Carbon and Nitrogen Stable Isotope Abundances and Soil Stoichiometry of *Zanthoxylum Planispinum* Var. *Dintanensis* Plantations at Different Ages

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Research Article

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

Posted Date: August 26th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-838535/v1

Abstract

Background and aims

Understanding the relationship between carbon, nitrogen and their stable isotope ^{13}C, ^{15}N and soil stoichiometry may assist to reveal the distribution pattern and stability mechanism of nutrient elements in karst ecosystem.

Methods

Four plantations of *Zanthoxylum planispinum* var. *dintanensis* (5–7, 10–12, 20–22 and 30–32 years) in the karst plateau gorge area of Guizhou Province, China, were selected as the research objects to clarify the variation characteristics and interaction effects of leaf, litter, soil C, N and their isotopes with plantation age, and to explore the relationship between soil stoichiometry and the ^{13}C, ^{15}N of *Zanthoxylum planispinum* var. *dintanensis* plantation.

Results

(1) the ^{13}C in leaf, litter and soil were − 28.04‰±0.59‰, -26.85‰±0.67‰ and − 19.39‰±1.37‰, respectively, correspondingly, the contents of ^{15}N were 2.01‰±0.99‰, 2.91‰±1.32‰ and 3.29‰±0.69‰, respectively. The contents of the ^{13}C and ^{15}N can be rank ordered as soil > litter > leaf; (2) with the increase of plantation age, the soil ^{13}C decreased; the leaf and litter ^{15}N increased first then decreased; the litter ^{13}C and soil ^{15}N did not vary significantly; (3) the litter layer positively correlated to soil ^{13}C, and negatively correlated to ^{15}N; (4) redundancy analysis showed that soil microbial biomass carbon (MBC) and bacteria/fungi (BAC/FUN) were the dominant factors affecting C and N isotope natural abundances.

Conclusions

This study indicated that the species and acidity of soil microbial can affect the C and N isotope natural abundance.

Introduction

The composition of forest C, N and stable isotope is a result of the cooperation of plant and environment. ^{13}C, ^{15}N are comprehensive indicators of C and N cycle in terrestrial ecosystem, the temporal and spatial differentiation, as well as their relationship with environment can reveal the source of plant nutrient elements (Zhou et al. 2014; Liu et al. 2016). The balance and cycling law of multiple elements can be studied through stoichiometry (Fan et al. 2015), as it analyzes the interaction of multiple elements in ecosystem and clarifies the cycling process and isotope fractionation mechanism of C and N (Collins et al. 2008; Wang et al. 2015). Discussion of the major cause of the differentiation of ^{13}C and ^{15}N in different forest components can assist to reveal the plantation nutrients distribution pattern and limitations, so as to provide theoretical support for explaining the mechanism of C and N cycle and isotope fractionation in karst ecosystem.

In recent years, scholars have carried out a series of researches on variations of ^{13}C and ^{15}N with plantation age. For example, the study of Wang et al. (2019) showed that the ^{13}C and ^{15}N in leaves of young *Cunninghamia lanceolata* was lower than that of mature forest, which was consistent with the results of studies on *Ulmus pumila* and *P. sylvestris* var. *mongolica* plantations (Tanaka-Oda et al. 2010; Song et al. 2015), yet opposite to the study on *Caragana intermedia* (Liu et al. 2016); Zheng et al.(2015) showed that the variation law of ^{13}C and ^{15}N in *Cunninghamia lanceolata* leaves were inconsistent, the content of ^{13}C were relatively low in 3- and 8-year old plantations, and the content of ^{15}N was significantly higher than that of other stands. These facts suggested the dynamic adjustment of ^{13}C and ^{15}N in different plantations. Factors such as species, growth and environment change affected the resource utilization strategy. Soil was an important part of terrestrial ecosystem, its stoichiometry characteristics were affected by plantation age, forest structure and habitat (Lucas-Borja et al. 2016). Soil stoichiometric coupling and microbial metabolism could affect vegetation nutrient uptake patterns and forest ecological effects, resulting in different C and N isotope fractionation. Wang et al. (2019) discovered that the alleviation of soil N restriction and exacerbation of P restriction could promote the fractionation of plant N isotope; Zhao et al. (2019) found that high C/N ratio could increase the rate of microbial decomposition, accelerate the loss of ^{12}C and thus enrich the ^{13}C in soil. Lorenz et al. (2020) proposed that microbes could optimize their resource utilization strategy according to litter quality, nutrients utilization efficiency and restriction status. In conclusion, soil stoichiometry, microorganism and soil fertility are closely related. Yet the variation of forest ^{13}C and ^{15}N with plantation age and the mechanism of soil stoichiometry driving C and N isotopes fractionation are still uncertain and need to be further studied.

Zanthoxylum planispinum var. *dintanensis* is a variety of *Zanthoxylum planispinum*, unique in 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 (Yang et al. 2016), thus being considered as a suitable species for karst desertification restoration. However, the large-scale planting caused soil fertility decreasing and -fast aging with shortened vigorous fruit bearing period and rotation cutting period. Therefore, clarifying the causes of forest decline and soil quality decline is conducive to the formulation of sustainable management measures. At present, the research on *Zanthoxylum planispinum* var. *dintanensis* mainly includes cultivation and management, aging and degradation, improving product yield and quality, etc. (Li et al. 2008; Qu et al. 2020), few of which use stable isotope technology as a tool to analyze the variation of ^{13}C and ^{15}N with plantation age and its relationship with soil stoichiometry. In view of this, this paper selected 4 plantations of Zanthoxylum planispinum var. dintanensis at the age of 5–7, 10–12, 20–22 and 30–32 years as the objects to clarify the variation of C, N and their stable isotopes in leaves, litter and soil with plantation age, to explore the internal correlation between the indicators, and to clarify the mechanism of soil stoichiometry driving ^{13}C and ^{15}N fractionation. This current study is helpful to reveal the nutrient status of plantations and lay a scientific basis for formulating fertilization measures, optimizing stand structure and diagnosing degradation mechanism.

Material And Method

Overview of the study area

The research area was located in the Beipan river basin at the junction of Zhenfeng and Guanling in Guizhou, China (E105°41'30.09"N35°39'49.64"). The major characteristics of the research area were as follows: (1) Landform: the area belongs to Karst Plateau Canyon landform, with broken surface and undulating terrain, falling to an altitude range of 530–1473 m; (2) Dry and hot climate: it belongs to subtropical monsoon climate, the average annual precipitation is about 1100 mm, unevenly distributed. 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, 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% (Zou et al. 2019). The main vegetation is subtropical evergreen deciduous coniferous broad-leaved mixed forest; as an economic species, *Zanthoxylum planispinum* var. *dintanensis* plantation is mainly transplanted from seedlings and cultivated according to its characteristics, such as plantation age, canopy and phenology. A relatively stable artificial community has been formed.

Experiment design

Sample plot setting

In August 2020, by using space distribution to replace time distribution, the Zanthoxylum planispinum var. dintanensis plantations with similar site conditions such as altitude, 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 (ending fruit bearing period) and 30–32 years (senescence and death period), which were recorded as yd1-yd4 in turn. Because of the replanting in the cultivation process, it is more feasible to record plantation age as a range rather than specific values. 3 10 m × 10 m sample plots were set in each plantation age group, with a buffer zone > 5 m between each sample plot (Table 1).

Table 1
The basic situation of sample plot

Plot No	Age	Average crown width/m	Height/m	Vgetation coverage/%	Density / (plant/hm^2)	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.						

Sample collection and processing

Select 5 well developed representative plants in each sample plot, collect 30 leaves from east, south, west, and north of the canopy, mix them and seal the samples in nylon bags. In each sample plot, 3 1m × 1m small quadrats were evenly arranged along the diagonal, from which samples were collected from the fully decomposed layer, semi decomposed layer and not decomposed layer and mixed evenly. At the sample collection sites, 0–20 cm soil samples were collected along a “S” shape route 30–50 cm away from the base of the tree. About 0.5 kg fresh weight soil sample was acquired according to “quartering method” from each spot. A total of 12 soil samples were uniformly mixed and brought back to our lab. The leaves and litters were dried to constant weight at 60°C for 30 minutes after being killed out at 105°C, then the samples were crushed, mixed and reserved; the visible gravel, root system, animal and plant debris were removed from the soil. A part of the fresh soil was stored at 4°C for the purpose of determining the microbial quantity and biomass. After the remaining soil was naturally dried, 95% of the samples were ground and passed through a 0.15 mm sieve.

Sample determination

C, N, ^{13}C and ^{15}N in leaves, litter and soil were determined by element analyzer stable isotope mass spectrometer (Vario ISOPOTE Cube-Isoprime, Elementar company) at the third Marine Research Laboratory of the natural resources sector. The isotopic ratio was measured in thousandth unit (‰), denoted by δ.

Soil organic carbon (SOC) and total nitrogen (TN) were determined by potassium dichromate oxidation external heating method and Kjeldahl method respectively (Bao et al., 2000). Soil microbial biomass carbon and nitrogen (MBC and MBN) were measured by chloroform fumigation. MBC was determined by chloroform fumigation-K_2SO_4 extraction-TOC analyzer, MBN was determined by chloroform fumigation-K_2SO_4 extraction-potassium persulfate oxidation method. The bacteria, fungi and actinomycetes in soil were determined by beef peptone culture method, potato glucose agar culture method and Gao's No.1 (culture methodmicrobiology laboratory, Nanjing Institute of soil research, Chinese Academy of Sciences, 1985) and the dilution plate counting method was used.

Statistical analysis

The data were sorted and analyzed by Microsoft Excel 2010 and SPSS 20.0. One-way ANOVA and least significant difference (LSD) were used to test the differences of C, N, their isotopes and soil stoichiometry of *Zanthoxylum planispinum* var. *dintanensis* plantations at different ages. The significant and extremely significant levels were $p = 0.05$ and 0.01, respectively. Data in figures and table were 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 *Zanthoxylum planispinum* var. *dintanensis* plantation ^{13}C and ^{15}N and soil stoichiometry were analyzed by Canoco 5 software (Redundancy analysis, RDA). The correlation heat map of each index of plantation was drawn by using R language.

Results

The characteristics of *Zanthoxylum planispinum* var. *dintanensis* C, N, and their stable isotopes at different plantation ages

The C and ^{13}C in leaves of the 4 plantation age groups did not have significant differences, indicating that the water usage efficiency did not vary significantly with plantation age; The leaf N ranged from 24.95 g/kg to 34.75 g/kg, showing a decreasing trend with the increase of plantation age; ^{15}N ranged from 0.86‰ to 3.20‰, showing an first increasing and then decreasing trend with plantation age, indicating that the N use efficiency of leaves in different stands was different. The litter C and N were 413.35-349.65 and 31.2-18.05 g/kg, respectively, which were significantly higher in 5–7 and 10 – 2 years than in 20–22 and 30–32 years, indicating that litter with smaller leaf age was easier to decompose; litter ^{13}C ranged from – 25.96‰ to -27.67‰, and there was no significant difference among the four plantation age groups; ^{15}N in 10–12 years old group was the highest (4.15‰±0.92‰), which increased first and then decreased with the increase of plantation age; soil C ranged from 9.1 g/kg to 16 g/kg, and decreased with the increase of plantation age; Soil N, ^{13}C and ^{15}N had no significant difference among the 4 plantation age groups, indicating that the soil nutrient pattern was less likely to change with plantation age (Fig. 1).

Relationship between *Zanthoxylum planispinum* var. *dintanensis* plantation C, N and their stable isotopes

The results of correlation and thermogram analysis (Table 2 and Fig. 2) showed that there were no significant correlations among leaf C, litter ^{13}C and other indexes. Leaf N and soil ^{13}C and C, N showed extremely significant ($P < 0.01$) and significant positive correlation ($P < 0.05$), indicating that soil can affect leaf nutrients to a certain extent. The litter layer was positively related to soil ^{13}C and negatively related to soil ^{15}N. Litter N was the only one showed significant positive correlation with soil ^{13}C (no significant correlation observed in others), indicating the interaction between litter and soil could be either promoting or inhibiting. Leaf ^{13}C was significantly positively correlated to liter $\delta^{15}N$ and negatively correlated to soil ^{15}N, implying there was a coupling relationship among leaves, litter and soil.

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

Index	LEC	LECI	LEN	LENI	LIC	LICI	LIN	LINI	SOC	SOCI	SON
LECI	0.055	1									
LEN	-0.108	-0.240	1								
LENI	-0.584	0.439	-0.474	1							
LIC	-0.043	0.256	0.260	-0.307	1						
LICI	-0.100	-0.246	0.624	-0.429	0.855**	1					
LIN	-0.392	0.391	0.706	-0.021	0.595	0.676	1				
LINI	-0.205	0.714*	0.007	0.275	0.727*	0.575	0.606	1			
SOC	-0.132	-0.388	0.792*	-0.614	0.400	0.666	0.534	-0.059	1		
SOCI	-0.193	-0.179	0.939**	-0.384	0.274	0.666	0.724*	0.167	0.747*	1	
SON	-0.280	-0.279	0.774*	-0.484	0.400	0.648	0.628	0.041	0.976**	0.778*	1
SONI	-0.180	-0.721*	0.054	0.041	-0.286	-0.019	-0.369	-0.484	0.181	-0.045	0.074

LEC: Leaf carbon content; LECI: Leaf ^{13}C vale; LEN: Leaf nitrogen content; LENI: Leaf ^{15}N value; LIC: Litter carbon content; LICI: Litter ^{13}C value; LIN: Litter nitrogen content; LINI: Litter ^{15}N value; SOC: Soil carbon content; SOCI: Soil ^{13}C value; SON: Soil nitrogen content; SONI: Soil ^{15}N value; * indicates significant correlation (P<0.05); ** indicates extremely significant correlation (P<0.01), the same below.

The impact of soil stoichiometry on plantation C, N and their stable isotopes

By running RDA on soil stoichiometry and different stands components, we reached the conclusion that soil stoichiometry can interpret the plantation structure (Table 4). The soil stoichiometry interpreted 90.75% and 4.82% variations on the first and second axis, indicating they are good indicators of the relationship between stands and soil stoichiometry.

Table 3
Importance sequencing and Duncańs test of physicochemical factors

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.

Table 4
Redundancy analysis of the component content in 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

According to the two-dimensional diagram of redundancy analysis on plantation components and soil stoichiometry (Fig. 3), MBC, BAC/FUN, TN, LIN, SOCI, LEN, LICI and SOC/TN, MBC, LECI, LEC were positively correlated, BAC/FUN, TN and LEC, LEC and MBN and LIN, SOCI, LEN were negatively correlated; the angles between MBC and LINI and BAC/FUN and SOCI was small, showing a strong positive correlation; SOC was negatively correlated with LENI, but not significantly correlated with other soil stoichiometry. As shown in Table 3, the physical and chemical variables can be rank ordered as MBC > BAC/FUN > SOC/TN > MBN > SOC > TN according to their importance (the information they contained for interpreting stands structure). The influence levels were all significant while the highest value went to MBC.

Discussion

The abundance and internal connection of ^{13}C and ^{15}N in *Zanthoxylum planispinum* var. *dintanensis* plantation at different plantation age

The content of ^{13}C in plant leaf is positively correlated to water use efficiency (Chen et al. 2011). The results of current study demonstrated that the water use efficiency did not significantly varied with plantation age, possibly resulting from the trade-off of the resource acquisition and usage in the plantation (Heberling et al. 2012). Next, the plants need to enhance their resource competition ability to survive in the vulnerable karst environment, which makes the water use efficiency critical and hardly showing differences. The leaf ^{15}N in 10–12 years group was significantly higher than that of the other 3 age groups, probably due to the tremendous needs of N in the vigorous fruit bearing period, which stimulated the root system to transfer more N to leaves for synthesis of photosynthetic products, thus meeting the high metabolism requirement. The leaf ^{15}N increased first and then decreased with plantation age, which was inconsistent with Wang et al.'s research (2019). The inconsistency was caused by the different N isotope fractionation speed due to different photosynthetic type of different species.

There were no significant differences in soil and litter ^{13}C in the 4 plantation age groups, because the organic matter mainly came from litter (Peri et al. 2012). Balesdent et al. (1993) found that soil ^{13}C was not significantly positively correlated to litter $\delta^{13}C$, which was consistent with our results, indicating that soil cannot fully inherit leaf ^{13}C, even if we don't consider the C isotope fractionation in the litter decomposition process. Soil ^{13}C is a result of the mixing of new and old C, an effect called isotopic mixing (Liao et al. 2006). The results of Buchmann et al. (1997) and Farquhar et al. (1989) showed that the soil $\delta^{13}C$ usually fall in the range of 1.0–3.0‰, a value higher than 3.0‰ indicates that the organic matter input into the soil may be a mixture of C_3 and C_4 plants. The average variation of ^{13}C in current study was 7.46‰, indicating that the vegetation in this area may have changed greatly, soil organic matters were simultaneously affected by *Zanthoxylum planispinum* var. *dintanensis* and other plants containing high ^{13}C, which is a reasonable result of agricultural transformation. The current study also showed that the $\delta^{15}N$ in *Zanthoxylum planispinum* var. *dintanensis* plantation was not varied significantly with plantation age, which is inconsistent with the results of Zheng et al.'s research, saying that soil δ^{15}N varied with *Caragana intermedia* plantation age in Fujian area (Zheng et al. 2015). The reason is that the structure of *Zanthoxylum planispinum* var. *dintanensis* plantation is simple, with few species of understory plants and small amount of surface litter. It is also related to the frequent interference of human activities such as chemical fertilizer, insecticide and herbicide application.

The ^{13}C in *Zanthoxylum planispinum* var. *dintanensis* leaf was significantly negatively correlated to N content, which is consistent with Tsialtas et al.'s (2001) results, yet opposite to Zhang et al.'s (2015) research, suggesting that the soil nutrients supply in different environment is different, and so is the resource utilization strategy of plants. The reason is that the leaf N can regulate the stomatal density, higher leaf N content promotes the absorption of CO_2, increases plant photosynthesis rate, and decreases the ration of intracellular and extracellular CO_2 concentration (C_i/C_a), thus increasing ^{13}C (Macfarlane et al. 2007; Diefendorf et al. 2010). The research area is a barren karst region, where needs supplementary fertilization for plant growth. Modern agriculture emphasizes the supplement of N and P, which leads to more uptake of N into leaves, increases stomatal density and C_i/C_a ratio, thus decreasing ^{13}C. Our results also showed that leaf ^{13}C was significantly positively and negatively correlated to litter ^{15}N and soil ^{15}N, respectively, indicating that there was a certain coupling relationship among leaves, litter, and soil. A possible reason could be that ecosystem C and N cycles are closely coupled, the potential of C fixing is greatly limited by the capability of soil providing N (Li et al. 2012; Zechmeister-Boltenstern et al. 2015); meanwhile, it may be related to the mechanism of nutrient reabsorption and the isotope fractionation happened in the process of plant nutrient cycling. However, due to the large number of influencing factors and limited measurement indicators, the reason for the weak inheritance cannot be clarified. Further research is needed in the future.

The driving mechanism of soil stoichiometry to plantation C and N isotopes fractionation

Soil stoichiometry links the chemical cycles in different ecosystem parts, reflecting the flowing of elements (Yang et al. 2018), 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 cycle and internal stability (Zhou et al. 2014; Mooshammer et al. 2014). The contents of soil elements can affect the testing results and restrict the application of stable isotope technology in soil C and N cycles. Stevenson et al.'s study (2010) indicated that the soil C/N was significantly negatively correlated to ^{15}N. The reason was that the biological activity of microbes in soil with different C/N were different, which led to different fractionation speed and degree in the process of mineralization. Generally, the growth of microorganism is limited by N content in high C/N soil, thus weakening the ^{15}N fractionation in the mineralization process; on the other hand, under low C/N condition, the growth of microorganism is limited by C content, thus strengthening the N decomposition in the process of mineralization (Collins et al. 2008). The current study showed that there was a weak correlation between soil C/N and ^{15}N, which was not completely consistent with previous studies. A possible explanation was that the small amount of litter in plantation, the strong human interference, and the high concentration allelochemicals secreted by *Zanthoxylum planispinum* var. *dintanensis* partially inhibited the microbial activity. Our research also demonstrated the negative correlation between soil C/N and ^{13}C. The reason is that the decomposition of organic matter by soil microoganisms is limited by the quality of substrate, the decomposition rate of C and the degree of C isotope fractionation are relatively low in soil with high C/N (Xu et al. 2012; Zhao et al. 2019), thus presenting a smaller ^{13}C value. Wang et al. (2015) reached similar conclusion, nonetheless, Peri et al. (2012) found that soil C/N did not affect the soil ^{13}C in their study of the primeval forests in southern Patagonia. A possible reason could be that the climate was different in each research area, and so was the litter type and amount, which urged the plants to adopt different resource utilization and adaptation strategies. Soil C and N are indispensable elements for plant survival, therefore it is scientifically feasible to use C/N to determine the composition characteristics of the soil ^{13}C and ^{15}N, though it is not the only criterion. In the future, coupling study with other factors should be carried out for comprehensive evaluation.

As the most active part of the soil organic matters (Arunachalam et al. 1999), biomass can establish good connections with ^{13}C and ^{15}N through the decomposition of organic matter and microbial activity. Our results showed that soil MBC was positively correlated to soil ^{13}C, which is related to the isotope fractionation in the process of microorganism decomposition (Billings & Richter,2006). During the process, ^{12}C enters the released CO_2 preferentially, and the heavier ^{13}C more likely enters the soil microorganism biomass (de Rouw et al. 2015) before its returning to the soil organic matter and enrichment in soil. Relevant research have shown that soil ^{13}C is positively correlated with organic C (Wynn & Bird, 2008). When the decomposition of organic C speeds up, more $^{12}CO_2$ will be released from the soil system, thus resulting the enrichment of ^{13}C in soil (Wynn et al. 2007). Inconsistent with these results, the organic C did not show significant correlation with ^{13}C in the current study, indicating that the soil organic C in our research area had no significant influence on C isotope fractionation. Possible reasons could be: in order to improve the economic value of the *Zanthoxylum planispinum* var. *dintanensis* plantation, pruning need to be carried out in winter and summer, thus reducing the litter return and nutrients; the unique soil structure, the nutrients loss pathway, and the soil barrenness are all related. In conclusion, litter and microorganisms are important sources of soil nutrients, which should be further protected for improvement of the soil quality.

Differences between artificial and natural ecosystems

In the artificial forest and secondary forest systems, the content of soil C decreased gradually from leaf, litter, to soil (Table 5). The reason is that leaf is the main place of photosynthesis and C accumulation, before its aging and withering, the old leaf needs to transfer a part of the nutrients to new leaves or perennial tissues (Brant & Chen, 2015; Lu et al. 2017). The leaf ^{13}C content in artificial forest is significantly higher than that of secondary forest, indicating that *Zanthoxylum planispinum* var. *dintanensis* plantation has higher water use efficiency. This is because the application of pesticides and chemical fertilizers inhibits the soil microbial activity; in addition, the small amount of litter results in low soil nutrients compensation, leading to even lower N content and higher environment stress (Table 5). Plants reduce N use by improving water use in the environment of low N (Zhou et al. 2016).

Table 5
Differences between artificial ecosystem and natural ecosystem

Forest type/Index	Artificial forest				Secondary forest (Wu et al.,2021)			
	C (g/kg)	N (g/kg)	^{13}C (‰)	$\delta^{15}N$ (‰)	C (g/kc)	N (g/kg)	^{13}C (‰)	^{15}N (‰)
Leaf	407.1 ± 6.1a	29.2 ± 4.7a	-19.4 ± 1.4a	2.0 ± 1.0ab	438.2 ± 32.8a	20.9 ± 8.8a	-29.2 ± 1.7ab	-0.1 ± 2.3b
Litter	384.2 ± 31.5b	25.1 ± 6.9a	-28.0 ± 0.6ab	2.9 ± 1.3a	421.4 ± 36.8a	15.7 ± 4.0a	-26.8 ± 4.9a	0.8 ± 2.0-b
Soil	10.6 ± 3.9c	1.8 ± 0.6b	-26.9 ± 0.7ab	3.3 ± 0.7a	107.8 ± 56.9b	10.6 ± 8.2a	-24.9 ± 2.4a	7.3 ± 2.1a

Data represent means ± SD (n = 3). Different letters indicate significant differences at P< 0.05 using Pearson correlation analysis test.

In artificial and natural ecosystems, ^{15}N distribution increases with the increase of leaves, litter, and soil, which is consistent with the results of Perakis et al. (2015). The soil $\delta^{15}N$ in secondary forest is significantly higher than that of artificial forest, indicating that isotope fractionation occurred in the processes of plant metabolism and the transformation and transportation of N in plants. The fractionation is more intense in secondary forest. Possible reasons could be that the soil N availability of plantation is affected by the amount of fertilizer, it decreases with the increase of fertilizer amount. By contrast, in natural ecosystem, the soil N availability is relatively high, the N cycle is more open, and more ^{14}N gas is released; moreover, natural ecosystem is less suffered from human activities, the biodiversity is high, the fractionation of soil N isotope is more intense, and the $\delta^{15}N$ is more enriched in it.

To sum up, affected by microoganisms, climates and many other factors, the ^{13}C and ^{15}N distribution law and nutrients allocation are different in different ecosystems. Generally, the growth rate of *Zanthoxylum planispinum* var. *dintanensis* in the artificial ecosystem is slightly higher because of the better management and fertilization measures applied for the purpose of maximizing the ecological and economic benefits of the plantation However, there are still some problems in plantation, such as single stand, low stability in the ecosystem and serious diseases and insect pests, which limit its sustainable development. Therefore, stable isotope technology can be used to explore the natural abundance characteristics of ^{13}C and ^{15}N and study the nutrients restriction status in different tree species, select suitable interplanting mode, enrich the plantation litter types, enhance the stability of ecosystem, thus providing scientific basis for delaying the growth decline of *Zanthoxylum planispinum* var. *dintanensis* plantation.

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Figures

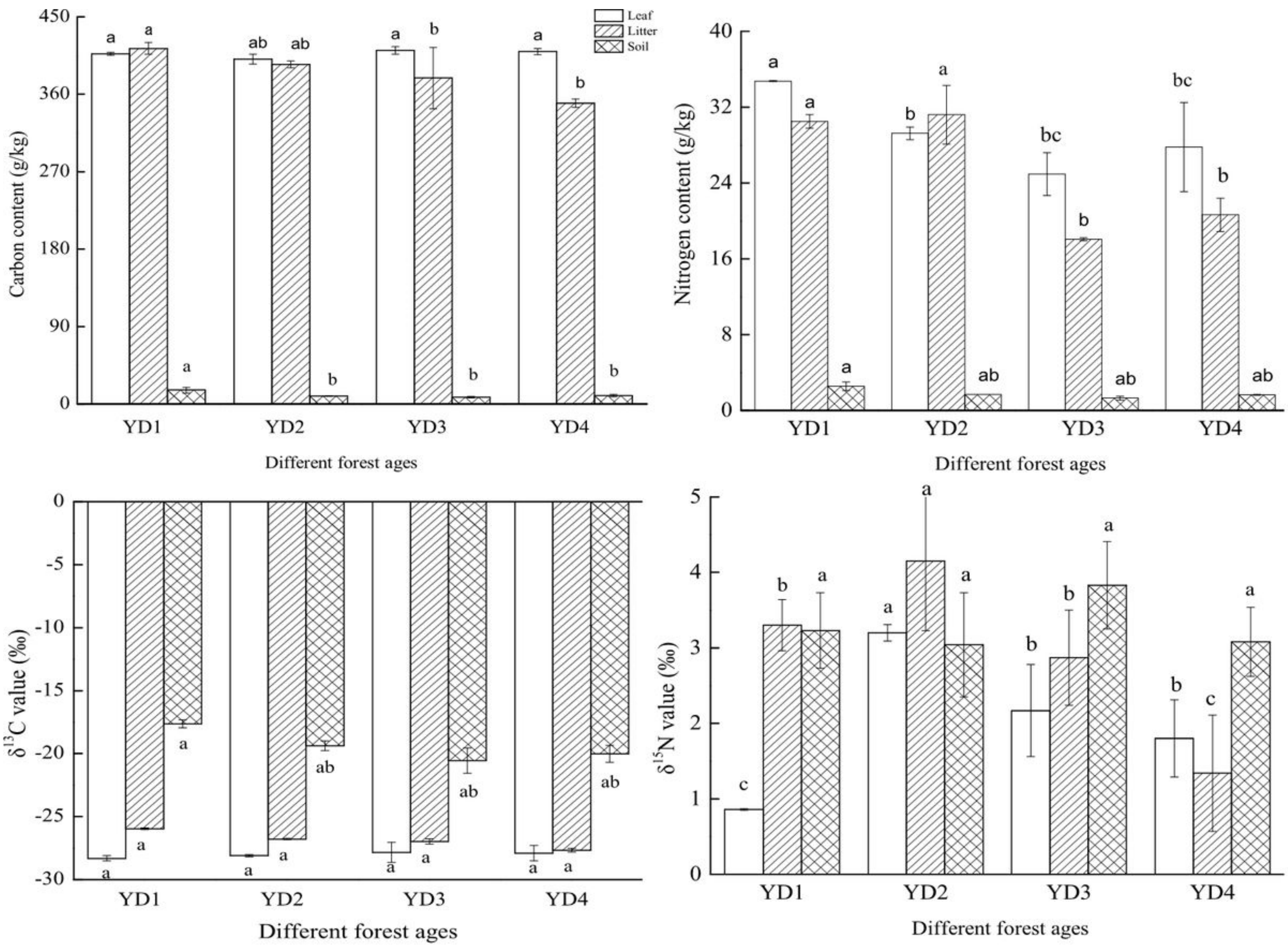

Figure 1

The C and N and isotopic characteristics of Zanthoxylum planispinum var. dintanensis with different ages. Date represent means ± SD (n=3). Different letters indicate significant differences among the age of stand (P0.05). YD1, YD2, YD3 and YD4 respective represent four different forest ages (5-7, 10-12, 20-22 and 30-32).

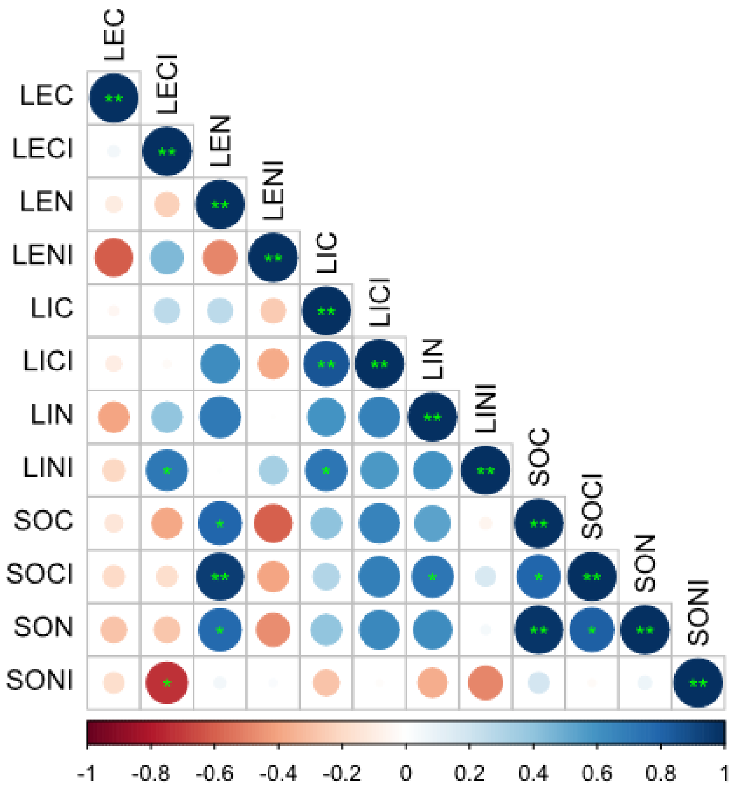

Figure 2

The heat map of C, N and isotope correlation in Zanthoxylum planispinum var. dintanensis plantation

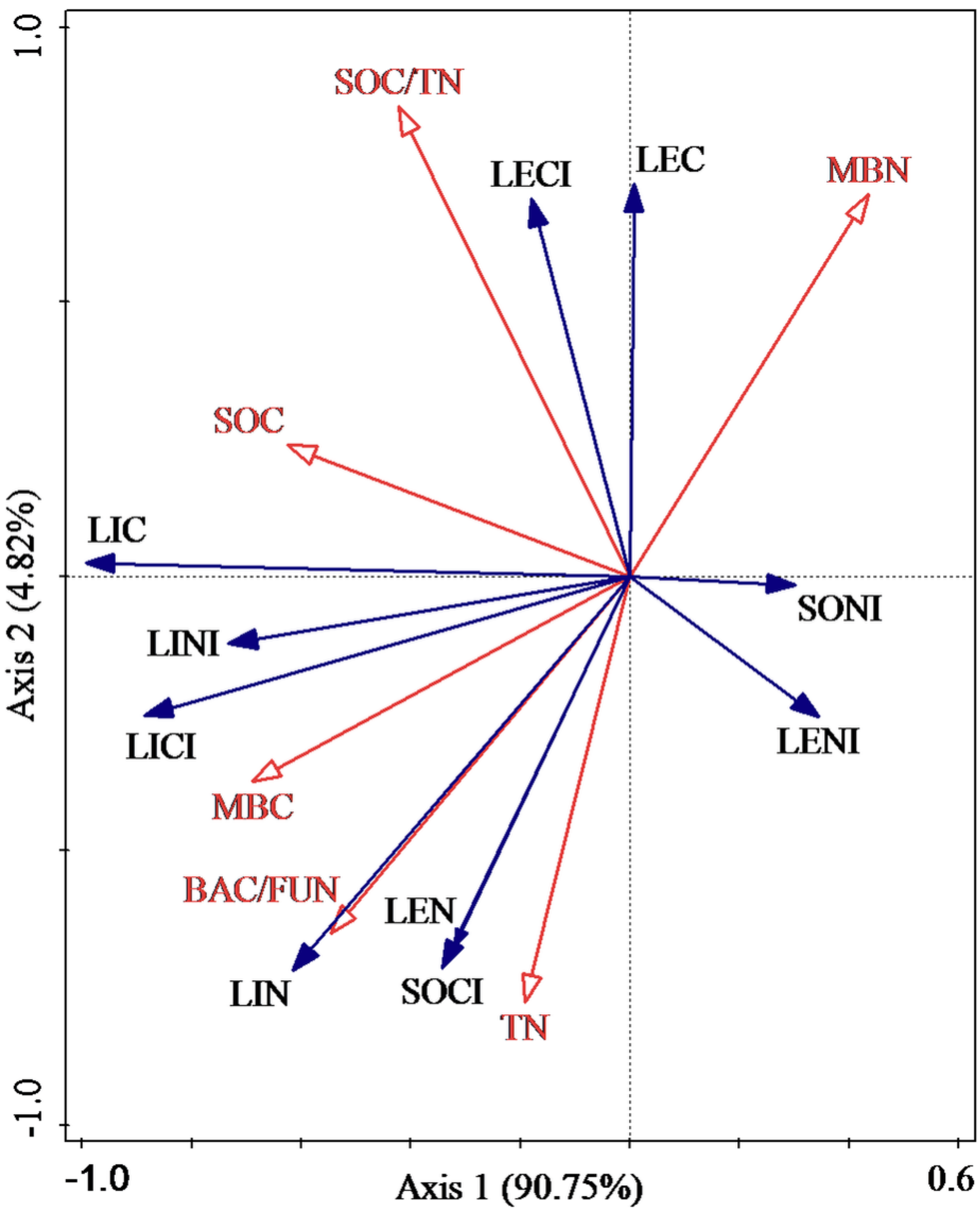

Figure 3

Redundancy analysis of artificial forest of C, N, 13C, 15N and soil stoichiometry in plantation. Axis 1 and Axis 2 represents the first axis and the second axis.