MULTIPROXY ANALYSIS (PHYTOLITHS, STABLE ISOTOPES, AND C/N) AS INDICATORS OF PALEOENVIRONMENTAL CHANGES IN A CERRADO SITE, SOUTHERN BRAZIL

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ABSTRACT – Cerrado vegetation is associated with the semi-humid climate of the Central Brazil highlands. The presence of this vegetation in small and sparse areas in Paraná, Southern Brazil, can be associated with its past climate conditions. Paleoenvironmental changes of Cerrado vegetation in the Campo Mourão region (24º06'S - 52º23'W) are discussed in this study. The environmental changes were characterized using proxy data that includes stable isotopes and phytolith analyses in peaty sediments. Two drill cores obtained in alluvial plains were analyzed. Results were compared with the isotopic record (δ^{13}C) from a trench in the Cerrado Ecological Station. Based on the results, we can infer that in ~48,800±270 yrs BP, the vegetation was mainly composed of grasses and at ~42,280 cal yrs BP a wetter climate allowed the expansion of arboreal vegetation. During the Middle Holocene (~7280 cal yrs BP), a drier period is also reported. Current climatic conditions (wet) were established since ~3280 cal yrs BP, after which the data suggests the expansion of subtropical forests over the Cerrado.

Keywords: Holocene, phytoliths, Pleistocene, stable isotopes.

RESUMO – A ocorrência de vegetação de cerrado está associada ao clima semiúmido e ao Planalto Central do Brasil. A presença dessa vegetação em pequenas e esparsas áreas no Estado do Paraná, Sul do Brasil, está associada às condições climáticas pretéritas. Mudanças paleoambientais da vegetação do Cerrado na região do Campo Mourão (24º06′S - 52º23'O) são discutidas neste trabalho. As alterações ambientais foram caracterizadas utilizando dados proxy, que incluem isótopos estáveis e análise de fitólitos em sedimentos turfosos. Foram analisados dois testemunhos sedimentares obtidos em planícies aluviais. Os resultados foram comparados com o registro isotópico (δ^{13}C) de uma trincheira na Estação Ecológica do Cerrado. Com base nos resultados, podemos inferir que em ~48.800 anos AP, a vegetação era composta principalmente por gramíneas seguidas por um período provavelmente mais úmido com expansão da vegetação arbórea ~42.280 anos cal. AP. Um período mais seco até o Holoceno Médio (~7.280 anos cal. AP) também é relatado. As condições climáticas modernas (úmidas) foram estabelecidas desde ~3.280 anos cal. AP; desde então é observado o avanço da vegetação arbórea sobre o Cerrado.

Palavras-chave: Holoceno, fitólitos, Pleistoceno, isótopos estáveis.

INTRODUCTION

The Cerrado is a xeromorphic vegetation characteristic of a semi-humid climate and is often classified as "Brazilian Savanna" (Eiten, 1972). This vegetation is easily found in the central Brazilian highlands, covering ~2 million km², (23% of the Brazilian territory) and is only exceeded by the Amazon biome (Klink & Machado, 2005). The Cerrado vegetation has more than 7,000 known species of vascular plants, and about 44% is endemic flora. For this reason, the Cerrado is considered the richest tropical savanna in the world, with considerable ecological importance (Eiten, 1972; Pinheiro & Monteiro, 2010).

Nevertheless, the Cerrado vegetation in the southern part of Brazil is poorly known, and some hypotheses about its presence are linked with its past environment. Maack (1949) suggested that the Cerrado vegetation that expanded during a dry/less humid climate occurred almost entirely on the South American continent in the Late Pleistocene. Recently, the use of multiproxy data in sedimentary cores, such as phytolith analyses (*e.g.* Piperno & Becker, 1996; Alexandre *et al.*, 1999; Borba-Roschel *et al.*, 2006; Coe *et al.*, 2013; Calegari *et al.*, 2013, 2015), palynology (*e.g.* Behling, 1998, 2002, 2006; Behling & Negrelle, 2001; Behling & Safford, 2010; Cohen *et al.*, 2012), stable isotopes (*e.g.* Pessenda *et al.*, 1996, 1998a, b; Freitas *et al.*, 2001), and others, have provided significant information about the past environment changes in different biomes in Brazil.

Carbon isotope signatures ($\delta^{13}C$) from the soil organic matter (**SOM**) has been widely regarded as a useful marker of environmental change, providing relevant proxy information about the characteristics of past environments in the Cerrado (Ledru *et al.*, 1996; Pessenda *et al.*, 1998a, 2004; Cohen *et al.*, 2012). Through stable carbon isotope analysis, it is possible to distinguish the C_3 ($\delta^{13}C$= -32 through -20‰) and C_4 ($\delta^{13}C$= -17 through -9‰) photosynthetic pathway in plants. The composition of $\delta^{13}C$ in C_3 and C_4 plants differ in approximately 14‰ (Boutton, 1991). Furthermore, the use of $\delta^{13}C$ has been combined with C/N in many studies to distinguish aquatic and terrestrial materials preserved in peat sediments (Meyers, 1994; Zani *et al.*, 2012). On the other hand, the C/N ratio is obtained from Total Organic Carbon (**TOC**) and the Total Nitrogen (**TN**), which helps to distinguish organic matter produced from freshwater phytoplankton (C/N: 4.0–10.0) and terrestrial plants (C/N: ≥12) (Meyers, 1994; Zani *et al.*, 2012).

Recently, the phytolith analysis has played an important role in paleoenvironmental reconstruction (Piperno & Becker, 1996; Alexandre *et al.*, 1999; Borba-Roschel *et al.*, 2006). Furthermore, the phytolith analysis can also be associated with stable carbon isotope analysis (Coe *et al.*, 2013; Calegari *et al.*, 2013). The formation of phytoliths ($SiO_2.nH_2O$) starts when silica (Si) is absorbed by plants in a soluble form (H_4SiO_4) from groundwater by the roots system and is accumulated as opal in micrometer shapes especially in leaves (Twiss *et al.*, 1969; Piperno, 1989; Kealhofer & Piperno, 1998). As an inorganic material, phytoliths are relatively stable in soils and sediments for thousands of years and for this reason they are an important tool for paleoclimatic and paleoenvironmental reconstructions (Piperno & Becker, 1996; Alexandre *et al.*, 1999; Borba-Roschel *et al.*, 2006; Coe *et al.*, 2013; Calegari *et al.*, 2013).

This paper aims to identify and track paleoenvironmental changes in the Cerrado vegetation from the Campo Mourão region, using a multi-proxy approach, including radiocarbon dating, phytolith analysis, stable isotopes ($\delta^{13}C$ and $\delta^{15}N$), and elementary data (TOC, TN, and C/N).

MATERIAL AND METHODS

Study site

The Campo Mourão plateau is composed by the basalts known as Serra Geral Formation. The formation of oxisols occurs mainly in areas of flattened relief and nitosols in areas with a steeper slope. Both types of soils have been widely used for intensive commercial agriculture since the decade of 1970. In the floodplains, where the terrains remain saturated in water practically all year, the formation of hydromorphic soils occurs, usually covered with peaty sediments (Luz & Parolin, 2013). The regional climate is classified as Cfb type, humid subtropical, with hot summers and concentrated rains without a dry season, according to the Köppen-Geiger classification. The average temperature in summer is higher than 22°C and in winter below 18°C, with an average precipitation of ~1,750 mm/yr (Andrade & Néry, 2003). Campo Mourão Municipality can be considered a transitional vegetation area, with the presence of the native Araucaria Forest, the Semi-deciduous Forest, and the Cerrado (Figure 1).

Floristic composition and carbon isotope signature of modern plants

Floristic identification of the dominant species in a radius of ~200 m from the sampling sites was performed by the herbarium staff (**HCF**) of the Universidade Tecnológica Federal do Paraná (**UTFPR**). In the core site, the vegetation is composed mainly by the following families: Acanthaceae, Asteraceae, Blechnaceae, Convolvulaceae, Cyperaceae, Fabaceae, Melastomataceae, Piperaceae, Poaceae, Pontederiaceae, Pteridaceae, Rubiaceae, Sapindaceae, Selaginellaceae, Thelypteridaceae, Woodsiaceae, and Xyridacea. Samples of leaves and stems of the most representative species were sent to the Center for Nuclear Energy in Agriculture (¹⁴C Laboratory, CENA), University of São Paulo, for carbon isotope determination.

Sampling and analytical procedures

Three sampling points were analyzed, two peaty cores in a transitional area of Araucaria Forest and a trench at the Cerrado Ecological Station (**CES**). The peaty sediment cores were collected using a vibro-core system (Martin *et al.*, 1995) with a 6 m long and 10 cm diameter aluminum tube, at the Ranchinho (**RRC**, 24º06'43"S - 52º23'04"W) and the Água dos Papagaios River (**APC**, 24º05'52"S - 52º23'44"W) alluvial plain. Both cores were 110 cm long and were collected
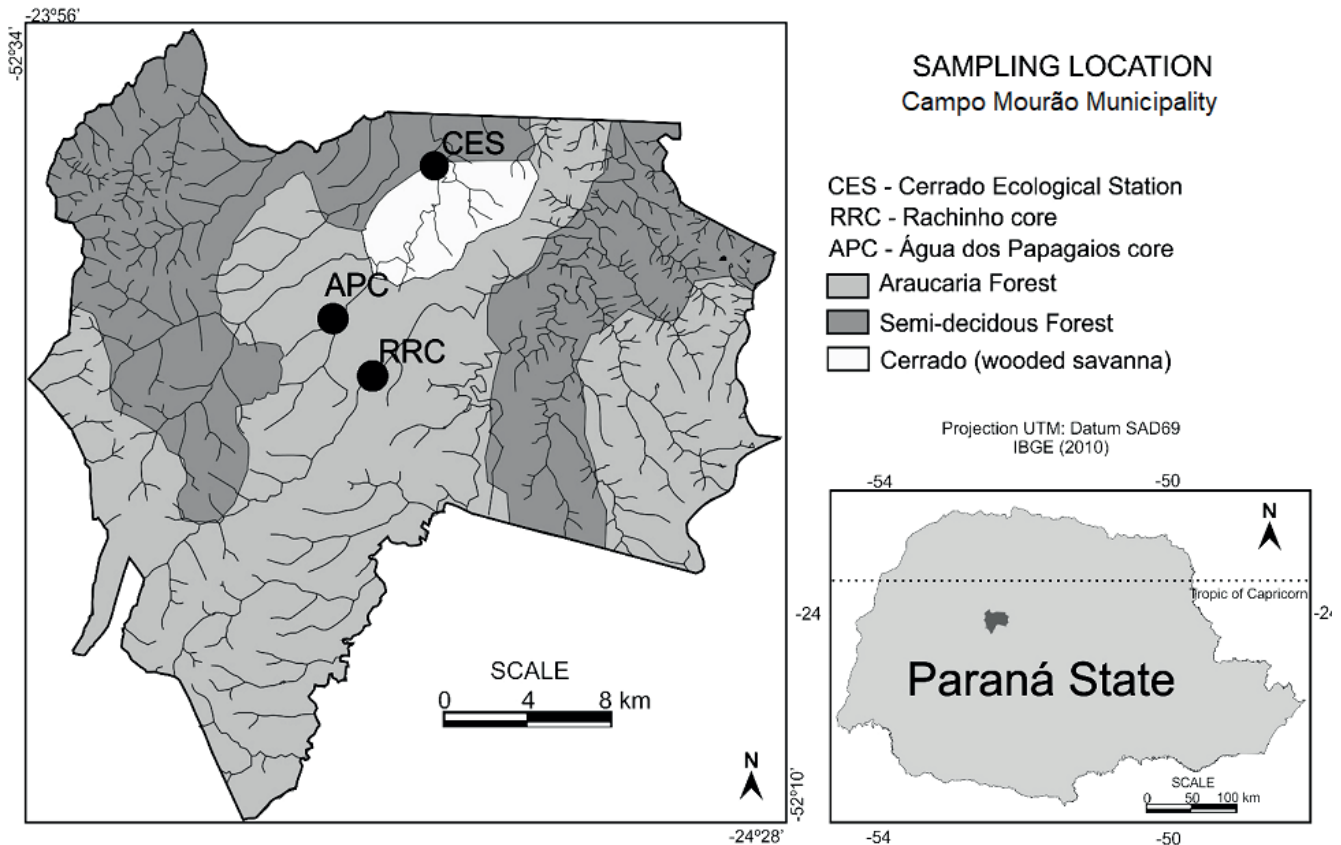
Figure 1. Sampling location in the Campo Mourão Municipality.

on the right margin in the middle of the alluvial plain. The cores were subsampled (5 cm³) in 3 cm intervals in the RRC and 4 cm intervals in the APC for phytolith analysis. Soil samples from the Cerrado Ecological Station (**CES**) were obtained at 10 cm intervals up to 370 cm depth for carbon isotope analysis ($\delta^{13}C$) and a charcoal sample for ^{14}C dating. Samples of CES trench were also sent to the ^{14}C Laboratory of CENA.

Carbon and nitrogen elementary and isotope analysis

Subsamples of the peaty cores (10 cm³) were dried at 60°C for 24 hours and sent for C and N elementary and isotope analysis. The $\delta^{13}C$, $\delta^{15}N$, Total Organic Carbon – TOC (%), Total Nitrogen – TN (%) analyses were carried out at the Center for Applied Isotopes Studies (**CAIS**) of the University of Georgia (USA) and at the ^{14}C Laboratory of CENA (Table 1). The isotope composition ($\delta^{13}C$ and $\delta^{15}N$), with analytical precision of ±0.2‰, are obtained through the following equations:

$$(1) \ \delta^{13}C \ (‰) = [R_{sample1}/R_{standard} -1] \times 1000$$

$$(2) \ \delta^{15}N_{sample} = [R_{sample2} - R_{air}/ R_{air}] \times 1000$$

where: $R_{sample1}$ and $R_{sample2}$ are, respectively, the $^{13}C/^{12}C$ and ratio $^{15}N/^{14}N$ of the sample, $R_{standard}$ refers to the $^{13}C/^{12}C$ ratio of the standard and R_{air} refers to the $^{15}N/^{14}N$ ratio of the atmospheric air (Pessenda *et al.*, 1996, 1998a,b).

The radiocarbon dating was carried out at the AMS Laboratory of the Center for Applied Isotope Studies - CAIS (Table 1). For dating calibration, we used the software Calib 7.1 (Stuiver, *et al.*, 2017). The ages were calibrated using the Intcal13 calibration curve, with 2σ error (Reimer *et al.*, 2013). One of the five radiocarbon ages was not able to be calibrated because it was out of range.

Phytolith extraction, counting, and classification

Phytoliths were extracted at the Paleoenvironmental Studies Laboratory (**LEPAFE**), following a modified protocol of Faegri & Iversen (1975): (i) Samples of 1 cm³ were collected in each 3 cm (RC, n=38 samples) and 4 cm (APC, n=28 samples). In a Becker, 20 ml of hydrochloric acid (10% concentration) were added to the samples to remove carbonates; (ii) the sediment samples were heated up to boil in a hotplate (2 hours to 60° C) with 50 ml of potassium hydroxide (10 %); (iii) the samples were centrifuged (500 RPM for 3 minutes) with distilled water for 3 times; (iv) afterwards, we added zinc chloride solution with 2.4 g/cm³ density; (v) the samples were centrifuged (500 RPM for 3 min); (vi) the supernatant containing the particles with density less than 2.4 g/cm³ was removed to separate phytoliths from other minerals; (vii) later, the samples were centrifuged (500 RPM for 3 minutes) with distilled water until the total elimination of the chloride; (viii) For each sample, 5 slides were made with 50 μl of sample material and covered using Entellan®.

Table 1. Samples analyzed in each sediment core. **Abbreviations: CAIS,** Center for Applied Isotopes Studies, University of Georgia, USA; **CENA,** Stable Isotope Laboratory, Center for Nuclear Energy in Agriculture, University of São Paulo, Brazil; **TN,** Total Nitrogen; **TOC,** Total Organic Carbon.

Sample (cm)	^{14}C Dating	δ^{13}C (‰)	δ^{15}N (‰)	TOC (%)	TN (%)	CAIS	CENA
Ranchinho River Core (RRC)							
0		x	x	x	x		x
10–07		x	x	x	x		x
19–16		x	x	x	x		x
28–25		x		x	x	x	
34–31		x	x	x	x		x
45	x	x				x	
58–55		x		x	x	x	
67–64		x	x	x	x		x
79–75		x		x	x	x	
88–85		x	x	x	x		x
97–94	x	x				x	
Água dos Papagaios Core (APC)							
All samples		x	x	x	x		x
45	x					x	
75	x					x	
Trench at Cerrado Ecological Station (CES)							
All samples		x					x
120–130	x					x	

Two hundred phytolith grains were counted in each sample and classified. We followed the International Code for Phytoliths Nomenclature 1.0 (**ICPN**) (Madella *et al.*, 2005). The ICPN uses as morphotype descriptors: a) the shape; b) the texture and/or ornamentation and c) the anatomical origin. For phytolith morphology identification, we used other studies published in the scientific literature, *e.g.* Twiss *et al.* (1969), Rapp & Mulholland (1992), Meunier & Colin (2001), Piperno (2006) and Coe & Osterrieth (2014). In addition, we used the reference collection of the Paleoenvironmental Studies Laboratory (LEPAFE).

Phytolith concentration for each sample was established by counting the absolute number of morphotypes viewed in three arbitrary transects. The microscopic slides were catalogued and stored in LEPAFE by the codes: Col. Agrícola (**APC**) (L163.C16; L164.C16 and L165.C16) and Rio Ranchinho (**RRC**) (L147.C14; L148.C14; L149.C14 e L150.C14). TiliaGraph® software was used for the graphical reproduction of the results and to load the cluster analysis used for establishing the phytolith zones.

RESULTS

Floristic and isotope signature of modern plants

Herbaceous species are predominant in the study area: *Melica sarmentosa* Nees., *Pseudechinolaena polystachya* (Kunth) Stapf with some arboreal species, *Acisanthera variabilis* (Mart. and Schrank) Triana, *Tibouchina cerastifolia* Cogn., *Mimosa pilulifera* Benth. Isotopic signals of the modern plants in the study area (Table 2) reflect the mixture of C_3 and C_4 plants. The isotopic signal of -20.17‰ obtained from the litter sample located in the RRC support this value.

^{14}C dating

Four sedimentary units were dated in the sedimentary cores, and one age was obtained in the soil profile at the CES site (Table 3). RRC (95 cm) was dated 48,800 ± 270 yrs BP and ~42,183 cal yrs BP (45 cm). APC was dated ~7280 cal yrs BP (75 cm) and ~3282 cal yrs BP (45 cm). CES soil profile was dated to ~5820 cal yrs BP (120–130 cm).

Table 2. Floristic composition of the study area and their respective δ¹³C and photosynthetic pathway.

Family	Specie	δ¹³C (‰)	Photosynthetic pathway
Annonaceae	*Anona coriacea* Mart.	-29.91	C_3
Arecaceae	*Butia paraguayensis* (Barb. Rodr.) L. H. Bailey	-29.1	C_3
Cucurbitaceae	*Cayaponia espelina* (Silva Manso) Cogn.	-31.93	C_3
Cyperaceae	*Rhynchospora corymbora* (L.) Britton.	-28.77	C_3
Erythroxylaceae	*Erythroxylum suberosum* St. Hil.	-30.37	C_3
Fabaceae	*Anadenanthera falcata* (Benth.) Altschul	-30.07	C_3
Melastomataceae	*Leandra lacunosa* Cogn.	-29.83	C_3
Myrtaceae	*Myrcia rastrata* DC.	-31.48	C_3
Poaceae	*Pennisetum purpureum* Schumach	-12.3	C_4
Poaceae	*Trichachne insularis* (L.) Nees	-12.63	C_4
Poaceae	*Bracharia decumbens* Stapf	-12.3	C_4
Poaceae	*Dactyloctenium aegyptium* (L.) Willd.	-13.59	C_4
Polypodiaceae	*Polypodium*	-29.28	C_3
Smilacaceae	*Smilax campestris* Grisebach.	-28.64	C_3
Vochysiaceae	*Vochysia tucanorum* Mart.	-30.65	C_3

Table 3. ¹⁴C dating of selected samples. **Abbreviations: APC**, Água dos Papagaios core; **CES**, Cerrado Ecological Station; **RRC**, Ranchinho Core; **UGAMS – AMS Laboratory**, University of Georgia, USA.

Lab Code (UGAMS)	Sample	Depth (cm)	Sample Material	Age (yrs BP)	Error	2-σ range (cal yr BP)	Median calibrated age (cal yrs BP)
10581	APC	45	Sediment	3060	25	3208 – 3356	~3282
10580	CES	120	Charcoal	5060	30	5738 – 5902	~5820
10582	APC	75	Sediment	6340	25	7239 – 7322	~7280
11842	RRC	45	Sediment	37,920	160	41,895 – 42,477	~42,183
11843	RRC	95	Sediment	48,800	270	*	*

*Out of range (Reimer *et al.*, 2013).

Elementary and isotopic analysis of C and N

The concentrations of TOC ranged from 7.8% (87 cm) to 26.98% (8 cm) in the RRC, 0.48% (110 cm) to 27.68% (15 cm) in the APC, and 0.28% (370 cm) to 2.1% (1 cm) in the CES. The TN ranged from 0.84% (77 cm) to 15.15% (8 cm) in the RRC and 0.04% (110 cm) to 1.86% (15 cm) in the APC (Tables 4–6). In the RRC the relation between δ¹³C and C/N suggest the presence of algae matter in the entire core. In contrast, APC presents more C_4 terrestrial plants in sediments older than ~7280 cal yrs BP and a tendency to harbor algae in younger sediments (Figure 2). The C/N values ranged between 1.42 (0 cm) to 15.61 (27 cm) in the RRC and 12.03 (110 cm) to 56.47 (80 cm) in the APC. The δ¹³C values ranged from -20.17‰ (base layer) to -14.09‰ (77 cm) in the RRC, -19.2‰ (10 cm) to -14.24‰ (110 cm) in the APC, and -18.94‰ (A horizon) to -14‰ (320 cm) in the CES. The δ¹⁵N ranged from 2.49‰ to 18.9‰ at the APC site and from 2.78‰ to 11‰ at the RRC site.

Phytolith zones

The RRC phytolith assemblage is mainly composed by Elongate, Parallelepipedal bulliform and Cuneiform bulliform morphotypes (Figures 3–4). A greater diversity of morphotypes was reported from 25-cm-depth toward the top, with the presence of short cell morphotypes, such as Bilobate, Saddle, Rondel, Cross, and Trapeziform. The phytolith assemblage of APC presents greater diversity of morphotypes in relation to the RRC, with a proper distribution of short cell forms in the sedimentary profile (Figures 3–4).

Ranchinho River Core – RRC. Four phytolith zones were defined in the RRC (Figure 4, Appendix 1):

- Zone I (110–50 cm) was dated ~48,800 ± 270 yrs BP (95 cm). Parallelepipedal bulliform, Cuneiform bulliform and Elongate psilate were the most representative morphotypes of this zone. These morphotypes were also more weathered than the morphotypes found in the superficial layers.
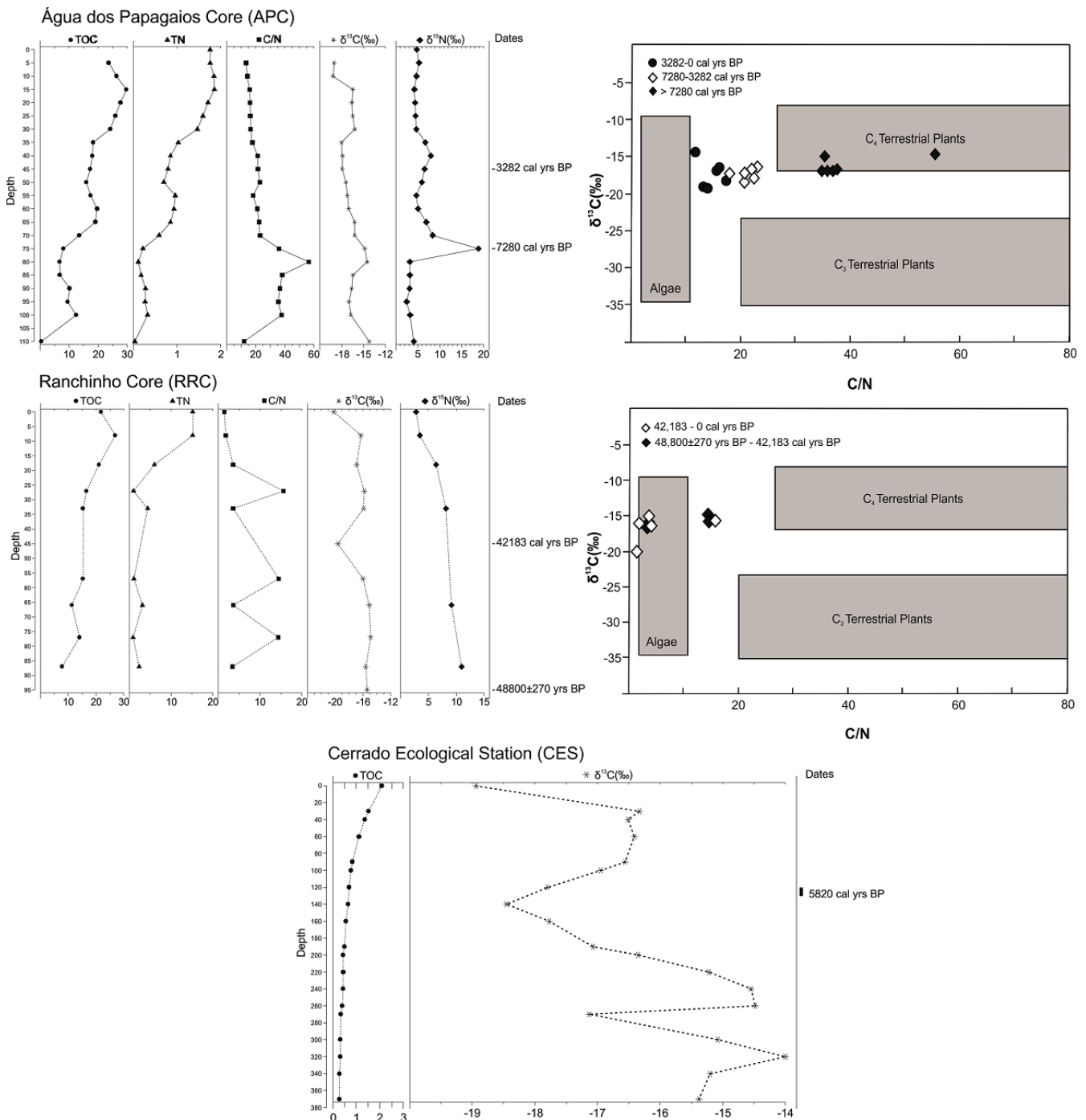
Figure 2. Elementary and isotopic analyses of C and N (**TOC**, Total Organic Carbon; **TN**, Total Nitrogen; C/N, $\delta^{13}C$ and $\delta^{15}N$), and $\delta^{13}C$ and C/N correlation.

- Zone II (50–40 cm) was dated ~42,183 cal yrs BP at 45 cm depth. In this zone, the reduction of Elongate and Parallelepipedal bulliform morphotypes and the occurrence of Globular echinate and Globular psilate were observed, associated with Arecaceae, Bromeliaceae, and ligneous dicotyledons.

- In Zone III (40–20 cm), the reduced occurrence of globular morphotypes was noted, with the presence of more robust forms (Parallelepipedal bulliform, Cuneiform bulliform and Elongate).

- In Zone IV (20–0 cm), the phytolith assemblage becomes more varied, with the presence of Bilobate and other short cells morphotypes (Cross, Rondel, and Trapeziform) associated with the presence of Poaceae without water stress. Globular morphotypes occur again.

Água dos Papagaios Core (APC). Four phytolith zones were also established in APC (Figure 4, Appendix 2):

- Zone I (110–100 cm). The most representative phytoliths were the short cells (Bilobate, Saddle, and Rondel);

Table 4. Values of C (%), N (%), δ13C, δ15N, and C/N (Água dos Papagaios).

Depth (cm)	C (%)	δ¹³C	N (%)	δ¹⁵N	C/N
0	-	-	1.76	4.77	-
5	23.62	-19.04	1.76	5.32	13.42
10	26.36	-19.2	1.85	4.72	14.23
15	29.64	-16.48	1.86	4.25	15.90
20	27.68	-16.62	1.71	4.4	16.23
25	25.93	-16.5	1.59	4.48	16.32
30	24.19	-16.21	1.47	4.68	16.46
35	18.33	-18.03	1.03	6.75	17.76
40	18.04	-17.90	0.85	7.99	21.34
45	17.25	-17.93	0.80	6.56	21.53
50	16.00	-17.44	0.70	5.95	22.96
55	17.45	-17.22	0.96	4.7	18.22
60	19.67	-17.06	0.93	5.09	21.10
65	19.08	-16.23	0.85	6.97	22.34
70	13.55	-16.23	0.59	8.42	23.01
75	8.06	-14.85	0.22	18.9	35.96
80	6.78	-14.52	0.12	3.19	56.47
85	6.86	-16.47	0.18	3.2	38.13
90	10.22	-16.64	0.28	3.15	36.48
95	9.59	-17.01	0.27	2.49	35.53
100	12.49	-16.76	0.33	3.26	37.83
110	0.48	-14.24	0.04	4.13	12.03

Table 5. Values of C (%), N (%), δ13C, and C/N (Ranchinho).

Depth (cm)	C (%)	N (%)	δ¹³C	C/N
28–25	16.48	1.01	−15.8	15.61
43–40	–	–	−19.6	–
58–55	15.28	1.10	−16	14.46
79–75	14.05	0.84	−14.9	14.36
97–94	–	–	−15.4	–

characteristic of Poaceae, with a low incidence of bulliform forms (Parallelepipedal and Cuneiform).

- Zone II (100–60 cm), 7280 cal yrs BP at 75 cm depth, shows a progressive increase in forms of bulliform (Parallelepipedal and Cuneiform). Zone III (60–35 cm), ~3282 cal yrs BP at 45 cm, presents a reduction of morphotypes Parallelepipedal bulliform and Cuneiform bulliform, with a progressive increase of short cells. A reduction in the occurrence of Globular echinate and lobular psilate (characteristics of Arecaceae, Bromeliaceae, and ligneous dicotyledons) was also observed, suggesting the predominance of Poaceae.

- Zone IV (35–0 cm) presents modern vegetation characteristics, composed mainly by small morphotypes (Poaceae), with minor variations in the composition of phytoliths. At 15 cm depth, there is a slight increase in the occurrence of Parallelepipedal and Cuneiform bulliform forms.

Table 6. Values of C (%) and δ13C (Cerrado Ecological Station).

Depth (CM)	C (%)	$\delta^{13}C$
0–10	2.10	-18.94
30–40	1.52	-16.33
40–50	1.37	-16.51
60–70	1.12	-16.41
90–100	0.84	-16.56
100–110	0.78	-16.95
120–130	0.69	-17.80
140–150	0.66	-18.45
160–170	0.56	-17.77
190–200	0.50	-17.07
200–210	0.44	-16.35
220–230	0.45	-15.22
240–250	0.44	-14.55
260–270	0.41	-14.48
270–280	0.35	-17.13
300–310	0.33	-15.08
320–330	0.33	-14.00
340–350	0.29	-15.20
370–380	0.28	-15.38
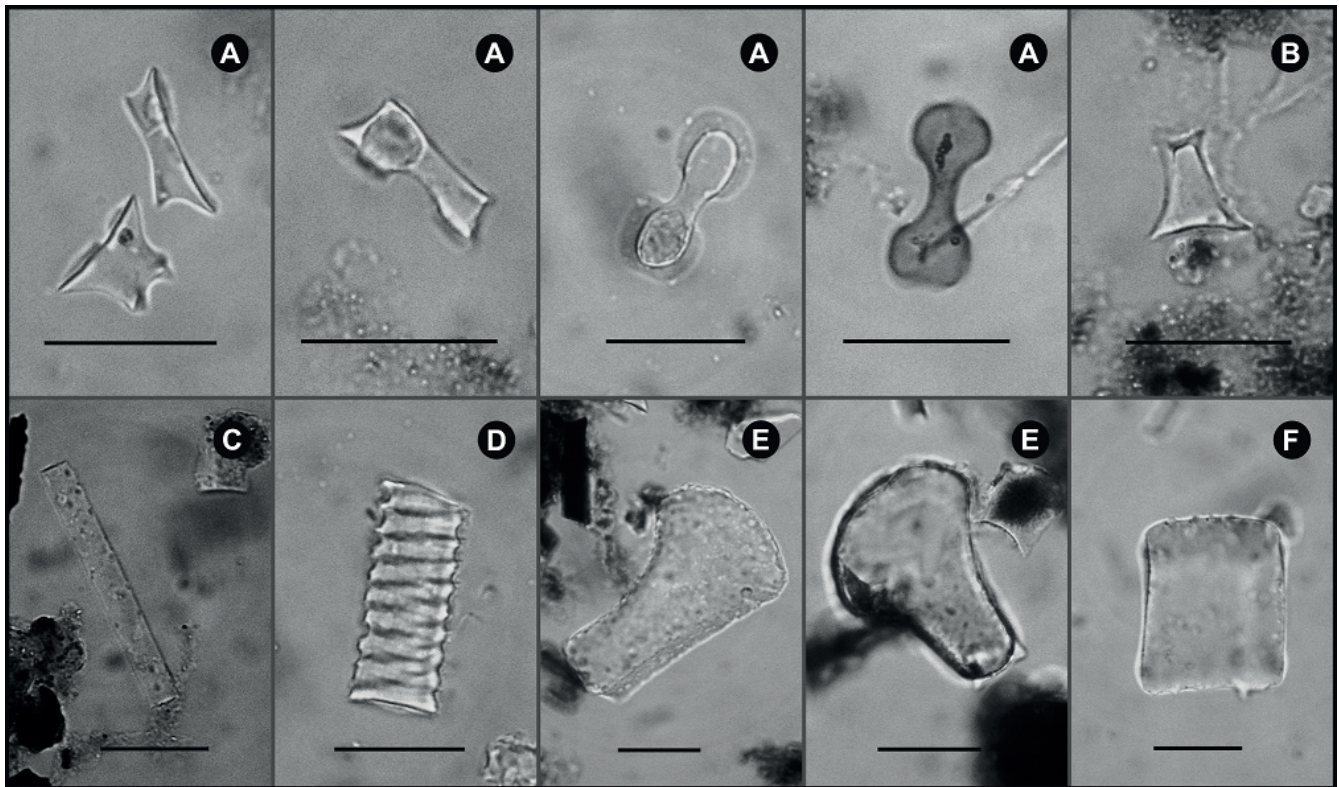
Figure 3. Main phytolith morphotypes classified from the peaty sediment samples: **A**, Bilobate; **B**, Rondel; **C**, Elongate psilate; **D**, Cylindrical sulcate tracheid; **E**, Cuneiform bulliform; **F**, Parallepipedal bulliform. Scale bars = 25 µm.

APC - Água dos Papagaios core
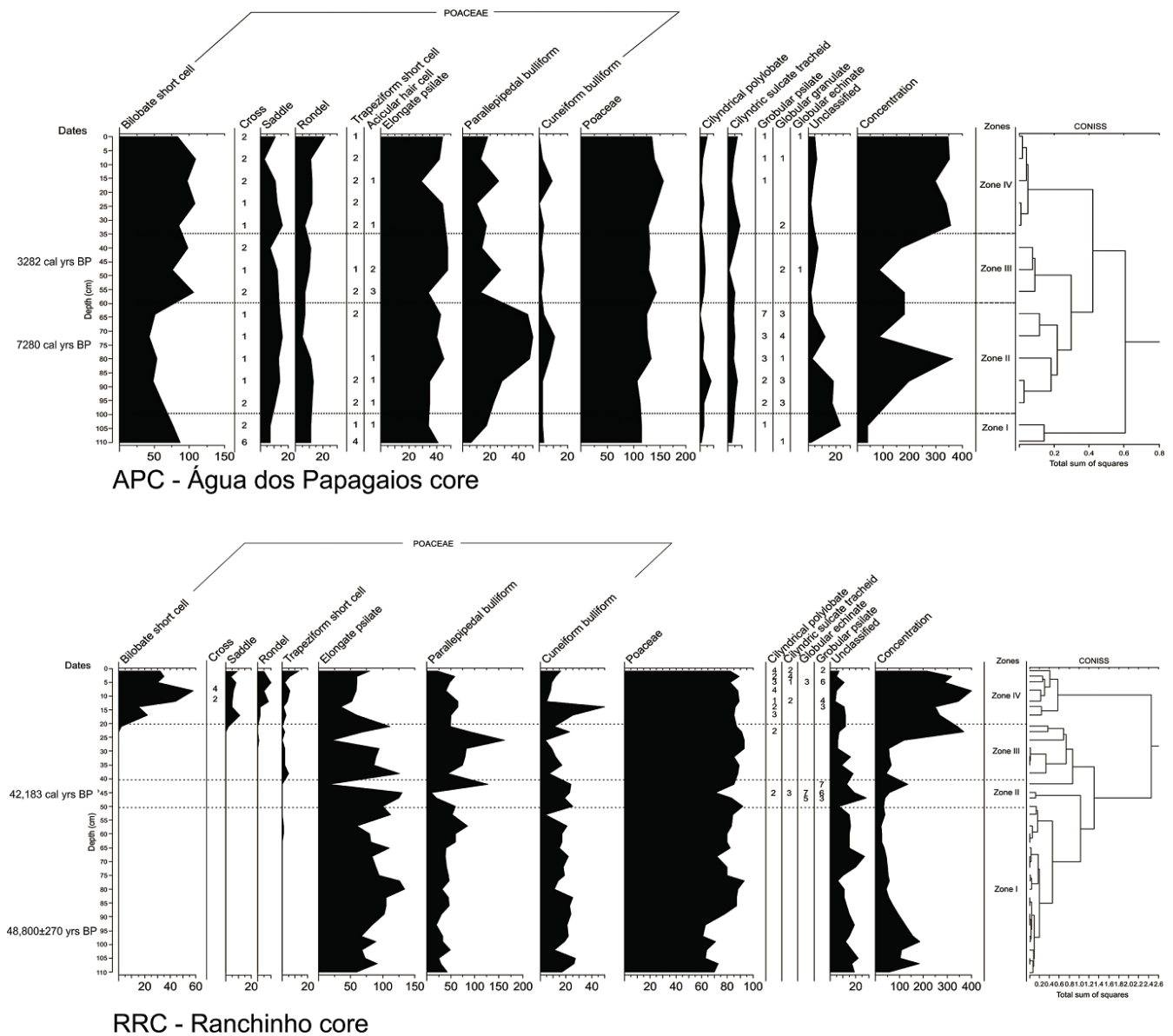

RRC - Ranchinho core

Figure 4. Diagram of phytolith morphotypes and zones identified in the sedimentary profiles.

DISCUSSION

Unclassified morphotypes, as well as the corrosion of phytolith surfaces decreased, progressively towards the top. This result may be related to the natural process of phytolith dissolution, where only robust forms resist destruction (Alexandre *et al.*, 1997; Barboni *et al.*, 1999; Borba-Roschel *et al.*, 2006; Coe *et al.*, 2013). According to Cabanes & Shahack-Gross (2015), partial dissolution of phytoliths may be related to the bulk ratio of the individual morphotype.

Phytolith assemblages, $\delta^{13}C$ and $\delta^{15}N$ values, TN, TOC and C/N in Zone I of the RRC, dated 48,800 ± 270 yrs BP reflects abundance in C_4 plants. The higher presence of bulliform morphotypes is probably associated with hydric stress (Parry & Smithson, 1958). Bremond *et al.* (2005) found that the silicification of the bulliform cells is related

to the hydric stress in Poaceae and Cyperaceae. Furthermore, higher silicification of bulliform cells is also associated to leaf aging (older leaves deposit more silica in bulliform cells than young leaves), and to transpiration rate (higher transpiration, higher silicification) (Takeoka *et al.*, 1984; Fernández Honaine & Osterrieth, 2012). The C/N and $\delta^{15}N$ data indicate the phytoplankton is an important source for the origin of the sediment organic matter. However, even in dry periods, the Ranchinho River alluvial plain probably helped to maintain wet conditions in the sampling site. Similarly, Behling (2002) also noticed a drier phase ~48,000 yrs BP, with the expansion of grasses under colder conditions in the Southern and Southeastern Brazil.

In the RRC Zone II there are more occurrences of globular morphotypes. According to the literature, Globular echinate is produced mainly by Arecaceae (Piperno & Jones, 2003;

Bremond *et al*., 2005; Rasbold *et al*., 2011) and Bromeliaceae (Kealhofer & Piperno, 1998). Globular psilate was recorded for Euphorbiaceae and Proteaceae by Mercader *et al*. (2009) and by Raitz (2012) for pteridophytes, Orchidaceae, Meliaceae, Lauraceae and Salicaceae, and in fewer instances for the family Poaceae. All of these vegetation occurrences are associated with a humid climate. More depleted $\delta^{13}C$ values of ~-19‰, suggest a more important presence of C_3 plants in a mixture with C_4 plants (Pessenda *et al*., 1996, 1998), probably associated with the expansion of the arboreal vegetation over the C_4 herbs/ grasses due to a more humid climate than in the previous period. The C/N values are lower (~14.4) in the sediment organic matter, which also indicates an increase of precipitation (Meyers, 1994). This interpretation agrees with Behling (2006), who suggested wetter climatic conditions from ~42,840 to ~41,470 yrs BP in Cambará do Sul/RS (Southern Plateau).

In the zone IV of the RCC the addition of the Bilobate morphotype is associated with Poaceae and Panicoideae, adapted to higher humidity or available moisture in the soil (Barboni *et al*., 1999, Bremond *et al*., 2005). The association of these morphotypes with the decrease of bullifoms and globular accretion reaffirm the interpretation of increased humidity in the uppermost 20 cm of the core. The C/N <5 ratio indicates a high influence of organic matter of aquatic origin. The concentration of phytoliths becomes more expressive (>300 phytoliths), and there is a more significant deposition of bulliforms (predominance of Parallelepipedal) in zone II of the APC core. The $\delta^{13}C$ values suggest the dominance of C_4 grasses adapted to drier conditions, and at ~7280 cal yrs BP, 75 cm depth, an abrupt increase in the C/N value (~56.47) is indicative of an input of terrestrial C_4 plant organic matter in association with the increase of $\delta^{15}N$ and its highest value (~18.9‰) since the early Holocene at the APC site. Isotopic data progressively changing to more depleted values of $\delta^{13}C$

(-18.45‰) up to ~7280 yrs cal BP in APC indicates a mixture of C_3 and C_4 plants and is probably related to a more humid climate. The $\delta^{15}N$ values (~5‰) suggest the presence of phytoplankton in the sediment organic matter (Meyers 1994, 2006; Meyers & Ishiwatari, 1993).

Pessenda *et al*. (1996) reported a drier climate with the predominance of C_4 plants from the Late Pleistocene (~10,530 cal yrs BP) to the Middle Holocene in an oxisol profile in the region of Londrina (Northern Paraná State), ~200 km from the study area. It is probably associated with the Pleistocene expansion of Cerrado. Gouveia *et al*. (2002) reported the presence of C_4 plants in the Upper Pleistocene of Jaguariaíva (Paraná State), progressively changing with a mixture of C_3 plants in the Lower Holocene. A similar tendency is observed at CES, as the dominance of C_4 plants (~-14‰ to -15.38‰) from the lowermost layer up to 300 cm depth is probably associated with a drier climate.

The presence of a drier period up to ~7000 yrs BP is also attested by other studies conducted near the study area (*e.g.* Stevaux, 2000; Behling, 2006; Parolin *et al*., 2008; Guerreiro *et al*., 2013, and others) (Figure 5). The modern predominant humid climate conditions were established ~4000-3000 cal yrs BP in the northern Paraná (Pessenda *et al*., 1996, 1998a) and São Paulo states (Pessenda *et al*., 1996, 1998a; Gouveia *et al*., 2002; Scheel-Ybert *et al*., 2003). Behling (1998) noticed the expansion of Araucaria over areas previously dominated by grasses, ~2872 yrs cal BP in a more humid climate in the region of Campos Gerais, Paraná.

Stevaux (2000) and Parolin *et al*. (2008) registered a drier period between 3500–1500 yrs BP in the Paraná River floodplain, located ~200 km from our study site. However, this drier period was not observed in this study. In the same paper, Stevaux (2000) stated that the current conditions of climate and vegetation were established from 1500 yrs BP onward.

(1) Bigarella & Andrade-Lima (1982); (2) Jabur (1992), Stevaux (1994); (3) Melo *et al*. (2003); (4) Pessenda *et al*. (2004); (5) Moro *et al*. (2004); (6) Stevaux (2000); (7) Rezende (2010); (8) Guerreiro *et al*. (2013); (9) Alcantara-Santos *et al*. (2014)
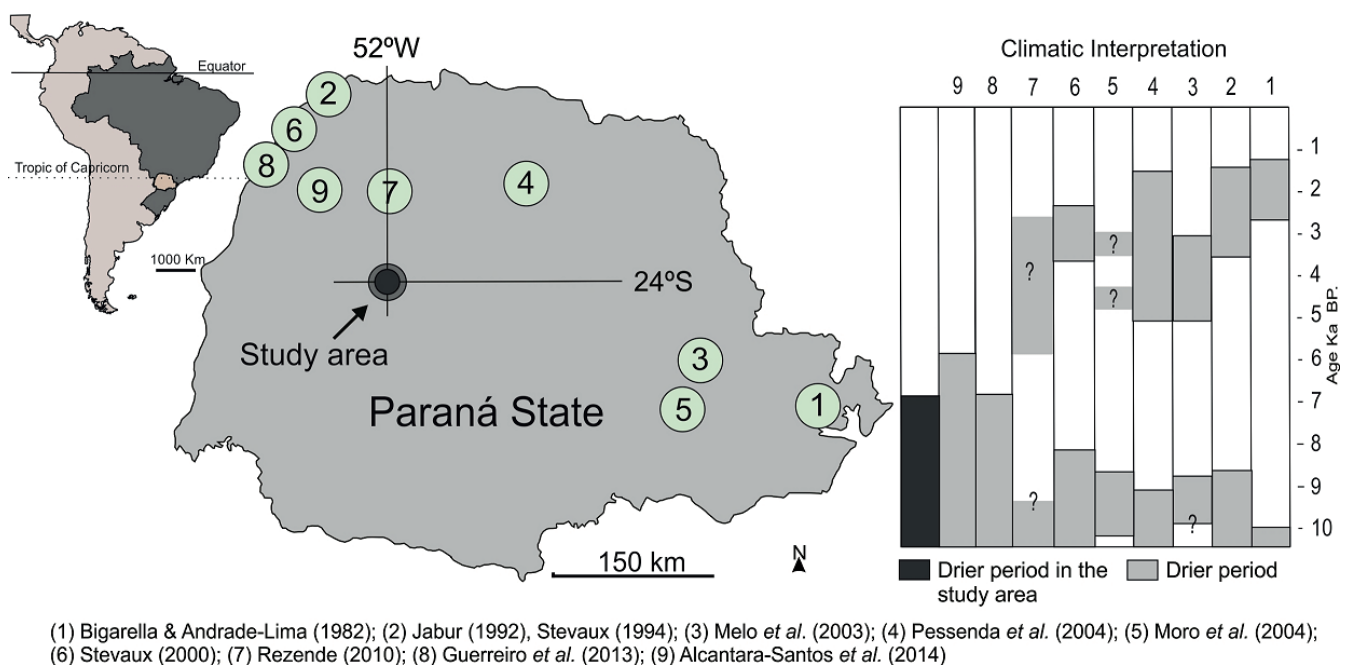
Figure 5. Climatic interpretation given by this study compared with selected studies in the Paraná State during the Holocene.

CONCLUSIONS

The application of phytolith analysis and isotopic and elementary C and N data has provided strong evidence for environmental changes since the Late Pleistocene in the Campo Mourão region. The results reflect the presence of grasses in the sampling location since ~48,800 years BP, including the Last Glacial Maximum, and suggesting the presence of Cerrado since the Late Pleistocene. In general, we can infer that at ~48,800 yrs BP, the vegetation was composed mainly of Poaceae, with an expansion of ligneous plants in a wetter phase ~42,280 cal yrs BP. In the Água dos Papagaios core, a drier period was identified during the Middle Holocene (~7280 cal yrs BP). The present climatic conditions were established at 3282 cal yrs BP with short variations in the phytolith composition, elementary, and isotopic data. In summary, since the Early Holocene, the arboreal vegetation has increasingly expanded over the Cerrado areas in the last ~3282 cal yrs BP. Our results corroborate Maack (1949)'s hypothesis, which proposes that the Cerrado origin was related to the drier climate conditions during the Late Pleistocene.

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Appendix 1. Phytoliths counting table of the Ranchinho core.

Depth (cm)	Bilobate	Saddle	Rondel	Cross	Cuneiform bulliform	Paralelepipedal bulliform	Cilindric polylobate	Cylindrical sulcate tracheid	Trapeziform polylobate	Elongate psilate	Acicular hair cell	Globular echinate	Globular granulate	Globular psilate
0	32	9	5	0	16	23	4	2	12	80	1	0	0	2
4	35	4	7	0	12	59	2	4	5	59	0	0	0	0
7	30	8	10	0	9	48	3	1	2	60	0	0	2	4
10	58	6	5	4	8	40	4	0	0	59	0	0	0	0
13	44	5	8	2	5	65	1	2	4	52	0	2	0	2
16	15	5	2	0	50	65	2	0	0	35	0	0	3	0
19	22	11	2	0	25	50	3	0	1	60	0	0	0	0
22	2	2	0	0	11	49	0	0	0	110	0	0	0	0
25	0	0	0	0	23	90	2	0	0	70	0	0	0	0
28	0	0	0	0	4	162	0	1	0	21	0	0	0	0
31	0	0	0	0	9	82	0	0	0	95	0	0	0	0
34	0	0	0	0	12	80	0	0	0	90	0	0	0	0
37	0	0	0	0	17	75	0	1	2	85	0	0	0	0
40	0	0	0	0	7	44	0	0	5	126	2	0	0	0
43	2	0	1	0	4	147	0	0	0	16	0	0	0	1
46	0	0	0	0	23	10	2	3	0	130	0	7	0	6
49	0	0	0	0	19	20	0	0	0	125	0	5	0	3
52	0	0	0	0	25	60	0	0	0	100	0	0	0	0
55	0	0	0	0	4	53	0	0	0	110	0	0	0	0
58	0	0	0	0	21	85	0	0	0	60	0	0	1	0
61	0	0	0	0	0	0	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0	0	0	0	0	0	0
67	0	0	0	0	15	40	2	0	1	110	0	0	2	0
70	0	0	0	0	22	39	0	0	0	84	0	0	0	0
73	0	0	0	0	0	0	0	0	0	0	0	0	0	0
76	0	0	0	0	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	14	47	0	0	0	126	0	0	0	0
82	0	0	0	0	11	32	0	0	0	134	0	0	0	0
85	0	0	0	0	25	45	0	0	2	105	0	0	0	0
88	0	0	0	0	23	46	0	0	0	105	0	0	0	0
91	0	0	0	0	24	31	0	0	0	102	0	0	0	0
94	0	0	1	0	21	20	0	1	0	81	0	0	0	0
97	0	0	0	0	22	33	0	0	0	67	0	0	0	0
100	0	0	0	0	19	33	0	0	0	90	0	0	0	0
103	0	0	0	0	11	49	0	0	2	68	0	0	0	0
106	0	0	0	0	27	26	0	0	0	73	0	0	0	0
109	0	0	0	0	26	29	0	0	0	92	0	0	0	0
112	0	0	0	0	17	43	0	0	1	60		0	0	0

Appendix 2. Phytoliths counting table of the Água dos Papagaios core.

Depth (cm)	Bilobate	Sadlle	Rondel	Cross	Cuneiform bulliform	Paralelepipedal bulliform	Cilindric polylobate	Cylindrical sulcate tracheid	Trapeziform polylobate	Elongate psilate	Acicular hair cell	Globular echinate	Globular granulate	Globular psilate
2	83	11	21	2	0	18	5	7	1	44	0	1	0	1
6	123	1	9	1	0	10	0	4	1	42	0	0	1	2
10	94	6	13	2	4	15	3	3	3	42	0	0	0	0
14	77	12	17	1	17	27	1	4	2	27	1	0	0	1
18	117	9	7	2	0	24	1	2	2	31	0	0	1	0
22	115	11	5	0	0	9	2	5	1	49	0	0	0	0
26	100	13	18	1	0	11	4	5	3	39	0	0	0	0
30	93	5	4	0	1	18	2	14	3	53	0	0	1	0
34	76	26	10	1	5	16	0	4	1	38	2	0	1	2
38	111	8	6	1	0	10	4	8	0	37	0	0	0	0
42	84	6	16	2	1	17	1	1	0	58	0	0	0	0
46	63	5	10	1	5	52	3	1	1	42	2	1	2	1
50	88	19	9	1	0	2	4	1	1	54	1	0	0	0
54	111	17	8	3	0	4	4	6	2	31	1	0	0	0
58	100	9	5	0	1	22	2	4	2	37	5	0	0	0
62	55	10	7	0	3	60	0	2	1	40	0	0	0	4
66	47	18	6	2	2	33	0	6	2	46	0	0	2	7
70	50	9	5	0	11	46	3	4	0	48	0	0	1	1
74	35	23	5	1	11	53	2	5	0	32	0	0	1	4
78	49	11	11	1	14	52	3	4	0	43	1	0	3	2
82	58	15	11	1	0	44	1	4	0	47	1	0	1	2
86	48	21	14	2	1	10	9	3	3	42	1	0	0	0
90	47	7	12	0	2	45	6	10	1	27	1	0	3	1
94	44	9	14	0	2	35	7	6	2	33	0	0	3	0
98	67	9	10	0	2	26	5	5	1	40	0	0	0	1
102	61	13	8	0	0	28	1	4	1	38	1	0	1	0
106	93	1	13	4	3	6	4	4	1	30	1	0	0	0
110	87	7	11	6	3	6	1	3	4	41	0	0	0	2