Effects of Different Land Use Types and Restoration Patterns on the C:N:P Ratio of Soil Enzyme Activities in Karst Regions: Postprint

Authors: Runyang Zhang, 钱前, Liu Kunping, Liang Yueming, Zhang Wei, Jin Zhenjiang, Pan Fujing

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Abstract

To investigate the effects of different land use types and ecological restoration patterns on soil enzyme activities and their C:N:P ratios in karst regions, this study selected three land use types [degraded disturbed land, grassland, and fruit tree (loquat) forest land] and four restoration patterns (evergreen arbor forest, deciduous arbor forest, evergreen-deciduous mixed forest, and naturally restored forest) at the long-term positioning observation and experimental site of the Huanjiang Karst Ecosystem Observation and Research Station of the Chinese Academy of Sciences in Huanjiang County, Guangxi, and analyzed the relationship between the activities of four soil enzymes [β-1,4-glucosidase (βG), β-1,4-N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP), and alkaline phosphatase (ALP)] and the variations in C:N:P ratios with soil ecological factors. The results showed that: (1) The four enzyme activities in restoration pattern soils were all higher than those in land use types. Among different land use types, grassland exhibited higher activities of the four enzymes, as well as higher C:P and N:P ratios, than the other two land use types. Among different restoration patterns, the βG and ALP enzyme activities in deciduous arbor forest were significantly higher than those in naturally restored forest and evergreen arbor forest, the NAG enzyme activity in evergreen arbor forest was significantly higher than that in the other three restoration patterns, while the enzyme activity C:P ratio in deciduous arbor forest and the enzyme activity N:P ratio in evergreen-deciduous mixed forest were significantly lower than those in the other three restoration patterns. Additionally, vector angle analysis of enzymatic stoichiometric ratios revealed that all land use types and restoration patterns were phosphorus-limited. (2) The four enzyme activities were all significantly positively correlated with soil organic carbon (SOC), ammonium nitrogen (NH4+-N), and nitrate nitrogen (NO3-N) contents, and significantly negatively correlated with total phosphorus (TP) content; βG enzyme activity was also significantly positively correlated with available phosphorus (AP) content, and ALP enzyme activity was significantly positively correlated with total nitrogen (TN) content. (3) Redundancy analysis (RDA) showed that soil TP, NH4+-N, NO3-N, and AP contents explained 38.3%, 9.5%, 9.3%, and 8.0% of the variations in soil enzyme activities and C:N:P ratios, respectively. In summary, soil phosphorus limitation was ubiquitous across different land use and restoration patterns in karst regions, implying that phosphorus occurrence and transformation during land use development and restoration are key focuses for soil quality improvement. Additionally, since grassland, evergreen-deciduous mixed forest, and deciduous arbor forest exhibited higher soil enzyme activities, C:P ratios, and available phosphorus contents compared with other land use and restoration patterns, it indicates that grasses and deciduous plants may have positive effects on soil quality improvement during karst land use and ecological restoration processes.

Full Text

Effects of Different Land Use and Ecological Restoration Types on Soil Enzymatic C:N:P Ratios in a Karst Ecosystem

ZHANG Runyang[1,4], QIAN Qian[1,4], LIU Kunping[2], LIANG Yueming[3], ZHANG Wei[2], JIN Zhenjiang[1,4], PAN Fujing[1,4*]

[1]Guangxi Key Laboratory of Theory and Technology for Environmental Pollution Control, College of Environmental Science and Engineering, Guilin University of Technology, Guilin 541004, Guangxi, China
[2]Huanjiang Observation and Research Station for Karst Ecosystems, Chinese Academy of Sciences, Huanjiang 547100, Guangxi, China
[3]Key Laboratory of Karst Dynamics, Ministry of Natural Resources & Guangxi Zhuang Autonomous Region, Institute of Karst Geology, Chinese Academy of Geological Sciences, Guilin 541004, Guangxi, China
[4]Collaborative Innovation Center for Water Pollution Control and Water Safety in Karst Area, Guilin 541004, Guangxi, China

Abstract: To investigate the effects of different land use types and ecological restoration patterns on soil enzyme activities and their C:N:P ratios in karst regions, this study selected three land use types (degraded disturbed land, pasture grassland, and orchard forest [loquat]) and four restoration patterns (evergreen forest, deciduous forest, evergreen-deciduous mixed forest, and natural restoration forest) at the long-term monitoring experimental site of the Huanjiang Karst Ecosystem Observation and Research Station in Huanjiang County, Guangxi. We analyzed the activities of four soil enzymes [β-1,4-glucosidase (βG), β-1,4-N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP), and alkaline phosphatase (ALP)] and their C:N:P ratios in relation to soil ecological factors. The results showed that: (1) All four enzyme activities were higher in restoration patterns than in land use types. Among land use types, pasture grassland exhibited the highest activities of the four enzymes, as well as the highest enzymatic C:P and N:P ratios. Among restoration patterns, the deciduous forest showed significantly higher βG and ALP activities than the natural restoration and evergreen forests, while the evergreen forest had significantly higher NAG activity than the other three restoration patterns. The enzymatic C:P ratio of the deciduous forest and the enzymatic N:P ratio of the mixed forest were significantly lower than those of the other three restoration patterns. Vector angle analysis of enzymatic stoichiometric ratios revealed that all land use and restoration types were phosphorus-limited. (2) All four enzyme activities were significantly positively correlated with soil organic carbon (SOC), ammonium nitrogen (NH_4^+-N), and nitrate nitrogen (NO_3^--N), but significantly negatively correlated with total phosphorus (TP). βG activity was also significantly positively correlated with available phosphorus (AP), while ALP activity was significantly positively correlated with total nitrogen (TN). (3) Redundancy analysis (RDA) showed that soil TP, NH_4^+-N, NO_3^--N, and AP explained 38.3%, 9.5%, 9.3%, and 8.0% of the variation in soil enzyme activities and C:N:P ratios, respectively. In conclusion, soil phosphorus limitation is widespread across different land use and restoration patterns in karst areas, indicating that phosphorus retention and transformation should be prioritized in soil quality improvement during land development and restoration. Additionally, pasture grassland, mixed forest, and deciduous forest showed higher soil enzyme activities, C:P ratios, and AP contents compared to other land use and restoration types, suggesting that forage and deciduous plants may play positive roles in improving soil quality during karst land use and ecological restoration.

Keywords: karst ecosystem, land use, land restoration, soil enzyme activity, enzymatic stoichiometry

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1.1 Study Area Overview

The Huanjiang Karst Ecosystem Observation and Research Station of the Chinese Academy of Sciences (108°18 -108°19 E, 24°43 -24°44 N, average elevation 228.5–337.8 m) is located in Huanjiang Maonan Autonomous County, Guangxi Zhuang Autonomous Region. Situated north of the Tropic of Cancer and on the southeastern edge of the Yunnan-Guizhou Plateau, the region experiences a typical subtropical monsoon climate. The average outdoor temperature in July is 29°C, with an annual mean temperature of 15.4-22.4°C. Annual sunshine hours total 4,422, solar radiation reaches 98.89 $kJ \cdot cm^{-2}$, the frost-free period extends to 290 days, and mean annual precipitation ranges from 1,400 to 1,500 mm, with 70% concentrated between April and September.

Prior to 1985, the study area experienced frequent burning and grazing, leading to severe rocky desertification. After local residents relocated in 1985, the degraded ecosystem gradually recovered. In 2004, a slope cultivation observation platform was established on a hillside with relatively uniform soil and vegetation to simulate anthropogenic disturbance and ecological restoration, creating long-term controlled experimental plots of similar area and slope. The specific treatments for the three land use types (degraded disturbed land, pasture grassland, orchard forest) and four restoration patterns (evergreen forest, deciduous forest, evergreen-deciduous mixed forest, natural restoration forest) are detailed in Table 1. Bedrock outcrop in the study area is less than 10%, gravel coverage ranges from 30% to 60%, and surface soil contains approximately 10–30% rock fragments by volume. Soils are primarily alkaline lime soils formed from dolomite weathering, with average thickness increasing from 10–30 cm at the upper slope to 50–80 cm at the lower slope.

1.2 Sampling Method

In July 2020, we selected three land use types and four restoration patterns at the slope cultivation observation platform as study sites. Each type constituted one runoff plot, with station slope runoff plots having a projected area of approximately 100 m × 10 m, spanning from mid-slope to foot-slope positions. We established three 10 m × 10 m quadrats at upper, middle, and lower positions in each plot type, yielding three replicate quadrats per type with intervals of at least 20 m between quadrats.

Within each quadrat, three sampling points were set up. At each point, soil was collected using a five-point sampling method within a 3 m × 3 m area. Soils from each sampling point were mixed to form one composite sample, resulting in three composite samples per quadrat. Consequently, each land use type or restoration pattern yielded nine composite soil samples, totaling 63 soil samples. Stones and roots were removed within 4 hours of collection, and soils were passed through a 10-mesh sieve before being divided into two equal portions. The first portion was stored at 4°C for determination of soil extracellular enzyme activities, ammonium nitrogen, and nitrate nitrogen. The second portion was air-dried, ground, and passed through 20-mesh and 100-mesh sieves for soil nutrient analysis.

1.3 Soil Nutrient Determination

Soil pH was measured using a pH meter (Leici PHS-2F) at a soil:water ratio of 1:2.5. Soil organic carbon (SOC) was determined by dichromate oxidation with sulfuric acid heating, followed by titration with ferrous sulfate solution. Total nitrogen (TN) was measured using the semi-micro Kjeldahl digestion method, with nitrogen concentration in digests analyzed by flow injection analyzer (AA3HR). Total phosphorus (TP) was determined by NaOH fusion, with digests color-developed using molybdenum-antimony reagent and measured by UV-Vis spectrophotometer (PerkinElmer L60200060). Ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) were extracted with KCl solution and measured by flow injection analyzer (AA3HR). Available phosphorus (AP) was extracted with Na_2HCO_3 solution, color-developed with molybdenum-antimony reagent, and measured by UV-Vis spectrophotometer (PerkinElmer

L60200060).

1.4 Soil Enzyme Activity Determination

Soil enzyme activities were measured using the MUB fluorometric method. Fresh soil (1 g) was placed in a 500 mL sterile glass bottle with lid, and 125 mL of sterilized, cooled sodium acetate or sodium bicarbonate buffer was added to create a soil suspension. The suspension was homogenized using a blender and vortexed to maintain uniformity. Aliquots of the suspension were pipetted into 96-well microplates, which were incubated at 20°C in darkness for 4 hours. After incubation, 10 L of NaOH solution (1 $mol \cdot L^{-1}$) was added to each well to terminate the reaction, and fluorescence was measured using a microplate reader (Synergy H4). Enzyme activity was expressed in units of $nmol \cdot g^{-1} \cdot h^{-1}$.

1.5 Data Processing

Enzymatic C:N:P ratios were calculated as ln(βG):ln(NAG+LAP):ln(ALP). Vector length (Vector L) and vector angle (Vector A) of enzymatic stoichiometric ratios were calculated according to Moorhead et al. (2016):

Vector L = $\{[\ln(\beta G)/\ln(NAG+LAP)]^2 + [\ln(\beta G)/\ln(ALP)]^2\}^1/^2$
Vector A = Degrees$\{ATAN2[\ln(\beta G)/\ln(ALP), \ln(\beta G)/\ln(NAG+LAP)]\}$

Where Vector L indicates the degree of microbial carbon limitation, and Vector A indicates the degree of microbial nitrogen or phosphorus limitation. Deviation of Vector A from 45° suggests N or P limitation, with greater upward deviation indicating stronger P limitation and greater downward deviation indicating stronger N limitation.

All variables were tested for normal distribution. One-way ANOVA and least significant difference (LSD) multiple comparisons (SPSS 22.0) were used to analyze the effects of land use and restoration patterns on soil enzyme activities and physicochemical properties, with figures generated using Origin 2018. Spearman correlation analysis (SPSS 22.0) examined relationships between soil enzyme activities, stoichiometric ratios, and physicochemical properties. Redundancy analysis (RDA, Canoco 5.0) quantified the contribution of soil environmental factors to variations in enzyme activities and stoichiometric ratios.

2.1 Soil Nutrient Characteristics Under Different Land Use and Restoration Patterns

Among the three land use types, SOC and TP contents showed no significant differences. Degraded disturbed land had significantly higher TN content than other land use types, while NO_3^--N content was lowest. Pasture grassland exhibited the highest AP and NH_4^+-N contents, with AP being 13.03% and

96.76% higher than in degraded disturbed land and orchard forest, respectively, and NH_4^+-N being 78.31% and 51.29% higher. Orchard forest had the highest NO_3^--N content, exceeding degraded disturbed land and pasture grassland by 180.48% and 31.91%, respectively.

Among the four restoration patterns, SOC and TP contents showed no significant differences. Deciduous forest had the highest NO_3^--N content, exceeding evergreen forest, mixed forest, and natural restoration forest by 39.60%, 34.59%, and 51.13%, respectively. Mixed forest had the highest TN and NH_4^+-N contents, with NH_4^+-N being 42.82% higher than in natural restoration forest, which had the lowest NH_4^+-N and AP contents. Mean values of SOC, TN, TP, NH_4^+-N, and AP for the three land use types showed no significant differences from those of the four restoration patterns.

2.2 Variations in Soil Enzyme Activities and C:N:P Ratios

Among land use types, pasture grassland showed significantly higher NAG and LAP activities than other types, while orchard forest had significantly lower NAG activity and degraded disturbed land had significantly lower βG activity. ALP activity showed no significant differences among land use types. Among restoration patterns, deciduous forest exhibited significantly higher βG activity than natural restoration and evergreen forests. Evergreen forest had significantly higher NAG activity than the other three restoration patterns. LAP activities in evergreen forest and mixed forest were significantly higher than in deciduous and natural restoration forests. ALP activities in deciduous and mixed forests were significantly higher than in evergreen and natural restoration forests. Mean enzyme activities across restoration patterns were significantly higher than those across land use types.

Enzymatic C:N:P ratios differed significantly between land use types and restoration patterns ($P<0.05$). Among land use types, degraded disturbed land had the lowest enzymatic C:N:P ratios. Pasture grassland showed the highest enzymatic C:P and N:P ratios, with its enzymatic C:N ratio being 12.48% higher than that of degraded disturbed land. Orchard forest had the highest enzymatic C:N ratio, 12.92% higher than degraded disturbed land.

Among restoration patterns, evergreen forest had the highest enzymatic C:P and N:P ratios. Deciduous forest showed relatively high enzymatic C:N and C:P ratios but significantly lower enzymatic N:P ratio than other restoration patterns. Mixed forest had relatively low enzymatic ratios, while natural restoration forest had relatively high ratios. Vector length (Vector L) of enzymatic ratios in orchard forest was significantly higher than in other land use types. In restoration patterns, Vector L in deciduous forest and natural restoration forest was significantly higher than in other patterns. Vector angles (Vector V) for all land use and restoration types exceeded 45°.

2.3 Relationships Between Soil Enzyme Activities, C:N:P Ratios, and Physicochemical Factors

Correlation analysis revealed that βG activity was significantly positively correlated with AP, NH_4^+-N, and NO_3^--N (P<0.01). NAG and LAP activities were significantly positively correlated with NH_4^+-N and NO_3^--N, while LAP activity was also significantly positively correlated with TN (P<0.01). All four enzyme activities were significantly negatively correlated with TP. ALP activity was significantly positively correlated with NH_4^+-N. Enzymatic C:N ratio was significantly positively correlated with AP and pH (P<0.01) but significantly negatively correlated with NH_4^+-N. Enzymatic C:P ratio was significantly positively correlated with AP and NO_3^--N (P<0.01) but significantly negatively correlated with NH_4^+-N. Enzymatic N:P ratio was significantly positively correlated with NH_4^+-N (P<0.01) and significantly negatively correlated with TP. Soil enzyme activities and stoichiometric ratios were closely related to physicochemical factors across different land use and restoration patterns.

Redundancy analysis showed that soil physicochemical properties explained 63.3% of the total variation in soil enzyme activities and stoichiometric ratios, with the first axis explaining 65.8% and the second axis 0.81% of the variation. TP (F=37.9, P=0.002), NH_4^+-N (F=11.0, P=0.002), NO_3^--N (F=12.9, P=0.002), and AP (F=13.4, P=0.002) were significant influencing factors, explaining 38.3%, 9.5%, 9.3%, and 8.0% of the variation, respectively.

3.1 Variations in Soil Enzyme Activities and C:N:P Ratios and Their Influencing Factors

Soil enzyme activities and stoichiometric ratios are influenced by changes in soil physicochemical properties, which are closely associated with land use conversion and vegetation type changes. In this study, mean enzyme activities under restoration patterns were significantly higher than those under land use types, with substantial variations observed among different land use and restoration patterns. Two primary factors may explain these differences: (1) disturbance intensity. Evergreen forest, deciduous forest, mixed forest, and natural restoration forest experienced less anthropogenic disturbance compared to orchard forest, pasture grassland, and degraded disturbed land. Consequently, improved litter return and fine root biomass input increased the availability of plant-derived nutrients for soil microorganisms, affecting nitrification and denitrification processes and the rate of enzyme release by phosphorus-solubilizing bacteria, thereby enhancing enzyme activities. (2) Plant physiological and ecological characteristics. Pasture grassland exhibits high aboveground biomass and well-developed root systems, while deciduous species primarily adopt resource-use efficiency strategies with short annual growth cycles and high nutrient turnover and leaf renewal rates. Given the high pH of the experimental site, phosphorus tends to form complexes unavailable to plants and microorganisms, leading to higher organic matter stability and reduced nitrogen and phosphorus availability. To acquire more nutrients, plants secrete substantial organic matter to soil microorganisms, promoting microbial growth and enzyme secretion to enhance nutrient availability.

3.2 Effects of Land Use and Restoration Patterns on Soil Enzyme Activities and C:N:P Ratios

This study found that among land use types, pasture grassland exhibited higher NAG and LAP activities and enzymatic N:P ratios compared to degraded disturbed land and orchard forest. Soil nutrient availability and biological properties are recognized as important factors influencing soil enzyme activities and stoichiometric ratios. Two mechanisms may explain these variations: (1) phosphorus limitation effects. Vector angle analysis indicated that all land use types suffered from phosphorus deficiency. Pasture grassland showed higher enzymatic C:P and N:P ratios and smaller vector angles than other land use types, suggesting reduced phosphorus limitation. Plants regulate microbial enzyme production to alter nutrient availability, and the significantly higher AP content in pasture grassland supports this trend, likely attributable to fertilization during plot establishment that alleviated phosphorus limitation. (2) nitrogen content effects. With phosphorus limitation alleviated, nitrogen becomes more prominent in biological processes. Most soil nitrogen originates from organic matter decomposition, which explains the significant positive correlation between NAG and LAP activities and SOC. Pasture grassland' s well-developed root system with numerous fine roots, particularly at root tips, secretes more enzymes and organic matter to support microbial proliferation and hydrolytic enzyme production for organic matter decomposition. NH_4^+-N derives from TN mineralization, and pasture grassland' s strong NH_4^+-N adsorption capacity provides greater nitrogen supply than other land use types, resulting in significant positive correlations between NAG/LAP activities and NH_4^+-N. In summary, pasture grassland demonstrates superior nutrient cycling compared to other land use types.

Among restoration patterns, deciduous forest and mixed forest showed higher βG and ALP activities, lower N:P ratios, and larger vector angles, indicating more severe phosphorus limitation than other patterns. This may be related to the presence of deciduous plants for two reasons: (1) physiological characteristics. Deciduous plants exhibit high growth rates, productivity, and photosynthetic rates, demanding substantial soil nutrients. When nutrient availability is low, microorganisms secrete more enzymes to enhance nutrient supply. (2) litter return. Deciduous forest and mixed forest had higher litterfall than other patterns, providing abundant organic matter for nutrient acquisition. Soil nutrients largely derive from organic matter decomposition and mineralization, processes mediated by hydrolytic enzymes. The significant positive correlation between enzyme activities and SOC supports this mechanism. In conclusion, deciduous plants are more responsive to environmental changes and more beneficial for soil C, N, and P sequestration during karst vegetation restoration.

3.3 Driving Factors of Enzyme Activity and C:N:P Ratio Changes

This study identified TP, NH_4^+-N, NO_3^--N, and AP as significant factors influencing soil enzyme activities and stoichiometric ratios, with TP having the greatest impact (importance value = 37.9%). Soil nitrogen and phosphorus have been confirmed as key factors affecting enzyme activities and C:N:P ratios. For instance, Xing et al. (2012) found that NAG activity was significantly correlated with NH_4^+-N in different vegetation communities on the Loess Plateau, while Yang et al. (2021) reported that NH_4^+-N and NO_3^--N affected NAG and LAP activities by influencing nitrogen-fixing microorganisms in maize fields in Qinghai. Although TP represents total soil phosphorus storage and does not fully reflect phosphorus supply levels, its proportional relationship with TN and SOC makes it an important factor for enzyme activities. Given the phosphorus limitation in the study area, AP reflects phosphorus availability, and phosphatase accelerates the transformation of organic phosphorus to inorganic forms for plant uptake. Furthermore, RDA indicated that seven physicochemical properties (SOC, TP, TN, AP, NH_4^+-N, NO_3^--N, pH) explained 65.1% of the variation in soil enzyme activities and stoichiometric ratios across land use and restoration patterns. These environmental factors represent the primary drivers of enzyme activity variation and warrant greater attention in karst land use and ecological restoration management.

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4 Conclusions

Based on our investigation of soil enzyme activities and C:N:P ratios under different land use and ecological restoration patterns, we conclude that: (1) Mean soil enzyme activities under restoration patterns exceeded those under land use types; (2) Vegetation in the study area was primarily limited by soil phosphorus, with changes in enzyme activities being critical for phosphorus transformation; (3) Pasture grassland and deciduous plants had stronger effects on soil enzyme activities and C:N:P ratios than other land use and restoration patterns.

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