karst trough valley. – Forests 16(2): Article 2. https://doi.org/10.3390/f16020279.

[16] Geekiyanage, N., Goodale, U. M., Cao, K., Kitajima, K. (2019): Plant ecology of tropical and subtropical karst ecosystems. – Biotropica 51(5): 626-640. https://doi.org/10.1111/btp.12696.

[17] Gu, X., He, H., Tao, Y., Jin, Y., Zhang, X., Xu, Z., Wang, Y., Song, X. (2017): Soil microbial community structure, enzyme activities, and their influencing factors along different altitudes of Changbai Mountain. – Acta Ecologica Sinica 37(24): 8374-8384.

[18] Gunn, J. (ed.) (2004): Encyclopedia of Caves and Karst Science. – Routledge, New York. https://doi.org/10.4324/9780203483855.

[19] Han, R., Zhang, Q., Xu, Z. (2024): Soil organic nitrogen variation shaped by diverse agroecosystems in a typical karst area: evidence from isotopic geochemistry. – PeerJ 12: e17221. https://doi.org/10.7717/peerj.17221.

[20] Huang, S., Huang, D., Wu, Q., Hou, M., Tang, X., Zhou, J. (2020): Effect of environmental C/N ratio on activities of lignin-degrading enzymes produced by Phanerochaete chrysosporium. – Pedosphere 30(2): 285-292. https://doi.org/10.1016/S1002-0160(17)60391-6.

[21] Huang, Y., Dai, Z., Lin, J., Qi, Q., Luo, Y., Dahlgren, R. A., Xu, J. (2021): Contrasting effects of carbon source recalcitrance on soil phosphorus availability and communities of phosphorus solubilizing microorganisms. – Journal of Environmental Management 298: 113426. https://doi.org/10.1016/j.jenvman.2021.113426.

[22] Idris, A. D., Bello, A. B., Hussaini, I. M., Umar, U. A., Abdulrahim, U. (2024): Soil Microbial Enzymes and Applications. – In: Aransiola, S. A., Atta, H. I., Maddela, N. R. (eds.) Soil Microbiome in Green Technology Sustainability. Springer Nature, Cham, pp. 429-461. https://doi.org/10.1007/978-3-031-71844-1_18.

[23] Krishna, M. P., Mohan, M. (2017): Litter decomposition in forest ecosystems: a review. – Energy, Ecology and Environment 2(4): 236-249. https://doi.org/10.1007/s40974-017-0064-9.

[24] Li, J., Niu, X., Wang, P., Yang, J., Liu, J., Wu, D., Guan, P. (2023): Soil degradation regulates the effects of litter decomposition on soil microbial nutrient limitation: evidence from soil enzymatic activity and stoichiometry. – Frontiers in Plant Science 13. https://doi.org/10.3389/fpls.2022.1090954.

[25] Li, T., Wang, R., Cai, J., Meng, Y., Wang, Z., Feng, X., Liu, H., Turco, R. F., Jiang, Y. (2021): Enhanced carbon acquisition and use efficiency alleviate microbial carbon relative to nitrogen limitation under soil acidification. – Ecological Processes 10(1): 32. https://doi.org/10.1186/s13717-021-00309-1.

[26] Liao, Q., Long, C., Xue, F., Xiong, L. (2021): Effect of different terrains in Maolan karst forest on soil enzyme activity and plant diversity. – Journal of Northeast Forestry University 49(10): 117-121: 126. https://doi.org/10.13759/j.cnki.dlxb.2021.10.020.

[27] Liu, G., Wang, H., Yan, G., Wang, M., Jiang, S., Wang, X., Xue, J., Xu, M., Xing, Y., Wang, Q. (2023): Soil enzyme activities and microbial nutrient limitation during the secondary succession of boreal forests. – Catena 230: 107268. https://doi.org/10.1016/j.catena.2023.107268.

[28] Ma, R., Hu, F., Liu, J., Wang, C., Wang, Z., Liu, G., Zhao, S. (2020): Shifts in soil nutrient concentrations and C:N:P stoichiometry during long-term natural vegetation restoration. – PeerJ 8: e8382. https://doi.org/10.7717/peerj.8382.

[29] Ma, Y., Zhang, C., Yang, H., Xu, Y., Chen, Y., Ning, J. (2023): The characteristics of soil C, N and P and stoichiometric ratios as affected by land-use in a karst area, Southwest China. – Land 12(6): Article 6. https://doi.org/10.3390/land12061126.

[30] Manzoni, S., Trofymow, J. A., Jackson, R. B., Porporato, A. (2010): Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter. – Ecological Monographs 80(1): 89-106. https://doi.org/10.1890/09-0179.1.

[31] Morales-Urrea, D., López-Córdoba, A., Contreras, E. M. (2023): Inactivation kinetics of horseradish peroxidase (HRP) by hydrogen peroxide. – Scientific Reports 13(1): 13363. https://doi.org/10.1038/s41598-023-39687-1.

[32] Pansu, M., Gautheyrou, J. (eds.) (2006): Handbook of Soil Analysis: Mineralogical, Organic and Inorganic Methods. – Springer, Berlin. https://doi.org/10.1007/978-3-540-31211-6_15.

[33] Peng, Y., Duan, Y., Huo, W., Zhang, Z., Huang, D., Xu, M., Wang, X., Yang, X., Wang, B., Kuzyakov, Y., Feng, G. (2022): C:P stoichiometric imbalance between soil and microorganisms drives microbial phosphorus turnover in the rhizosphere. – Biology and Fertility of Soils 58(4): 421-433. https://doi.org/10.1007/s00374-022-01633-0.

[34] Qiao, Y., Lan, J., Lei, J., Wang, X., Miao, S. (2025): Enzyme activity and microbial resource limitation mediated the soil priming effect in response to straw C components in clay and loam. – Journal of Soils and Sediments 25(1): 252-262. https://doi.org/10.1007/s11368-024-03947-y.

[35] Shen, F., Wu, J., Fan, H., Liu, W., Guo, X., Duan, H., Hu, L., Lei, X., Wei, X. (2019): Soil N/P and C/P ratio regulate the responses of soil microbial community composition and enzyme activities in a long-term nitrogen loaded Chinese fir forest. – Plant and Soil 436(1): 91-107. https://doi.org/10.1007/s11104-018-03912-y.

[36] Sistla, S. A., Schimel, J. P. (2012): Stoichiometric flexibility as a regulator of carbon and nutrient cycling in terrestrial ecosystems under change. – New Phytologist 196(1): 68-78. https://doi.org/10.1111/j.1469-8137.2012.04234.x.

[37] Song, Z., Zuo, X., Zhao, X., Qiao, J., Ya, H., Li, X., Yue, P., Chen, M., Wang, S., Medina-Roldán, E. (2024): Plant functional traits mediate the response magnitude of plant-litter-soil microbial C: N: P stoichiometry to nitrogen addition in a desert steppe. – Science of the Total Environment 915: 169915. https://doi.org/10.1016/j.scitotenv.2024.169915.

[38] Wang, H., Wu, J., Li, G., Yan, L. (2020): Changes in soil carbon fractions and enzyme activities under different vegetation types of the northern Loess Plateau. – Ecology and Evolution 10(21): 12211-12223. https://doi.org/10.1002/ece3.6852.

[39] Wang, J., Wen, X., Zhang, X., Li, S., Zhang, D.-Y. (2018): Co-regulation of photosynthetic capacity by nitrogen, phosphorus and magnesium in a subtropical karst forest in China. – Scientific Reports 8(1): 7406. https://doi.org/10.1038/s41598-018-25839-1.

[40] Wang, L., Hamel, C., Lu, P., Wang, J., Sun, D., Wang, Y., Lee, S.-J., Gan, G. Y. (2023a): Using enzyme activities as an indicator of soil fertility in grassland—an academic dilemma. – Frontiers in Plant Science 14. https://doi.org/10.3389/fpls.2023.1175946.

[41] Wang, W., Peng, Y., Chen, Y., Lei, S., Wang, X., Farooq, T. H., Liang, X., Zhang, C., Yan, W., Chen, X. (2023b): Ecological stoichiometry and stock distribution of C, N, and P in three forest types in a karst region of China. – Plants 12(13): Article 13. https://doi.org/10.3390/plants12132503.

[42] Wu, J., Wang, H., Li, G., Hou, F., Xu, G. (2024a): Effects of nitrogen forms on soil enzyme activities in a saline-alkaline grassland. – Ecology and Evolution 14(10): e70501. https://doi.org/10.1002/ece3.70501.

[43] Wu, J., Zeng, Q., Mei, K., Lin, H., Xie, H., Liu, Y., Xu, J., Chen, Y. (2024b): Soil phosphorus availability regulates the response of soil enzyme activity and enzymatic stoichiometry to litter addition in a subtropical forest. – Chinese Journal of Plant Ecology 48(2): 242. https://doi.org/10.17521/cjpe.2022.0430.

[44] Wu, P., Zhou, H., Cui, Y., Zhao, W., Hou, Y., Tan, C., Yang, G., Ding, F. (2022): Stoichiometric characteristics of leaf, litter and soil during vegetation succession in Maolan National Nature Reserve, Guizhou, China. – Sustainability 14(24): Article 24. https://doi.org/10.3390/su142416517.

[45] Xu, H., Qu, Q., Li, G., Liu, G., Geissen, V., Ritsema, C. J., Xue, S. (2022a): Impact of nitrogen addition on plant-soil-enzyme C–N–P stoichiometry and microbial nutrient limitation. – Soil Biology and Biochemistry 170: 108714. https://doi.org/10.1016/j.soilbio.2022.108714.

[46] Xu, H., Qu, Q., Wang, Z., Xue, S., Xu, Z. (2022b): Plant-soil-enzyme C-N-P stoichiometry and microbial nutrient limitation responses to plant-soil feedbacks during community

succession: a 3-year pot experiment in China. – Frontiers in Plant Science 13. https://doi.org/10.3389/fpls.2022.1009886.

[47] Xu, M., Wenjie, L., Wang, J., Zhu, Y., Feng, Y., Yang, G., Zhang, W., Han, X. (2022c): Soil ecoenzymatic stoichiometry reveals microbial phosphorus limitation after vegetation restoration on the Loess Plateau, China. – Science of the Total Environment 815: 152918. https://doi.org/10.1016/j.scitotenv.2022.152918.

[48] Yang, S., Shi, Z., Zhang, M., Li, Y., Gao, J., Wang, X., Liu, D. (2021): Stoichiometry of carbon, nitrogen and phosphorus in shrub organs linked closely with mycorrhizal strategy in northern China. – Frontiers in Plant Science 12. https://doi.org/10.3389/fpls.2021.687347.

[49] Ye, H., Hu, Y., Zhu, D., Zheng, S., Tang, X., Wu, J., Guo, S. (2024): Effects of forest conversion on the stocks and stoichiometry of soil carbon, nitrogen, and phosphorus at a county scale in subtropical China. – Forests 15(9): Article 9. https://doi.org/10.3390/f15091515.

[50] Yu, W., Wang, C., Cornelissen, J. H. C., Ye, X., Huang, Z., Wang, D., Liu, G. (2025): Litter position and stoichiometry regulate plant litter decomposition and nitrogen release in terrestrial ecosystems. – Catena 256: 109093. https://doi.org/10.1016/j.catena.2025.109093.

[51] Zeng, Q., Chen, Z., Tan, W. (2021): Plant litter quality regulates soil eco-enzymatic stoichiometry and microbial nutrient limitation in a citrus orchard. – Plant and Soil 466(1): 179-191. https://doi.org/10.1007/s11104-021-05025-5.

[52] Zhang, X., Heděnec, P., Yue, K., Ni, X., Wei, X., Chen, Z., Yang, J., Wu, F. (2024): Global forest gaps reduce litterfall but increase litter carbon and phosphorus release. – Communications Earth & Environment 5(1): 1-10. https://doi.org/10.1038/s43247-024-01453-0.

[53] Zheng, H., Liu, Y., Zhang, J., Chen, Y., Yang, L., Li, H., Wang, L. (2018): Factors influencing soil enzyme activity in China's forest ecosystems. – Plant Ecology 219(1): 31-44. https://doi.org/10.1007/s11258-017-0775-1.

[54] Zhu, X., Fang, X., Xiang, W., Chen, L., Ouyang, S., Lei, P. (2023): Vegetation restoration drives dynamics of soil nitrogen content and availability in the subtropics. – Catena 220: 106720. https://doi.org/10.1016/j.catena.2022.106720.