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## **Dissolution does not affect grass phytolith assemblages**

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### **Abstract**

Dissolution is one among several taphonomical processes that may bias paleoenvironmental, paleoclimatic or taxonomic interpretation of phytolith assemblages. To improve our understanding of dissolution on grass phytoliths, we studied systematic changes of surface features, morphotype assemblages, and dissolution rates of phytoliths extracted from two grass species *Hyparrhenia involuocrata* (Panicoideae), *Nastus borbonicus* (Bambusoideae), one soil from La Réunion Island (approximate mean age  $\leq 800$  yr), and three paleosols from Ethiopia (approximate age of 4.4 million years). We used heavy-liquid to extract phytoliths, and 1% Na<sub>2</sub>CO<sub>3</sub> to perform partial dissolution experiments. Physicochemical surface properties, morphotypes, and assemblages were analyzed using optical and scanning electron microscopy, laser diffraction, and X-ray diffractometry. Our results show that 1) phytoliths from different grass species may have different dissolution rates: phytoliths from the leaves of *Hyparrhenia involuocrata* (Panicoideae) are more prone to dissolution than those from *Nastus borbonicus* (Bambusoideae). 2) Silicon (Si) released by phytolith assemblages (i.e., phytolith dissolution rate) decreases as follows: plant > soil > paleosol. 3) Dissolution leads to cavity formation on phytolith surfaces and disappearance of fragile silica particles. 4) Partial dissolution does not significantly change percentages of common grass phytolith

30 morphotypes in a given assemblage. These results provide a benchmark for assessing the reliability  
31 of paleoenvironmental reconstructions using grass phytolith assemblages from buried soils and  
32 sediments.

33

34 **Keywords:** weathered phytolith, dissolution, stability, pH, paleoenvironment

35

## 36 **1. Introduction**

37 Phytoliths are extensively used in paleobotany (e.g., Piperno and Pearsall, 1998; Prasad et al.,  
38 2011), archaeobotany (e.g., Ball et al., 2016; Lu et al., 2009, 2016), paleoenvironmental and  
39 paleoclimatic studies (e.g., Arráiz et al., 2017; Issaharou-Matchi et al., 2016; Nogué et al., 2017;  
40 Yost et al., 2021; Zhang et al., 2020). They are produced in stems and leaves of many plants (Hodson  
41 et al., 2005), and are particularly abundant, taxonomically diagnostic, and precisely classified within  
42 grasses (Poaceae) (Piperno, 2006). Grass phytolith assemblages can provide detailed information  
43 about the composition of past grass flora (e.g., Bremond et al., 2008; Cordova et al., 2011; Fredlund  
44 and Tieszen, 1994). Phytolith ratios, particularly those including grass silica short cells (GSSCs),  
45 have been used as climatic proxies to track paleoclimate changes (e.g., Aleman et al., 2014; Nogué  
46 et al., 2017; Zhang et al., 2020).

47 Using buried soil or sedimentary phytolith assemblages to reconstruct past environments assumes  
48 that they reflect some of their source vegetation characteristics (e.g., tree cover density, grass  
49 subfamily dominance, grass drought stress), despite dissolution and concentration mechanisms  
50 known to affect phytoliths in litters, soils, surface waters and sediments (Alexandre et al., 2011;  
51 Hyland et al., 2013; McCune et al., 2013). In terrestrial ecosystems, phytoliths can be highly soluble  
52 and recycled (e.g., Meunier et al., 2022), with concentrations in soils decreasing with increasing  
53 depth: highest at the surface, decreasing below about 30-80 cm, and stabilizing in deeper horizons  
54 (Alexandre et al., 1997; White et al., 2012), implying the occurrence of phytolith dissolution and/or  
55 translocation during pedogenesis (Alexandre et al., 2011).

56 Phytoliths are among the fastest dissolving silicate constituents in soil at pH > 4; pH > 8-9  
57 amplifies their solubility (Frayssé et al., 2009), making the phytolith preservation problematic in  
58 alkaline soil and sediments (e.g., Arráiz et al. 2017; Liu et al., 2019; Yost et al., 2021). Conversely,

59 phytoliths may accumulate in acidic environments (Meunier et al., 1999; Nguyen et al., 2019), and  
60 preserve well in sedimentary contexts oversaturated with silica (e.g., Novello et al., 2015). Several  
61 experimental tests [demonstrated that phytoliths from fresh plants are more soluble than those from](#)  
62 [fossil plants](#) (Cabanes et al., 2011; Cabanes and Shahack-Gross, 2015), [supporting](#) the hypothesis  
63 that only a portion of phytoliths incorporated into soil [are](#) preserved [for](#) long periods. These tests  
64 also [indicated](#) that thin and flat phytoliths with large [surface](#) area relative to volume (e.g., Double-  
65 peaked husk phytoliths from rice, Papillate phytoliths from sedges) are more [likely](#) to dissolve than  
66 thick, densely silicified particles such as grass silica short cell phytoliths (e.g., Bartoli and Wilding,  
67 1980; Cabanes et al., 2011), [suggesting that preferential dissolution](#) may be [problematic](#) because  
68 paleovegetation inferences rely on the relative abundance of phytolith morphotypes.

69 In soils and sediments, phytoliths do not always [have](#) a smooth, pristine surface. [They often](#)  
70 exhibit cavities that are round, deep and large, [suggesting](#) dissolution pits. Phytoliths with numerous  
71 cavities are particularly abundant at lower soil profile [depths](#), even [under](#) soil with acidic to neutral  
72 conditions (Borrelli et al., 2010; Riotte et al., 2018). Dissolution processes may affect the surface  
73 of phytoliths to [the point](#) that taxonomic identification is impossible (e.g., Arráiz et al., 2017;  
74 Cabanes et al., 2011; Yost et al., 2021).

75 Given [that](#) grass phytoliths [are](#) exceptional tools [used](#) in paleoecology and archaeobotany to  
76 document past grass communities, changes in herbivore diets (e.g., Ciochon et al., 1990), early  
77 domestication of cereals (e.g., Iriarte, 2003; Zhao and Piperno, 2000), changes in agricultural  
78 practices (e.g., irrigation; Rosen and Weiner, 1994), as well as understanding the contribution of  
79 plants to the global silicon cycle (Alexandre et al., 1997, 2011; Blecker et al., 2006; Borrelli et al.,  
80 2010), [improved understanding of effects of dissolution and other taphonomic processes on grass](#)  
81 [phytolith assemblages is crucial](#).

82 To improve our understanding of the impact of dissolution processes on grass phytolith  
83 morphotypes and assemblages, we [performed](#) laboratory experiments simulating alkaline  
84 dissolution. Phytolith sensitivity to dissolution was evaluated by measuring Si release [from](#)  
85 phytoliths [extracted](#) from two tropical grass species, one sub-modern soil sample with abundant  
86 bamboo phytoliths (Meunier et al., 1999), and Pliocene paleosol samples containing high grass and  
87 palm (Arecaceae) phytoliths (WoldeGabriel et al., 2009). For the first time, effects of partial

88 dissolution on phytolith surfaces [are](#) described and quantified for different morphotypes [originating](#)  
89 from different silicification processes (Hodson, 2019). [Impacts](#) on phytolith assemblages [were](#) also  
90 assessed through morphotype counts and quantification of altered surfaces.

## 92 **2. Materials and methods**

### 93 *2.1 Plant, soil and paleosol samples*

94 Plant phytoliths were extracted from two grass species: a specimen of *Hyparrhenia involucreta*  
95 (Panicoideae) collected in 2018 [as part](#) of the HUMI-17 project at the Nalohou grassland site [at](#) the  
96 AMMA-CATCH observatory ([www.amma-catch.org](http://www.amma-catch.org)) in Benin (9°44'N, 1°36'E) (Outrequin,  
97 [2022](#)), [and](#) a specimen of *Nastus borbonicus* (Bambusoideae) collected [from](#) La Réunion Island  
98 (21°07'S, 55°32'E) (Meunier et al., 1999).

99 The soil sample was collected at 20 cm [depth](#) in the M horizon of an [Andosol](#) developed on the  
100 west side of La Réunion Island, [which is mostly](#) covered by bamboo forests; [estimated sample age](#)  
101 [is](#) 745 years (charcoal <sup>14</sup>C age, Meunier et al., 1999). [Paleosol](#) samples (SA18, SA28, and SA52)  
102 with relatively abundant phytoliths were obtained from the Sagantole Formation in the Middle  
103 Awash Valley of Ethiopia and are dated ca. 4.4 Ma (10.5°N, 40.5°E, WoldeGabriel et al., 2009).

### 105 *2.2 Phytolith extraction*

106 Plant phytoliths were extracted using [a wet oxidation method](#) (Alexandre et al., 1997). [Leaves](#)  
107 were separated from stems, washed, dried at 50°C, and cut into small pieces. Milli-Q ultrapure water  
108 was used for rinsing. Dried pieces of leaves were soaked in 1N HCl and heated at 80°C for 2 h,  
109 [rinsed](#), soaked in H<sub>2</sub>SO<sub>4</sub> (95%) and heated at 80°C for > 8h. To improve oxidation of organic matter,  
110 samples were soaked again in H<sub>2</sub>O<sub>2</sub> (30%, at 80°C) until the supernatant became clear. Samples  
111 were then rinsed, centrifuged (10 min at 4000 rpm), and dried before storage. [Plant phytolith content](#),  
112 expressed as a percentage relative to plant leaf dry biomass (% DW), [was 4.4%](#) in *Hyparrhenia*  
113 *involucreta* and 12.8% in *Nastus borbonicus* (Table S1).

114 [Chemical](#) extraction may [cause some](#) dissolution (Crespin et al., 2008), [so](#), we used the same  
115 protocol for all our samples to avoid [the](#) bias shown by Cabanes et al. (2011), who used [dry ashing](#)  
116 and acid digestion.

117 [Soil and paleosol phytoliths were extracted using the protocol of Alexandre et al. \(1997\)](#). No  
118 treatment was needed for the [La Réunion soil sample because](#) it is almost exclusively composed of  
119 phytoliths (Meunier et al., 1999). [Phytoliths were extracted from paleosol samples using](#) 1N HCl  
120 heated at 80°C for 2 h to remove carbonates and 30% H<sub>2</sub>O<sub>2</sub> under constant heat (80°C) to remove  
121 organic matter. Clays were removed by decantation, and minerals by densimetric separation using  
122 ZnBr<sub>2</sub> heavy liquid with density 2.3 g/cm<sup>3</sup>.

123 After extraction, [phytolith extracts were weighed and three](#) to five permanent slides were  
124 prepared with Canada Balsam for microscopic observation. The remaining [extract](#) was used for  
125 dissolution experiments and scanning electron microscope (SEM) observations.

### 127 2.3 Phytolith dissolution experiments and Si measurement

128 Phytoliths were [subjected](#) to partial dissolution using 1% Na<sub>2</sub>CO<sub>3</sub> solution. Sixty mg dried  
129 phytolith powder were added to 100 ml 1% Na<sub>2</sub>CO<sub>3</sub> solution (pH = 11.2) in a polypropylene tube  
130 with caps slightly loosened to vent gases, and placed in a shaker bath at 85 °C for digestion. To stop  
131 the reaction, the tube was cooled in a water bath at room temperature. To measure dissolved Si, 1ml  
132 liquid aliquot was added to 9 ml 0.021 N HCl in 10 ml polypropylene tubes, [washed in](#) 0.1mol/l  
133 HCl ([twice](#)) and Milli-Q ultrapure water ([twice](#)), centrifuged for 10 min at 4000 rpm, and dried at  
134 70°C for [weighing and phytolith observation](#).

135 [Dissolution times](#) had to be adjusted for each sample (Table S1). At ½ hour, phytoliths were  
136 partially dissolved in *Hyparrhenia* and *Nastus*, while they were barely affected in the soil and  
137 paleosol samples. [Dissolution times were](#) increased for the soil (up to 4h) and paleosol samples (up  
138 to 10 days) to obtain [noticeable](#) dissolution. We duplicated the plant and La Réunion soil samples  
139 to test the reproducibility of the dissolution experiment and error values were calculated as the  
140 standard deviation between duplicates. For the paleosol samples, instead of duplicating the  
141 experiments, which was not possible due to the small amount of material available, we chose three  
142 different samples (SA18, SA28, SA52) of similar age (WoldeGabriel et al., 2009) [and treated them](#)  
143 [separately](#). [Because they originated](#) from the same geological Formation but from different localities  
144 within the same paleontological area where paleovegetation was likely different.

145 [Concentration of dissolved Si \(DSi, mg L<sup>-1</sup>\) in solution was measured using molybdenum blue](#)  
146 [colorimetry with](#) Spectroquant reactants (Merck), which allows monitoring the phytolith dissolution  
147 process [with coefficient of variation](#) ± 1.4%. Absorption was detected at 820 nm with a Jasco V-  
148 650 Spectrometer. Calibration lines ( $R^2 \geq 0.999$ ) were calculated using dilute solutions from a  
149 standard Si solution at 1g L<sup>-1</sup> (Plasma CAL).

150 [Percent phytolith dissolution](#) was estimated using two methods: [1\) calculating](#) Si loss in solution  
151 according to the following equation:

$$\text{Phy}_{\text{loss-DSi}} (\%) = \text{DSi (mg L}^{-1}\text{)} \times 0.1 \text{ (L)} \times 10 / \text{initial phytolith weight (mg)} / 0.42 \times 100$$

152  
153  
154  
155 where 10 is the dilution of 1 ml liquid aliquot in 9 ml 0.021 M HCl, 0.1 (liter) is the volume of  
156 Na<sub>2</sub>CO<sub>3</sub> solution, in which the sample was digested, and 0.42 corresponds to 42% mass of Si in  
157 phytoliths; [2\) estimating the percentage of phytoliths dissolved based on phytolith mass](#) remaining  
158 after dissolution:

$$\text{Phy}_{\text{loss-phy}} (\%) = (\text{initial phytolith mass (mg)} - \text{final phytolith mass (mg)}) / \text{initial phytolith mass (mg)} \times 100$$

159  
160  
161  
162  
163 Data are presented in [Table S1](#) for all experiments. [However, after 10 days, solution volume](#) was  
164 different from the initial volume, so  $\text{Phy}_{\text{loss-DSi}}$  could not be calculated accurately.

#### 165 166 *2.4 Counting, observation and properties of phytolith assemblages*

167  
168 [Identification, observation, and counting of phytoliths was performed](#) at ×400 and ×600  
169 magnification [with an](#) optical microscope. Phytolith names follow the International Code for  
170 Phytolith Nomenclature 2.0 ([Neumann et al, 2019](#)). We identified and counted the most common  
171 and stable morphotypes present in soil and paleosol phytolith assemblages. SADDLE (SAD),  
172 BILOBATE (BIL) and RONDEL (RON) are GSSCs diagnostic of Poaceae. BULLIFORM FLABELLATE  
173 (BUL-FLA) [is](#) diagnostic of Poaceae and Cyperaceae. SPHEROID ECHINATE and SPHEROID

174 DECORATED (SPH) are diagnostic of Areaceae and woody taxa, respectively. Other common  
175 morphotypes such as ELONGATE (ELO), ACUTE BULBOSUS (ACU-BUL) and BLOCKY (BLO) have  
176 no taxonomical value. We did not count rare morphotypes and fragile particles such as small and  
177 thin ( $< 5 \mu\text{m}$ ) silica pieces with ill-defined shapes, and silicified stomata. More than 500 phytoliths  
178 were counted over several slides [and phytolith morphotype percentages were calculated.](#)

179 [Exact Clopper-Pearson confidence intervals \(95% CIs\) were calculated for each phytolith](#)  
180 [morphotype percentage](#) (Suchéras-Marx et al., 2019). [Contingency table analysis, with  \$p\$  values](#)  
181 [from the chi-squared distribution and from a permutation test with 9999 replicates, was used to test](#)  
182 [whether](#) the dissolution [significantly](#) altered phytolith assemblages (Davis, 1986). [The null](#)  
183 [hypothesis  \$H\_0\$  is that assemblages are similar at  \$p > 0.05\$](#)  (i.e. [dissolution is not significant](#)). When  
184  $p \leq 0.05$ , changes in [assemblages](#) can be confirmed. Both 95% CIs [on proportions](#) and Chi-square  
185 tests [on counts](#) were calculated using PAST (Hammer et al., 2001).

186 Several terminologies have been used to describe weathered phytoliths. Here we used ‘cavity’  
187 (Lisztes-Szabó et al., 2020), which is equivalent to ‘pits’ or ‘pitted surface’ (Cabanes et al., 2011)  
188 and ‘cratered phytoliths’ (Kaczorek et al., 2019). To evaluate weathering intensity, we classified  
189 unweathered phytoliths as ‘smooth’ when the surface was smooth and devoid of cavities, ‘irregular’  
190 when the surface looked dark and rough without evident cavities under the microscope, ‘moderately  
191 weathered’ when cavities occupied less than 50% of the phytolith surface, and ‘highly weathered’  
192 when cavities could be observed on more than 50% of the phytolith surface.

193 In addition to [light](#) microscopy, we carried out a grain size analysis of each pure phytolith sample  
194 obtained from the plants before and after dissolution experiments using laser diffraction  
195 (Mastersizer 3000, Malvern Panalytical) to [test](#) the hypothesis that fragile silica particles [and/or](#)  
196 smaller phytoliths [were](#) preferentially dissolved. [Phytolith samples were dispersed in pure water](#)  
197 [without additional physical \(crushing\) treatment to avoid breaking silica particles and magnetically](#)  
198 [agitated](#) to keep them in suspension during measurement. Analyses were made in triplicate and  
199 averaged.

200 We also performed X-ray diffraction (PANalytical X’Pert Pro X-ray diffractometer; Co anti-  
201 cathode, 40KV; 40mA) on [ground](#) phytolith powders [to analyze](#) mineral composition of plant  
202 *Nastus borbonicus*, La Réunion soil, and the paleosol SA18. Phytoliths were mounted on stubs

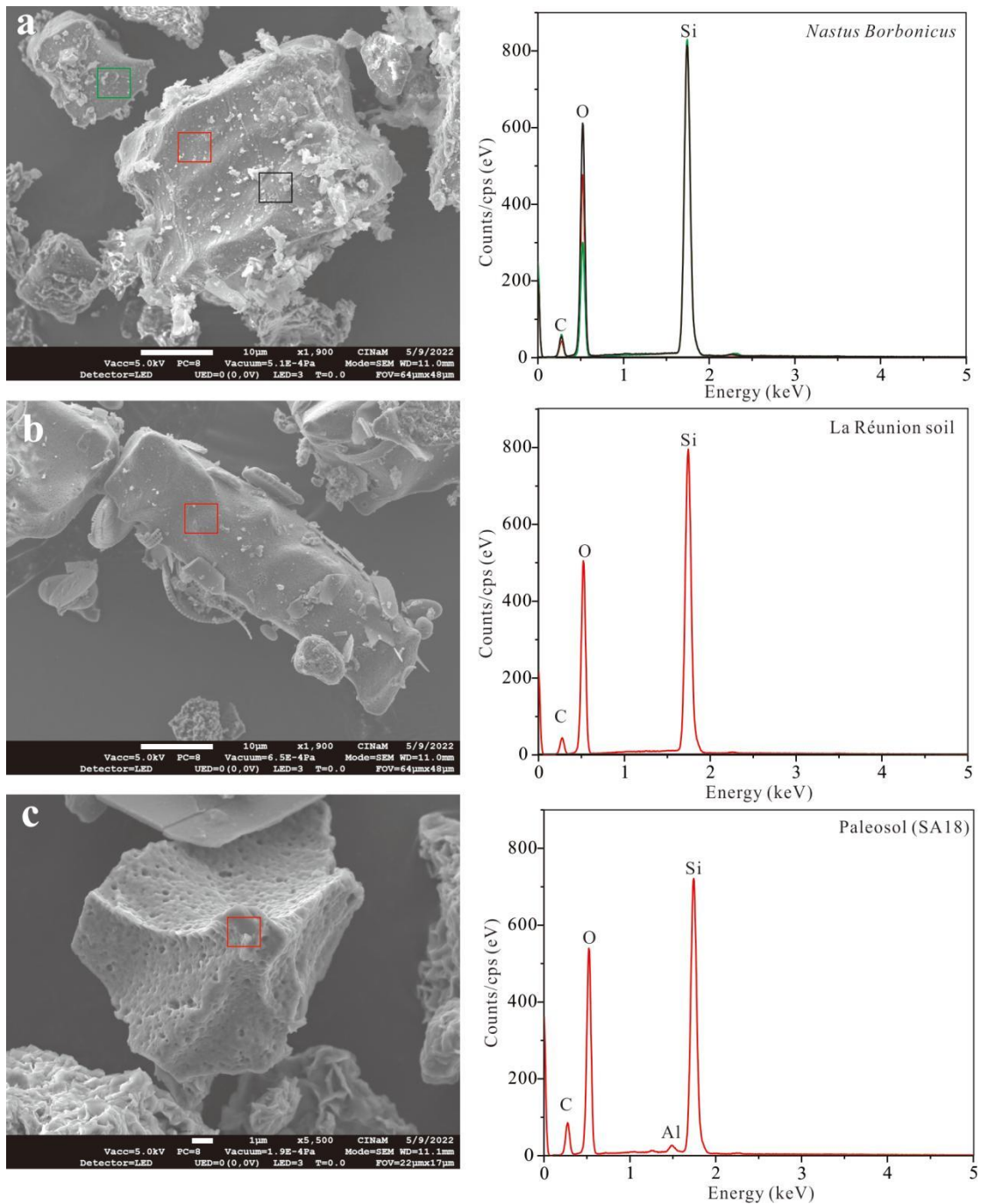
203 using double-sided carbon tape for further surface analysis using scanning electron microscopy  
204 equipped with energy dispersive X-ray (EDX) for localized element analysis (SEM, JEOL JSM-  
205 7900 F × Flash 60-Brüker).

206

### 207 **3. Results**

#### 208 *3.1 Physical, mineralogical and chemical characteristics resulting from partial dissolution*

209 Before dissolution tests we observed that phytoliths from plants, soil, and paleosol samples  
210 exhibit important differences at different levels. First, XRD analysis showed mineral composition  
211 of phytolith assemblages extracted from *Nastus borbonicus* leaves and La Réunion soil peak at 2-  
212 theta value 25°, indicating only opal-A with no well-defined crystalline structure (Figs. S1a-b).  
213 Pliocene paleosols, on the other hand, include small amounts of crystalline mineral residues such as  
214 quartz, smectite, cristobalite and tridymite despite the careful extraction protocol (Fig. S1c). Second,  
215 Si and O were detected in all phytolith samples, but Al only on paleosol phytoliths (Fig. 1). C  
216 detected in all samples likely originated from the C coating used prior to SEM observations. Third,  
217 differences were found in grain-size distributions of pure phytoliths extracted from plants. Phytolith  
218 size distributions in undissolved assemblages of *Hyparrhenia involucrata* ranged from 1 to 200 µm,  
219 showing two overlapping populations centered at 2 µm and 10 µm, while *Nastus borbonicus*  
220 phytoliths ranged from 2 to 2,000 µm, showing two populations centered at 10-20 µm and 500 µm  
221 (Fig. 2). We assume that particles >300 µm in the latter sample probably correspond to aggregates  
222 because phytoliths of this size were not observed in microscopy.



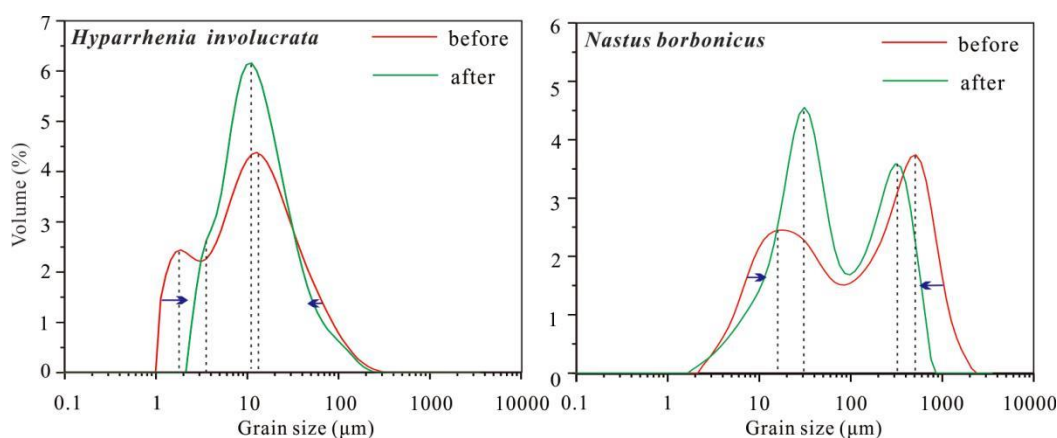
223

224 Fig. 1. SEM images and Energy dispersive X-ray spectroscopy of phytoliths before dissolution from a) *Nastus*  
 225 *borbonicus* leaves, b) La Réunion soil, and c) paleosol SA18.

226

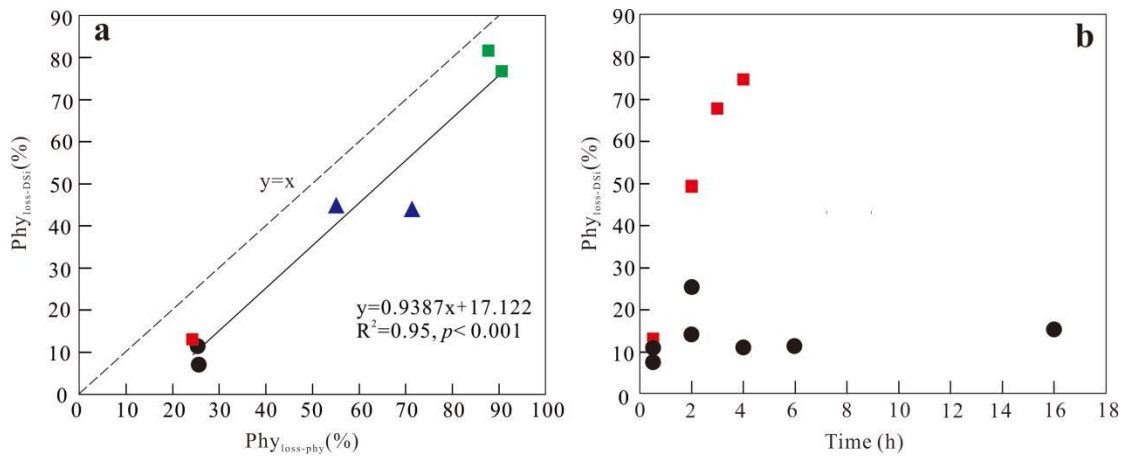
227 After partial dissolution treatment, grain-size distributions were less widespread (Fig. 2) mostly  
 228 due to disappearance of particles < 2-5  $\mu\text{m}$  originally seen in the assemblage from *Hyparrhenia*  
 229 *involucrate* (Fig. S2), leaving only a narrow, monomodal distribution centered at 10  $\mu\text{m}$ . The *Nastus*  
 230 phytolith assemblage distribution remained bimodal but the low size peak was slightly shifted  
 231 toward larger sizes, showing a new mode at 30  $\mu\text{m}$ , likely due to dissolution of thin and small silica

232 particles that were present before the dissolution step (Fig. S2). [Large phytolith aggregates](#)  
 233 [comprising the upper end of the \*Nastus borbonicus\* size distribution showed lower maximum size](#)  
 234 [after dissolution, likely because of aggregate separation.](#)



235  
 236 Fig. 2 Changes in the grain size distribution of phytolith assemblages from plants before and after dissolution.

237  
 238 [Loss of phytoliths resulting from the dissolution experiments was](#) also shown by values of  $Phy_{loss-phy}$   
 239  $Phy_{loss-DSi}$  (Fig. 3a), [which](#) showed a strong positive correlation ( $R^2 = 0.95$ ,  $p < 0.001$ ).  
 240 Systematically lower values of  $Phy_{loss-DSi}$  compared to  $Phy_{loss-phy}$  indicate [larger](#) loss when  
 241 considering phytolith mass [for the calculation, possibly due to phytolith losses occurring during](#)  
 242 [processing](#), particularly when the solution is removed after alkaline extraction. After ½ hour of  
 243 [dissolution](#),  $Phy_{loss-DSi}$  was 44% to 80% for plants (phytoliths from leaves of *Hyparrhenia*  
 244 *involucreta* dissolved more than those [from](#) *Nastus borbonicus*), and about 10% for soil and  
 245 paleosols samples (Fig. 3b). The difference between  $Phy_{loss-DSi}$  to  $Phy_{loss-phy}$  [was least for](#)  
 246 *Hyparrhenia involucreta* (+13%), [followed by](#) *Nastus borbonicus* (+44%), La Réunion soil (+90%),  
 247 [and](#) paleosols (+170%). By extending dissolution [times](#), we observed different behaviors between  
 248 La Réunion soil and Pliocene paleosols. After 4 h,  $Phy_{loss-DSi}$  was around 75% for La Réunion soil,  
 249 close to results at ½ h for *Hyparrhenia involucreta* leaves. However, paleosol phytoliths remained  
 250 [little](#) dissolved even after 16 h ( $Phy_{loss-DSi}$  around 10%).



251

252 Fig. 3. Dissolution experiments of phytoliths in 1% NaCO<sub>3</sub> (60mg/100ml). a) Correlation between the percentages  
 253 of phytolith loss from mass differences (Phy<sub>loss-phy</sub>) and from Si measurement in solution (Phy<sub>loss-DSi</sub>) after ½ hour;

254 b) Phy<sub>loss-phy</sub> vs time. Green square = Hyparrhenia involucrata leaf, blue triangle = Nastus borbonicus leaf, red

255 square = La Réunion soil, black round = Pliocene paleosol.

256

257

### 258 3.2 Visible dissolution features

259 Microscopic observation before dissolution showed differences on surfaces of phytoliths from  
 260 plant, soil, and paleosol samples and as well as among phytoliths within a given assemblage.

261 Phytoliths extracted from living plant tissues exhibited mostly smooth surfaces devoid of cavities.

262 Spheres of around 100 nanometers were observed on the surface of some morphotypes using SEM  
 263 (Fig. S3). On some BLOCKY and ELONGATES, however, rough and dark features over the whole

264 phytolith surface were observed (Fig. 4). Some morphotypes in the La Réunion soil sample

265 exhibited cavities (Fig. 4). In paleosol samples SA18 and SA28, coalescent cavities were seen on

266 most morphotypes (Fig. 5).



20 µm

267

268

Fig. 4 Surface properties changes of different phytolith morphotypes from plant and soil samples.

269

Before dissolution, plant phytolith surfaces are smooth or irregular and some cavities can be observed on soil

270

phytoliths, but after [dissolution](#) phytolith surfaces exhibit more cavities which have been categorized into

271

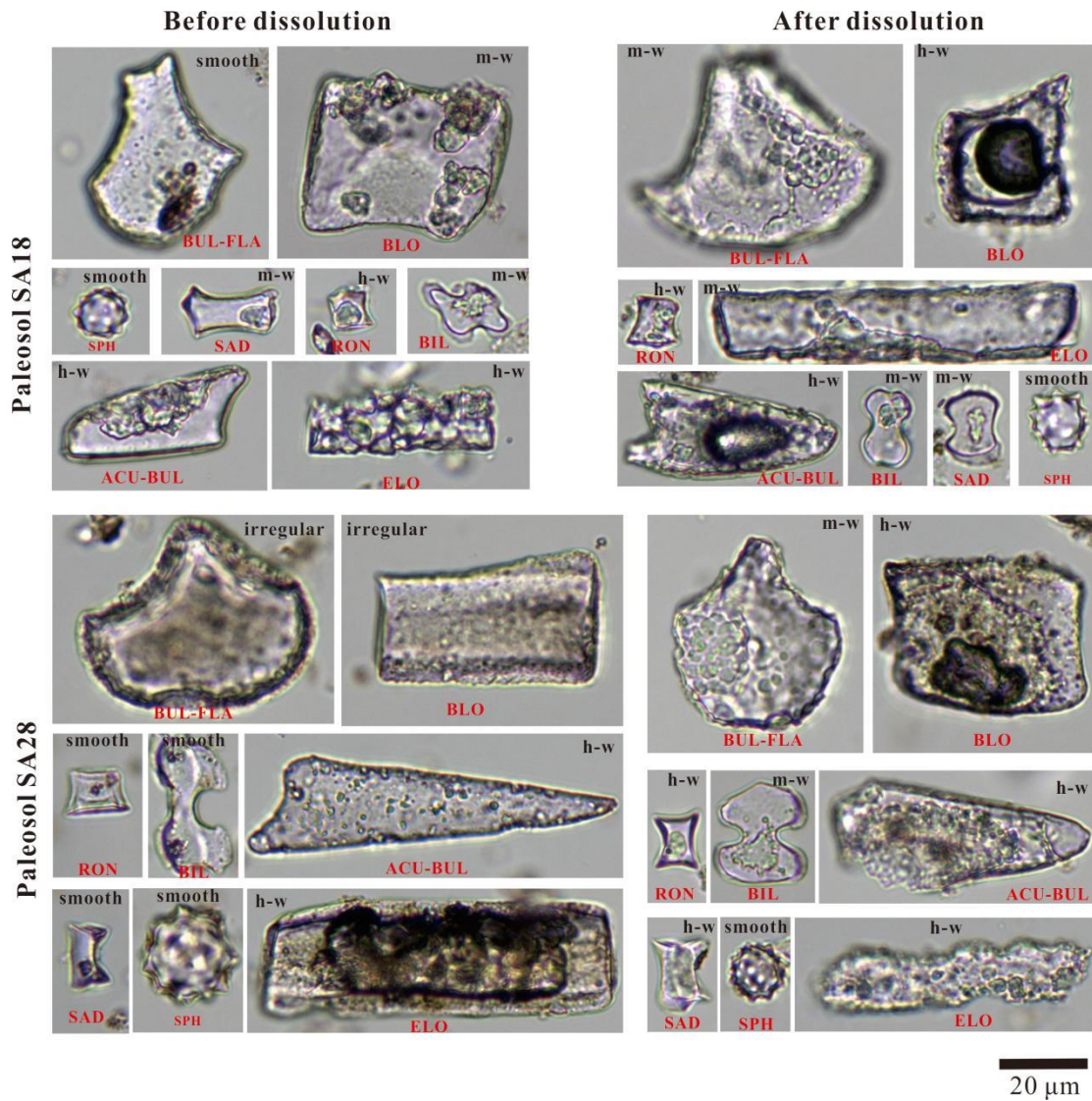
moderately weathered (% cavities < 50%) or highly weathered (% cavities > 50%). m-w: moderately weathered, h-

272

w: highly weathered.

273

274 After partial dissolution [in 1% Na<sub>2</sub>CO<sub>3</sub>](#), we observe that thinly silicified cell walls, cuticles,  
 275 shapeless and indefinite pieces of silica, as well as the silicified stomata present in plant phytolith  
 276 samples have mostly disappeared ([Fig. S2](#)), while larger and more numerous cavities up to 20-30  
 277 μm diameter have appeared on surfaces of all plant, soil and paleosol phytoliths ([Figs. 4-5](#)).  
 278 Dissolution altered surface of [most](#) grass morphotypes [in paleosols](#) but [had](#) little impact on  
 279 SPHEROIDS produced by Arecaceae (palms) ([Fig. 5](#)).



280

20 μm

281 Fig. 5 [Effects of dissolution on surface properties](#) of different phytolith morphotypes from two Pliocene paleosol  
 282 samples.

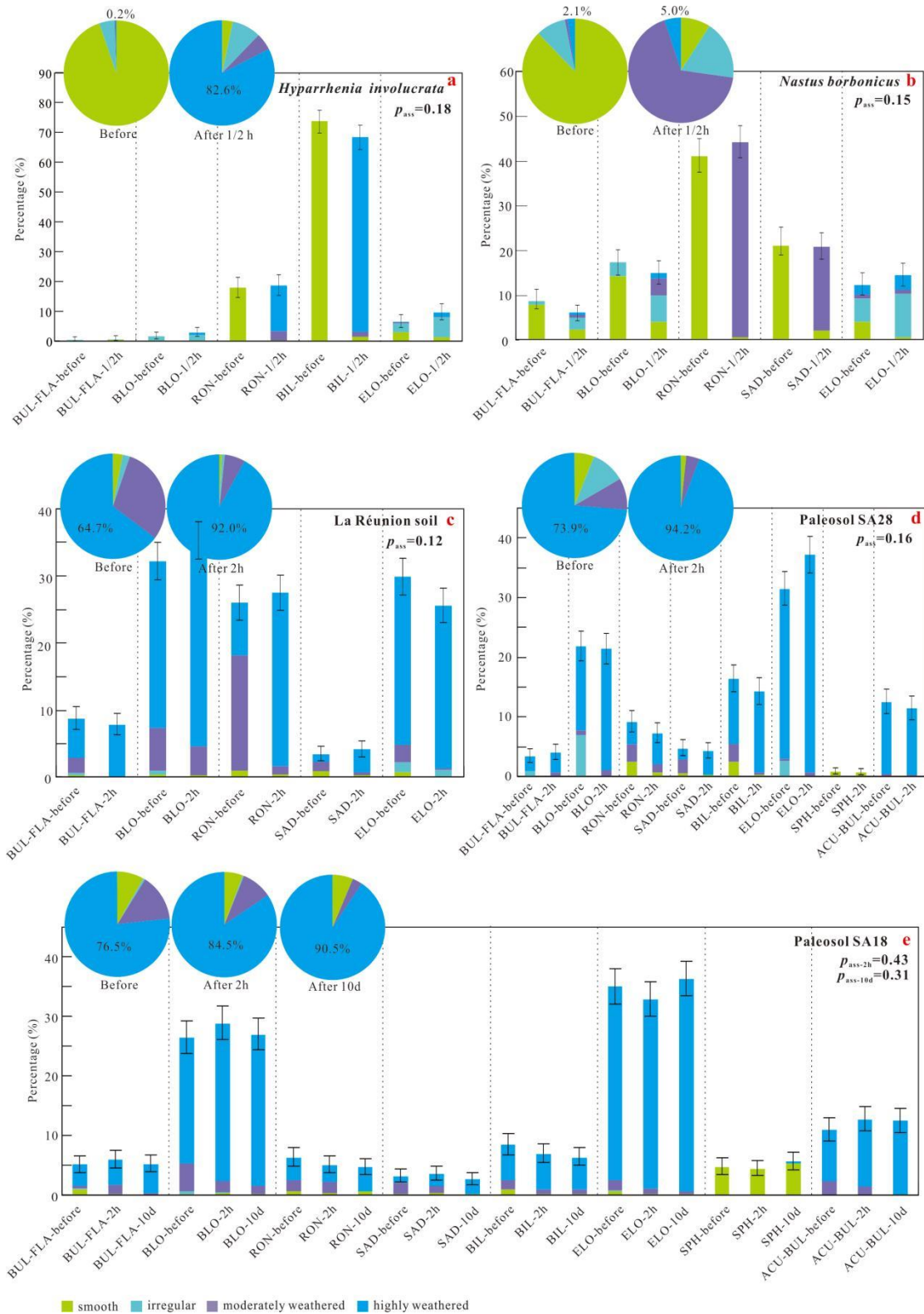
283 Before dissolution, paleosol phytolith surfaces already exhibited cavities, but after dissolution cavities are more  
 284 numerous and larger. m-w: moderately weathered, h-w: highly weathered.

285

286 *3.3 Impact of partial dissolution on the phytolith assemblages*

287 After the partial dissolution experiments, well-defined phytolith morphotypes BUL-FLA, BLO,  
288 RON, SAD, BIL, ELO, ACU-BUL and SPH were still numerous and recognizable enough to  
289 identify and count despite sometimes highly weathered surfaces. Percentages of irregular,  
290 moderately and highly weathered morphotypes increased in all plant, soil and paleosol samples. In  
291 plant samples, moderately and highly weathered morphotypes represented 0-3% before dissolution,  
292 and up to 87% in *Hyparrhenia* and 72% in *Nastus* after partial dissolution, with almost no RON,  
293 BIL, SAD and ELO left intact (Figs. 6a-b).

294 Soil and paleosol phytoliths exhibited some cavities before dissolution; after dissolution relative  
295 proportions of highly weathered morphotypes increased >27% in La Réunion soil and >20% in  
296 paleosol sample SA28 (Figs. 6c-d). Increasing dissolution time increased the proportion of highly  
297 weathered morphotypes in paleosol sample SA18 from 76% to 84% and then to 90% (Fig. 6e).  
298 However, most SPH phytoliths were remained intact (Figs. 6d-e). As shown by overlapping  
299 percentage values and error bars, there were no significant differences between relative abundance  
300 of morphotypes in any assemblage, dissolution also did not alter assemblages according to a chi-  
301 squared test ( $p > 0.05$ ) before and after partial dissolution (Fig. 6), but phytolith surfaces changed,  
302 becoming more deeply perforated (Figs. 4-5).



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Fig. 6 Phytolith assemblages of the main morphotypes before and after partial dissolution experiments according to morphotypes (bar diagrams) and features on their surfaces (pie diagrams). Phytolith surfaces are defined as smooth, irregular, moderately weathered, and highly weathered. In each graph, the pie diagram at the far left shows proportions before dissolution times of ½ h for plants, 2 h for soil, and 2h and 10 days for paleosol samples.

308  $p > 0.05$  means no significant changes in phytolith assemblages ( $p_{\text{ass}}$ ) before and after partial dissolution.

## 310 Discussion

### 312 4.1 Effects of dissolution on phytoliths

313 Our analysis [showed that phytolith dissolution in 1% alkaline solution](#) produces cavities [on](#)  
314 [phytolith surfaces similar](#) to features occurring naturally, as well as to features [observed](#) during  
315 dissolution experiments using different solutions (Cabanes et al., 2011; Kaczorek et al., 2019;  
316 Lisztes-Szabó et al., 2020), [suggesting that our experiments mimic](#) natural dissolution processes  
317 occurring in various sedimentary and pedological contexts. [Dissolution of amorphous silica also](#)  
318 [occurs in acidic conditions, though acidic conditions compared to alkaline conditions are more](#)  
319 [favorable to phytolith preservation. Moreover, many soil phytoliths are likely to be](#) preserved in  
320 organo-mineral aggregates, [which we did not address in this study](#).

321 We observed that thin and small silica particles, very abundant in leaves of *Hyparrhenia*  
322 *involucrata* and *Nastus borbonicus*, [disappeared after experimental dissolution \(Fig S2\), more so](#) in  
323 the phytolith assemblage of *Hyparrhenia* compared to *Nastus* (Figs. 2-3). [Comparing](#) grain size  
324 evolution of the two plant phytolith assemblages showed [that](#) dissolution of the smallest particles  
325 (< 2mm) and disaggregation of the [largest](#) aggregates (> 1000 mm) favors dominance of 10 mm  
326 particles in the *Hyparrhenia* assemblage and 30 mm and 300 mm particles in the *Nastus* assemblage.

327 Dissolution creates cavities [whose](#) size increases with increasing time of exposure to the alkaline  
328 solution. ELONGATE, BLOCKY and BULLIFORM [morphotypes](#) exhibited more highly pitted surfaces  
329 in soils and sediments than SADDLES and RONDELS, [a phenomenon](#) observed elsewhere (e.g.,  
330 Alexandre et al., 1999; Meunier et al. 1999, Osterrieth et al., 2009), [perhaps because](#) pits and cavities  
331 may be a feature of weathering that reflects a particular silica structure or density of various  
332 [phytolith morphotypes](#). SAD, RON and BIL originate [in silica cells, accumulating silica](#) in the cell  
333 lumen, while ELO, BUL-FLA and BLO originate from cell walls and include a greater number of  
334 various organic molecules, [likely weakening the silica structure](#) (Kumar et al., 2016, 2017a, b).

### 336 4.2 Stability of phytoliths in soils and paleosols

337 [Our finding](#) that plant phytoliths dissolve more easily than soil and paleosol phytoliths (Fig. 3) is  
338 consistent with previous findings (Cabanes et al., 2011; Fraysse et al. 2009; Meunier et al. 2014),  
339 [also](#) observed for other biogenic silica particles such as diatoms that dissolve faster when [compared](#)  
340 [to those](#) extracted from sediments (Van Cappellen et al., 2002). Fraysse et al. (2006) showed that  
341 the specific surface area of phytoliths extracted from *Nastus* is  $159.5 \text{ m}^2\text{g}^{-1}$ , much higher than  $5.18$   
342  $\text{m}^2\text{g}^{-1}$  measured for La Réunion soil phytoliths (Fraysse et al., 2006). This difference likely results  
343 from [transformation](#) of the siliceous structure of phytoliths during pedogenesis, sedimentation or  
344 burial, leading to more resistance to dissolution. Unfortunately, [we could not observe](#) transformation  
345 of biogenic opal-A into more stable forms of amorphous silica (Kastner and Gieskes, 1983) [in](#)  
346 paleosol samples due to the dominance of quartz peaks in the XRD spectra. Al, [commonly observed](#)  
347 [on soil phytolith surfaces](#) (Bartoli and Wilding, 1980; Cornelis et al., 2011; Van Cappellen et al.,  
348 [2002](#)), detected on phytolith surfaces of paleosol samples (Fig. 1) may also decrease phytolith  
349 sensitivity to dissolution. [Al deposition may occur](#) during pedogenesis or sedimentation as  
350 suggested by Bartoli (1985), although Al [has been](#) detected in phytoliths extracted from plants  
351 (Carnelli et al., 2002). Finally, the low dissolution rate of paleosol phytoliths [compared](#) to plant and  
352 soil phytoliths may [be](#) accentuated by the presence of low solubility crystalline aluminosilicate and  
353 quartz particles remaining in the sample (Fraysse et al., 2009).

#### 354

#### 355 4.3 Assessment of phytolith-based paleo-environment reconstruction

356 [We found](#) that while the fragile silica particles almost totally disappeared (Fig. S2), relative  
357 abundances of the main phytolith types in [an](#) assemblage are not affected by dissolution (Fig. 6).  
358 This finding [matches](#) Cabanes and Shahack-Gross (2015), [who](#) showed that only double peaked  
359 husk, long cell wavy, parallelepipedal elongate rugulate [phytoliths](#) from rice inflorescences and  
360 papillates from sedges ([fragile morphotypes not commonly](#) preserved in soils) showed significant  
361 [proportion](#) change after partial dissolution, while relative abundances of morphotypes such as  
362 BULLIFORM and GSSCs remained constant. [These results strongly indicate that](#) past vegetation  
363 reconstructions from phytolith assemblages and phytolith indices [are robust](#) (Diester-Haass et al.  
364 1973; Twiss, 1992).

365 La Reunion soils [probably](#) developed under long-term bamboo vegetation with *Nastus*

366 *borbonicus* as dominant species. Meunier et al. (1999) showed that RONDELS and SADDLES (named  
367 square and round in Meunier et al. (1999)) occurred in higher proportions in the leaves of *Nastus*  
368 compared to the surface soil, [suggesting selective dissolution of these morphotypes](#). Yost et al.  
369 (2021) showed that GSSC phytoliths disappeared prior to ELONGATE and BULLIFORM phytoliths in  
370 a continuous sediment core from Lake Baringo, Kenya. [They](#) proposed a conceptual model for  
371 biogenic silica dissolution succession, [suggesting](#) that phytoliths, diatoms, and sponge spicules  
372 exhibit more cavities and even disappear when pH increases [and](#) also shows that short cells  
373 disappear at lower pH than BUL-FLA, BLO and ELO.

374 In our experiments, where we maintained pH at 11.2 (at 85 °C), all morphotypes including short  
375 cells, ELO, BLO and BUL-FLA are still present and some do not even show cavities (e.g., SPH).  
376 Proportions within the GSSCs and between GSSCs, ELO, BLO, and BUL-FLA are also maintained.  
377 [Our study does not confirm selective dissolution of common phytolith](#) morphotypes or the model  
378 of silica dissolution succession proposed by Yost et al (2021). [However, if dissolution experiments](#)  
379 [had continued considerably longer, we cannot reject they may in fact have been able to confirm the](#)  
380 [model of Yost et al. \(2021\)](#). Apart from dissolution, soil and sediment phytolith assemblages can be  
381 affected by physical erosion and selective translocation inside the soil column (Alexandre et al.,  
382 1997; Alexandre et al., 2011; Kaczorek et al., 2019). GSSCs with smaller volume are [more easily](#)  
383 translocated to deeper layers (Liu et al., 2019) [and](#) exported by erosion, [likely explaining](#) the over-  
384 representation of long and/or [large](#) particles in some sedimentary levels and soil layers.

385 [Our results indicate](#) that although dissolution [kinetics differ between](#) phytolith assemblages,  
386 relative proportions of phytolith [morphotypes typically](#) used for paleoenvironmental reconstruction  
387 stay stable. [Phytolith assemblages showing dissolution features can](#) still provide significant  
388 qualitative information. [Other taphonomic processes such as translocation and erosion that](#) may  
389 [modify](#) phytolith assemblages extracted from soils and sediments should be addressed in future  
390 studies.

391

## 392 **Conclusion**

393 Our systematic analysis, [examining effects](#) of partial alkaline dissolution on plant, soil and  
394 paleosol phytolith assemblages by considering phytolith surface features, assemblage changes and

395 Si release, shows that dissolution leads to formation of pits and cavities, an increase in number and  
396 size of cavities on phytolith [surfaces](#), as well as rapid loss of thin silica particles such as silicified  
397 stomata cells and cell walls. The order of [the dissolution rate of phytoliths](#) is plant > soil > paleosol.  
398 [Pedogenic](#) and burial processes lead to stable phytolith assemblages. Dissolution does not  
399 significantly change relative proportions of stable phytolith morphotypes within an assemblage,  
400 [supporting](#) the robustness of past vegetation interpretations inferred from phytolith assemblages.

401

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408

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