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

2

3

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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_{org}) and
118 carbonates (C_{CaCO₃}) 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 H₂O₂. Complete dispersion was achieved with Na-
123 hexametaphosphate (20 g L⁻¹) 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%, HNO₃, 65%, and HClO₄, 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_{org} 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

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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 _{org} (g kg ⁻¹)	C _{CaCO₃} (g kg ⁻¹)	N (g kg ⁻¹)	Clay (%)	Silt (%)	Sand (%)
Reference	6.2 ^b (0.11)	98.9 ^b (26.04)	31.36 ^b (8.31)	3.77 ^b (4.67)	3.73 ^b (0.09)	24.30 (3.07)	27.40 (2.90)	52.72 (6.02)
Protective	6.7 ^{ab} (0.16)	546.7 ^a (125.85)	56.37 ^b (16.86)	19.53 ^a (6.18)	5.73 ^b (1.15)	30.46 (1.45)	22.77 (0.98)	46.76 (0.77)
Graveyard	7.2 ^a (0.22)	568.3 ^a (153.98)	148.16 ^a (34.32)	23.63 ^a (8.42)	14.83 ^a (3.54)	23.58 (8.21)	31.30 (1.59)	45.13 (6.71)
<i>F</i> _{2,6}	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

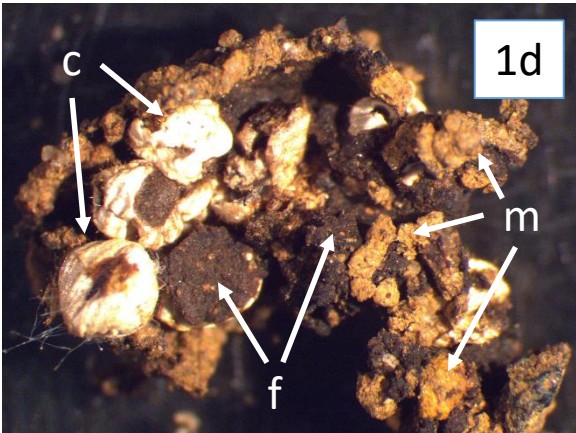
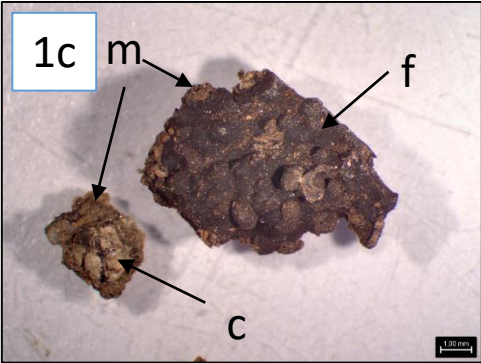


Fig 2. SEM Reference soil

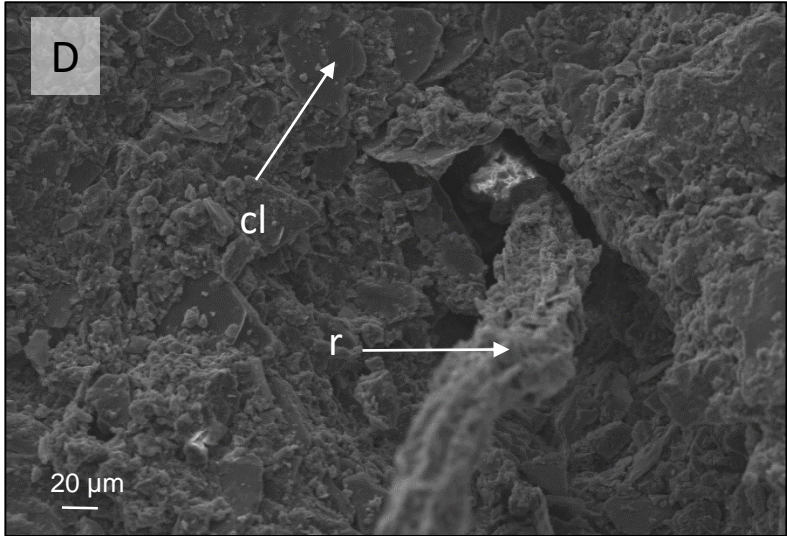
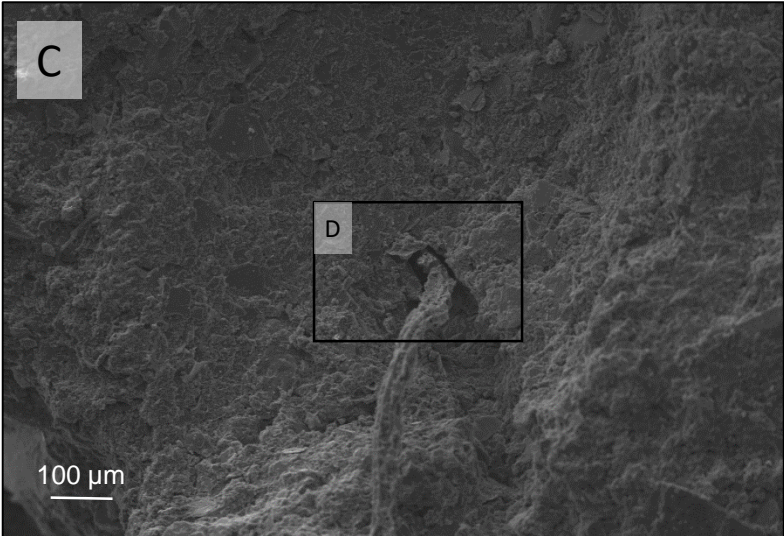
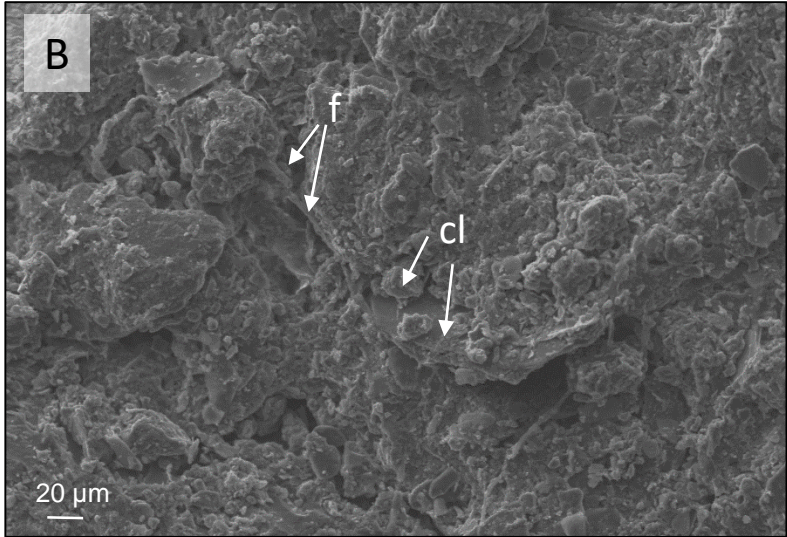
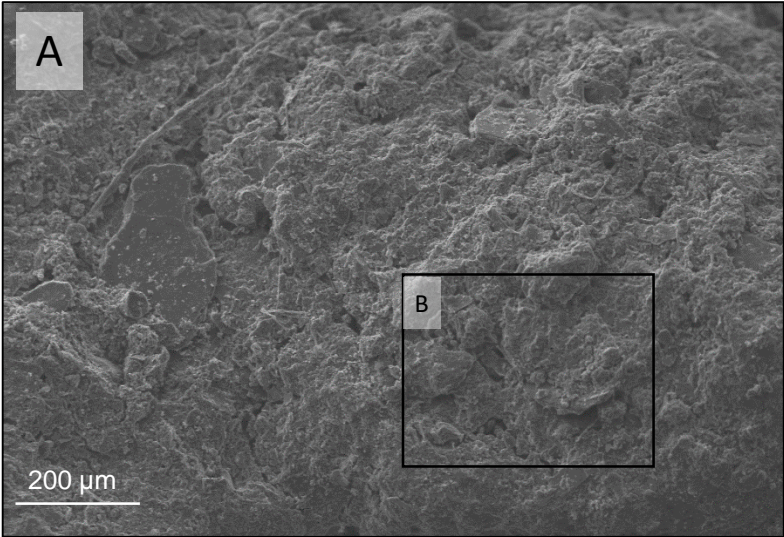


Fig 3. SEM protective sheeting

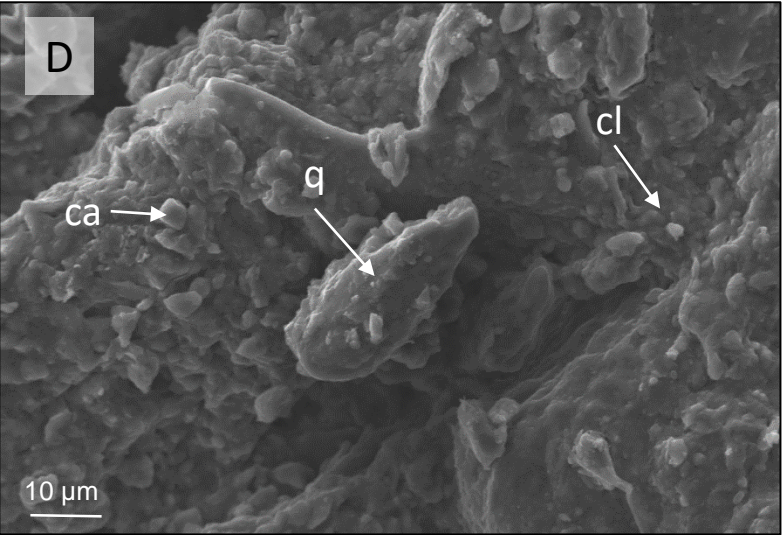
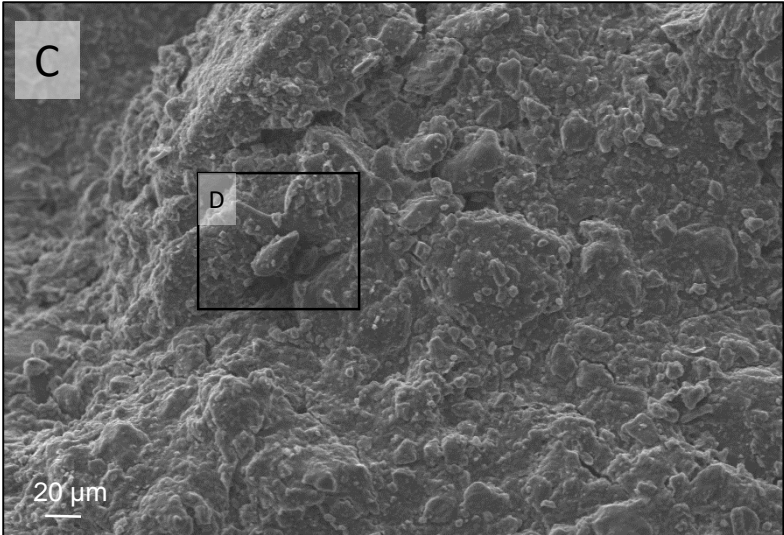
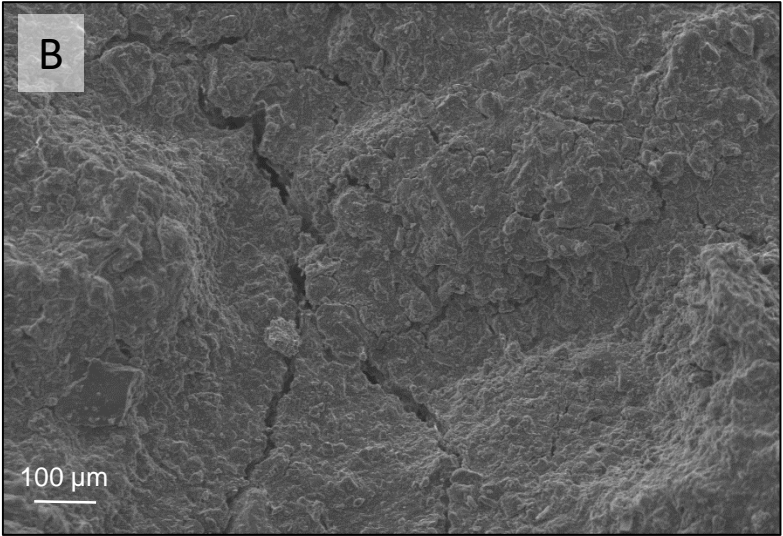
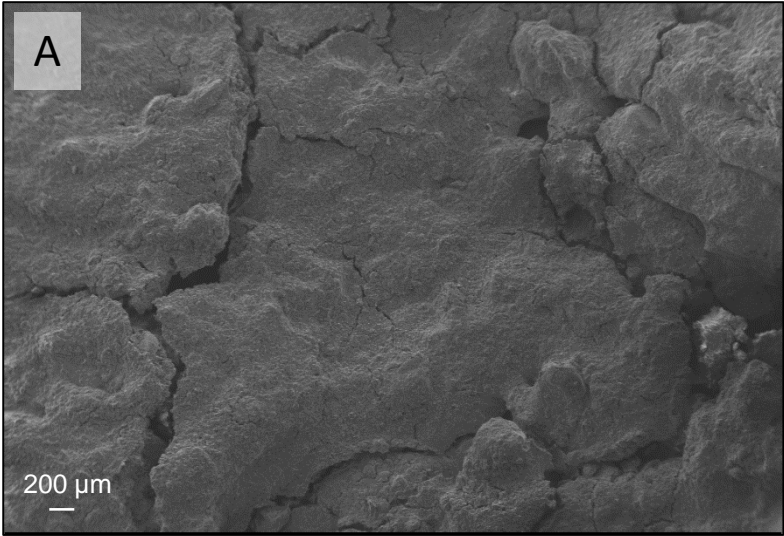


Fig 4. SEM graveyard sheeting

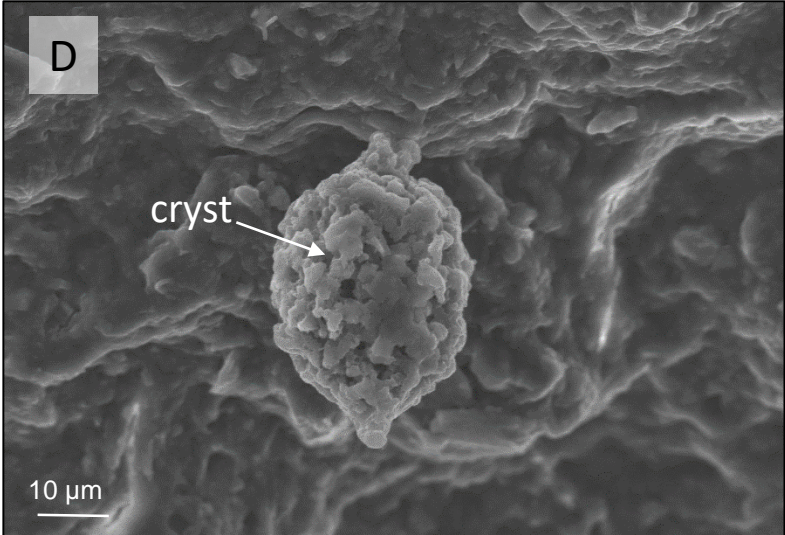
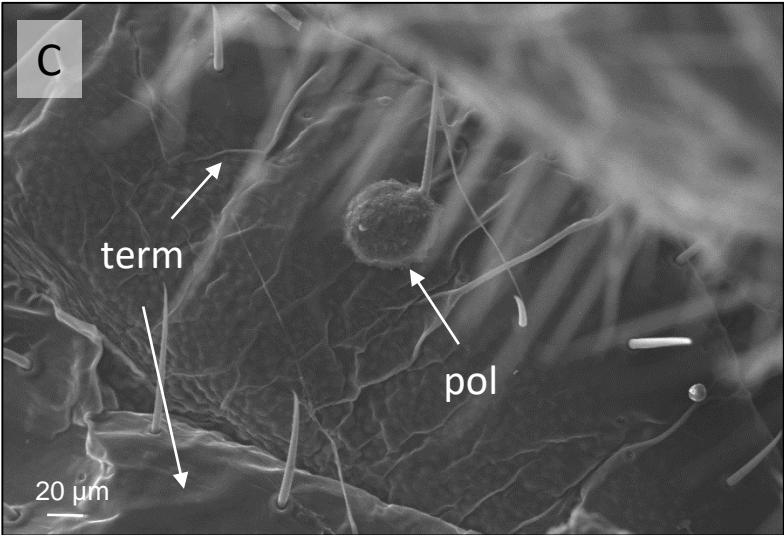
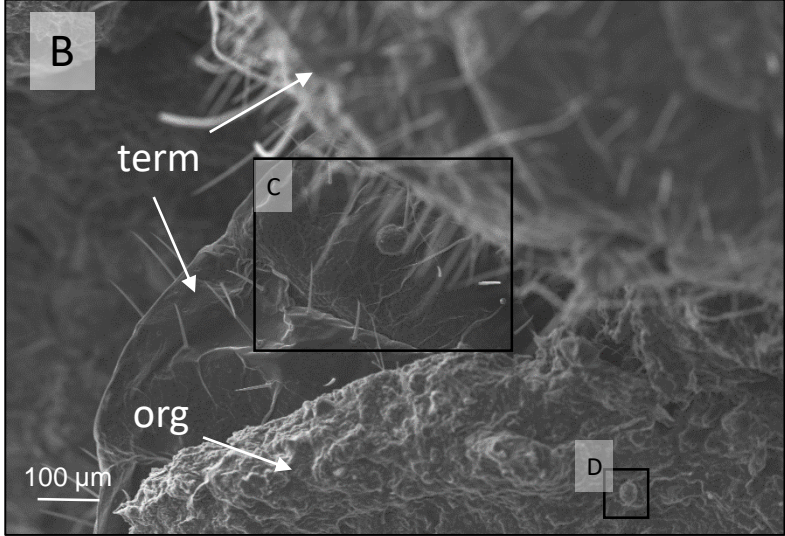
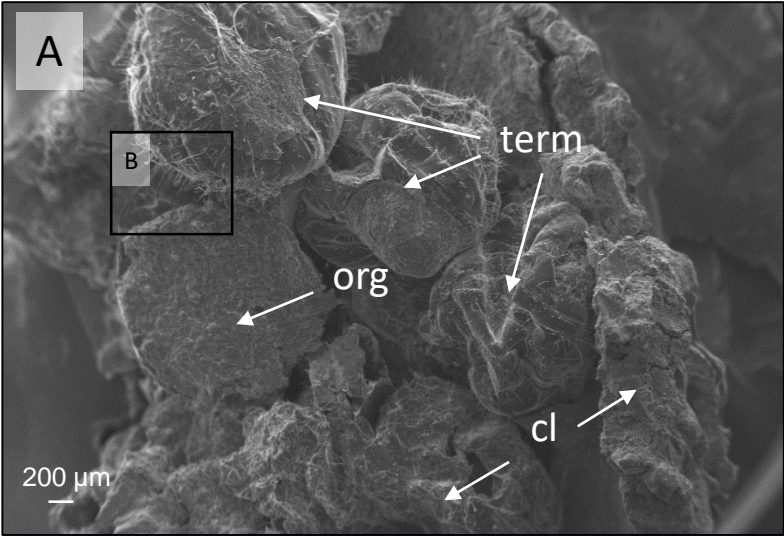


Figure 5

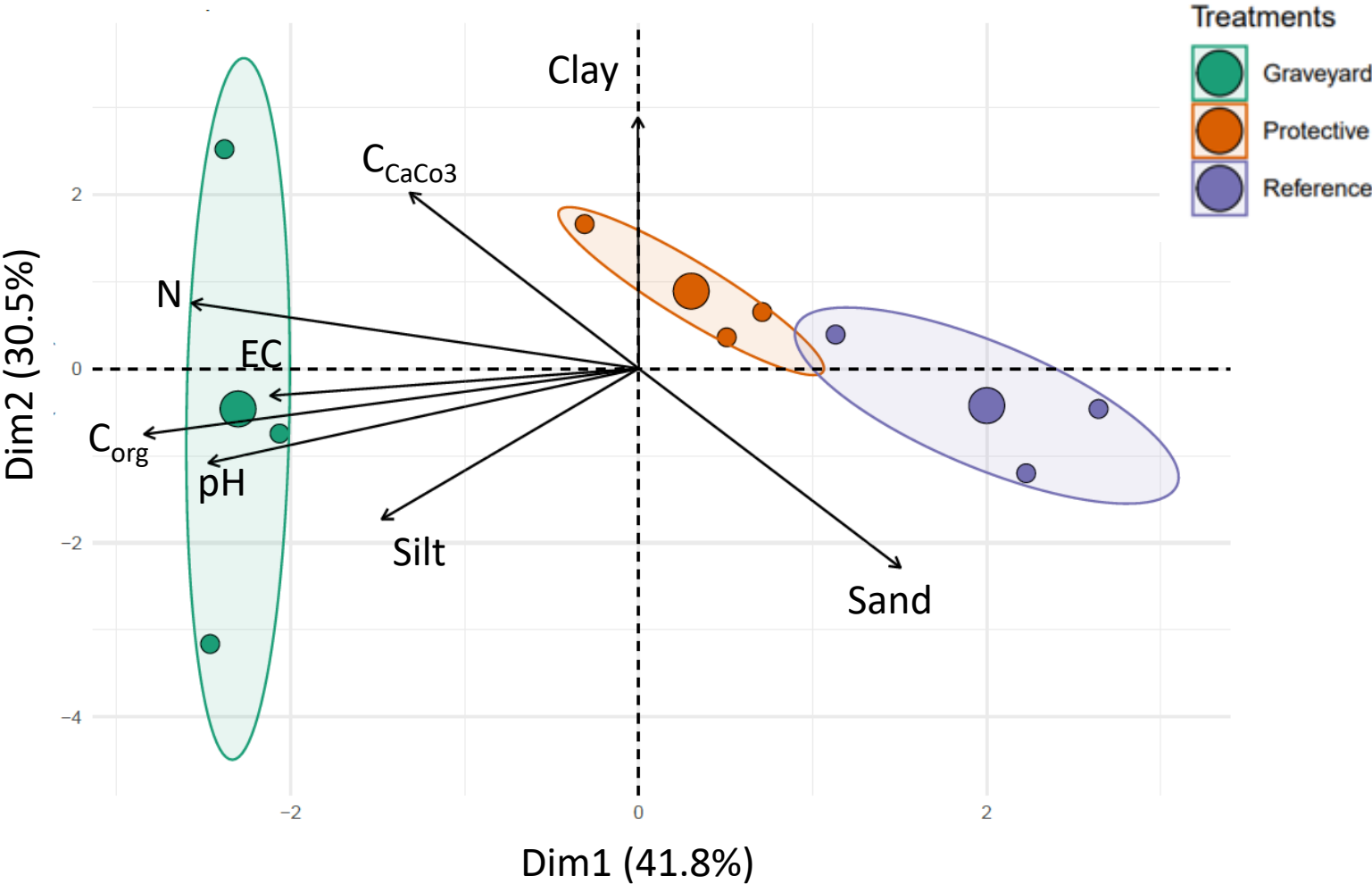
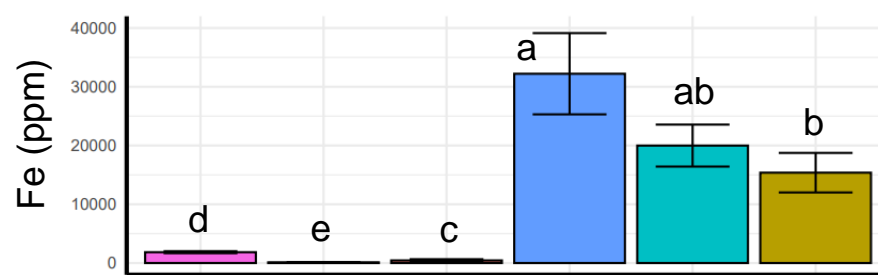
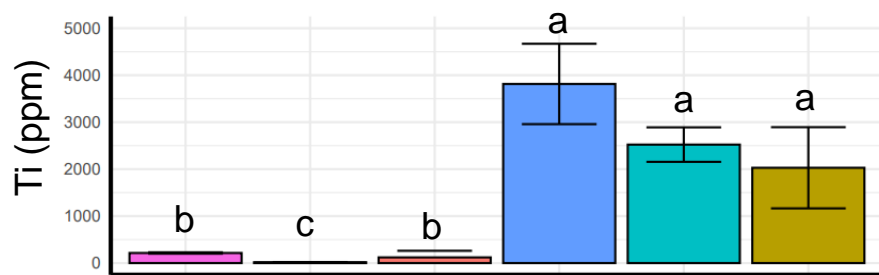
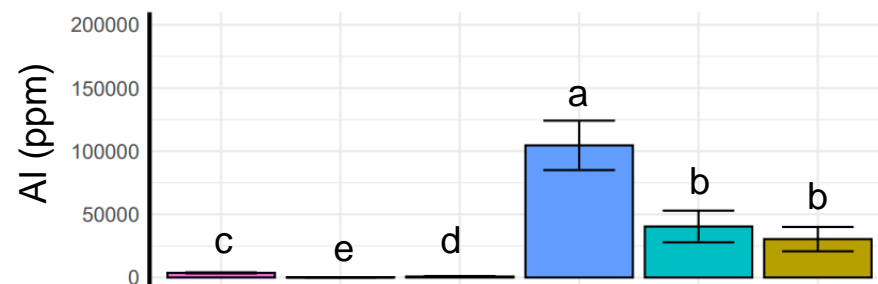
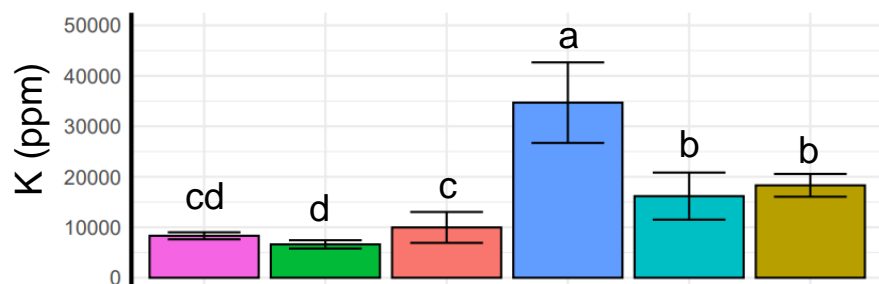
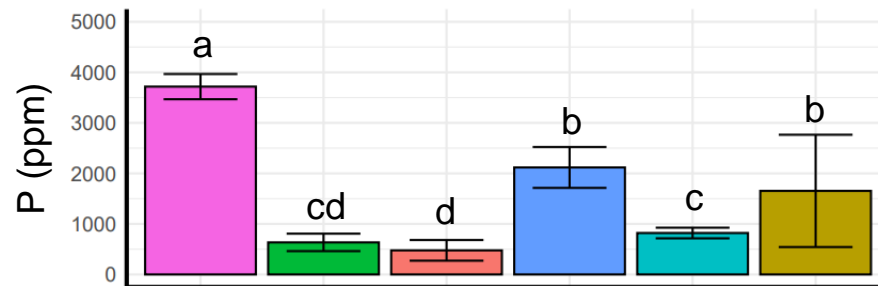
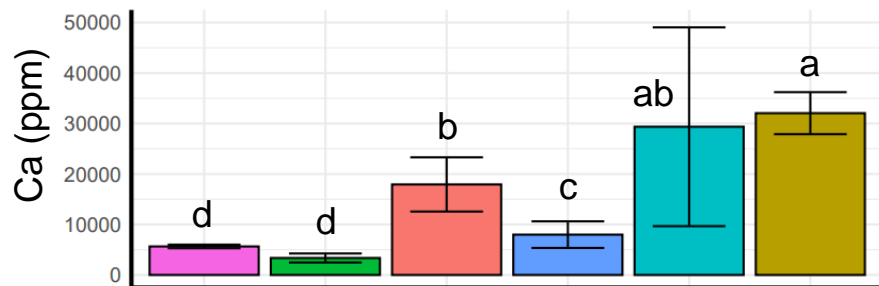
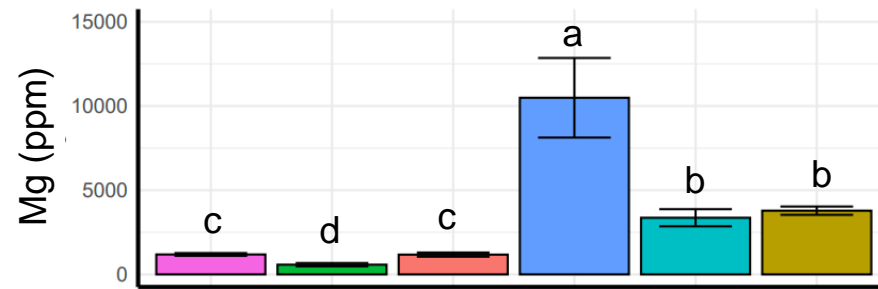
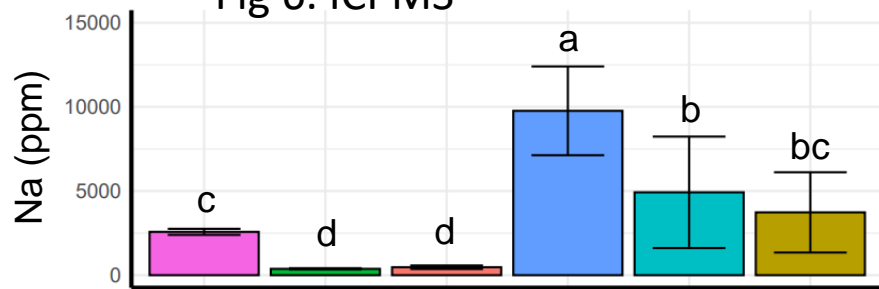


Fig 6. ICPMS



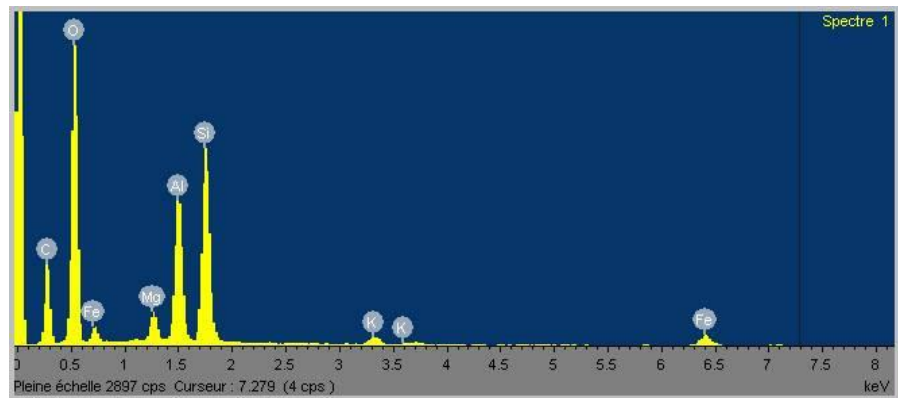
Termite Straw Wood Reference Protective Graveyard

Biomass Soil

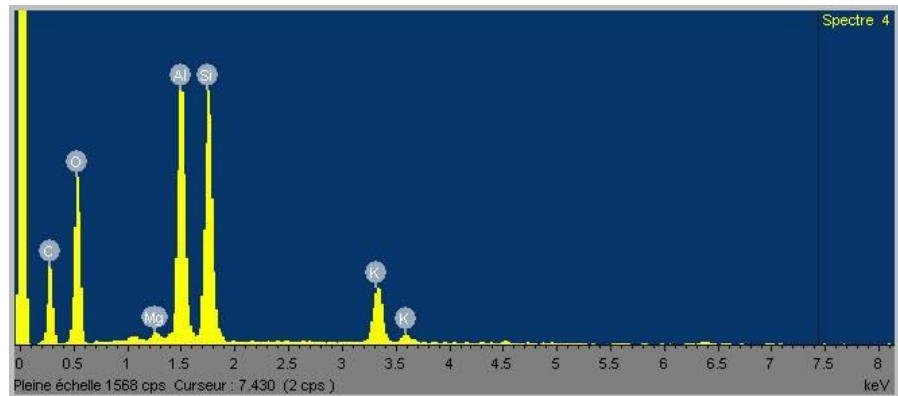
Termite Straw Wood Reference Protective Graveyard

Biomass Soil

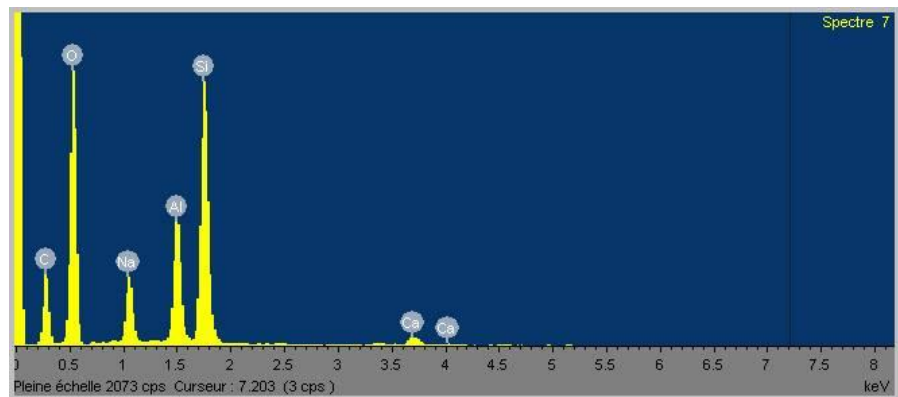
Suppl. file 1.



Smectite type

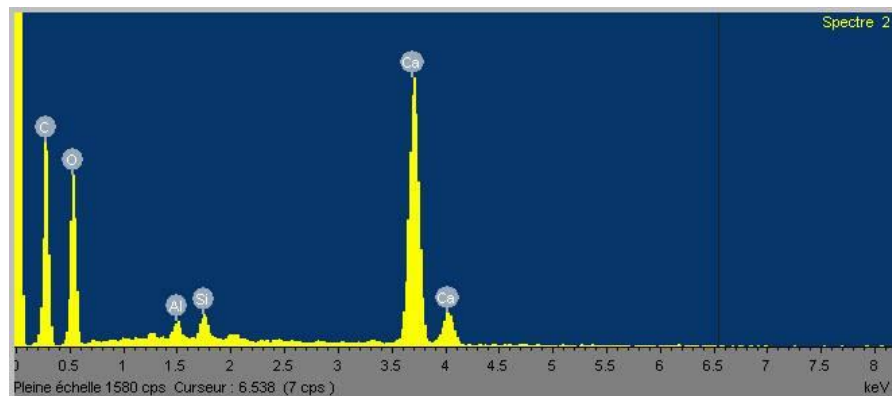


Illite/mica type

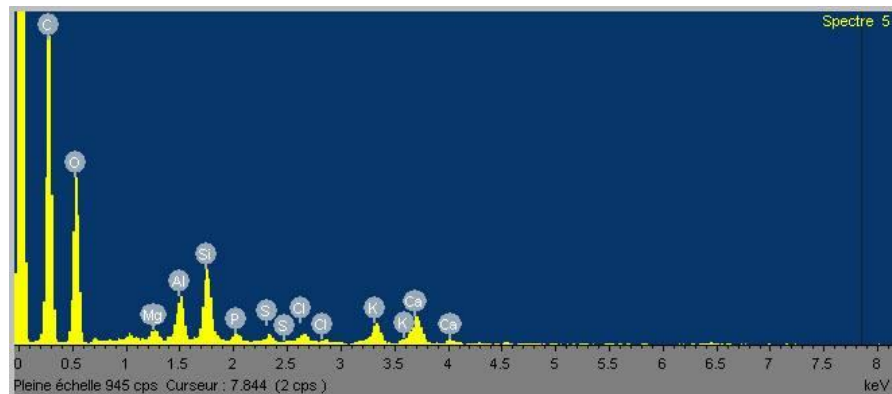


Na-Feldspar type

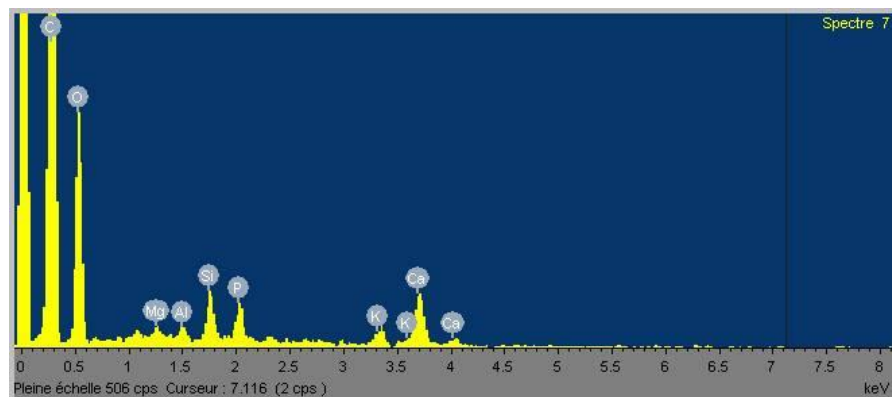
Suppl. file 2.



Calcium carbonate type



Organic matter



Organic matter

Suppl. file 3.

