Effects of Plant Root Exudate Enzyme Activity on Rhizosphere Soil Enzyme Activity and Nutrients in Karst Regions: Postprint

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Abstract

To investigate the effects of plant root exudate enzyme activities on rhizosphere soil enzyme activities and nutrients in karst regions, we measured the activities of β-1,4-glucosidase (βG), β-N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP), and acid phosphatase (ACP) in root exudates and rhizosphere soils, as well as soil carbon, nitrogen, and phosphorus contents, at the community level across four vegetation restoration stages (herb-shrub grassland, shrubland, shrub-arbor forest, and arbor forest), and analyzed their relationships. The results revealed that the activities of the four enzymes in both rhizosphere soils and root exudates were significantly higher in the later stages of vegetation restoration than in the earlier stages; the C:P and N:P ratios of enzyme activities in root exudates in arbor forest were significantly higher than those in other vegetation restoration stages, whereas the opposite pattern was observed for the enzyme activity ratios in rhizosphere soils. Correlation analysis showed that root exudate enzyme activities were significantly positively correlated with the corresponding soil enzyme activities; compared with root exudate enzyme activities, the degree of correlation between soil enzyme activities and related nutrients was significantly enhanced. Additionally, the activities of βG, NAG, and LAP in both rhizosphere soils and root exudates were significantly positively correlated with rhizosphere soil organic carbon (SOC) and total nitrogen (TN), while ACP enzyme activity in both rhizosphere soils and root exudates was significantly positively correlated with rhizosphere soil available phosphorus (AP). These results indicate that vegetation restoration has a positive effect on enhancing root exudate enzyme and soil enzyme activities. Root exudate enzymes serve as a beneficial supplement to soil enzymes, playing an active role in promoting carbon, nitrogen, and phosphorus nutrient cycling. Regulating root exudate secretion and their enzyme activities may provide a new perspective for vegetation restoration in karst ecosystems.

Enzyme Activities of Plant Root Exudates Affect Rhizosphere Soil Enzyme Activities and Nutrients in Karst Areas

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Abstract: To explore the effects of plant root exudate enzyme activities on rhizosphere soil enzyme activities and nutrients in karst ecosystems, we measured the activities of β-1,4-glucosidase (βG), β-N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP), and acid phosphatase (ACP) in both root exudates and rhizosphere soils across four vegetation restoration stages: shrub-grassland, shrubland, shrub-arbor forest, and arbor forest. We also analyzed soil carbon, nitrogen, and phosphorus contents and their relationships with enzyme activities. Our results revealed that the four enzyme activities in both rhizosphere soils and root exudates were significantly higher in the later stages of vegetation restoration than in the early stages. The C:P and N:P ratios of root exudate enzyme activities in arbor forest were significantly higher than those in other stages, whereas the opposite pattern was observed for soil enzyme activity ratios. Correlation analysis showed that root exudate enzyme activities were significantly positively correlated with their corresponding soil enzyme activities. Compared with root exudate enzyme activities, the correlations between soil enzyme activities and related nutrients were significantly stronger. Additionally, βG, NAG, and LAP activities in both rhizosphere soils and root exudates were significantly positively correlated with soil organic carbon (SOC) and total nitrogen (TN), while ACP activity was significantly positively correlated with available phosphorus (AP). These results indicate that vegetation restoration positively enhances both root exudate and soil enzyme activities. Root exudate enzymes serve as a valuable supplement to soil enzymes and play an active role in promoting carbon, nitrogen, and phosphorus nutrient cycling. Regulating root exudation and its enzyme activities may provide new insights for vegetation restoration in karst ecosystems.

Keywords: karst ecosystem, vegetation restoration, root exudates enzyme activity, soil enzyme activity, soil nutrients

Introduction

Root exudates are organic compounds released by plant roots into the surrounding medium during growth and development (Vives-Peris et al., 2020). As a medium for material and energy exchange between plants, soil, and microorganisms, root exudates play an irreplaceable role in alleviating environmental stress, improving soil structure, and mobilizing soil nutrients (Cai et al., 2021). Root exudates are typically divided into low-molecular-weight and high-molecular-weight compounds, with the latter primarily including extracellular enzymes (Wen et al., 2022). Influenced by root physiological activities and environmental stress, plants often secrete large quantities of enzymes into their external growth environment, such as acid phosphatase (Zhang et al., 2007; Ma et al., 2018). Previous studies have noted that higher enzyme activity in the rhizosphere compared with bulk soil depends not only on microbial activity but also on root contributions (Zhang et al., 2019). Consequently, enzymes secreted by plant roots are considered an important source of extracellular enzymes in soil (Egamberdieva et al., 2010).

Soil enzymes are proteins with biological catalytic capacity in soil (Jing et al., 2018) and represent one of the most active soil components. Enzymes in soil (derived from roots, microorganisms, and other sources) are major factors in soil organic matter decomposition and nutrient cycling (Peng et al., 2016; Zi et al., 2018) and characterize soil metabolic processes (Gianfreda, 2015). Soil enzymes can accelerate organic matter decomposition, release nutrients fixed in soil organic matter, and provide available nitrogen and phosphorus for microorganisms and plants (Sun, 2014). Research has shown that phosphatase activity on root surfaces and in the rhizosphere plays a greater role than phosphatase activity adsorbed on clay particles and organic matter outside the rhizosphere (Liu, 2017), as phosphatase mineralizes organic phosphorus and promotes the release of available phosphorus. Therefore, enzymes contained in root exudates constitute an important component of soil enzymes and play a crucial role in maintaining carbon, nitrogen, and phosphorus nutrient balance in terrestrial ecosystems.

Southwest China's karst region represents a major ecologically fragile area in China (Wang et al., 2019). Due to its unique aboveground-underground dual structure, soil easily creeps on smooth rock surfaces, leading to nutrient loss and rocky desertification (Yuan, 2008; Zhang et al., 2011; Wang et al., 2019). Additionally, because the region's karst system is dominated by carbonate rocks rich in calcium, which readily combines with organic matter and phosphorus to form stable compounds, the release of nitrogen and phosphorus nutrients is difficult, resulting in low nutrient availability (Hu et al., 2012; Hu & Pan, 2020).

Studies have shown that soil nitrogen supply is low during early vegetation restoration stages in karst areas, while phosphorus supply becomes limiting during later stages (Liang et al., 2017). Vegetation restoration has demonstrated significant effects on soil quality recovery, markedly improving soil physical, chemical, and biological properties (Nadal-Romero et al., 2016; van Hall et al., 2017). In karst regions, many studies have focused on the effects of soil enzymes on soil nutrients. As vegetation restoration progresses, differences in nutrient limitation likely affect the types and quantities of enzymes released by plant roots at different restoration stages, influencing soil nutrient cycling. However, the variation characteristics of enzyme activities in root exudates across different vegetation restoration stages in karst areas remain unclear, as do their effects on soil enzyme activities and nutrient availability. Therefore, investigating the relationship between root exudate enzymes and soil enzymes and their role in soil nutrient cycling is important for deepening our understanding of root-soil ecological processes in karst regions.

This study examined four vegetation restoration stages—shrub-grassland, shrubland, shrub-arbor forest, and arbor forest—by measuring the activities of β-1,4-glucosidase (βG), β-N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP), and acid phosphatase (ACP) in both soils and root exudates, as well as soil organic carbon, total nitrogen, total phosphorus, and available phosphorus contents. We analyzed relationships among these indicators to explore how plant root exudate enzyme activities affect rhizosphere soil enzyme activities and nutrients in karst areas. Our findings will help improve the theoretical research system of rhizosphere eco-enzymology in karst regions and provide scientific evidence for vegetation ecosystem restoration.

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1. Materials and Methods

1.1 Study Area and Plot Setup

The study area is located in the karst ecosystem of the Lijiang River basin in Guilin, Guangxi, China, within the geographic range of 109°36’50 –111°29’30 E, 24°15’23 –26°23’30 N. The region has a subtropical monsoon climate with an annual average temperature of 18.8 °C and precipitation of 1,915.2 mm. The rainy season is hot and wet, while the dry season is cool with less rainfall. Rainfall from April to August accounts for 70% of the annual total, concentrated mainly between mid-June and mid-July (Hui et al., 2015).

We selected four vegetation restoration stages as study objects: shrub-grassland, shrubland, shrub-arbor forest, and arbor forest, in typical karst areas of the Lijiang River basin. Shrub-grassland and shrub-arbor forest were located at the Maocun Karst Experimental Site in Chaotian Township, Lingchuan County; shrubland was at the Yaji Experimental Site in Chaoyang Township, Qixing District; and arbor forest was at Dongguazhai, Baisha Town, Yangshuo County. The soil type in the study area is limestone soil developed from carbonate rocks.

The shrub-grassland had a restoration age of 15 years with dominant species including *Neyraudia reynaudiana* and *Miscanthus floridulu*; shrubland had a restoration age of 30 years with dominant species including *Loropetalum chinense*, *Millettia pulchra*, and *Bauhinia championii*; shrub-arbor forest had a restoration age of 45 years with dominant species including *Loropetalum chinense*, *Pyracantha fortuneana*, *Sageretia rugosa*, and *Leucaena leucocephala*; and arbor forest had a restoration age of 60 years with dominant species including *Quercus glauca*, *Aidia cochinchinensis*, *Tilia tuan*, and *Sapium rotundifolium*.

In each of the four vegetation restoration stages, we selected slopes with the same aspect and established sampling plots at middle and lower slope positions. For shrub-grassland, shrubland, and shrub-arbor forest, we designed six 10 m × 10 m plots per stage; for arbor forest, we designed six 20 m × 20 m plots.

1.2 Sample Collection and Processing

Rhizosphere soil and root exudate samples were collected in May 2020. In each vegetation restoration stage, we randomly selected six trees per plot to collect exudates and excavate rhizosphere soil, totaling $4 \times 6 = 24$ samples. Rhizosphere soil was collected first, followed by root exudates. Soil samples were collected using the shaking method (Liang et al., 2017). At three positions around each tree, we excavated intact root systems from the 0–20 cm soil layer, gently shook the roots to remove bulk soil without roots, then collected soil adhering within 0–5 mm of the roots as rhizosphere soil. The three subsamples were mixed to represent the plot sample. After removing stones, plant roots, and animal residues, the soil was divided into two portions: one stored at 4 °C for enzyme activity determination, and the other passed through a 10-mesh sieve, air-dried, and then passed through 20-mesh and 100-mesh sieves for physicochemical property analysis.

Root exudates were collected following the method of Phillips et al. (2008). After excavating three intact root systems from each tree, we removed soil debris from the root surfaces with tweezers, then rinsed the roots with carbon-free nutrient solution (containing 0.1 $\mathrm{mmol \cdot L^{-1}}$ KH_2PO_4, 0.5 $\mathrm{mmol \cdot L^{-1}}$ NH_4NO_3, 0.2 $\mathrm{mmol \cdot L^{-1}}$ $MgSO_4$, 0.2 $\mathrm{mmol \cdot L^{-1}}$ K_2SO_4, and 0.3 $\mathrm{mmol \cdot L^{-1}}$ $CaSO_4$). The cleaned roots were buried back in soil for 24 h to allow recovery from potential damage or stress during excavation and washing. After 24 h, the roots were excavated and rinsed with carbon-free nutrient solution. A 100 ml sterile syringe tip was sealed with Parafilm to prevent solution leakage, filled with 100 ml carbon-free nutrient solution. Cleaned roots were carefully placed into the syringe, the barrel opening was sealed with Parafilm to prevent leakage and contamination, and the syringe was buried in soil for collection. After 24 h, the syringe was excavated, the connected roots were cut, and the roots were placed in clean envelopes, brought back to the laboratory, and oven-dried to measure dry weight. After recording the solution volume, the three root exudate samples from the same tree were filtered through a 0.22 m membrane into a 300 ml plastic bottle, immediately placed in a cooler, brought back to the laboratory, and stored at -20 °C.

1.3 Enzyme Activity and Soil Property Measurements

1.3.1 Enzyme Activity Determination Enzyme activities of βG, NAG, LAP, and ACP were measured using microplate fluorometry. Substrates were: 4-Methylumbelliferyl-β-D-glucoside for βG; 4-Methylumbelliferyl-N-acetyl-β-D-glucosaminide for NAG; L-Leucine-7-amido-4-methylcoumarin hydrochloride for LAP; and 4-Methylumbelliferyl-phosphate for ACP. The standard was 4-Methylumbelliferone.

For soil enzyme activity, 0.4 g fresh soil was weighed into a 100 ml sterilized glass bottle with lid, 50 ml sterilized sodium acetate or sodium bicarbonate buffer solution was added, and the soil suspension was homogenized with a high-speed homogenizer and vortexed. Using a pipette, 200 L of soil suspension was transferred to a 96-well microplate, then 50 L of buffer, standard, or substrate was added. The microplate was incubated in darkness at 20 °C: βG, NAG, and ACP for 4 h, LAP for 18 h. After incubation, 10 L NaOH ($1\ \mathrm{mol} \cdot \mathrm{L}^{-1}$) was added to each well to terminate the reaction, and fluorescence was measured with a microplate reader at excitation wavelength 365 nm and emission wavelength 450 nm. After correction for negative controls and quenching, enzyme activities were expressed as $\mathrm{nmol} \cdot \mathrm{h}^{-1} \cdot \mathrm{g}^{-1}$ (Pan et al., 2016; Chen et al., 2018).

For root exudate enzyme activity, after filtration, 200 L of root exudate solution was pipetted into a 96-well microplate, and the subsequent steps followed the same method as for soil enzyme activity determination.

1.3.2 Soil Physicochemical Properties Soil organic carbon (SOC) was determined using the $K_2Cr_2O_7 + H_2SO_4$ oxidation method. Soil total nitrogen (TN) was measured by infrared digestion followed by flow injection analysis (FIAstar 5000, FOSS, Hillerød, Denmark) (Zhang et al., 2013). Soil total phosphorus (TP) was determined by infrared digestion followed by molybdenum blue colorimetry using a UV spectrophotometer. Soil available phosphorus (AP) was extracted with $NaHCO_3$ solution and measured by molybdenum blue colorimetry using a UV spectrophotometer (Liang et al., 2021).

1.4 Data Analysis

βG enzyme is related to carbon cycling; NAG and LAP enzymes are related to nitrogen cycling; ACP enzyme is related to phosphorus cycling (Sinsabaugh & Shah, 2012; Cui et al., 2018). Enzyme activity C:N ratio was calculated as lnβG:ln(NAG+LAP), C:P ratio as lnβG:ln(ACP), and N:P ratio as ln(NAG+LAP):ln(ACP) (Sinsabaugh et al., 2009). These ratios reflect the relative rates of carbon, nitrogen, and phosphorus cycling in soil and reveal important indicators of soil nutrient cycling.

Original data were statistically analyzed using Excel 2021. One-way ANOVA was performed using SPSS 26.0 to compare differences in root exudate enzyme activities, soil enzyme activities, and their ratios among different vegetation restoration stages. Origin 2021 software was used for graphing. Pearson correlation analysis was employed to analyze correlations between root exudate enzyme activities, soil enzyme activities, their ratios, and soil nutrients. Canoco 5 software was used for redundancy analysis (RDA) to determine the influence of soil environmental factors on root exudate and soil enzyme activities and their ratios.

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2. Results

2.1 Soil Nutrient Characteristics Across Vegetation Restoration Stages

Soil SOC and TP contents differed significantly across vegetation restoration stages, while soil TN and AP contents showed no significant differences between shrub-grassland and shrubland (Table 1). Arbor forest had the highest soil TN, TP, and AP contents; shrub-arbor forest had the highest soil SOC content; shrub-grassland had the lowest soil SOC and TP contents; and shrub-grassland and shrubland had the lowest soil TN and AP contents. Overall, except for soil SOC, soil TN, TP, and AP contents increased with vegetation succession.

Table 1 Soil physical and chemical properties at different vegetation restoration stages

<table>
<tr><th>Recovery phase</th><th>SOC (g · kg^{-1})</th><th>TN (g · kg^{-1})</th><th>TP (g · kg^{-1})</th><th>AP (mg · kg^{-1})</th></tr>
<tr><td>Shrub-grassland</td><td colspan="4">30.61$±7.57*d*|3.21±0.69*c*|0.22±0.05*d*|3.95±0.24*c*||*Shrubland*|48.89±4.56*c*|3.96±0.64*bc*|0.37 *arborforest*|61.15±6.60*a*|4.43±0.75*b*|0.43±0.02*b*|5.77±0.37*b*||*Arborforest*|55.93±8.63*b*|5.98</td></tr>
</table>

Note: Different lowercase letters in each row indicate significant differences between different vegetation restoration stages (P<0.05).

2.2 Enzyme Activities and Ratios in Root Exudates and Soils

Among soil enzyme activities across vegetation restoration stages, LAP and ACP activities in arbor forest were significantly higher than in other stages, while shrub-grassland showed the lowest enzyme activities. βG and NAG activities in arbor forest and shrub-arbor forest were significantly higher than in shrubland and shrub-grassland (Figure 1 [Figure 1: see original paper]A). For root exudate enzyme activities, βG, NAG, LAP, and ACP activities in arbor forest were significantly higher than in other stages, while shrub-grassland showed the lowest βG and ACP activities (Figure 1B). Overall, soil enzyme activities were significantly higher than root exudate enzyme activities, and both increased from early to late vegetation restoration stages.

As shown in Figure 2 [Figure 2: see original paper], for soil enzyme activity ratios across vegetation restoration stages, the C:N ratio in shrubland was significantly higher than in shrub-grassland; shrub-grassland showed the highest C:P and N:P ratios, while arbor forest showed the lowest. For root exudate enzyme ratios, the C:N ratio in shrubland was significantly higher than in shrub-grassland; arbor forest showed the highest C:P and N:P ratios, while shrub-grassland showed the lowest C:P ratio and shrubland showed the lowest N:P ratio. Except for shrub-grassland, root exudate enzyme C:N ratios were higher than soil enzyme C:N ratios, while root exudate enzyme C:P and N:P ratios were lower than soil enzyme C:P and N:P ratios.

Figure 1 [Figure 1: see original paper] Changes of enzyme activities in root exudates and soils in different vegetation restoration stages

Note: Different lowercase letters represent significant differences in root exudates and soil enzyme activities among different vegetation restoration stages ($P<0.05$). βG: β-1,4-Glucosidase; NAG: β-N-acetyl-glucosaminidase; LAP: Leucine aminopeptidase; ACP: Acid phosphatase.

Figure 2 [Figure 2: see original paper] Changes in the ratio of enzymes between root exudates and soil in different vegetation restoration stages

Note: Different lowercase letters represent significant differences in root exudates and soil enzyme activity ratios among different vegetation restoration stages ($P<0.05$). Enzyme activity C:N ratio: lnβG:ln(NAG+LAP); Enzyme activity C:P ratio: lnβG:ln(ACP); Enzyme activity N:P ratio: ln(NAG+LAP):ln(ACP); QC: shrub-grassland; QM: shrubland; GQ: shrub-arbor forest; QM: arbor forest.

2.3 Correlations Among Root Exudate Enzymes, Soil Enzymes, and Soil Nutrients

Correlation analysis revealed that βG, NAG, LAP, and ACP activities in root exudates were significantly positively correlated with their corresponding soil enzyme activities (Figure 3 [Figure 3: see original paper]). Root exudate βG, NAG, and LAP activities were significantly positively correlated with soil SOC, TN, TP, and AP ($P<0.01$). Root exudate ACP activity was significantly positively correlated with soil AP ($P<0.05$). Soil βG, NAG, LAP, and ACP activities were significantly positively correlated with soil SOC, TN, TP, and AP ($P<0.01$). Root exudate enzyme activity C:P and N:P ratios were significantly positively correlated with soil SOC, TN, TP, and AP ($P<0.01$), whereas soil enzyme activity C:P and N:P ratios were significantly negatively correlated with soil SOC, TN, TP, and AP ($P<0.01$) (Table 2).

RDA analysis, with root exudate and soil enzyme activities and their ratios as response variables and soil factors as explanatory variables, showed that the first axis explained 56.5% of the variation and the second axis explained 6.32%. AP and TP were identified as key environmental factors affecting both enzyme activities and their ratios during vegetation restoration (Figure 4 [Figure 4: see original paper]).

Figure 3 [Figure 3: see original paper] Relationship between root exudates enzymes and soil enzymes

Table 2 Correlation between the enzymatic activities and ratios of root exudates and soil and soil factors

Variable	SOC	TN	TP	AP
Root exudates βG enzyme	0.776**	0.743**	0.863**	0.828**
Root exudates NAG enzyme	0.667**	0.666**	0.712**	0.860**
Root exudates LAP enzyme	0.616**	0.703**	0.708**	0.838**
Root exudates ACP enzyme	0.479*	0.596**	0.427*	0.657**
Root exudates enzyme activity C:N ratio	0.827**	0.735**	0.893**	0.752**
Root exudates enzyme activity C:P ratio	0.607**	0.629**	0.636**	0.774**
Root exudates enzyme activity N:P ratio	0.819**	0.704**	0.839**	0.813**
Soil βG enzyme	0.709**	0.740**	0.849**	0.774**
Soil NAG enzyme	0.665**	0.731**	0.818**	0.791**
Soil LAP enzyme	0.827**	0.850**	0.917**	0.956**
Soil ACP enzyme	-0.624**	-0.723**	-0.760**	-0.699**
Soil enzyme activity C:N ratio	-0.832**	-0.769**	-0.843**	-0.834**
Soil enzyme activity C:P ratio	-0.624**	-0.723**	-0.760**	-0.699**
Soil enzyme activity N:P ratio	-0.832**	-0.769**	-0.843**	-0.834**

Note: indicates P<0.05; ** indicates P<0.01.*

Figure 4 [Figure 4: see original paper] Redundancy analysis of root exudates and soil enzyme activities and their ratios with soil environmental factors

Note: Root βG: Root exudates βG enzyme; Root NAG: Root exudates NAG enzyme; Root LAP: Root exudates LAP enzyme; Root ACP: Root exudates ACP enzyme; Root C:N: Root exudates enzyme activity C:N ratio; Root C:P: Root exudates enzyme activity C:P ratio; Root N:P: Root exudates enzyme activity N:P ratio; Soil βG: Soil βG enzyme; Soil NAG: Soil NAG enzyme; Soil LAP: Soil LAP enzyme; Soil ACP: Soil ACP enzyme; Soil C:N: Soil enzyme activity C:N ratio; Soil C:P: Soil enzyme activity C:P ratio; Soil N:P: Soil enzyme activity N:P ratio; AP: Soil available phosphorus; TP: Soil total phosphorus; SOC: Soil organic carbon; TN: Soil total nitrogen.

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3. Discussion

3.1 Variation in Root Exudate and Rhizosphere Soil Enzyme Activities and Ratios Across Vegetation Restoration Stages

Across different vegetation restoration stages, root exudate enzyme activities in later stages were greater than in early stages. This likely represents an evolutionarily stable strategy for plants to enhance nutrient availability. Previous studies have shown that karst ecosystems are limited by nitrogen and phosphorus or other nutrients during early vegetation restoration, while phosphorus becomes limiting during later stages (Zhang et al., 2015). In this study, ACP activity in arbor forest was significantly higher than in other stages, suggesting that under phosphorus-deficient conditions, roots—the most metabolically active plant parts (Bell et al., 2014)—increase extracellular enzyme release to accelerate nutrient cycling and obtain higher mineral nutrient content.

The four soil enzyme activities showed the same pattern as root exudate enzyme activities, being greater in later restoration stages than in early stages. This phenomenon is related to differences in plant species and nutrient release among vegetation stages (Pan et al., 2020). Generally, tree species can regulate rhizosphere nutrients through litter decomposition, thereby affecting soil enzyme activity (Snajdr et al., 2013). Our vegetation survey revealed that early-stage shrub-grassland was dominated by *Neyraudia reynaudiana* and *Miscanthus floridulu*, with relatively simple plant species, less litter for decomposition, low plant productivity, and slow soil nutrient recovery, resulting in lower soil enzyme content than other stages. In contrast, late-stage arbor forest was dominated by deciduous trees such as *Quercus glauca*, *Tilia tuan*, and *Sapium rotundifolium*. Deciduous trees primarily enhance resource use efficiency (Jiang et al., 2021), and the large plant biomass under forest canopy produces abundant litter with high nutrient content. As litter is the main nutrient source for soil microorganisms, it stimulates microbial extracellular enzyme secretion (Wei et al., 2019), thereby increasing soil enzyme activity in arbor forest. This study also found that arbor forest had the lowest soil enzyme activity C:P and N:P ratios, while shrub-grassland had the highest. This is because microbial decomposition of litter is the main source of soil nitrogen (Li et al., 2018). Early restoration stages have sparse litter layers, low vegetation coverage, and weak water retention capacity, making nitrogen loss more susceptible to subsurface flow (Song et al., 2017). However, in later stages, phosphorus is strongly adsorbed by calcium from weathered bedrock (Vitousek et al., 2010), resulting in lower soil enzyme activity C:P and N:P ratios in arbor forest. In summary, positive vegetation succession enhanced both root exudate and soil enzyme activities.

3.2 Effects of Root Exudate Enzyme Activity on Rhizosphere Soil Enzyme Activity

This study found that βG, NAG, LAP, and ACP activities in root exudates were significantly positively correlated with their corresponding soil enzyme activities, indicating that root exudate enzymes and soil enzymes are complementary, with root exudate enzymes promoting increased soil enzyme activity and thereby enhancing soil nutrient supply to plants. The rhizosphere is the narrow soil zone surrounding roots that is strongly influenced by root and microbial activities (Jones et al., 2004), and roots can directly release enzymes into soil to supplement soil enzyme content under certain conditions. Koranda et al. (2011) reported that protease activity was enhanced in rhizosphere soil compared with bulk soil, and Pan et al. (2020) also found that βG and NAG activities were significantly higher in rhizosphere soil than in bulk soil. Therefore, in the rhizosphere, plant roots release enzymes to increase soil enzyme content.

Table 2 shows that correlations between soil enzyme activities and related nutrients were significantly stronger than those between root exudate enzyme activities and nutrients, indicating that root-secreted enzymes enhance the ability of soil enzymes to release available carbon, nitrogen, and phosphorus compounds from complex organic matter. Xiao et al. (2014) demonstrated that under phosphorus deficiency, macadamia seedlings secrete large amounts of acid phosphatase to mobilize organic phosphorus and release phosphate ions, thereby improving soil phosphorus nutrition. In this study, the region experienced severe nitrogen limitation during early restoration and phosphorus limitation during later stages. Shrub-grassland showed higher root exudate NAG enzyme secretion than shrubland, while arbor forest roots actively released ACP to cope with nutrient deficiency. Therefore, enzymes in root exudates serve as a valuable supplement to soil enzymes, significantly enhancing soil enzyme function.

3.3 Relationships Among Root Exudate Enzymes, Rhizosphere Soil Enzymes, and Soil Nutrients

This study found that βG, NAG, and LAP activities in both soil and root exudates were significantly positively correlated with soil SOC and TN. Previous research has shown close relationships between soil organic matter and hydrolases, with positive correlations between organic matter and soil enzyme activities (Sui et al., 2009; Tang et al., 2020). Soil enzymes are produced by microorganisms and plant root exudates (Liu et al., 2020), and nitrogen is an essential element for enzyme synthesis. TN can increase fine root biomass, promote rhizosphere microbial growth, and enhance related soil enzyme activities (Tu et al., 2012), indicating that during nutrient cycling, positive nitrogen release by soil enzymes adapts to heterogeneous habitat conditions and partly determines the quantity of enzymes produced by microorganisms (Luo et al., 2017).

Root exudate ACP activity was significantly positively correlated with soil AP. RDA analysis identified AP as one of the key environmental factors affecting both enzyme activities during vegetation restoration. This is because enhanced root acid phosphatase secretion is a physiological plasticity mechanism for plants to cope with phosphorus deficiency. This enzyme participates in the mobilization and utilization of rhizosphere organic phosphorus (Zhu et al., 2022) and represents the most important biological strategy for obtaining phosphate ions from organic molecules. Acid phosphatase is produced not only by microorganisms such as mycorrhizal and saprotrophic fungi or bacteria (Margalef et al., 2021) but also released by plant roots. Karst regions have scarce soil phosphorus supply (Pan et al., 2011). While plants can directly absorb inorganic soluble phosphate, large amounts of organic phosphorus such as phosphate esters and anhydrides cannot be directly utilized (Zhou et al., 2021). Utilization of soil organic phosphorus requires hydrolysis by phosphatases. The primary role of plant acid phosphatase is to decompose organic phosphorus substrates in the soil environment, releasing inorganic phosphorus that can be directly absorbed by plants, thereby increasing soil phosphorus availability (Miller et al., 2001; Zhang, 2014). Studies have shown that under low phosphorus conditions, root-secreted acid phosphatase activity increases significantly (Liang et al., 2005; Xie et al., 2005). Additionally, soil ACP activity was significantly positively correlated with soil AP, possibly because acid phosphatase in root exudates increased soil acid phosphatase content, further promoting the transformation of soil organic phosphorus and increasing soil AP content. Research indicates that mycorrhizal plants can release more enzymes through improved nutrition or expanded root systems, and enhancing soil phosphatase activity is important for mobilizing soil phosphorus (Hu et al., 2019). Root-produced eco-enzymes can also enter soil after root death (Rillig et al., 2007), altering carbon, nitrogen, and phosphorus cycling enzyme levels. Therefore, root exudate enzymes are key drivers of soil nutrient cycling, and enzymes released by plant roots can directly participate in the effective transformation of soil nutrient elements, timely secreting extracellular enzymes to obtain required nutrients.

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4. Conclusion

Vegetation restoration significantly affected root exudate and soil enzyme activities, with positive vegetation succession enhancing both. Root exudate enzyme activities were significantly positively correlated with corresponding soil enzyme activities, indicating that root-secreted enzymes increased rhizosphere soil enzyme activity. Plant root exudate enzyme activities showed significant positive correlations with both total and available nutrient contents, demonstrating that root exudate enzyme activity plays an active role in improving soil nutrient availability. Regulating root exudation and its enzyme activities is important for enhancing soil nutrient availability in karst ecosystems and may provide a new perspective for vegetation restoration in these fragile environments.

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Note: Figure translations are in progress. See original paper for figures.