


RESEARCH ARTICLE

Insights into growth, ring formation and maximum ages of Brazil nut trees (*Bertholletia excelsa*) using ^{14}C dating and tree-ring analysis

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Abstract

The Brazil Nut tree (*Bertholletia excelsa*, Lecythidaceae) is a species of considerable historical, economic and ecological importance in South America. Radiocarbon dating indicates some individuals can live from hundreds to more than 1000 years, which means they have the potential to reconstruct deep time growth patterns and their relationship to anthropogenic management or climate change from pre-colonial to present times. However, age estimates vary considerably amongst trees dated with different methods (i.e. tree-ring analysis, radiocarbon-dating, and repeated diameter measurements). Here we analyze living Brazil Nut trees growing in four distinct regions across the Brazilian Amazon using two dating methods: tree-ring counting and radiocarbon dating. Our results show that the congruence between the two methods varies amongst regions, and the highest congruence is found at the site of Tefé, Amazonas. This region features archaeological sites with anthropogenic *Terra Preta* soils, and is known for its long-term human forest management. This management likely enhanced light and nutrient availability, which possibly enabled the trees to grow at higher rates and form annual rings. Our findings highlight the need for better understanding of the growth of Brazil Nut trees for ecological research, but also the potential of dendrochronology for exploring climate change and human-forest interactions in the Amazon Basin.

Introduction

Dendrochronological studies can contribute to topics as diverse as tree ecology and physiology, climate change, human forest management, and carbon cycling (Amoroso et al. 2017). The study of growth ring patterns, alongside radiocarbon dating and stable isotope analysis, can provide multiple insights into the history of individuals and communities of trees that can be of wide-reaching relevance (Andreu-Hayles et al. 2015; Baker et al. 2017; Cintra et al. 2021). Although dendrochronological approaches in tropical regions have been employed for a century (Worbes 2002), their validity has been questioned due to a lack of marked seasonality in the tropics. Nevertheless, the use of bomb curve radiocarbon measurements to confirm annual ring formation has stimulated a number of recent studies on tropical species (i.e. Giraldo Giraldo et al. 2023; Andreu-Hayles et al. 2015; Baker et al. 2017; Herrera Ramirez et al. 2017;). Despite growing interest, however, dendrochronological studies in the tropics still face major challenges relating to the distinctiveness of tree-ring boundaries, the occurrence of missing rings, and the determination of the annual nature of tree-rings (Baker et al. 2017; Herrera-Ramirez et al. 2017).

Although the crossdating technique, which matches growth patterns among multiple individuals, is a paramount principle in tree-ring science, the use of ^{14}C dating has shown that even successfully crossdated trees can present a mismatch when the two independent dating methods are compared (Herrera-Ramirez et al. 2017). As a result, for each studied species, it is critical to build a firm understanding of the relationship between growth rings and chronological time in order to harness the benefits of dendrochronological approaches. In this study we seek to advance such methodological knowledge about dendrochronology in relation to the species *Bertholletia excelsa* Bonpl., Lecythidaceae.

Radiocarbon dating is a widely-applied method for obtaining direct ages for organic materials. For timescales of decades to thousands of years, ^{14}C dating is based on the principle of the natural decay of ^{14}C to ^{14}N , and the use of calibration curves to correct for well-documented variations in atmospheric ^{14}C over time (Taylor 2000). Studies reporting radiocarbon dates from tree disks and cores have demonstrated that some tropical trees can live many hundreds to more than a thousand years (Chambers et al. 1998; Vieira et al. 2005). Most important for our methodological approach, however, is that, for the last 60 years, radiocarbon dating can be used to accurately estimate the year of ring formation, as demonstrated in previous studies (Andreu-Hayles et al. 2015; Baker et al. 2017). From the late 1950s to early 1960s, tropospheric ^{14}C levels almost doubled due to the large-scale thermonuclear bomb tests conducted during the Cold War Era, followed by a sharp decrease after the Test Ban Treaty of 1963 (Levin et al. 2022), such that the C fixed by trees from the atmosphere in a given year should have a specific ^{14}C signature (Santos et al. 2022). The visual identification of tree-rings jointly applied with “bomb peak” ^{14}C analysis has been important for determining the growth periodicity of tropical trees and evaluating the annual nature of tree rings (Lisi et al. 2001; Andreu-Hayles et al. 2015; Santos et al. 2015; Ohashi et al. 2016; Linares et al. 2017; Herrera-Ramirez et al. 2017; Santos et al. 2022; Durgante et al. 2023). Studies designed in this way have shown the latitudinal congruence between ^{14}C levels in trees across the tropics (Andreu-Hayles et al. 2015), the dendrochronological potential of tropical species (Linares et al. 2017), and provided evidence for local fossil CO_2 emissions (Santos et al. 2022). Such studies have also highlighted the risk of assuming dendrochronological results for anatomically complex species without validation by independent dating methods (Herrera-Ramirez et al. 2017). Further, even for species that, in some locations have had their annual tree-ring formation periodicity confirmed by independent dating methods, like tree-ring analysis and radiocarbon dating, this cannot be generalized as the same methods can produce incongruent results in other regions (i.e. Baker et al. 2017).

Here, we study the Brazil Nut tree *Bertholletia excelsa*, one of the most iconic terra-firme (non-flooded) upland trees in the Amazon forest. Archaeological evidence shows that Brazil Nut was exploited by human societies in the Amazon as early as 11,000 years ago (Roosevelt et al. 1999) and that stands of the species (‘castanhais’) often occur close to archaeological sites with *Terra Preta* (Amazonia Dark Earth) (Clement et al. 2015; Thomas et al. 2015). *Terra Preta* are soils enriched with phosphorus, calcium, magnesium, and other nutrients, and are the result of human landscape management and long-term sedentary occupation (Neves et al. 2003; Arroyo-Kalin et al. 2012; Glaser and Birk. 2012), which has also partially influenced the distribution and abundance of several plant species considered “useful” to humans (Levis et al. 2017).

The Brazil Nut is the 178th most common of the ~200 hyper-dominant species that make up 50% of the trees of the Amazon Basin (ter Steege et al. 2013), and its seeds are an important economic product in South American countries today (Guariguata et al. 2017). Its abundance, fast growth and wood density make it important in terms of biomass stocks and production in Amazonian forests (Fauset et al. 2015). The tree-rings of *Bertholletia excelsa* are characterised by a repeated pattern of alternating fiber and parenchyma bands (Brienen and Zuidema 2005; Schöngart et al. 2015), that become narrower towards the ring boundary. This pattern is common to other Lecythidaceae that occur in Amazonian floodplain forests (Worbes 2002). The occurrence of anatomical factors that can impact tree-ring identification, such as wedge, false, and/or discontinuous rings, is common for tropical species (Brienen and Zuidema 2006). Further, there is a large variation in annual growth rates for this species (Schongart et al. 2015; Caetano Andrade et al. 2019; Scoles and Gribel 2021), which translates into variations in the

width of the rings. The main driver for this variation is arguably the availability of sunlight (Scoles and Gribel 2021), although other factors such as P, Zn, Cu, clay, and water content in soils have also been shown to have a positive impact on the diameter growth of the Brazil Nut (Costa et al. 2022). Temporal changes in resource availability may also cause the Brazil Nut trees to undergo sustained growth changes (release and suppression events), which provide information on forest dynamics and canopy ascension strategies (Brienen and Zuidema 2006; Schongart et al. 2015), as well as records of past human forest management (Caetano Andrade et al. 2019).

Studies have reported the age and growth rates for Brazil Nut trees using different methods, such as repeated diameter measurements (Locatelli et al. 2005), radiocarbon dating (Camargo et al. 1994; Vieira et al. 2005), dendrometer bands (Kainer et al. 2006) and tree-ring analysis (Brienen and Zuidema 2006; Schöngart et al. 2015; Caetano-Andrade et al. 2019). Although its annual ring formation has been confirmed by counting rings of trees with known age (Schöngart et al. 2015), studies have indicated a considerable difference between the maximum ages reported for these trees ranging from 400 to 500 years (Camargo et al. 1994; Zuidema and Boot 2002; Brienen and Zuidema 2006; Schöngart et al. 2015; Caetano-Andrade et al. 2019) to up to 1000 years (Vieira et al. 2005). Estimates of average diameter growth rates derived by different methods also vary considerably (Schöngart et al. 2015), from 0.06 cm/year (Vieira et al. 2005) up to 2.55 cm/year (Locatelli et al. 2005), and it is not clear whether these large differences are due to methodological problems, varying site conditions (i.e. climate, soils) and/or management practices. Therefore, in this study we (i) exploit the ^{14}C bomb peak to compare independent chronological methods (visual tree-ring identification and ^{14}C dating), (ii) evaluate the periodicity of tree-ring formation in living Brazil Nut tree individuals growing at four sites scattered across the Amazon Basin, (iii) evaluate the potential for developing chronologies for the species, and (iv) present ^{14}C dates for large living Brazil Nut trees to add new data to the debate as to which time-depth dendrochronological studies can be applied for this species. With this, we aim to further explore the potential of this species for climatological and human forest management studies.

Materials and Methods

Description of Sites and Sampling

We sampled living trees from Brazil Nut stands in *Tapirapé Aquiri National Forest* (hereinafter TAP), *Tefé National Forest* (TEF), *Jaú National Park* (JAU), and *Jamanxim National Forest* (JAM) (Figure 1). Brazil Nut stands are clustered stands of Brazil Nut trees, commonly referred to as “castanhais” or “bolas,” interspersed with areas of forest with very few or no Brazil Nut trees (Mori and Prance 1990; Peres and Baider 1997; Baider 2000; Zuidema 2003; Salomão 2009; Scoles and Gribel 2011; Thomas et al 2015). The study sites are all located in the Brazilian Amazon, between latitudes 7°11'S and 1°48'S and longitudes 65°00'W and 50°04'W. The great distance between the sites was designed so as to test the methodology across the wide distribution range of this long-lived pioneer and to assess different geographical, edaphic and climatic contexts (Mori and Prance 1990; Salomão 2009; Zuidema 2003). At each site, we collected trees from two separate Brazil Nut stands (i.e. TAP1, TAP2, TEF1, TEF2, . . .) located from 5 to 60 km apart, and numbered the trees according to the order in which they were sampled during the field campaign (i.e. TAP1_01, TAP1_02, . . .). The reason we chose trees from two Brazil Nut stands at each site was to cover a larger area with our sampling.

TAP is in Pará state and covers an area of approximately 196,504 ha (MMA 2006). The vegetation in the TAP is dominated by submontane ombrophilous forest and ombrophilous alluvial forest, which together cover more than 50% of the park (MMA 2006). TAP is in an area granted to a mining company, and the Brazil Nut stands are occasionally visited by the *Xikrin do Catete* Indigenous People who own a territory close to the park. There are occurrences of patches of *Terra Preta* and other archaeological sites in the region (Silveira et al. 2008), but not specifically where the trees were sampled. JAM is in the municipality of Novo Progresso, Pará state, and its area of approximately 1,301,120 ha is located on the border of the deforestation arc, in the midst of a land related conflict situated between protected areas

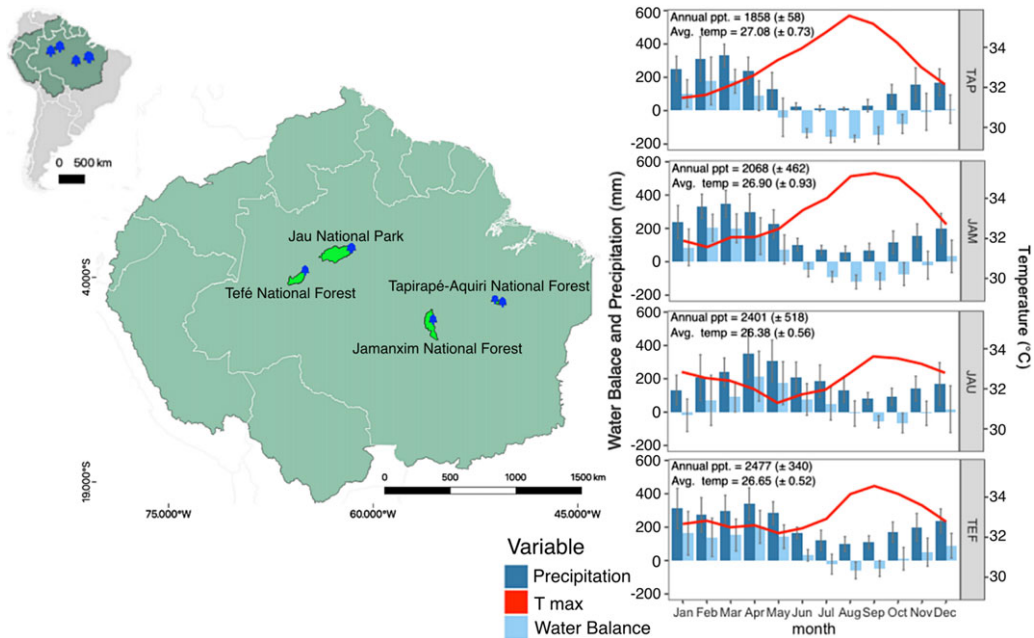


Figure 1. Map of the Amazon biome showing the Brazil Nut (*Bertholletia excelsa*) sampling sites within the Brazilian system of conservation units, with average annual and monthly rainfall, average temperatures, monthly maximum temperatures and monthly Water Balance of each conservation unit. The bars indicate the standard deviations.

and cattle ranchers (Nascimento et al. 2019). The vegetation found in JAM is represented by open Ombrophilous Forest, dense Ombrophilous Forest and dense Ombrophilous Alluvial Forest (ICMBio 2010). Currently there is no management carried out in the Brazil Nut stands at this site, and no traces of *Terra Preta* were found in our soil sampling (e.g., dark coloration, presence of archaeological artefacts). TEF is in the municipality of Tefé, Amazonas state, and has an area of 865,126 ha (ICMBio 2014). Its vegetation consists of dense ombrophilous forest, mostly in the form of terra-firme forest, but *igapó* flooded forest is also present. TEF1 and TEF 2 have undergone continuous forest management since the mid-20th century, according to the local residents (Shepard and Ramirez 2011). TEF1 and TEF 2 trees are growing on an archaeological site, with *Terra Preta* with ceramics, as well as other “useful” species such as the rubber tree (*Hevea brasiliensis*, Euphorbiaceae), caiaué (*Elaeis oleifera*, Arecaceae), and açai (*Euterpe precatoria*, Arecaceae) (Lopes 2020). JAU is the world’s third largest park of tropical forest, with an area of 2,272,000 ha, in the Amazonas state between the municipalities of Novo Airão and Barcelos, in the lower Rio Negro (MMA 2019). The vegetation of JAU is formed by *terra firme*, *campinaranas* (Amazonian white sand ecosystem), and *igapó* forests (FVA 1998). The Brazil Nut stands at this site are occasionally managed by the very low density local population and some of our soil sampling points indicate the occurrence of *Terra Preta*, although the trees are not growing directly on archeological sites.

Sampling was non-destructive and obtained cores of up to 1.08 m in length. Cores were obtained using a manual increment drill (Haglöf, Sweden) up to 50 cm for the smallest trees and with a customised mechanised drilling system, which consists of a large drill (up to 1.5 m) connected to a gasoline-powered drilling machine and a support that attaches to the tree (Caetano Andrade et al. 2021), for the larger trees. In order to compare the dates derived from the visual identification of the rings to those obtained from radiocarbon measurements, ten trees were sampled at each site, consisting of five coming from each sampled Brazil Nut stand (five in TAP1, five in TAP2, five in TEF 1, and so on). Two to three cores were collected from each of the sampled trees, except for two trees (one from TAP and one from TEF) that had only one core included because of damage. The list of the trees is provided in

Supplementary Material 1. In total, 40 trees were analyzed (five per Brazil Nut stand \times two stands per site \times four sites), from which one core was selected, and three rings (formed after the 1950s) were extracted for radiocarbon analysis, totaling 120 rings analyzed. In addition to these 40 analyzed trees, we selected another five large trees (one in TAP, one in JAM, one in JAU and two in TEF), from which we counted the rings and measured the radiocarbon of the innermost part of the collected cores, in order to not only compare the dates derived from the visual tree-ring identification with the radiocarbon measurements, but also to access information on the age of these large individuals to further advance knowledge of the longevity of Brazil Nut.

We collected information on Diameter at Breast Height (DBH), liana index, illumination index (adapted from Brienen et al. 2010), and the presence or absence of termites for each tree to provide insights into the present competitive status of the trees within the Brazil Nut stands (Caetano-Andrade et al. 2019). The Brazil Nut trees included in our sampling were all growing at or above the canopy (receiving full sunlight over the crown), and about half of the trees had lianas, albeit at low levels. All sites are situated in the same ^{14}C calibration zone (Post-bomb atmospheric SH3 curve) according to the recently updated hemispheric and global radiocarbon data sets (Hogg et al. 2020; Hua et al. 2022). We obtained measurements of the monthly precipitation, solar irradiance, maximum temperatures, potential evapotranspiration from the closest climatic station for each site and calculated the Water Balance (the difference between monthly precipitation and potential evapotranspiration) (Supplementary Material 2). The climate data were downloaded from the National Institute of Meteorology (INMET)–Brazil, available at <https://portal.inmet.gov.br>. Adjacent to the trees, we also collected soil samples, which had their physical (% of Silt, Sand, Clay) and chemical (pH, Ca, Mg, Al, K, P, Fe, Zn, Mn, C, Organic Matter) properties measured (Supplementary Material 3). Three soil samples were collected at 0–20 cm depth per hectare where the trees are located. These soil samples were then pooled to form a composite sample. Soil results for each hectare were used in further analysis associated with trees growing on the same hectare from which the soil was collected.

Tree-Ring Analysis

The tree core samples were glued to a wooden support, sanded to a 600 grit size, and scanned at 1200 DPI. Tree rings were visually identified and the cores from the same tree were compared to verify the quality of visual identification and measured to an accuracy of 0.001 cm using the software Image J (Abràmoff et al. 2004). Ring width series within and between trees were statistically analyzed using standard dendrochronological procedures. The series from each site were first visually and statistically crossed with COFECHA (Holmes 1983; Grissino-Mayer 2001) and the Dpl-R package for R (Bunn 2008; Bunn 2010; RStudio Team 2020) to identify possible errors on the tree-ring series. We fitted a cubic smoothing spline with a 32-year 50% frequency response in the tree-ring series to reduce non-climatic variation and applied a non-robust autoregressive model to remove the autocorrelation. The resulting individual standard and residual series were used to build a mean chronology calculated with a biweight robust mean (Cook 1990). We also calculated the Expressed Population Signal (EPS), which indicates the strength of the unknown population signal from a tree ring dataset (Wigley et al. 1984), and the Subsample Signal Strength (SSS), which estimates the loss of explanatory power as the number of tree-ring records decreases back in time (Wigley et al. 1984).

Radiocarbon Analysis and Comparison between Dating Methods

The 120 rings selected for comparison between visual dating and radiocarbon dating were expected to have formed after the “bomb peak” (~1965) according to the tree-ring counting. We chose to analyze the radiocarbon of rings visually dated to the post-bomb period due to the characteristic shape of the ^{14}C bomb peak that facilitates high-precision dating (Linares et al. 2017). The selected rings were removed

from the core with a small chisel and chipped into small pieces (< 1mm). They were then placed in individual test tubes and had their structural alpha-cellulose extracted using a standard Acid Base Acid (ABA) + Bleach procedure. The procedure is described in Supplementary Material 4 and is based on the principles of cellulose extraction employed in similar research (i.e. Gaudinski et al. 2005; Hammerschlag et al. 2019). When it was not possible to sample a single ring due to its narrow width, we took wood from neighbouring rings until we had enough wood for cellulose extraction. We have noted the rings collected in these cases in Supplementary Material 5. All samples were subjected to the same lab protocols, including the reference materials. We included the post-AD 1950 ^{14}C barley mash (FIRI-J) and blank (HTC) standards in every batch for quality control and background correction (Boaretto et al. 2002; Santos et al. 2020, 2021). As the samples were previously glued to a wooden support with adhesive in aqueous dispersion based on vinyl polyacetate (PVAC), we measured the effect of the glue with the standards (FIRI-J and HTC), as well as the pure glue without any chemical treatment. These tests indicated that the glue was completely removed from the samples during our chemical treatment and so does not influence the radiocarbon measurements of the rings (Supplementary Material 6).

Chemically treated samples were converted to graphite and measured by ^{14}C -AMS at the Radiocarbon Laboratory at the Max Planck Institute for Biogeochemistry in Jena, Germany (Steinhof et al. 2017). We obtained calibrated Radiocarbon date (cal. AD) ranges (calendar year intervals calibrated with ± 2 sigma error) for each tree ring with OxCal (Ramsey 2009) using the post-bomb atmospheric SH3 calibration curve (Hua et al. 2022). To compare data between visual tree-ring counts and cal. ^{14}C dates, we first selected a date from the cal. ^{14}C interval range to use as a proxy. For this, we first checked whether the visually dated tree-rings fit their respective cal. AD interval ranges. In cases where the dates coincided, we assumed the value of the cal. AD to be the same as the visually counted tree-ring. Otherwise, we selected the cal. AD value from the interval range that most closely approximated the age estimated by the ring count. For example, if the cal. AD interval for a given tree-ring ranges from 2001 to 2003, and the visually dated tree-ring is assigned to the year 2005, we considered the cal. ^{14}C value as 2003 for that ring. We refer to the age difference between the two dating methods as the “Age Deviation,” which represents the minimum number of years for a given visually dated tree-ring to fit its respective cal. ^{14}C interval range. In the cases where the dates coincide, the Age Deviation equals to zero.

Estimation of Growth Rate and Tree Age

With the selected cal. AD values and the position they occupy in the core, we calculated the annual average increments in DBH for each tree. In order to do this, we adopted the strategy proposed by Vieira et al. (2005) for those cases where they estimated tree growth rates based on post-bomb ^{14}C measurements only. That is, we extrapolated the average growth rates for the trees based on the most recent rates of increment. As proposed by Vieira et al. (2005), we used a linear trend between the cal. AD values of the tree-rings along the tree radii, in relation to the radial distances of these rings transformed into DBH. That is, based on the current DBH of the trees we projected the diameter they had at each position from which a sample for ^{14}C dating was taken. This linear trend is not meant to be a regression, as there are only three points per tree. Rather, it is practically the same as taking the cal. AD values at two points of the tree’s radii and calculating the difference in age in relation to the distance between these points, assuming a linear growth. Such a methodology can be problematic in tree ring studies and long-term growth monitoring which show that Brazil Nut presents variations in growth rates throughout their lives (Brienen et al. 2010; Schongart et al. 2015; Caetano Andrade et al. 2019; Scoles and Gribel 2021). Nevertheless, we adopted this method for calculating the ^{14}C based average growth because (i) post-bomb ^{14}C dating yields the most accurate age estimates in comparison to pre-bomb dates that fall between ≈ 1650 and 1950 AD, in light of the wide range of ages given by the ^{14}C calibration curve, which make it difficult to obtain precise ages for calculating the average growth (Worbes 2002; Vieira et al. 2005), and (ii) we can compare and discuss the estimates between the methods employed here and with the existing literature (i.e. Vieira et al. 2005). We also calculated the

average annual growth in diameter based on the tree-ring measurements, which consists in averaging the measurements of the tree-rings visually identified. To compare the average growth based on both methods, we calculated the average growth based on the tree-ring measurements for the post-bomb period (from 1965 onwards).

The age of trees was estimated by dividing the tree diameter at DBH by the average annual increment, as determined by cal. AD measurements. For tree-ring based age estimations, the age was obtained by counting rings when the pith of the tree was present in the core or, by extrapolating the average growth when the pith was not present, and dividing it by the missing distance to the centre of the tree, considering the DBH measured in the field. To understand the factors affecting the age mismatch between the two age approaches, we conducted multiple linear regression analysis of the Age Deviation values of the rings located furthest inside (most distant from the bark) of each core, because they showed the highest deviation values. For this we considered the average growth based on both chronological methods, the precipitation ranges for each site (the difference between the highest and lowest average monthly rainfall), the Water Deficit (average annual cumulative negative difference between precipitation and potential evapotranspiration), the monthly average maximum temperatures, the duration of the dry period (number of months with negative difference between precipitation and potential evapotranspiration), the average monthly solar irradiation, and all of the measured soil parameters [% silt, % sand, % clay, pH, Ca, Mg, Al, K, P, Fe, Zn, Mn, C, Organic Matter (O.M.)]. The variables with the greatest correlation with Age Deviation were selected using the “dredge” function in R package “MUMIn” (Multi-Model Inference) (Barton and Barton 2015), that is used to perform automated model selection by fitting all possible combinations of predictors to a given response variable, and then ranking the models based on the Akaike Information Criterion (AIC). We then visualised the selected model output with Principal Component Analysis (PCA). We initially included all sites in the first PCA. However, as the trees from TEF showed almost no Age Deviation, we decided to exclude TEF and conduct another selection of model and visualise it in a second PCA.

Radiocarbon and Tree-Ring Analysis of Large Brazil Nut Trees

To obtain age estimates for the largest individuals, we separately analyzed five large trees from across the four sites (one in TAP, one in JAM, one in JAU and two in TEF), with a mean DBH of 197 cm (ranging from 148.2 to 290.3). For these trees we only took a radiocarbon measurement from the innermost part of their cores. Accurate counting of all rings up to the innermost portion of the cores, where radiocarbon datings were carried out, was not possible, as the cores showed zones with indistinguishable rings due to fire scars, damage to the bark, damage during the sampling process, rotting, and twisting of the wood. Regardless, we calculated the average width of the conspicuous tree-rings of each core and drew on this value to estimate the number of rings of the indistinguishable zones up to the innermost position of the cores, so we could compare the ages based on both methods. It is important to note that the innermost portion of the cores are not necessarily the pith of the trees, but rather the innermost portion of the wood that could be sampled. The length of the cores used for ^{14}C dating from these large trees, as well as the average growth based on tree-ring measurements, and the estimated ages are shown in Supplementary Material 7. These samples were subjected to the same cellulose extraction procedures as the other samples in this study, but the cal. AD values were obtained using the SHCal20 calibration curve (Bronk Ramsey 2021; Hogg et al. 2020).

Results

Tree-Ring Analysis

The distinctiveness of tree rings in our samples was clear overall (Figure 2 a, b). For some individuals, however, we identified zones in the wood with hard-to-distinguish ring boundaries, especially in parts of

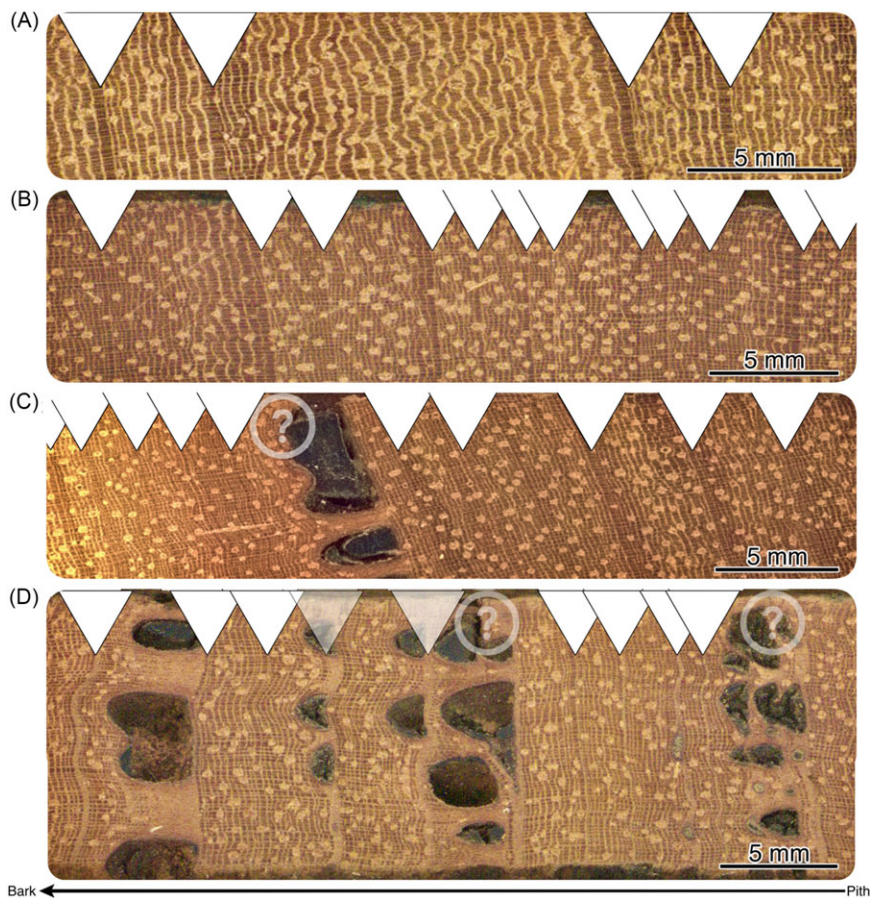


Figure 2. Tree rings of *Bertholletia excelsa*, defined by an alternating pattern of fiber (dark tissue) and parenchyma (light tissue), and tree-ring boundaries indicated by arrows. (A) and (B) show easily distinguishable tree-rings, (C) and (D) show scars caused by fire or injuries to the bark, making tree-rings hard to distinguish.

the wood produced after damage (Figure 2 c, d). These scars were mainly caused by injuries to the cambium as a result of traditional cultural human actions (e.g. bark extraction for medical purposes) or from natural or human-induced fires. Some tree-rings, however, also showed low distinctiveness in parts of the wood with no apparent damage. The two or three radii extracted from each individual were successfully cross-dated within the same tree for the majority of individuals. This diminished the uncertainties produced by rings that were not very well delimited and helped us to identify false and missing rings. The average series intercorrelation within cores of the same trees were higher than 0.4 for more than half of the individuals, overlapping up to 183 years (Supplementary Material 1). These values are commonly found amongst tropical and subtropical tree species with complex wood anatomies (Quesada-Román et al. 2022).

Our Cross-dating of the trees growing within each site and within the Brazil Nut stands of each site (TAP 1, TAP2, TEF1 . . .) did not produce the statistical confidence needed to build up chronologies for dendroclimatological analysis. However, we did further explore the ring-width series of TEF1 (n of cores = 9) and TEF2 (n of cores = 10), which showed modest intercorrelations of 0.27 and 0.22, spanning the periods 1936–2018, and 1921–2018, respectively. The running correlation values for TEF1 and TEF2 were not higher than 0.3 (20-year time window, with 10-year lags) across the chronologies (Supplementary Material 8). The EPS statistics were low for TEF1, but consistently higher

than 0.5 for TEF2, considering a 20-year time window with 10-year lags, and the SSS statistics was higher than 0.85 for TEF1 and TEF2 from 1971 and 1944, respectively (Supplementary Material 9). For the periods with $SSS > 0.85$, only the residual and standard chronologies produced for TEF 1 showed significant correlation ($r = -0.3$, $p < 0.05$) with monthly rainfall (Supplementary Material 10).

Radiocarbon Dates and Comparison between Methods

From the 120 tree-rings analyzed, 38 (31.6%) had Dendrochronological dated ages that fell outside of the cal. ^{14}C date ranges and thus presented an Age Deviation. At the level of individuals, 22 of the 40 trees (55%) presented at least one mismatch between measured tree-rings and cal. ^{14}C years, with 20% deviating only in one of the three tree-rings analyzed. Figure 3a shows the regressions between the visually dated tree-ring ages and their respective selected cal. AD values for each site. It also shows a black line with slope = 1, along which the values would be expected to fall if all of the dates matched perfectly. TEF showed the best match between the cal. AD values and the visually dated tree-ring ages, with only three individuals presenting Age Deviation. For the trees growing at TAP, JAU, and JAM, the mismatch between the dates was somewhat higher due many to missing rings (Figure 3a), which caused the regression line to be below the black line. Furthermore, the slope of the regressions from TAP, JAU, and JAM assumed values greater than one, which means that the older the tree-rings, the greater the Age Deviation, likely associated with the effect of accumulating mismatch. In Supplementary Material 5 we present the fraction [$F^{14}\text{C}$ values ($\pm 2\sigma$)], the cal. ^{14}C interval ranges in which we indicated the selected cal. AD values used in our analysis, and the ages based on the tree-ring counts for each tree-ring.

In Figure 3b we present the data for each tree in the form of $F^{14}\text{C}$ (fraction modern ^{14}C) content of the tree-rings versus their tree-ring dated ages. When the two dating estimates coincide they fall along the Post-Bomb SH3 Radiocarbon Calibration Curve ($F^{14}\text{C} \pm 2\sigma \times$ calendar ages) (Hua et al. 2021). In the lower part of Figure 3b, eight samples presented very low $F^{14}\text{C}$ values, so their calibrated dates fell beyond the Post-Bomb Curve. The cal. AD values selected for these samples were considered using the same criteria as for the other samples, which involves taking the closest age so that their visually dated ages fit their respective cal. ^{14}C interval ranges. This meant that the cal. AD values for these samples fell between 1953 and 1956, which is the limit between the pre- and post-bomb period. As a result, although the Age Deviation for these tree-rings was large, we decided not to exclude these results. The only exception was TAP2_27, which had a cal. AD of over eight hundred years old, which we suspect to be the result of a problem during the lab procedures, and we are running a new analysis to verify.

Average annual growth increment estimates based on cal. ^{14}C values were lower than those based on visual tree-ring measurements for the post-bomb period (from 1965 onwards) for all sites, except for TEF (Table 1). At TEF there was practically no Age Deviation, except for three samples that presented a small deviation (one, two and three years, respectively) which, unlike the other sites, presented younger ages for cal. ^{14}C compared to their visually dated ages. This means that ^{14}C -based growth estimates are higher for TEF compared to estimates based on tree-ring measurements for the post-bomb period. For TAP, JAM and JAU the tree-ring cal. AD values were older than their respective visually dated ages, which produced lower growth estimates. Age estimates presented in Table 1 show older ages based on cal. ^{14}C , except for TEF, for the same reasons mentioned above. The values presented for age estimation based on visual tree-ring dating considered all tree-rings measured in the cores, and not just the post-bomb period, as described in the methods. There was no significant difference between the estimates of average growth and ages if we consider only the tree-rings that showed no Age Deviation. For the tree-rings that showed Age Deviation, the difference between the estimates was significant ($p < 0.05$). The tree-rings that showed Age Deviation occurred mainly at TAP, JAM and JAU (Figure 3).

The first PCA was performed with all sites and six variables, which were selected based on the AIC (Figure 4a). These included the average growth based on the tree-ring measurements and soil content of Ca, Fe, Al, and O.M. The selected model has a R^2 value of 0.48 and an adjusted R^2 of 0.40, ($p < 0.01$).

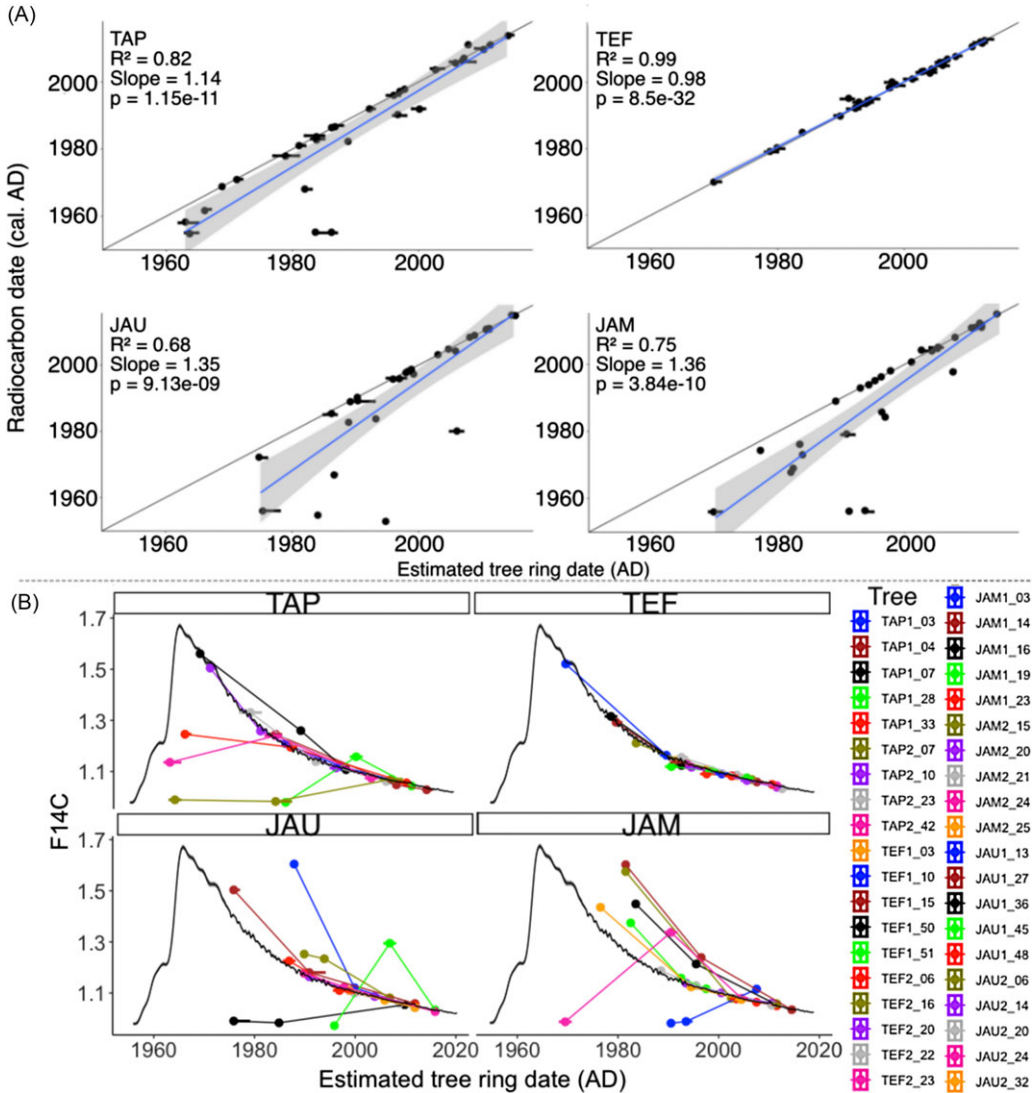


Figure 3. (A) Relationship between the tree-ring dates and the radiocarbon dates (cal. AD) for each site where Brazil Nut (*Bertholletia excelsa*) was collected in Tapirapé Aquiri National Forest (TAP), Tefé National Forest (TEF), Jau National Park (JAU), and Jamanxim National Forest (JAM), in Brazilian Amazonia. The horizontal lines in the dots indicate when more than one tree-ring was sampled for radiocarbon analysis. The black line indicates the slope = 1, along the values would be expected to fall if the dates matched perfectly, the blue line is the linear regression between cal. AD measurements and visually dated ages, and the grey area is the standard deviation. (B) For each site, the SH3 Post-Bomb Radiocarbon Calibration Curve (Hua et al. 2021) is plotted ($F^{14}C \pm 2\sigma$) alongside the estimated tree-ring dates (AD) and their respective $F^{14}C$ values. The three samples from each tree are connected by a line for easy identification.

The first two components of the PCA accounted for 63.3% of the variance. The variables that contributed the most to the first component (PC1), which explained 37.0% of the variance, were Al, Fe, Ca, and O.M. PC1 revealed a separation between the trees from TEF and JAU sites and those of the JAM and TAP sites. The variables that contributed the most to PC2, which explains 26.3% of the

Table 1. Mean diameter at breast height (DBH), tree-ring-based annual growth increment, ¹⁴C-based annual growth increment from the post bomb period (1965–2018), tree-ring-based age estimate and ¹⁴C-based age estimate for the Brazil Nut trees (*Bertholletia excelsa*) in the Tapirapé Aquiri National Forest (TAP), Tefé National Forest (TEF), Jau National Park (JAU) and Jamanxim National Forest (JAM) in Brazilian Amazonia. All means are followed by their respective standard deviations and the sample minimum and maximum

Site (n of trees)	DBH (cm)	Post-bomb tree-ring based annual growth (cm/year)	¹⁴ C based annual growth (cm/year)	Tree-ring based age estimate (years)	¹⁴ C based age estimate (years)
TAP (10)	138.08 ± 48.43 (75.2–239.4)	0.61 ± 0.11 (0.26–1.35)	0.51 ± 0.09 (0.13–1.05)	256 ± 41 (73–450)	356 ± 63 (72–696)
TEF (10)	91.27 ± 30.66 (63.4–163.0)	0.91 ± 0.05 (0.59–1.29)	1.27 ± 0.21 (0.67–2.45)	98 ± 12 (58–180)	92 ± 17 (31–203)
JAU (10)	92.33 ± 36.07 (36.3–160.1)	0.72 ± 0.05 (0.42–0.98)	0.47 ± 0.09 (0.10–1.16)	145 ± 22 (59–258)	263 ± 58 (80–606)
JAM (10)	95.58 ± 38.75 (45.7–167.2)	0.78 ± 0.08 (0.41–0.98)	0.61 ± 0.15 (0.31–1.45)	141 ± 29 (46–329)	290 ± 84 (39–718)

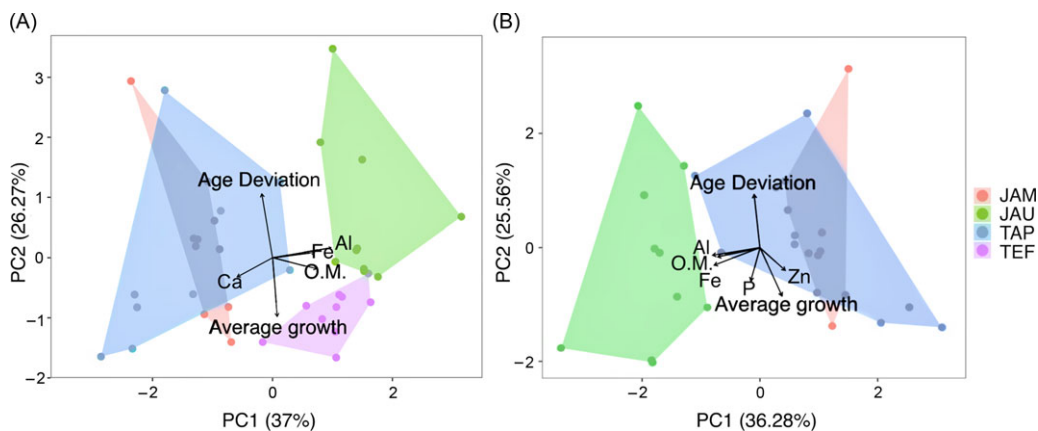


Figure 4. Principal Component analysis of the Brazil Nut trees (*Bertholletia excelsa*) in the Tapirapé Aquiri National Forest (TAP), Tefé National Forest (TEF), Jaú National Park (JAU) and Jamaxim National Forest (JAM) in the Brazilian Amazon. (A) PCA including all sites (TAP, TEF, JAU, and JAM) based on the multiple linear model with the best AIC in relation to the Age Deviation. Each point represents a tree-ring. (B) PCA excluding the TEF site, based on the multiple linear model with variables most related to Age Deviation for TAP, JAU, and JAM. The tree-rings of each sampling site are outlined with a convex hull. The vectors represent the direction in which the independent variables have the highest information.

variance, were mainly the Age Deviation and the average growth (Figure 4a). In our second PCA (Figure 4b), after excluding TEF from the analysis, we selected another multiple linear model containing variables that were the most related to Age Deviation for TAP, JAU and JAM. These variables included the average growth, Fe, Al, O.M., Zn, and P. This model had an R^2 value of 0.55 and an adjusted R^2 of 0.43, ($p < 0.01$), and the variance explained by the first two principal components was 61.8%. The variables that contributed the most to PC 1 were Al, Fe and O.M., and PC1 separated the trees from the JAU site from those of the JAM and TAP sites. The contribution of PC2, explaining 25.6% of the variance, was mainly driven by Age Deviation, average growth, and P (Figure 4b).

Age and Growth Estimates for the Large Trees

The five large trees that had the innermost portion of their cores dated by radiocarbon dating, showed average growth based on tree-ring measurements ranging from 0.213 to 0.641 cm per year (Supplementary Material 7). The length of these cores ranged from 75.8 to 108 cm. TEF2_60, TAP1_30, and TEF2_61 presented younger cal. AD values, with a wide range of possible calibrated (SHCal20, $\pm 2\sigma$) dates, spanning from the late 18th century to the early 20th century (Figure 5). The probability distribution of the cal. AD values for JAU1_33 and JAM1_21 ranges from 1505 to 1665 calAD (Figure 5), which indicates that these individuals are nearly four centuries old according to their cal. AD values alone. The tree-ring ages for the innermost tree-rings of the cores fit within their respective cal. AD limits for the trees from JAU and TEF sites. The tree-ring age of JAM1_21 is two years younger than the limit of its cal. AD, indicating the occurrence of missing rings (Supplementary Material 7). For TAP1_30, the tree-ring age falls between the cal. AD values, but does not fit the probability distribution interval. The ages of these trees, estimated by the increments based on the visually dated tree-rings, ranged from 137 to 681 years (Figure 5).

OxCal v4.4 Bronk Ramsey (2020); Atmospheric data from Hogg et al (2020)

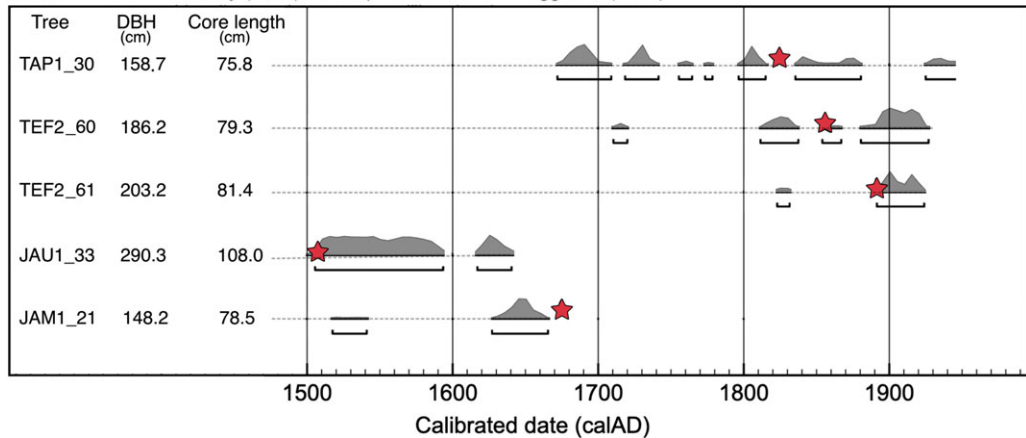


Figure 5. Ages of the innermost part of the cores of Brazil Nut trees (*Bertholletia excelsa*) collected in the Tapirapé Aquiri National Forest (TAP), Tefé National Forest (TEF), Jau National Park (JAU) and Jamaxim National Forest (JAM) in Brazilian Amazonia, calibrated with SHCal20 (Hogg et al. 2020) in the software OxCal v4.4 (Bronk Ramsey 2020). The position in the core where the samples were taken is represented by the Core Length. The grey areas represent the probability distribution (94.5%, 2σ) of the calibrated ages, and the red stars represent the estimated ages based on the visual identification of the tree-rings of each individual. This image was adapted from OxCal v4.4 (Bronk Ramsey 2020).

Discussion

The Age Deviation presented by some trees within and between sites in this study highlights the complexity of ring formation in tropical trees, even for the same species growing in distinct regions of the Amazon basin. Although problematic anatomical features in wood, such as false and discontinuous rings, are common in tropical species (Brienen and Zuidema 2006), the Age Deviation found in some trees is unlikely to be purely anatomical in origin as the difference in dating between two well-marked rings in the same sample sometimes exceeded more than ten years. From the 120 analyzed samples, 38 samples showed mismatches (31.6%). At the level of individuals ($n=40$), only 4 trees (10%) presented mismatches in all rings, with most of these coming from the JAM site (20% of the trees from this site). In other words, at least 90% of all analyzed trees have evidence of annual ring formation (with at least one sampled tree-ring showing congruence between dating methods). Therefore, we believe that the main problem lies in the correct identification of tree rings, as radiocarbon dating suggests higher ages than those derived from tree rings in most cases (Figure 3). There can be errors in the correct anatomical identification of tree rings, especially individuals that present slow growth rates, as they have narrower tree-ring boundaries and probably more consecutive missing rings. Frequently occurring scars, mainly injuries caused by bark removal or by fire, can also lead to dating errors. For Brazil Nut tree-rings analyzed from individuals of known age (Schöngart et al. 2015), it is clear that the rings associated with injuries are not always of an annual nature. As indicated, the mismatch between the two dating techniques is negatively correlated with diameter growth increments (slow-growing trees have a greater Age Deviation), likely resulting in the formation of missing or wedge rings. Wedge rings would explain the fact that in most trees that show incompatibilities, at least one sample shows congruence between the two dating methods. Wedge rings are often the result of limited growing conditions that may be related to severe weather events, limited light conditions or other factors, such as fire recovery or damage to bark (Lorimer et al. 1999; Novak et al. 2011; Novak et al. 2016). Finally, although we did not detect a synchronous pattern in our samples that would suggest the mismatch could be a product of the

atmospheric ^{14}C signal, it is worth mentioning that the existing post-bomb calibration curves are constructed with some uncertainty, especially for the Amazon region (Hua et al. 2021).

On the other hand, soil attributes such as P, Zn, Cu, clay, and water content have a positive impact on the growth in diameter of Brazil Nut trees (Costa et al. 2022), and our analysis also indicates that higher levels of nutrients, such as P, associated with other benefits of forest management (mainly light availability), contribute to annual ring formation, as is the case for the trees growing at TEF. This might also be a product of the occurrence of *Terra Preta* at the TEF site, since the presence of this highly fertile anthropogenic soil is an important factor for the growth of Brazil Nut trees (Simões et al. 2015; Silva et al. 2018). The reduction of limiting factors in the growth of this species, such as competition for light and nutrients, might allow greater sensitivity to climatic factors and the formation of annual rings stimulated by rainfall seasonality. The Brazil Nut tree shows marked differences in growth in relation to light exposure and management (Scoles and Gribel 2021; Costa et al. 2022), which we believe is a key factor in understanding the differences between our sites. The trees from TEF, which showed practically no deviation between the dating methods and higher average growth, present management conditions similar to trees in plantation conditions analyzed by Schöngart et al. (2015), in which the number of tree rings coincided with the age of planting and where tree-ring series between trees were successfully cross-dated. Unlike the Brazil Nut stands at JAU, TAP, and JAM, which eventually undergo management during seed collection, the rapid growth of TEF is likely related to the fact that this site has undergone continuous management for at least the last eighty years (Shepard and Ramirez 2011).

Practices such as removing lianas, which increases light on the canopy and therefore enhances fruit production (Kainer et al. 2006, Shepard and Ramirez 2011), and opening of the area for planting are reported to have taken place since the early 1940s (Shepard and Ramirez 2011) and are still observed at the site today along with manioc farming and the management of other species. Fruit production may also be a contributing factor in the non-formation of annual tree-rings. Hadad et al. (2021), for example, showed that during years of seed maturation and dispersal, the growth of female *Araucaria araucana* was significantly reduced. Although *Araucaria* is phylogenetically distinct from *Bertholletia excelsa*, further investigation comparing annual increment rates with fruit production may reveal whether this factor also plays a role in Brazil Nut growth. It may be that there are changes in carbon allocation between growth and reproduction for Brazil Nut trees, which can produce more than two hundred woody fruits per tree (Ribeiro et al. 2014; Rockwell et al. 2015), that can weigh more than a kilogram each. Overall the fact that active management on good soils seems to be the main force behind the lack of Age Deviation at TEF, as it promotes higher growth rates, suggests the interesting scenario that trees growing on sites with long histories of human occupation and management will not only be of importance for dendrochronological study of past anthropogenic forestry activities, but also potentially for producing the best climatic records, due to the fact that these trees growing under forest management are also the ones that show annual rings.

The dynamics by which Brazil Nut ascends to the canopy greatly influences its growth, something observed both in plantation monitoring (Scoles and Gribel 2021) and through growth release events observed in tree-ring analysis (Brienen and Zuidema 2006; Schöngart et al. 2015; Caetano Andrade et al. 2019). Even trees established in the canopy can undergo sudden changes in growth when they benefit from intensified management (Caetano Andrade et al. 2019). This reveals the enormous potential that this species has for investigating forest disturbances, as has been done by Baker et al. (2005), linked not only to human management, but to other factors such as extreme weather events (i.e., floods, droughts), and blowdowns that occur stochastically in the Amazon (Marra et al. 2014). In addition, disturbances related to severe climate conditions showed a remarkable reduction in diameter growth for a Brazil Nut population in Central Amazonia in the 1860s (Caetano-Andrade et al. 2019), which might be the result of pluriannual drought conditions, shown by tree-ring based reconstruction of precipitation (Granato-Souza et al. 2019, 2020). Unlike the study of Herrera-Ramirez et al. (2017), in the Atrato River (Colombia), our study demonstrates a low discrepancy between dating methods for the bomb period for TEF, however our crossdating attempt was unsuccessful. The small number of samples, the impossibility to get cross-sections, and the great sensitivity to changes in light conditions are the

most likely reasons why the dendrochronological results for TEF are not statistically significant for dendroclimatic reconstructions. However, the annual nature of the tree-rings at this site indicates its potential for climatic analysis, although a higher number of samples are necessary for properly accessing this potential.

The time-span over which forest management and climatic influences on growth can be studied through Brazil Nut dendrochronology depends inherently on what age Brazil Nut trees can achieve (at least in the absence of rings from preserved, dead trees). In Camargo et al. (1994), extrapolation based on the growth increment rate of a Brazil Nut individual (440 ± 60 years, and DBH of 233 cm) yielded an estimate of 1000 years for other large Brazil Nut trees occurring at the same site, in Para state, Brazil (Camargo et al. 1994). Similarly, radiocarbon based increment rates and age estimates in Vieira et al. (2005) yielded ages ranging from 668 to 996 years for Brazil Nut trees occurring in Acre state, Brazil. However, we observed that in trees where visually dated tree-rings were congruent with their cal. AD values, extrapolation of growth rates based only on radiocarbon dating can provide ages both higher or lower than those based on tree-ring estimates. This is because estimations based solely on the post-bomb growth rates consider a relatively short period in the tree's life, and not their entire life span, which means, for example, that they do not account for the fact that the diameter of a tree grows at faster rates when the tree is young and at slower rates with older age (Speer 2010). Therefore it is risky to assume an average growth increment rate for the entire life of the tree based only on the post-bomb growth rate estimates, especially as the species can present large variations in growth rates (Schöngart et al. 2015; Caetano Andrade et al. 2019; Scoles and Gribel 2021; Costa et al. 2022). Furthermore, for the period between cal. AD 1650 and cal. AD 1950, there is a lot of uncertainty produced by the radiocarbon calibration curve due to the variation in the cosmogenic production of ^{14}C in this period, which makes accurate radiocarbon age estimations difficult (Worbes 2002), although some approaches, such as “wiggle-match dating” for age modelling, have been successfully applied for dating wood from this period in South Africa (Norstrom et al. 2005).

In this study, the cal. AD interval ranges of the innermost portion of the cores taken from the five large trees (Figure 5), together with their visually dated tree-ring age estimates, highlights the importance of advancing the field of tropical dendrochronology through multidisciplinary and multiproxy approaches (i.e., ^{14}C , X-ray, dendrochronology, direct measurements), as a single method alone is not always accurate (Schöngart et al. 2015; Baker et al. 2017; Santos et al. 2022). The use of other isotopes, such as $\delta^{18}\text{O}$ for example, can provide a parallel line of information about growth periodicity in tropical trees (Baker et al. 2015; Ohashi et al. 2016). Three of the large trees analyzed here showed values ranging between cal. AD 1650 and cal. AD 1950, and the other two presented older cal. AD ranges, between cal. AD 1505 (517 years) and cal. AD 1665 (357 years) (Figure 5, Supplementary Material 7). The longest core analyzed in this study (JAU1_33, 108 cm long) had its innermost portion estimated at 510 years by the visual identification of tree-rings, which fits at the beginning of its cal. ^{14}C interval range. The maximum ages reported here are within the range of those reported for tropical angiosperms elsewhere (Brienen et al. 2016; Schöngart et al. 2017), as well as for other climate zones, based on tree-ring analyzes (Lara and Villalba 1993; Brown 1994; Lusk 1999; Schweingruber and Wirth 2009; Morales et al. 2012; Stahle et al. 2016; Di Filippo et al. 2015; Locosselli et al. 2020). However, radiocarbon dating suggests higher maximum ages for some angiosperms, especially in the tropics (Chambers et al. 1998; Kurokawa et al. 2003; Vieira et al. 2005; Patrut et al. 2018). Overall, we present the oldest direct evidence of age produced by the joint use of dating methods for Brazil Nut, although ^{14}C dates have documented ages of around a thousand years for this species (Vieira et al. 2005).

Regardless of whether older living Brazil Nuts are found in future, if we consider the average growth increment to the centre of the oldest tree analyzed here, JAU1_33, the estimated age for this tree is 681 years. This means that its lifespan covered significant historical and climatic events over the last half millennium, highlighting the potential of dendrochronological studies of Brazil Nut to explore changes in human management as well as long-term climatic and environmental change. So far, no long and statistically robust tree-ring chronologies have been built with this species, despite several attempts

(Schöngart et al. 2015; Caetano Andrade et al. 2019). Although some tree-ring series show matches of cross-dating, as shown in this study, at sites with the potential to construct long tree-ring chronologies (i.e., with old trees and slow diameter growth), our results indicate problems of dating, probably due to frequently occurring wedge rings (or missing rings) and errors in the correct identification of tree-ring boundaries by macroscopic wood anatomy. To build up a chronology, many cores must be sampled many trees at a given location, and ideally also from cross-sections from harvested/dead trees, when available. At sites with favourable growing conditions, such as TEF, the potential to construct tree-ring chronologies is higher, however, the chronologies will hardly span more than two centuries, as trees that grow fast yield lower ages, even for large trees.

Conclusions

Radiocarbon dating and tree-ring counting showed significant variation in the growth rates of *Bertholletia excelsa* across the Brazilian Amazon basin. Low growth rates associated with poor light and edaphic conditions are related to the occurrence of missing and wedge rings. On the other hand, trees growing on highly fertile *Terra Preta* soils, under constant management conditions, grow more and have fewer missing rings. Radiocarbon estimates of growth increments and ages suggest that ages estimated by tree-rings are underestimated for this species. However, assuming growth rates with the bomb period alone may not reflect the growth over the entire life of the tree, and the uncertainties produced by ^{14}C variation in the calibration curve make it hard to accurately date the age of individuals between cal. AD 1650 and cal. AD 1950 by direct radiocarbon measurement alone, making the application of age modeling approaches important for future work. Nevertheless, even though our measurements of Age Deviation reveal that it is hard to accurately date the tree-rings for some trees and, consequently, to perform dendroclimatological analysis, where rings could be demonstrated to be annual we could access the potential of crossdating the individuals. The oldest estimate for an age reconstructed by independent methods in this study is 681 years, for a tree with 290 cm DBH, and represents an advance in the discussion about the maximum ages that this species can reach. Although more work is needed to understand the growth and ring production of Brazil Nut across its range in the Amazon Basin, this study demonstrates the value of these trees not only as key species in Amazonian ecosystem functioning and carbon cycling, but also as long-term recorders of past human activities and climatic and environmental change.

Supplementary material. To view supplementary material for this article, please visit <https://doi.org/10.1017/RDC.2024.39>

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