



**HAL**  
open science

## Termite graveyards. Hidden geochemical patches?

Pascal Jouquet, Angélique Bultelle, Irina Djouraev, Sandrine Caquineau,  
Vincent Hervé, Mireille Vasseur-Cognet

► **To cite this version:**

Pascal Jouquet, Angélique Bultelle, Irina Djouraev, Sandrine Caquineau, Vincent Hervé, et al.. Termite graveyards. Hidden geochemical patches?. Soil Biology and Biochemistry, 2022, 170, pp.108678. 10.1016/j.soilbio.2022.108678 . hal-03701957

**HAL Id: hal-03701957**

**<https://hal.science/hal-03701957>**

Submitted on 22 Jul 2024

**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 4.0 International License

1                   **Termite graveyards. Hidden geochemical patches?**

2

3

4   **Pascal Jouquet<sup>a,\*</sup>, Angélique Bultelle<sup>a</sup>, Irina Djouraev<sup>b</sup>, Sandrine Caquineau<sup>b</sup>, Vincent**  
5   **Hervé<sup>c</sup>, Mireille Vasseur-Cognet<sup>a,d</sup>**

6

7   <sup>a</sup> Sorbonne Université, UPEC, CNRS, IRD, INRA, Institut d'Ecologie et des Sciences de  
8   l'Environnement, iEES-Paris, FEST, 75005 Paris, France

9   <sup>b</sup> LOCEAN (Laboratoire d'Océanographie et du Climat : Expérimentations et approches  
10   numériques), IRD-CNRS-Sorbonne Université-MNHN, IRD France-Nord, 93143 Bondy  
11   Cedex, France

12   <sup>c</sup> Institut de Recherche sur la Biologie de l'Insecte, UMR7261, CNRS-University of Tours,  
13   Parc Grandmont, 37200 Tours, France

14   <sup>d</sup> INSERM, Paris, France

15

16

17   **Corresponding author:** Sorbonne Université, UPEC, CNRS, IRD, INRA, Institut d'Ecologie  
18   et des Sciences de l'Environnement, iEES-Paris, FEST, 75005 Paris, France

19   Email address: *pascal.jouquet@ird.fr* (P. Jouquet)

20 **Highlights**

- 21 • Termite graveyards constitute a new type of soil biogenic structure.  
22  
23 • Both sheeting types are enriched in carbon and calcium in comparison with the refer-  
24 ence soil.  
25  
26 • High calcium content in sheeting is explained by the toxicity of this element at high  
27 concentration.  
28  
29 • Ca is excreted in soil in the form of carbonates in saliva, urine or faeces  
30  
31

32 **Abstract**

33 Entombment, or the production of graveyards for the disposal of dead bodies, is not only a  
34 practice of human societies but is also observed in nature, including among small  
35 invertebrates such as termites. While the influence of termites on soil dynamics has largely  
36 been studied in comparing the specific properties of their mounds and protective sheeting with  
37 those of the surrounding soil, the properties of their graveyards have never been described  
38 before. Using incipient colonies of *Macrotermes natalensis* reared in a controlled  
39 environment, we showed that graveyard sheeting was characterized by a much higher C  
40 content in comparison with the reference soil and protective sheeting (4.7-fold increase). As a  
41 consequence, a slight increase in the C:N ratio was measured from 8 in the reference soil to  
42 10 in graveyard sheeting. No changes in soil particle size fractions were measured. However,  
43 lower Fe and Al contents were measured in sheeting, and micrographs obtained from  
44 scanning electron microscopy revealed the presence of calcium carbonate, or calcium oxalate  
45 crystals, in sheeting, as well as the presence of organic substances and salt crystals covering  
46 termite corpses, most likely for controlling the spread of pathogens. The presence of calcium  
47 carbonates and/or calcium oxalate was explained by the very high Ca content within termite  
48 bodies. Therefore, this study shows that termite graveyards are likely to constitute unexplored  
49 patches of nutrients in soil.

50

51 **Keywords.** Fungus-growing termites, Macrotermitinae, corpses, calcium, carbonates,  
52 sheeting, aggregates

53

54

**55 1. Introduction**

56 At the landscape scale, fungus-growing termites impact the cycling of nutrients while they  
57 forage and consume plant residues when they are preyed upon by other organisms and  
58 through the construction of nest structures made of soil minerals mixed with saliva and  
59 occasionally faeces (King, 2016). While the first two processes are important, the influence of  
60 the last (i.e., the construction of soil-nest structures) remains questionable. Indeed, termites  
61 build two types of soil structures (mounds and sheeting), and both have very different  
62 dynamics and impacts. Although termite mounds might be seen as patches of nutrients  
63 favouring bacterial, plant and animal diversity (Fleming and Loveridge, 2003; Choosai et al.,  
64 2019; Okullo et al., 2013; Baker et al., 2020) and therefore key ecological functions such as  
65 herbivory and hydrology (Chen et al., 2019; Grant and Scholes, 2006; Levick et al., 2010a,b),  
66 these structures are very stable and resistant, leading to a temporary immobilization of  
67 nutrients. Conversely, sheeting made by termites to protect themselves against predators and  
68 desiccation (i.e., Harit et al., 2017) while they forage on the ground or on woody materials is  
69 very fragile and unstable, leading to a rapid redistribution of minerals and nutrients to the  
70 surrounding environment soil. However, their properties are very close to those of the  
71 surrounding soil (Harit et al., 2017; Jouquet et al., 2022), and their impact on the cycling of  
72 nutrients is considered to be limited.

73 While termites have a high nutrient use efficiency (Hungate, 1941; Higashi et al.,  
74 1992; Ohkuma, 2003; Vesala et al., 2019), organic matter and nutrients can also return to the  
75 system via the mortality of individuals. Indeed, like many other social insects, termites  
76 segregate sick or dead individuals from the colony through different behaviours, including  
77 necrophagy (i.e., cannibalism), necrophoresis (i.e., the removal of dead bodies from the nest)  
78 and entombment (i.e., the burial of corpses or ‘mummification’), for hygienic purposes  
79 (Pearce, 1987; Sun et al., 2013, 2018). This “way to deal with the dead” (*sensu* Sun et al.,

80 2013) can therefore lead to the formation of specific waste chambers or “termite graveyards”  
81 (Chouvenc et al., 2012; Sun et al., 2013). These disposals host specific microbial populations  
82 that can constitute a risk of pathogenicity outbreak, which are avoided by grooming the dead  
83 and/or burying them. If this behaviour was described, the importance of this specific  
84 concentration of dead individuals, carcasses and faeces has never been considered from a soil  
85 and nutrient point of view, although it is likely that the return of these nutrients to the soil  
86 system might be important for plants (Noble et al., 1989).

87 As already mentioned, the impact of termite corpse management on soil properties  
88 remains unknown, most likely because of the difficulty in sampling such areas in the field.  
89 Colonies of *Macrotermes natalensis* established and reared in the laboratory since 2016 (Séité  
90 et al., 2022) show that termites build different soil sheeting on the ground. Some are used to  
91 cover the litter they consume (the soil or protective sheeting *sensu stricto*) while others cover  
92 dead individuals (hereafter called ‘graveyard’ sheeting). In this study, these two soil types  
93 were sampled and their physical and chemical properties were compared to those of the soil  
94 given to termites for building their nests. We hypothesized that protective sheeting had very  
95 similar properties to the parent soil, while graveyard sheeting was enriched in organic matter  
96 and nutrients.

97

## 98 2. Materials and Methods

### 99 2.1. Soil material

100 Protective and graveyard sheeting materials were sampled from three different incipient  
101 colonies of *M. natalensis* in mesocosms (60 x 80 cm) located in a breeding room with  
102 controlled conditions (28°C, 85% relative humidity, and 12:12 photoperiod) described in  
103 Séité et al. (2022) (Figure 1). Colonies were 4 years old and developed in a soil collected in  
104 Affieux, France. Colonies were fed twice a week with small branches of birch and wild oat

105 collected in private spaces in the Ile-de-France and Occitanie regions, respectively. Both soil-  
106 sheeting types were carefully collected on the soil surface with a spatula and forceps to avoid  
107 the presence of plant material and dead bodies (n = 2-3 per colony, ~20-30 g in total). Their  
108 physical and chemical properties were compared to those of the parental soil (reference) given  
109 to termites stored in dry conditions in the laboratory.

110

## 111 *2.2. Physical and chemical analyses*

112 Physical and chemical properties were measured from soil samples previously air-dried  
113 during four days (n = 3 for each soil category). Soil aggregates were observed with a scanning  
114 electron microscope (SEM) (Zeiss EVO LS15) at 15 kV on carbon coated samples.  
115 Identification of the particles constituting these aggregates was achieved from their elemental  
116 spectrum obtained with an energy dispersive X-ray microprobe (EDX, Oxford Instruments,  
117 INCA Energy 350) coupled to the microscope. Concentrations in organic carbon (C<sub>org</sub>) and  
118 carbonates (C<sub>CaCO<sub>3</sub></sub>) were measured with an elemental analyser (Thermo Flash HT, Thermo  
119 Fisher Scientific Inc., Waltham, USA) before and after carbonates removal using diluted HCl  
120 at 2%. The presence of carbonates was also visually estimated after the application of HCl  
121 drops on soil samples (FAO, 2006). Soils were sieved in water after soil organic matter  
122 (SOM) destruction with H<sub>2</sub>O<sub>2</sub>. Complete dispersion was achieved with Na-  
123 hexametaphosphate (20 g L<sup>-1</sup>) and ultrasonication during 30 min. Three soil particle size  
124 classes were considered: clay (<2 µm), silt (2–50 µm) and sand (50–2000 µm). Soil pH and  
125 electrical conductivity (EC) were determined in soil/water suspension (soil:solution = 1:5).

126 Concentrations of Na, Mg, Ca, P, K, Al, Ti and Fe in soil samples (reference,  
127 protective and graveyard sheeting) and in the biomass of termite workers (composite samples  
128 from 20 to 25 minor and major workers, with n = 3 per colony), wood and wild oat were  
129 analyzed by inductively coupled plasma mass spectrometry (ICP–MS) (Agilent 7500) after

130 hot-plate acid digestion in polytetrafluoroethylene (PTFE) vessels. The acids employed (HF,  
131 40%, HNO<sub>3</sub>, 65%, and HClO<sub>4</sub>, 70%) permitted to eliminate OM and mineralize silicates.

132

### 133 *2.3. Statistical analyses*

134 One-way analysis of variance (ANOVA) and least significant difference (LSD) tests were  
135 performed to assess differences between means. Prior to the ANOVAs, data were log-  
136 transformed (when required) to achieve homogeneity of variances and normality, which were  
137 confirmed using Levene and Shapiro-Wilk tests. Pairwise comparisons were made with  
138 Kruskal-Wallis tests with a false discovery rate correction when ANOVA assumptions were  
139 not met. Principal component analysis (PCA) and Monte Carlo permutation tests (999  
140 permutations) were also carried out from soil physical and chemical properties (i.e., electrical  
141 conductivity, organic C content, particle size distribution and % of soil > 100 μm).  
142 Differences among treatments were declared significant at the < 0.05 probability level. All  
143 statistical calculations were carried out using R version 3.5.1 (<https://www.r-project.org/>) and  
144 using “ade4”, “car”, “ggplot2” and “factoextra” packages.

145

## 146 **3. Results**

### 147 *3.1. Soil surface feature changes observed by SEM micrographs*

148 SEM micrographs of the soil surface features are shown in **Figs. 2–4**. Reference soil  
149 aggregates were characterized by a granular surface with a porosity sealed by the presence of  
150 clay minerals displaying a chemical composition of kaolinite, smectite, mica, and Na-feldspar  
151 and quartz (see appendix 1) (**Figs. 2 A-D**). Small filaments, possibly hyphae, and large roots  
152 were also observed. The surface of protective sheeting was smoother than that of the reference  
153 soil (**Figs. 3 A-B**). The soil surface was also sealed by clay minerals associated with quartz  
154 and small calcium carbonate or calcium oxalate concretions (**Figs. 3 C-D**). Graveyard

155 sheeting was made of soil particles and organic substances covering termite corpses (Figs. 4  
156 A-B). In addition to clay mineral particles, very specific spherical elements were observed  
157 with chemical signatures suggesting the presence of plant debris, fungal spores and/or pollen  
158 (Fig. 4 C) and salt crystals (Fig. 4 D) (see appendix 2).

159

### 160 *3.2. Major physical and chemical changes between soil types*

161 Protective and graveyard sheeting had very different properties than the reference soil (Figure  
162 5). The main differences between soil types were mostly related to chemical changes. Indeed,  
163 no significant differences in soil particle size fractions could be measured between the  
164 reference soil and the two soil-sheeting types ( $P > 0.05$  for all the size fractions, Table 1).  
165 Conversely, the two soil-sheeting types were characterized by a much higher electrical  
166 conductivity (almost 5-fold) in comparison with the reference soil. Significant differences in  
167 Corg content were also measured between graveyard sheeting and both the reference soil and  
168 the protective sheeting ( $P > 0.05$  between both). The soil pH was also significantly higher in  
169 graveyard sheeting than in the reference soil, and protective sheeting had intermediate pH  
170 values that were not significantly different from those of the two other soil types. Graveyard  
171 sheeting was also enriched in N in comparison with reference and protective sheeting ( $P >$   
172  $0.05$  between reference and protective sheeting). Finally, although no reaction occurred  
173 following the application of HCl drops to the soil samples, a higher  $C_{CaCO_3}$  content (~5.5-fold)  
174 was measured in both sheeting types than in the reference soil.

175

### 176 *3.3. ICP-MS analysis*

177 The total amounts of Na, Mg, Ca, P, K, Al, Ti and Fe were analysed by ICP-MS (Figure 6).  
178 Concerning the soil material, the reference soil was characterized by higher concentrations of  
179 Na, Mg, Al, and K but significantly lower concentrations of Ca than the two soil-sheeting

180 types ( $P < 0.05$  in all cases). The two sheeting types had similar properties, with the exception  
181 of the P content, which was significantly higher in the reference soil and in the graveyard  
182 sheeting ( $P > 0.05$  between both) than in the protective sheeting. Soil materials were  
183 characterized by higher amounts of Na, Mg, K, Al, Ti and Fe than termite and plant materials.  
184 Although the concentration of Ca was low in termite biomass and straw ( $P > 0.05$  between  
185 both), the amount of Ca was significantly higher in wood than in the reference soil ( $P < 0.05$ )  
186 and as high as that in the protective sheeting ( $P > 0.05$ ). Moreover, the amount of P was  
187 higher in the termite body than in the other soil and biomass materials.

188

## 189 **4. Discussion**

### 190 *4.1. Incorporation of organic matter within soil and graveyard sheeting*

191 If termites have a significant impact on soil structure when they build their mound nests (Holt  
192 & Lepage, 2000; Abe et al., 2011; Jouquet et al., 2011), their impact is more limited with soil  
193 sheeting (Jouquet et al., 2022) and totally unknown with graveyard sheeting. A recent meta-  
194 analysis showed that soil sheeting built by fungus-growing termites has very similar  
195 properties to the surrounding soil (Harit et al., 2017; Jouquet et al., 2022), although it is  
196 almost always slightly enriched in clay particles and enriched in C when the soil they use  
197 contains less than 2% C. In our study, no difference in soil particle size fractions could be  
198 observed between soil types, and only graveyard sheeting was enriched in  $C_{org}$  in comparison  
199 with the reference soil.

200 Termites are known to use two main strategies for building soil sheeting (Harit et al.,  
201 2017; Jouquet et al., 2022): they either select clay particles and/or incorporate organic matter  
202 in the form of saliva. Our findings suggest that workers of *Macrotermes natalensis* use the  
203 second strategy for building protective sheeting, perhaps because they prefer using clay for  
204 the construction of their permanent nest structures that host the colony members (see

205 [appendix 3](#)). In their synthesis, [Jouquet et al. \(2022\)](#) suggested that the enrichment in C in soil  
206 protective sheeting occurs only when the topsoil contains less than 2% C. Above this  
207 threshold, soil sheeting is expected to have a lower C content than the bulk soil. These results  
208 were not confirmed in our study which evidenced similar C<sub>org</sub> contents between protective  
209 sheeting and the reference soil despite the high C content in the reference soil (i.e. > 2 %).  
210 This apparent contradictory result can, however, easily be explained by the specific breeding  
211 conditions of colonies. In nature, soil sheeting can be made from soil sampled at different soil  
212 depths, including from the deeper soil layers (e.g., the B horizon or deeper) where the soil is  
213 impoverished in C. In our study, termites were grown in homogeneous soil media without  
214 specific soil layers as those observed in the field. Since termites did not modify the particle  
215 size distribution, the soil they used was likely to reflect the properties of the bulk soil, with the  
216 exception of roots and small filaments that were observed in the reference soil but not  
217 sampled by termites for building soil sheeting.

218 Graveyard sheeting contained almost 5 times more C than protective sheeting and the  
219 reference soil. As explained previously, this increase in C was, however, probably  
220 underestimated due to the experimental conditions of this study. In nature, termites build soil  
221 sheeting from soil that has been collected at several cm depths, which is often depleted in C in  
222 comparison to the surface, whereas such a gradient did not exist in our experiment. Therefore,  
223 we assume that this 5-fold increase was likely to be underestimated, and the consequences of  
224 such an addition of organic matter on C-depleted soil remain to be defined. The production of  
225 graveyard sheeting is explained by the need for termites to avoid the spread of opportunistic  
226 pathogens within the colony ([Chouvenc et al., 2012](#); [Sun et al., 2018](#)). The behaviour  
227 associated with the removal and burying of dead bodies has been described earlier (e.g.,  
228 [Chouvenc et al., 2012](#); [Neoh et al., 2012](#); [Sun et al., 2018](#)), with corpses first moved away by  
229 workers and then covered by saliva and buried with soil particles coated by saliva. In our

230 study, corpses were associated with soil particles and dark substances considered to be faeces.  
231 As evidence with other termite species, the secretion of saliva and excretion of feces were  
232 likely to play the role of antimicrobial compounds, thus protecting the colony from the spread  
233 of pathogenic fungi (Pearce, 1987; Chouvenec and Su, 2010). Indeed, fungus-growing termites  
234 (guts and cuticles) as well as fungus combs are known to harbour diverse *Actinobacteria*  
235 with antimicrobial activities (Visser et al., 2012; Murphy et al., 2021). Therefore, a third  
236 strategy should be added to the two already proposed by Harit et al. (2017) (i.e., the  
237 enrichment in clay or C to ensure the stability of soil sheeting): the incorporation of organic  
238 matter, mostly faeces, to protect the colony from the spread of pathogens (Cole et al., 2021).

239

#### 240 *4.2. Geochemical changes associated with soil and graveyard sheeting*

241 The important increases in organic matter were associated with a slight increase in the C:N  
242 ratio (8.4, 9.8 and 10.0, for the reference, protective and graveyard sheeting, respectively).  
243 This increase in organic matter was associated with an enhancement in soil electrical  
244 conductivity in protective and graveyard sheeting in comparison with the reference soil. These  
245 changes were also associated with increases in soil pH, although they were nonsignificant in  
246 the case of protective sheeting. This increase could be attributed to the incorporation of  
247 organic matter within the soil sheeting but also to the presence of calcium carbonate granules  
248 and/or calcium oxalate crystals that were observed in the soil and graveyard sheeting. The  
249 presence of calcium carbonates observed with the SEM was confirmed by the measure of  
250 higher  $C_{CaCO_3}$  contents in both sheeting types, but not with the HCl test. The accumulation of  
251 carbonates has been evidenced in termite constructions (e.g., Watson, 1974; McComie and  
252 Dhanarajan, 1993; Hesse, 1955; Liu et al., 2007; Sako et al., 2009; Muon et al., 2022) and is  
253 usually considered to have a pedogenic origin (Mujinya et al., 2011). However, since calcium  
254 carbonates were almost absent from the reference soil (i.e., < 1%) and because a high content

255 of Ca was measured in wood and sheeting, our results suggest biogenic incorporation of Ca  
256 by termites. Indeed, insects are able to regulate their internal concentrations of metals  
257 (Stewart et al., 2011). While calcium plays a key role in insect physiology as a second  
258 messenger in cells and underpins the operation of the nervous system and musculature (Dowe,  
259 2017), it is toxic at high concentrations and needs to be eliminated (Clark, 1958; Ntukuyoh et  
260 al., 2012; Hervé et al., 2016; Suryavanshi et al., 2016). Therefore, regarding the higher  
261 concentrations in  $C_{CaCO_3}$  in sheeting than in the reference soil, we consider that excess Ca  
262 ingested by termites during the consumption of wood was eliminated via the production of  
263 sheeting. Because Ca mostly accumulates within Malpighian tubules (Stewart et al., 2011), its  
264 elimination is likely to occur through the secretion of urine. However, this hypothesis would  
265 not support the general assumption that fungus-growing termites only incorporate saliva into  
266 their nests and protective sheeting (Holt and Lepage, 2000; Jouquet et al., 2011). Therefore,  
267 more research is needed to identify the mechanisms by which termites enrich their  
268 constructions in calcium, particularly if it occurs via the incorporation of urine. In insects,  
269 calcium can accumulate in the form of spherites, concretions or calcium oxalate crystals in the  
270 lumen of the Malpighian tubules (Clarck, 1958; Dowe, 2017; Reynolds et al., 2021),  
271 potentially explaining the presence of small calcium carbonate or calcium oxalate crystals in  
272 both sheeting types. The formation of carbonates from calcium oxalate requires a pH of 8.4,  
273 which was not reached in soil and graveyard sheeting. Therefore, further research is needed to  
274 determine if the oxidation of calcium oxalate occurred in the termite body; for instance in the  
275 Malpighian tubules or in the paunch of termites where the pH can reach 9.5 (Anklin-  
276 Mühlemann et al., 1995), or if it is a process occurring at a very small scale within soil pellets  
277 (e.g., Kim and Or, 2019).

278 In addition to impacting the cycling of nutrients and the dynamics of C in soil, our  
279 study also showed an effect of termites on the cycling of P. As evidenced with termite

280 mounds, the amount and availability of P in fungus-growing termite constructions are highly  
281 variable (e.g., [Lopez-Hernandez et al., 2006](#); [Tilahun et al., 2012](#)). In our study, an  
282 impoverishment in P was measured in protective sheeting in comparison with the reference  
283 soil and the graveyard sheeting. Although our study does not allow us to determine the  
284 mechanisms associated with this reduction in P in soil, it is likely that it reflects the need for  
285 termites to conserve this essential element (e.g., for the synthesis of nucleotides and  
286 phospholipids), as shown by the high concentration of P found in termite biomass.

287 This study also showed the presence of fungal spores and plant residues, which could  
288 be attributed to grains of pollens and/or phytoliths or a mixture of undecomposed organic  
289 debris associated with clay minerals in termite sheeting. While we cannot reject the  
290 hypothesis that this material was brought attached to the corpses, we consider that it is more  
291 likely that it corresponds to undigested plant material ejected by termites within faeces and/or  
292 saliva. This hypothesis is in line with the presence of phytoliths and Si available found in  
293 termite mound soils ([Jouquet et al., 2020](#)), which are only made of small aggregates glued  
294 together with saliva. While silicon is not included in the list of elements essential for plants,  
295 there is growing evidence that it has beneficial effects on the resistance of crops to  
296 environmental stresses ([Datnoff et al., 2001](#); [Liang et al., 2015](#); [Tombeur et al., 2021](#)).  
297 Therefore, together with the incorporation of organic matter and nutrients, the presence of Si-  
298 organic elements in graveyard sheeting is likely to locally constitute an attractive resource to  
299 plants.

300

#### 301 *4.3. Conclusion*

302 Without considering predation, the death of termite workers and soldiers is a frequent event in  
303 a colony, especially because they have a short lifespan (< 1 year, [Jemielity et al., 2005](#)) and  
304 because they have risky activities and are subjected to diseases ([Sun et al., 2018](#)). However,

305 the influence of the burial of corpses on the cycling of nutrients remains unknown. One of the  
306 most striking results of this study is that graveyard sheeting is enriched in organic matter and  
307 nutrients (without including termite corpses), as well as undigested plant material. In  
308 particular, the presence of Ca and/or Si within graveyard sheeting is also likely to positively  
309 influence soil fertility and the cycling and protection of C in soil. More generally, this study  
310 revealed the potential importance of the termite necrobiome (Benbow et al, 2019) for soil  
311 properties. It also highlighted a new facet of the role of termites in ecosystem functioning: in  
312 addition to being among the main terrestrial decomposers, their own decomposition in  
313 dedicated areas appeared to be a significant process for soil biogeochemistry.

314 Obviously, the fact that samples came from colonies reared in captivity under  
315 controlled conditions requires confirmation of our findings in the field. Indeed, social insects  
316 often show plastic responses for the management of corpses (Sun et al., 2018). Therefore, the  
317 fact that the specific rearing conditions constitute an artefact cannot be rejected and it is  
318 possible that necrophoresis is preferred over entombment in open areas because it is less  
319 costly in terms of labour force and energy (Sun et al., 2018). Additionally, the influence of  
320 termite species, feeding habits and nesting ecology of termites would need to be considered as  
321 variable when studying corpse management (Neoh et al, 2012). Therefore, further studies are  
322 needed to determine whether this study displays a hidden process occurring in the field and, if  
323 so, its importance to soil biogeochemical cycling.

324

#### 325 **Author contributions**

326 PJ and AB planned and designed the experiment. PJ, AB, ID and SC carried out the research  
327 and data analyses. PJ, SC, VH and MVC wrote the manuscript.

328

#### 329 **Declaration of competing interest**

330 The authors declare that they have no known competing financial interests or personal  
331 relationships that could have appeared to influence the work reported in this paper.

332

### 333 **Acknowledgments**

334 This project was supported by the French National Institute for Research for Development  
335 (IRD). Soil analyses and SEM observations were performed on the ALYSES facility (IRD-  
336 UPMC) which was supported by grants from the Région Ile de France. M. V-C was supported  
337 by the International Human Frontier Science Program RGP0060/2018. We are particularly  
338 grateful to Corinne Rouland, David Sillam-Dussès, Alain Robert and Roland Lupoli for the  
339 establishment of the incipient colonies, and to Garance Néollier, Hanane Aroui, Lotfi Smaili,  
340 Thierry Henry-des-Tureaux, Féthiyé Cetin and Magloire Mandeng-Yogo, for their technical  
341 supports in the laboratory.

342 **References**

- 343 Abe, S.S., Watanabe, Y., Onishi, T., Kotegawa, T., Wakatsuri, T., 2011. Nutrient storage in  
344 termite (*Macrotermes bellicosus*) mounds and the implications for nutrient dynamics in a  
345 tropical savanna Ultisol. *Soil Science and Plant Nutrition* 57, 786-795.
- 346 Anklin-Mühlemann, R., Bignell, D.E., Veivers, P.C., Leuthold, R.H., Slaytor, M., 1995. Mor-  
347 phological, microbiological and biochemical studies of the gut flora in the fungus-growing  
348 termite *Macrotermes subhyalinus*. *Journal of Insect Physiology* 41, 929-940.
- 349 Baker, C.C., Castillo Vardaro, J.A., Doak, D.F., Pansu, J., Puissant, J., Pringle, R.M., Tarnita,  
350 C.E., 2020. Spatial patterning of soil microbial communities created by fungus-farming  
351 termites. *Molecular Ecology* 29, 4487-4501.
- 352 Benbow, M.E., Barton, P.S., Ulyshen, M.D., Beasley, J.C., DeVault, T.L., Strickland, M.S.,  
353 Tomberlin, J.K., Jordan, H.R., Pechal, J.L., 2019. Necrobiome framework for bridging de-  
354 composition ecology of autotrophically and heterotrophically derived organic matter. *Eco-  
355 logical Monographs* 89, e01331.
- 356 Chen, C., Wu, J., Zhu, X., Jiang, X., Liu, W., Zeng, H., Meng, F.R., 2019. Hydrological  
357 characteristics and functions of termite mounds in areas with clear dry and rainy seasons.  
358 *Agriculture, Ecosystems & Environment* 277, 25-35.
- 359 Choosai, C., Mathieu, J., Hanboonsong, Y., Jouquet, P., 2009. Termite mounds and dykes are  
360 biodiversity refuges in paddy fields in north-eastern Thailand. *Environmental Conservation*  
361 36, 71-79.
- 362 Chouvinc, T., Robert, A., Semon, E., Bordereau, C., 2012. Burial behaviour by dealates of  
363 the termite *Pseudacanthotermes spiniger* (Termitidae, Macrotermitinae) induced by  
364 chemical signals from termite corpses. *Insectes Sociaux* 59, 119-125.

- 365 Chouvenc, T., Su, N-Y., 2010. Apparent synergy among defense mechanisms in subterranean  
366 termites (Rhinotermitidae) against epizootic events: Limits and potential for biological  
367 control. *Journal of Economic Entomology* 103, 1327-1337.
- 368 Cole, M. E., Ceja-Navarro, J. A., Mikaelyan, A., 2021. The power of poop: Defecation  
369 behaviors and social hygiene in insects. *PLoS pathogens*, 17(10), e1009964.
- 370 Datnoff, L. E., Seebold, K.W., Correa-V, F.J., 2001. The use of silicon for integrated disease  
371 management: reducing fungicide applications and enhancing host plant resistance. *Studies*  
372 *in Plant Science* 8, 171-184.
- 373 Dow, J.A.T., 2017. The essential roles of metal ions in insect homeostasis and physiology.  
374 *Current Opinion in Insect Science* 23, 43-50.
- 375 FAO IUSS Working Group WRB, 2006. World reference base for soil resources 2006. World  
376 Soil Resources Reports No. 103. FAO, Rome, 145 pp.
- 377 Fleming, P.A., Loveridge, J.P., 2003. Miombo woodland termite mounds: resource islands for  
378 small vertebrates? *Journal of Zoology* 259, 161-168.
- 379 Grant, C.C., Scholes, M.C., 2006. The importance of nutrient hot-spots in the conservation  
380 and management of large wild mammalian herbivores in semi-arid savannas. *Biological*  
381 *Conservation* 130, 426-437.
- 382 Harit, A., Shanbhag, R., Chaudhary, E., Cheik, S., Jouquet, P., 2017. Properties and  
383 functional impact of termite sheetings. *Biology and Fertility of Soils* 53, 743-749.
- 384 Hervé, V., Junier, T., Bindschedler, S., Verrecchia, E., Junier, P., 2016. Diversity and ecology  
385 of oxalotrophic bacteria. *World Journal of Microbiology and Biotechnology* 32, 28.
- 386 Hesse, P.R., 1955. A Chemical and Physical Study of the Soils of Termite Mounds in East  
387 Africa. *Journal of Ecology* 43, 449-461.
- 388 Higashi, M., Abe, T., Burns, T.P., 1992. Carbon-nitrogen balance and termite ecology.  
389 *Proceedings of the Royal Society of London. Series B: Biological Sciences* 249, 303-308.

- 390 Holt, J.A., Lepage, M., 2000. Termites and soil properties. *Termites: evolution, sociality,*  
391 *symbioses, ecology*, Springer: 389-407.
- 392 Hungate, R.E., 1941. Experiments on the nitrogen economy of termites. *Annals of the*  
393 *Entomological Society of America* 34, 467-489.
- 394 Jemielity, S, Chapuisat, M, Parker, J, Keller, L., 2005. Long live the queen: studying aging in  
395 social insects. *Age* 27, 241–248.
- 396 Jouquet, P., Traoré, S., Choosai, C., Hartmann, C., Bignell, D., 2011. Influence of termites on  
397 ecosystem functioning. *Ecosystem services provided by termites. European Journal of Soil*  
398 *Biology* 47, 215-222.
- 399 Jouquet, P., Jamoteau, F., Majumdar, S., Podwojewski, P., Nagabovanalli, P., Caner, L.,  
400 Barboni, D., Meunier, J-D., 2020. The distribution of Silicon in soil is influenced by  
401 termite bioturbation in South Indian forest soils. *Geoderma* 372, 114362.
- 402 Jouquet, P., Harit, A., Hervé, V., Moger, H., Carrijo, T., et al., 2022. The impact of termites  
403 on soil sheeting properties is better explained by their feeding and building strategies.  
404 *Geoderma* 412, 115706.
- 405 Kim, M., Or, D., 2019. Microscale pH variations during drying of soils and desert biocrusts  
406 affect HONO and NH<sub>3</sub> emissions. *Nature Communications* 10, 3944.
- 407 King, J.R., 2016. Where do eusocial insects fit into soil food webs? *Soil Biology and*  
408 *Biochemistry* 102, 55-62.
- 409 Levick, S.R., Asner, G.P., Chadwick, O.A., Khomo, L.M., Rogers, K.H., Hartshorn, A.S., et  
410 al. 2010a. Regional insight into savanna hydrogeomorphology from termite mounds.  
411 *Nature Communications* 1, 1-7.
- 412 Levick, S.R., Asner, G.P., Kennedy-Bowdoin, T., Knapp, D.E. 2010b. The spatial extent of  
413 termite influences on herbivore browsing in an African savanna. *Biological conservation*  
414 143, 2462-2467.

- 415 Liang, Y., Nikolic, M., Bélanger, R., Gong, H., Song, A., 2015. Silicon in agriculture.  
416 Dordrecht: Springer. doi 10: 978-994.
- 417 Liu, X., Monger, H.C., Whitford, W.G., 2007. Calcium carbonate in termite galleries –  
418 biomineralization or upward transport? *Biogeochemistry* 82, 241-250.
- 419 Lopez-Hernandez, D., Brossard, M., Fardeau, J.-C., Lepage, M., 2006. Effect of different  
420 termite feeding groups on P sorption and P availability in African and South American  
421 savannas. *Biology and Fertility of Soils* 42, 207-214.
- 422 McComie, L.D., Dhanarajan, G., 1993. The physical and chemical composition of mounds of  
423 *Macrotermes carbonarius* (Hagen) (Termitidae, Macrotermitinae), in Penang, Malaysia.  
424 *Journal of Soil Science* 44, 427-433.
- 425 Mujinya, B.B., Mees, F., Boeckx, P., Bodé, S., Baert, G., Erens, H., Delefortrie, S., Verdoodt,  
426 A., Ngongo, M., Van Ranst, E., 2011. The origin of carbonates in termite mounds of the  
427 Lubumbashi area, D.R. Congo. *Geoderma* 165, 95-105.
- 428 Muon, R., Ket, P., Sebag, D., Aroui, H., Podwojewski, P., Hervé, V., Ann, V., Jouquet, P.,  
429 2022. The importance of bioturbation by termites in Cambodian paddy fields varies with  
430 the amount of sand in soil. *Applied Soil Ecology*, accepted.
- 431 Murphy, R., Benndorf, R., de Beer, Z. W., Vollmers, J., Kaster, A. K., Beemelmans, C.,  
432 Poulsen, M., 2021. Comparative Genomics Reveals Prophylactic and Catabolic  
433 Capabilities of Actinobacteria within the Fungus-Farming Termite Symbiosis. *Msphere* 6,  
434 e01233-20.
- 435 Neoh, K.B., Yeap, B.K., Tsunoda, K., Yoshimura, T., Lee, C.Y., 2012. Do termites avoid  
436 carcasses? Behavioral responses depend on the nature of the carcasses. *PLoS One* 7,  
437 e36375.

- 438 Noble, J.C., Diggle, P.J., Whitford, W.G., 1989. The spatial distributions of termite  
439 pavements and hummock feeding sites in a semi-arid woodland in eastern Australia. *Acta*  
440 *Oecologica / Oecologia Generalis*, 355-376.
- 441 Ntukuyoh, A.I., Udiong, D.S., Ikpe, E., Akpakpan, A.E., 2012. Evaluation of nutritional value  
442 of termites (*Macrotermes bellicosus*): soldiers, workers, and queen in the Niger Delta  
443 region of Nigeria. *International Journal of Food Nutrition and Safety* 1, 60-65.
- 444 Ohkuma, M., 2003. Termite symbiotic systems: efficient bio-recycling of lignocellulose.  
445 *Applied Microbiology and Biotechnology* 61, 1-9.
- 446 Okullo, P., Greve, P.M., Moe, S.R., 2013. Termites, large herbivores, and herbaceous plant  
447 dominance structure small mammal communities in savannahs. *Ecosystems* 16, 1002-  
448 1012.
- 449 Pearce, M.J., 1987. Seals, tombs, mummies, and tunnelling in the drywood termite  
450 *Cryptotermes* (Isoptera, Kalotermitidae). *Sociobiology* 13, 217-226.
- 451 Reynolds, C.J., Turin, D.R., Romero, M.F., 2021. Transporters and tubule crystals in the  
452 insect Malpighian tubule. *Current opinion in insect science* 47, 82-89.
- 453 Sako, A., Mills, A.J., Roychoudhury, A.N., 2009. Rare earth and trace element geochemistry  
454 of termite mounds in central and northeastern Namibia: Mechanisms for micro-nutrient  
455 accumulation. *Geoderma* 153, 217-230.
- 456 Séité, S., Harrison, M.C., Sillam-Dussès, D., et al., 2022. Lifespan prolonging mechanisms  
457 and insulin upregulation without fat accumulation in long-lived reproductives of a higher  
458 termite. *Communications Biology* 5, 44.
- 459 Stewart, A.D., Anand, R.R., Laird, J.S., Verrall, M., Ryan, C.G., de Jonge, M.D., ... &  
460 Howard, D.L., 2011. Distribution of metals in the termite *Tumulitermes tumuli* (Froggatt):  
461 two types of Malpighian tubule concretion host Zn and Ca mutually exclusively. *PLoS One*  
462 6, e27578.

- 463 Sun, Q., Haynes, K.F., Zhou, X., 2013. Differential undertaking response of a lower termite to  
464 congeneric and conspecific corpses. *Scientific Reports* 3, 1650.
- 465 Sun, Q., Haynes, K.F., Zhou, X., 2018. Managing the risks and rewards of death in eusocial  
466 insects. *Philosophical Transactions of the Royal Society B: Biological Sciences* 373,  
467 20170258.
- 468 Suryavanshi, M.V., Bhute, S.S., Bharti, N., Pawar, K., Shouche, Y.S., 2016. Eubacterial  
469 diversity and oxalate metabolizing bacterial species (OMBS) reflect oxalate metabolism  
470 potential in *Odontotermes* gut. *Journal of Pure and Applied Microbiology* 10, 2035-2044.
- 471 Tilahun, A., Kebede, F., Yamoah, C., Erens, H., Mujinya, B.B., Verdoodt, A., Van Ranst, E.,  
472 2012. Quantifying the masses of *Macrotermes subhyalinus* mounds and evaluating their  
473 use as a soil amendment. *Agriculture Ecosystems & Environment* 157, 54-59.
- 474 Tombeur, F., Roux, P., Cornelis, J.T., 2021. Silicon dynamics through the lens of soil-plant-  
475 animal interactions: perspectives for agricultural practices. *Plant and Soil* 467, 1-28.
- 476 Vesala, R., Harjuntausta, A., Hakkarainen, A., Rönholm, P., Pellikka, P., Rikkinen, J., 2019.  
477 Termite mound architecture regulates nest temperature and correlates with species  
478 identities of symbiotic fungi. *PeerJ* 6, e6237.
- 479 Visser, A.A., Nobre, T., Currie, C.R., Aanen, D.K., Poulsen, M., 2012. Exploring the  
480 potential for actinobacteria as defensive symbionts in fungus-growing termites. *Microbial*  
481 *ecology* 63, 975-985
- 482 Watson, J., 1974. Calcium carbonate in termite mounds. *Nature* 247, 74-74.

483 **Table 1.** Soil physical and chemical analyses of the reference soil, protective sheeting and soil used for burying termite corpses (graveyards).

484 Values in parenthesis are standard errors (n = 3). For each variable, values with similar letters are significantly similar at  $P \geq 0.05$ .

485

	pH	EC ( $\mu\text{S cm}^{-1}$ )	C <sub>org</sub> (g kg <sup>-1</sup> )	C <sub>CaCO<sub>3</sub></sub> (g kg <sup>-1</sup> )	N (g kg <sup>-1</sup> )	Clay (%)	Silt (%)	Sand (%)
Reference	6.2 <sup>b</sup> (0.11)	98.9 <sup>b</sup> (26.04)	31.36 <sup>b</sup> (8.31)	3.77 <sup>b</sup> (4.67)	3.73 <sup>b</sup> (0.09)	24.30 (3.07)	27.40 (2.90)	52.72 (6.02)
Protective	6.7 <sup>ab</sup> (0.16)	546.7 <sup>a</sup> (125.85)	56.37 <sup>b</sup> (16.86)	19.53 <sup>a</sup> (6.18)	5.73 <sup>b</sup> (1.15)	30.46 (1.45)	22.77 (0.98)	46.76 (0.77)
Graveyard	7.2 <sup>a</sup> (0.22)	568.3 <sup>a</sup> (153.98)	148.16 <sup>a</sup> (34.32)	23.63 <sup>a</sup> (8.42)	14.83 <sup>a</sup> (3.54)	23.58 (8.21)	31.30 (1.59)	45.13 (6.71)
<i>F</i> <sub>2,6</sub>	7.52	5.24	17.84	9.81	7.58	0.54	4.61	0.59
<i>P</i> -values	0.023	0.048	0.003	0.013	0.023	0.606	0.061	0.586

**Fig. 1.** Illustration of a *Macrotermes natalensis* incipient colony raised for 4 years in the laboratory and in the second plan general view of termite colonies (1a), the protective sheeting used to forage and protect termites while they feed on wood debris and wild oat (1b) and graveyard sheeting covering termite corpses (c) (1c and 1d). Note that graveyard sheeting is made of soil (m) and dark organic substances (f).

**Fig. 2.** SEM micrographs of the reference soil aggregates made of quartz grains, mica, filaments (f) and roots (r) associated with clay minerals (cl).

**Fig. 3.** SEM micrographs of soil sheeting (internal face) made of quartz grains (q) embedded in a matrix of clay particles (cl) and calcium carbonate granules (ca).

**Fig. 4.** SEM micrographs of graveyards sheeting (internal face) made of quartz grains embedded in a matrix of clay particles (cl) and organic substances (org). Bodies of termite workers (term) are present, as well as organic substances with the signature of pollen (pol) and salt crystals (cryst).

**Fig. 5.** Biplot showing the principal component analysis (PCA) from variables describing the soil physical and chemical properties of the termite protective and graveyard sheeting and the reference soil. Variables are  $C_{org}$ ,  $C_{CaCO_3}$  and N contents, the clay, silt and sand fractions, electrical conductivity (EC) and pH.

**Fig. 6.** Total Na, Mg, Ca, P, K, Al, Ti and Fe contents in the termite body, plants (straw or wood) and in the reference soil, protective sheeting and graveyard sheeting measured by ICP-MS.

**Appendix 1 and 2.** Examples of energy dispersive X-ray spectra results showing the presence of smectite-type clay, mica, feldspar, calcium carbonates and organic matter containing important proportions of Si, Al, Mg and Ca.

**Appendix 3.** Illustration showing the internal structure of a termite nest chamber from a dead colony of *M. natalensis*. Note the specific aspect of the chamber wall containing a high proportion of small particles (photo by P. Jouquet, 2021).

Figure 1

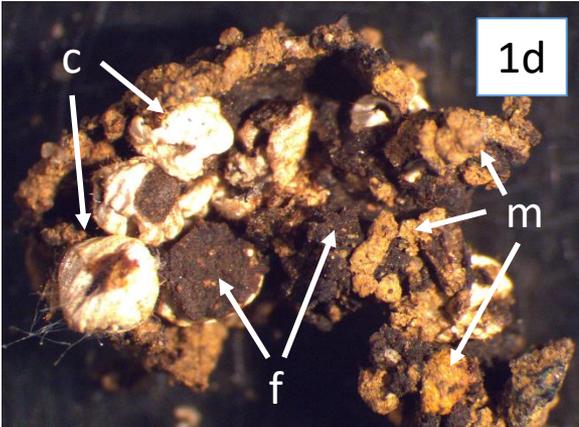
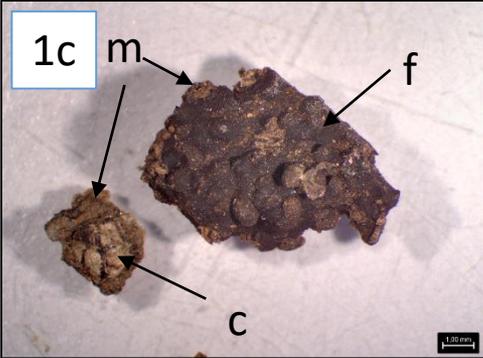


Fig 2. SEM Reference soil

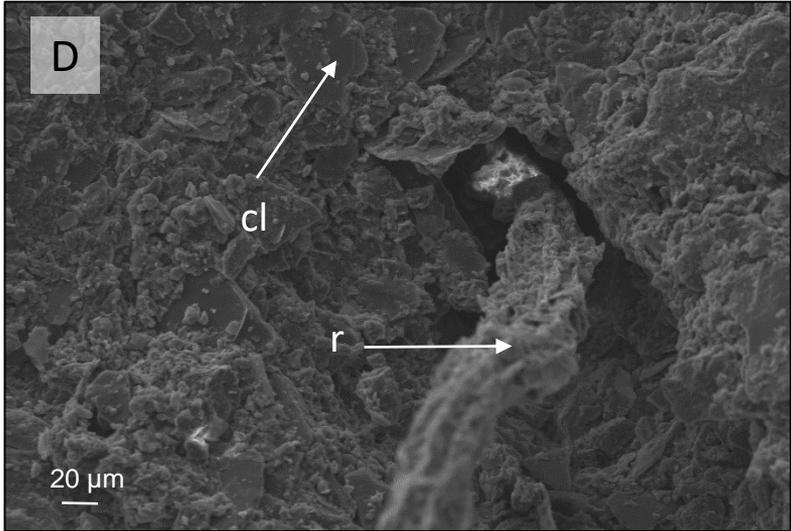
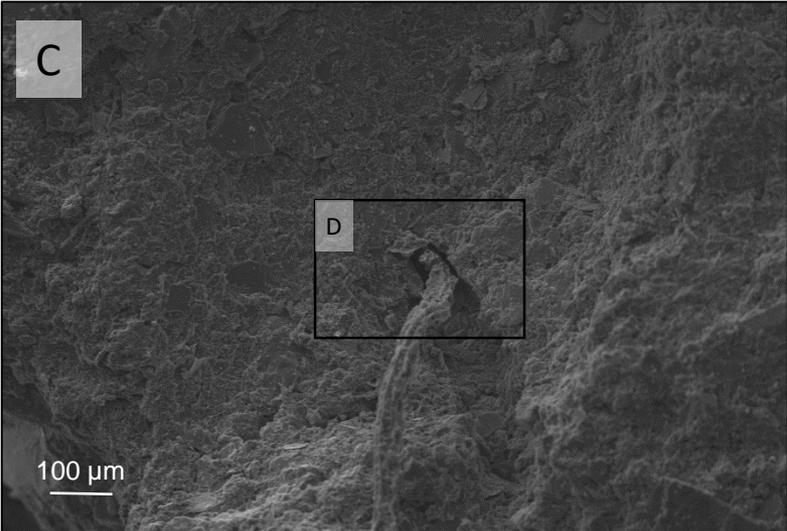
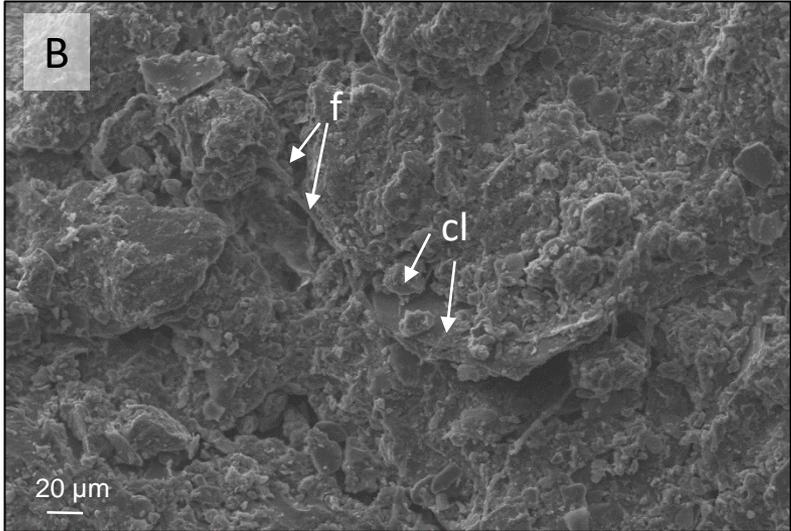
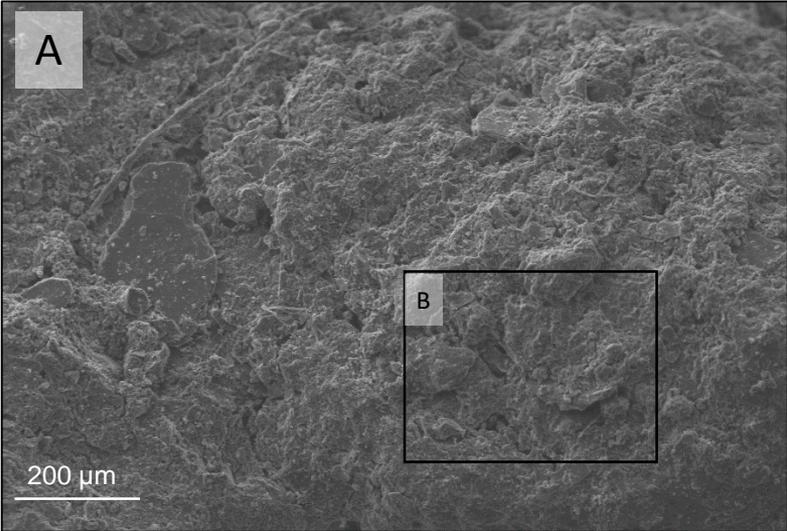


Fig 3. SEM protective sheeting

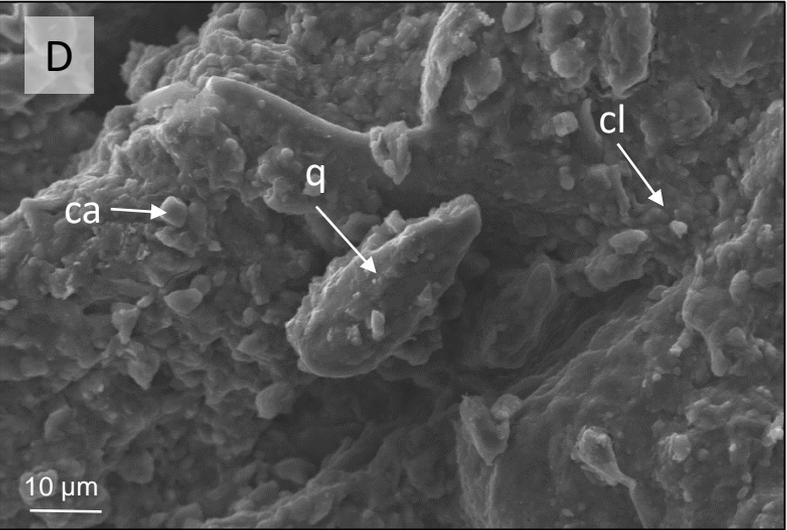
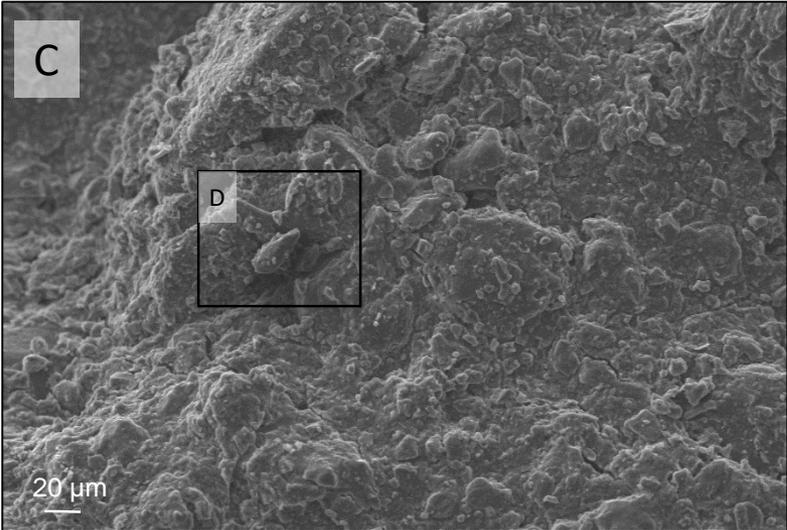
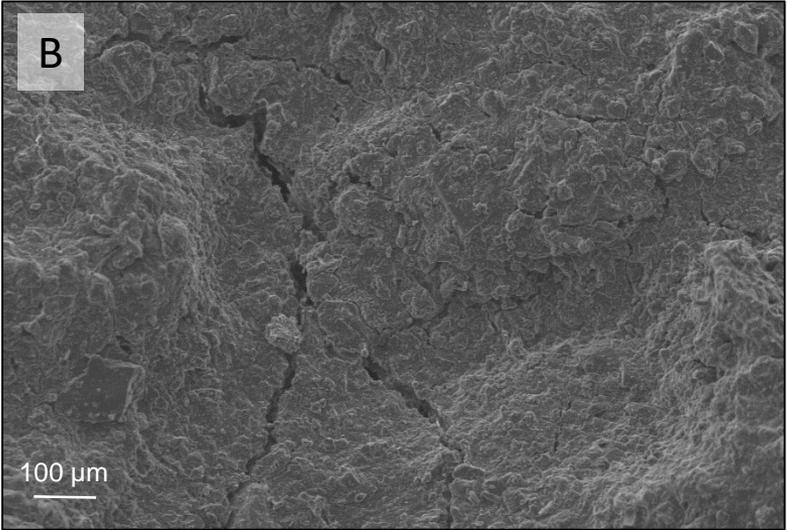
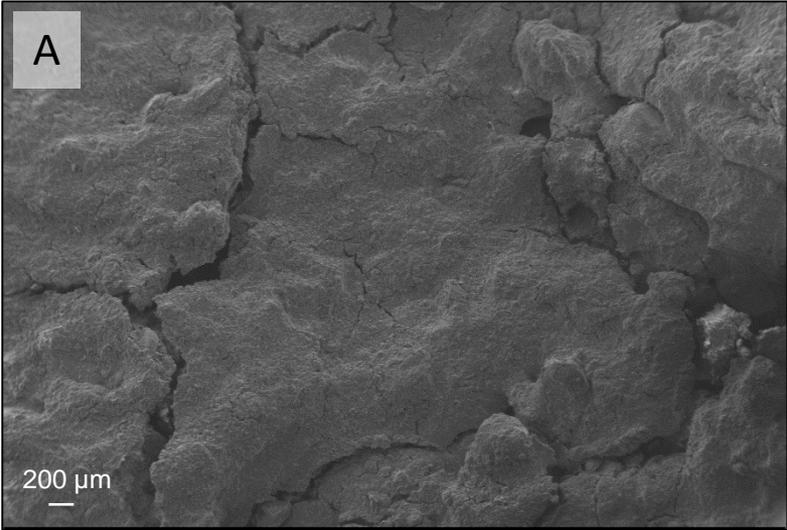


Fig 4. SEM graveyard sheeting

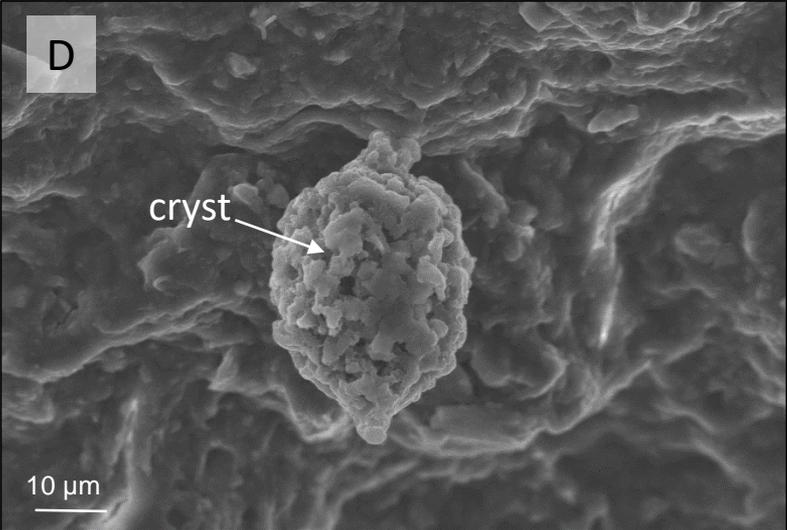
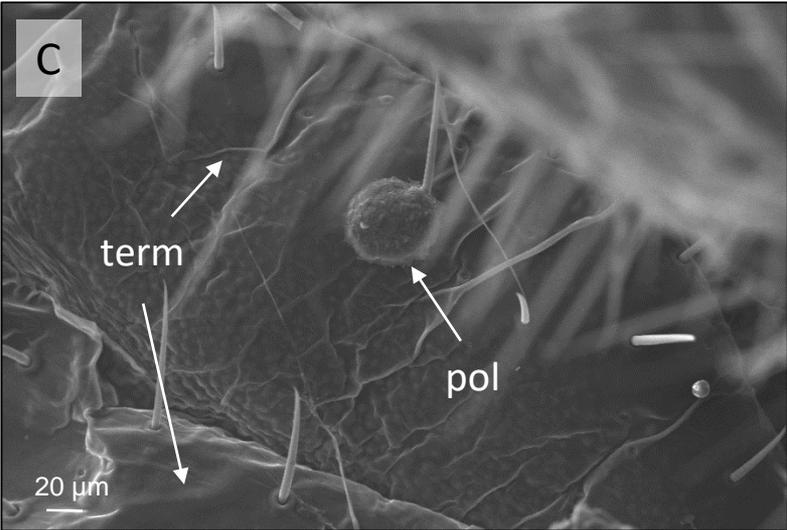
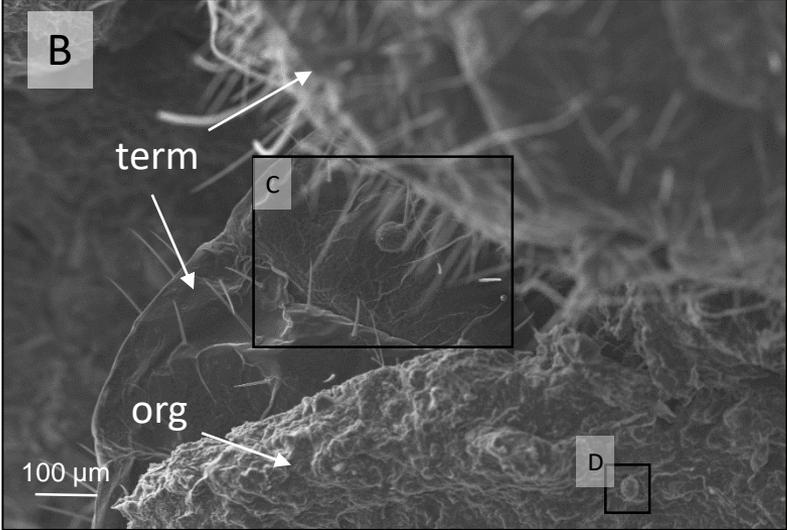
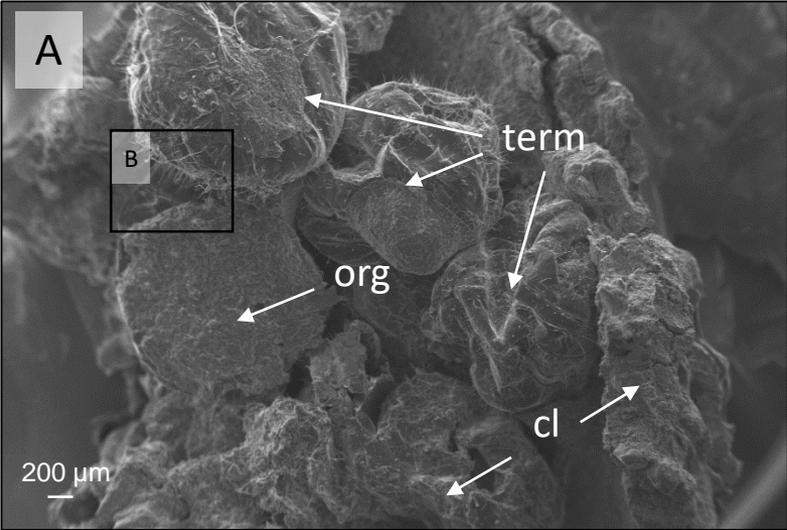


Figure 5

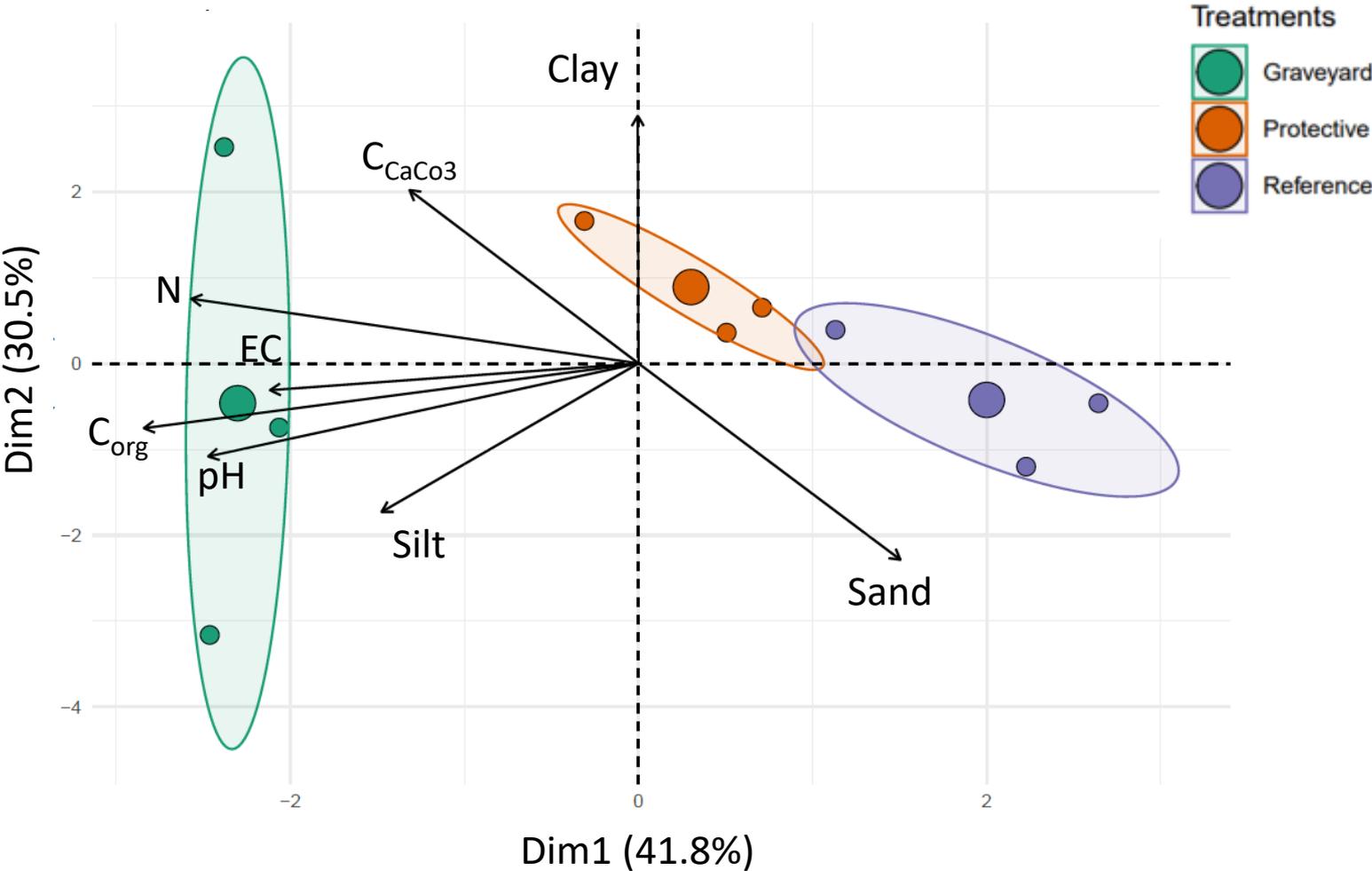
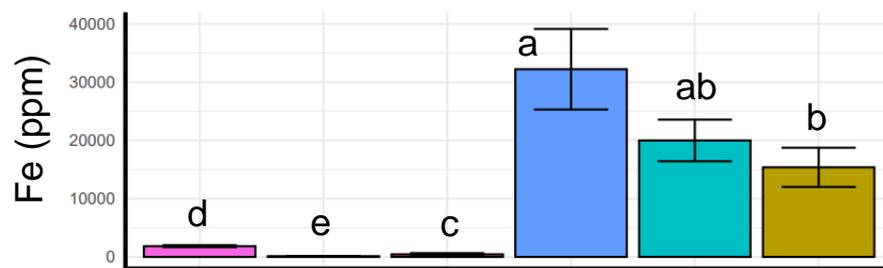
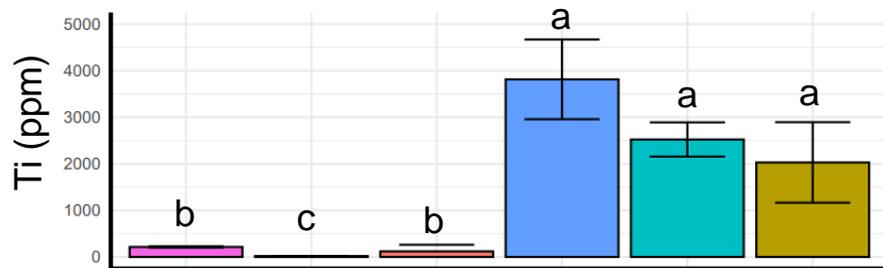
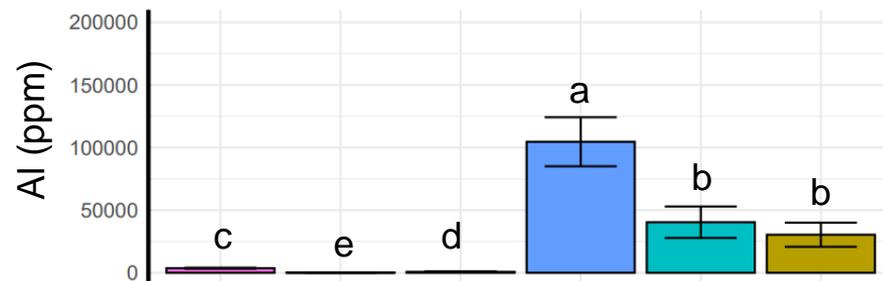
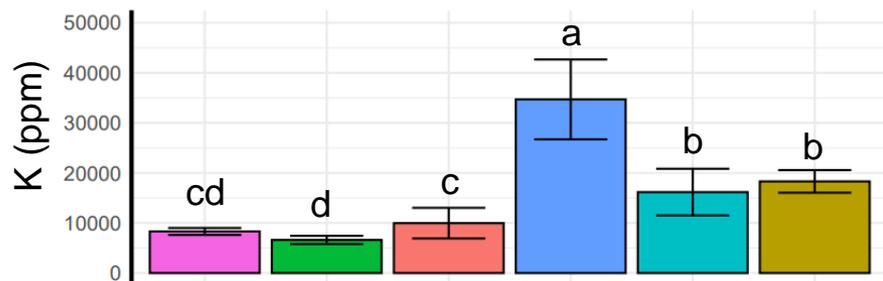
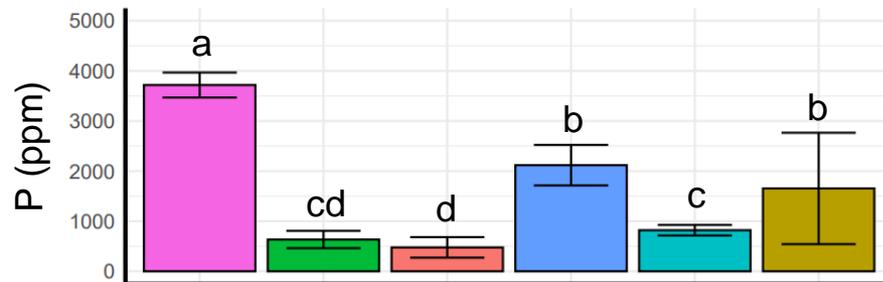
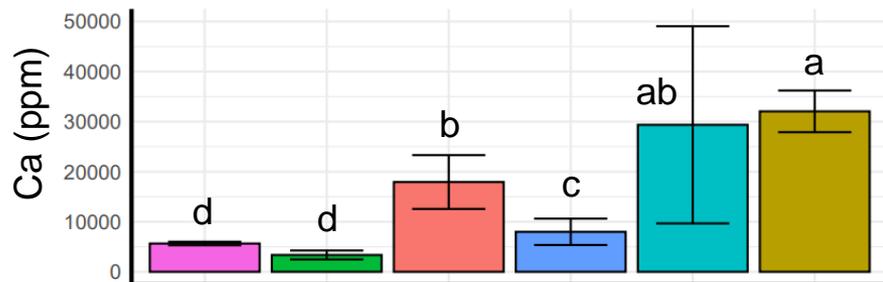
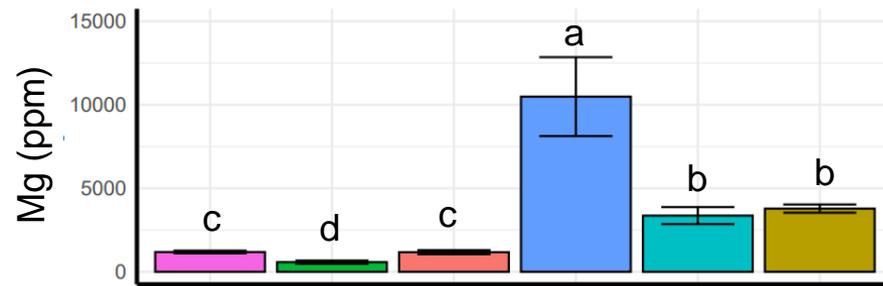
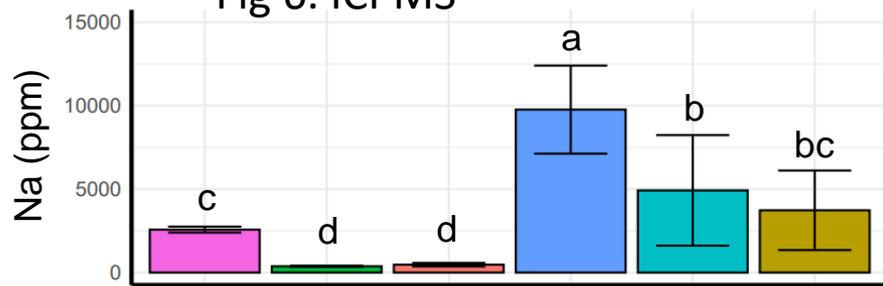


Fig 6. ICPMS



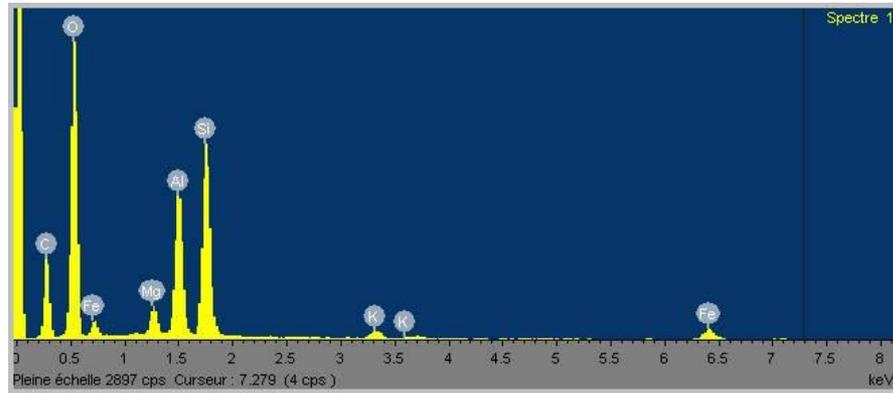
Termite Straw Wood Reference Protective Graveyard

Biomass Soil

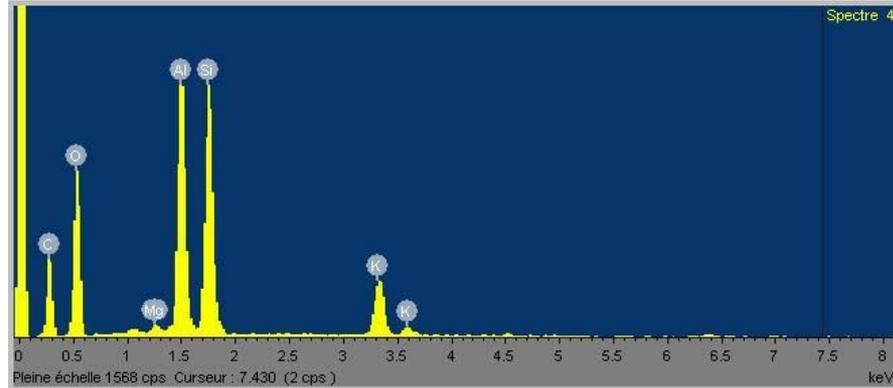
Termite Straw Wood Reference Protective Graveyard

Biomass Soil

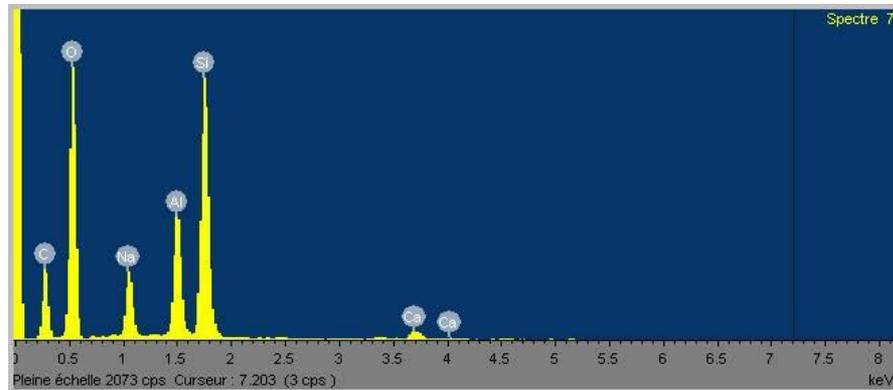
Suppl. file 1.



Smectite type

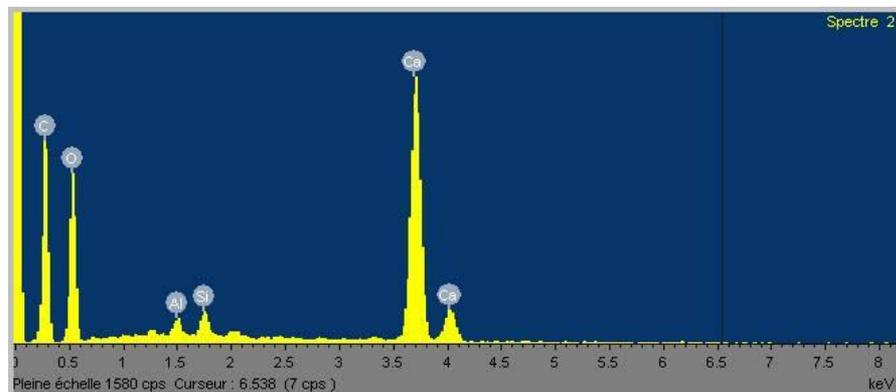


Illite/mica type

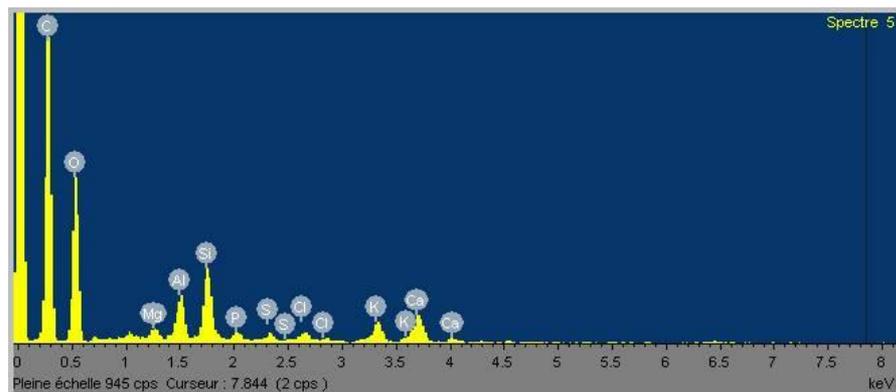


Na-Feldspar type

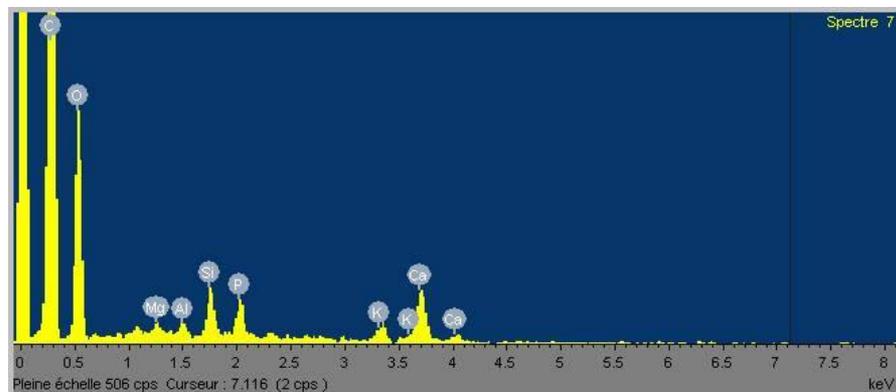
Suppl. file 2.



Calcium carbonate type



Organic matter



Organic matter

Suppl. file 3.

