






# Phytolith records in organic soils: Evidence of environmental changes in the southern Brazilian highlands

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**ABSTRACT:** Soils preserve numerous proxies for paleoenvironmental reconstruction, including phytoliths, which are highly resistant to weathering and can be preserved over long periods. This study investigates the environmental history and soil formation processes in the highlands of southern Brazil through the analysis of organic soils and phytolith records. Our research focuses on the Serra Geral formation, where soils are deeply influenced by environmental factors such as climate, vegetation, and human activities. Four soil profiles in the states of Santa Catarina and Rio Grande do Sul were studied. We reconstructed the paleoenvironmental conditions using a multiproxy approach that includes phytolith analysis,  $\delta^{13}\text{C}$  isotopes, and C/N ratios. The combined proxies identified three phytolith zones in each profile, forming three paleoenvironmental phases related to soil formation. These phases represent distinct environmental moments: the first phase, related to a colder and drier environment with  $\text{C}_3$  vegetation dominance; the second phase, associated with higher temperatures, elevated humidity, fewer trees, and an expansion of  $\text{C}_4$  grasses; and the third phase, characterized by an overall decrease in temperature and humidity variation in the study sites, with predominant grasslands and herbaceous marsh presence in specific areas. This study aligns with previous regional research, offering information on environmental changes and their impact on soil formation. Results enhance the understanding of the soils from high-altitude grasslands, contributing valuable data on paleoenvironmental changes in southern Brazil.

**Keywords:** soil formation, Histosols, soil organic carbon, climate change.

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## INTRODUCTION

Soils are dynamic and integral components of the landscape. Environmental changes, particularly in factors that influence soil formation such as climate, vegetation, and human activities, drive pedogenetic processes, leading to distinct soil properties. As these changes are recorded in the soil, they are considered archives of paleoenvironmental changes (Janzen, 2016; Targulian and Goryachkin, 2019).

In addition to soil properties that can be used to interpret the formation environment, soils can preserve numerous proxies used for studies of vegetation and climate changes and paleoenvironmental reconstruction. Among these are phytoliths ( $\text{SiO}_2 \cdot n\text{H}_2\text{O}$ ), amorphous inorganic structures formed by the polymerization of silicon (Si), occurring both intracellularly and extracellularly in plant tissues throughout the Plantae kingdom (Piperno, 2006; Katz, 2018). These structures are resistant to weathering and are often preserved as fossil records after the death and decomposition of the plant tissues in which they formed. Due to their high resistance to natural destructive forces, they can be preserved for long periods in various terrestrial and aquatic environments (Piperno, 2006; Alexandre et al., 2011; Strömberg et al., 2013; Calegari et al., 2022). Phytolith analysis alongside other indicators such as  $\delta^{13}\text{C}$  isotopes and the C/N ratio constitutes an excellent multiproxy analysis for observing paleoenvironmental changes that has been used in multiple studies (Augustin et al., 2014; Coe et al., 2018; Luz et al., 2019; Silva Neto et al., 2020, 2024; Marcolin et al., 2023; Paisani et al., 2023).

Since phytoliths originate from plant tissues, they are better preserved in soils that also preserve organic matter, such as organic soils. In Brazil, these soils cover between 0.03 to 0.07 % of the land surface and are predominantly distributed in the South and Southeast regions (Anjos et al., 2015). These soils form exclusively under two different conditions that limit decomposition: conditions of low oxygen availability in soils with permanent water saturation and conditions of low temperatures that slow down organic matter degradation (Silva Neto et al., 2023). Thus, besides being important carbon reservoirs, organic soils are important for paleoenvironmental studies as they can provide significant insights into a given region vegetation and past climate dynamics.

South Brazilian highlands have ideal conditions for the formation of these soils, specifically under the subtropical forests and "campos de altitude" environments. The "campos de altitude" (high-altitude grasslands) are a phytophysognomy observed in the highlands of Brazil, rich in species of grasses and small bushes, thriving in mountainous environments and lower temperatures in South and Southeast Brazil (Ferrão and Soares, 1989; Safford, 1999a,b; Behling, 2002). These grasslands usually occur in a mosaic with Araucaria forests, forming the predominant vegetation of the South and Southeast Brazilian highlands (Behling and Oliveira, 2017). Several paleoenvironmental reconstruction studies have been conducted in the South and Southeast Brazilian highland region (Roth and Lorscheitter, 1991; Behling, 2002; Behling et al., 2004; Behling and Safford, 2009; Leonhardt and Lorscheitter, 2010; Behling and Oliveira, 2017; Perin et al., 2021), these authors identified in soils rich in organic matter a registry of the late Paleocene-Holocene transition, observing a change from the drier and colder climate of the Paleocene epoch to the climate we observe today in the region. An interesting environmental period was also observed during this transitional phase, where a more humid and warmer period prevailed between the transition of the Paleocene to the Holocene.

The studied soils are located in the eastern region of the Serra Geral formation, in the Southern region of Brazil. Warmer climates originating from northern Brazil and cold climates from southern South America influence the region, making it a sensitive area for environmental changes (Leonhardt and Lorscheitter, 2010), and a reliable source of archived paleoenvironmental changes. This paper seeks to correspond with other studies in the region to better comprehend the past environment, the occurrence of vegetation and climatic changes archived in these soils through phytoliths and other proxies, and

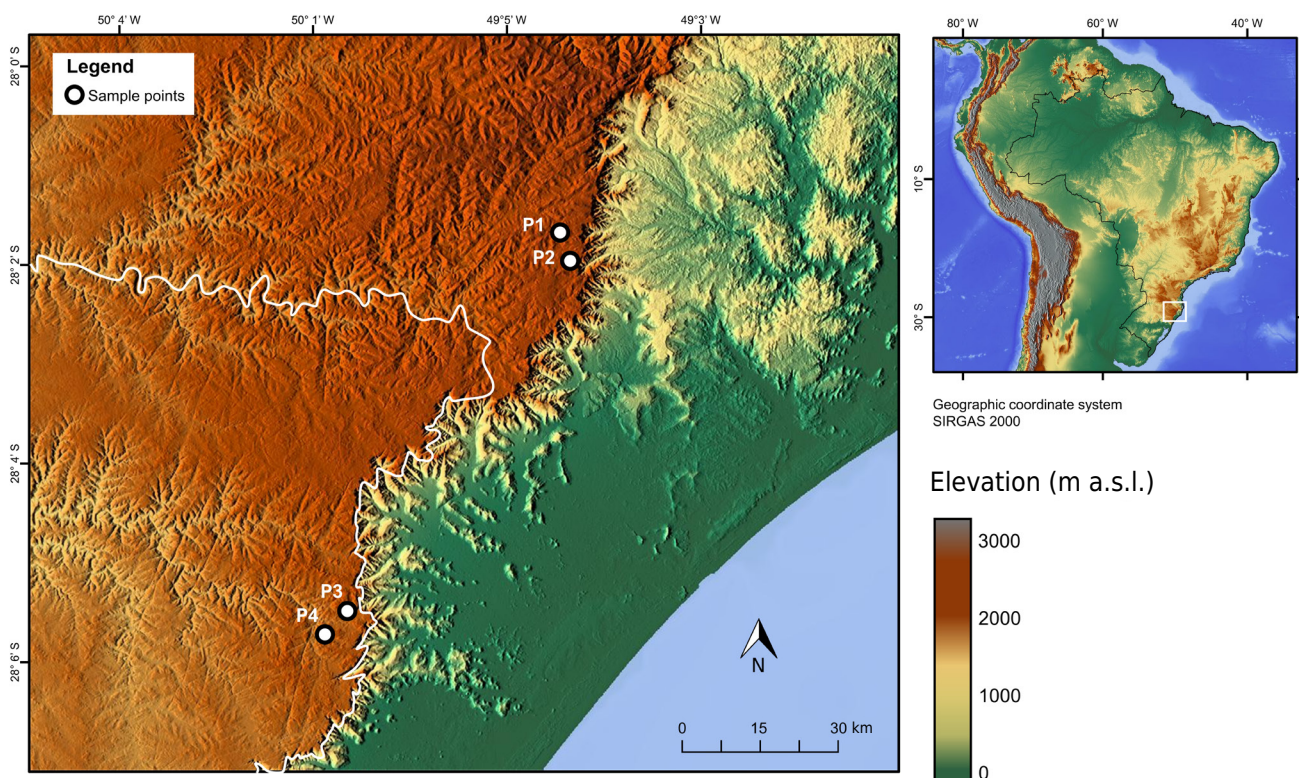
to better understand the impact those environmental changes had on the formation of these soils with accumulated organic matter.

## MATERIALS AND METHODS

### Study sites

Four soil profiles were studied, located in the Serra Geral Formation in the southern region of Brazil. Profiles P1 and P2 were located in the municipality of Bom Jardim da Serra, located in the state of Santa Catarina. Profiles P3 and P4 were located in the municipality of Cambará do Sul, located in the state of Rio Grande do Sul (Figure 1). The landscape position of the profiles is as follows: P1 – Shoulder; P2 – Backslope; P3 – Summit; P4 – Toeslope. All areas are characterized by a subtropical climate (Cfb) according to Köppen climate classification system, with an average annual precipitation between 1,480 and 1,700 mm, and an average annual temperature between 15 and 15.4 °C (maximum of 22.3 °C and minimum of 8.5 °C) (Ferreira et al., 2016; Castiglio et al., 2021)

The areas where this study is located are on a plateau composed of basalts known as the Serra Geral Formation, which forms the South Brazilian highlands (elevations between 500 and 1,200 m a.s.l.) and some higher-elevation mountains (>1,200 m a.s.l.) (Frank et al., 2009). All profiles were collected in sites of "campos de altitude" (high elevation grasslands) with gallery forests alongside small water courses, all located >1000 m above sea level (Table 1). These grasslands grow in mountainous environments, occurring on highlands in South and Southeast Brazil (Safford, 2007). They are rich in endemic species, containing Poaceae and Cyperaceae species as well as small shrubs from the Melastomataceae, Ericaceae, Eriocaulaceae, Asteraceae, and Verbenaceae families (Safford, 1999a,b; Behling, 2002).



**Figure 1.** Locations of the soil profile in the states of Santa Catarina and Rio Grande do Sul, showcasing the mountainous region in which they are located.

**Table 1.** Identification, location, and altitude of the studied soil profiles

Identification	State	Coordinates	Altitude	Mean temperature	Mean precipitation
			m	°C	mm
Profile 1 (P1)	SC	28° 22' 19.00" S, 49° 33' 53.70" W	1.379,7	15,4	1480
Profile 2 (P2)	SC	28° 24' 16.60" S, 49° 33' 20.70" W	1.433,1	15,4	1480
Profile 3 (P3)	RS	29° 4' 1.14" S, 49° 57' 42.66" W	1.025,72	15	1700
Profile 4 (P4)	RS	29° 4' 55.32" S, 50° 0' 17.76" W	1.079,28	15	1700

SC: Santa Catarina; RS: Rio Grande do Sul.

### Soil sampling and analysis

Soil profiles were described according to Ditzler et al. (2017) and sampled conventionally for recognizable genetic horizons for soil physical and chemical characterization. Additionally, samples were collected at regular depths of 0.05 m intervals in the peat soil layer until reaching the mineral horizon for the determination of total C and N (using a CHN elemental analyzer), stable carbon isotope ratios ( $\delta^{13}\text{C}$ ), and phytolith analysis. Physical and chemical characterization of the samples was performed according to Teixeira et al. (2017). The parameters measured were: exchangeable  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{Al}^{3+}$  extracted by 1 mol  $\text{L}^{-1}$  of KCl, assimilable phosphorus (P), exchangeable potassium ( $\text{K}^{+}$ ) and sodium ( $\text{Na}^{+}$ ) determined by colorimetry and flame photometry, and extractable acidity ( $\text{H}^{+}\text{Al}$ ) and hydrogen ( $\text{H}^{+}$ ) assessed in a 0.025 mol  $\text{L}^{-1}$  calcium acetate solution. Organic horizons were analyzed for fiber concentration (unrubbed and rubbed), sodium p YRophosphate-extractable color (SPEC), and classified according to the von Post decomposition scale (Santos et al., 2018).

### Soil phytoliths and Isotopic composition ( $\delta^{13}\text{C}$ )

Phytolith extraction was performed by calcination in a muffle furnace, following Piperno (2006), a method for phytolith extraction in organic soils. Morphotypes were identified from slides and observed at 400 × magnification using a Zeiss Axioskop 40 optical microscope, following the International Code for Phytolith Nomenclature - ICPN 2.0 (Neumann et al., 2019). A minimum of 200 morphotypes with taxonomic or environmental significance were counted in each soil sample. After identification, the phytoliths were grouped according to the morphological patterns of the source plants. These groups are:

- $\Sigma$  Poaceae: All morphotypes produced exclusively by grasses, including those with no taxonomic significance (ELO\_ENT, ACU\_BUL, BUL\_FLA, BLO, TRZ, RON, SAD, Cone-shaped) (Alexandre et al., 1997; Piperno, 2006; Barboni et al., 2007);
- Pooid: RON and TRZ (Twiss, 1992);
- Chloridoid: SAD (Twiss, 1992);
- Cyperaceae: Cone-shaped (Piperno, 2006); and
- Eudicots: SPH\_ORN (Bremond et al., 2005; Piperno, 2006).

Phytolith indices were calculated, including Tree Cover Density (D/P) (Bremond et al., 2008), Climate Index (IC) (Twiss, 1992), and Water-Stress Index (Bi) (Bremond et al., 2005). Adaptations were made to these formulas, as some morphotypes used in their calculations were not found in this study. As such, the morphotypes CRO, POL and BIL used to calculate the D/P and Ic indexes were removed from the equations 1, 2 and 3.

$$D|P = \frac{SPH\_ORN}{(RON + TRZ + SAD)} \quad \text{Eq. 1}$$

$$Ic \% = \frac{(TRZ + RON)}{(SAD + RON + TRZ)} \quad \text{Eq. 2}$$

$$Bi = \frac{BUL\_FLA + BLO}{(RON + TRZ + SAD + ACU\_BUL)} \quad \text{Eq. 3}$$

Morphotypes names follow the code defined by the ICPN 2.0 (Neumann et al., 2019). SPH\_ORN - Spheroid Ornate; RON - Rondel; TRZ - Trapezoid; SAD - Saddle; BUL\_FLA - Bulliform Flabellate; BLO - Blocky; ACU\_BUL - Acute Bulbosus; ELO\_ENT - Elongate Entire; Morphotypes without a defined code by the ICPN remain as their full name.

Isotope ratios were quantified using an isotope ratio mass spectrometer (IRMS) (Delta V Advantage) paired with an elemental analyzer (Flash EA 2000), both manufactured by Thermo Fisher Scientific (Bremen, Germany). This analysis was conducted at the Carbon and Nitrogen Biotransformation Research Laboratory (LABCEN) of the Federal University of Santa Maria, Rio Grande do Sul, Brazil. Elemental composition (SOC) was expressed as a percentage of dry weight, and the isotopic composition ( $\delta^{13}\text{C}$ ) was measured relative to the Vienna Pee Dee Belemnite (VPDB) standard, expressed in parts per thousand (‰) with a standard deviation of 0.2 ‰ (Boutton et al., 1998).

### Data analysis

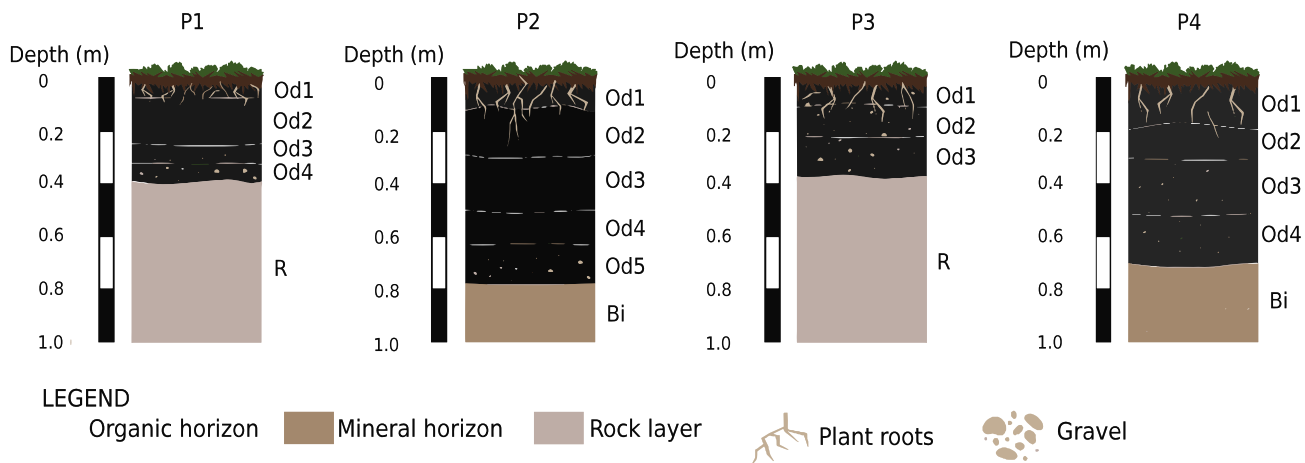
Phytolith data were evaluated using a stratigraphic diagram in C2 (Juggins, 2007), creating a graphical representation of variable values relative to profile depth. The results from the phytolith analysis were assessed through multivariate statistical analysis. In each profile, a constrained similarity-clustering analysis (UPGMA algorithm with Euclidean similarity index) conducted in TiliaGraph and CONISS (Grimm, 1987) enabled the definition of phytolith zones. Once defined, another cluster analysis was performed on all zones to separate them into phases further, enhancing the interpretation of different paleoenvironmental and paleoclimatic periods. Phytolith indexes, phytolith counts, and  $\delta^{13}\text{C}$  isotope values were utilized as variables for all analyses.

## RESULTS

### Soil morphology and general properties

Studied soils have organic horizons (Histols) developing directly over the bedrock (P1 and P3) or on top of mineral horizons (Figure 2). In P1 and P3, the organic horizons reached depths of 0.40 and 0.37 m, respectively. Profiles P2 and P4 presented a mineral horizon below the organic horizons and were the deepest profiles, with organic horizon depths of 0.77 and 0.70 m, respectively. Dark colors indicate a high organic matter content. Color in all profiles was exclusively 10 YR hue, with a constant value and chroma of 2/1 in the organic horizons. Soil structure is predominantly granular, indicating high biological activity, with a moderate to strong degree of development (Table 2).

All organic horizons had a low fiber content, indicating well to almost completely humified organic matter with little to no apparent plant structure, ranging from H8 in the most superficial horizons to H9 in the deeper layers, according to the von Post method for determining the degree of peat humification (Santos et al., 2018). Rubbed Fiber (RF) content presented the highest value of 15.0 % in the most superficial layer of P3, with a steady decline in value as depth increased (Table 3). The highest content of RF was recorded in P3, with a mean value of  $9.1 \pm 5.4$  %, followed by P2, P4, and P1, with values of  $7.1 \pm 2.3$  %,  $6.7 \pm 2.2$  %, and  $5.2 \pm 1.6$  %, respectively.



**Figure 2.** Soil morphology of the studied soil profiles.

**Table 2.** Main morphological characteristics of the soil profiles

Horizon	Layer	Munsell color chart	Soil structure <sup>(1)</sup>	Consistence		Transition	Texture class	von post	
		Moist		Dry <sup>(2)</sup>	Moist <sup>(3)</sup>			Scale	Class
m									
P1		Organossolo Fólico Sáprico lítico (Folic Histosol)							
Od1	0.00-0.06	10 YR 2/1	2, f., gr.	S	FR	Smooth and clear	Organic	H8	Sapric
Od2	0.06-0.25	10 YR 2/1	3, m., gr.	S	FR	Smooth and clear	Organic	H9	Sapric
Od3	0.25-0.32	10 YR 2/1	3, f/m., gr.	S	FR	Smooth and gradual	Organic	H9	Sapric
Od4	0.32-0.40	10 YR 2/1	3, m., gr/abk.	S	FR	-	Organic	H9	Sapric
P2		Organossolo Fólico Sáprico cambissólico (Folic Histosol)							
Od1	0.00-0.15	10 YR 2/1	2, f., gr.	S	FR	Smooth and clear	Organic	H8.	Sapric
Od2	0.15-0.32	10 YR 2/1	2, f., gr.	S	FR	Smooth and gradual	Organic	H8	Sapric
Od3	0.32-0.50	10 YR 2/1	3, f., gr.	S	FR	Smooth and diffuse	Organic	H8	Sapric
Od4	0.50-0.64	10 YR 2/1	3, f., gr.	S	FR	Smooth and diffuse	Organic	H8	Sapric
Od5	0.64-0.77	10 YR 2/1	3, f., gr.	S	FR	Smooth and clear	Organic	H9	Sapric
Bi	0.77-1.00+	10 YR 5/4	2, m., abk.	SH	FR	-	Sandy clay loam	-	-
P3		Organossolo Fólico Sáprico lítico (Folic Histosol)							
Od1	0.00-0.10	10 YR 2/1	2, f., gr.	S	FR	Smooth and clear	Organic	H8	Sapric
Od2	0.10-0.22	10 YR 2/1	2, f/m., gr.	S	FR	Smooth and clear	Organic	H8	Sapric
Od3	0.22-0.37	10 YR 2/1	3, f/m., abk/b.s.	S	FR	Smooth and gradual	Organic	H8	Sapric
P4		Organossolo Fólico Sáprico cambissólico (Folic Histosol)							
Od1	0.00-0.17	10 YR 2/1	2, m., gr.	S	FR	Smooth and clear	Organic	H9	Sapric
Od2	0.17-0.32	10 YR 2/1	2, m., gr.	S	FR	Smooth and gradual	Organic	H9	Sapric
Od3	0.32-0.51	10 YR 2/1	3, /m., abk.	S	FR	Smooth and gradual	Organic	H9	Sapric
Od4	0.51-0.70	10 YR 2/1	3, f., abk.	S	FR	Smooth and clear	Organic	H9	Sapric
Bi	0.70-1.00+	10 YR 5/6	2, m., abk.	SH	FR	-	Sandy clay loam	-	-

<sup>(1)</sup> Structure: 2: Moderate grade; 3: strong grade; f: fine size; m: medium size; gr: granular structure; abk: angular blocky structure. <sup>(2)</sup> Dry consistence: S: soft; SH: slightly hard. <sup>(3)</sup> Moist consistence: FR: Friable.

**Table 3.** Chemical properties of the soil profiles

Horizon	pH(H <sub>2</sub> O)	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	S	Al	H+Al	T	V	P	C	N	C:N	RF
						cmol <sub>c</sub> dm <sup>-3</sup>				%	mg dm <sup>-3</sup>	g kg <sup>-1</sup>			%
P1	Organossolo Fólico Sáprico lítico (Folic Histosol)														
Od1	5.1	1.2	0.5	0.07	0.21	1.98	2.5	19.7	21.6	9.1	1.7	180.3	9.7	14.2	7
Od2	5.1	0.7	0.2	0.05	0.12	0.99	3.1	29.5	30.4	3.3	0.6	161.4	8.0	19.9	5.8
Od3	5.4	0.3	0.2	0.03	0.04	0.58	2.2	19.5	20.1	2.9	0.1	93.2	5.0	18.5	5
Od4	5.3	0.3	0.4	0.04	0.06	0.82	2.7	22.1	22.8	3.6	0.1	97.5	3.4	42.7	3
P2	Organossolo Fólico Sáprico cambissólico (Folic Histosol)														
Od1	4.5	0.4	0.4	0.08	0.18	1.00	7.9	59.5	60.5	1.8	3.8	238.7	18.2	19.9	10.3
Od2	4.7	0.1	0.1	0.04	0.07	0.34	7.1	69.4	69.3	0.5	0.5	224.1	15.8	20.5	8
Od3	4.6	0.2	0.1	0.03	0.03	0.15	5.1	51.1	51.1	0.2	0.2	208.8	12.7	18.9	7.3
Od4	4.8	0.2	0.1	0.02	0.02	0.17	7.3	45.7	45.8	0.4	0.3	153.8	12.5	12.4	5.7
Od5	4.8	0.1	0.1	0.02	0.02	0.15	7.7	33.7	33.8	0.4	0.3	100.6	9.2	20.8	4
Bi	4.7	0.1	0.1	0.02	0.02	0.24	2.1	12.3	12.5	1.9	0.1	43.9	3.1	14.2	-
P3	Organossolo Fólico Sáprico lítico (Folic Histosol)														
Od1	4.5	0.3	0.1	0.07	0.12	0.55	5.7	36.9	37.4	1.5	3.1	179.4	13.9	12.3	15
Od2	4.4	0.2	0.2	0.06	0.1	0.55	6.1	43.1	43.6	1.2	2.2	199.2	11.1	21.1	8
Od3	4.6	0.1	0.1	0.04	0.05	0.29	6.5	38.7	39.1	0.7	0.5	134.4	9.8	17.2	4.3
P4	Organossolo Fólico Sáprico cambissólico (Folic Histosol)														
Od1	5.2	0.2	0.3	0.06	0.14	0.76	4.2	34.7	35.4	2.2	1.3	212.2	12.3	16	9
Od2	5.2	0.1	0.2	0.04	0.04	0.35	3.3	28.4	28.7	1.2	0.4	126.8	9.1	12.2	7.3
Od3	5.1	0.1	0.1	0.02	0.02	0.19	3.6	29.7	29.9	0.7	0.1	113.7	6.1	21	6.8
Od4	5.0	0.1	0.1	0.02	0.02	0.16	4.4	25.2	25.1	0.6	0.1	91.1	5.9	24.1	3.8
Bi	4.9	0.1	0.1	0.02	0.02	0.24	2.1	12.3	12.4	1.1	0.1	43.9	3.1	14.2	-

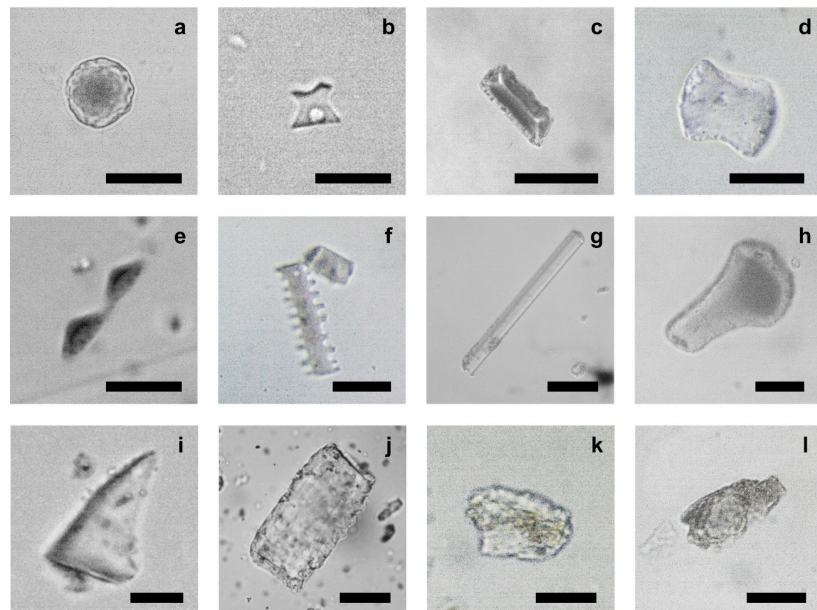
C: Total Organic Carbon. C/N: Soil organic carbon and soil nitrogen ratio. RF: percentage of rubbed fibers.

According to the Brazilian Soil Classification System (Santos et al., 2018), the studied soils are classified as Organossolos Fólicos Sápricos (Folic Histosols; IUSS Working Group WRB, 2022), due to the presence of organic horizons consisting of well-aerated organic material with low content of plant fibers and formed in cool climates or at high elevations. Profiles P1 and P3 differ by presenting lithic contact (underlying intact rocks), while in P2 and P4, the organic horizons are formed over mineral horizons (incipient B horizon).

Regarding chemical properties, all soils are acidic ( $\text{pH} = 4.9 \pm 0.3$ ) with low basic cation content ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^{+}$ , and  $\text{K}^{+}$ ), presenting the highest nutrient contents in the surface horizons. All organic horizons had soil organic carbon (C) greater than  $80 \text{ g kg}^{-1}$ , meeting the criterion for identifying organic soils in the Brazilian Soil Classification System (Santos et al., 2018). Profile P2 had the highest content, with a mean value of  $185.2 \pm 56.3 \text{ g kg}^{-1}$ , followed by P3 ( $171.2 \pm 32.1 \text{ g kg}^{-1}$ ), P4 ( $135.9 \pm 38.7 \text{ g kg}^{-1}$ ), and P1 ( $133.1 \pm 41.5 \text{ g kg}^{-1}$ ). Associated with high C contents, H+Al values were high ( $33.9 \pm 11.9 \text{ cmol}_c \text{ kg}^{-1}$ ), resulting in high T values ( $34.4 \pm 10.2 \text{ cmol}_c \text{ kg}^{-1}$ ) and low base saturation ( $\text{V}\% = 1.8 \pm 1.3$ ). Aluminum ( $\text{Al}^{3+}$ ) levels were also elevated, with a mean of  $4.6 \pm 1.9 \text{ cmol}_c \text{ kg}^{-1}$ . Nitrogen (N) contents averaged  $10.2 \pm 3.2 \text{ g kg}^{-1}$ , and the C:N ratio ranged from 12.2 to 42.7.

### Soil phytoliths and carbon isotope ( $\delta^{13}\text{C}$ )

Soil phytolith assemblage (Figures 3 and 4) consisted of morphotypes and plant families as follows: RON and TRZ (Poaceae Pooideae) (Twiss, 1992); SAD (Poaceae Chloridoid) (Twiss, 1992); ELO\_ENT, BUL\_FL, and BLO (Poaceae); ACU\_BUL (Poaceae and some Arecaceae); Cone-shaped (Cyperaceae) (Piperno, 2006); Rectangular (Poaceae and woody Eudicotyledons) (Piperno, 2006); SPH\_ORN (woody Eudicotyledons) (Bremond et al., 2005; Piperno, 2006).

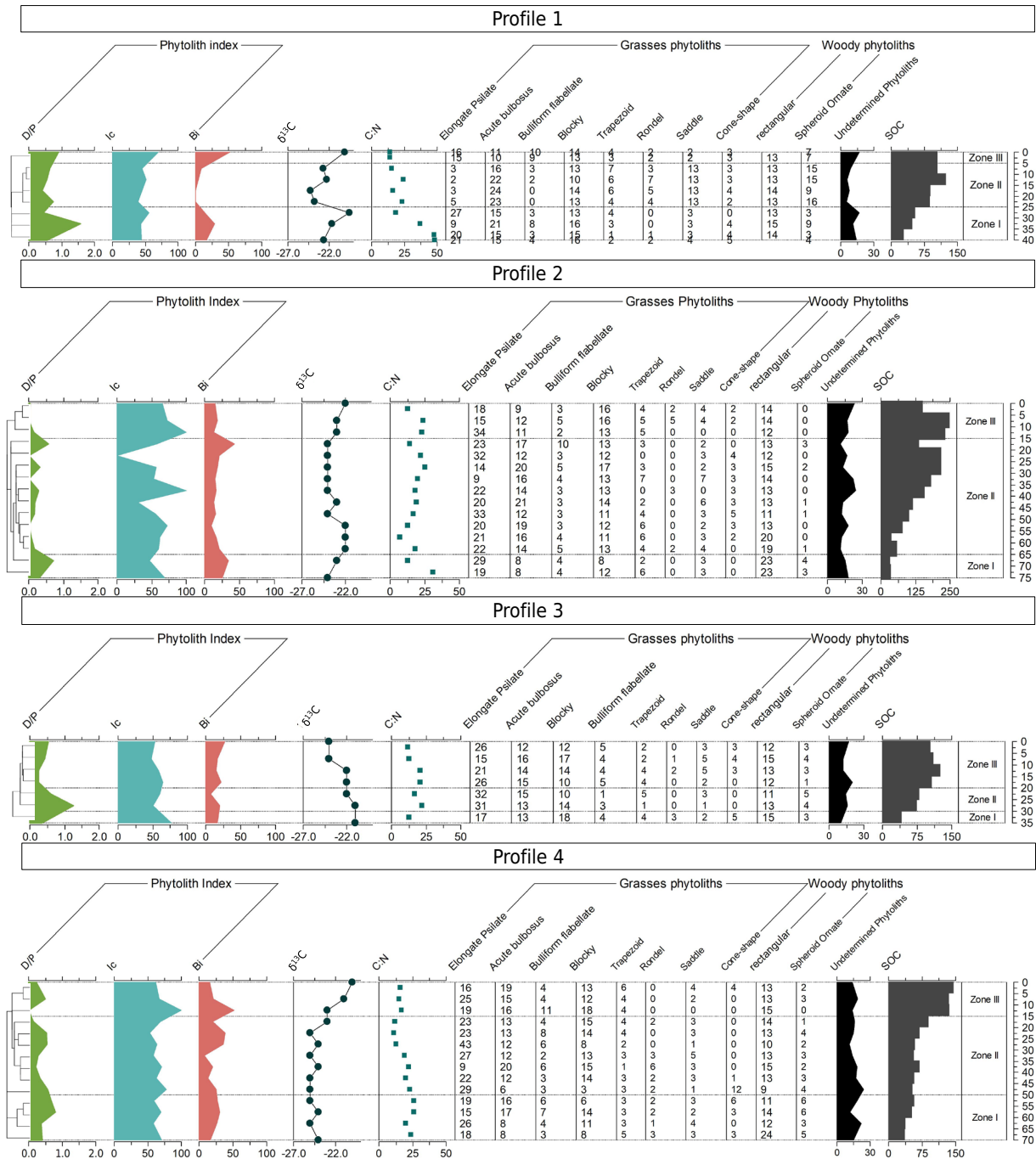


**Figure 3.** Main identified morphotypes: a – Spheroid Ornate (SPH\_ORN); b – Rondel (RON); c – trapezoid (TRZ); d – Saddle (SAD); e – Cone-Shape; f – Elongate Dendritic (ELO\_DEN); g – Elongate Entire (ELO\_ENT); h – Bulliform Flabellate (BUL\_FLA); i – Acute Bulbosus (ACU\_BUL); j – Rectangular; k and l – examples of unidentifiable phytoliths observed.

The  $\delta^{13}\text{C}$  isotope values ranged from -19.41 to -24.62 ‰ ( $-22.60 \pm 1.42$  ‰) across all soils. In P1, more enriched isotope values were found in the basal layer ( $-21.06 \pm 1.55$  ‰), followed by a depletion in the middle layer ( $-23.97 \pm 0.26$  ‰), and again more enriched values at the surface ( $-21.39 \pm 1.30$  ‰). This indicates a predominance of mixed C3 and C4 vegetation with an increase in C3 plants. Profiles P2 and P4 showed a trend of enrichment from the basal layer ( $-23.36 \pm 1.09$  ‰) to the surface ( $-20.55 \pm 1.14$  ‰), indicating an increase in C4 plants in predominantly C3 vegetation. Profile P3 was the only to show a depletion of  $\delta^{13}\text{C}$  from the base ( $-20.83 \pm 0.72$  ‰) to the surface ( $-23.47 \pm 0.22$  ‰), indicating an increase of C3 vegetation on a C3/C4 mixed area.

Cluster analysis applied to the absolute phytolith count and their indexes separated the profiles into three zones each (Figure 3). Another cluster analysis, conducted with the mean values of the phytolith counts and indexes of all profile zones, further separated them into what can be considered three different paleoenvironmental phases. Phase 1 (Ph1) includes zones I of P1, P2, and P3, and zones I and II of P4; Phase 2 (Ph2) includes zone II of P1 and P2, and zones II and III of P3; and Phase 3, encompassing zones III of P1, P2, P4. Zone I of P3 was included in Ph1, however, this zone can be further separated into Phase 1.2, as it registered due to some slight differences in the phytolith assembly archived in it.

Climate (Ic) and water-stress (Bi) indexes displayed an average value of  $58.8\% \pm 3.8$  and  $24.6 \pm 5.8\%$ , respectively, in Ph1;  $52.7\% \pm 5.9$  (Ic) and  $14.3\% \pm 2.9$  (Bi) in Ph2;  $75.5\% \pm 2.8$  (Ic) and  $28.8\% \pm 12.22$  (Bi) in Ph3. These values indicate a colder and not very dry climate in Ph1 relative to Ph2, followed by a warming period with a wetter and cooler climate in Ph2, followed by a gradual cooling in evolving towards modern conditions of a warmer and drier climate (Ph3). In relation to the tree cover index (D/P), apart from P1, all profiles showed a decrease in value from the base to the top of the soil, evidence of a predominance of grasses and a small decline of woody arboreal of shrub vegetation existent in the study area, with a decline of 0.77 (Ph1) to 0.51 (Ph2), and 0.39 (Ph3). Profile P1 showed different D/P values compared to the other profiles, presenting 0.86, 0.59, and 0.90 in Ph1, Ph2, and Ph3.1, respectively. Profile P1 registered a highly elevated Bi index (52 %) in zone I, in contrast to zone II (3 %), showing a much higher water-stress level compared to past moments.



**Figure 4.** Diagram containing Phytolith indexes D/P, IC (%) and BI (%); isotope  $\delta^{13}C$  (‰); C:N ratio; phytolith assemblage (%); and Soil Organic Carbon ( $g\ kg^{-1}$ ). It is important to highlight that the percentage of undetermined phytoliths in the total sum of the classified phytoliths.

Regarding the total phytolith count, the morphotypes with no specific taxonomic significance (ELO\_ENT, ACU\_BUL, BLO, BUL\_FLA, and Rectangular) showed a semi-constant value through all phases (maximum 4 % difference). The Ph1 manifested the highest percentage of undetermined phytoliths, possibly corresponding to Ph1 being the oldest phase and located in the deepest part of the profile. As found in those profiles, chemical conditions encountered in soils with high organic content and high acidity also affect the stability and resistance of phytoliths. The Ph3 presented the most RON morphotypes identified, indicating a cooler climate compared to the other phases.

## DISCUSSION

Cluster analysis of the phytoliths, indexes, and  $\delta^{13}\text{C}$  isotope of all zones allowed the characterization of three possible paleo-environmental phases (Figure 5). Each phase represents a different environmental setting during the formation process of the profiles. Phase 1 is likely the oldest and was identified in the deepest parts of all profiles. The Ph1 can be interpreted as a slightly colder and drier environment compared to the subsequent Ph2, indicated by an average Ic ( $58.8\% \pm 3.8$ ) and Bi ( $24.65\% \pm 5.83$ ), which are higher than those of the following phase. Vegetation was predominantly C3, as indicated by the  $\delta^{13}\text{C}$  isotope values ( $-23.56\text{‰} \pm 1.05$ ) and was mainly composed of grasses and shrubs, with possibly small pockets of forests, as observed by a higher D/P medium value ( $0.77\% \pm 0.24$ ) than following phases. This data suggests that a campos vegetation, likely with gallery forests, was predominant during this phase. Medium C/N ratio in this phase ( $19.57 \pm 6.20$ ) indicates slightly decomposed organic matter.

Roth and Lorscheitter (1991) (Parque Nacional de Aparados da Serra), Behling and Pillar (2007) (Cambará do Sul), and Spalding and Lorscheitter (2015) (São Francisco de Paula) studied different bogs in South and Southeast Brazilian highland areas with similar properties and proximity to this study. These authors identified a similar paleo-environmental moment as Ph1, depicting the late Pleistocene ( $< \pm 12,000$  years BP) scenario in the region. They describe a cold and arid climate prevailing during the end of the Pleistocene and the last glacial age, with a predominant campos vegetation attributed to these cold and dry times, alongside herbaceous marshes.

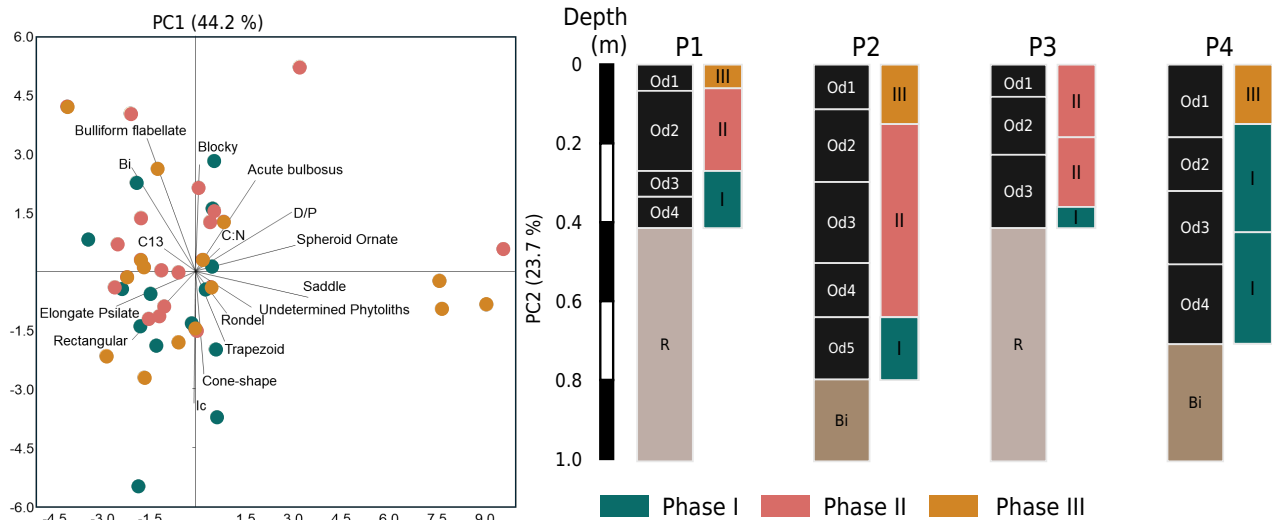
As the transition from the Pleistocene to the Holocene began and the last glacial age came to an end, several authors observed a brief warming event with increased humidity in various bogs in the South and Southeast Brazilian highlands (Roth and Lorscheitter, 1991 - Cambará do Sul; Leal and Lorscheitter, 2007; Behling and Safford, 2009 - Serra dos Órgãos; Leonhardt and Lorscheitter, 2010 - São Francisco de Paula; Spalding and Lorscheitter, 2015 - São Francisco de Paula; Behling and Oliveira, 2017 - Serra do Tabuleiro). Similar signs of slight warming can be observed in Phase 2 (P1, P2, and P3), succeeding Ph1. This phase coincides with the transitional period of the Late Pleistocene - Holocene warming event noted by other authors.

Decrease in the Ic index ( $52.7\% \pm 5.9$ ) observed in Ph2, along with a reduction in the TRZ ( $3.67\% \pm 2.20$ ) and RON ( $0.66\% \pm 1.23$ ) morphotypes, indicates a possible small elevation in temperature and a decline in the Pooideae subfamily. Pooideae is a temperate, arctic-dominant grass subfamily (Schubert et al., 2018). Decline of the Pooideae, coupled with an increase in ELO\_ENT ( $22.29\% \pm 7.57$ ) and ACU\_BUL ( $16.20\% \pm 2.89$ ), as well as the enrichment of the  $\delta^{13}\text{C}$  isotope ( $-22.17\text{‰} \pm 1.36$ ), suggests an expansion of other grass families at the expense of the cool temperate Pooideae subfamily and a small increase in C4 vegetation, likely due to the regional climate warming (Figure 6).

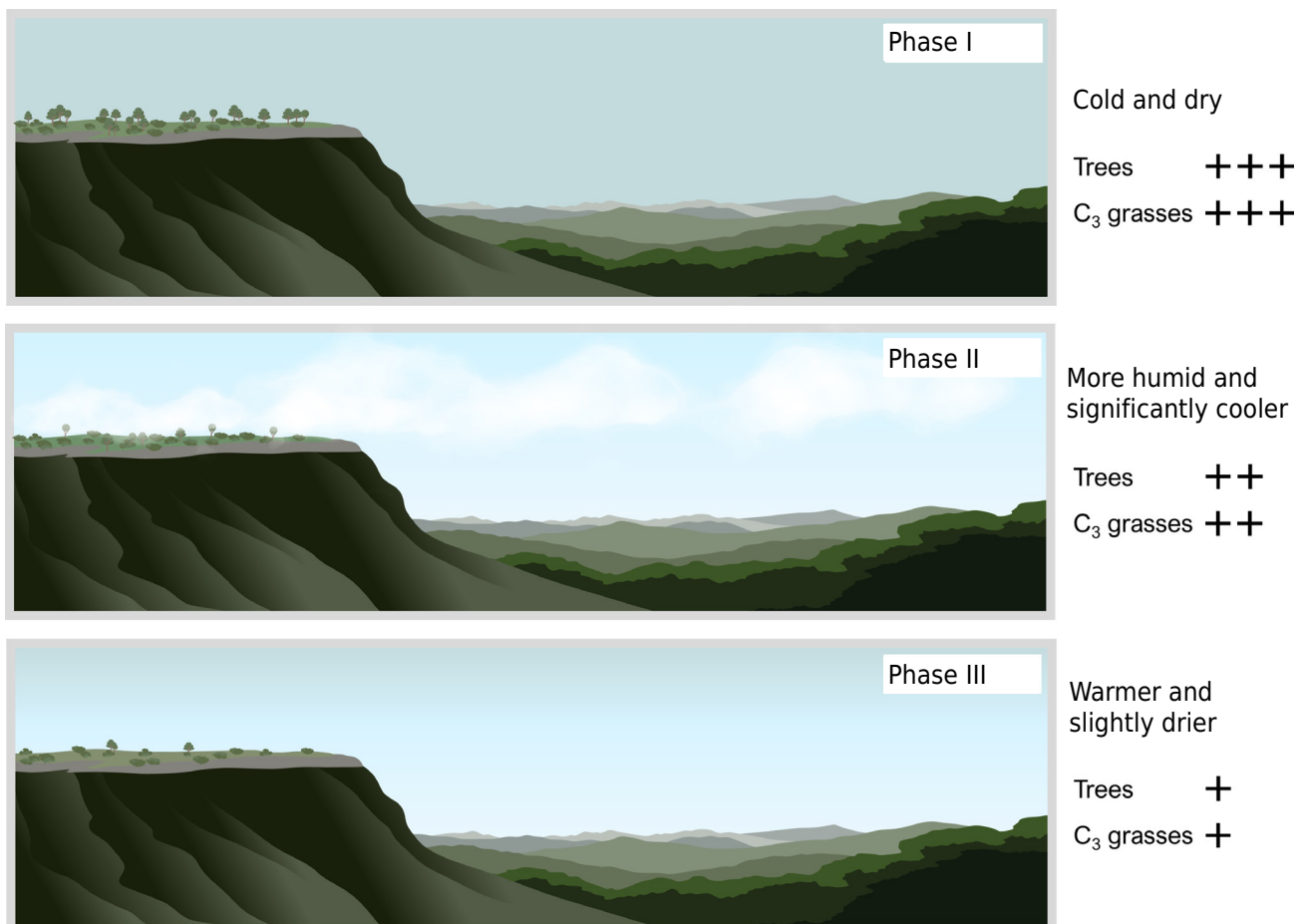
A decrease of 10 % in Bi ( $14.3\% \pm 2.9$ ) was recorded, indicating less water stress and providing evidence of a more humid climate. The C/N ratio recorded a slight increase from the previous phase ( $21.1 \pm 9.9$ ), suggesting either a decrease in humidity or a greater input of terrestrial C4 plant organic matter (Meyers, 1994; Luz et al., 2019). Given that other proxies indicated a period of increased temperature and humidity ( $< \text{Ic}$ ;  $< \text{Bi}$ ) and an expansion of C4 grasses (enrichment of  $\delta^{13}\text{C}$  isotope), the rise in the C/N ratio is most likely related to the increase in temperature and the spread of C4 vegetation. This period portrays a significant increase in temperature and humidity at the end of the Pleistocene and the beginning of the Holocene (Berglund and Ralska-Jasiewiczowa, 1986; Behling and Oliveira, 2017). During this phase, the study area was dominated by grasslands, with some areas of herbaceous marsh and forest vegetation.

After Ph2, a cooling episode was recorded in Ph3 across all profiles. The Ph3 can be associated with the Greenlandian age at the start of the Holocene, to the most recent

environmental situation of the studied region. This phase indicates an overall cooler climate with much more frequent water stress, shown by an overall increase in the Ic index ( $75.56 \% \pm 2.7$ ) and Bi index ( $28.8 \% \pm 12.2$ ), as well as a decrease in the C/N ratio ( $17.9 \pm 4.4$ ) compared to Ph2.



**Figure 5.** Representation of the zones and phases of each profile, and PCA representing the environmental phases.



**Figure 6.** Schematic diagram of the studied area, and environmental interpretation of the vegetation history.

The  $\delta^{13}\text{C}$  isotope values recorded for Ph3 ( $-20.8\text{‰} \pm 0.5$ ) suggest a predominance of C3 vegetation, with a bigger contribution of C4 compared to the prior phases due to more enriched isotope values. This is likely due to a much drier situation in Ph3, aligning with the increase of 14.4 % of the Bi index. Several authors observed a cooling of the climate in the early stages of the Holocene (Leonhardt and Lorscheitter, 2010; Spalding and Lorscheitter, 2015; Behling and Oliveira, 2017; Luz et al., 2019), right after the warming event at the Pleistocene-Holocene transition (identified in Ph2), similar to what is recorded in Ph3. These authors noted an expansion of other ecosystems onto the campos, but no such expansion was recorded in this study. The data suggest that campos has remained the prevalent vegetation throughout the formation of the profiles and continues to be the local vegetation of the study sites.

Leonhardt and Lorscheitter (2010) and Spalding and Lorscheitter (2015) documented a dry phase in the Greenlandian period in southern Brazil, near the second study site, followed by a slow increase in humidity until the present day. This transition can be observed in P4 at the start of Ph3 (0.10-0.15 m), where Bi indicates a very dry environment (52.31 %), followed by an increase in moisture up to the present (0.00-0.10 m) ( $18.63\% \pm 3.41$ ). This transition was also registered in P3 but on a lesser scale, as indicated by the decline of Bi from the start of the phase (0.15-0.20 m) (22.64 %) to the middle of the phase (0.05-0.15 m) ( $16.67\% \pm 1.12$ ).

Profile P1 recorded the lowest values of Bi ( $3.25\% \pm 4.19$ ) in Zone II (0.05-0.25 m), indicating a period of greater humidity. The  $\delta^{13}\text{C}$  isotope values ( $-23.23\text{‰} \pm 0.90$ ) align with the index data, indicating abundant C<sub>3</sub> vegetation with minimal water stress. A sponge spicule was found in Zone II of P1, another indicator of soils with abundant water. This data suggests that P1 was likely under a gallery forest during the early stages of Ph3, probably near a water body. In Zone III (0.00-0.05 m) at the most superficial layer of the profile, a sudden extreme increase in water-stress levels was recorded by an increase in Bi (52.17 %), suggesting a significant decrease in humidity from Zone II to Zone III. This decrease is also indicated by the enrichment of the  $\delta^{13}\text{C}$  isotope ( $-20.09\text{‰}$ ), showing a more mixed vegetation of C3 and C4 plants. This data can most likely be attributed to deforestation by human activity, suggesting a disruption or termination of the water course of the gallery forest, forcing a change in the vegetation to one more resilient to environmental change.

Although  $^{14}\text{C}$  dating was not used in this study, it is possible to associate the ages of each phase by evaluating and comparing them with other studies conducted in the region with similar properties. It is important to note that several authors recorded different dates for each period observed in this study; however, those ages are considerably close, allowing for an approximation of the ages of the phases in this study. The Ph1 can be related to the late Pleistocene scenario ( $< \pm 12,000$  years BP) with a climate colder and drier than the other phases. The vegetation is predominantly C3 grasses with pockets of forests or other types of woody vegetation, probably covering water bodies. Transitional period between the late Pleistocene and the Greenlandian age ( $\pm 12,000 - 10,000$  years BP) can be associated with Ph2, documenting a period of increased temperature and humidity. This period also exhibited a slight decrease in ligneous vegetation and the Pooideae subfamily and an overall increase in other grass subfamilies. The Ph3 can be related to the start of the Holocene to the present day ( $> \pm 10,000$  years BP). This phase detected an overall temperature and humidity decrease compared to the previous phase. In Camará do Sul (P3 and P4), a short and sudden arid period was noticed at the beginning of the phase, followed by an increase in humidity once again. Predominant vegetation in Ph3 is grasses, with a small presence of ligneous plants representing gallery forests. As in the other phases, this indicates that campos remained the prevalent ecosystem from the formation of these soil profiles to today.

Regarding soil genesis, P1 and P3 are the shallowest profiles in terms of depth, with P1 recording only two paleoenvironmental phases. According to the Hillslope Profile Position (Ditzler et al., 2017), P1 and P3 are located at the shoulder and the summit of their respective topographies (Figure 4). These profiles are in areas with higher chances of material loss through aeolian and water erosion, making them shallower compared to soils in lower segments (Kämpf and Curi, 2015). Since P1 is at the summit, there is no possibility of material deposition from other areas, meaning all environmental records studied in this profile were deposited from the area where P1 is located. Profiles P2 and P4 are positioned at lower segments of their respective topographies, with P2 on the backslope and P4 at the toeslope position of the hill. These are the deepest profiles in locations where material deposition from higher segments of the hill is easier. This positioning also means they are less precise in paleoenvironmental reconstruction, as recently deposited material can mix with older material in the profile, adding another set of proxies with different environmental significance than what is already archived in the profile. The lack of the middle phase (Ph2) in P4 can be explained by its position on the hill (toeslope). Some of the horizons may have originated from colluvial sediments of the upper parts of the hill, mixing new material with what may have been Ph2. As the toeslope segment receives more material than other segments, it is more prone to such mixing. The lack of evidence of the most recent phase (Ph3) on P3 may also be related to its positioning on the topography of the slope, as it is located in the summit position; it is likely that soil erosion is responsible for material loss at the surface of the profile.

This study reveals the paleoenvironmental dynamics and soil genesis in four profiles in the highlands of southern Brazil. Despite the lack of richness in details, phytoliths have been compared to other studies in the field, like palynology; they prove to be great environmental indicators, especially if worked together with other indicators like  $\delta^{13}\text{C}$  isotope and others that were not used in this study, such as  $^{14}\text{C}$  dating. This study produced content that helps clarify the region environmental conditions in the past, as well as its impact on the formation of the soil, thereby complementing previous regional studies with similar objectives.

## CONCLUSION

Phytolith analysis along  $\delta^{13}\text{C}$  proved to be an efficient proxies for environmental reconstruction and contributed to a better understanding of the variations in climate and vegetation these profiles witnessed during formation and the impact these environmental changes had in the soil formation process. The analysis of phytoliths,  $\delta^{13}\text{C}$  isotope indices, and soil chemical properties identified three distinct paleoenvironmental phases. The first phase (Ph1) can be associated with a colder and drier environment at the end of the Pleistocene, dominated by C3 grass vegetation with small pockets of forest. The second phase (Ph2) can be related to an increase in temperature and humidity during the transition from the Pleistocene to the Holocene, with an expansion of C4 grasses. The third phase (Ph3) is characterized by an overall decrease in temperature, a drier environment at the start of the phase, and a slow increase in humidity until the end at the surface of the profile, and a dominant grassland vegetation with the presence of woody plants in specific areas. Topographic position of the profiles influenced the depth and precision of the paleoenvironmental records, highlighting the importance of erosion and material deposition in soil formation. These findings contribute to understanding environmental changes and landscape evolution in the highlands of southern Brazil.


## DATA AVAILABILITY




The data will be provided upon request.




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