



Cold and humid Atlantic Rainforest during the last glacial maximum, northern Espírito Santo state, southeastern Brazil

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ABSTRACT

The Atlantic Rainforest, covering the area from northern Espírito Santo to Southern Bahia states, has been considered as a stable forest during Pleistocene Glacial times. Aside from modelling and phylogenetic studies, this hypothesis has never been tested with empirical, fossil pollen, paleo-vegetation data. By using palynology, radiocarbon dating, and carbon and nitrogen elemental and isotopic analyses of organic matter, we reconstructed the vegetation dynamics and inferred climatic changes since the Late Pleistocene in the centre of this global biodiversity hotspot. Our results show that the forest biome was resilient to Last Glacial Maximum - LGM conditions, but that the floristic composition differed from that of today. Since the late glacial, the floristic composition of the dense forest changed from cold- to warm-adapted taxa. Structural, as well as floristic, changes occurred during drier conditions of the early and middle Holocene, with an opening of the forest, providing insights into the type of vegetation impacts that might be expected under predicted future drought.

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1. Introduction

A number of theories have been proposed over the years to explain the exceptionally high levels of endemism (Prance, 1982, 1987; Mori et al., 1981), biodiversity (Smith, 1962), and botanical uniqueness (Mori et al., 1981) of Brazil's Atlantic Rainforest. From Northern Espírito Santo to Southern Bahia, the Atlantic Rainforest was considered stable during the last glacial period of the Pleistocene, referred to as the Bahia forest refuge (Prance, 1982) or as the most "historically stable region of Atlantic forest" (Carnaval and Moritz, 2008). Today it is considered a global biodiversity hotspot (Carnaval et al., 2009).

In this context, paleoenvironmental studies have contributed to

understanding the response of the Atlantic Rainforest to Quaternary climate change, especially with respect to the long-standing debate over the rainforest refugia hypothesis, i.e. the degree to which tropical rainforest was replaced by savanna during glacial periods.

A wide range of climatic conditions span the length of Brazil's Atlantic Forest, which has been divided into three zones: North Atlantic Forest (NAF; 5°–15°S), Central Atlantic Forest (CAF; 15°–23°S), and South Atlantic Forest (SAF; 23°–30°S) (Ledru et al., 2017). The southern area hosts species which are mainly adapted to cool wet climates, such as *Araucaria angustifolia*. The central area, where the study area is located, is composed of coastal forest and inland areas are dominated by semi-deciduous forests, whereas the northern area represents contains moist semi-deciduous forests restricted to the coastal zones, lowlands and mountaintops (Ledru et al., 2016, 2017).

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The response of the Atlantic Rainforest to LGM conditions is poorly understood due to the paucity of empirical palaeovegetation data, most of which comes from ecotonal areas rather than core areas of rainforest. At forest-savanna ecotones of central and western regions of the Atlantic Forest, fossil pollen data show that the Last Glacial Maximum (LGM) was characterized by the expansion of grasslands and savannas at the expense of forest (Behling and Lichte, 1997; Stevaux, 2000; Ledru et al. 2005, 2009), corroborated by stable carbon isotope analyses of soil profiles (Gouveia et al., 2002; Pessenda et al., 2004; Saia et al., 2008). However, there is some pollen data to suggest that some ecotonal forests may have survived as micro-refugia during the LGM, where microclimates remained sufficiently humid (De Oliveira, 1992; Cruz Junior et al., 2006, 2007; Pessenda et al., 2009).

Ecological niche models and phylogenetic studies of small animals suggest that the central core area of the Atlantic Rainforest biome (northern Espírito Santo to southern Bahia states) persisted as a major Pleistocene forest refuge. It has been argued that this long-term rainforest stability explains the exceptionally high levels of biodiversity and endemism in this central core rainforest area (Carnaval and Moritz, 2008; Carnaval et al., 2009; Leite et al., 2016). However, empirical palaeovegetation data are absent from this central core rainforest area, and are needed to test these modelling and phylogenetic studies that this region constituted a major Pleistocene rainforest refuge.

This paper presents the first continuous late Quaternary palaeovegetation record (based on fossil pollen and stable isotope data) from the central core area of Atlantic Rainforest, spanning the last 30,000 years. The key aim is to examine the response of the Central Atlantic Rainforest to glacial and Holocene climate change.

1.1. Regional settings

At the northern coast of Espírito Santo state, Brazil, between Doce and São Mateus rivers, two ecological reserves of Atlantic Rainforest, Vale Nature Reserve (VNR) and Sooretama Biological Reserve (ReBio Sooretama) cover an area of approximately 50,000 ha of preserved vegetation (Fig. 1).

The sampling point, known as Brejo do Louro (BL), comprises a herbaceous, slightly depressed, bog surrounded by dense Atlantic Rainforest, ~33 km far from the current coastline, ~45 m above sea-level (m.a.s.l), and located at 19°06'32.2"S, 40°01'53.8"W. The elevation difference between the bog and the adjacent forest is ~4–5 m. The bog becomes flooded to a depth of ca. 0.5 m during the rainy season, creating an anoxic, water-logged environment, suitable for peat development and preservation of organic remains.

Beneath the peat, the substrate contains coarse-grained sand, classified as Spodosol (Santos et al., 2013), based on maps of soil distribution and previous research (Schiavo et al., 2020) in the study area. Spodosols are usually found in cold climates, such as northern Europe and Central Asia, but can be present in tropical regions too. In Brazil, Spodosols are mainly present in coastal environments (Gomes et al., 2007; Oliveira et al., 2010; Coelho et al., 2010) and parts of Amazonia, such as the upper Rio Negro basin (Mafra et al., 2002). These soils are relatively young, nutrient poor, and strongly influenced by water table fluctuations (Calegari et al., 2017).

The regional climate is strongly seasonal, classified as "Aw" - tropical humid - in the Köppen System (Köppen, 1948), with an average annual temperature ranging between 20 and 26 °C. The mean annual precipitation is 1215 mm (data obtained at RNV

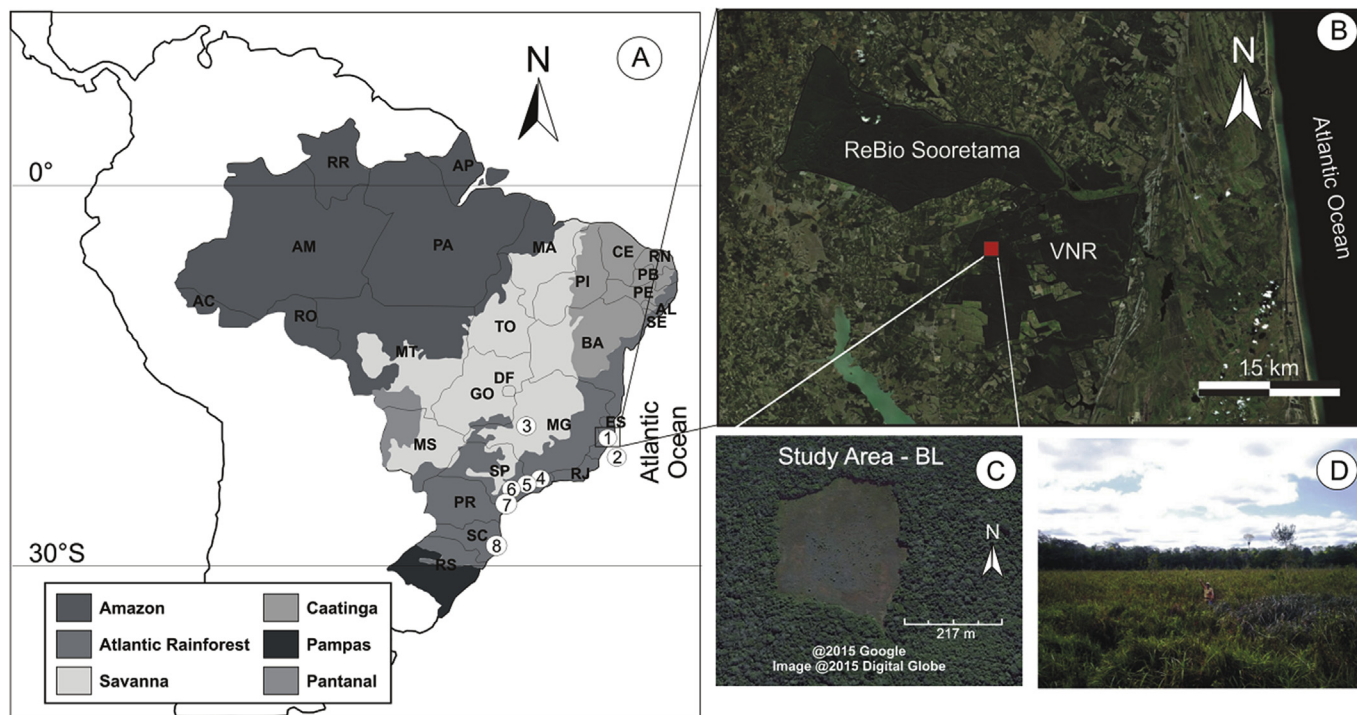


Fig. 1. Study area. A) Map of Brazil showing the distribution of biomes and the study area. The numbers indicate paleoenvironmental records of southern and southeastern Brazil. 1. Linhares (ES): Buso Junior et al., (2013), 2019; Buso Junior (2015). 2. Marine Core GeoB3202-1 (ES): Behling et al., (2002). 3. Salitre (MG): Ledru (1996). 4. Jacaré (SP): Garcia et al. (2004). 5. Colônia Crater (SP): Ledru et al. (2009). 6. Curucutu (SP): Pessenda et al. (2009). 7. Cardoso Island (SP): Pessenda et al. (2012). 8. Botuvera Cave (SC): Cruz Junior et al., (2005), 2006. The red dot indicates the location of the Brejo do Louro (BL) study site. B) Image obtained from Google Earth showing BL bog surrounded by dense forest. C) Aerial photograph of BL. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

meteorological station), largely concentrated during austral summer, controlled by the South America Monsoon System (SAMS) and the South Atlantic Convergence Zone (SACZ) (Carvalho et al., 2004; Garreaud et al., 2009). The dry season occurs during austral winter, from May to September. The other two important air masses that bring humidity to this region are the South Atlantic Trade Winds and the Atlantic Polar Front (Dominguez et al., 1992). The annual rainfall increases and the seasonality decreases from Espirito Santo to Southern Bahia, sustaining warm and humid coastal rainforest (Oliveira-Filho and Fontes, 2000).

Vegetation of the region has diverse phytophysiognomies of the Atlantic Rainforest *latu sensu* domain (Rizzini, 1997), largely comprising *Tabuleiro* forest (1), grassland (2) and a narrow strip of mangroves along the coast;

- (1) Characterized by a canopy around 30 m, containing emergent trees up to 40 m (Rizzini, 1997:30). It also has a lower arboreal stratum, 20 m in height, with a herbaceous stratum below. Some characteristic *taxa* have disjunct distributions with the Amazon biome (Mori et al., 1981), such as *Parkia pendula*, *Swartzia polyphylla*, *Lycania cymosa* and *Anthodiscus amazonicus*. species endemic to the *Tabuleiros* forest include *Hydrogaster trinervis*. *Tabuleiro* forest occurs from Rio de Janeiro to Pernambuco, but the central core area lies in our study area, from northern Espirito Santo to Southern Bahia, above the Neogene deposits of the *Barreiras* Formation.
- (2) Also called *campos nativos* or *mussununga* vegetation, growing in Spodosol patches which become flooded during the rainy season (IBGE, 1987). *Campos nativos* range from grasslands to woodlands, with sparse trees due to poor soil-water retention (Sporetti-Junior et al., 2012). Includes stress tolerant and mesic species within the Poaceae, Melastomataceae, Bromeliaceae, Bignoniaceae, and Myrtaceae families (Candido et al., 2019).

2. Material and methods

2.1. Sampling and substrate features

A 123-cm long core was collected in the middle of Brejo do Louro bog using a vibro-corer aluminium tube system. The water table was right below the bottom of the core tube. At the ^{14}C Laboratory of CENA/USP, substrate samples were systematically subsampled at 2 cm intervals for laboratory analyses and described according to variations in colour, grain size, and texture. Roots, charcoal and plant fragments were also recorded and collected. For grain-size analysis, sub-samples were sent to the Geosciences Institute at the University of São Paulo (USP), where analysis was undertaken using a Laser Diffraction Particle Size Analyzer Malvern Mastersizer - 2000.

2.2. ^{14}C dating and age-depth model

Fourteen substrate organic matter samples were submitted for accelerator mass spectrometer (AMS) radiocarbon dating at the University Federal Fluminense Radiocarbon Laboratory (LACUFF) and Centre for Applied Isotope Studies at the University of Georgia, USA (UGAMS). Samples were physically and chemically treated according to Pessenda et al. (2009). Radiocarbon dates were reported as ^{14}C yr (1 σ) BP (Before, 1950 AD) and in calibrated years as cal yr (2 σ) BP (Reimer et al., 2013) and each sample normalized to isotope fractionation ($\delta^{13}\text{C}$) with respect to VPDB (Vienna Pee Dee Belemnite Standard) in the conventional δ (‰) notation, with standard deviation of $\pm 0.2\text{‰}$. The Bayesian age-depth model of the

BL core was constructed using the Bacon R package v.2.3.5 (Blaauw and Christen, 2011) and Intcal13 calibration curve (Reimer et al., 2013). All results and interpretations are based on the mean calibrated age (mean cal yr B.P.).

2.3. C and N analyses

Elemental and isotopic analyses of total carbon and nitrogen of organic matter were performed at 2 cm resolution and bulk substrate samples pre-treated physically and chemically according to Pessenda et al. (1996). The most representative modern plant species around the sampling point were also analysed to determine their stable carbon isotope signature. Modern plants collected around the sampling point were washed in deionized water, dried at 40 °C, and grinded. Plant and substrate subsamples were then sent to the Stable Isotope Laboratory of CENA/USP (São Paulo, Brazil), and analyses were carried out on an elemental analyzer attached to a Mass Spectrometry ANCA SL 2020 of Europa Scientific. Total Carbon and Nitrogen are expressed as percentages of dry weight. $\delta^{13}\text{C}$ are measured with respect to VPDB and using the conventional δ (‰) notation, with standard deviation of $\pm 0.2\text{‰}$.

2.4. Palynology

Subsamples of 2 cm³ were taken at 2-cm intervals and processed with the addition of two spikes of exotic *Lycopodium clavatum* spores (Batch number: 177,745, Lund University, 2008) to determine pollen and spore concentrations (Colinvaux et al., 1999). The samples were sieved (0.210 mm) before the chemical treatment, due to the high concentration of coarse-grained sand. The procedure was based on the addition of HF to remove minerals, KOH and acetolysis to remove humic acids and the organic contents of the palynomorphs. For each sample, at least 300 terrestrial pollen grains (trees, shrubs and herbs) were counted using ZEISS photomicroscopes at 1000x. Non-terrestrial taxa, fern spores and algae were not included in the total pollen sum. Identification was based on the pollen reference collection of the ^{14}C Laboratory (CENA/USP) and from the University of Reading, UK. Pollen diagrams were plotted using TILIA and TGView 1.7.16 and CONISS - cluster analysis by similarity index - to calculate the zone boundaries (Grimm, 1992).

3. Results and discussions

3.1. ^{14}C dating

The ^{14}C dating results of the BL core (Table 1) revealed ages ranging from ~35,910 cal yr. BP (119 cm) to ~2140 cal yr. BP (11 cm), and substrate accumulation rates ranging from 0.007 mm/yr (49–45 cm) to 0.44 mm/yr (53–49 cm). Fourteen radiocarbon dates from a 123 cm core enabled a robust chronology to be obtained. Age inversions occurred between 71 (~20,880 cal yr. BP), 61 (~22,020 cal yr. BP) and 57 cm (~20,940 cal yr. BP). All the samples received the same chemical treatments, and both LACUFF and UGAMS laboratories were inter-calibrated (Macario et al., 2013).

The age inversions may be due to erosion processes and/or bioturbation during the soil formation (Boulet et al., 1995). Furthermore, the content of organomineral complexes in the samples may have influenced the radiocarbon dating, since organic clay complexes tend to rejuvenate soil organic matter formation (Scharpenseel and Becker-Hedemann, 1992). A similar interpretation can be found at Nativo do Flamengo, 11 km from BL (Buso Junior et al., 2019). Also, we cannot discard a depositional hiatus between 53 and 49 cm (19,310 to 13,740 cal yr BP), and 45 to 41 cm

Table 1
AMS radiocarbon ages and accumulation rates of the BL.

Laboratory Number	Depth (cm)	Sample	Age conventional (¹⁴ C yr. BP)	Age (cal yr. BP, 2σ)	Mean calibrated age (cal yr. BP)	Deposition time (mm/yr)
UGAMS21574	11	Substrate	2120 ± 25	2002–2283	2140	0.05
LACUFF140148	21	Substrate	6374 ± 35	7253–7417	7300	0.02
UGAMS21576	41	Substrate	8430 ± 30	9466–9533	9500	0.09
LACUFF160058	45	Substrate	11,835 ± 79	13,467–13,792	13,650	0.009
LACUFF160037	49	Substrate	11,915 ± 81	13,555–13,988	13,740	0.44
LACUFF160041	53	Substrate	16,004 ± 92	19,035–19,563	19,310	0.007
LACUFF160062	57	Substrate	17,356 ± 104	20,636–21,278	20,940	0.02
UGAMS21577	61	Substrate	18,160 ± 45	21,830–22,212	22,020	0.04
LACUFF160048	71	Substrate	17,312 ± 101	20,590–21,202	20,880	-
UGAMS21578	81	Substrate	21,430 ± 55	25,606–25,904	25,760	0.02
LACUFF160052	85	Substrate	23,148 ± 165	27,143–27,701	27,440	0.02
LACUFF160034	95	Substrate	27,448 ± 262	30,944–31,741	31,300	0.03
UGAMS15864	103	Substrate	29,210 ± 120	33,077–33,760	33,460	0.04
LACUFF160038	119	Substrate	32,024 ± 416	34,949–36,848	35,910	0.06

UGAMS, University of Georgia, USA; and LACUFF, Fluminense Federal University, Brazil.

(13,650–9500 cal yr BP), probably due to erosion processes associated with the water table movements in the bog.

3.2. Substrate features

No substrate structures were visible along the core. Medium grained sand (>50%) containing organic matter dominates the profile from 123 to 20 cm, >35,910–7300 cal yr BP (Fig. 2). The range of sand compounds varies from 83 to 90%, and from 50 to 80% along the 119 - 75 cm (35,910 to 24,260 cal yr BP – estimated age) and 75 - 0 cm (<24,620 cal yr BP – estimated age) intervals, respectively; followed by silt (5–30%). From ~16 cm depth to the core top (<4000 cal yr BP), vegetal remains and roots were preserved, probably due to the proximity to the water table most of the year.

Considering the depression where the sampling site is located, and the relative abundance of mud along the stratigraphic profile, this deposit was probably formed by a vertical accretion in a lake over at least the last 36,000 years. This environment favored the continuous accumulation and preservation of pollen grains. However, post-depositional processes may have increased the sand/mud ratio along the core, since previous research has shown that such deposits undergo podsolization (Santos et al., 2004). This process causes weathering and migration of aluminium, iron, and organic matter along the profile, with the formation of a cemented B spodic horizon characteristic of Spodosols (Santos et al., 2009;

Schiavo et al., 2020). The organic matter content, its quality, and mobilization play an important role in the weathering of minerals and the transfer of metal ions (Fritsch et al., 2009, 2011; Nikodem et al., 2013). This process may have favored the proportional increase in the sand fraction in the studied deposits, where kaolinite (Si₂Al₂O₅), which constitutes the silt/clay fraction, is more easily weathered. The sandy fraction represented by quartz (SiO₂) grains is more resistant to physical and chemical weathering. The time required for the formation of Spodosols may require between 300 and 3000 years, depending on the vegetation and climatic conditions (Nikodem et al., 2013).

3.3. Late Pleistocene and Holocene vegetation

3.3.1. Late Pleistocene

Despite the substantial concentration of organic matter and TOC values around 12%, palynomorphs were not preserved in the substrate dating from ~35,900 to ~33,460 cal yr BP (119–103 cm; 0.06 mm/yr; Fig. 2). The absence of palynomorphs may be due to leaching of pollen grains caused by fluctuations in the water table, as proposed for phytoliths at this site (Calegari et al., 2017).

Carbon isotope values (δ¹³C) with a mean value of −28‰, typical of C₃ plants, indicate the presence of forest and/or C₃ grasses at the site since at least ~33,460 cal yr BP (103 cm). Tree and shrub taxa (~85%) dominate the pollen spectra throughout most of the Pleistocene sequence of the core (Fig. 2). *Tapirira* was the most abundant

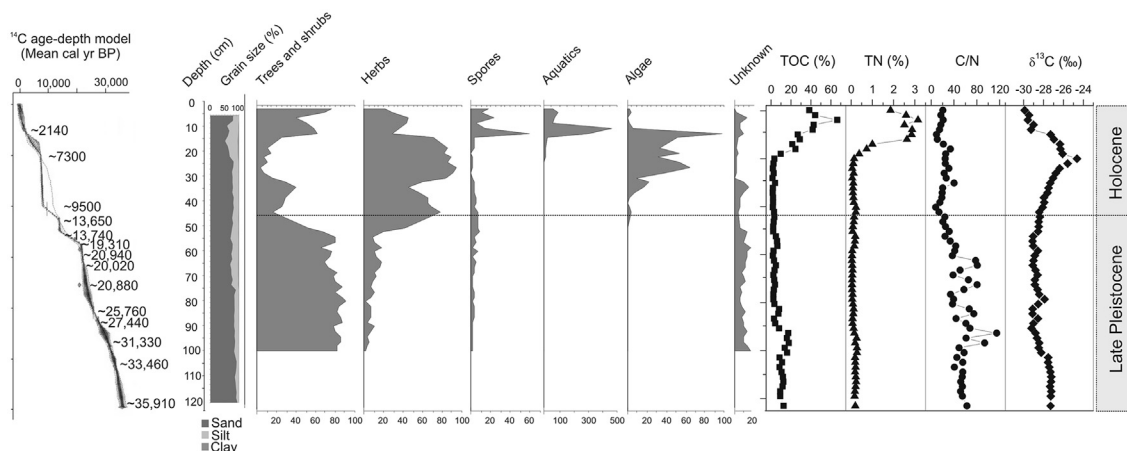


Fig. 2. ¹⁴C age-depth model (mean cal yr BP), grain size (%), pollen and spores groups percentage, total organic carbon (TOC), total nitrogen (TN), C/N, and ¹³C values.

(~21%), probably due to its relatively high pollen dispersal (Behling and Negrelle, 2006), followed by *Symplocos* (~12%) and *Ilex* (~10%). Together with *Podocarpus* (~4%), the pollen assemblage indicates the presence of plant communities very different from the those of the present (Fig. 3). *Symplocos*, *Ilex*, and *Podocarpus* are strongly related to high altitudes and relatively low temperatures in South and Southeastern Brazil (Oliveira-Filho and Fontes, 2000). Also, *Podocarpus* is a conifer generally indicative of cold and moist climates (Ledru et al., 2009). Furthermore, pollen traps from the Atlantic Rainforest of Santa Catarina state (Southern Brazil), a region characterized as meso-thermic with no frosts and very humid without a dry season, yield similar pollen assemblages to those found at BL from ~33,460 to ~13,740 cal yr BP (103–49 cm; 0.04–0.44 mm/yr): *Tapirira* (24.9%), *Alchornea* (12%), *Rapanea* (6.8%), *Sloanea* (5.4%), *Ilex* (0.9%) and *Podocarpus* (0.8%) (Behling and Negrelle, 2006).

Typically, forest tree taxa such as *Laplacea* (4%), *Eriotheca* (3%), *Virola* (2%), and *Sloanea* (~1%) indicate the presence of dense forest near BL (Fig. 3). The rare pollen type *Glycidendron* (present up to ~1%), a genus which currently has a disjunct distribution between the Amazon and Atlantic Rainforest (Buso Junior et al., 2013), indicates that humid tropical forest has occupied the region since at least ~25,760 cal yr BP (81 cm) (Fig. 3).

Despite the increase in Poaceae (from 7% to 37%) and decrease in arboreal/shrub taxa between ~25,760 and ~13,740 cal yr BP (81–49 cm; 0.02–0.44 mm/yr), the high abundance of arboreal taxa (90–65%) and the depleted mean value of $\delta^{13}\text{C}$ (~-28‰) signify closed-canopy tropical forest and humid climate conditions during the LGM (Fig. 2).

Two different core sections have low substrate accumulation rates (Table 1), one spanning almost 6000 years, where mean calibrated ages vary from 19,310 (53 cm) to 13,740 cal yr BP (49 cm), with accumulation rates of 0.007 mm/year, and another of 4000 years from 13,650 (45 cm) to 9500 (41 cm) cal yr BP, with accumulation rates around 0.009 mm/year and an age inversion at 71 cm (20,880 cal yr BP). Similarly, stratigraphic records indicate low sedimentation rates and age inversions for the end of the Late Pleistocene and the beginning of the Holocene in Southeast Brazil, between 23 and 12 kyr (Ledru et al., 1998; Behling and Negrelle, 2001; Behling et al., 2002; Pessenda et al., 2009). In the study area, the palynology and depleted mean value of $\delta^{13}\text{C}$ ~-28‰ attest to the dominance of forest and C₃ grasses during these periods,

signifying a humid climate (Fig. 2). Based on that climatic inference, the low accumulation rate is probably associated with the erosion processes caused by leaching of surface water and the water table movement. Despite the low activity found in similar substrates (Buurman and Jongmans, 2005), the bioturbation process (Gouveia and Pessenda, 2000) associated with the age inversion cannot be discarded.

3.3.2. Holocene vegetation

Between 13,740 and 7300 cal yr BP (49–21 cm; 0.44–0.02 mm/yr), herbaceous plants dominate the palynological records (up to 95%), and the $\delta^{13}\text{C}$ values range from ~-28‰ to ~-24‰, indicating a mixture of C₃ and C₄ sources and isotope enrichment between ~9500 cal yr BP (41 cm) and ~7300 cal yr BP (21 cm) (Fig. 2). Poaceae comprises up to ~87% of the pollen assemblage, followed by Cyperaceae (5%–8%). Phytoliths from BL also indicate the presence of grasses (up to 43%), with a mixture of C₄ (Panicoid 2.4%) and C₃ herbaceous plants (Poooid 5.4%) during the same period (Calegari et al., 2017). The genera *Tapirira*, *Ilex*, and *Symplocos*, which were highly dominant throughout the Pleistocene section of the core, decrease to negligible/low percentages of only 0.4, 1.4, and 6%, respectively, between 13,740 and 7300 cal yr BP (49–21 cm; 0.44–0.02 mm/yr). However, the presence of *Laplacea*, *Eriotheca*, *Virola*, and *Sloanea* pollen, even at low abundance, indicate that forest was present between 13,740 and 7300 cal yr BP (49–21 cm; 0.44–0.02 mm/yr) (Fig. 3).

From ~7300 cal yr BP (21 cm) to present, peat formation occurred. The TOC increases (up to 66%), the $\delta^{13}\text{C}$ ranges from ~-24‰ (~7300 cal yr BP; 21 cm) to ~-30‰ (present) and C/N x $\delta^{13}\text{C}$ (Figs. 2 and 4) indicates the presence of freshwater phytoplankton, due to the water layer that maintains local humidity and creates an anoxic environment, minimizing organic carbon degradation and supporting peat preservation. Palynological data show that the algae *Zygnema* (6–58%) and *Spirogyra* (0.3–6.7%) were abundant (Fig. 3).

From ~4000 (15 cm) to 970 cal yr BP (7 cm), pollen grains from herbaceous plants inside the bog, such as Poaceae (11%), Cyperaceae (7%), and Asteraceae (7%) were less abundant (between 25 and 32%). Tree and shrub pollen taxa around the bog reached ~55–70% abundance, comprising Melastomataceae/Combretaceae (~16%), Myrtaceae (~9%), *Alchornea* (~5%), *Tapirira* (~2%) and *Cordia* (~2%). The abundance of Moraceae/Urticaceae pollen (4–23%)

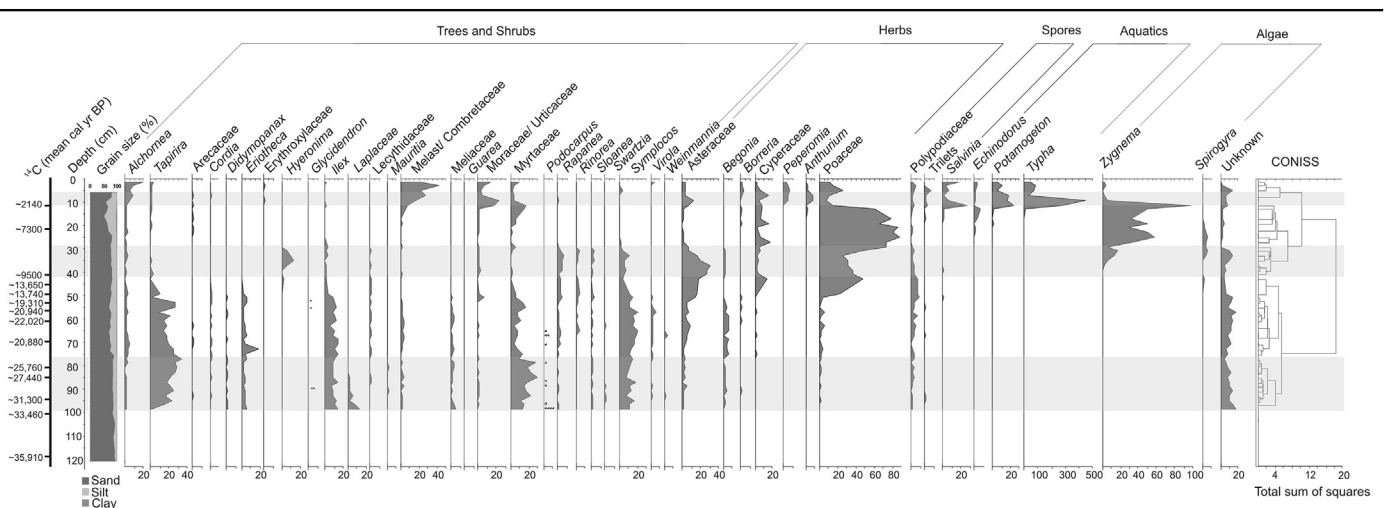


Fig. 3. ^{14}C ages, grain size (%), and taxa percentage from BL core. White and gray horizontal bars indicate zones generated by CONISS.

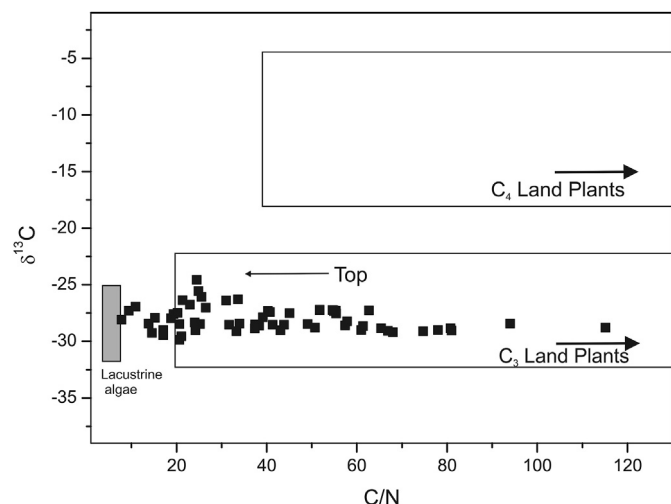


Fig. 4. Correlation between $\delta^{13}\text{C}$ and C/N and their meaning (Meyers, 2003): C_4 l and plants, C_3 l and plants, and Lacustrine algae.

indicates that forest vegetation was relatively near the bog. The abrupt peaks in abundance of *Typha* (250%), *Potamogeton* (18%), and *Salvinia* (11%) indicate local colonization of the bog (Fig. 3).

From ~970 cal yr BP (7 cm) to present, the vegetation comprised sparsely distributed small trees and shrubs (~50–73%); e.g. Melastomataceae/Combretaceae (15–42%), *Alchornea* (4–22%), Myrtaceae (3–8%), *Tapirira* (1–4%) and *Cordia* (0–1%). These families and genera are found around BL, as transitional vegetation between the bog and the dense forest. Poaceae (13–25%) and Cyperaceae (2–7%) (predominantly C_3 taxa), as well as some aquatic (70–90%) plants, such as *Typha* (55–76%), *Potamogeton* (6–10%) and *Echinodorus* (1–6%), which are prevalent during the rainy season, reflect a very local signal. Despite their low abundance, pollen of *Virola* (~3%) and *Chrysophyllum* (0.3%) signify closed-canopy forest near to BL, which surrounds the site today (Fig. 3).

3.4. Modern vegetation

The modern dominant vegetation within the bog BL is mainly composed of shrubs and herbs with $\delta^{13}\text{C}$ values varying from -20.6‰ (*Andropogon bicornis*) to -29.8‰ (*Cyperus* sp.) and small trees such as *Alchornea triplinervia*, $\delta^{13}\text{C}$ -29.3‰ (Fig. 5). The only dominant grass species, *Andropogon bicornis*, has the C_4 photosynthetic cycle ($\delta^{13}\text{C}$: -20.6‰). The range of isotopic values for C_3 plants may be attributed to the water layer present in the bog, since the high humidity is suitable for its development and establishment.

3.5. Paleoenvironmental interpretation

3.5.1. Late Pleistocene

Palynological and carbon isotopic data indicate the presence of a cold-adapted, dense forest near the BL bog from ~33,460 cal yr BP to ~25,760 cal yr BP (103–81 cm; 0.04–0.02 mm/yr). In Colônia crater, São Paulo State, Ledru et al. (2009) recorded a pollen assemblage associated with a cold forest during the time interval between 30 and 23 k cal yr BP, which reflects forest expansion and a cold and wet climate. Similar climate conditions were also characterized in Salitre, Minas Gerais State (Ledru et al., 1996), and in the marine core GeoB3202-1 (21°37'S/39°58'W; Behling et al., 2002) for the same period, and those data are comparable to BL.

From ~25,760 to ~13,740 cal yr BP (81–49 cm; 0.02–0.44 mm/yr)

the slight increase of herbs probably occurred due to C_3 Poaceae/Cyperaceae locally occupying the bog, with carbon isotope values $\sim -28\text{‰}$ (Fig. 2). In the Colônia crater, the biodiversity index decreased, and open vegetation dominated by Poaceae replaced the rainforest between 23 and 12 k yr BP. During the LGM dry climatic conditions were predominant (Ledru et al., 2009). Such climatic conditions were also characterized by other palynological records in southeastern Brazil (Ledru et al., 1996, 1998; Behling et al., 2002).

Despite the marked changes in floristic composition of the vegetation between the LGM and present at BL, we infer stability of the forest biome throughout the Late Pleistocene, since ~33,460 cal yr BP, signifying humid climatic conditions. The maintenance of forest vegetation since the Late Pleistocene in northern Espírito Santo was also noted by Buso Junior et al. (2013). According to the authors, $\delta^{13}\text{C}$ analysis of soil organic matter at the forest and grassland sites suggest the dominance of C_3 plants and humidity conditions since ~17,000 cal yr BP.

The BL data for the Late Pleistocene is consistent with data obtained from noble gases from Piauí state (Stute et al., 1995), which suggests a cooling of 5 °C during the LGM across the Americas between 40°N and 40°S. Speleothem studies show that the LGM was wet in Southeastern Brazil (Cruz Junior et al., 2005, 2006; 2007; Cheng et al., 2012), due to the expansion of ice sheets in the Northern Hemisphere, causing a southward shift in the Intertropical Convergence Zone (ITCZ) (Wang et al., 2004) and strengthening of the South America monsoon system. In agreement with moister conditions indicated by these speleothem data, Ledru et al. (2016) cited the presence of semideciduous and evergreen forests at low latitudes during the late glacial, albeit with a floristic composition different from that of today's forests.

Similar data was also recorded for the Serra do Mar State Park – Curucutu and the Cardoso Island (Pessenda et al., 2009, 2012), São Paulo state, southeastern Brazilian coast. Between 22,780 and 12,000 cal yr BP Curucutu site was characterized by a forested landscape with montane species such as *Araucaria*, *Podocarpus*, and *Weinmannia*, an increase of algal spores and a more significant contribution of C_3 grasses, which suggest a cold and humid climate

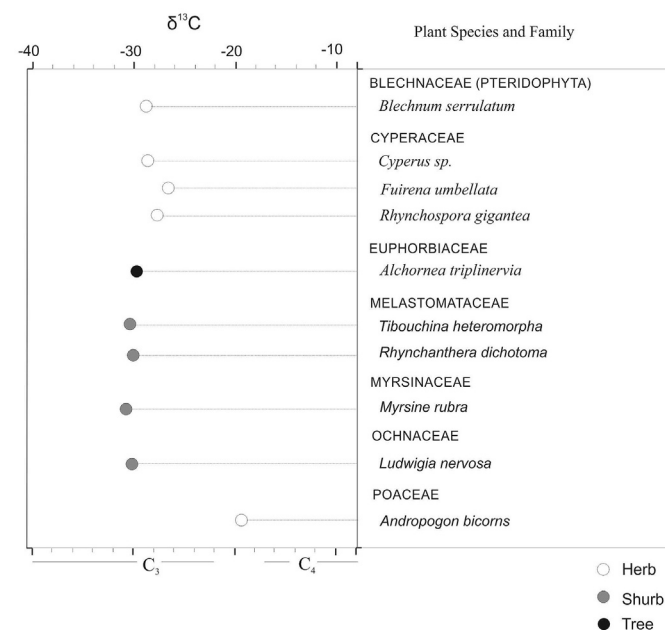


Fig. 5. Most representative plants collected around the sampling point, the isotopic value ($\delta^{13}\text{C}$), and habit.

(Pessenda et al., 2009). The same line of reasoning applies to Cardoso Island, ~170 km of Curucutu, where the pollen record indicated a cool climate forest in the period from ~29,500 to ~23,000 cal yr BP (Pessenda et al., 2012).

Speleothems at the Botuverá cave, southern Brazil, recorded higher $\delta^{13}\text{C}$ and low stalagmite growth rates between 116 and 19 k cal yr BP and reflect a significant cooling during the glacial period, probably due to incursions of polar cold air over the southern hemisphere (Cruz et al., 2006). In the Linhares region, northern Espírito Santo, Buso Junior et al. (2013) postulated that polar air masses may have reached the studied area during the Late Pleistocene and early Holocene, which allowed the maintenance of the forest due to frontal precipitation and a humid climate.

3.5.2. Holocene

From 13,740–7300 cal yr BP (49–21 cm; 0.44–0.02 mm/yr), the dominance of herbaceous plants, carbon isotope enrichment (up to ~ -24‰) (Fig. 2), and phytolith results (Calegari et al., 2017) together indicate the presence of both C_3 and C_4 grasses, due to a less humid climate than that of the Pleistocene. However, the presence of *Zygnema* and *Spirogyra* algae at this time (Fig. 3) may be indicative of a highly seasonal climate, with well defined wet and dry seasons. Even so, this opening of vegetation recorded at the bog probably did not occur in the surrounding forest, as the presence of *Virola*, *Laplacea* and *Sloanea* pollen, even in low abundance, signify continued presence of dense forest (Fig. 3).

A palynological study by Buso Junior (2015) of Lake Canto Grande, north of Espírito Santo and 13 km from BL, revealed that the species composition of the forest around the lake between 11,000 and 7700 cal yr BP was markedly different from that of the modern *Tabuleiro's* forest, probably due to a less humid climate. The relatively dry period in the region may have influenced the sedimentation of sand-rich sediments and alluvial processes (Lorente et al., 2018). The same environmental context was observed by Garcia et al. (2004) at Jacaré deposits, São Paulo State, where the authors recorded an increase in Poaceae pollen and drop in the sedimentation rate between ~8000 and ~5000 cal yr BP, reflecting a less humid climate.

At Nativo do Flamengo, 11 km from BL, herbaceous taxa dominate the record and pollen of *Rheedia brasiliensis*, a species characteristic of sandy soils of dry and seasonally flooded areas, may indicate a less humid climate in the region before 7000 yr BP (Buso Junior et al., 2019). In addition, depleted $\delta^{13}\text{C}$ values, the dominance of C_3 plants, and the presence of freshwater sponge spicules, are suggestive of a more humid climate and the establishment of the wetland around 7000 years BP, which is also consistent with the BL record.

A sea-level transgression was recorded in Brazil during the Holocene, beginning at ~6500 ^{14}C yr BP (~7400 cal yr BP) and peaking at ~5000 ^{14}C yr BP (~5700 cal yr BP) (Suguio and Martin, 1981; Suguio et al., 1985; Angulo et al., 2006). This record is in accordance with the peat formation and the presence of freshwater phytoplankton found at BL around ~7300 cal yr BP. As the bog is located ~45 m. a.s.l, far away from the coast (~33 km), and with no link to the tidal channel, all the water sources for BL come from the rain and the water table, with hydrological variations mainly driven by climatic change and influenced by the water base level during Holocene sea-level rise.

Climate changes reported during the Late Holocene have contributed to the local moisture increase at BL due to the intensification of the South America Monsoon System over the last ~4000 cal yr BP, caused by the increase of summer insolation (Cruz Junior et al., 2005), corroborated by many previous studies throughout the Atlantic Rainforest (Behling et al., 1997; Ledru et al.,

2005; Pessenda et al., 2009, 2010). According to Buso Junior et al. (2013), the modern climate in the Linhares region was established in the last 4000 years. The abundance of *Typha*, *Potamogeton*, and *Salvinia*, together with the correlation between C/N versus $\delta^{13}\text{C}$, probably indicates flooding episodes at BL between ~2140 (11 cm) and ~970 (estimated age; 7 cm) cal yr BP (Figs. 3 and 4).

The pollen record shows the establishment of current vegetation inside the bog ~823 cal yr BP (5 cm – estimated age), comprising small trees and shrubs sparsely distributed among Poaceae and Cyperaceae herbs, aquatic plants during the rainy season, and the *Tabuleiro's* forest surrounding the area. Artificial pollen traps installed for 20 months at Nativo da Gavea, which has a vegetation structure similar to that of BL, and located 13 km to the southeast (19°12'29"S/39°57'46"W), have similar pollen assemblages to those of the surface samples from BL. At Nativo da Gavea tree and shrub pollen dominates (60%), herb pollen is abundant (31%), while pollen of aquatic herbs is absent, probably because, unlike BL, this site does not become flooded during the rainy season (Buso Junior, 2015). At Nativo do Flamengo, pollen and sponge-spicule analyses also show that current environmental conditions were established ~950 cal yr BP (Buso Junior, 2015; Buso Junior et al., 2019).

Paleodata from BL have shown the long-term stability of the central core region of the Atlantic rainforest biome under LGM climatic conditions, confirming the modelling and phylogenetic studies, despite changes to its floristic composition. Our data reveal a forest with cold- and humid-adapted species during the last glacial period, and a herb-dominated bog under less humid climate conditions of the early to mid-Holocene. The current floristic composition of the central Atlantic rainforest only became established in the Late Holocene.

In contrast to the glacial refugium model (Haffer and Prance, 2002) and the “Historically stable forest” model (Carnaval and Moritz, 2008), our results indicate that the floristic composition and biodiversity of the central Atlantic Rainforest has not remained stable since the Pleistocene, but has instead undergone significant, climate-driven change, with no analog assemblages in the Pleistocene. However, the forest biome has remained intact and shown resilience to Pleistocene-Holocene climate change. The late Quaternary history of this complex tropical ecosystem in northern Espírito Santo state highlights its importance as a biodiversity hotspot and a key priority for conservation.

4. Conclusions

Pollen and isotope data from a 30,000 year late Quaternary substrate sequence at Brejo do Louro bog in the central Atlantic Rainforest of Brazil show that the forest biome has remained intact throughout this time, but has experienced significant floristic changes between warm- and cold-adapted species. Drier climatic conditions during the Early and Mid- Holocene led to more open vegetation within the bog, but the surrounding *Tabuleiro's* rainforest remained intact. The current floristic composition of the central Atlantic rainforest became established during the Late Holocene. Considering the past environmental changes in the Atlantic forest, our data can highlight the importance of forest conservation to maintain the biodiversity of this globally important biodiversity hotspot in the face of future climate change.

Authors' individual contributions

Mariah Izar Francisquini – Investigation, Conceptualization, Writing - original draft, Visualization, Project administration. Flávio Lima Lorente - Writing - review & editing. Luiz Carlos Ruiz Pessenda

– Supervision, Conceptualization, Writing - review & editing. Antônio Alvaro Buso Jr. - Writing - review & editing. Francis Edward Mayle - Supervision, Writing - review & editing. Marcelo Cancela Lisboa Cohen - Writing - review & editing. Marlon Carlos França - Writing - review & editing. José Albertino Bendassolli – Resources. Paulo César Fonseca Giannini – Resources. Jolimar Antonio Schiavo - Resources. Kita Macario - Resources

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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