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Combining phytoliths and $\delta^{13}C$ matter in Holocene palaeoenvironmental studies of tropical soils: An example of an Oxisol in Brazil

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ABSTRACT

Many plants deposit the soluble silica absorbed from the soil as monosilicic acid (H_4SiO_4) in and between their cells, generating bodies of opal silica ($SiO_2 \cdot nH_2O$) called phytoliths. Although phytoliths are susceptible to dissolution under extreme pH conditions, they generally do remain in the soil for long periods of time and can help in the reconstruction of past vegetation and climates. In the present study, phytolith analysis was used to reconstruct the palaeoenvironmental conditions that contributed to the pedogenetic processes, the deposition of organic matter and its stabilization in a very thick (>1 m) umbric epipedon of a Humic Hapludox profile from Minas Gerais State (Brazil). The results from the phytolith assemblages were also compared to the fractions and isotopic data of soil carbon of the same profile. The result from studying these two palaeoenvironmental proxies together has shown that the environment under which the umbric epipedon was formed was a mixture of vegetation with predominance of C_3 plants in mesothermic conditions and with little variation in humidity since Middle Holocene.

1. Introduction

In tropical and sub-tropical areas of Brazil, there are Humic Oxisols with thick epipedons (A horizon >100 cm) overlying the diagnostic Bw horizons. The epipedons have dark colour (value and chroma < 4) and significant content of organic carbon in the deeper layers. The genesis and palaeoenvironmental significance of these soils are not completely understood. Most authors have suggested that they are relic soils in landscapes that had a favourable climate for organic carbon accumulation (Nakashima, 1973; Queiroz Neto and Castro, 1974; Kampf and Klamt, 1978; Lepsch and Buol, 1986; Silva and Vidal-Torrado, 1999). Carbon was then preserved due to several specific soil and environmental factors such as high acidity, low-base saturation, relatively cold climate, stable geomorphic surfaces and accumulation of charred material (charcoal) that had been partially altered, decomposed into microparticles, and distributed in the soil by biological activity. The study of Humic Oxisols with thick epipedons is particularly interesting because of the significant amount of accumulated organic carbon (300 t/ha in the first 1 m), five times higher than in other Oxisol classes, providing the potential to study palaeoenvironmental reconstruction in Brazilian tropical areas. In many of these Oxisols, some considered as polycyclic soils (Lepsch and Buol, 1986), past climate changes may have been recorded.

The intensity of climate variability occurring in Brazil between glacial and interglacial periods influenced the weathering and pedogenesis rates of soils as well as the regional floristic cover distribution. This took place throughout the Quaternary by quick and recurring expansions and retractions of forests at the expense of more open vegetation such as the *Campo* (grassland savannah), the *Caatinga* (open arboreal savannah) and the *Cerrado* (closed arboreal savannah) (Martinelli et al., 1996; Pessenda et al., 1996, 1998, 2005). There have been several isotopic and pollen studies aimed at defining the boundaries between forest and open vegetation (see for example, Absy et al., 1991; Oliveira, 1992; Ledru et al., 1994; Pessenda et al., 1996, 2004; Gouveia et al., 1997). Pollen studies, $\delta^{13}C$, and ^{14}C dating provided evidence that in northeast and southeast Brazil, climate oscillations during the Quaternary were not always synchronous (Scheel-Ybert et al., 2003).

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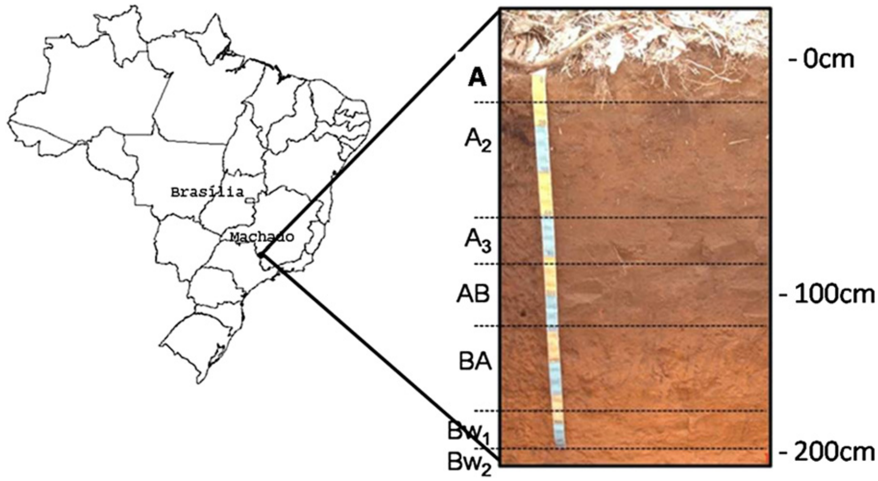

Fig. 1. Humic Hapludox profile showing the very thick umbric epipedon, (Machado, Minas Gerais State, Brazil).

The Humic Oxisols (*Latossolos húmicos*) cover an area of 144,000 km^2 of the Brazilian territory (FAO-UNESCO, 1981). They are most commonly found in the south and southeast (tropical and sub-tropical environments) on elevated topography, indicative of ancient erosional surfaces, and show an abundant presence of macroscopic soil charcoals at great depth in the profile, together with high contents of organic carbon in the umbric epipedon. These characteristics are not compatible with the current warmer and more humid climate (Lepsch and Buol, 1986). In this respect, the hyper-developed umbric epipedon can be seen as an important carbon reservoir and a characteristic feature of the soil. The study of this soil type is of fundamental importance to understand the pedogenetic processes in tropical and sub-tropical areas of the South American continent as well as changes in vegetation cover.

Opal phytoliths are a potential source of information for reconstructing structural changes in vegetation and the edaphic conditions of soil formation through time, and can be important in oxic environments, offsetting the paucity of fossil pollen (Argant, 1990; Alexandre et al., 1997; Bremond et al., 2005). Soils in tropical areas generally show much higher silica concentrations (of which phytoliths are an important proportion) than soils in temperate areas (Piperno, 2006). In Brazil, however, there are surprisingly very few phytolith studies. Kondo and Iwasa (1981) investigated the phytolith assemblages of an Oxisol, locally called *Terra Preta de Índio* (black earth-like anthropogenic soil) in the Amazon region, detecting changes in the environmental conditions during the formation of the soils. Alexandre et al. (1999) studied the phytolith assemblage of an Oxisol situated at the boundary between forest and *Cerrado* in Salitre (Minas Gerais State) in southeastern Brazil. The phytolith analysis, together with isotope data from soil organic matter, highlighted a savannah phase (drier climate during the middle Holocene between 5500 and 4500 BP) that was followed by two periods with more developed tree communities, the first between ca. 4000 and 3000 BP and the second after ca. 970 BP. Borba-Roschel et al. (2006) published the results of a comparison between phytolith assemblages obtained from the modern vegetation and a phytolith sequence obtained from a peat profile located in a Brazilian *Cerrado* at Uberaba (Minas Gerais State). Given the potential that opal phytoliths and $\delta^{13}C$ offer to palaeoenvironmental reconstruction, the objective of this study was to analyze the combined records to reconstruct the palaeovegetation, and palaeo-edaphic/climatic condition that lead to the development of a very thick umbric epipedon in Brazilian oxisols.

2. Regional setting

For this study, a representative Humic Oxisol pedon class, very deep and rich in organic carbon, classified as Humic Hapludox was selected. The soil (Fig. 1) is located in Machado, southern

Table 1
Sample list, soil properties of each examined sample.

Horiz.	Depth cm	Munsell colour	Clay	Silt	Sand Fine	Coarse	C-Total	Bulk density	pH (H_2O)
			g kg^{-1}					t m^{-3}	(1:2.5)
Ap	0–10	5 YR 3/2	455	139	142	264	47.5	0.8	4.2
A2	10–20						53.7		
	20–30						83.8		
	30–40	5 YR 2.5/2	498	176	173	153	57.3	0.8	4.7
	40–50						39.4		
	50–60						40.0		
A3	60–70						32.9		
	70–80						25.5		
	80–90	5 YR 2.5/2	567	131	140	162	28.4	0.9	5.0
AB	90–100						26.5		
	100–110	5 YR 2.5/2	540	176	120	164	23.5	1.0	5.0
	110–120		0				22.9		
BA	120–140	5 YR 4/4	598	147	137	119	18.7	1.1	4.9
Bw1	140–170	5 YR 4/6	624	136	134	107	12.5	1.1	5.5
Bw2	170–200+	5 YR 5/8	650	88	151	112	9.3	1.0	5.3

Table 2
AMS radiocarbon assays.

Material dated	Depth (cm)	Conventional ^{14}C Age BP	2-sigma ^{14}C Cal age BP
Charcoal[a]	20–40	210 ± 30	180 ± 36
Charcoal[a]	100–120	10,320 ± 120	12,131 ± 428

[a] UGAMS – The University of Georgia – Center for Applied Isotopes Studies.

Table 3
Phytolith indices.

Sample/Depth (cm)	Indices		
	Iph*100	IC*100	D/P
F1 (0–10)	0	16.1	0.2
F2 (10–20)	27.8	21.4	0.1
F3 (20–30)	56.7	20.8	0.1
F4 (30–40)	18.8	27.7	0.01
F5 (40–50)	47.1	34.0	0.01
F6 (50–60)	46.9	31.5	0.1
F7 (60–70	66.1	24.2	0.05
F8 (70–80)	62.2	27.2	0.03
F9 (80–90)	90.2	13.0	0.1
F10 (90–100)	72	30.5	0.03
F11 (100–110)	45.2	19.7	0.1

Minas Gerais State, at 1155 m asl. The topography of the region varies from undulating to mountainous relief. Bedrock comprises gneiss and migmatite rocks belonging to the Guaxupé Complex. The climate in the region is a Cwb type (moderate humid sub-tropical) according to the Köppen classification, with mean temperatures in the warmest and coldest months of 22° and 15 °C. There are two well-defined seasons (warm and humid summer; cold and dry winter), with a mean annual rainfall of around 1500 mm. The soils region moisture regime is udic and the temperature regime is isothermic. The studied soil is located on the higher areas (higher morphologies) where old erosion vestiges are still evident, possibly the South American or Velhas Surface (King, 1956), and it has a clay texture. The water pH increases from 4.2 to 5.5 from the surface to the bottom of the profile (Table 1). The local vegetation is composed of a sub-perennial tropical forest with occurrence of *Cerrado* patches (Silva and Vidal-Torrado, 1999), and the region is an ecotone between these two formations.

3. Material and methods

3.1. Soil description, physical properties and sampling

The profile was cut to a depth of 200 cm on the upper part of a slope, and the soil exposed was described according to Santos et al. (2006). Loose samples were collected of each soil horizons. Samples were air-dried, and particle size analysis was carried out after dispersion with 0.01 mol L^{-1} NaOH solution according to Embrapa (1997). Soil bulk density was calculated using the amount of dry soil (grams) contained in 100 cm^3 cylinders collected in triplicate from the field (Embrapa, 1997).

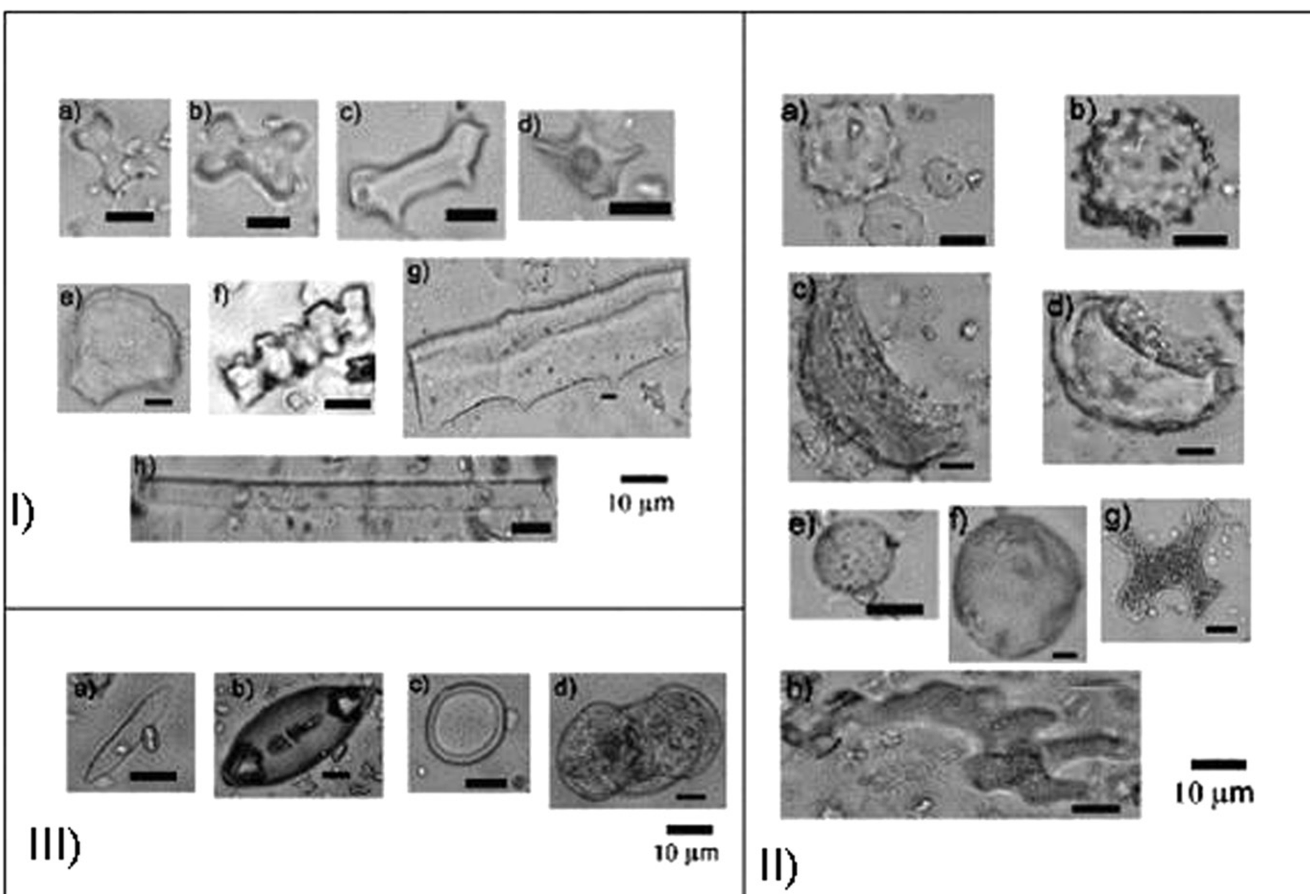

Fig. 2. Mircrophotography of Phtyoliths. I) Poaceae phytoliths morphotypes found in the umbric epipedon (in %). a,b) bilobates; c) bilobate - *Chusquea*type (cf. Montti et al. 2009); d) rondel; e) bulliform; f,g) different types of elongate morphologies.; II) a, b) globular echinate (Arecaceae morphotypes); c, d) crater-shaped (Araucariaceae morphotypes); e, f) globular and g,h) irregularly shaped (both Dicotiledoneae morphotypes).III) Photomicrographs of other silica bodies and pollen found in the umbric epipedon. Pictures were taken at 400× magnification.

3.2. Soil organic matter – SOM

The samples were taken in 10 cm increments to a depth of 110 cm. The total organic carbon and $\delta^{13}C$ analyses were carried out at the Stable Isotopes Laboratory of the Centre for Nuclear Energy in Agriculture (CENA-University of Sao Paulo - Brazil). The Total carbon results are expressed in grams per kilogram of dry matter ($g\ kg^{-1}$) for each horizon (Table 1) and the ^{13}C results are expressed as $\delta^{13}C$ using the conventional δ (‰) notation, with an analytical precision of ±0.2‰:

$$\delta^{13}C\left(‰\right) = \left[R_{sample}/R_{standard} - 1\right]1000$$

Where: R_{sample} refers to the $^{13}C/^{12}C$ ratio of the sample and $R_{standard}$ refers to the $^{13}C/^{12}C$ ratio of the standard.

Soil organic matter AMS dating was performed at the AMS Laboratory of Centre for Applied Isotopes Studies, University of Georgia, United States, on charcoal fragments physically separated from the soil by hand-picking and transformed in CO_2 by combustion at ^{14}C Laboratory of CENA-USP (Brazil). Radiocarbon ages are reported as ^{14}C (1σ) BP (present is AD 1950) normalized to a $\delta^{13}C$ of −25‰ VPDB and in calibrated years as cal (2σ) BP using the program CALIB 5.0. Two sections of the epipedon, 20–40 cm and 100–120 cm, were sampled, as the objective was the understanding of umbric epipedon genesis (Table 2).

3.3. Phytolith sampling and extraction

The phytolith samples were taken in parallel to the SOM ones at 10 cm increments to a depth of 110 cm. The presence of biological channels (bioturbation) is common in Oxisol pedons, indicating possible mixture of materials and suggesting upward movement and translocation of materials along the profile. However, during sampling the areas of the soil section with minimum bioturbation features were selected to avoid mixed material that would result in inadequate samples.

Phytoliths were extracted from 4 g of dry soil. The organic matter was oxidized with hydrogen peroxide at 30%, first at room temperature and then heated to 70 °C, followed by carbohydrate and iron oxides removal using sodium citrate-bicarbonate-dithionite according to Mehra and Jackson (1960). The density separation of phytoliths from the resulting fraction was performed using sodium polytungstate with a density of 2.3, according to Madella et al. (1998). The recovered fraction, which included phytoliths, diatoms and other silicate bodies, was mounted with immersion oil on microscope slides for 3D observations with a Zeiss Axioscope microscope at 400× magnification.

3.3.1. Phytolith classification and counting

For each sample at least 300 phytoliths with taxonomic significance were counted. A transect in each slide was counted to determine the proportion of phytoliths and other particles in the fraction obtained after heavy liquid separation, according to Carnelli (2002). The identified phytoliths were named following the International Code for Phytolith Nomenclature (Madella et al., 2005) and then grouped in accordance to their taxonomical significance (Mulholland, 1989; Twiss, 1992; Fredlund and Tieszen, 1994; Alexandre et al., 1997; Alexandre et al., 1999; Runge, 1999; Parr and Watson, 2007):

(a) Poaceae (grasses)
  Pooideae or pooid (trapeziform and rondel)
  Panicoid or panicoid (bilobate and cross)
  Chloridoideae or chloridoid (sadde)
(b) Arecaceae/palms (gloubar echinate)
(c) Dicotiledoneae/woody (globular, plate, papillae, irregular)
(d) Araucariaceae (crater-shaped cell)

For the interpretation of palaeoenvironmental conditions, the following phytolith indices were calculated (Table 3):

The Humidity-Aridity Index (Iph) (Diester-Haas et al., 1973; Alexandre et al., 1997) is based on the ratio of chloridoid *versus* chloridoid and panicoid phytoliths. High *Iph* values suggest open woodlands and/or grasslands dominated by xerophytic Chloridoideae, indicating dry edaphic and/or climatic conditions. Conversely, low *Iph* values indicate the predominance of mesophytic Panicoideae, suggesting more humid conditions (Alexandre et al., 1997).

The Climatic Index (Ic) (Twiss, 1987, 1992) is the ratio of pooid *versus* the sum of pooid, chloridoid and panicoid morphotypes. High values indicate the predominance of C_3 Pooideae grasses, suggesting cold climatic conditions (Twiss, 1992).

The Tree Cover Density Index (D/P) (Alexandre et al., 1997, 1999; Barboni et al., 1999) consists of the *D/P* ratio, where *D* is the number of dicotyledon phytoliths (globular morphotypes) and P is the number of Poaceae phytoliths (pooids, chloridoids, panicoids, trichomes and bulliforms). High values indicate open vegetation, adapted to warm and dry climates, as in the African tropical and intertropical zones; lower values indicate forest vegetation with warm and wet climates (Alexandre et al., 1997, 1999; Barboni et al., 1999).

3.4. Statistical analysis

Two forms of statistical analysis were applied to over phytolith data: Cluster Analysis (CA) and Principal Component Analysis

Table 4
Phytolith morphotype frequencies from the hyper-developed umbric epipedon.

Sample/ Depth (cm)	Angiospermae																	
	Monocotiledoneae																	
	Poaceae/Grasses																	
	Panicoideae		Pooideae		Chloridoideae		Cylindric		Elongate		Bulliform		Trichome		Clavate		Hair	
		%		%		%		%		%		%		%		%		%
F1 (0-10	41	12	35	10	0	0	7	2	50	15	128	38	7	2	0	0	0	0
F2 (10-20)	13	4	28	10	5	2	6	2	64	21	69	23	0	0	15	5	10	3
F3 (20-30)	13	4	32	11	17	6	8	3	52	18	70	24	0	0	5	2	14	5
F4 (30-40)	13	4	33	10	3	1	1	0.3	53	16	64	20	0	0	12	4	5	2
F5 (40-50)	18	5	66	19	16	5	3	1	62	18	87	25	0	0	18	5	4	1
F6 (50-60)	17	5	57	16	15	4	1	1	58	17	89	26	0	0	12	4	0	0
F7 (60-70	20	6	53	16	39	12	1	0.3	66	20	106	32	0	0	18	5	0	0
F8 (70-80)	14	4	58	18	23	7	1	0.3	60	19	112	35	0	0	24	7	5	2
F9 (80-90)	4	1	29	10	37	12	0	0	32	11	137	45	0	0	7	2	16	5
F10 (90-100)	7	2	71	21	18	5	0	0	47	14	135	41	0	0	26	8	2	1
F11 (100-110)	17	5	39	12	14	5	0	0	63	20	118	38	1	0.3	0	0	9	3

[a] The number of taphonomised phytoliths is not considered when calculating the total sum of phytoliths.

(PCA). For both analyses, Ward's method was applied and for the CA the Euclidian distance was used. The clustering was based on binary grouping according to the degree of similarity between the samples (Beebe and Kowalski, 1987). The dataset were expressed as percentages.

The dataset was not normalized because the analyses were run on the original correlation matrix expressed in percentages. Ward's method and the squared Euclidean distance were used for CA.

4. Results

4.1. Soil morphology

The studied pedon is deep (for other profiles in this area it is between 700 cm and 1000 cm), and extremely well-drained. It has a minimum 220 cm thick dark and organic carbon-rich A horizon including well expressed transitional AB and BA horizons, classified as humic by the SiBCS (Embrapa, 2006) and a very thick umbric epipedon by Soil Taxonomy (USDA, 1999). A recent 10 cm deep deposit of reworked material (colluvium) was identified in the upper epipedon. However, as suggested by the soil position in the landscape (upper third of the slope) and the morphology and Ti/Zr ratio (Calegari, 2008), the colluvial process was not the primary factor causing the formation of this very thick epipedon. The Ti/Zr ratio shows that the establishment and development (thickening) of the epipedons occurred after the older colluvial deposition.

Macroscopic charcoal fragments were identified in the pedon (between 20–40 and 100–120 cm) and dated at 180 ± 36 cal BP and 12,131 ± 428 cal BP (Table 1). These ages are consistent with those found by Silva and Vidal-Torrado (1999) in another Machado (MG) soil located not far from the profile studied in this work. The soil charcoals represent a period of fires associated with drier climatic spells during the Holocene (Pessenda et al., 1996, 2004).

The highest total carbon amount of 83.82 g kg^{-1} was found at the top of the A2 horizon, between 20 and 30 cm depth (Table 2). Below 40 cm, total carbon values gradually decrease. Kaolinite (Kt) was the main clay mineral in all soils where XRD analysis was done, followed by gibbsite (Gb) (Marques et al., 2011).

The bulk density in the umbric epipedon is lower than in the Bw (Table 1). This trend may be associated with the variations in texture and soil structure between horizons. The clay content ranged (top to bottom) from 498 g kg^{-1} to 650 g kg^{-1}, and these values are typical for this type of soil. Generally, the Humic Oxisol varies from clayey to very clayey as a result of intense desilicification that precedes organic carbon accumulation (Marques et al., 2011). In this epipedon there are clear vertical channels (pedotubules). They are filled with small to very small granular aggregates, well-rounded and reddish/dark materials originating from overlying horizons. This type of bioturbation has been considered to be the result of intense activity of termites (Marques et al., 2011). These features are particularly well-preserved in Brazilian Ferralsols with high clay content (Silva and Vidal-Torrado, 1999; Marques et al., 2011).

4.2. Phytolith assemblages and $\delta^{13}C$ values

The main phytolith types that were identified and counted are presented in Fig. 2 and Table 4. The amount of phytoliths per gram of soil is variable, with two important peaks at 30–40 cm and at 100–110 cm (Table 4).

Hierarchical cluster and principal components (PCA) analyses were performed on the phytolith assemblages expressed in percentages. The dendrogram shows that the samples cluster in three groups (Fig. 3) that are in agreement with the pedological horizons previously defined by morphological conformity (colour, texture, structure, porosity, etc.). Group I is composed by the samples from the horizons AB, A3 and the base of A2, group II is composed by the upper part of the A2 horizon, and group III corresponds to the modern samples from the top of the soil.

The PCA first axis accounts for a 33.2% of the total variance and it is defined by bulliform, Dicotiledoneae, Chloridoideae, Panicoideae and Pooideae phytoliths for the positive values and palm and Araucaria phytoliths for the negative values (Fig. 4). This axis can be considered a proxy for the temperature, because the Araucaria forest ecological limit in central Brazil is within a mean winter temperature of <10° (Ledru, 1993).

The PCA second axis expresses 27% of the total variance, and it is defined by Dicotiledoneae and Panicoideae for the positive values and Chloridoideae and Pooideae for the negative values. This axis can be considered a proxy for humidity, varying between mesic to xeric conditions. Woody/Dicotiledoneae plants as well as Panicoideae grasses need higher humidity while Choridoideae are more adapted to dry conditions.

On the basis of the similarity expressed by the statistical analysis, three phytolith zones were identified (Fig. 5), from bottom to top:

Zone I (from ca. 12,131 ± 428 cal BP to ca. 6000 cal BP), subdivided as:

Sub-zone Ia: This sub-zone includes horizon AB and sub-horizon A3 (110–70 cm). The soil has a dark reddish brown color (5 YR – 2.5/2), clay texture (Table 1) and a large amount of vertical pedotubules that extend through the transition between AB and BA horizons. Charcoal fragments were found in large quantities along this transition. The phytolith assemblages are dominated by Poaceae morphotypes that represent, as an average, 88% of the identified assemblage (Table 2). Dicotiledoneae (globular

Angiospermae								Gymnospermae		Not identified		Taphonomised[a]		Amount of Phytolith/g.soil
Monocotiledoneae		Dicotiledoneae (trees and shrubs only)						Araucariaceae Araucaria						
Arecaceae Palms Globular Echinate		Globular psilate/rugose		Papilla		Irregular		Crater-shaped						
	%		%		%		%		%		%		%	
18	5	42	12	2	1	0	0	0	0	11	3	33	10	12,800,000
71	24	8	3	2	1	3	1	5	2	3	1	23	8	2,997,069
52	18	8	3	2	1	6	2	6	2	9	3	52	18	69,226,667
135	41	1	0	4	1	0	0	1	0.3	2	1	17	5	122,716,000
35	15	2	1	5	1	7	2	3	1	6	2	6	2	95,480,000
58	17	16	5	0	0	11	3	1	0.3	5	1	6	2	36,055,556
11	3	10	3	8	2	0	0	0	0	0	0	10	3	27,463,333
11	3	7	2	0	0	1	0.3	0	0	6	2	16	5	35,200,000
24	8	16	5	1	0	0	0	0	0	0	0	20	7	38,390,000
7	2	6	2	2	1	0	0	0	0	7	2	14	4	77,000,000
21	7	29	9	0	0	6	0	0	0	0	0	37	12	316,800,000

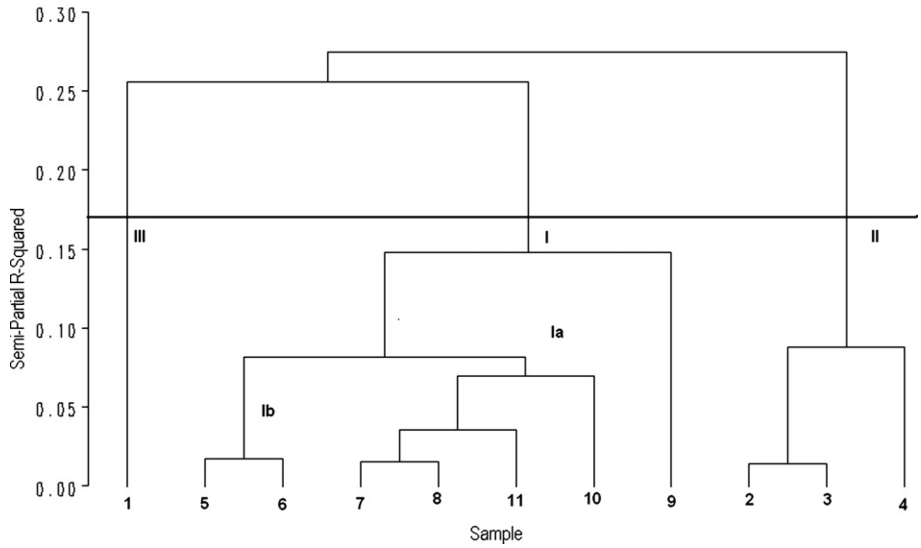

Fig. 3. Cluster analysis (Ward method) for the phytoliths assemblage of the umbric epipedon.

morphotypes) frequency ranges between 2% and 9%, and palms (Arecaceae – globular echinate morphotypes) have values between 2% and 8%. Iph is more than 45%; Ic is less than 30% (with the lowest value of 19% at the bottom); D/P varies between 0.19 and 0.03 (Table 3). Between 120 cm and 70 cm $\delta^{13}C$ values indicate a small but progressive isotopic depletion from −23.2‰ to −24.2‰, suggesting a mixture of C_3 and C_4 plants but with predominance of C_3 (most likely woody elements) (Pessenda et al., 1996, 1998; Gouveia et al., 1999, 2002).

Sub-zone Ib corresponds to the top of sub-horizon A3 and the bottom of A2 (70-40 cm). Darker colour and a large amount of charcoal fragments characterize this zone. Biological activity is also intense and pedotubules and galleries filled with material of lighter colour (probably from the Bw horizon) are common. The sub-zone is separated on the basis of the frequency of globular echinate phytoliths (15%–415%) produced by palms (Arecaceae) as well as the appearance of crater-shaped phytoliths (1%–2%), produced by Araucaria trees. However, the phytolith assemblage is still dominated (76%) by Poaceae phytoliths (of which 5% are Panicoideae, 18% are Pooideae and 4% are Chloridoideae). Dicotiledoneae types account for 6% of the assemblage. The charcoal fragments found in this sub-zone are at similar depth as those found by Silva and Vidal-Torrado (1999) in a nearby soil with similar characteristics and with an age of 6103 ± 113 cal BP The index Iph is 47%–48%, Ic varies between 28% and 34%, and D/P varies between 0.01 and 0.1 (Table 3). A $\delta^{13}C$ value of −23.4‰, presents the same tendency of sub-zone Ia, and suggests a mixture of C_3 and C_4 plants, with predominance of C_3 (Fig. 6).

Zone II corresponds to the A2 sub-horizon (40-10 cm) Colour and texture are similar to those of sub-horizon A3, although the structure is slightly more granular. Phytoliths assemblage showed a very noticeable decrease in Poaceae morphotypes (the sum of Pooideae, Cloridoideae and Panicoideae becomes only 17% of the assemblage) (Table 2). In this zone the Araucariaceae types are more common, reaching a frequency of 2% but the Palms (Arecaceae) types show a more considerable increase, reaching 41% of the phytolith sum at the bottom of this zone. The values of the phytolith indices Iph are generally less than 20% with the exception of the 40-30 cm sample (56.9%); Ic less than 30% and D/P less than 0.1 (Table 3). The $\delta^{13}C$ value (−23.0‰) is a mixture of C_3 and C_4 plants, with predominance of C_3 and the more depleted values between 40 cm and 20 cm (−25.0‰) suggest a progressive predominance of arboreal vegetation. Several diatom exoskeletons (Fig. 2-III) and Pteridophytae spores were observed in the microscope slides, all indicating more humid environmental conditions. Charcoal fragments lines as well as sparse fragments were observed in the upper

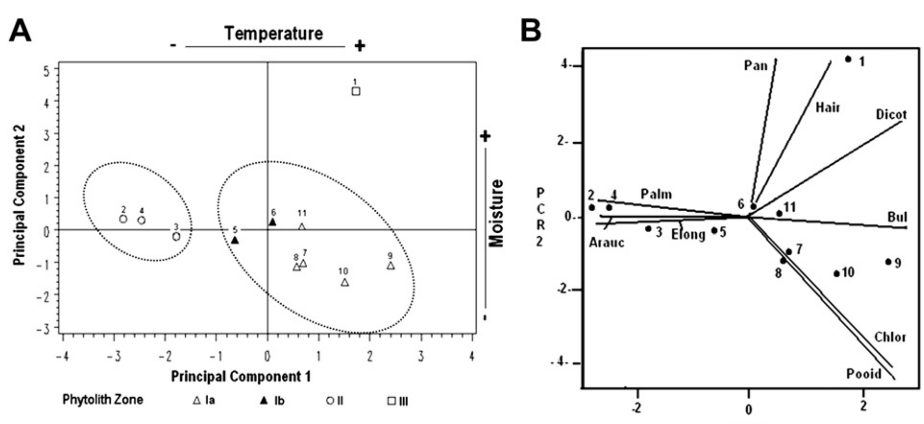

Fig. 4. Principal component analysis of the phytoliths assemblage from the umbric epipedon. (A) Ellipses and points dispersion diagram for each phytolith zone. (B) Principal Components Regression.

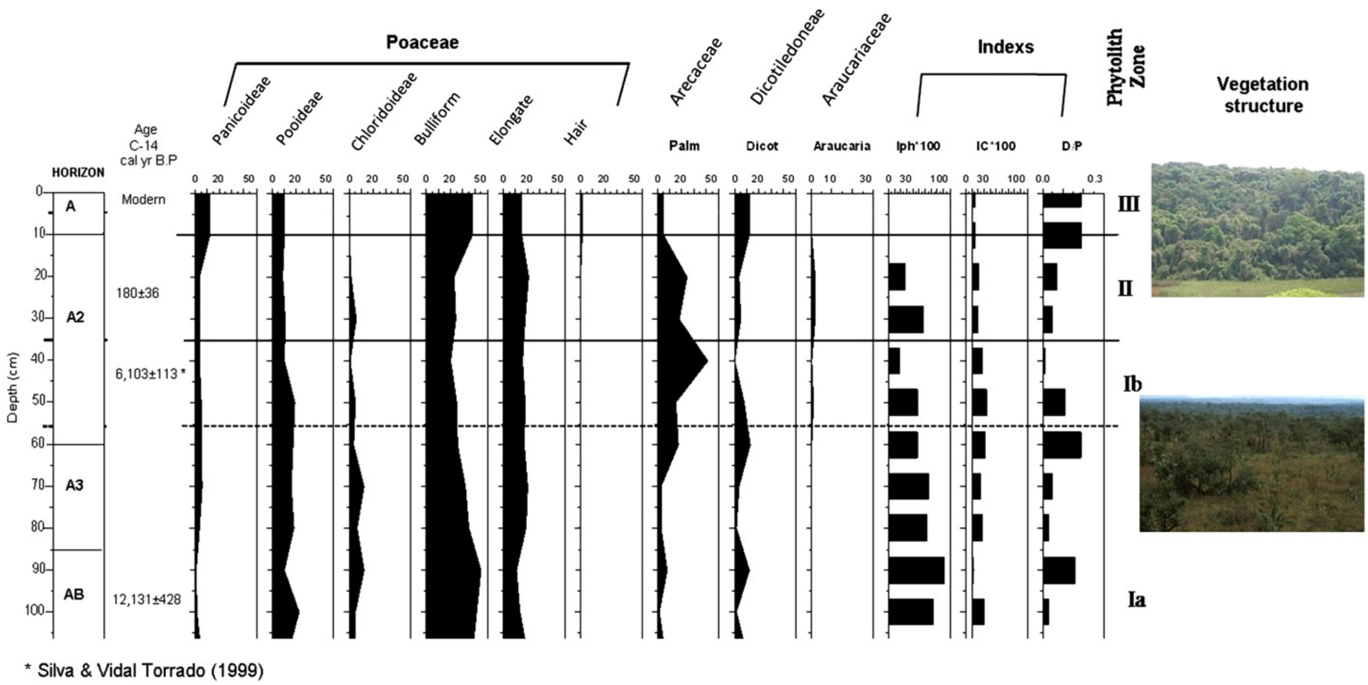

Fig. 5. Phytolith spectrum and indexes from the umbric epipedon.

part of this zone (between 30 and 20 cm), and the charcoal was dated 180 ± 36 cal BP, probably reflecting anthropogenic activities during the colonial period (Silva and Vidal-Torrado, 1999).

Zone III corresponds to the deposit of the current vegetation (an open forest) and shows $\delta^{13}C$ value of −23.0‰. The charcoal fragments found in this zone were dated to the modern age, after 1950.

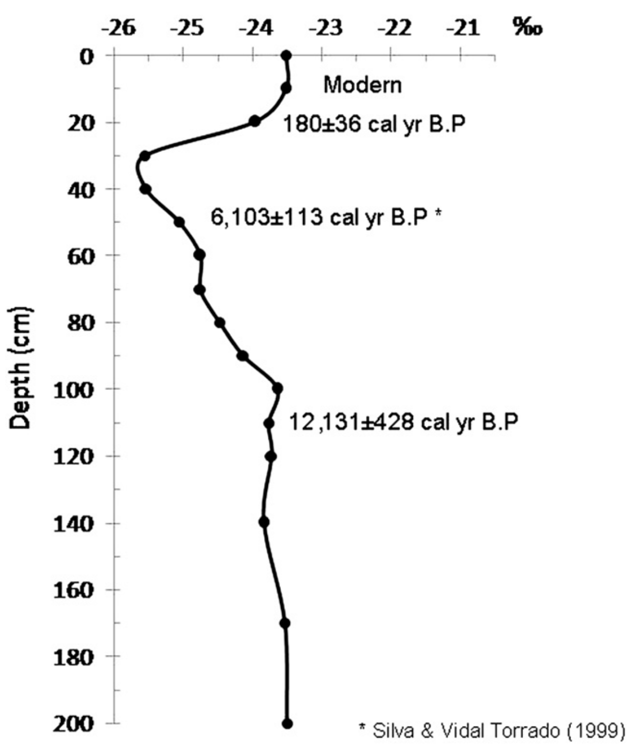

Fig. 6. Range of $\delta^{13}C$ values in the umbric epipedon.

In this zone an increase of Poaceae phytoliths in comparison with Zone II was observed. The phytolith indices are: Iph 0, Ic 16.1% and D/P 0.2 (Table 3 and Fig. 6).

5. Discussion

The pedogenetic history of Brazilian Oxisols seems to have recorded some climatic variations as well as erosion cycles since the Neogene (Müggler, 1998; Schaefer, 2001). However, the results suggest low climatic variability since 12,000 cal BP, as evident by the isotopic signal of the organic matter accumulated in the humic horizon, derived from plant formations composed by a mixture of C_3 and C_4 plants. The interactions between different forms of

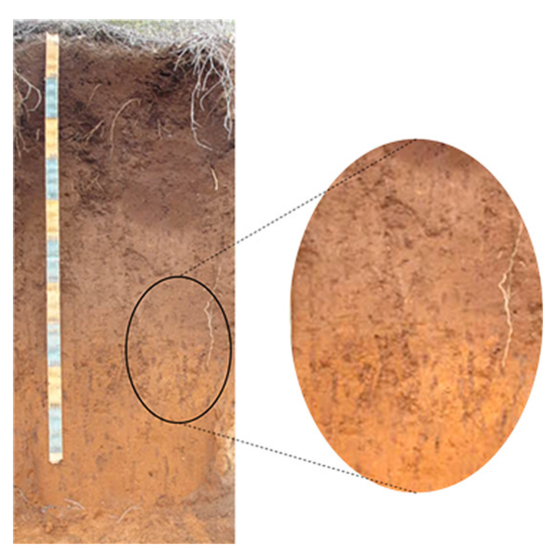

Fig. 7. Details of the biological channels in transitions horizons AB/BA of Humic Hapludox profile (Machado, Minas Gerais State, Brazil).

carbon and poorly crystalline aluminum represent a mechanism of carbon protection in the humic epipedon that results in higher OM accumulation (Marques et al., 2011).

The signal in Zone I (between 12, 131 ± 428 cal BP and 6103 ± 113 cal BP), the base of the humic horizon (umbric epipedon), is of an open savannah with arboreal elements and a grass cover mainly composed of C_3 taxa. This vegetation was associated with a drier-than-present climate, also identified in the region by Ledru (1993), Ledru et al. (1994), Alexandre et al. (1999) and Pessenda et al. (1996, 2004). The first indication of a more significant abundance of arboreal elements in the vegetation cover and of an increase in humidity is observed from around 6000 cal BP (50–60 cm depth – top of Zone Ib) with the appearance of Araucaria phytoliths (crater-shaped).

The low values of the D/P index in Zone I are similar to the ones from shrub steppes observed in Africa by Barboni et al. (1999). Alexandre et al. (1999) interpret the *D*/*P* values between 1 and 1.3 in an Oxisol from Salitre (Minas Gerais, Brazil), more or less contemporary to Zone I of the current profile, as open vegetation with some trees and shrubs. These data are also in agreement with the results presented by Silva and Vidal-Torrado (1999). This zone has a common presence of biological channels (bioturbation) between 120 and 90 cm (Fig. 7). The occurrence of bioturbation in Brazilian Oxisols, particularly those with high clay content, is rather common because these soils can preserve these features for long periods (Boulet et al., 1995; Behling et al., 1998; Gouveia and Pessenda, 2000; Schaefer, 2001). The linear increase with depth of the corroded phytoliths together with the decrease of $\delta^{13}C$ and organic carbon content are consequence of prolonged pedogenetic processes. These characteristics are common in many tropical and sub-tropical soils (Parton et al., 1987) and are observed in the majority of the isotopic datasets from Brazilian soils (Vidotto, 2008).

In Zone II, the phytolith assemblages show an increase of the crater-shaped (Araucariaceae) and of globular echinate (Arecaceae – palms) morphotypes together with a decrease of grass (Poaceae) phytoliths as well as an isotopic depletion (Fig. 6). The signal from this trend is reinforced by the presence of *Dicksonia sellowiana* Hook. spores and diatom exoskeletons. This evidence points to an increase in humidity from around 6000 cal BP that was already observed at the top of Zone I. The current results are in agreement with earlier studies that highlighted a humid phase in Central Brazil during the Mid-Late Holocene with the establishment of climatic conditions similar to the present and the formation of forest vegetation (Oliveira, 1992; Ledru, 1993; Pessenda et al., 1996, 1998, 2004; Scheel-Ybert et al., 2003).

Finally, the phytoliths from Zone III represent the modern day vegetation, an ecotone of Sub-perennial Tropical Forest and *Cerrado* (BRASIL, 1983; Silva and Vidal-Torrado, 1999) with species typical of *Cerrado* such as *Casearia sylvestris* Sw. and *Piptadenia gonoacantha* (Mart.) Macbr. The presence of charcoal fragments (180 ± 36 cal BP) and charred phytoliths indicate fire events during the 18th century. Silva and Vidal-Torrado (1999) interpreted these last fire episodes as resulting from the initial occupation of the area during the colonial period.

6. Conclusions

This is the first time that an Oxisol with a humic horizon has been studied in detail using two powerful and complementary palaeoenvironmental techniques. The association of $\delta^{13}C$ values and phytolith records has shown that it is extremely important to rely on a combined set of evidence to refine understanding of environmental and climatic evolution of tropical and sub-tropical areas. The poor pollen preservation in this kind of terrestrial record makes it even more fundamental to move to an interdisciplinary approach and to take advantage of all the available records preserved in these deposits.

On the basis of $\delta^{13}C$ and phytolith records, it is likely that the organic matter accumulated in the humic horizon of this profile derived from plant formations composed by a mixture of C_3 and C_4 plants, with a certain increase in C_3 plant contribution from the Middle Holocene. The index values characterizing the studied soil are similar to those of soils from African tropical regions (Alexandre et al., 1997; Barboni et al., 1999, 2007) and to other studies in Brazil (Alexandre et al., 1999). They indicate dry to humid mesothermic climatic conditions suitable for the development of a mixture of vegetation with C_3 and C_4 plants, but with a predominance of C_3 plants. Despite the general agreement with the values from other geographical areas, it is necessary to improve the accuracy of the indices for the tropical and sub-tropical vegetation of South America.

Finally, there is also the need for additional studies of phytolith assemblages from tropical modern plants in South America to obtain a more precise taxonomic understanding of the phytolith assemblages that will allow distinguishing vegetation variability in relation to topography, humidity and temperature.

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References

Absy, M.L., Cleef, A.M., Fournier, M., Martin, L., Servant, M., Sifeddine, A., Ferreira da Silva, M., Soubies, F., Suguio, K., Turcq, B., Van Der Hammen, T., 1991. Mise en évidence de quatre phases d'ouverture de la forêt dense dans le sud-est de l'Amazonie au cours des 60,000 derniéres anneés. Premiérecomparaison avec d'autres régions tropicales. Comptes Rendus de l'Academie des Sciences de Paris 312 (II), 673–678.

Alexandre, A., Meunier, J.-D., Lczine, A.-M., Vincens, A., Schwartz, D.A., 1997. Phytoliths: indicators of grassland dynamics during the late Holocene in intertropical Africa. Palaeogeography, Palaeoclimatology, Palaeoecology 136, 213–229.

Alexandre, A., Meunier, J.-D., Mariotti, A., Soubies, F., 1999. Late Holocene phytolith and carbon-isotope record from a Latosol at Salitre, south-central Brazil. Quaternary Research 51, 187–194.

Argant, J., 1990. Climat et environnement au Quaternaire dans le Bassin du Rhône d'après les données palynologiques. Document Laboratoire Géologique de Lyon, Lyon. n.111, p.199.

Barboni, D., Bonnefille, R., Alexandre, A., Meunier, J.D., 1999. Phytoliths as paleoenvironmental indicators, West Side middle Awash Valley, Ethiopia. Palaeogeography, Palaeoclimatology, Palaeoecology 152, 87–100.

Barboni, D., Bremond, L., Bonnefille, R., 2007. Comparative study of modern phytolith assemblages from inter-tropical Africa. Palaeogeography, Palaeoclimatology, Palaeoecology 246, 454–470.

Beebe, K.R., Kowalski, B.R., 1987. An introduction to multi-variate calibration and analysis. Analytical Chemistry 59, 1007A–1017A.

Behling, H., Lichte, M., Miklós, A.W., 1998. Evidence of a forest free landscape under dry and cold climatic conditions during the last glacial maximum in the Botucatu region (São Paulo State), Southeastern Brazil. In: Rabassa, J., Salemme, M. (Eds.), Quaternary of South America an Antarctic Peninsula, vol. 11. A.A. Balkema, Rotterdam, pp. p99–110.

Borba-Roschel, M., Alexandre, A., Varajao, A.F.D.C., Meunier, J.D., Varajao, C.A.C., Colin, F., 2006. Phytoliths as indicators of pedogenesis and paleoenvironmental changes in Brazilian cerrado. Journal of Geochemical Exploration 88 (1/3), 172–176. Amsterdam.

Boulet, R., Pessenda, L.C.R., Telles, E.C.C., Melfi, A., 1995. Une evaluation de la vitesse de l'accumulation de matière par la faune du sol à partir de la datation dês charbones et d'humine du sol. Exemple dês versants du lac Campestre, Salitre,

Minas Gerais, Brasil. In: Comptes Rendus de L'Academie dês Sciences, vol. 320, pp. 287–294. Série IIA, Paris.
BRASIL. Ministério das Minas e Energia. Secretaria Geral, 1983. Projeto RADAM-BRASIL. Folhas SF23/24, Rio de Janeiro/Vitória: Geologia, geomorfologia, pedologia, vegetação e uso Potência da Terra. Rio de Janeiro: Ministério das Minas e Energia, 1983. 780p. (Levantamento dos Recursos Naturais, 32).
Bremond, L., Alexandre, A., Hély, C., Guiot, J., 2005. A phytolith index as a proxy of tree cover density in tropical areas: calibration with Leaf Area Index along a forest–savanna transect in southern Cameroon. Global and Planetary Change 45 (4), 277–293.
Calegari, M.R., 2008. Ocorrência e Significado paleoambiental do horizonte A húmico em Latossolos. Escola Superior de Agricultura Luiz de Queiroz, Piracicaba. 259p (Tese de Doutorado).
Carnelli, A., 2002. Long term dynamics of the vegetation at the subalpine-alpine ecocline during the Holocene: comparative study in the Aletsch region, Val d'Arpette, and Furka Pass (Valai, Switzerland). Terre & Environnement 40, 349.
Diester-Haas, L., Schrader, H.J., Thiede, J., 1973. Sedimentological and paleoclimatological investigations of two pelagic ooze cores off Cape Barbas, North-West Africa. Meteor Forsch-Ergebnisse, C 16, 19–66.
Embrapa, 1997. Empresa Brasileira de Pesquisa Agropecuária – Embrapa. In: Manual de métodos de análises de solo, vol. 2. Centro Nacional de Pesquisa de Solos, Rio de Janeiro, p. 212.
Embrapa, 2006. Empresa Brasileira de Pesquisa Agropecuária – Embrapa. Centro Nacional e Pesquisa em Solos. Sistema Brasileiro de Classificação de Solos, Brasilia: Embrapa-SP; Rio de Janeiro. 306p.
FAO-UNESCO, 1981. Soil Map of the World (1:5.000.000) v.1–10. UNESCO, Paris.
Fredlund, G.G., Tieszen, L.L., 1994. Modern phytolith assemblages from the North American great Plains. Journal of Biogeography 21, 321–335.
Gouveia, S.E.M., Pessenda, L.C.R., 2000. Datation par Le ^{14}C de Charbons inclus dans lê sol pour l'etude du role de la remontée biologique de matiére et du colluvionnement dans la formation de latosols de l'etat de São Pauloi, Brésil. In: Comptes Rendus de L'Académie dês Sciences, vol. 330, pp. 133–138. Série IIA, Paris, n 2.
Gouveia, S.E.M., Pessenda, L.C.R., Aravena, R., Boulet, R., Roveratti, R., Gomes, B.M., 1997. Dinâmica de vegetações durante o Quaternário recente no sul do Amazonas indicada pelos isótopos do carbono (^{12}C, ^{13}C e ^{14}C). Geochimica Brasiliensis 11 (3), 355–367.
Gouveia, S.E.M., Pessenda, L.C.R., Boulet, R., Aravena, R., Scheel-Ybert, R., 1999. Isotopos do carbono dos carvões e da matéria orgânica do solo em estudos de mudanças de vegetação e clima no Quaternário e da taxa de formação de solos do estado de São Paulo. Anais da Academia Brasileira de Ciências 71 (4), 969–980.
Gouveia, S.E.M., Pessenda, L.C.R., Aravena, R., Boulet, R., Scheel-Ybert, R., Bendassoli, J.A., Ribeiro, A.S., Freitas, H.A., 2002. Carbon isótopos in charcoal and soils in studies of paleovegetation na climate changes during the late Pleistocene and the Holecene in the southeast and centerwest regions of Brazil. Global and Planetary Change 33 (1–2), 95–106. Amsterdam.
Kampf, N., Klamt, E., 1978. Mineralogia e Gênese de Latossolos (Oxisols) e Solos Podzólicos da Região Nordeste do Planalto Sul-Riograndense. Revista Brasileira de Ciência do Solo, Viçosa 2, 68–73.
King, L.C., 1956. A geomorfologia do Brasil Oriental. Revista Brasileira de Geografia 18 (2), 147–265. Rio de Janeiro.
Kondo, R., Iwasa, Y., 1981. Opal phytoliths in humic yellow laterosols and yellow laterosols in the Amazon region. Research Bulletin Obihiro University 12, 231–239.
Ledru, M.-P., 1993. Late quaternary environmental and climatic changes in Central Brazil. Quaternary Research 39, 90–98.
Ledru, M.P., Behling, H., Fournier, M., Martin, L., Servant, M., 1994. Localisation de la forêt d'Araucaria du Brésil au cours de l'holocène – implications paléoclimatiques. Comptes Rendus de l'Académie des Sciences, Sciences de la vie 317 (III), 517–521.
Lepsch, I.F., Buol, S.W., 1986. Oxisol-landscape relationship in Brazil. In: International Soil Classification Work Shop, 13, Campinas. 1986. Part1: papers. Campinas: SNLCS/EMBRAPA. University of Puerto Rico, USA, pp. 174–189.
Madella, M., Powers-Jones, A.H., Jones, M.K., 1998. A Simple method of Extraction of opal phytoliths from Sediments using a Non-Toxic heavy liquid. Journal of Archaeological Science 25, 801–803.
Madella, M., Alexandre, A., Ball, T., 2005. International Code for phytolith Nomenclature 1.0. Annales of Botany 96 (2), 253–260.
Marques, F.A., Calegari, M.R., Vidal Torrado, V., Buurman, P., 2011. Relationship between soil oxidizable carbon and physical, chemical and mineralogical properties of Umbric Ferralsons. Revista Brasileira de Ciência do Solo 35, 25–40.
Martinelli, L.A., Pessenda, L.C.R., Espinoza, E., Camargo, P.B., Telles, E.C., Cerri, C.C., Victoria, R.L., Aravena, R., Richey, J., Trumbore, S., 1996. Carbon-13 variation with depth in soils of Brazil and climatic change during Quaternary. Oecologia 106, 376–381.
Mehra, O.P., Jackson, M.L., 1960. Iron oxide removal from soils and clays by a dithionite–citrate system buffered with sodium bicarbonate. Clays and Clay Minerals 7, 317–327.
Montti, L., Honaine, M.F., Osterrieth, M., Ribeiro, D.G., 2009. Phytolith analysis of Chusquea ramosissima Lindm. (Poaceae: Bambusoideae) and associated soils. Quaternary International 193 (1-2), 80–89.
Müggler, C.C. 1998. Polygenetic oxisols on Tertiary surfaces, Minas Gerais, Brazil-soil genesis and landscape development. Thesis (PhD), The Netherlands: Wageningen Agricultural University, Wageningen, 185p.
Mulholland, S.C., 1989. Phytolith shape frequencies in North Dakota grasses: a comparison to general patterns. Journal of Archaeological Science 16, 489–511.
Nakashima, P., 1973. Estudo das formações superficiais na área de Campinas – Viracopos (SP): suas relações com a evolução geomorfológica e os solos. 149p. Dissertação, Mestrado na área de Geografia Física, Faculdade de Filosofia, Letras e Ciências, Universidade de São Paulo, São Paulo.
Oliveira, P.E., 1992. A Palynological record of late quaternary vegetation and climatic change in southeastern Brazil, PhD Dissertation, Ohio State University, Columbus, 238p.
Parr, J., Watson, L., 2007. Morphological characteristics observed in the leaf phytoliths of selected Gymnosperms of eastern Australia. In: Madella, M., Zurro, D. (Eds.), Plants, People and Places- Recent Studies in Phytoliths Analysis. Oxbow Books, p. 272.
Parton, W.J., Schimel, D.S., Cole, C.V., Ojima, D.S., 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. Soil Science Society of America Journal 51, 1173–1179.
Pessenda, L.C.R., Aravena, R., Melfi, A.J., Telles, E.C.C., Boulet, R., Valencia, E.P.E., Tomazello, M., 1996. The use of carbon isotopes (C-13, C-14) in soil to evaluate vegetation changes during the Holocene in Central Brazil. Radiocarbon 38 (2), 191–201.
Pessenda, L.C.R., Gomes, B.M., Aravena, R., Ribeiro, A.S., Boulet, R., Gouveia, S.E.M., 1998. The carbon isotope record in soils along a forest–cerrado ecosystem transect: implications for vegetation changes in the Rondonia state, southwestern Brazilian Amazon region. The Holocene 8 (5), 631–635.
Pessenda, L.C.R., Gouveia, S.E.M., Aravena, R., Boulet, R., Valencia, E.P.E., 2004. Holocene fire and vegetation changes in southeastern Brazil as deduced from fossil charcoal and soil carbon isotopes. Quaternary International 114, 35–43.
Pessenda, L.C.R., Gouveia, S.E.M., Freitas, H.A., Ribeiro, A.S., Aravena, R., Bendassoli, J.A., Ledru, M.P., Scheel-Ybert, R., 2005. Isótopos do carbono e suas aplicações em estudos paleoambientais. In: De Oliveira, P., Suguio, K., Gouveia, C. (Eds.), O Quaternário No Brasil. Holos, São Paulo, pp. 75–93.
Piperno, D.R., 2006. Phytoliths: a Comprehensive Guide for Archaeologists and Paleoecologists. AltaMira Press, Lanham MD.
Queiroz Neto, J.P., Castro, S.S., 1974. Formações Superficiais e Latossolos Vermelho-Amarelo Húmico na Área de Bragança Paulista. Estado de São Paulo, Brasil. In: Congresso Brasileiro de Geologia, vol. 18, pp. 65–83. Porto Alegre. Anais. Porto Alegre: SBG.
Runge, F., 1999. The opal phytolith inventory of soil in central Africa – quantities, shapes, classification, and spectra. Review of Palaeobotany and Palynology 107, 23–53.
Santos, R.D., Lemos, R.C., Santos, H.G., Ker, J.C., Anjos, L.H., 2006. In: Viçosa, M.G (Ed.), Manual de descrição e coleta de solo no campo, vol. 5. Sociedade Brasileira de Ciência do Solo, p. 100.
Schaefer, C.E.R., 2001. Brazilian latosols and their B horizon microstructure as long-term biotic constructs. Australian Journal Soil Research 39, 909–926. Melbourne.
Scheel-Ybert, R., Gouveia, S.E.M., Pessenda, L.C.R., Aravena, R., Coutinho, L.M., Boulet, R., 2003. Holocene palaeoenvironmental evolution in the São Paulo State (Brazil) based on anthracology and soil $\delta^{13}C$ analysis. The Holocene 13 (1), 73–81.
Silva, A.C., Vidal-Torrado, P., 1999. Gênese dos Latossolos Húmicos e sua relação com a evolução da paisagem numa área cratônica do Sul do Minas Gerais. Revista Brasileira de Ciência do Solo 23, 329–341.
Twiss, P.C., 1987. Grass opal phytoliths as climatic indicators of the Great Plains Pleistocene. In: Johnson, W.C. (Ed.), Quaternary Environments of Kansas. Kansas Geological Survey Guidebook, vol. 5, pp. 179–188.
Twiss, P.C., 1992. Predicted world distribution of C3 and C4 grass phytolihs. In: Rapp Jr., G., Mulholland, S.C. (Eds.), Phytolith Systematics. Emerging Issues, Advances in Archaeological and Museum Science, vol. 1. Plenum Press, pp. 113–128.
USDA, 1999. Soil Taxonomy. A Basic System of Soil Classification for Making and Interpreting Soil Surveys. In: Soil Survey Staff, Handbook, second ed., vol. 436. United States Government Printing Office, Washington, DC.
Vidotto, E., 2008. Reconstrução paleoambiental (vegetação e clima) no Parque Estadual da Ilha do Cardoso – SP durante o Quaternário tardio. Doutorado em Energia Nuclear na Agricultura e no Ambiente. Centro de Energia na Agricultura, Universidade de São Paulo, Piracicaba. 210p. Tese.