Carbon and Nitrogen Stable Isotope Characteristics of Vegetation Succession Stages in the Karst Plateau Canyon Region of Beipanjiang Town, Southwest Guizhou (Postprint)

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Abstract

To investigate the variation characteristics and intrinsic relationships of carbon and nitrogen (C, N) and stable carbon and nitrogen isotopes ($ $13C, $ $15N) during forest succession, this study examined forest plant communities at four successional stages (grass-shrub, shrub, arbor-shrub, and arbor) in the karst plateau-canyon region, measuring C, N, and stable isotope values in leaf-litter-soil systems and analyzing their interactive effects among different layers. The results showed: (1) The $ $13C values of leaf, litter, and soil in the karst forest were -31.31‰ to -28.23‰, -29.96‰ to -20.07‰, and -26.83‰ to -21.14‰, respectively, while the corresponding $ $15N values were -3.41‰ to 1.54‰, -2.61‰ to 0.99‰, and 5.36‰ to 8.63‰, respectively, with soils generally exhibiting an enrichment effect; (2) With succession, leaf $ $13C values and soil $ $15N values first decreased and then increased, while soil and litter $ $13C values showed a decreasing trend, and leaf and litter $ $15N values showed no obvious patterns; (3) The leaf-soil $ $15N values were lowest at the arbor-shrub stage, indicating that the ecosystem had a lower degree of N saturation and relatively deficient N content at this stage; (4) Strong correlations were observed between C, N, and stable isotopes in leaf-soil systems, indicating that nutrient cycling between them was closely related with significant inhibitory or promoting effects. In summary, when restoring ecosystems in this region, tree species with high water use efficiency such as Lindera pulcherima, Triadica rotundifolia, and Cladrastis platycarpa should be selected to enhance the ecosystem' s self-regulation capacity for resource utilization and nutrient absorption.

Full Text

Carbon and Nitrogen Stable Isotopes of Vegetation Succession Stages in the Karst Plateau Gorge Area of Beipanjiang, Southwestern Guizhou

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Abstract

To explore the variation characteristics and intrinsic relationships of carbon (C) and nitrogen (N) contents and their stable isotopes (δ^{13}C and δ^{15}N) during forest succession, this study examined forest plant communities at four successional stages (herb-shrub, shrub, tree-shrub, and tree) in a karst plateau gorge area. We measured C, N, and stable isotope values in leaves, litter, and soil, and analyzed their interactive effects across different compartments. The results showed: (1) δ^{13}C values in the leaf-litter-soil continuum of karst forests ranged from -31.31‰ to -28.23‰, -29.96‰ to -20.07‰, and -26.83‰ to -21.14‰, respectively, with corresponding δ^{15}N values of -3.41‰ to 1.54‰, -2.61‰ to 0.99‰, and 5.36‰ to 8.63‰, demonstrating overall enrichment effects in soil; (2) With succession, leaf δ^{13}C and soil δ^{15}N values first decreased then increased, while soil and litter δ^{13}C values showed decreasing trends, and leaf and litter δ^{15}N values exhibited no clear patterns; (3) The lowest leaf-soil δ^{15}N values occurred at the tree-shrub stage, indicating lower N saturation and relative N deficiency in the ecosystem at this stage; and (4) Strong correlations between leaf-soil C, N, and their stable isotopes indicated tightly coupled nutrient cycling with significant inhibitory or promotional effects. These findings suggest that for ecosystem restoration in this region, species with high water-use efficiency such as *Lindera pulcherima*, *Triadica rotundifolia*, and *Cladrastis platycarpa* should be selected to enhance the ecosystem' s self-regulation capacity for resource utilization and nutrient absorption.

Keywords: δ^{13}C value, δ^{15}N value, leaf-litter-soil continuum, forest succession, carbon and nitrogen cycle, karst plateau

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Introduction

Carbon and nitrogen are essential life elements, and their biogeochemical cycling processes have long been central focuses of ecological research (Xiong et al., 2016). The δ^{13}C and δ^{15}N composition in plant-soil systems can accurately record the integrated effects influencing plant metabolism and ecosystem C and

N cycling (Yao et al., 2012; Liu et al., 2018), revealing plant response and adaptation mechanisms to specific environmental resource utilization strategies. Stable C and N isotope technology offers advantages including rapid detection, accurate results, and no temporal limitations (Zheng and Wang, 2009), making it an ideal method for studying C and N cycling during forest succession.

In recent years, δ^{13}C and δ^{15}N values have been widely used to indicate plant water-use efficiency and ecosystem C and N cycling mechanisms (Zheng et al., 2018; Diao et al., 2019). Studies by Si et al. (2017) and Twohey et al. (2018) found that leaf δ^{13}C values can characterize plant water-use efficiency and carbon fixation water costs, with their composition revealing integrated characteristics of carbon fixation. Yao et al. (2012) demonstrated that plant-soil δ^{15}N values can serve as indicators of ecosystem N saturation levels and long-term trends in N cycling. Clearly, stable isotope technology has opened new windows for studying chemical element cycling in ecosystems. As the primary carriers of C and N cycling in ecosystems (Zhang et al., 2018), the isotopic characteristics of the leaf-litter-soil continuum can precisely depict environmental information changes related to forest plant community succession and clarify variations in ecosystem structure and function. Therefore, studying the fundamental characteristics and intrinsic relationships of C, N, and their stable isotopes among these three components helps elucidate the patterns of C and N cycling characteristics and nutrient distribution in karst plateau gorge forest ecosystems along successional gradients.

Currently, research on karst forest succession has primarily focused on ecological stoichiometric characteristics, soil physicochemical properties, and soil microorganisms (Sheng et al., 2015; Pi et al., 2016; Wu et al., 2019). However, studies employing C and N stable isotope methods to investigate karst forest succession processes and patterns remain scarce. Understanding of δ^{13}C and δ^{15}N variation characteristics during karst ecosystem succession is still incomplete, particularly regarding the distribution patterns of δ^{13}C and δ^{15}N values in the leaf-litter-soil continuum during forest succession and how they indicate ecological effects, all of which require in-depth research.

Based on this context, this study examined plant communities at different successional stages in a karst plateau gorge area of Guizhou Province using stable C and N isotope methods. By measuring C, N, and stable isotopes in leaf-litter-soil across four successional stages and analyzing their interactive effects, we attempted to address two scientific questions: (1) Investigate the characteristics of C, N, and their stable isotopes in leaf-litter-soil across different karst forest successional stages to clarify how forest δ^{13}C and δ^{15}N values change with succession; and (2) Explore the intrinsic relationships between C, N, and their stable isotopes in the leaf-litter-soil continuum during forest succession to reveal variation patterns in plant resource utilization and adaptation strategies, providing theoretical support for diagnosing nutrient status and developing sustainable management measures for karst forest ecosystems.

1. Materials and Methods

1.1 Study Area

The study area is located in Beipanjiang Town, Zhenfeng County, Qianxinan Prefecture, Guizhou Province (105°38 11 E, 25°40 16 N), characterized by typical karst plateau gorge landforms with fragmented surfaces and large topographic relief, with elevations ranging from 370 to 1,473 m. The region has a subtropical monsoon climate with mean annual precipitation of 1,100 mm, temporally concentrated from May to October, resulting in severe seasonal drought. The annual accumulated temperature is 6,542.9 °C, with mean annual temperature of 18.4 °C, maximum and minimum temperatures of 32.4 °C and 6.6 °C, respectively, showing distinct wet-dry and cold-hot seasons with abundant light and heat resources. Soils are primarily limestone soils with shallow profiles and low fertility, and high bedrock exposure rates. Vegetation consists mainly of subtropical evergreen-deciduous coniferous-broadleaf mixed forests. Due to human disturbance, primary vegetation has been largely destroyed, with secondary vegetation and plantations dominating (Du et al., 2017). Prominent ecological problems include severe soil erosion and fragile ecosystems.

Dominant species at the herb-shrub stage include *Vitis heyneana*, *Phyllanthus urinaria*, and *Indigofera amblyantha*. The shrub stage is dominated by *Lindera pulcherima*, with *Itea yunnanensis* and *Pistacia weinmanniifolia* also present. The tree-shrub stage features *Celtis sinensis*, *Alangium kurzii*, and *Alchornea davidii*. The tree stage is dominated by *Triadica rotundifolia* and *Cladrastis platycarpa*, with *Choerospondias axillaris* and *Broussonetia papyrifera* also distributed.

1.2 Experimental Design and Sampling

Based on comprehensive field surveys conducted in July-August 2019 and referencing the vegetation successional stage classification method for karst areas by Lu et al. (2015), plant communities were divided into four successional stages: herb-shrub, shrub, tree-shrub, and tree (Table 1). Three 20 m × 20 m survey plots were established at each successional stage (at 810-850 m elevation with minimal differences in longitude, latitude, and slope, and consistent aspect). Due to the special topography of fragmented surfaces and shallow soils in karst areas, plot spacing was set at >10 m. Shrub and herb survey quadrats of 10 m × 10 m and 1 m × 1 m were established, respectively, with four shrub sub-quadrats and four herb sub-quadrats in each plot. Woody plants with height <2 m were recorded as shrubs. Tree species names, height, diameter at breast height, and crown width; shrub species names, height, ground diameter, and coverage; and herb species names, coverage, and average height were recorded. Environmental factors including longitude, latitude, elevation, slope, and aspect were also documented.

1.3 Sample Collection

Based on plot surveys, species with importance values >0.2 were considered dominant. Five dominant individuals were randomly selected in each plot, and healthy, mature leaves without pests or diseases were collected from five directions (east, south, west, north, and center), mixed to form one composite sample and placed in nylon bags. Undecomposed, semi-decomposed, and fully decomposed litter layers were collected along an S-shaped route in each plot (fully decomposed litter was identified as unidentifiable material not yet forming humus), combined into one mixed sample and placed in nylon bags. Simultaneously, 0-20 cm soil samples were collected at the litter collection sites, combined into mixed soil samples, and approximately 0.5 kg of fresh soil was retained using the quartering method. A total of 12 samples each of plant leaves, litter, and soil were collected across the four successional stages (4 stages × 3 replicate plots).

1.4 Laboratory Analysis

After transport to the laboratory, leaf and litter samples were oven-dried at (65$±$2) °C to constant weight, then ground and sieved. Soil samples were cleaned of roots, litter, stones, and animal residues, air-dried indoors, ground with a grinder, and sequentially passed through 2.00 mm and 0.15 mm sieves before being stored in sealed glass bottles for determination of C and N contents and stable isotope natural abundances.

Measurements of C, N, δ^{13}C, and δ^{15}N in leaves, litter, and soil were completed at the Third Institute of Oceanography, Ministry of Natural Resources using an elemental analyzer-stable isotope ratio mass spectrometer system (Vario ISOPOTE Cube-Isoprime, Elementar). Isotope ratios are expressed in per mil (‰) using δ notation. δ^{13}C values were referenced to the PDB international standard and calculated as:

$$\delta^{13}C(\%) = \left[\frac{R(^{13}C/^{12}C_{sample})}{R(^{13}C/^{12}C_{PDB})} - 1\right] \times 1000$$

where $R(^{13}C/^{12}C_{VPDB})$ is the C isotope ratio of the international standard PDB (Peedee Belemnite). The analytical precision for δ^{13}C values was $±$0.2‰. δ^{15}N values were referenced to atmospheric N_2 and calculated as:

$$\delta^{15}N_{air}(\%) = \left[\frac{R(^{15}N/^{14}N_{sample})}{R(^{15}N/^{14}N_{air})} - 1\right] \times 1000$$

where $R(^{15}N/^{14}N_{air})$ is the N isotope ratio of atmospheric N_2. The analytical precision for δ^{15}N values was $±$0.25‰.

1.5 Data Analysis

One-way ANOVA was used to test differences in C and N concentrations and δ^{13}C and δ^{15}N ratios among different forest successional stages in leaf-litter-soil compartments. Least significant difference (LSD) tests were performed for multiple comparisons, and Pearson correlation analysis was used to examine relationships among parameters. Data processing, analysis, and graphing were completed using Microsoft Excel 2010, SPSS 20.0, and Origin Pro 2018 software.

2. Results

2.1 Leaf C and N Contents and δ^{13}C and δ^{15}N Characteristics Across Successional Stages

Leaf C content was lowest at the shrub stage (402.55 $g \cdot kg^{-1}$), indicating lower organic matter content and implying relatively weak potential C storage. Leaf δ^{13}C values were lowest at the tree-shrub stage (-31.31‰), showing significant differences from herb-shrub, shrub, and tree stages ($P<0.05$), and exhibited a decreasing then increasing trend with succession, suggesting that plant water-use efficiency underwent partial adjustment during succession. Leaf N and δ^{15}N values ranged from 11.97 to 29.35 $g \cdot kg^{-1}$ and -3.41‰ to 1.52‰, respectively, both being lowest at the tree-shrub stage and showing no clear patterns with succession. Overall, leaf C, N, and δ^{13}C and δ^{15}N values were greater in early than in late successional stages (Figure 1 [Figure 1: see original paper]).

2.2 Litter C and N Contents and δ^{13}C and δ^{15}N Characteristics Across Successional Stages

Litter C content was highest at the tree-shrub stage (475.9 $g \cdot kg^{-1}$), significantly higher than at herb-shrub, shrub, and tree stages, indicating that tree-shrub communities facilitate nutrient return to the ecosystem. Litter N content was lowest at the tree-shrub stage (10.55 $g \cdot kg^{-1}$), with no significant differences between herb-shrub and tree stages or between shrub and tree stages ($P>0.05$). Litter δ^{13}C values ranged from -29.96‰ to -20.07‰, being highest at the herb-shrub stage and decreasing with succession, suggesting that litter decomposed more readily in early succession. Litter δ^{15}N values ranged from -2.61‰ to 0.99‰, showing alternating positive and negative values with succession, indicating high variability in this parameter (Figure 2 [Figure 2: see original paper]).

2.3 Soil C and N Contents and δ^{13}C and δ^{15}N Characteristics Across Successional Stages

Soil C and N contents ranged from 57.3 to 147.65 $g \cdot kg^{-1}$ and 5.45 to 16.15 $g \cdot kg^{-1}$, respectively, being highest at shrub and tree stages, followed by tree-shrub stage, and lowest at herb-shrub stage, indicating tight coupling between C and N cycling. Soil δ^{13}C values ranged from -26.83‰ to -21.14‰, decreasing with succession and significantly higher at the herb-shrub stage than at other stages, implying more thorough soil organic matter decomposition. Soil δ^{15}N values ranged from 5.36‰ to 8.625‰, decreasing then increasing with succession. Overall, soil showed greater ^{15}N enrichment, indicating enhanced ^{15}N fractionation during soil mineralization with forest succession (Figure 3 [Figure 3: see original paper]).

2.4 Correlations Among C and N Contents and δ^{13}C and δ^{15}N Values in Leaf-Litter-Soil

As shown in Table 2 , strong correlations existed between leaf-soil C, N, and their isotopes, indicating tight linkages in plant-soil nutrient cycling with significant synergistic or trade-off effects. Litter N, δ^{13}C, and δ^{15}N showed no significant correlations with other indicators, while litter C was significantly and extremely significantly negatively correlated with soil N and δ^{15}N (-0.777, -0.845), respectively, suggesting stronger interactions between litter and soil than between litter and leaves.

3. Discussion

3.1 Isotope Characteristics of the Leaf-Litter-Soil Continuum

Leaf δ^{13}C values across the four successional stages ranged from -31.31‰ to -28.23‰, being more positive than those in non-karst forests at Dinghushan (Table 3). This difference arises because the study area is a karst plateau gorge, while Dinghushan is underlain by Devonian thick-layer sandstone and sandy shale in a non-karst region with primarily latosolic red soils that have better water retention. Additionally, Dinghushan receives higher precipitation (1,955 mm) than the study area (1,100 mm), resulting in richer soil moisture. Furthermore, the unique aboveground-underground binary structure of karst landforms leads to lower water availability, which is negatively correlated with water-use efficiency and leaf δ^{13}C values (Xu et al., 2017). Therefore, we infer that lower water availability in this region resulted in more positive leaf δ^{13}C values. Leaf δ^{15}N values ranged from -3.41‰ to 1.54‰, being more positive than those in coastal sandy artificial forests (Table 3), indicating relatively higher N saturation and richer N content in this forest ecosystem. This occurs because the karst plateau gorge has fragile habitats with relatively scarce resource supply, and plants adapt to this special environment by enhancing resource competition ability and allocating more nutrients to organ construction.

Litter δ^{13}C values across the four successional stages ranged from -29.96‰ to -20.07‰, more positive than those of dominant species litter in secondary forests of northwestern Guizhou (Table 3). This may result from differences in litter types, microbial quantity and activity, as well as high heterogeneity in water availability and rock exposure rates among different sample areas, affecting litter decomposition rates and extent. Litter δ^{15}N values ranged from -2.61‰ to 0.99‰, higher than results from Qingzhen, Guizhou (Table 3), because the study area has implemented forest restoration and protection measures including afforestation and returning farmland to forest, leading to positive plant community succession, more complex ecosystem structure, diversified surface litter composition, increased microbial quantity, accelerated litter decomposition, and increased ammonia volatilization enriched in ^{15}N, resulting in higher surface litter ^{15}N values (Pan et al., 2011; Luo et al., 2014).

Soil δ^{13}C values across the four successional stages ranged from -26.83‰ to -21.14‰, more positive than those in alpine meadows (Table 3), because different study areas have large temperature differences. Alpine meadows have a plateau continental climate, while the study area has a subtropical monsoon climate with significantly higher temperatures that promote soil microbial activity and accelerate soil organic matter decomposition (Zhou et al., 2019), leading to higher soil δ^{13}C values. Soil δ^{15}N values ranged from 5.36‰ to 8.63‰, higher than those in Dinghushan forest soils (Table 3), which relates to microbial decomposition of litter compensating soil nutrients. Collins et al. (2008) found that under microbial C limitation, ^{15}N fractionation during soil mineralization is enhanced. Our results show extremely significant negative correlation between litter C and soil δ^{15}N values, consistent with this conclusion. In karst plateau gorge forests, although litter accumulation gradually increases, the total amount remains low, and the C quantity replenished to soil is still lower than in non-karst areas. Microorganisms are primarily C-limited, mineralization process ^{15}N fractionation is enhanced, and soil δ^{15}N values increase.

3.2 Relationships Between δ^{13}C, δ^{15}N and Elemental Correlations with Community Succession

Significant variation in C content among different compartments may result from changes in plant community structure, forest microclimate, and litter input, output, and decomposition status during succession, leading to different nutrient quantities returned to soil (Xiong et al., 2016). Additionally, close linkages exist between ecosystem C and N cycling, with C sequestration potential largely limited by soil N supply capacity (Li et al., 2018). Under the combined effects of community structure, environmental factors, and N supply capacity, forest C content variation is particularly pronounced. Furthermore, we found weak patterns in leaf-litter-soil δ^{13}C and δ^{15}N with succession, indicating isotopic fractionation during forest succession. This occurs because surface vegetation types, hydrothermal conditions, and ecosystem nutrient allocation patterns differ significantly with succession. To adapt to the environment and maintain survival, plants adopt corresponding resource utilization and adaptation strategies. Accompanying changes in resource use strategies and survival modes, plant photosynthesis and respiration also change synchronously, affecting forest C and N isotopic fractionation mechanisms and resulting in varied patterns of δ^{13}C and δ^{15}N values across components during succession. However, how plant metabolism drives C and N isotopic fractionation requires further research.

Our results also showed lowest leaf $\delta^{13}C$ values at the tree-shrub stage. In mixed tree-shrub forests, the upper canopy of tall trees is lush, and its leaves block some light from entering the forest, reducing light intensity and temperature inside the forest (Liu et al., 2014), decreasing photosynthetic capacity, increasing atmospheric CO_2 concentration within the forest, leading to higher Ci/Ca ratios and lower $\delta^{13}C$ values. At the species level, leaf $\delta^{13}C$ values can characterize long-term plant water-use efficiency (Yu et al., 2008), indicate carbon fixation water costs, and evaluate plant adaptability to adverse environments (Hussain et al., 2018). Generally, higher leaf $\delta^{13}C$ values indicate higher water-use efficiency and lower water costs for carbon fixation (Yu et al., 2008), meaning less water consumption for fixing the same amount of carbon. As shown in Figure 1, dominant species such as *Lindera pulcherima*, *Triadica rotundifolia*, and *Cladrastis platycarpa* in the study area had relatively high leaf $\delta^{13}C$ values, indicating that tree and shrub stage species have higher water-use efficiency, lower water costs for photosynthetic carbon fixation, and stronger adaptability to water-stressed habitats, making them suitable species for ecosystem restoration.

Previous studies indicate that leaf and soil $\delta^{15}N$ values can indicate ecosystem N saturation levels, with higher leaf-soil $\delta^{15}N$ values indicating greater proximity to N saturation (Pardo et al., 2006; Zheng et al., 2015). Our results showed higher leaf and soil $\delta^{15}N$ values at the shrub stage, indicating relatively higher N saturation at this stage. This may be because N cycling is more active and N output is higher at this stage, accompanied by N nitrification and NO_3^- leaching (Huang et al., 2013; Zheng et al., 2015), causing more depleted N to leave the ecosystem and resulting in elevated soil and leaf $\delta^{15}N$ values. Meanwhile, leaves showed ^{15}N depletion relative to soil because lighter ^{14}N is preferentially utilized by plant roots, while heavier ^{15}N accumulates in cells as unused carbonate (Craine et al., 2015) and is expelled from the body through root exudates into the soil medium, making soil $\delta^{15}N$ values more positive. In summary, species such as *Lindera pulcherima*, *Triadica rotundifolia*, and *Cladrastis platycarpa* are more suitable for forest ecosystem restoration in karst plateau gorge areas.

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