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Short Communication

Radial variation of carbon and nitrogen isotopes in *Dinizia excelsa* Ducke (Fabaceae) wood

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ABSTRACT

Dinizia excelsa Ducke (Fabaceae) is an Amazonian tree species of high commercial value, therefore, it is among the species most frequently seized by the Brazilian federal police due to illegal logging. The isotopic analysis technique can help determine the origin of tropical woods and speed up their traceability, thus curbing illegal exploitation. However, the study of stable isotopes in high-value Amazonian wood species has only been applied to a few species. We aimed to evaluate the radial variation of the isotopes ^{13}C and ^{15}N in the wood of *D. excelsa* adult trees. Fifty-six radial wood samples were analyzed using an elemental analyzer to determine the isotopic composition of $\delta^{13}C$ and $\delta^{15}N$. The absolute values of $\delta^{15}N$ ranged between -0.54 and 1.60‰, and those of $\delta^{13}C$ ranged between -27.88 and -26.57‰. It was observed that samples taken from heartwood provided more homogeneous data, and that there is greater variation of $\delta^{15}N$ and $\delta^{13}C$ in the regions closest to the pith and bark. Hence, this study provides important information for refining sampling techniques to determine the origin of wood, contributing to the development and enforcement of strategies against the illegal exploitation of this important Amazonian tree species.

Keywords: Isotopic composition, stable isotopes, traceability, tropical wood.

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The Amazon rainforest is an ecosystem rich in biodiversity, intensely threatened by deforestation and forest degradation; the latter affecting 34 million hectares of the Brazilian Amazon between 1992 and 2014, surpassing deforestation and creating important implications for biodiversity, carbon emissions, and energy balance (Matricardi *et al.*, 2020). Among the types of forest degradation, logging stands out, as it is responsible for degrading 1.8% (11,970 hectares) of the Amazon Forest between 2001 and 2018 (Lapola *et al.*, 2023).

One of the main problems related to logging in the Amazon is the illegality of this practice. Surveys conducted by the Institute of Man and Environment of the Amazon (Imazon) indicate that approximately 40% of the wood extracted from the Amazon is not authorized (Imazon, 2023), turning this into one of the most lucrative environmental crimes. It is estimated that the direct costs of illegal logging in the Brazilian, Bolivian, and Peruvian Amazon range between USD 558 and 639 million per year (Gutierrez-Velez & Macdicken, 2008).

The illegal timber trade threatens biodiversity conservation, harms local and global economies, and affects sustainable forest management (Hoare & Uehara, 2022). Despite the great impact associated with this practice, efforts from law enforcement to reduce this trade are often limited by a lack of independent tools available to verify the true origin of the wood (Boeschoten *et al.*, 2023). The main types of fraud related to this trade consist of false declarations of origin and irregularities in the forest inventory of Forest Exploitation Authorizations (Brancalion *et al.*, 2018; Camargo *et al.*, 2022).

One method that has been widely applied in verifying the origin of various products is the use of stable isotopes, which has shown successful results in determining the origin of marijuana (Shibuya *et al.*, 2006; Hurley *et al.*, 2010), food (Chesson *et al.*, 2010; Muñoz-Redondo *et al.*, 2021; Li *et al.*, 2023), and animals (Cerling *et al.*, 2006; Hanson *et al.*, 2013; Hobson & Wassenaar, 2018). However, its application in determining the provenance of tropical wood is still quite limited, with few studies reporting varied results, some promising (Watkinson *et al.*, 2022; Kafino *et al.*, 2024) and others not (Paredes-Villanueva *et al.*, 2022; Boeschoten *et al.*, 2023).

Despite positive results, the isotopic tool for accurately determining wood origin is still under development, and broader studies are required for a variety of issues, including how isotopes vary among individuals of the same species, among different species, across different geographic points of occurrence, and even along the diameter of an individual (Leavitt, 2010; Albano, 2022; Camargo *et al.,* 2022). In the latter case, it can be especially useful, as understanding how this variation is spatially distributed in the tree can help carrying out sampling that represents all isotopic variation that exists throughout the diameter, contributing to obtaining more accurate origin attribution results.

Dinizia excelsa Ducke (Fabaceae) is one of the largest trees in the Amazon, known as angelim-vermelho, with great economic potential mainly attributed to wood production, being ranked among the most traded species in Brazil (Ibama, 2023). Its wood is considered heavy and very resistant, factors that contribute to its widespread commercialization and use in civil, naval, and furniture construction. It is estimated that in the last ten years, approximately 7 million m^3 of this wood had been commercialized (Ibama, 2023), without considering the volume extracted illegally. This situation contributes to this species being listed in the IUCN Red List of Threatened Species and imposes the need for measures that contribute to its protection.

This study aimed to evaluate the radial variation of the isotopes ^{13}C and ^{15}N in the wood of *D. excelsa*, a heavily exploited Amazonian timber species, to optimize sampling and isotopic analysis. For this, isotopic analyses were carried out on the wood for carbon and nitrogen, and the variation of isotopic values was evaluated along the radial direction of the disk. Such information can provide subsidies to refine wood traceability techniques based on stable isotope analysis.

Fifty-six radial perforations were carried out on a disk taken from the base of the shaft, whose origin was the management plan of the Sustainable Forest Management Company Mil Madeiras Preciosas, in Itacoatiara, Amazonas, Brazil (02º 30' S, 03º 00' S, 59º 00 ' W, and 58º30'W). Voucher material number 1025 was deposited on Coleção Dr. Valmir Souza de Oliveira – Xiloteca da UFAM, Manaus – AM. For sampling, eight collection points (positions) were defined along the radial sample, approximately 3 cm apart (Figure 1). At each position, seven wood samples were collected transversely by using an impact drill (Makita 40V Li-Ion 140Nm). The wood flake samples were then dried in an oven for 24 hours at 40°C. After drying, the samples were ground into a fine powder using a bench-top vibratory mill (MM 400 – Retsch) for 1 minute. After pulverization, the samples were dried again in an oven for 2 hours at 60°C.

For the analysis of the isotopic ratios of the elements C and N in *D. excelsa* wood, duplicate readings were taken for each of the 56 samples. From each sample, two measurements of 5 mg of pulverized wood were weighed and placed in tin capsules. Subsequently, the samples were analyzed in an Isotope Ratio Mass Spectrometer (Delta VTM – Isotope Ratio Mass Spectrometer from Thermo Fisher Scientific) coupled to an elemental analyzer at the Technical Scientific/Forensic Laboratory of Stable Isotopes from the Superintendence of Amazonas Federal Police.

To analyze $\delta^{13}C$ and $\delta^{15}N$ from organic samples of *D. excelsa*, the values obtained was normalized from Vienna Pee Dee Belemnite (VPDB) scales and atmospheric air, respectively, from the calibration of gases with international certificates IAEA-602 and IAEA-N2 at the Brazilian Federal Police Laboratory of Amazonas (SR/PF/AM). The isotopic ratio (R) was expressed as the ratio between the rare isotope and the most abundant one ($^{13}C/^{12}C$; $^{15}N/^{14}N$) between the analyzed sample and the standard, represented by the notation "delta" (δ) with values in parts per thousand (‰), in which δ = ((R sample/R standard)-1) x 1000 (Sulzman, 2007). All statistical tests were performed using the software MINITAB ® 14. For the analysis, the data were grouped according to the position as presented in Figure 1 and tested according to the element ($\delta^{13}C$ or $\delta^{15}N$).

Descriptive statistics were used to report the isotopic values of $\delta^{13}C$ and $\delta^{15}N$. A normality test was performed to verify the data distribution, as well as a homogeneity of variances test to verify data equivalence. ANOVA was performed to investigate differences between the means of the isotopic values of $\delta^{13}C$ and $\delta^{15}N$ between the different positions along the radial diameter of the sample.

The results show a greater difference between the means of the $\delta^{15}N$ samples compared to the $\delta^{13}C$ means (Tables 1 and 2), showing that the sampling position influenced the total values obtained, mainly for $\delta^{15}N$.

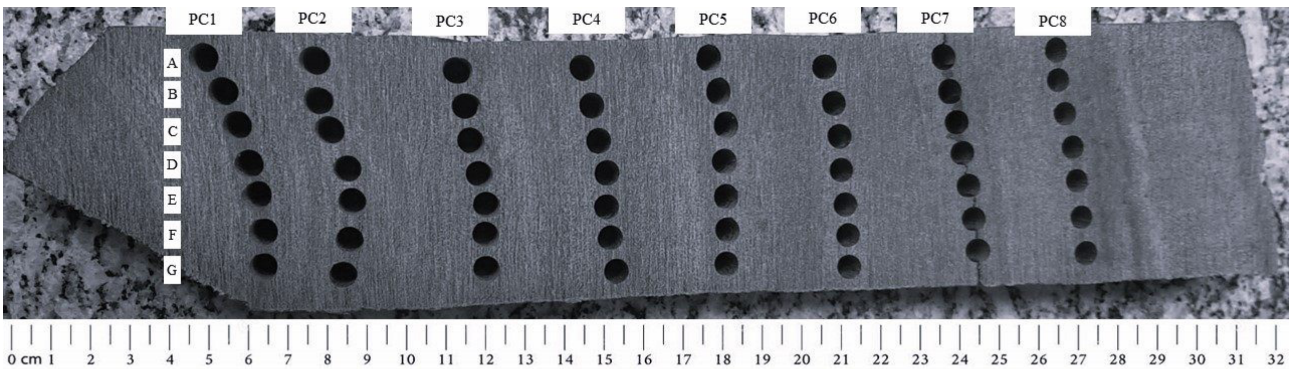

Figure 1. Sampling scheme for analyzing the variation of isotopic concentration of C and N in *D. excelsa* wood. Radial sample containing 32 cm in length. (PC = Position 1-8 from pith to bark). The letters A to G represent the seven repetitions made at each position. Code PC1 to PC8 are the positions sampled from pith to bark.

Table 1. Descriptive statistics (Mean, StDev = standard deviation, Min = minimum, Max = maximum, and CV = coefficient of variation) of $\delta^{15}N$ isotopic values in the wood of *D. excelsa*. PC = position (Figure 1).

Variable	Mean (‰)	StDev	Min (‰)	Max (‰)	CV(%)
PC1	1,40	0,13	1,26	1,60	9
PC2	0,49	0,10	0,30	0,60	20
PC3	-0,10	0,19	-0,35	0,15	-178
PC4	-0,12	0,14	-0,30	0,08	-121
PC5	-0,23	0,16	-0,44	0,03	-71
PC6	-0,26	0,14	-0,54	-0,13	-53
PC7	-0,10	0,24	-0,46	0,33	-239
PC8	0,10	0,16	-0,24	0,25	170

Table 2. Descriptive statistics (Mean, StDev=standard deviation, Min=minimum, Max = maximum, and CV = coefficient of variation) of $\delta^{13}C$ isotopic values in the *D. excelsa* wood. PC = position (Figure 1).

Variable	Mean (‰)	StDev	Min (‰)	Max (‰)	CV(%)
PC1	-27,60	0,08	-27,71	-27,47	0
PC2	-27,17	0,30	-27,49	-26,65	-1
PC3	-26,64	0,06	-26,75	-26,57	0
PC4	-26,99	0,04	-27,05	-26,95	0
PC5	-26,71	0,03	-26,76	-26,66	0
PC6	-27,10	0,07	-27,16	-27,00	0
PC7	-27,83	0,04	-27,88	-27,76	0
PC8	-27,60	0,04	-27,66	-27,56	0

The ANOVA test showed that the means of δ15N isotopic values between the different positions were statistically different ($p<0.05$) (Figure 2). Regarding the variation of δ15N concentrations in the radial direction of *D. excelsa* wood, positions closest to the pith and bark showed the highest values for heavy nitrogen (positions 1, 2, and 8) (Table 1). The sample's more central positions showed a tendency for stabilization in the δ15N isotopic values.

Regarding δ13C, all isotopic values showed a normal distribution, and the variance analysis showed a significant difference ($p<0.01$) in the δ13C isotopic values between different positions (Figure 3). The positions closest to the pith and bark showed the lowest δ13C isotopic values (Table 2), while more central positions also tended to show greater stability, especially positions 3, 4, and 5, located between 14 and 20 cm in depth from the bark.

The absolute values of δ15N ranged between -0.54 and 1.60‰ (0.1±0.6), and those of δ13C ranged between -27.88 and -26.57‰ (-27.2±0.4), with δ15N showing greater variation between positions. Ometto *et al.* (2006), investigating the stable isotopic composition of carbon and nitrogen in wood samples collected in ZF2, located in the Manaus region, Amazonas, observed an isotopic composition of δ13C -28.3±1.5‰ and δ15N 4.6±2.0‰, with nitrogen showing greater variation and higher values than those observed in this study.

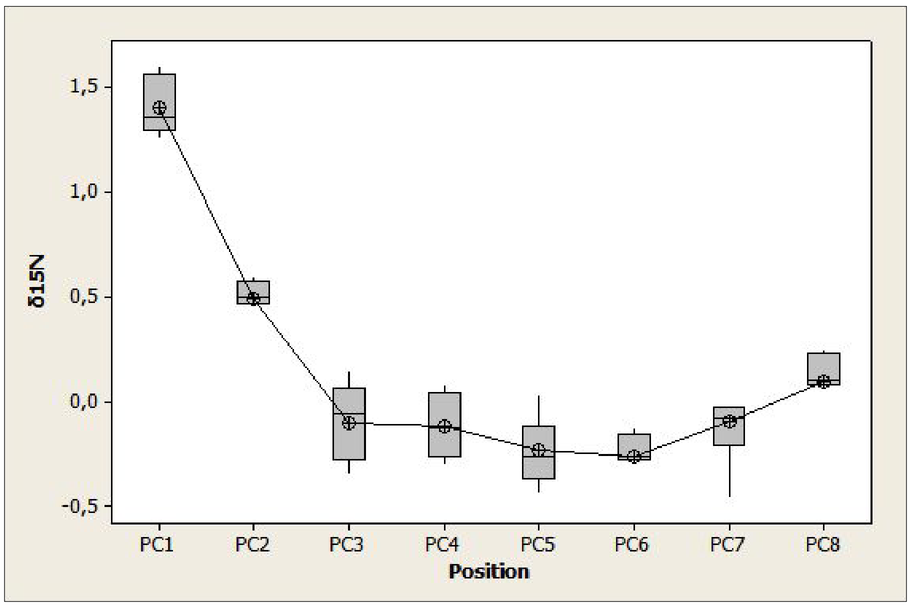

Figure 2. Radial variation of δ15N isotopic values in *D. excelsa* trees wood (PC = Position 1-8 from pith to bark).

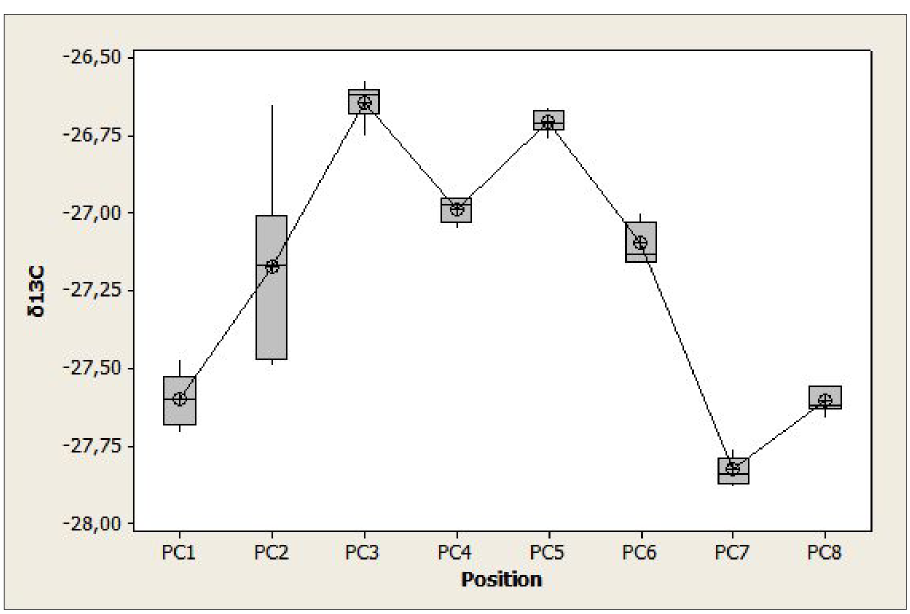

Figure 3. Radial variation of δ13C isotopic values in *D. excelsa* wood trees (PC = Position 1-8 from pith to bark).

As observed, the isotopic values of $\delta^{15}N$ and $\delta^{13}C$ from the area closest to the pith and bark showed greater variation compared to samples from the central portion, which showed greater stabilization. Recent studies with tropical species have found that isotopic values of ^{13}C, ^{18}O, and ^{2}H in samples collected from the heartwood and sapwood areas showed little isotopic variation, unlike samples from the bark and pith (Camargo *et al.,* 2022). Albano (2022) observed the same result when analyzing the radial isotopic variation of ^{13}C, ^{18}O, and ^{2}H in eucalyptus wood, finding that samples collected from heartwood and sapwood areas represented better the isotopic values to this species.

Therefore, our results suggest that samples collected from these positions can provide more homogeneous data. Considering the implementation of the methodology shown above for collecting wood samples for isotopic analysis, these results provide important information to be able to define a homogeneous position for sampling. Thus, the strategy of sampling from 5 cm depth proves efficient in obtaining representative data of the tree's radial isotopic variability.

This study demonstrated for the first time the radial variation of $\delta^{15}N$ isotopic values of *D. excelsa* wood, a tropical species of commercial interest, and provided important information for refining sampling techniques to determine the origin of the tropical wood. However, it is important to consider factors such as limitations in the study sampling, the relatively new methodology used, which is still under development, and, in the context of isotopic analysis in wood, factors such as the type of material collected and the treatment of the samples may influence the results. Therefore, further studies are needed, using a wider sample size, as well as other methodologies aimed at making the analyses safer and allowing greater extrapolations. Despite this, our results provide valuable contributions, such as the efficiency of using material collected from 5 cm depth, especially in the central regions of the heartwood that demonstrate greater homogeneity. This may be especially useful in studies focused on wood traceability, since understanding the spatial isotopic variation can help obtain more accurate data for origin determination and curb illegal trade, with less influence from growth trends.

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Authors Contribution

Daiane Monteiro de Oliveira: Conceptualization and visualization, Methodology, Data curation, formal analysis, validation, and writing – original draft, Investigation, Writing – review and editing; Veridiana Vizoni Scudeller: Conceptualization and visualization, Methodology, Data curation, formal analysis, validation, and writing – original draft, Investigation, Writing – review and editing; Marciel José Ferreira: Conceptualization and visualization, Methodology, writing – original draft, Writing – review and editing; Maria Teresa Gomes Lopes: Supervision, Resources, Writing – review and editing; Santiago Linorio Ferreyra Ramos: Investigation, Writing, review, and editing; Helinara Lais Vieira Capucho: Investigation, Writing – review, and editing; José Haroldo de Oliveira: Methodology, Investigation, Writing – review, and editing; Larissa Sousa da Silva: Investigation, Writing, review, and editing; José Guilherme Cavalcante Freitas: Investigation, Writing, review, and editing; Gabriel Assis Pereira: Conceptualization and visualization, Methodology, Writing – review and editing; Mario Tomazello-Filho: Conceptualization and visualization, Methodology, Writing – review and editing.

Conflicts of interest

The authors declare no conflict of interest (personal, scientific, commercial, political, or financial).

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