



HAL
open science

Dissolution does not affect grass phytolith assemblages

Hongye Liu, Jean-Dominique Meunier, Olivier Grauby, Jérôme Labille, Anne Alexandre, Doris Barboni

► **To cite this version:**

Hongye Liu, Jean-Dominique Meunier, Olivier Grauby, Jérôme Labille, Anne Alexandre, et al.. Dissolution does not affect grass phytolith assemblages. *Palaeogeography, Palaeoclimatology, Palaeocology*, 2023, 610, pp.111345. 10.1016/j.palaeo.2022.111345 . hal-03962098

HAL Id: hal-03962098

<https://hal.science/hal-03962098v1>

Submitted on 17 Jul 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

Dissolution does not affect grass phytolith assemblages

Hongye Liu^{a, b, *}, Jean-Dominique Meunier^a, Olivier Grauby^c, Jérôme Labille^a, Anne Alexandre^a, Doris Barboni^{a, d}

^a CEREGE, Aix-Marseille Université, CNRS, IRD, INRAE, Aix en Provence, France

^b State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Wuhan
430074, China

^c Aix-Marseille Université, CNRS, CINaM, Marseille, Cedex 09, France

^d Institut Français de Pondichéry, UMIFRE 21, UAR 3330 « Savoirs et Mondes Indiens », Pondicherry, India

* Corresponding author: hoyeliu@cug.edu.cn – Hongye LIU, China University of Geosciences,
Lumo Rd. 388, Wuhan 430074, China.

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 ≤ 800 yr), and three paleosols from Ethiopia (approximate age of 4.4 million years). We used heavy-liquid to extract phytoliths, and 1% Na₂CO₃ 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) 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 ¹⁴C 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 H₂SO₄ (95%) and heated at 80°C for > 8h. To improve oxidation of organic matter,
110 samples were soaked again in H₂O₂ (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% H₂O₂ under constant heat (80°C) to remove
121 organic matter. Clays were removed by decantation, and minerals by densimetric separation using
122 ZnBr₂ heavy liquid with density 2.3 g/cm³.

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% Na₂CO₃ solution. Sixty mg dried
129 phytolith powder were added to 100 ml 1% Na₂CO₃ 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 \(DSi, mg L⁻¹\) 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⁻¹ (Plasma CAL).

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

$$153 \text{Phy}_{\text{loss-DSi}} (\%) = \text{DSi (mg L}^{-1}\text{)} \times 0.1 \text{ (L)} \times 10 / \text{initial phytolith weight (mg)} / 0.42 \times 100$$

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 Na₂CO₃ 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:

$$160 \text{Phy}_{\text{loss-phy}} (\%) = (\text{initial phytolith mass (mg)} - \text{final phytolith mass (mg)}) / \text{initial phytolith mass}$$
$$161 (\text{mg}) \times 100$$

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.

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 µm) 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 H0 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

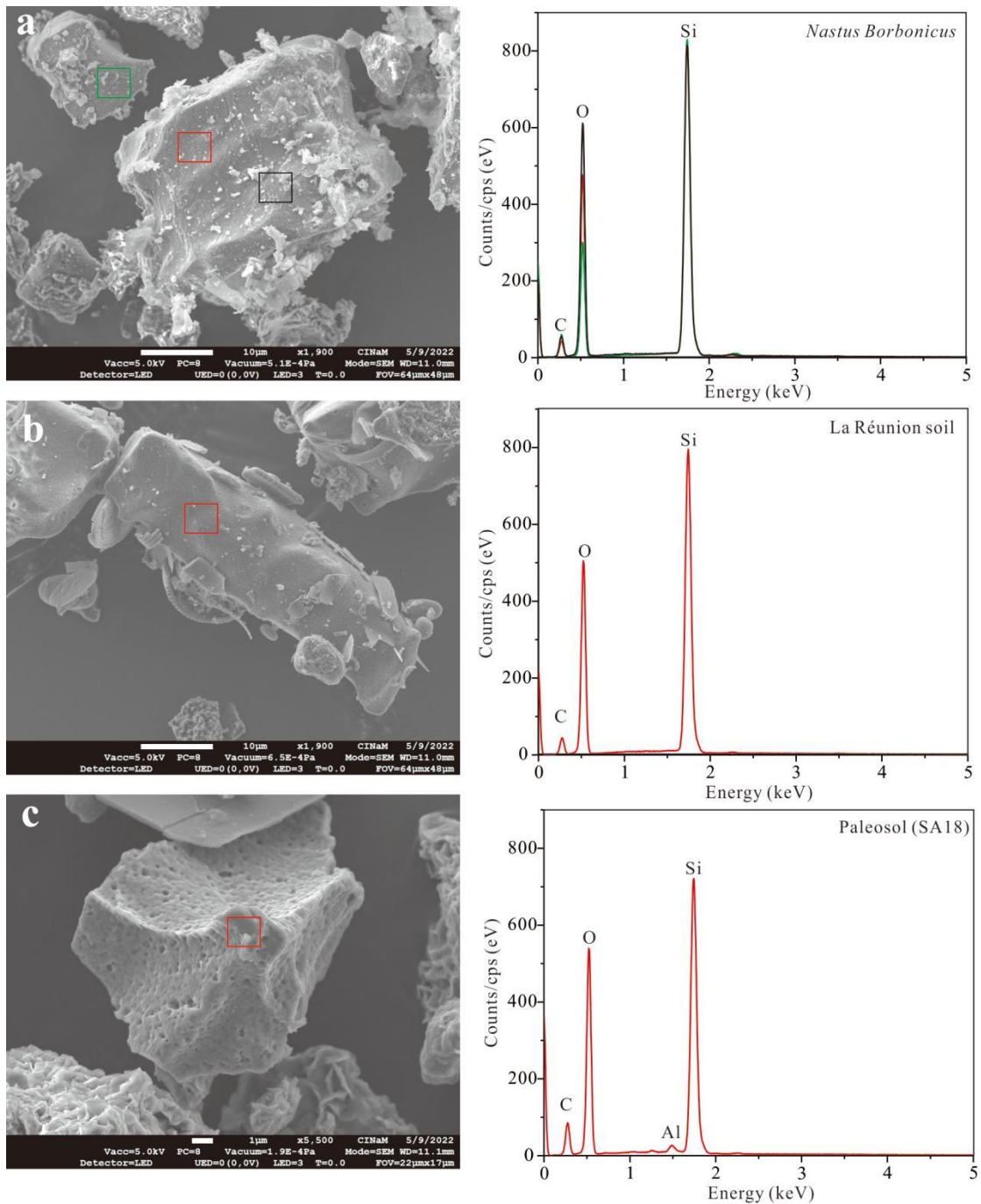
203 using double-sided carbon tape for further surface analysis using scanning electron microscopy
204 equipped with energy dispersive X-ray (EDX) for localized element analysis (SEM, JEOL JSM-
205 7900 F × Flash 60-Brüker).

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.



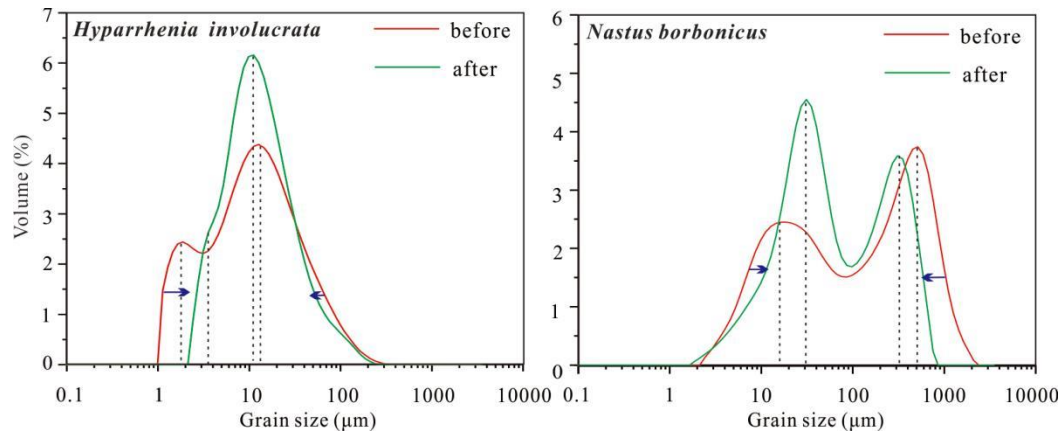
223

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

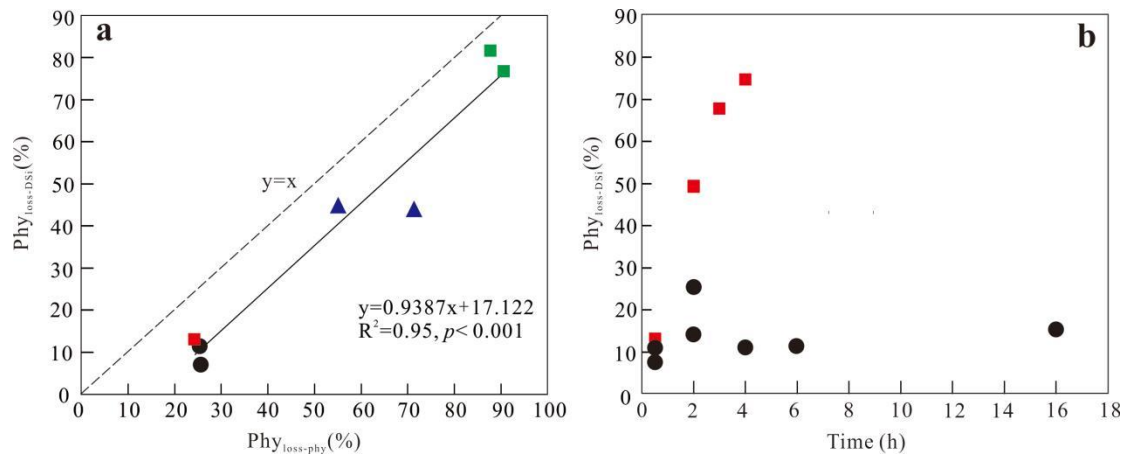
227 After partial dissolution treatment, grain-size distributions were less widespread (Fig. 2) mostly
 228 due to disappearance of particles < 2-5 μm originally seen in the assemblage from *Hyparrhenia*
 229 *involucrate* (Fig. S2), leaving only a narrow, monomodal distribution centered at 10 μm . The *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 μm , 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% NaCO₃ (60mg/100ml). a) Correlation between the percentages
 253 of phytolith loss from mass differences (Phy_{loss-phy}) and from Si measurement in solution (Phy_{loss-DSi}) after ½ hour;

254 b) Phy_{loss-phy} 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

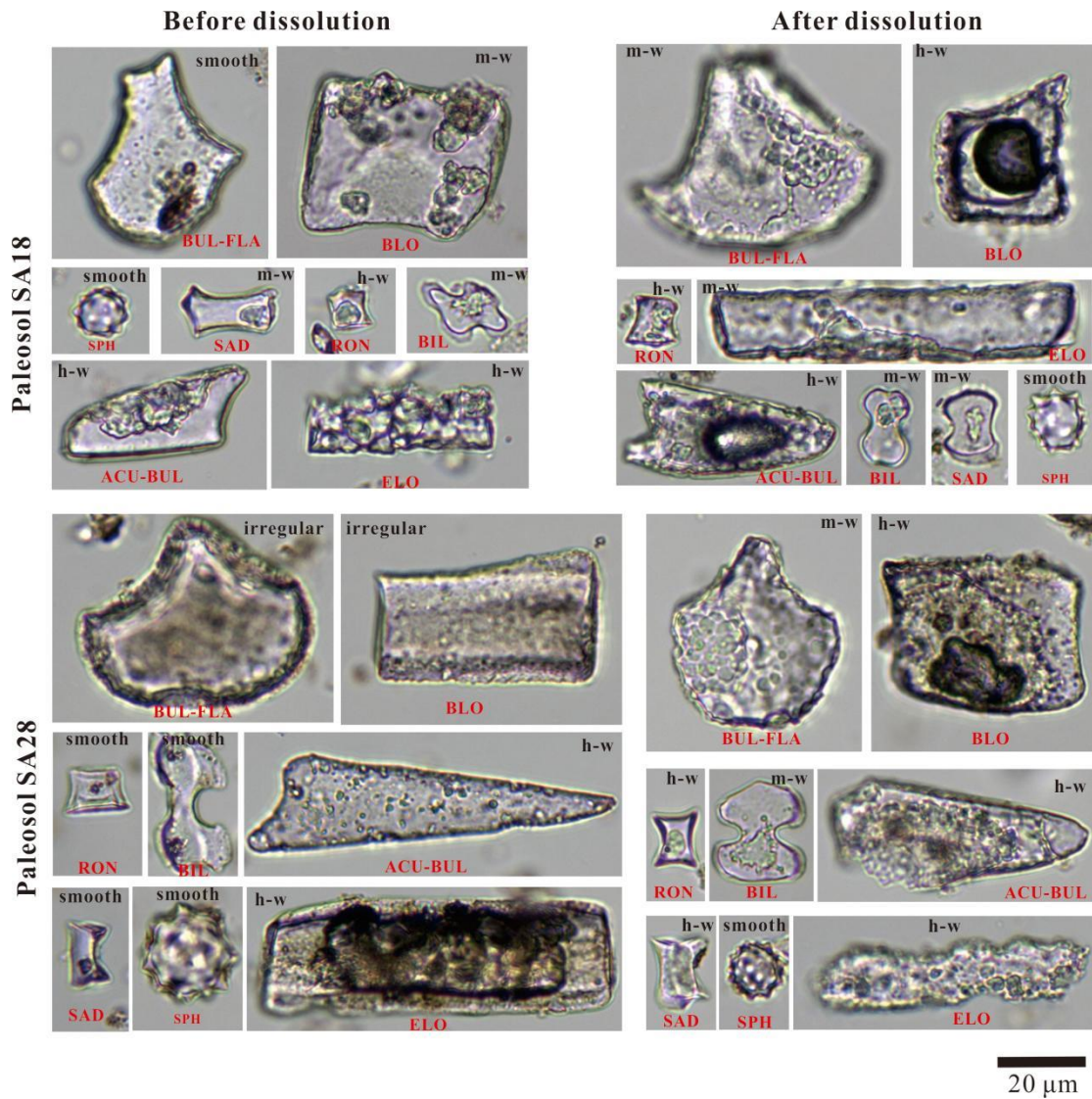
moderately weathered (% cavities < 50%) or highly weathered (% cavities > 50%). m-w: moderately weathered, h-

272

w: highly weathered.

273

274 After partial dissolution [in 1% Na₂CO₃](#), 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.

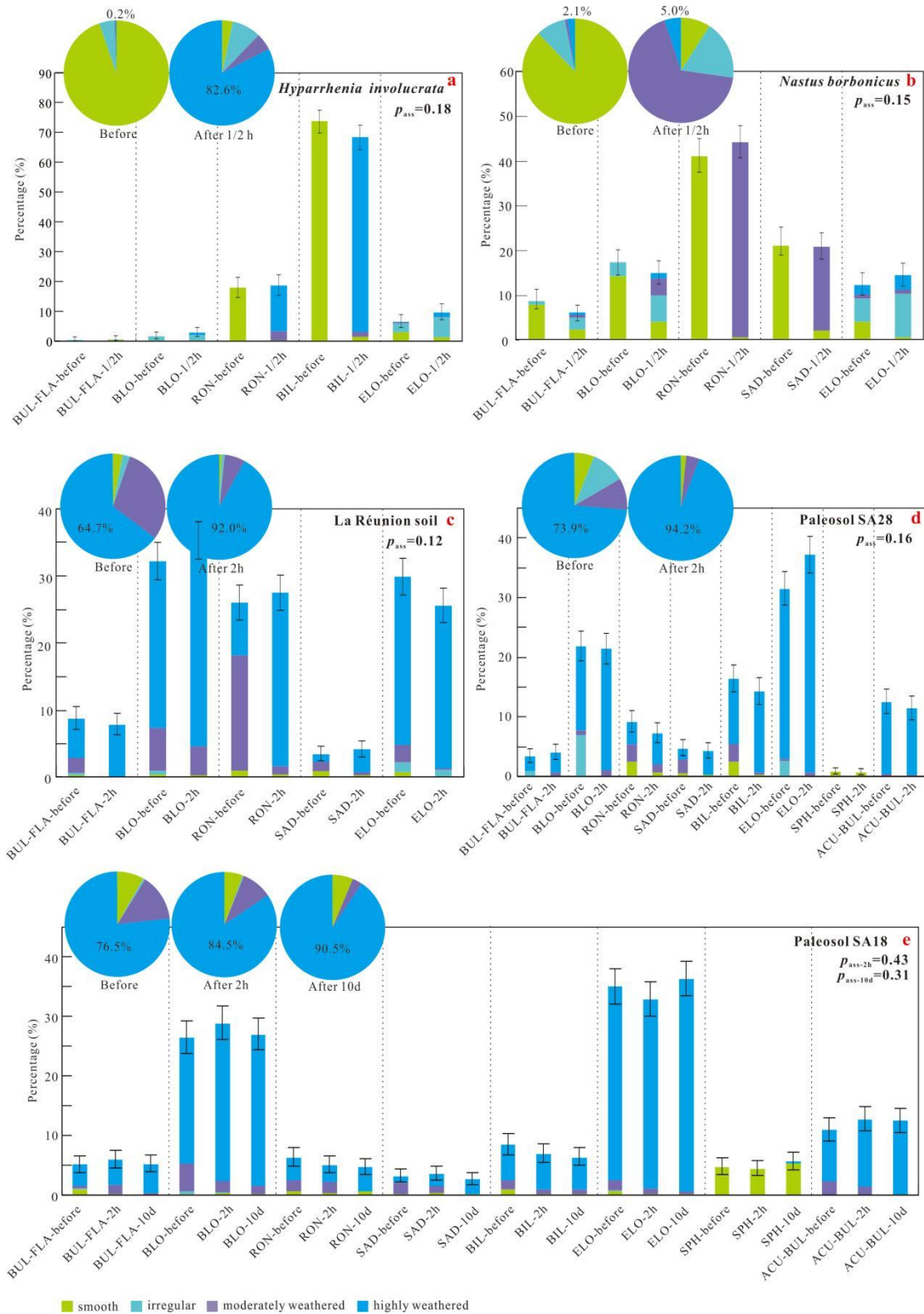
283 Before dissolution, paleosol phytolith surfaces already exhibited cavities, but after dissolution cavities are more
 284 numerous and larger. m-w: moderately weathered, h-w: highly weathered.

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).



303

304

305

306

307

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_{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 m^2g^{-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 release₂ 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 assemblage₂.
400 [supporting](#) the robustness of past vegetation interpretations inferred from phytolith assemblages.

401

402 **Acknowledgments**

403 This research has benefited from the support of the China Scholarship Council through the
404 provision of financial support to HL and the support of CEREGE APIC fund to DB and JDM for
405 carrying the experiments. We thank Daniel Borschneck, Yves Noack, Baptiste Suchéras-Marx,
406 Sandrine Conrod, Christine Pailles and Jean-Charles Mazur at CEREGE for their helpful
407 contribution.

408

409 **References**

- 410 Alexandre, A., Bouvet, M., and Abbadie, L., 2011. The role of savannas in the terrestrial Si cycle:
411 A case-study from Lamto, Ivory Coast. *Global and Planetary Change* 78, 162-169.
- 412 Alexandre, A., Meunier, J.D., Colin, F., Koud, J.M., 1997. Plant impact on the biogeochemical cycle
413 of silicon and related weathering processes. *Geochimica et Cosmochimica Acta* 61, 677-682.
- 414 Alexandre, A., Meunier, J.D., Mariotti, A., Soubies, F., 1999. Late Holocene Phytolith and Carbon-
415 Isotope Record from a Latosol at Salitre, South-Central Brazil. *Quaternary Research* 51, 187-
416 194.
- 417 Aleman, J.C., Canal-Subitani, S., Favier, C., Bremond, L., 2014. Influence of the local environment
418 on lacustrine sedimentary phytolith records. *Palaeogeography, Palaeoclimatology,*
419 *Palaeoecology* 414, 273-283.
- 420 Arráiz, H., Barboni, D., Ashley, G.M., Mabulla, A., Baquedano, E., Domínguez-Rodrigo, M. 2017.
421 The FLK Zinj paleolandscape: Reconstruction of a 1.84Ma wooded habitat in the FLK Zinj-
422 AMK-PTK-DS archaeological complex, Middle Bed I (Olduvai Gorge, Tanzania),
423 *Palaeogeography, Palaeoclimatology, Palaeoecology* 488, 9-20.

424 Ball, T., Chandler-Ezell, K., Dickau, R., Duncan, N., Hart, T.C., Iriarte, J., Lentfer, C., Logan, A.,
425 Lu, H., Madella, M., Pearsall, D.M., Piperno, D.R., Rosen, A.M., Vrydaghs, L., Weisskopf, A.,
426 Zhang, J., 2016. Phytoliths as a tool for investigations of agricultural origins and dispersals
427 around the world. *Journal of Archaeological Science* 68, 32-45.

428 Bartoli, F., 1985. Crystallochemistry and surface properties of biogenic opal. *The European Journal*
429 *of Soil Science* 36, 335-350.

430 Bartoli, F., Wilding, L.P., 1980. Dissolution of Biogenic Opal as a Function of its Physical and
431 Chemical Properties. *Soil Science Society of America Journal* 44, 873-878.

432 Blecker, S., McCulley, R., Chadwick, O., Kelly, E., 2006. Biologic Cycling of Silica across a
433 Grassland Bioclimate sequence, *Global Biogeochemical Cycles* 20, GB3023.

434 Borrelli, N., Alvarez, M.F., Osterrieth, M.L., Marcovecchio, J.E., 2010. Silica content in soil
435 solution and its relation with phytolith weathering and silica biogeochemical cycle in Typical
436 Argiudolls of the Pampean Plain, Argentina-a preliminary study. *Journal of Soils and*
437 *Sediments* 10, 983-994.

438 Bremond, L., Alexandre, A., Wooller, M.J., Hely, C., Williamson, D., Schafer, P.A., Majule, A.,
439 Guiot, J., 2008. Phytolith indices as proxies of grass subfamilies on East African tropical
440 mountains. *Global and Planetary Change* 61, 209-224.

441 Cabanes, D., Shahack-Gross, R., 2015. Understanding fossil phytolith preservation: the role of
442 partial dissolution in paleoecology and archaeology. *PloS one* 10, e0125532.

443 Cabanes, D., Weiner, S., Shahack-Gross, R., 2011. Stability of phytoliths in the archaeological
444 record: a dissolution study of modern and fossil phytoliths. *Journal of Archaeological Science*
445 38, 2480-2490.

446 Carnelli, A.L., Madella, M., Theurillat, J.P., Ammann, B., 2002. Aluminum in the opal silica
447 reticule of phytoliths: a new tool in palaeoecological studies, *American Journal of Botany* 89,
448 346-351.

449 Ciochon, R.L., Piperno, D.R., Thompson, R.G., 1990. Opal phytoliths found on the teeth of the
450 extinct ape *Gigantopithecus blacki*: implications for paleodietary studies. *Proceedings of the*
451 *National Academy of Sciences, USA* 87, 8120-8124.

452 Cordova, C.E., Johnson, W.C., Mandel, R.D., Palmer, M.W., 2011. Late Quaternary environmental

453 change inferred from phytoliths and other soil-related proxies: case studies from the central
454 and southern Great Plains, USA. *Catena* 85, 87-108.

455 Cornelis, J.T., Delvaux, B., Georg, R.B., Lucas, Y., Ranger, J., Opfergelt, S., 2011. Tracing the
456 origin of dissolved silicon transferred from various soil-plant systems towards rivers: a review.
457 *Biogeosciences* 8, 89-112.

458 Crespín, J., Alexandre, A., Sylvestre, F., Sonzogni, C., Paillès, C., and Garreta, V., 2008. IR laser
459 extraction technique applied to oxygen isotope analysis of small biogenic silica samples,
460 *Analytical Chemistry* 80, 2372-2378.

461 Davis, J.C., 1986. *Statistics and Data Analysis in Geology*. John Wiley & Sons.

462 Diester-Haass, L., Schrader, H.J., Thiede, J., 1973. Sedimentological and palaeoclimatological
463 investigations of two pelagic-ooze cores off Cape Barbas, North-West Africa. *Meteor*
464 *Forschungsergebnisse: Reihe, C., Geologie und Geophysik* 16. pp. 19-66.

465 Fraysse, F., Pokrovsky, O.S., Schott, J., Meunier, J.D., 2006. Surface properties, solubility and
466 dissolution kinetics of bamboo phytoliths. *Geochimica et Cosmochimica Acta* 70, 1939-1951.

467 Fraysse, F., Pokrovsky, O.S., Schott, J., Meunier, J.D., 2009. Surface chemistry and reactivity of
468 plant phytoliths in aqueous solutions. *Chemical Geology* 258, 197-206.

469 Fredlund, G.G., Tieszen, L.L., 1994. Modern phytolith assemblages from the North American
470 Great Plains. *Journal of Biogeography* 21, 321-335.

471 Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: paleontological statistics software package
472 for education and data analysis. *Palaeontologia Electronica* 4, 1-9.

473 Hodson, M.J. 2019. The relative importance of cell wall and lumen phytoliths in carbon
474 sequestration in soil: a hypothesis. *Frontiers in Earth Science* 7, 167.

475 Hodson, M.J., White, P.J., Mead, A., Broadley, M.R., 2005. Phylogenetic variation in the silicon
476 composition of plants. *Annals of Botany* 96, 1027-1046.

477 Hyland, E., Smith, S.Y., Sheldon, N.D., 2013. Representational bias in phytoliths from modern soils
478 of central North America: Implications for paleovegetation reconstructions. *Palaeogeography,*
479 *Palaeoclimatology, Palaeoecology* 374, 338-348.

480 Iriarte, J., 2003. Assessing the feasibility of identifying maize through the analysis of cross-shaped
481 size and three-dimensional morphology of phytoliths in the grasslands of southeastern South

482 America. *Journal of Archaeological Science* 30, 1085-1094.

483 Issaharou-Matchi, I., Barboni, D., Meunier, J.D., Saadou, M., Dussouillez, P., Contoux, C., Zirihi-
484 Guede, N., 2016. Intraspecific biogenic silica variations in the grass species *Pennisetum*
485 *pedicellatum* along an evapotranspiration gradient in South Niger. *Flora-Morphology,*
486 *Distribution, Functional Ecology of Plants* 220, 84-93.

487 Kaczorek, D., Puppe, D., Busse, J., Sommer, M., 2019. Effects of phytolith distribution and
488 characteristics on extractable silicon fractions in soils under different vegetation-An
489 exploratory study on loess. *Geoderma* 356, 113917.

490 Kastner, M., Gieskes, J.M., 1983. Opal-A to opal-CT transformation: a kinetic study. *Developments*
491 *in Sedimentology* 36, 211-227.

492 Kumar, S., Milstein, Y., Brami, Y., Elbaum, M., Elbaum, R., 2016. Mechanism of silica
493 deposition in sorghum silica cells, *New Phytologist* 213, 791-798.

494 Kumar, S., Soukup, M., Elbaum, R., 2017a. Silicification in Grasses: Variation between Different
495 Cell Types, *Frontiers Plant Science* 8, 438.

496 Kumar, S., Elbaum, R., 2017b. Interplay between silica deposition and viability during the life
497 span of sorghum silica cells, *New Phytologist* 217, 1137-1145.

498 Lisztes-Szabó, Z., Filep, A.F., Csík, A., Pető, Á., Kertész, T.G., Braun, M., 2020. pH-dependent
499 silicon release from phytoliths of Norway spruce (*Picea abies*). *Journal of Paleolimnology* 63,
500 65-81.

501 Liu, L., Li, D., Jie, D., Liu, H., Gao, G., Li, N., 2019. Translocation of phytoliths within natural Soil
502 profiles in Northeast China. *Frontiers in Plant Science* 10, 1254.

503 Lu, H., Zhang, J., Liu, K.B., Wu, N., Li, Y., Zhou, K., Ye, M., Zhang, T., Zhang, H., Yang, X.,
504 Shen, L., Xu, D., Li, Q., 2009. Earliest domestication of common millet (*Panicum miliaceum*)
505 in East Asia extended to 10,000 years ago. *Proceedings of the National Academy of Sciences,*
506 *USA* 106, 7367-7372.

507 Lu, H., Zhang, J., Yang, Y., Yang, X., Xu, B., Yang, W., Tong, T., Jin, S., Shen, C., Rao, H., Li, X.,
508 Lu, H., Fuller, D.Q., Wang, L., Wang, C., Xu, D., Wu, N., 2016. Earliest tea as evidence for
509 one branch of the Silk Road across the Tibetan Plateau. *Scientific Reports* 6, 18955.

510 McCune, J.L., Pellatt, M.G., 2013. Phytoliths of Southeastern Vancouver Island, Canada, and their

511 potential use to reconstruct shifting boundaries between Douglas-fir forest and oak savannah.
512 Palaeogeography, Palaeoclimatology, Palaeoecology 383-384, 59-71.

513 Meunier, J.D., Colin, F., Alarcon, C., 1999. Biogenic silica storage in soils. *Geology* 29, 835-838.

514 Meunier, J.D., Cornu, S., Keller, C., Doris, B., 2022. The role of silicon in the supply of terrestrial
515 ecosystem services. *Environmental Chemistry Letters* 20, 2109-2121.

516 Meunier, J.D., Keller, C., Guntzer, F., Riotte, J., Braun, J.J., Anupama, K., 2014. Assessment of the
517 1% Na₂CO₃ technique to quantify the phytolith pool. *Geoderma* 216, 30-35.

518 Neumann, K., Ball, T., Albert, R.M., Vrydaghs, L., Cummings, L.S., 2019. International Code for
519 Phytolith Nomenclature (ICPN) 2.0. *Annals of Botany* 124, 189-199.

520 [Nguyen, M.N., Dultz, S., Meharg, A.A., Pham, Q.V., Hoang, A.N., Dam, T.T.N., Nguyen, V.T.,](#)
521 [Nguyen, K.M., Nguyen, H.X., Nguyen, N.T., 2019. Phytolith content in Vietnamese paddy](#)
522 [soils in relation to soil properties. *Geoderma* 333, 200-213.](#)

523 Nogué, S., Whicher, K., Baker, A.G., Bhagwat, S.A., Willis, K.J., 2017. Phytolith analysis reveals
524 the intensity of past land use change in the Western Ghats biodiversity hotspot, *Quaternary*
525 *International* 437, 82-89.

526 Novello, A., Lebatard, A.E., Moussa, A., Barboni, D., Sylvestre, F., Bourlès, D. L., Paillès, C.,
527 Buchet, G., Decarreau, A., Durringer, P., Ghienne, J.F., Maley, J., Mazur, J.C., Roquin, C.,
528 Schuster, M., Vignaud, P., 2015. Diatom, phytolith, and pollen records from a ¹⁰Be/⁹Be dated
529 lacustrine succession in the Chad basin: insight on the Miocene-Pliocene paleoenvironmental
530 changes in Central Africa. *Palaeogeography, Palaeoclimatology, Palaeoecology* 430, 85-103.

531 Osterrieth, M., Madella, M., Zurro, D., Fernanda Alvarez, M., 2009. Taphonomical aspects of silica
532 phytoliths in the loess sediments of the Argentinean Pampas. *Quaternary International* 193, 70-
533 79.

534 Outrequin, C., 2022. The ¹⁷O-excess of plant silica: towards a new humidity indicator *Atmospheric*
535 *Discipline Science*. PhD thesis. Aix-Marseille University.

536 Piperno, D.R., Pearsall, D.M., 1998. The silica bodies of tropical American grasses: morphology,
537 taxonomy, and implications for grass systematics and fossil phytolith identification.
538 *Smithsonian Contributions to Botany* 85, 1-40.

539 Piperno, D.R., 2006. *Phytoliths: a comprehensive guide for archaeologists and paleoecologists.*

540 AltaMira Press, New York.

541 Prasad, V., Strömberg, C.A.E., Leache, A., Samant, B.R.P., Tang, L., Mohabey, D.M., Ge, S., Sahni,
542 A., 2011. Late Cretaceous origin of the rice tribe provides evidence for early diversification in
543 Poaceae.. *Nature Communications* 2, 480.

544 Riotte, J., Meunier, J.D., Zambardi, T., Audry, S., Barboni, D., Anupama, K., Prasad, S., Chmeleff,
545 J., Poitrasson, F., Sekhar, M., Braun, J.J., 2018. Processes controlling silicon isotopic
546 fractionation in a forested tropical watershed: Mule Hole Critical Zone Observatory (Southern
547 India). *Geochimica et Cosmochimica Acta* 228, 301-319.

548 Rosen, A.M., Weiner, S., 1994. Identifying ancient irrigation: a new method using opaline
549 phytoliths from emmer wheat. *Journal of Archaeological Science* 21, 125-132.

550 Saccone, L., Conley, D.J., Koning, E., Sauer, D., Sommer, M., Kaczorek, D., Blecker, S.W., Kelly,
551 E.F., 2007. Assessing the extraction and quantification of amorphous silica in soils of forest
552 and grassland ecosystems. *European Journal of Soil Science* 58, 1446-1459.

553 Suchéras-Marx, B., Escarguel, G., Ferreira, J., Hammer, Ø., 2019. Statistical confidence intervals
554 for relative abundances and abundance-based ratios: Simple practical solutions for an old
555 overlooked question. *Marine Micropaleontology* 151, 101751.

556 Twiss, P.C., 1992 Predicted world distribution of C3 and C4 grass phytoliths. In: Rapp G Jr,
557 Mulholland SC (eds) *Phytolith systematics: emerging issues*. Plenum Press, New York,
558 London, pp. 13-128.

559 Van Cappellen, P., Dixit, S., van Beusekom, J., 2002. Biogenic silica dissolution in the oceans:
560 Reconciling experimental and field-based dissolution rates. *Global Biogeochemical Cycles* 16,
561 23-21-23-10.

562 White, A.F., Vivit, D.V., Schulz, M.S., Bullen, T.D., Evett, R.R., Aagarwal, J., 2012. Biogenic and
563 pedogenic controls on Si distributions and cycling in grasslands of the Santa Cruz soil
564 chronosequence, California. *Geochimica et Cosmochimica Acta* 94, 72-94.

565 Wilding, L.P., Drees, L.R., 1974. Contributions of Forest Opal and Associated Crystalline Phases
566 to Fine Silt and Clay Fractions of Soils. *Clays and Clay Minerals* 22, 295-306.

567 WoldeGabriel, G., Ambrose, S.H., Barboni, D., Bonnefille, R., Bremond, L., Currie, B., DeGusta,
568 D., Hart, W.K., Murray, A.M., Renne, P.R., Jolly-Saad, M.C., Stewart, K.M., White, T.D.,

569 2009. The geological, isotopic, botanical, invertebrate, and lower vertebrate surroundings of
570 Ardipithecus ramidus. *Science* 326, 61-65.

571 Yost, C.L., Ivory, S.J., Deino, A.L., Rabideaux, N.M., Kingston, J.D., Cohen, A.S., 2021. Phytoliths,
572 pollen, and microcharcoal from the Baringo Basin, Kenya reveal savanna dynamics during the
573 Plio-Pleistocene transition. *Palaeogeography, Palaeoclimatology, Palaeoecology* 570, 109779.

574 Zhang, J., Lu, H., Jia, J., Shen, C., Wang, S., Chu, G., Wang, L., Cui, A., Wu, N., Li, F., 2020.
575 Seasonal drought events in tropical East Asia over the last 60,000 y. *Proceedings of The*
576 National Academy of Sciences, USA 117, 30988-30992.

577 Zhao, Z., Piperno, D.R., 2000. Late Pleistocene/Holocene environments in the Middle Yangtze
578 River Valley, China and rice (*Oryza sativa* L.) domestication: the phytolith evidence. *Geoar-*
579 chaeology: An International Journal 15, 203-222.

580