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The use of carbon isotopes (^{13}C,^{14}C) in different soil types and vegetation coverage in a montane atlantic forest region, Southeast Brazil

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ABSTRACT

The study of the paleoenvironment depends upon proxies of palaeovegetation associated with chronological records. Carbon stable isotopes in soil samples provide information on the past vegetation type due to differences in mass fractionation during photosynthesis. Radiocarbon measurements on soil organic matter may also have different behaviors, given the complexity of soils as mixtures of multiple sources. With the aim of investigating how different soils, under different vegetation coverages, may affect paleoenvironmental reconstructions, we have analyzed four soil profiles collected at the Itatiaia National Park, between Rio de Janeiro and Minas Gerais states in Brazil, in the context of the Atlantic Forest biome, at altitudes between 1898m and 2457m. Different chemical fractions of the bulk soil were separately ^{14}C dated for each sample depth. For the total soil and the non-hydrolyzable carbon, discrepancies in pMC values were mostly within 5% from the humin fraction values. Two Histosol profiles collected under forest vegetation on a hillside presented very different morphologies and chronologies, possibly related to colluvium effect, indicated by the deposition of originally older material. The results for a Histosol profile under grassland indicates that C3 plants were the dominant vegetation over most of the last 8000 years at the most distant location while a Cambisol profile under transitional vegetation shows variations, with C3 plants at ca. 2 kyr BP, switching to C4 before ca. 700 yr BP, suggesting anthropic influence.

1. Introduction

The Brazilian Atlantic forest occupies vast areas with distinct geographical and climatic characteristics. In Brazil, forest formations occur as Mixed Ombrophilous Forests, commonly in the southern highlands and with disjunctions in the Southeast and in neighboring countries such as Argentina and Paraguay. This type of forest often occurs interspersed with field/grassland areas, characterizing a system of mosaic vegetation (Klein, 1960; Roderjan et al., 2002; Kozera et al., 2006).

The Itatiaia National Park (INP) is an important environmental conservation unit located in Itatiaia massif, on the Mantiqueira mountain. It is located in the south and southwest regions of Minas Gerais and Rio de Janeiro states, respectively, covering an area of about 225.54 km^2. The vegetation is Atlantic Forest, with only 10% of its original vegetation remaining (Myers et al., 2000; Mittermeier et al., 2005). The INP was the first Brazilian ecological reserve and is considered a biotic and geomorphological patrimony (IBDF, 1982). The Atlantic Forest biome has high levels of biodiversity with unique species, being considered an important hotspot in the world with exceptional concentrations of endemic species (Myers et al., 2000), with large carbon storage capacity and the ability to recharge aquifers (Weissert and Disney, 2013; Cooper et al., 2015).

Until it lasts, it is crucial to study this rare environment and understand the carbon dynamics taking place over the last thousands of years. Paleoenvironmental reconstructions based on several proxies are able to provide information on the vegetation and climatic changes in specific ecosystems. The decomposition of plant and animal residues and the

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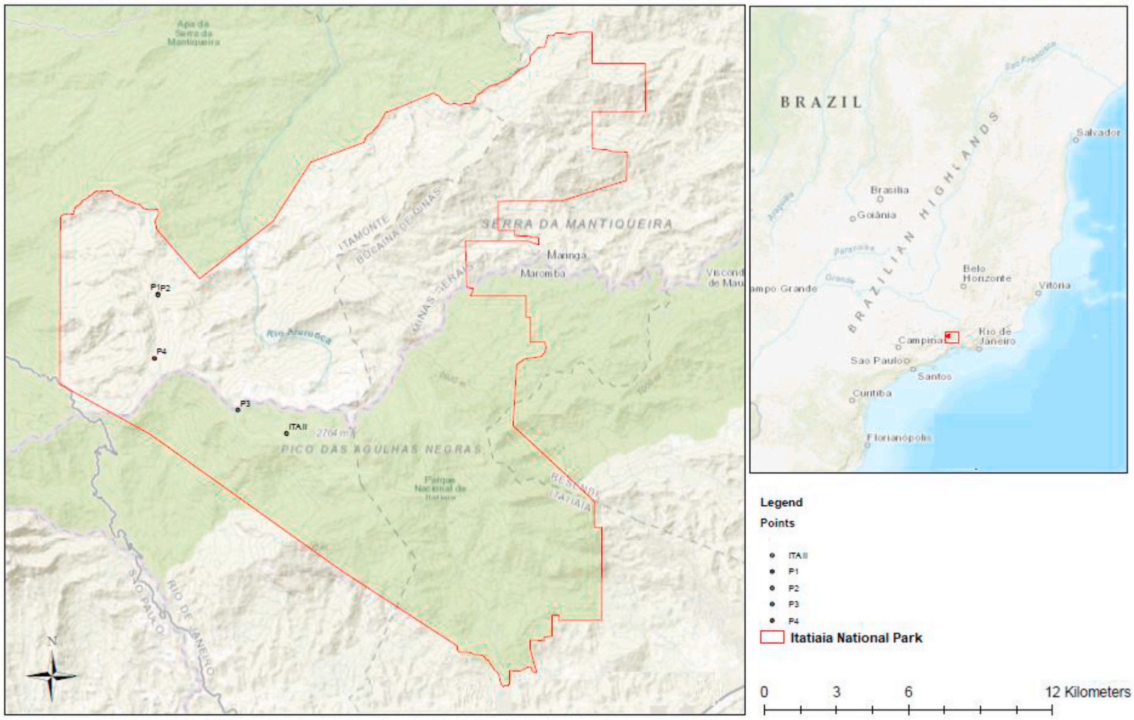

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Fig. 1. Location map of INP with sampling points.

Table 1
Vegetation, altitude, soil type and geographic coordinates of the soil sampling points.

Profiles	Vegetation	Altitude asl (m)	Type of soil	Coordinates
P1	FOREST	1898	Histosols (Typic Udifolists)	22°20′1.68"S 44°44′1.14"W
P2	FOREST	1912	Histosols (Typic Udifolists)	22°20′3.06"S 44°44′1.14"W
P3	FIELD/ Grassland	2452	Histosols (Typic Udifolists)	22°22′26.28"S 44°42′13.26"W
P4	TRANSITION	2160	Cambisol	22°22′26.28"S 44°42′13.26"W

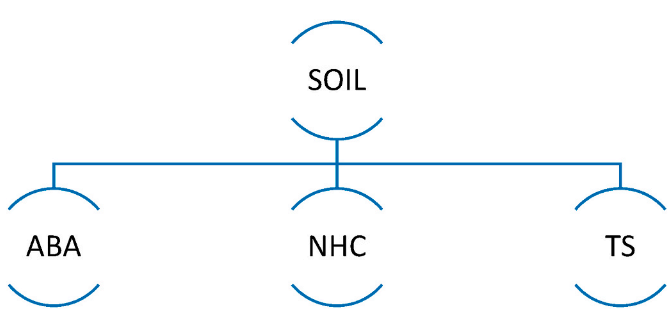

Fig. 2. Soil fractions for ^{14}C dating. *NHC- Non-hydrolisable carbon (HCL 1 mol L^{-1}). ABA – Humin (HCl/NaOH + $Na_4P_2O_7$/HCl), TS- Total soil (4% HCl).

activity of microorganisms over time is reflected in soil organic matter. The products of this activity are the so-called humic substances, a group of heterogeneous compounds (Stevenson, 1994) formed by complex chemical structures. The humic substances can be chemically divided according to their solubility into three different fractions: humic acids, fulvic acids and humin (Hayes, 1998). The proportion of such fractions can be related to the soil type and influenced by environmental conditions.

Vegetation coverage and humidity, for example, can be studied, for example, through phytolith analyses. The observation of biomineralized particles under the microscope allow for calculating phytolith indexes based on the frequency of different cell shapes in the soil profile that are characteristic of specific environmental conditions. Different plant types can also be distinguished by their stable carbon isotopes ratios ($\delta^{13}C$). Water dependent plants which perform the Calvin-Benson photosynthetic mechanism (Calvin and Benson, 1948), i.e. a cycle based on the reduction of CO_2 in 3-carbon phosphoglyceric acid via RuBisCO enzyme (ribulose bisphosphate carboxylase/oxygenase enzyme), such as woody plants, are called C3. Grasses, or C4 plants, on the other hand, use the Hatch-Slack cycle (Hatch and Slack, 1966), a process where CO_2 is reduced to aspartic acid or malic acid composed of 4 carbons, followed by the Calvin-Benson cycle.

The different photosynthesis processes for C3 and C4 plants cause different fractionation during carbon incorporation. Because of these differences it is possible to discriminate the vegetation by the $\delta^{13}C$ analyses of the soil organic matter - SOM (Martinelli et al., 1998; Pessenda et al., 2004), in the range of −32 e −21‰ for C3 plants and between −17‰ a −9‰ for C4 plants (Gleixner et al., 2002; Gordon and Goñi, 2003; Killops and Killops, 2004; Lamb et al., 2006). The pattern of each type of photosynthesis is significantly influenced by climatic conditions and the distribution of plants bounded by environmental standards (Ehleringer et al., 1997). Changes in vegetation during the Holocene period were recorded on carbon stable isotope ratios and have been reported for different Brazilian soils (Volkoff and Cerri, 1987; Victoria

Table 2
Conventional ^{14}C ages of soil fractions in distinct depths at P1, P2, P3 and P4 sampling points and at the peat record.

		P1		P2		P3		P4		ITA II	
FRACTIONS	DEPTH (cm)	^{14}C (yr BP)	pMC	^{14}C (yr BP)	pMC	^{14}C (yr BP)	pMC	^{14}C (yr BP)	pMC	^{14}C (yr BP)	pMC
TS	20–30	–	–	–	–	3195 ± 30	67.18 ± 0.22 (−3% ABA)	>MODERN	102.71 ± 0.27 (+2% ABA)	–	–
	40–50	2370 ± 20	74.44 ± 0.17 (+4% ABA)	1065 ± 25	87.6 ± 0.25 (+2% ABA)	5390 ± 30	51.11 ± 0.17 (−3% ABA)	605 ± 25	92.73 ± 0.24 (+1% ABA)	–	–
	80–90	–	–	–	–	–	–	1820 ± 20	79.75 ± 0.17 (−0.7% ABA)	–	–
	90–100	8040 ± 30	36.76 ± 0.13 (+4% ABA)	925 ± 35	89.13 ± 0.38 (+1% ABA)	7080 ± 35	41.43 ± 0.16 (+7% ABA)	–	–	–	–
	140–150	8734 ± 54	33.71 ± 0.22 (−11% ABA)	2360 ± 25	74.57 ± 0.29 (+1% ABA)	–	–	–	–		
ABA	20–30	–	–	–	–	2905 ± 30	69.64 ± 0.23	>MODERN	100.61 ± 0.26	–	–
	40–50	2415 ± 20	71.68 ± 0.21	1270 ± 20	85.39 ± 0.19	5090 ± 30	53.06 ± 0.18	700 ± 35	91.65 ± 0.36	–	–
	80–90	–	–	–	–	–	–	1870 ± 20	79.21 ± 0.19	–	–
	90–92	–	–	–	–	–	–	–	–	7010 ± 35	41.79 ± 0.18
	90–100	8475 ± 45	35.34 ± 0.19	1010 ± 20	88.19 ± 0.19	7610 ± 30	38.77 ± 0.14	–	–	–	–
	126–128	–	–	–	–	–	–	–	–	8560 ± 60	34.47 ± 0.23
	140–150	6730 ± 35	37.79 ± 0.19	2495 ± 20	73.30 ± 0.18	–	–	–	–	–	–
	170–178	–	–	–	–	–	–	–	–	8935 ± 50	32.89 ± 0.19
NHC	20–30	–	–	–	–	3280 ± 20	66.47 ± 0.15 (−5% ABA)	>MODERN	102.21 ± 0.21 (+2% ABA)	–	–
	40–50	2675 ± 25	74.04 ± 0.17 (+3% ABA)	1205 ± 15	86.08 ± 0.14 (+2% ABA)	5555 ± 30	50.08 ± 0.17 (−6% ABA)	575 ± 20	93.09 ± 0.22 (+2% ABA)	–	–
	80–90	–	–	–	–	–	–	1490 ± 20	83.09 ± 0.19 (+5% ABA)	–	–
	90–100	8355 ± 45	34.81 ± 0.17 (−1% ABA)	1000 ± 20	88.28 ± 0.21 (+0.1% ABA)	7225 ± 35	40.68 ± 0.16 (+5% ABA)	–	–	–	–
	140–150	7815 ± 45	43.28 ± 0.16 (+14% ABA)	2415 ± 20	74.02 ± 0.18 (−0.9% ABA)	–	–	–	–	–	–

*ABA - Humin (HCl/NaOH + $Na_4P_2O_7$/HCl), NHC - Non-hydrolysable carbon, TS - Total soil (4% HCl).
**For NHC and TS, ratios from pMC results over ABA are given.

et al., 1995; Desjardins et al., 1996; Pessenda et al., 1996a, 2001, 2004; Freitas et al., 2001). However, in order to synchronize environmental proxies, the best possible estimates for the deposition age should be made since, unlike for other materials, soils are not closed systems.

Understanding the dynamics of carbon in organic matter from different types of soils and determining the duration of humus formation is very important for evaluating ^{14}C dating of the soil (Lobo and Flexor, 1974). The inert fraction of the hummus, humin, is usually assumed to be the closest possible ^{14}C age of the deposition (Chichagova and Cherkinsky, 1993). However, there is no consensus in literature about the most appropriate chemical treatment to extract the fraction that would represent the "real age" of SOM deposition, with different protocols guiding the physical and chemical treatment of soils for carbon dating.

A common approach is to separate active and passive components using physical and chemical fractionation techniques (Campbell et al., 1967; Martel and Paul, 1974; Goh et al.,1984; Trumbore et al., 1989; Paul et al., 2001). However, different soil types and environmental conditions may interfere in soil development and, therefore, in carbon stabilization. For example, wetland areas with hydromorphism or upper mountain regions, whose low temperature conditions cause reduction of biological activity and favor the accumulation of organic material. The same way, different characteristics of each soil type are expected to enhance or decrease the contribution of younger carbon on the age of SOM. Histosols, for example, present high carbon contents and organic material in different stages of decomposition, having humic and fulvic acids as principal organic fractions (Pereira et al., 2005; Fontana et al., 2008; Santos et al., 2013), while Cambisol is a mineral soil presenting medium or fine texture and absence of significant pedogenetic development, and high content of primary minerals (Santos et al., 2013).

Aiming to verify whether significant changes in ^{14}C along soil profiles would take place, in this work we compare the results of different soil fractions for both, Histosols and Cambisols, under different native altitude vegetation coverages (forest, grassland and transition) in the Itatiaia National Park, southeastern Brazil.

2. Materials and methods

2.1. Site description

The area selected for sampling covers the upper part of the study site with approximately 2450m of altitude a.s.l. The average annual temperature is 11.4 °C, with January being the warmest month (average of 13.6 °C) and July the coldest month (average of 8.2 °C). In Itatiaia the vegetation differs along the mountain due to the position of the slopes and the altitudinal variation. The greatest diversity of species occurs in the vegetation of Atlantic Montane Forest, Upper Montane Atlantic Forest and Altitude Fields/Grasslands (Aximoff et al., 2014). The Montane Forest is located between 1300 and 2000 m altitude, and the altitude fields/grasslands from 2000 m (Safford, 1999).

In the field/grassland, four trenches were used for soil classification, and soil samples were collected from the profiles P1, P2, P3, and P4 (Fig. 1), with depths of 150 cm, 200 cm, 100 cm and 90m, respectively. The profiles P3 and P4 are shallower due to the presence of the soil rock at the respective depths.

Soil samples of 10 cm layers were collected to measure $\delta^{13}C$ and ^{14}C

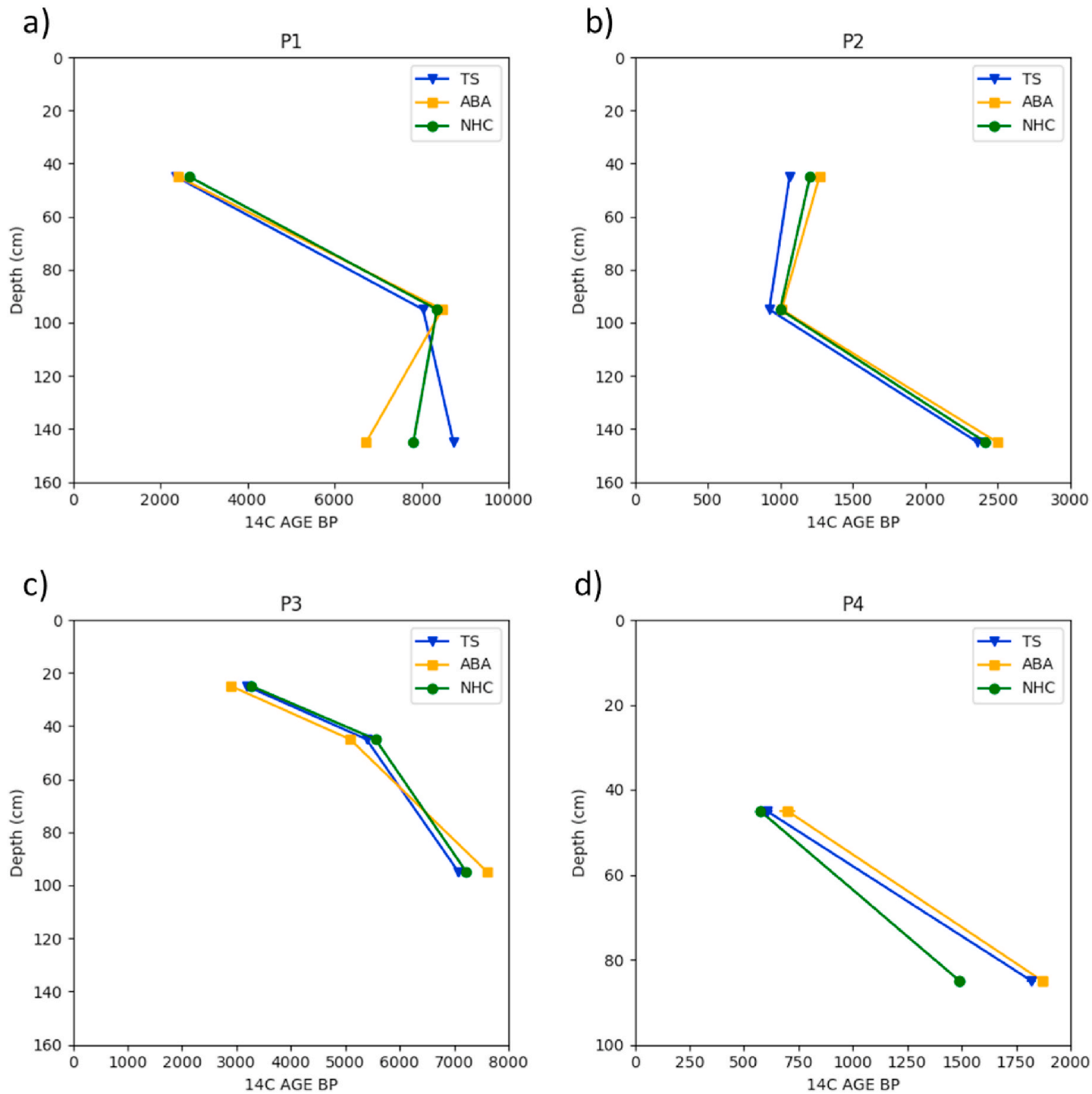

Fig. 3. Results of ^{14}C for the different soil fractions. a) for profiles P1; b) P2; c) P3; and d) P4.

on the three different native vegetation covers (Table1).

In addition, a ~170 cm peat testimony was collected at a peat/bogland (ITA II) located near P3 (S 22°22′55.02", W 44°41′7.95", 2410 m) by using a Russian sampler (Cohen et al., 2005).

2.2. Methodologies for elemental and isotopic analyses

The methods to obtain the different fractions of soil organic matter were adapted from the chemical pre-treatment protocols available in literature for soil dating (Pessenda et al., 1996b; Trumbore and Zheng, 1996; Paul et al., 2001). All soil samples of ~500g were physically pretreated with the removal of visible contaminants such as vegetal fragments, insects, etc. before chemical treatment, quartered with a Jones Riffle Splitter for homogenizing soil samples (Van Johnson and Maxwell, 1981; Schumacher et al.,1990) and sieved (0.212 mm) with an automatic shaker. During the chemical treatment, a centrifuge at 3200 RPM for 3 min was used for the separation of the supernatant. The samples were washed with ultrapure water (until pH was equal to 5) and residue was left to dry at 60 °C.

For the isolation of humin, an Acid-Base-Acid (ABA) chemical pre-treatment was used with a pretreated sample of ~2g. The purpose of the first treatment, with HCl 0.5 mol L^{-1} at 80 °C for 4 h, was to remove light materials such as fulvic acids. After the removal of the supernatant, $Na_4P_2O_7$ and NaOH 0.1 mol L^{-1} were used for 12 h for the solubilization of humic acids for a maximum of 3 times (supernatant should be clear). The last acid treatment was with HCl 3 mol L^{-1} between 90 °C and 100 °C for 12h, aiming for the removal of organic residues and contamination by atmospheric CO_2 (Pessenda et al., 1996b; Trumbore and Zheng 1996).

For the isolation of the non-hydrolysable carbon (NHC) from the 2g physically pretreated soil sample, the procedure was adapted from Paul et al. (2001), with 2 h treatments with HCl 1.0 mol L^{-1} at 90 °C, until the supernatant was clear or up to 5 times (Paul et al., 2001).

For the preparation of total organic matter (TS), a single reaction with HCl (0.1 mol L^{-1}) at room temperature with 2g physically pre-treated soil sample was performed, in order to eliminate eventual adsorbed carbonates (Pessenda et al. 2001). A scheme of the protocols for the fractions used in this study is shown in Fig. 2.

All the samples were combusted in independently sealed quartz tubes, purified and graphitized at the Fluminense Federal University

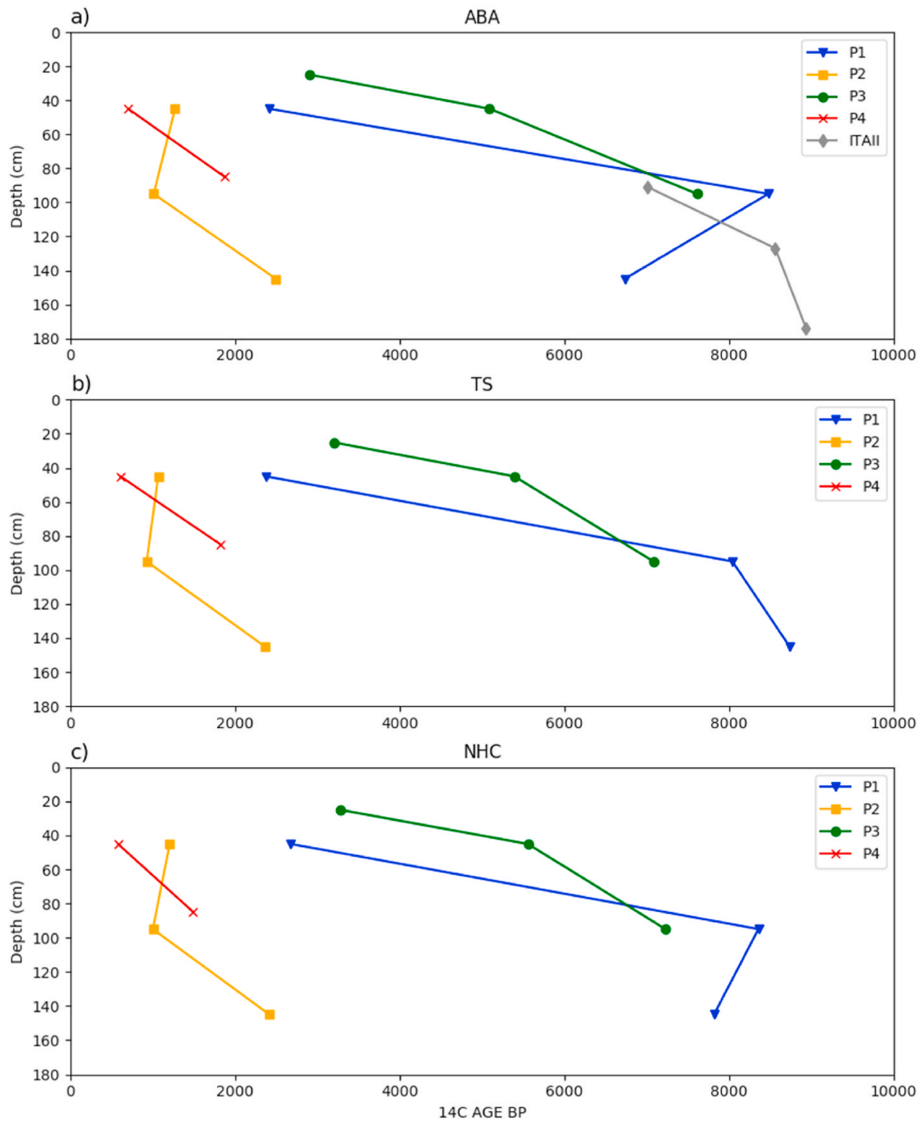

Fig. 4. Ages for each fraction of the profiles (P1, P2, P3, P4, and ITAII).

Radiocarbon Laboratory -LAC-UFF in Niterói, Brazil-(Macario et al., 2015) and sent to the National University of Australia (ANU) Radiocarbon Laboratory. The samples were analyzed in a Single Stage Accelerator Mass Spectrometer (SSAMS). Results are expressed in Radiocarbon years Before Present (BP) and percent modern carbon (pMC).

The stable carbon isotope ratios were measured at Laboratory of Radioecology and Environmental Change (LARA, in Niterói, Brazil) using an EA-IRMS system Thermo Fisher Scientific Co. This setup consists of a FlashEA2000, a MAS200R sample with 32 sample capacity, a ConFlo IV universal interface, which connects to the IRMS model DELTA V Advantage. Around 3–15 mg of physically pretreated sample were encapsulated and used, based on their carbon content, so that between 0.2 and 0.3 mg of carbon would be injected in the IRMS. Samples were measured with a group of empty capsules and certified standards. The empty capsules were used to subtract any possible influence of the tin capsules and the results were corrected from the standards measurements: Urea ($\delta^{13}C = -41.3 \pm 0.04$ VPDB), Caffeine ($\delta^{13}C = -27.771 \pm 0.043$ VPDB), Soil ($\delta^{13}C = -26.66 \pm 0.24$ VPDB) and Graphite ($\delta^{13}C = -16.049 \pm 0.035$ VPDB). The error associated is 0.3‰ for carbon stable isotopes measurements.

Besides the photosynthesis, other processes can cause fractionation during carbon incorporation, as erosion, decomposition and the Suess effect. In our study we simplify the ^{13}C of SOM assuming that the bulk carbon pool analyzed refers to the mass average of the hole pools of SOM integrated (Trumbore, 1997). The Suess effect progressively decreased the $\delta^{13}C$ on soil organic carbon, with 1.5‰ variation since the industrial revolution (Bird et al., 1996). Since the difference of $\delta^{13}C$ for the types of vegetation are more then 10‰ these influence should not be a problem.

3. Results and discussion

The results for the ^{14}C ages of the TS, ABA, and NHC fractions are shown in Table 2 and in Figs. 3 and 4, referring to the treatments used for soil samples of the four profiles and TS fraction ^{14}C ages for ITA II peat record. The $\delta^{13}C$ and the total carbon content (%) data are presented in Table 3 and in Figs. 5 and 6.

Chemically removing fulvic acids (FA) and humic acids (HA), is intended to isolate the most stable fraction: humin, often used to establish ^{14}C chronologies in environmental studies (Trumbore and Zheng, 1996; Pessenda et al., 2001). Since HA and FA fractions are soluble, they migrate from the surface for soil depth, which has residual modern carbon input, therefore masking the age of the original SOM that generated the soil. The expected results for the ^{14}C ages of different fractions in a well-developed non-disturbed soil would be such as ABA > NHC > TS. However, when analyzing the results, it is important to bear

Table 3
Values of $\delta^{13}C$ (‰) and total C (%).

Profiles	Depth (cm)	δ^{13}Ctot (‰) VPDB (±0.3‰)	%Ctot (±0.2%)
P1	(0–10)	−24.4	13.3
	(10–20)	−24.0	14.4
	(20–30)	−22.6	11
	(30–40)	−23.9	7.2
	(40–50)	−23.7	8
	(50–60)	−22.6	8
	(60–70)	−21.6	6
	(70–80)	−20.8	5.9
	(80–90)	−20.9	7.7
	(90–100)	−22.8	7.8
	(100–110)	−23.0	6.7
	(110–120)	−23.9	5
	(120–130)	−24.3	3
	(130–140)	−24.0	3
	(140–150)	−24.1	2.1
P2	(0–10)	−23.7	9.4
	(10–20)	−25.0	10
	(20–30)	−24.9	9.1
	(30–40)	−24.4	4.8
	(40–50)	−21.2	6.5
	(50–60)	−22.6	15.7
	(60–70)	−22.3	16.2
	(70–80)	−23.1	7.2
	(80–90)	−24.1	7.2
	(90–100)	−24.4	6.2
	(100–110)	−24.3	2.7
	(110–120)	−24.0	8.2
	(120–130)	−23.0	9.2
	(130–140)	−23.9	5.7
	(140–150)	−23.9	6.2
	(150–160)	−23.9	6.1
	(160–170)	−24.0	7.6
	(170–180)	−24.3	4.4
	(180–190)	−24.1	4.8
	(190–200)	−24.5	7.5
P3	(0–10)	−24.4	20.1
	(10–20)	−23.6	21.5
	(20–30)	−23.4	21.5
	(30–40)	−23.0	14.9
	(40–50)	−22.9	14.7
	(50–60)	−23.6	12.2
	(60–70)	−24.1	6.1
	(70–80)	−24.2	6.8
P4	(0–10)	−21.6	8.2
	(10–20)	−16.6	9.9
	(20–30)	−16.3	3.7
	(30–40)	−15.1	5.8
	(40–50)	−15.3	5.2
	(50–60)	−19.7	6.5
	(60–70)	−20.3	4.9
	(70–80)	−19.5	1.7
	(80–90)	−24.7	10.4

in mind that soils are open and very heterogeneous systems, with different pools, allowing C with very different ages even within the same soil depths (Balesdent et al., 2018). The impact of such mixture to the pMC of each fraction will depend on the age of the "contamination" and depth, in case of migration of humic substances. But also, the whole soil can be mobilized and deposited in other locations, creating apparent age inversions and/or gaps. In Table 2, discrepancies of pMC results for the NHC and TS fractions over the ABA results for the same depths are given. For most samples these discrepancies are below 5% and positive, indicating more recent contamination. Using ABA chemical treatment would probably contribute to increasing accuracy. On the other hand, given the magnitude of the uncertainties of the measurements, discrepancies of around 2% indicate statistically similar results, where any of these chemical treatments could be applied. Exceptions such as the results for profile P1, where TS reached 2000 ^{14}C yr older ages than the ABA fraction (Fig. 3a). For the P1 sample at 140–150 cm depth, not only ages are much older than for P2, but also the ABA fraction is the youngest between the three analyzed fractions (Fig. 3b), corroborating a

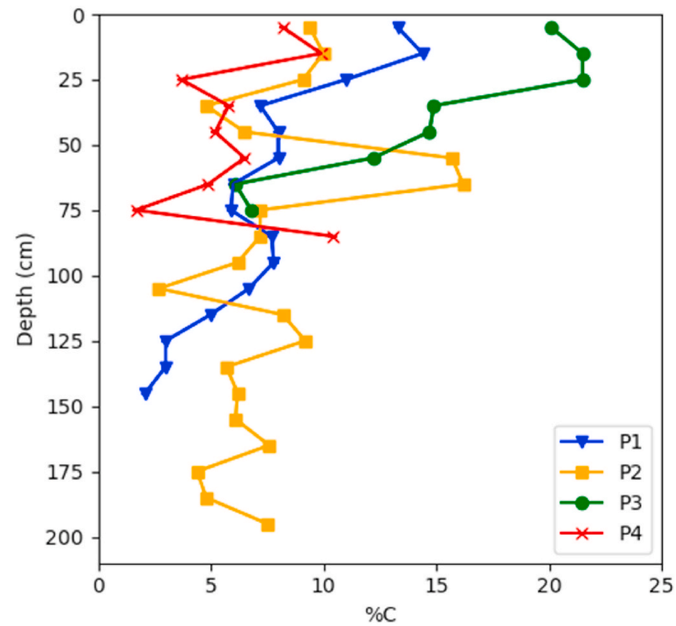

Fig. 5. Total carbon content (%C) for all soil profiles (P1, P2, P3, and P4).

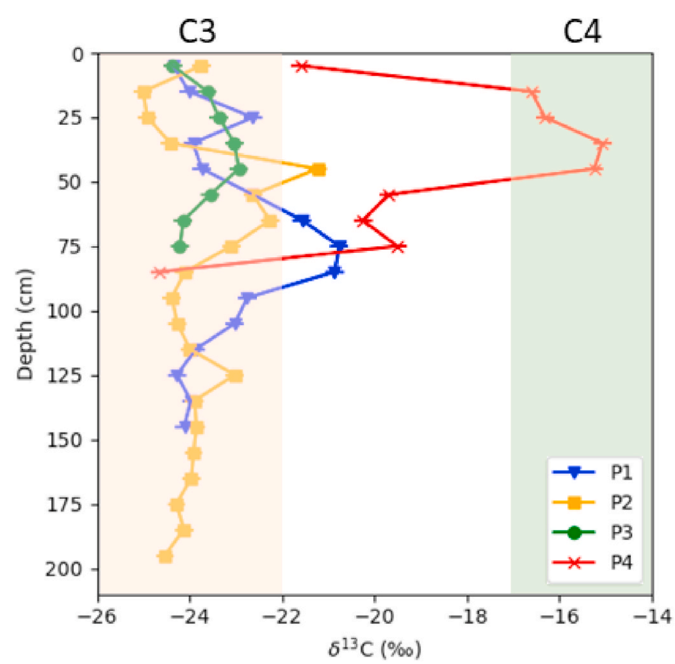

Fig. 6. The δ^{13}C values, along depth for all profiles (P1, P2, P3 e P4). The orange box corresponds to C3 plant and the green box to the C4 plant values. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

possible migration of older materials (pMC of NHC +14% and TS -11% than that of ABA fraction). Considering the humin (ABA) radiocarbon ages, expected to better represent the deposition events, the P1 profile (Fig. 4a) presents an age inversion, reaching ~ 8000 yr BP at 90–100 cm and ~7000 years BP at the profile base (140–150 cm). Both profiles are located on a hillside with 8–20% of slope, favoring a colluvium effect, caused by deposition of materials of various ages from higher parts. The morphology of P1 reinforces the interpretation of more recent deposition of originally older material, despite fairly developed. The P2 profile shares the same kind of soil, with the same vegetation coverage but is characterized by well-developed horizons, 14 m higher than P1. Very different radiocarbon ages between the two profiles stand out. Age inversions within P2 are statistically insignificant with its bottom layer reaching 2500 yr BP, a similar value to the upper layer of P1 (Fig. 4a).

P1 and P2 are both Histosols and both have forest coverage (Table 1), under very similar conditions, but they disclose the importance of sampling on the hilltop instead of on hillsides and looking for well-developed soils when aiming for palaeoenvironment reconstructions. It is interesting to notice how the discrepancy of different chemical fractions ^{14}C ages can contribute to the palaeoenvironmental reconstruction.

P3 profile with vegetation coverage typical of altitude fields such as herbs and shrubs, is the profile with the highest altitude (2457 m) at the top of the elevation. It presents older ^{14}C age in the depth 40–50 cm (~5500 yr BP) when compared to P1, P2 and P4.

P3 does not show ages inversion in the profile layers. The analyzed fractions (TS, ABA, NHC) presented discrepancies in pMC ranging from −6% to +7%, the ABA fraction being the youngest for depths from 20 to 40 cm and the oldest for 90–100 cm (Fig. 3c).

Results for profile P4 (Fig. 3d), a Cambisol with transition vegetation (trees and fields), located at 2154m altitude, with the youngest ages relative to the other points and without inversions, but also with similar ages for the organic fractions (TS, ABA, NHC) analyzed at different depths.

For ITA II, a bogland close to P3, the ABA fraction was analyzed. The results for depths of 90–92 cm, 126–128 cm, and 170–178 cm, respectively, were 7010 years BP, 8560 years BP, and 8935 years BP, with no inversions.

The organic soils are formed by the accumulation of organic matter favored by the low temperature, which reduces their transformation even under good drainage conditions, and subsequently it is possible that there is in this soil a greater amount of old organic C (humin), it has a carbon inventory in the older soil with longer residence time. In this place of study, INP, the high-altitude part is characterized by extreme climatic conditions, which corresponds to an upland sub-tropical climate.

Regarding the comparison of the ages of different soil fractions for each point, it is observed (Fig. 4) that in decreasing order from oldest to youngest at depth up to 50 cm was P3> P1> P2> P4 and in relation to depth up to 100 cm was approximately P1> P3 = ITA II > P4> P2. The soils samples under transition vegetation were the youngest, followed by the soils with forest vegetation up to 50 cm deep and lastly the soil over altitude field and peat bog. For most samples, the NHC fraction would give enough accuracy for ^{14}C ages.

All profiles show high content of carbon (Fig. 5), with the highest concentrations in the top layers, decreasing with depth as the classic trend observed for both organic and mineral soils (Pessenda et al., 2001; Freitas et al., 2001). The lowest carbon concentrations are found for the Cambisol, as expected.

Profiles P1 and P2 are Histosols under forest-type vegetation (C3 plants) and the $\delta^{13}C$ results (Table 3) supports the characterization of the forest vegetation cover. At specific depths the results indicate an excursion of the $\delta^{13}C$ results to more enriched values, characteristic of C3 and C4 plants mixing, possibly as a result of anthropic interference or of climatic variations. P1 has a mean value of $\delta^{13}C$ of −23.10‰ (reaching - 20.8‰ at 90 cm in depth) and P2 mean value of −23.78‰ (reaching −21.2‰ at 50 cm). The profile P3 is under altitude field with mean value of −23.78‰ and not much variation (ranging from −22.93‰ to −24.65‰), recording persistent C3 plants presence and, therefore, a sign of humid environment.

P4 profile is a Cambisol, which is considered an underdeveloped soil, very common in these high-mountain environments (Soares et al., 2016). The mean value of $\delta^{13}C$ of −18.77‰, ranging from −24.45‰ to −15.07‰, indicates a variation between C3 and C4 plants along the profile, (Pessenda et al., 2004). This profile was found in a transitional area, but with a cover of forest vegetation (shrubs and trees), can be observed at 80–90 cm depth a $\delta^{13}C$ more depleted with −24.7‰ indicating C3 plants (~1890 yrs BP ago), but as the depth decreases the $\delta^{13}C$ enriches to −15.3‰ with 40–50 cm depth indicative of C4 plants (~700 yrs BP) and decreases again until the top of the profile 0–10 cm with −21.6‰, indicative of a mixture of C3 and C4 plants and possible anthropic influence (Fig. 6).

Previous studies of vegetation in Itatiaia National Park made using phytolith indexes indicated open vegetation environments with a predominance of C3 plants, suggesting humid climate conditions, and corroborating the $\delta^{13}C$ isotopic values (Silva Neto et al., 2018).

In ITA II the phytolithic and isotopic analysis ($\delta^{13}C$) was performed and typical values of C3 plants (unpublished) were found as in Silva Neto et al. (2018), showing a predominance of morphotypes of Pooideae grasses, suggesting cold climate and open vegetation dominated by C3 plants.

It is important to observe that profiles P1 (forest), P2 (forest) and P4 (transition) are closer between each other and in similar altitudes, while P3 (field), where the oldest ages appear, reflecting the influence of the geographic position (more isolated) and the lesser possibility of anthropic influence to the vegetation and soils of the region.

4. Conclusions

In the context of the Itatiaia National Park, regarding the TS and NHC dated soil fractions, discrepancies in pMC values were most within 5% from the ABA fraction values. Therefore, for undisturbed systems either TS or NHC fractions could be used for stablishing an approximate chronology. On the other hand, evaluating the discrepancies between radiocarbon ages of the different chemical fractions contribute to a better interpretation of radiocarbon ages of soils as for paleoenvironmental reconstruction studies associating carbon stable isotopes ($\delta^{13}C$).

According to the $\delta^{13}C$ values, during the last 8000 yrs, the C3 plants (trees, shrubs and grasses) were the most representative vegetation. The $\delta^{13}C$ enrichment at intermediate depths for P1, P2 and P3 also suggest the presence of C4 plants during late Holocene, probably associated to less humid climate and/or anthropogenic influence in the region.

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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