Carbon and Nitrogen Stable Isotope Abundance and Soil Stoichiometry of Zanthoxylum planispinum var. dintanensis Plantations of Different Ages

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Abstract: Understanding the relationships between carbon; nitrogen, their stable isotopes δ13C and δ15N, and soil stoichiometry may further our understanding of the regulatory mechanisms of the soil quality index on the equilibrium on isotopic fractionation. Four plantations of Zanthoxylum plantispinum var. dintanensis (5–7, 10–12, 20–22 and 30–32 years) in the karst plateau gorge area, Guizhou Province, China, were selected to determine the variation characteristics and interactions between leaves, leaf litter, soil carbon (C), soil nitrogen (N) and their isotopes with plantation age, and to explore the relationship between soil stoichiometry and the isotopes δ13C and δ15N. The results were as follows: (1) the δ13C in leaves, litter, and soil were −28.04‰ ± 0.59‰, −26.85‰ ± 0.67‰, and −19.39‰ ± 1.37‰, respectively. The contents of δ15N were 2.01‰ ± 0.99‰, 2.91‰ ± 1.32‰, and 3.29‰ ± 0.69‰, respectively. The contents of δ13C and δ15N were ranked in the order, soil > litter > leaf. (2) With increasing plantation age, the soil δ13C decreased; the leaf and the litter δ15N increased first then decreased, and the litter δ13C and the soil δ15N did not vary significantly. (3) The litter layer was positively correlated with soil δ13C and negatively correlated to δ15N. (4) Redundancy analysis showed that the soil microbial biomass carbon (MBC) and the bacteria/fungi (BAC/FUN) were the dominant factors affecting the natural abundance of C and N isotopes.

Keywords: age; Zanthoxylum plantispinum var. dintanensis; leaf-litter-soil continuum; carbon and nitrogen stable isotope; soil stoichiometry; rocky desertification area

1. Introduction

The composition of forest carbon (C), nitrogen (N), and their stable isotopes is a result of the continuous recycling of nutrients between plants and their environments, Consequently, δ13C and δ15N are comprehensive indicators of the C and the N cycles in terrestrial ecosystems. Their temporal and spatial differentiation, as well as their relationships in the environment, can reveal the source of plant nutrient elements [1,2]. The degree of fractionation is a discrepancy due to different photosynthetic pathways in plants [3], the δ13C values of plants with C3, C4, and CAM are −35−−20‰, −15−−7‰, and −22−−10‰ [4], respectively, which can be used to identify plant photosynthetic types. The effect of atmospheric carbon dioxide (CO2) in the same photosynthetic pathway produces variations in the δ13C values within plants [5]. The balance and the cycling laws of multiple elements can be studied through stoichiometry [6], as it analyzes the interaction of multiple elements in an ecosystem, and it clarifies the cycling process and isotope fractionation mechanism of C and N [7,8]. Investigation into the main causes of the differentiation of δ13C and δ15N in different forests may help to understand the configuration and the restriction status of nutrient elements in a plantation. This, in turn, may provide theoretical support to explain the mechanism of C and N cycles and isotope fractionation in a karst ecosystem.
In recent years, scholars have studied variations in δ\textsuperscript{13}C and δ\textsuperscript{15}N values with plantation age. For example, the study of Wang et al. (2019) showed that δ\textsuperscript{13}C and δ\textsuperscript{15}N in leaves of young *Cunninghamia lanceolata* were lower than that of a mature forest [9], which was consistent with the results of studies on *Ulmus pumila* and *Pinus sylvestris* var. *mongolica* plantations [10,11], yet conflicted with the study on *Caragana intermedia* [2]. Zheng et al. (2015) showed that the C. *lanceolata* content of δ\textsuperscript{13}C was relatively low in 3- and 8-year-old plantations, and the content of δ\textsuperscript{15}N was significantly higher than that of other stands [12]. These results suggest that there is a dynamic adjustment of δ\textsuperscript{13}C and δ\textsuperscript{15}N in different plantations. Factors such as species, ages, and environmental changes affect resource use strategies. Soil is an essential part of a terrestrial ecosystem, and its stoichiometry characteristics are affected by plantation age, forest structure, and habitat [13]. Soil stoichiometric coupling and microbial metabolism could affect nutrient uptake patterns and forest ecological effects, resulting in different C and N isotope fractionation [1,9,14]. Wang et al. (2019) discovered that the alleviation of soil N limitation and the exacerbation of phosphorus limitation could promote the fractionation of plant N isotope [9]. The soil C/N ratio can represent the decomposition rate of organic N and microorganisms; the higher soil C/N ratio, the lower the decomposition rate of organic N; and the opposite is true for microorganisms [15]. In addition, it was found that microbes can optimize their resource utilization strategy according to litter quality, nutrient utilization efficiency, and restriction status, thus affecting the δ\textsuperscript{13}C and the δ\textsuperscript{15}N composition [16]. In conclusion, soil stoichiometry and microorganisms are tightly connected to δ\textsuperscript{13}C and δ\textsuperscript{15}N. Yet, the variation of forest δ\textsuperscript{13}C and δ\textsuperscript{15}N with plantation age and the mechanism of soil stoichiometry driving C and N isotope fractionation are still uncertain and need to be further studied.

*Zanthoxylum planispinum* var. *dintanensis* is a variety of *Zanthoxylum planispinum*, unique to Guizhou province, with characteristics of calcium preference, drought tolerance, and strong adaptability. It plays an important role in rocky desertification control, ecological function improvement, and soil erosion control [17]. *Z. planispinum* var. *dintanensis* is a suitable species for karst restoration. However, due to the continuous cropping obstacle, *Z. planispinum* var. *dintanensis* may experience accelerated aging, a vigorous fruit setting period, and a shortening of the rotation cutting period. At present, the research on *Z. planispinum* var. *dintanensis* has focused on cultivation, management, aging, degradation, improving product yield, and quality [18,19]. Stable isotope technology has not been widely used as a tool to analyze the variation of δ\textsuperscript{13}C and δ\textsuperscript{15}N in plantations of different ages and their relationship with soil stoichiometry. Plantation age, availability of soil C and N, and soil microbial communities could be key factors affecting the natural abundance of δ\textsuperscript{13}C and δ\textsuperscript{15}N. We hypothesized that: (1) the contents of δ\textsuperscript{13}C and δ\textsuperscript{15}N are significantly different in young and old plant tissues, and (2) soil δ\textsuperscript{13}C and δ\textsuperscript{15}N are negatively correlated with soil C/N ratio. Our study investigated if leaves, leaf litter, soil C, soil N, and stable isotopes varied with plantation age and the internal relationship among leaf litter, soil C, soil N, and stable isotopes. We also studied the driving mechanism of soil stoichiometry to δ\textsuperscript{13}C and δ\textsuperscript{15}N fractions, to further understand the C and the N cycles in a karst ecosystem. We elucidated the current status of the nutrient configuration in a plantation, formulated fertilizer application measures, optimized stand structure, and diagnosed degradation mechanisms.

### 2. Materials and Methods

#### 2.1. Overview of the Study Area

The research area is located in Beipan River Basin, Huajiang Grand Canyon, Guanling, Guizhou, China (E 105°41′30.09″, N 25°39′49.64″). The major characteristics of the research area are as follows. (1) Landform: the area belongs to Karst Plateau Canyon landform, with a broken surface and undulating terrain, and an altitude range of 530–1473 m. (2) A dry and hot climate, primarily a subtropical monsoon climate. The average annual precipitation is approximately 1100 mm; precipitation in May to October accounts for 83% of the annual total. The annual average temperature is 18.4 °C, with the highest and lowest temperatures...
of 32.4 °C and 6.6 °C, respectively. It is warm and dry in winter and spring, and humid and hot in summer and autumn. (3) Rocky desertification; the soil is mainly limestone and marl, and the exposed area of bedrock is more than 70% [20]. The main vegetation is subtropical evergreen deciduous coniferous broad-leaved mixed forest. *Zanthoxylum planispinum* var. *dintanensis* is the dominant tree species. The plantations were cultivated from transplanted seedlings, with a survival rate of 70% with replanting as necessary; no other dwarf crops were intercropped under the plantation. The management mode was mainly natural renewal with human interference. Generally, *Z. planispinum* var. *dintanensis* plantations grew well, in spite of partial degradations and yield reductions in the over-matured plantations (Figure 1).

![Schematic diagram of the study sample area.](image)

**Figure 1.** Schematic diagram of the study sample area.

### 2.2. Experimental Design

#### 2.2.1. Sample Plot Setting

By using space distribution to replace time distribution, the *Z. planispinum* var. *dintanensis* plantations with similar site conditions, such as altitude (621 ± 5 m), slope, aspect, and soil type were selected and divided into four age groups: 5–7 years (initial fruit bearing period), 10–12 years (vigorous fruit bearing period), 20–22 years (end fruit bearing period), and 30–32 years (senescence and death period), which were recorded as yd1–yd4, in turn. Plantation age was recorded as a range rather than specific values because of the replanting regime. Sample plots, 3 10 m × 10 m in size, were set in each plantation age group, with a buffer zone >5 m between each sample plot (Table 1).
Table 1. The basic information for each sample plot.

| Plot No | Age  | Average Crown Width/m | Height/m | Vegetation Coverage/% | Density/ikaika (Plant/hm²) | Yield (Plant/Kg) |
|---------|------|------------------------|----------|------------------------|----------------------------|------------------|
| YD1     | 5-7  | 2.5 × 3                | 2.7      | 100                    | 1150                       | 6-7              |
| YD2     | 10-12| 2.5 × 3                | 2.7      | 100                    | 1150                       | 7-8              |
| YD3     | 20-22| 3.5 × 3                | 3.5      | 90                     | 1000                       | 4-5              |
| YD4     | 30-32| 4 × 5                  | 4        | 75                     | 650                        | 1-1.5            |

YD1-YD4: Initial fruit bearing period, vigorous fruit bearing period, ending fruit bearing period, senescence, and death period, respectively.

2.2.2. Sample Collection

In August 2020, five well developed representative plants were selected from each sample plot. The leaves were sampled from the middle part of the canopy, and 30 matured leaves without diseases and pests were picked from east, west, south, and north of the canopy, then mixed and stored in nylon bags [21,22]. In the leaf acquisition area, 31 m × 1 m quadrats were evenly arranged along the diagonal, from which samples were collected from the fully decomposed layer, semi decomposed layer, and undecomposed layer, and then evenly mixed. Five samples were collected from the soil layer at a depth of 0-20 cm along an “S” curve in each sample plot, and then mixed into one sample. Approximately 0.5 kg of a fresh weight soil sample was acquired according to the quartering method from each spot. A total of 12 soil samples were uniformly mixed and brought back to the laboratory.

2.2.3. Sample Determination

The leaf and the litter were transported to the laboratory and washed clean with deionized water. After that, it was dried at 60 °C for 24–28 h and then cooled naturally. Finally, it was crushed with a grinder and screened through 0.2 mm sieves for storage. The visible gravel, roots, and animal and plant debris were removed from the soil. Some of the fresh soil was stored at 4 °C for the purpose of determining the microbial quantity and biomass. The remaining soil was naturally dried, 95% of the samples were ground and passed through a 0.15 mm sieve. The preprocessed samples were sent to the third Marine Research Laboratory of the natural resources sector for determination (Xiamen, China). Soil samples were acidified with 1 mol L⁻¹ hydrochloric acid, and carbonate was removed. The reaction time of the acidification process shall not be more than 6 h. The sample was stirred with glass rods every 1 h to ensure complete removal of inorganic carbon in the soil, then stirred and wash with deionized water three to four times to remove excess hydrochloric acid, and dried for reserve. C, N, δ¹³C, and δ¹⁵N in leaves, leaf litter, and soil were determined by an element analyzer stable isotope mass spectrometer (Flash EA 1112 HT- Delta V Advantages, Thermo Fisher Corporation, Waltham, MA, USA), and the measuring error was <0.05‰. The gas He flow rate was 90 mL·min⁻¹, with a reaction tube temperature of 960 °C and a column temperature of 50 °C. The δ¹³C and the δ¹⁵N values are expressed in thousand percentage units (‰), the composition is calculated as follows [1,23]:

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

In the formula, \( R(^{13}C/^{12}C_{\text{VPDB}}) \) represents the C isotope ratio of the international standard VPDB (Vienna Pee Dee Belemnite), the analysis accuracy of the δ¹³C value was ± 0.2 ‰.\n
\[
\delta^{15}N(\%) = \left[ \frac{R(^{15}N/^{14}N_{\text{sample}})}{R(^{15}N/^{14}N_{\text{air}})} - 1 \right] \times 1000 \tag{2}
\]

In the formula, \( R(^{15}N/^{14}N_{\text{air}}) \) represents the N isotope ratio of N₂ in the atmosphere, the analysis accuracy of the δ¹⁵N value was ± 0.25 ‰.
Soil organic carbon (SOC) and total nitrogen (TN) were determined by the potassium dichromate oxidation external heating method and the Kjeldahl method, respectively [24]. Soil microbial biomass carbon and nitrogen (MBC and MBN) were measured by chloroform fumigation. Treatment was done by chloroform fumigation followed by extraction with a 0.5 mol L\(^{-1}\) K\(_2\)SO\(_4\) solution; MBC was determined by a chloroform fumigation-K\(_2\)SO\(_4\) extraction-TOC analyzer, and MBN was determined by chloroform fumigation-K\(_2\)SO\(_4\) extraction-potassium persulfate oxidation method, where MBC and MBN calculate using the uniform transformation coefficients 0.45 and 0.54 [25].

Soil bacteria, fungi, and actinomycetes in soil were determined by the beef peptone culture method, the potato glucose agar culture method, and Gao’s No.1 [26]. The bacteria and actinomycetes were counted in a 30 °C incubator after 24 h and a 25 °C incubator for 72 h by diluted plate counting; the fungi were cultured in a 28 °C incubator for 96 h after counting, using the inverted plate method.

### 2.2.4. Statistical Analysis

The data were sorted and analyzed by Microsoft Excel 2010 and SPSS 20.0. The parameter sets were examined for normality; except for N content in leaves, the others parameters were all normally distributed. For N content in leaves, we performed a Kruskal–Wallis test. One-way ANOVA and least significant difference (LSD) were used to test the others parameters. The significant and highly significant levels were \( p = 0.05 \) and 0.01, respectively. Data in figures and tables are expressed in the form of mean ± standard deviation. Pearson correlation analysis was used to test the correlation between the indicators. Origin 8.6 software was used to depict figures. The relationships between \( Z. \) planispinum var. dintanensis plantation \( \delta^{13} \)C and \( \delta^{15} \)N and soil stoichiometry were analyzed by Canoco 5 software (Redundancy analysis, RDA).

### 3. Results

#### 3.1. The Characteristics of Zanthoxylum planispinum var. dintanensis C, N, and Their Stable Isotopes from Different Aged Plantations

There were no significant differences in C and \( \delta^{13} \)C content in leaves from the four plantation age groups, which indicate that water use efficiency did not vary significantly with plantation age. Leaf N ranged from 24.95 g kg\(^{-1}\) to 34.75 g kg\(^{-1}\), showing a decreasing trend with an increase in plantation age. \( \delta^{15} \)N in leaves ranged from 0.86‰ to 3.20‰, showing a first increasing and then decreasing trend with plantation age, which suggests that the N use efficiency of leaves in different stands was different. Leaf litter C and N were 413.35–349.65 and 31.2–18.05 g kg\(^{-1}\), respectively, which were significantly higher in 5–7- and 10–12-year-old plantations than in 20–22- and 30–32-year-old plantations, suggesting that the litter of young leaves decomposes more readily. Leaf litter \( \delta^{13} \)C ranged from −25.96‰ to −27.67‰, with no significant difference among the four plantation age groups. The 10–12-year-old plantation had the highest \( \delta^{15} \)N (4.15‰ ± 0.92‰), which increased first and then decreased with increasing plantation age. Soil C ranged from 9.1 g kg\(^{-1}\) to 16 g kg\(^{-1}\), and decreased with increasing plantation age. There were no significant differences in soil N, \( \delta^{13} \)C, and \( \delta^{15} \)N from the four plantation age groups, which suggests that soil nutrient patterns did not change with plantation age (Figure 2).

#### 3.2. Relationship between Zanthoxylum planispinum var. dintanensis Plantation C, N and Their Stable Isotopes

Table 2 shows that there was a highly significant \( (p < 0.01) \) and positive correlation \( (p < 0.05) \) between leaf N and soil \( \delta^{13} \)C and C, indicating that soil can affect leaf nutrient status. The litter layer was positively related to soil \( \delta^{13} \)C and negatively related to soil \( \delta^{15} \)N. There was a significant positive correlation between leaf litter N with soil \( \delta^{13} \)C, which suggested there may be promoting or inhibiting effects between litter and soil. Leaf \( \delta^{13} \)C was significantly positively correlated to leaf litter \( \delta^{15} \)N and negatively correlated to soil \( \delta^{15} \)N, indicating that leaves, leaf litter, and soil were coupled with each other.
Figure 2. The C, N and their stable isotope characteristics of *Zanthoxylum planispinum var. dintanensis* with different ages, in carbon content (a), nitrogen content (b), \( \delta^{13} \text{C} \) value (c) and \( \delta^{15} \text{N} \) value (d). Data represent mean ± SD (n = 3). The different letters indicate significant differences among the age (p < 0.05). YD1, YD2, YD3, and YD4 represent four different forest ages (5–7, 10–12, 20–22, and 30–32), respectively.

Table 2. The C, N and their stable isotope correlation in *Zanthoxylum planispinum var. dintanensis* plantation.

| Index          | C_leaf | \( \delta^{13} \text{C} \) leaf | N_leaf | \( \delta^{15} \text{N} \) leaf | C_litter | \( \delta^{13} \text{C} \) litter | N_litter | \( \delta^{15} \text{N} \) litter | C_soil | \( \delta^{13} \text{C} \) soil | N_soil |
|----------------|--------|---------------------------------|--------|-------------------------------|----------|---------------------------------|----------|-------------------------------|--------|---------------------------------|--------|
| \( \delta^{13} \text{C} \) leaf | 0.225  | −0.108                          | −0.584 | −0.043                        | −0.100   | −0.392                          | −0.205   | −0.132                        | −0.193 | −0.280                          | −0.180 |
| N_leaf         | 1      | −0.751 *                         | 0.044  | 0.260                         | 0.244    | 0.706                           | 0.210    | 0.054                         | 0.792 * | 0.580 *                         | 0.054  |
| \( \delta^{15} \text{N} \) leaf | 1      | −0.474                          | −0.307 | −0.307                        | −0.429   | −0.021                          | −0.064   | 0.400                         | 0.666  | 0.575                           | 0.041  |
| C_litter      | 1      | 1                               | 1      | 1                             | 1        | 1                               | 1        | 1                             | 1      | 1                               | 1      |
| \( \delta^{13} \text{C} \) litter | 1      | 1                               | 1      | 1                             | 1        | 1                               | 1        | 1                             | 1      | 1                               | 1      |
| N_litter      | 1      | 1                               | 1      | 1                             | 1        | 1                               | 1        | 1                             | 1      | 1                               | 1      |
| \( \delta^{15} \text{N} \) litter | 1      | 1                               | 1      | 1                             | 1        | 1                               | 1        | 1                             | 1      | 1                               | 1      |
| C_soil        | 1      | 1                               | 1      | 1                             | 1        | 1                               | 1        | 1                             | 1      | 1                               | 1      |
| \( \delta^{13} \text{C} \) soil | 1      | 1                               | 1      | 1                             | 1        | 1                               | 1        | 1                             | 1      | 1                               | 1      |
| N_soil        | 1      | 1                               | 1      | 1                             | 1        | 1                               | 1        | 1                             | 1      | 1                               | 1      |
| \( \delta^{15} \text{N} \) soil | 1      | 1                               | 1      | 1                             | 1        | 1                               | 1        | 1                             | 1      | 1                               | 1      |

Leaf C: Leaf carbon content; Leaf \( \delta^{13} \text{C} \): Leaf \( \delta^{13} \text{C} \) value; Leaf N: Leaf nitrogen content; Leaf \( \delta^{15} \text{N} \): Leaf \( \delta^{15} \text{N} \) value; Litter C: Litter carbon content; Litter \( \delta^{13} \text{C} \): Litter \( \delta^{13} \text{C} \) value; Litter N: Litter nitrogen content; Litter \( \delta^{15} \text{N} \): Litter \( \delta^{15} \text{N} \) value; Soil C: Soil carbon content; Soil \( \delta^{13} \text{C} \): Soil \( \delta^{13} \text{C} \) value; Soil N: Soil nitrogen content; Soil \( \delta^{15} \text{N} \): Soil \( \delta^{15} \text{N} \) value; * indicates a significant correlation (p < 0.05); ** indicates an extremely significant correlation (p < 0.01).
3.3. The Impact of Soil Stoichiometry on Plantation C, N, and Their Stable Isotopes

RDA analysis was conducted on soil stoichiometry and plantation components in the different plantation age groups. MBC/MBN was ignored due to its negligible influence. The soil stoichiometry interpreted 90.75% and 4.82% variations on the first and the second axis (Table 3), reflecting the strong connection between soil stoichiometry and some of the plantation components.

Table 3. Redundancy analysis of the component content in the plantation.

| Sorting Axis | Axis 1 | Axis 2 | Axis 3 | Axis 4 |
|--------------|--------|--------|--------|--------|
| Explains     | 90.75  | 4.82   | 1.79   | 0.54   |
| Pseudo-canonical correlation | 0.9937 | 0.9975 | 0.8307 | 0.9781 |
| Explained variation (cumulative) | 90.75  | 95.57  | 97.35  | 97.89  |
| Explained fitted variation (cumulative) | 92.65  | 97.57  | 99.36  | 99.34  |

The black arrows in Figure 3 indicate parts of the C and N components and their isotopes in the plantation, with the red arrows indicating the soil stoichiometry. According to the two-dimensional diagram of redundancy analysis on plantation components and soil stoichiometry, MBC, BAC/FUN, TN, leaf litter N, soil $\delta^{13}$C, leaf N, leaf litter $\delta^{13}$C, and SOC/TN, MBC, leaf $\delta^{13}$C, and leaf C were positively correlated. BAC/FUN, TN, and leaf C, leaf C and MBN and litter N, soil $\delta^{13}$C, and leaf N were negatively correlated. The angles between MBC and leaf litter $\delta^{15}$N, and BAC/FUN and soil $\delta^{13}$C were small, showing a strong positive correlation; SOC was negatively correlated with leaf $\delta^{15}$N, but not significantly correlated with other soil stoichiometry. As shown in Table 4, the physical and the chemical variables could be ranked in the order: MBC > BAC/FUN > SOC/TN > MBN > SOC > TN, although no significant influence was found.

Figure 3. Redundancy analysis of artificial forest C, N, $^{13}$C, $^{15}$N and soil stoichiometry in the plantation.
**Table 4.** Importance sequencing and Duncan’s test of soil stoichiometry.

| Index   | Order of Importance | Explains/% | $F$  | $p$  |
|---------|---------------------|------------|------|------|
| MBC     | 1                   | 44.1       | 4.7  | 0.072|
| BAC/FUN | 2                   | 17.6       | 3.0  | 0.144|
| SOC/TN  | 3                   | 15.1       | 1.8  | 0.234|
| MBN     | 4                   | 8.6        | 4.2  | 0.218|
| SOC     | 5                   | 6.7        | 1.2  | 0.368|
| TN      | 6                   | 5.8        | 1.1  | 0.416|

SOC: Soil organic carbon; TN: Soil total nitrogen; MBC: Soil microbial biomass carbon; MBN: Soil microbial biomass nitrogen; SOC/TN: Soil C/N ratio; BAC/FUN: Soil bacteria to fungi ratio, the same below.

**4. Discussion**

**4.1. The Abundance Characteristics of $\delta^{13}C$ and $\delta^{15}N$ in Zanthoxylum planispinum var. dintanensis Plantations of Different Plantation Age**

The greater the $\delta^{13}C$ value, the higher the water usage efficiency over a long period [27]. The results of our work indicated there was no significant difference in $\delta^{13}C$ among the four plantation age groups, indicating that the water usage efficiency did not vary with plantation age; this was a result of the balance between resource acquisition and spending balance in plantation trees [28]. This may be due to a high competition ability for consumable resources in the vulnerable karst habitat, which minimized age effects. Our results rejected the hypothesis that there was a significant difference in $\delta^{13}C$ between young and old plant tissues. Kieckbusch et al. (2004) [29] showed that there was no significant difference in the $\delta^{13}C$ composition between green leaves and aged leaves, while Lee et al. [30] (2000) showed a significant difference in the $\delta^{13}C$ composition between green leaves and yellow leaves in the two plants. Thus, the observed variation in $\delta^{13}C$ as leaves aged, and its physiological and ecological significance, still need further study. Under the influence of “canopy effect”, the closer to the soil surface, the smaller the $\delta^{13}C$ value in plant leaves, and the more obvious the dilution phenomenon [31]. Because the canopy of Z. planispinum var. dintanensis was relatively small and frequently trimmed, the canopy effect was weak. This meant that it was reasonable to sample middle canopy leaves as representative of the whole canopy. In future research, it will be advantageous to sample leaves from the upper, middle, and lower canopies, to further reveal the mechanisms of $\delta^{13}C$ fractionation.

The leaf $\delta^{15}N$ in the 10–12-year-old group was significantly higher than that of the other three age groups, probably due to high N demand during the vigorous fruit bearing period, which stimulated the root system to transfer more N to leaves for photosynthesis. Leaf $\delta^{15}N$ increased first and then decreased with plantation age, which was inconsistent with the research of Wang et al. (2019) [9]. This inconsistency may be due to different N isotope fractionation speeds in the photosynthetic processes of different species.

The $\delta^{13}C$ in soil and litter did not vary significantly with plantation age. This may be because the organic C in soil was derived primarily from litter. C input and output C from litter, combined with the decomposition of soil C, may have determined the characteristics of soil $\delta^{13}C$ [32]. Balesdent et al. (1993) found that soil $\delta^{13}C$ was positively correlated to leaf litter $\delta^{13}C$, although the results were not significant [33], which was consistent with our results, indicating that soil could not fully inherit leaf $\delta^{13}C$, even if C isotopic fractionation was not considered in the litter decomposition process. The decomposition rate of litter determines the flow direction of C. Because fresh organic C is more easily decomposed by microorganisms, the soil $\delta^{13}C$ value was the result of mixed new and old C in soil, demonstrating an isotope mixing effect [34–36]. The results of Buchmann et al. (1997) and Farquhar et al. (1989) showed that soil $\delta^{13}C$ generally fell within the range of 1.0–3.0‰; a value higher than 3.0‰ indicates that the organic matter input into the soil may derive from a mixture of C₃ and C₄ plants [3,37]. The average variation of $\delta^{13}C$ in our study was 7.46‰, which is significantly higher than 3.0‰, the reason is that the Z. planispinum var. dintanensis plantation was initially a mix of coniferous and broad-leaf trees, the soil organic matter would have been influenced by both. With the gradual increase in the atmospheric CO₂
concentration, its $\delta^{13}C$ value decreases continuously, and the amount of CO$_2$ released into the atmosphere by different ecosystems through respiration varies [5]. It is speculated that the $\delta^{13}C$ value of atmospheric CO$_2$ changes after agricultural transformation in this area. The variation law of the soil $\delta^{15}N$ presented in this study was inconsistent with that of the Chinese fir forest in Fujian district [9]. The reason was that there were fewer forest plant classes, little difference in litter regression and accumulation, and more intensive human interference in the Z. planispinum var. dintanensis plantation. Considerable research has shown that soil $\delta^{13}C$ and $\delta^{15}N$ increase with the soil profile [3,12,38,39]. However, this research was conducted in an area with shallow soil cover, and most soil is <20 cm thick and has a high gravel content, so the soil samples were not collected in this study, which limited the understanding of C and N cycles in the soil profile of the karst region. In the future, the soil layer should be divided into finer sublayers, and samples should be collected from different depths; for instance, from 0–2 cm, 2–5 cm, and 5–10 cm soil layers [5]; this will elucidate more clearly the soil C and N cycles and the varying mechanisms of $\delta^{13}C$ and $\delta^{15}N$ fractionations in space.

The $\delta^{13}C$ in Z. planispinum var. dintanensis leaves was significantly negatively correlated to N content, which is consistent with the results of Tsialtas et al. (2001) [40], yet conflicts with the findings of Zhang et al. (2015) [41]. This finding indicates that N acquisition is different in different environments, which affects the plant leaf $\delta^{13}C$ value. The reason is that leaf N can regulate stomatal density, and higher leaf N content promotes the absorption of CO$_2$, increases plant photosynthesis rate, and decreases the ratio of intracellular and extracellular CO$_2$ concentration ($C_i/C_a$), which leads to an increase in $\delta^{13}C$ [42,43]. Our research area was a barren karst region, where supplementary fertilization is needed for adequate plant growth. Modern agriculture emphasizes supplementation with N and p, which leads to greater N leaf uptake, increases stomatal density, and $C_i/C_a$ ratio, thus decreasing $\delta^{13}C$. The results of our study showed that leaf $\delta^{13}C$ was positively correlated to litter $\delta^{15}N$ and negatively correlated to soil $\delta^{15}N$, indicating the coupling relationship between leaves, leaf litter, and soil. The reason was that the C and N cycles in forest ecosystem went through the entire plant-litter-soil continuum. Moreover, the C cycle and N cycles were tightly coupled, and the potential for C fixation was heavily limited by the soil N supply [44,45]. The C and the N cycles in the forest ecosystem were regulated by environmental factors, leading to unique connections between continuums; in addition, nutrient reabsorption and allocation may cause isotope fractionation. However, due to the large number of influencing factors and limited measurement indicators, the reason for the weak inheritance could not be clarified. Further research is required.

4.2. The Driving Mechanism of Soil Stoichiometry to Plantation C and N Isotopes Fractionation

Soil stoichiometry links the chemical cycles in different parts of an ecosystem, reflecting the flowing of elements [46], indirectly regulating forest C and N isotopes fractionation via changing the coupling relationship between soil and microorganism stoichiometry. It is an important index for the evaluation of ecosystem element cycles and internal stability [1,47]. The contents of soil elements can affect these results and restrict the application of stable isotope technology in soil C and N cycles [15,48]. Stevenson et al. (2010) indicated that soil C/N was significantly negatively correlated to $\delta^{15}N$ [49]. The reason was that the biological activity of microbes in soil with different C/N were different, which led to different fractionation speeds and degree in the process of mineralization. Generally, the growth of microorganisms is limited by N content in high C/N soil, thus weakening the $\delta^{15}N$ fractionation in the mineralization process; on the other hand, under low C/N conditions, the growth of microorganisms is limited by C content, thus strengthening the N decomposition in the process of mineralization [7]. The current study showed that there was a weak correlation between soil C/N and $\delta^{15}N$, which was not completely consistent with previous studies [49]. A possible reason could be leaf litter type and quantity are lower in artificial forest, and human interference combined with a high concentration of allelochemicals secreted by Z. planispinum var. dintanensis inhibited the decomposition
of leaf litter and associated microbial activity, leading to a reduced recycling of nutrients. Our research also demonstrated the negative correlation between soil C/N and δ^{13}C. This was attributed to the low C/N soil SOC decomposing faster, more ^{12}CO_{2} being released from the soil, and the remaining soil C library enriched by ^{13}C [50]. Wang et al. (2015) reached a similar conclusion; nonetheless [8], Peri et al. (2012) found that soil C/N did not affect soil δ^{13}C in their study of the primeval forests in southern Patagonia [32]. A possible reason could be that the climate was different in each research area, leading to different leaf litter types and quantities, and plants adopt different resource utilization and adaptation strategies under different climatic conditions. Soil C and N are indispensable elements for plant survival and it is scientifically feasible to use C/N to determine the composition characteristics of soil δ^{13}C and δ^{15}N, although it is not the only criterion. In the future, research on coupling with other soil factors should be conducted to comprehensively evaluate soil quality and nutrient status.

As the most active part of soil organic matter [51], biomass can establish good connections with δ^{13}C and δ^{15}N through the decomposition of organic matter and microbial activity [52]. Our results showed that soil MBC was positively correlated to soil δ^{13}C, which is related to the isotope fractionation in the process of microorganism decomposition [53]. During the process, ^{12}C enters the released CO_{2} preferentially, and the heavier ^{13}C more likely enters the soil microbial biomass before returning to soil organic matter [54]. Relevant research has shown that soil δ^{13}C was positively correlated with organic C [55]. When the decomposition of organic C speeds up, more ^{12}CO_{2} will be released from the soil system, thus resulting in the enrichment of δ^{13}C in soil [56]. Inconsistent with these results, organic C did not show significant correlation with δ^{13}C in our study (Figure 3) indicating that the soil organic C in our research area had no significant influence on C isotope fractionation. This may be because pruning is carried out in winter and in summer to improve the economic value of the Z. planispinum var. dintanensis plantations, and this reduces the litter return and nutrients; on the other hand, the unique dual structure of karst leads to the aggravation of water loss and soil erosion. In conclusion, litter and microorganisms were important sources of soil nutrients, which should be protected to improve soil quality.

5. Conclusions

Different types of forest have a different natural abundance of δ^{13}C and δ^{15}N. In Z. planispinum var. dintanensis plantations, the soil δ^{13}C value gradually decreased, the δ^{15}N value of leaves and litter increased first and then decreased, and the remaining indicators did not change; the overall soil showed isotopic enrichment effects. There is a trade-off between plantation indicators between leaf δ^{13}C and δ^{15}N only, the parameters of other indicators are synergistic. There is a correlation between soil stoichiometry and Z. planispinum var. dintanensis plantations; soil MBC and BAC/FUN have a relatively strong driving effect on plantation C and N and their isotopes. Henceforth, attention should be paid to measures that can protect litter and soil microorganisms in order to retain the quality and the nutrient composition of plantation soil, and thus delay its decline.

Author Contributions: Conceptualization, Y.Y. and Y.W.; methodology, Y.Y.; software, Y.W.; validation, Y.Y. and Y.W.; formal analysis, Y.W.; investigation, Y.S. and Y.L.; resources, Y.Y.; data curation, Y.W.; writing—original draft preparation, Y.Y. and Y.W.; writing—review and editing, Y.W., Y.Y. and Y.S.; visualization, Y.L.; supervision, Y.L.; project administration. All authors have read and agreed to the published version of the manuscript.

Funding: Guizhou Province Science and Technology Support Plan Project (Qian-ke-he Zhicheng [2022] Yiban 103).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: No relevant datasets were used. Therefore, no data availability statement exists.

Conflicts of Interest: The authors declare no conflict of interest.
