



HAL
open science

Modified nest architecture and increased bioturbation in response to rise in temperature. A laboratory experiment with the ant species *Lasius niger*

Fátima García Ibarra, Pascal Jouquet, Nicolas Bottinelli, Angélique Bultelle, Thibaud Monnin

► To cite this version:

Fátima García Ibarra, Pascal Jouquet, Nicolas Bottinelli, Angélique Bultelle, Thibaud Monnin. Modified nest architecture and increased bioturbation in response to rise in temperature. A laboratory experiment with the ant species *Lasius niger*. 2023. hal-04282822

HAL Id: hal-04282822

<https://hal.science/hal-04282822v1>

Preprint submitted on 13 Nov 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.

1 Modified nest architecture and increased bioturbation in response to rise in
2 temperature. A laboratory experiment with the ant species *Lasius niger*

3

4 Fátima GARCÍA IBARRA*¹, Pascal JOUQUET^{1,2}, Nicolas BOTTINELLI^{1,3}, Angélique
5 BULTELE¹, Thibaud MONNIN¹

6

7 *Corresponding author

8 ¹ Sorbonne Université, Université Paris Cité, Université Paris Est Créteil, CNRS, INRAE, IRD,
9 Institut d'Ecologie et des Sciences de l'Environnement de Paris (UMR7618), 75005 Paris, France

10 ² Institut de Technologie du Cambodge, Russian Federation Bld, Phnom Penh, Cambodge

11 ³ Department of Soil Sciences, Soils and Fertilizers Research Institute (SFRI), Tù Liêm, Ha Noi,
12 Viet Nam

13 Authors Orcid number: FAGI: 0000-0002-1224-9394; NB: 0000-0003-4944-9696; PJ: 0000-0002-
14 4189-5133; AB: 0000-0003-0546-5408; TM: 0000-0003-3762-942X

15

16 ACKNOWLEDGEMENTS

17 This work was supported by the Mexican National Council of Science and Technology
18 (CONACyT) and the French National Research Institute for Sustainable Development (IRD, ARTS
19 program) through PhD grants awarded to Fatima Garcia Ibarra. We thank Iordan Helissey and Paula
20 Piseiro-Robic of the Specialized Imaging and Emergency Service of the Pitié-Salpêtrière hospital
21 in Paris, for facilitating our access to the medical scanner and carrying out the scanning of the soil
22 columns, and Pierre Fédérici for help in rearing the ants and the transportation of the soil columns.
23 We also thank the reviewers for their insightful and constructive comments.

24 CONFLICT OF INTEREST

25 We have no conflicts of interest to disclose.

26

27 AUTHOR CONTRIBUTIONS:

28 Fátima GARCÍA IBARRA, Pascal JOUQUET and Thibaud MONNIN conceived the ideas,
29 designed the methodology and led the writing of the manuscript; Fátima GARCÍA IBARRA and
30 Angélique BULTELLE carried out the experiment; Fátima GARCÍA IBARRA and Nicolas
31 BOTTINELLI analysed the CT Scan images; Fátima GARCÍA IBARRA analysed the data; all
32 authors contributed critically to the drafts and gave final approval for publication.

33

34 DATA AVAILABILITY STATEMENT:

35 Data are available upon request on DataSuds repository: <https://doi.org/10.23708/ZJTSDI> (Garcia
36 Ibarra, et.al., 2023).

37

38 ABSTRACT

39 1. Ants are important bioturbators that actively produce biopores and move soil particles.
40 They could be particularly affected by global warming as they are ectotherms.
41 Nevertheless, they can indirectly regulate their temperature, through changes in their
42 circadian cycles and the architecture of their nests (e.g., digging deep nests or using
43 insulating materials). Nest architecture has been considered an expanded functional trait of
44 ant colonies and thus sensitive to environmental changes such as increasing temperatures.
45 This work aimed to study the nest architecture of ants as a functional trait and its effects on
46 soil bioturbation. We hypothesized that, when exposed to increased surface temperatures,
47 ants would increase their excavation activities, build deeper nests, and alter the layout of

48 chambers to maintain their preferred temperature and humidity, thus enhancing soil
49 porosity.

50 2. We allowed 17 young *Lasius niger* ant colonies to excavate nests in soil columns exposed
51 to three surface temperatures (mild, n=5; medium, n=6; and high, n=6) for 100 days. We
52 measured the amount of soil excavated weekly, and took X-ray scans of the soil column on
53 days 7, 14, 28, and 88 to characterise the three-dimensional structure of the nests (depth,
54 shape, volume of chambers and tunnels). We then collected the colonies and measured their
55 growth during the experiment, and the size and weight of workers.

56 3. Ants reacted to surface temperature. Colonies exposed to medium and high temperatures
57 excavated larger and deeper nests than those exposed to mild temperature. Nests excavated
58 under high and medium temperatures had the same maximal depth, but chambers were
59 located deeper in the former, which were further characterised by the refiling of some of the
60 upper chambers. Colonies grew well in all treatment, although less under mild temperature.
61 They produced normal-sized workers despite differences in surface temperature. Overall,
62 these results suggest ants exposed to higher temperatures live in deeper chambers.

63 4. This study shows that surface temperature affects ant nest architecture, confirming its status
64 as extended phenotype and highlighting its flexibility over time, which has in turn
65 consequences on soil porosity.

66

67 INTRODUCTION

68 Global warming may particularly affect ectotherms including ants, since they largely depend on
69 external temperatures to regulate their own body temperature (Jørgensen et al., 2022). For instance,
70 increasing temperatures accelerate ant development (S. Porter & Tschinkel, 1993), yielding smaller
71 adults (Verberk et al., 2021), and increase their metabolism, i.e. their movements become faster
72 (Hulbert et al., 2008) and their food and oxygen consumption increase (Coenen-Stass et al., 1980).
73 Ants can thermoregulate to some extent, but this is an energy-consuming process that depends on

74 many factors such as population size, moisture and thermal conductivity of the material, and nest
75 size (Coenen-Stass et al., 1980; Kadochová & Frouz, 2013).

76 Most of the studies about temperature regulation in ant nests have focused on mounds or ant
77 hills (above-ground structures usually built with soil or organic materials). For example, the mound
78 nests of fire ants (*Solenopsis spp.*) are asymmetrical and their architecture changes seasonally to
79 increase the surface directly exposed to the sun (Vogt et al., 2008). Overheating during day and loss
80 of temperature during night are also avoided in the nests of *Acromyrmex heyeri* because of the
81 lower thermal diffusivity of their mounds that are thatched with plant fragments (Bollazzi & Roces,
82 2010). *Atta vollenweideri* nests are thermoregulated with a wind-ventilation system consisting of an
83 outflow of air through central tunnels coupled to an inflow through peripheral tunnels (Kleineidam
84 et al., 2001). *Formica polyctena* underground nests are covered with a dome of pine needles whose
85 decay, produced by the aerobic metabolism of microorganisms, warms the nest (Coenen-Stass et al.,
86 1980). In addition to the above mentioned thermoregulation mechanisms, ants exposed to increased
87 temperature may alter their circadian rhythm and forage for food at cooler times of the day (Lei et
88 al., 2021), or even relocate their nest to cooler microenvironments (Penick & Tschinkel, 2008).
89 Nevertheless, these mechanisms might cause negative interactions with other species or be
90 detrimental to the development of the colony (Adler & Gordon, 2003; Penick et al., 2017).

91 Another simple way to deal with raising temperatures may be to modify the underground
92 activities, e.g., excavate deeper nests and/or reshape tunnels and chamber connectivity as previously
93 documented in ants (Sankovitz & Purcell, 2021), earthworms (Gerard, 1977; Perel, 1977), termites
94 (Korb & Linsenmair, 1998) and other macroarthropods like beetles and flies (Villani & Wright,
95 1990). Such modifications of underground activities in response to environmental factors would
96 affect soil bioturbation, i.e., the dispersal and reorganization of soil particles and aggregates through
97 the activity of animals (Meysman et al., 2006; Bottinelli et al., 2015).

98 Soil bioturbation is a crucial process in soil formation and ecosystem functioning
99 (Wilkinson et al., 2009). Termites, earthworms, and ants are usually considered the most important

100 bioturbators, especially regarding their ability to produce soil biopores and biogenic structures
101 (Paton et al., 1995; Lavelle et al., 1997; Jouquet et al., 2006). Ants are likely to play a key role in
102 the dynamics of nutrients and water in the soils (Cammeraat & Risch, 2008; Benckiser, 2010; Finér
103 et al., 2013; Farji-Brener & Werenkraut, 2017; Sousa et al., 2021). Indeed, several ant species with
104 populous colonies build large underground nests, which are likely to increase soil macroporosity
105 (Frouz & Jilková, 2008) (e.g., *Atta* (Moreira et al., 2004) and *Acromyrmex* leafcutters (Verza et al.,
106 2020), *Formica* wood ants (Mikheyev & Tschinkel, 2004), *Aphaenogaster* (Richards, 2009) and
107 *Pogonomyrmex* seed harvesters (Tschinkel, 2004)). In addition, less populous but highly abundant
108 species such as *Lasius niger* or *Prenolepis imparis* can also be important to soil processes (Rasse &
109 Deneubourg, 2001; Tschinkel, 2003). Moreover, a meta-analysis showed that excavation activities
110 of ants in soils increase soil fertility through an augmentation in nutrient and cation contents
111 independently on their feeding type, the latitude or type of vegetation, which increases the
112 performance and fitness of plants (Farji-Brener & Werenkraut 2017). Although the importance of
113 ant bioturbation is well established (Tschinkel, 2021; Viles et al., 2021), the structure and properties
114 of the biopores they produce remain relatively understudied especially compared to those produced
115 by earthworms (e.g., Cheik et al., 2021; Pham et al., 2023).

116 Nest architecture has been studied by excavating colonies, sometimes by casting nests with
117 liquid plaster or aluminium (Tschinkel, 2010) prior to their excavation. This has shown that
118 underground nests of most ants have the same basic structure, consisting of chambers where brood
119 and workers are typically located, and that are connected by narrower tunnels (Mikheyev &
120 Tschinkel, 2004). The depths of the nests vary from few centimetres to several metres depending on
121 species and environmental conditions (Tschinkel, 2004; Tschinkel, 2021). Due to the technical
122 impossibility of studying how underground nest structure develops through time in the field, most
123 of the works exploring ant nests construction or evolution have used a two-dimensional approach,
124 in which ants dig their nests in soil a few cm thick placed between two glass plates (but see Minter
125 et al., 2012; Pinter-Wollman, 2015). An example is the study carried on by Sankovitz and Purcell

126 (2021) where *Formica podzolica* colony fragments collected at two different elevations were reared
127 in a 2D setup under two temperature regimes in a full factorial experiment, and differences between
128 nests were analysed. Nest architecture differed between the two temperature treatments, with
129 maximal complexity matching the original temperature of the colonies (i.e., ants from higher sites
130 built more complex nests under cool temperatures, whereas ants from lower sites had the opposite
131 response). These results are interesting yet should be treated with caution for the artificial approach
132 of the experimentation (i.e. only a small number of workers without brood nor the queen, two-
133 dimensional setup, and no temperature gradient in the soil).

134 During the last decades, X-ray computed tomography (X-ray CT) has been used as a non-
135 destructive method to investigate the effect of invertebrates like earthworms, termites, and beetles
136 on soil properties (Perna et al., 2008; Booth et al., 2020; Cheik et al., 2021). This study uses X-ray
137 CT to analyse the effects of increased surface temperatures (and different gradients across the soil)
138 on the nests excavated by complete young *L. niger* colonies. X-ray CT being non-destructive, also
139 allows repeated measures of colonies over time enabling the study of the temporal nest architecture
140 dynamics.

141 We hypothesized that ants react to increased surface temperatures by modifying the
142 architecture of their nest (digging a deeper nest and/or altering the layout of chambers) ensuring
143 suitable abiotic conditions (i.e. temperature, humidity) for the colony. For instance, higher
144 temperature accelerates development and results in the production of smaller adults in insects.
145 Producing smaller workers may be detrimental to colony success hence we expect workers to
146 excavate deeper nests with deeper chambers providing adequate temperature for brood to develop in
147 normal-sized workers. As a by-product of this increased excavation activity, we also expect soil
148 porosity to increase.

149

150 MATERIALS AND METHODS

151 *Study species*

152 *L. niger* (L.) is one of the most common species of ants in European urban areas. They are typically
153 found in habitats such as open areas with scattered plant cover, as gardens, meadows, and roadsides
154 (Czechowski et al., 2012), and they can change significantly the chemical and bacterial dynamics of
155 soils (Holec & Frouz, 2006). In addition, their claustral independent colony foundation facilitates
156 the rearing of new colonies, and the adult colonies are easy to maintain in the laboratory.

157 *Collection of colonies and rearing*

158 We collected 51 fertilized *L. niger* queens immediately after their mating flight in Paris in July
159 2021. This species uses claustral colony foundation to found new colonies. After the mating flight
160 the queen excavates a small nest and starts rearing the first generation of workers using her
161 metabolic reserves only, without ever leaving the safety of the nest. We set up the queens to found
162 colonies in the laboratory, in a windowless climatized room, by keeping ants in glass test tubes (10
163 cm length x 0.7 cm diam.) with a water reserve held by wet cotton. Once the queens had produced
164 cocoons, 18 queens were randomly selected for the experiment. The growth of their colonies was
165 boosted by providing each of them with the cocoons produced by two other queens. Note that this
166 procedure is not unnatural as *L. niger* colonies raid neighbouring colonies to steal and adopt their
167 brood as a mean to increase colony growth (e.g. Pollock & Rissing 1989, Sommer & Hölldobler
168 1995). Workers started hatching after three months. Colonies were then removed from the tubes and
169 installed in 12.5 cm length x 12.5 cm width x 5.5 cm height clear plastic boxes. They were fed with
170 a mix of equal parts of honey and apple puree, fresh mealworms, and a balanced protein gel twice a
171 week, with water provided *ad libitum* by a test tube filled with water and plugged with cotton. The
172 experiment started seven months later (February 2022). At that time, colonies contained in average
173 106 ± 27 (mean \pm SD) workers, the queen and brood.

174

175 *Experimental design*

176 Twenty-one polyvinyl chloride (PVC) tubes (30 cm height x 20 cm diameter) had the bottom closed
177 with a nylon cloth (mesh size = 150 μm) to retain soil but allow water to pass through. They were
178 filled to a height of 24 cm with air-dried soil previously sampled in the topsoil layer (0-20 cm) of
179 the park of the French National Research Institute for Sustainable Development (IRD) campus in
180 Bondy (7.5 Km Northeast of Paris), France. The soil had a neutral pH ($\text{pH}_{\text{H}_2\text{O}} = 7.2$) and was sandy
181 (70% sand, 15% silt, and 15% clay) with 4.5% organic carbon. It was sieved at 2 mm to discard
182 large size particles and roots, and then manually compacted to reach the bulk density found in the
183 field (1.08 g cm^{-3}), stepwise in 3 cm layers to reach 24 cm height. The soil column was afterward
184 watered to reach 70% of the field capacity ($0.14 \text{ g H}_2\text{O g}^{-1} \text{ soil}$) following Weil & Brady (2016).

185 To avoid border effects affecting the shape of the excavated nest, e.g. tunnels following the
186 side of the PVC tubes, a plastic lid with a central opening was placed on the soil surface. The side
187 of the lid was glued to the side of the PVC tubes to force ants to dig through the lid central opening
188 only, i.e. in the centre of the soil surface. The lid had numerous perforations (10 mm diameter each)
189 totalling $\frac{1}{4}$ of its surface, to allow watering of soil and gaseous exchanges between the soil and the
190 atmosphere. The lid was underlined by a nylon fabric that prevented ants from digging through the
191 perforations. A short plastic tube connected the central opening of the lid to a tall plastic petri dish
192 (8.5 cm diameter x 5 cm height) whose bottom had been drilled. The petri dish allowed providing
193 food and water to the colony as well as collecting the excavated soil for weighing (Fig S1).

194 The 18 colonies were randomly assigned to high (T_{high}), medium (T_{medium}), and mild
195 temperature (T_{mild}) treatments ($n = 6$ for each treatment). The surface of the soil columns was heated
196 using 40 W UV lamps placed 20 or 40 cm above the soil surface (T_{high} and T_{medium} , respectively) or
197 fluorescent lights 50 cm above the soil surface (T_{mild}). Soil temperature was monitored in two
198 columns per treatment using two iButton temperature sensors on the surface and at ~ 10 cm depth.
199 To avoid any effect of the sensors on the nest structure, they were placed on the edge of the column.
200 None of the ant nests reached an iButton during the experiment. This protocol was successful in
201 producing three treatments with significantly different temperatures. Specifically, daytime average

202 temperatures (i.e., while the lamps were on) on the soil surface were 22.2 ± 1.1 , 35.9 ± 5.8 and 47.9
203 $\pm 10.7^\circ\text{C}$ for T_{mild} , T_{medium} , and T_{high} treatments, respectively (mean \pm SD, $F_{2,7497} = 83$, $P < 0.001$,
204 T_{mild} vs T_{medium} , $P < 0.001$, T_{mild} vs T_{high} , $P < 0.001$, T_{medium} vs T_{high} , $P < 0.001$), and night-time average
205 temperatures (when the lamps were off) were 21.5 ± 1.1 , 24.8 ± 5.4 and $28.4 \pm 11.1^\circ\text{C}$ ($F_{2,7527} = 584.5$,
206 $P < 0.001$, T_{mild} vs T_{medium} , $P < 0.001$, T_{mild} vs T_{high} , $P < 0.001$, T_{medium} vs T_{high} , $P < 0.001$). As expected,
207 this difference markedly declined in the soil, with a mid-depth daytime temperature of 22.2 ± 1.1 ,
208 25.5 ± 2.1 , and $26.2 \pm 2.4^\circ\text{C}$ for T_{mild} , T_{medium} , and T_{high} treatments, respectively ($F_{2,2469} = 567.4$, $P <$
209 0.001 , T_{mild} vs T_{medium} , $P < 0.001$, T_{mild} vs T_{high} , $P < 0.001$, T_{medium} vs T_{high} , $P < 0.001$), and night-time
210 temperature of 21.8 ± 1.0 , 23.8 ± 2.1 and $24.6 \pm 2.4^\circ\text{C}$ ($F_{2,2487} = 270.1$, $P < 0.001$, T_{mild} vs T_{medium} ,
211 $P < 0.001$, T_{mild} vs T_{high} , $P < 0.001$, T_{medium} vs T_{high} , $P < 0.001$). Because one colony died during the
212 experiment, the number of replicates was $n = 5$ for T_{mild} , and $n = 6$ for T_{medium} , and T_{high} . The impact
213 of ants on water evaporation was also monitored using soil columns without ants as control ($n = 1$
214 per treatment). All colonies were kept with a light/dark 12h/12h schedule for 100 days.

215 Ants were put in the plastic box connected to the top of the soil column four days before
216 turning on the lamps, to allow them to start digging under the same conditions and not under the
217 stress of excess heat. The start of the experiment was marked by the lighting of the lamps.

218

219 *Soil bioturbation by ants*

220 To assess the underground excavation activity, the amount of soil excavated by ants and deposited
221 on the ground was collected. This assessment, may underestimate the underground activity as it has
222 been reported that ants refill chambers and tunnels according to their space necessities taking the
223 soil previously deposited out of the nest (Römer & Roces, 2015). However, such remodelling of the
224 nest in our experiment may be revealed by the tomography if refilled spaces differ in density from
225 the surrounding soil. The excavated soil was collected weekly, dried at 80°C for 48h and weighted.
226 Soil columns were also weekly weighed for measuring water loss and watered to maintain soil

227 moisture at 70% of the field capacity to allow the development of ants, without promoting the
228 development of anoxic bacteria. The amount of evaporated water (i.e. the added water to keep 70%
229 field capacity), was calculated as follows:

$$\text{Calculated column weight} = \text{Previous week column weight} - (\text{Excavated soil} \times 70\% \text{ field capacity})$$

$$H_2O_{\text{evaporated}} = \text{Calculated column weight} - \text{real weight}$$

230

231 On days 7, 14, 28, and 88 of the experiment, soil columns were scanned using a medical
232 Computed X-ray Tomograph (IQon - Spectral CT, Philips) at the Pitié-Salpêtrière Hospital in Paris
233 to acquire a set of 0.8 mm thick images with a pixel size of 0.45 mm. The X-ray beam was operated
234 at 58 mA and 120 kV. Images (16-bit DICOM format, 512 x 512 pixels) were obtained and
235 subsequently transformed into 8-bit TIFF format and rendered isotropic of 0.45 mm. Images were
236 processed and quantified with ImageJ software version 1.53s (Schindelin et al., 2012) and
237 visualized with Avizo software version 2021.2 (ThermoScientific).

238 Once the images were pre-processed, the soil volume was defined using the ROI manager
239 tool and its volume was measured using the Volume Fraction tool on ImageJ. The level of grey of
240 the histograms was bimodal, hence the Otsu automatic thresholding method was applied before
241 segmentation. The excavated nests on the other hand, were selected by applying a 5-pixel 3D ball
242 opening, 8-connection fill holes, 10-pixel 3D ball opening, and a 17-pixels 3D disc erosion and
243 removing small spots 3D (100 pixels) on Avizo, after confirming that these parameters were the
244 most suitable for all the images. Nest volume was calculated with Volume Fraction as well, and
245 MorphoLibJ plugin was used to further describe their i) shape: sphericity index (i.e., the ratio of the
246 squared volume over the surface area, such that ratio of a ball equals one), Euler number (an
247 indicator of topology corresponding to the number of objects minus the number of holes in the
248 object), number of pores; ii) position: maximal depth, ellipse elevation (orientation of the inertia
249 ellipse in degrees); and iii) diameter: the maximal radius of the inscribed ball.

250 Chambers and tunnels (Figs 1, S2, S3, S4), have different shapes (i.e., bulbous for the
251 former, and tubular for the latter). To evaluate them independently, we used filter tools on Avizo,
252 which allowed us to select them precisely. Chambers were separated from the nests by using the
253 Avizo Label analysis tool in mode 3D and Analysis filter tool, until the selected volume best fitted
254 most of our observations (filter: $\text{Breadth } 3d < 7$ and $\text{Breadth } 3d / \text{Length } 3d < 0.8$, where breadth
255 (width) and length are geometrical descriptors of the chambers). Tunnels were obtained by
256 subtracting the chambers from the nests. The mean thickness (diameter of the greatest sphere that
257 fits within) of both tunnels (1.68 to 3.67 mm) and chambers (4.41 to 7.61 mm) were calculated with
258 ImageJ BoneJ plugin.

259 During the image processing, we noticed a third level of grey on some images (Fig S5),
260 intermediate between those corresponding to the soil (higher density) and the nests (density equal to
261 zero). It represented structures less dense than the soil and herein suspected to correspond to refilled
262 parts of the nests i.e. denoting nest remodelling. Prior to segmentation, a 3D non-local means filter
263 of three pixels was applied to reduce noise and scatter. Avizo manual thresholding (110, 160) and a
264 manual 3D ball opening were used to ensure the desired objects were segmented (Fig S2 to S5).
265 Volume was measured with the Volume Fraction tool on ImageJ. All volumes (i.e. nests, chambers,
266 tunnels, and refilled chambers) were transformed into percentages (structure volume / soil volume
267 x100).

268 To assess the distribution of soil excavated along the entire column, the pore area was
269 measured by counting voxels per slice with 2D Analyse particles tool on ImageJ and multiplying by
270 voxel size (0.45 mm). Next, this porosity was transformed in percentage (pore area / soil area x
271 100). To analyse the nest structure, a skeleton was obtained using the Avizo AutoSkeleton tool, and
272 the 3D Centroid path tortuosity (that considers tortuosity as a path formed by the centroids of the
273 objects on each plan, compared to the distance between its ends along the Z-axis) was applied to
274 measure tortuosity and coordination number (number of branches connected to each node).

275

276 *Growth of colonies*

277 At the end of the experiment, the soil columns were opened, and the colonies recollected, to
278 measure the effects of the temperature treatments on colony growth, worker size and weight. The
279 number of workers was counted to measure colony growth throughout the duration of the
280 experiment. Moreover, 10 workers per colony were placed in 70% ethanol and their morphological
281 traits were measured. Head length and Weber's length (the diagonal length of the mesosoma, from
282 anterior edge of the pronotum to the posterior corner of the metapleuron, Weber, 1938), were
283 measured and summed as indicator of the individual size. In addition, 10 other workers per colony
284 were chill killed at -20°C for 12 hours, dried in a stove at 40°C for 48 hours, and weighed using a
285 Sartorius Cubis microbalance.

286

287 *Statistical analysis*

288 Data were analysed using R (version 4.0.0) software. Differences among treatments for water
289 evaporation, soil excavated, number, size and weight of ants were analysed using one-way Anova
290 and Tukey tests, after verifying the normality (Shapiro-Wilk test, rstatix package (Kassambara,
291 2021)) and homogeneity of variances (Levene's test, rstatix package) of the data. Data from the
292 tomography analyses were analysed using a PCA (factoextra package (Kassambara & Mundt,
293 2020)) to determine which traits of the nests contributed the most to total variance. Depth (D),
294 volume of nests (V_{nest}), chambers (V_{Ch}), tunnels (V_{Tu}) and refilled chambers (V_{Re}), coordination
295 number, and tortuosity were considered suitable for further description and analysed using two-way
296 (treatment, time) mixed Anova (rstatix package) after verifying the absence of significant outliers,
297 normality, homogeneity of variances, sphericity of variance (Maulchy's test) and homogeneity of
298 covariances (Box's M test), to assess the effect of treatment and time. Differences among groups
299 were analysed with one-way Anova (when an interaction between main factors was present) and
300 Bonferroni adjusted pairwise comparisons.

301

302 RESULTS

303 *Soil bioturbation*

304 Excavation activity was similar across treatments during the first half of the experiment. It was
305 highest at the onset of the experiment (time 0) and then gradually decreased. A late increase in
306 excavation (after day 50) occurred for T_{medium} , but not for T_{high} and T_{mild} (Fig 2A). The total amount
307 of soil excavated (Fig 2B) was significantly higher at T_{medium} (26.4 ± 12.1 g, mean \pm SD) than at
308 T_{mild} (12.8 ± 4.7 g), with T_{high} having intermediate values (19.5 ± 8.3 g, $F_{2,14} = 3.06$, $P < 0.05$).

309 From the beginning of the experiment, ants produced nests whose maximum depth did not
310 change with time ($F_{3,64} = 2.56$, $P > 0.05$, Figs 3, S2 to S4). No nest reached the bottom of the soil
311 column, in any of the treatment. However, differences in temperature led to differences in nest
312 depth, with shallower nests for T_{mild} than T_{medium} and T_{high} (13.7 ± 3.5 for T_{mild} vs. 17.2 ± 3.2 and
313 19.3 ± 2.2 cm for T_{medium} and T_{high} , respectively $F_{2,14} = 7.99$, $P < 0.05$, T_{mild} vs T_{medium} , $P < 0.01$, T_{mild}
314 vs T_{high} , $P < 0.001$, T_{medium} vs T_{high} , $P < 0.05$).

315 Porosity profiles showed the vertical distribution of chambers and tunnels (Fig 3). The peak
316 of soil porosity (% of empty volume compared to soil volume) was located deeper under increased
317 temperature, ranging from 7.6 to 13.1 and 16.9 cm depths for T_{mild} , T_{medium} and T_{high} , respectively
318 (Fig S9).

319 The Principal Component Analysis (PCA) of the variables describing nest architecture (Fig
320 4, see also Tables S1 and S2) showed that the first axis described 40.9% of the total variance and
321 corresponded mainly to a gradient of size, with smaller nests towards higher values and bigger nests
322 toward lower values. This gradient also corresponds to nests development, with younger nests
323 towards higher values and older nests towards lower values. The second axis described 14.9% of
324 the whole variance and corresponded to a shape and position gradient. T_{medium} and T_{high} were better
325 described by size related variables (i.e. volume of nests, volume of chambers, volume of tunnels,

326 surface area (number of pixels corresponding to the external boundary of the 3D nest)) than by
327 shape or position related variables (i.e. number of pores, sphericity index). Conversely, T_{mild} was
328 better explained by shape than by size related variables. Nests excavated under T_{mild} differed from
329 those under T_{medium} and T_{high} . Nests under T_{medium} and T_{high} were similar at days 7, 14 and 28, but less
330 so at day 88 where the overlap in characteristics was lower. Within each treatment, nests differed
331 little between days 7 and 14, and increasingly differed from day 28 onwards, with nests becoming
332 deeper. This temporal change was less evident under T_{mild} than under T_{medium} and T_{high} , with nest
333 characteristics overlapping largely over days under T_{mild} , but not under T_{medium} and T_{high} .

334 Nest volume (V_{nest}) was affected by the interaction between the time and the temperature
335 treatments ($F_{3,24} = 3.28$, $P < 0.001$). There was no difference among treatments until day 88 when
336 the nests at T_{mild} were smaller than those at T_{medium} ($F_{2,14} = 5.20$, $P < 0.05$, Fig S6). Nests of colonies
337 at T_{high} showed intermediate volumes compared to the other two treatments. Chambers volume
338 (V_{Ch}) showed the same trend as the nest volume, i.e. it was affected by the interaction between the
339 time and temperature ($F_{3,24} = 3.67$, $P < 0.001$, Fig S6). Only on day 88, chambers in T_{medium} were
340 larger than T_{mild} whereas T_{high} had intermediate volumes ($F_{2,14} = 5.71$, $P < 0.05$, Fig S6). Tunnels
341 volume (V_{Tu}) increased over time ($F_{1,26} = 0.26$, $P < 0.001$), without influence of the temperature
342 ($F_{2,14} = 0.26$, $P > 0.05$, Fig S6).

343 The volume of refilled chambers (V_{Re}) was significantly larger for T_{high} than T_{medium} ($F_{2,14} =$
344 8.62 , $P = 0.011$) and T_{mild} ($F_{2,61} = 8.62$, $P = 0.006$) on day 28 (Fig S7). A similar trend was observed
345 on day 88 but was not statistically significant. The porosity profiles of the refilled chambers (Fig 5)
346 showed that they were mostly located in the first cm of the soil columns. The highest peak of V_{Re}
347 was observed on day 88 at 3.69, 6.98 and 5.04 cm depth for T_{mild} , T_{medium} , and T_{high} , respectively.

348 The tortuosity of the nest system was affected neither by the sampling time nor by the
349 temperature treatments ($F_{6,42} = 0.344$, $P > 0.05$). Coordination number did not present any changes
350 amongst treatments at any moment of the experiment ($F_{3,26} = 0.965$, $P > 0.05$).

351

352 *Water evaporation*

353 Water evaporation showed an effect of temperature, as soil columns exposed to T_{mild} lost less water
354 (185.2 ± 60.1 ml) than those exposed to T_{medium} (261.1 ± 75.5 ml) and T_{high} (293.1 ± 84.0 ml) during
355 the whole experiment ($F_{2,17} = 134.56$, $P < 0.001$). The evaporation rate of T_{medium} and T_{high}
356 treatments were similar to one another from the start of the experiment until day 79, date after
357 which T_{high} showed higher evaporation than T_{medium} ($F_{2,238} = 3.19$, $P < 0.001$, Fig S8). The presence
358 of ants had no effect on water evaporation since no difference occurred between soil columns with
359 and without ants in any of the treatments ($F_{2,167} = 0.21$, $P > 0.05$), however these results should be
360 treated with caution due to low number of replicates ($n=1$, per treatment).

361

362 *Development of the colonies*

363 The colonies exposed to T_{mild} (253 ± 122 workers, mean \pm SD) grew less than those under T_{medium}
364 and T_{high} (503 ± 93 workers, $F_{2,14} = 10.45$ $P < 0.05$, Fig S9, Table S3). Temperature had no effect on
365 the size of workers (1.67 ± 0.10 mm, mean \pm SD, $F_{2,167} = 0.37$, $P > 0.05$, Fig S9), but ants exposed
366 to T_{mild} were significantly heavier (0.25 ± 0.08 mg, mean \pm SD) than those exposed to T_{medium} and
367 T_{high} (0.18 ± 0.06 mg, $F_{2,167} = 19.67$, $P < 0.001$, Fig S10).

368

369 DISCUSSION

370 *Soil bioturbation by ants*

371 Ant activity is known to increase with the temperature in the field, i.e., during spring and summer
372 (Andrew et al., 2013; Nobua-Behrmann et al., 2017). Although this general trend was confirmed by
373 our experiment, we also found that soil excavation did not increase proportionally to the increase in
374 temperature (i.e., no difference between T_{medium} and T_{high} , Fig 2). However, ants seem to be located

375 proportionally deeper in the nests with increasing temperatures (Fig 3). We assume that the
376 modification of the nest architecture provided suitable conditions of temperature and humidity, so
377 that the ants did not need to excavate further to survive and develop. Also, the average daytime
378 surface temperature on T_{high} (47.9°C) was above their critical thermal maximum (CT_{max} , *i.e.*, the
379 temperature at which the individuals no longer control their locomotion (Lutterschmidt &
380 Hutchison, 1997)). At this temperature, ants could be at risk of thermal stress if the duration of
381 exposure was too long or if they were close to their point of desiccation. The CT_{max} of *L. niger*
382 measured through a stepwise method in our laboratory was 47.5°C, hence workers could be active
383 outside, *e.g.* for foraging and rejecting excavated soil, but not for too long or solely at night when
384 the temperatures decreased (Lei et al., 2021).

385 Soil excavation by *L. niger* occurred very rapidly in our experiment. Nests had already
386 reached their maximal depths at the time of the first X-ray scan at day 7, and they were later
387 enlarged by excavating chambers. Excavation in all treatments occurred with a phase of intense
388 digging (between days 1 and 28) followed by a phase of lower activity (between days 28 and 88,
389 Fig 2A). These results are comparable to the findings of Rasse & Deneubourg (2001) and Toffin et
390 al. (2009), who described an initial phase of maximal and fast digging activity (amplification
391 phase), followed by a second phase (saturation phase) when the activity ceases almost completely.
392 However, our results show that ants also respond to increased temperatures by modifying their
393 excavation process, since ants exposed to moderately increased temperatures (T_{medium}) intensified
394 their excavation after being almost inactive (around day 50, Fig 2A) while the others kept their
395 digging activity low. We could assume that the temperature stimulated the oviposition of the queen,
396 as previously described for other species like *Solenopsis invicta* (Abril et al., 2008; Asano &
397 Cassill, 2012). As expected, we also found that ants exposed to room temperature produced the
398 most superficial nests among the three treatments. Ants exposed to medium and high temperatures
399 built nests of similar maximal depth. However, chambers were distributed differently along the
400 depth of the nest. They were located deeper under high temperatures than under medium

401 temperatures (Fig 3), hinting that the higher the temperature on the surface, the deeper the ants were
402 located. In ant species living belowground, queens, workers, and brood are primarily located in
403 intermediate and lower chambers inside the nest, while upper chambers are empty, used for
404 stocking or seasonally used for warming up cocoons (Mikheyev & Tschinkel, 2004; Kadochová &
405 Frouz, 2013). As such, ants likely continually reshape their nests by refilling the less crowded upper
406 chambers, leaving the bottom, more populous part of the nest, untouched. In our study, we showed
407 that changes in surface temperature could significantly influence the dynamics of the nest chambers.
408 Indeed, we found that ants exposed to higher temperatures responded by filling back some of their
409 most shallow galleries (Fig 5). Although these structures were small (~2% of the total nest volume),
410 they were significantly larger for T_{medium} and T_{high} , in comparison with T_{mild} . The underground
411 movement of soil material carried by ants has been previously reported by Tschinkel and Seal
412 (2016) who showed that 17% of the soil excavated by the gardening ant, *Trachymyrmex*
413 *septentrionalis* was deposited underground. Also, Römer & Roces (2014) reported that in a 2D
414 setup, workers of *Acromyrmex lundii* adjust the size of chambers and tunnels depending on the
415 number of inhabiting workers and the presence of brood and fungus by either digging or refilling
416 spaces using pellets of soil previously removed from the nest.

417 Despite changes in soil porosity, no difference in soil water evaporation could be measured
418 between the columns with ants and those without ants in all three treatments. These results did not
419 confirm other previously reported findings that suggested that ants decrease (Li et al., 2017) or
420 increase (Woodell and King, in 1991; Blomqvist *et al.*, 2000) water evaporation from soil. Our
421 results could be explained by the specific conditions of our experiment. Nests had a single small
422 opening, and the total porosity due to nests was very low (less than 0.5% of the column volume).
423 Also, our soil columns were only 24 cm deep, and the differences in soil water evaporation among
424 treatments (Fig S8) most likely stemmed from the increased temperatures, that intensify the
425 atmospheric evaporative demand (i.e., drying, or evaporating power of the atmosphere)
426 (Vicente- Serrano et al., 2020), rather than from the ants' burrowing activities. Soil humidity is an

427 important factor in nest excavation. Ants burrowing activity depends on the grain size and level of
428 soil moisture (i.e. ants tend not to dig when the soil is fully wet and when it is completely dry
429 (Monaenkova et al., 2015)). Our results show that ants respond to warming by modifying nest
430 architecture, presumably setting up chambers at the depth providing adequate temperature for brood
431 development. We kept humidity constant in our experiment; however, soil humidity may also be
432 affected by global changes so that ants may have to compromise between nest architecture
433 optimising one factor or the other.

434

435 *Influence of changes in surface temperature on colony development*

436 This study dealt with the influence of an increase in temperature and ant colony development.
437 Colonies grew under the three temperature treatments, which suggests that the nests and food
438 provided adequate conditions for colony development. However, colonies reared under room
439 temperature (T_{mild} , 22.2°C during daytime) produced fewer workers than those reared under higher
440 temperatures (35.9 and 47.9°C). This may be due to room temperature being lower than the natural
441 temperature since *L. niger* typically occurs in open environments where the sun heats the soil, often
442 above this temperature. Colonies produced normal-sized workers under the three temperatures (3.5-
443 5.0 mm length (Seifert, 2018)), despite ant development being sensitive to temperature (i.e. high
444 temperatures promote maturity at smaller size in ectotherms, Atkinson, 1994, Verberk et al., 2021).
445 Given that nest architecture was affected by soil surface temperature, one may hypothesize that ants
446 modified nest architecture so that it provided adequate abiotic environment (temperature, humidity)
447 to allow for the development of brood into normal-sized workers. Indeed, ant nests provide
448 protection against predators but also against environmental hazards (Porter, 1988; Porter &
449 Tschinkel, 1993), and it is well known that ants place brood of various developmental stages at
450 varied nest depths to provide it with optimal environment (Penick & Tschinkel, 2008). This
451 modification of the nest requires additional soil excavation and is energetically costly. Therefore,
452 species with shallow nests (i.e. more exposed to increased surface temperature) or with small

453 colony size or with foundation of new colonies by a solitary queen (i.e. less capable of supporting
454 the cost of extra excavation) may be more affected by increased temperature than species with deep
455 nests, large populations or foundation of new colonies by colony fission (Cronin et al., 2013). For
456 instance, queens founding new colonies solitarily could excavate deeper nests to provide optimal
457 conditions to their brood, but this extra energetic expenditure would presumably decrease the
458 number of workers they would produce, hence negatively affect the incipient colony survival and
459 growth. Alternatively, they could not adjust nest depth and their brood would develop at a higher
460 temperature than normal and presumably yield smaller workers with lower foraging efficiency and
461 lifespan, again presumably negatively affecting the incipient colony survival and growth. In
462 comparison, queens founding new colony by colony fission would be less affected as the workers
463 accompanying the queen carry out nest excavation.

464 In our study, increased temperatures seem to allow the growth of the colonies. Overall, the
465 only negative effect that we could observe in ants exposed to high temperatures (T_{medium} and T_{high})
466 was the reduction of worker body mass compared to those reared under room temperature (T_{mild} , Fig
467 S8). This may result from increased metabolic costs during worker development in warm
468 environments, as previously reported in ants (Kaspari, 2005). This could decrease workers
469 longevity and foraging efficiency, for instance by lowering their competitiveness against larger
470 workers of other colonies or species. Alternatively, it may be that workers reared under T_{mild} were
471 heavier than those exposed to high and medium temperatures because the brood grew slower (as
472 colonies grew less) and worker stored food resources as fat bodies.

473

474 *Global warming and its consequences on nest and colony*

475 Our study, as well as several others (Jenkins et al., 2011; Verberk et al., 2021; Parr & Bishop, 2022;
476 Nascimento et al., 2022), support the idea that ants would be affected by the augmentation of global
477 temperatures. At first glance, ants successfully mitigated the effect of high temperatures by

478 excavating deeper nests as colonies grew well and produced normal-sized workers under high
479 temperatures. However, our experiment focused on nest architecture did not include the effects of
480 temperature on worker longevity or foraging (direct effects such as heat stress and desiccation,
481 indirect effects such as food availability and competition). In addition, it lasted 100 days and longer
482 exposure to high temperature may reveal accumulated effects, which are important when evaluating
483 thermal risk (Jørgensen et al., 2021).

484 It has been suggested that ants of temperate and cold environments, such as our model
485 species *L. niger*, are more resistant to global warming because they already deal with strong
486 temperature variations between summer and winter (Diamond et al., 2012; Andrew et al., 2013).
487 Our results support this hypothesis as *L. niger* showed resistance to high temperatures through nest
488 plasticity. However, heat induced changes in *L. niger*'s nest architecture may modify physical soil
489 structure as this species is highly abundant in temperate environments (Czerwinski et al., 1971;
490 Holec & Frouz, 2006) and contributes to the movement of soil particles and the dynamic of water
491 (Cerdà & Jurgensen, 2008). Moreover, modifications in colony development could have important
492 ecological consequences due to its prevalence as topsoil predator that feeds on invertebrates, seeds,
493 and honeydew (Czerwinski et al., 1971), and its dominance in initial stages of ecological succession
494 (Dauber & Wolters, 2005). Our results need to be confirmed under natural conditions as our setup
495 could not exactly mimic the natural temperature dynamics. For instance, soil humidity was kept
496 constant, and temperature rapidly rose and fell (lamps on or off) whereas variation is more gradual
497 in nature. However, rapid fluctuations in temperature will be increasingly common as global
498 warming increases the intensity and frequency of heat-waves, which reduced the foraging
499 efficiency of soil-dwelling ants living in temperate environments (Andrew et al., 2013).

500 Carbon dioxide is expected to rise in the atmosphere as a consequence of climate change.
501 Its effects on ants are not well understood yet (Parr & Bishop, 2022) and we did not measure it in
502 our experiment. Nevertheless, it could affect nest architecture. Ants cope with levels of CO₂ through
503 ventilation systems and moving the brood to shallower chambers where the levels are lower. For

504 example, the leafcutter ants *A. lundii* prefers to excavate soils with lower content of CO₂, which
505 could explain why they excavate superficial chambers probably to suit the needs of the fungus
506 inside and brood the nest (Römer et al., 2018). Nevertheless, these effects seem to depend on the
507 size of the colony, as experiments with fewer individuals of *Formica pallidefulva* did not show any
508 difference on excavation (Mikheyev & Tschinkel, 2004). We show that *L. niger* ants respond to
509 heat by adjusting nest architecture in the laboratory, but global warming is a complex phenomenon
510 with multiple effects (heat, humidity, gases, etc) that need to be investigated in several ant species
511 to see how they will respond to these changes.

512

513 CONCLUSION

514 Our hypothesis that ants modify the architecture of their nest as a response to temperature was
515 confirmed, since most architectural features and digging activities were increased by increased
516 temperatures, probably to provide an adequate environment to the colony. Also, ants were able to
517 produce normal-sized workers despite the high temperatures. As such, our study supports the idea
518 that ant nest architecture is a plastic response to environmental changes (i.e., ant nest as an extended
519 phenotype construction, sensu Jouquet et al., 2006; Richards, 2009; Sankovitz & Purcell, 2021).
520 Interestingly, bioturbation was not proportional to the increase in temperature. Therefore, we
521 foresee two different possibilities: a) our experiment reached a temperature threshold above which
522 the effects on nest architecture and bioturbation are no longer discernible, or b) if exposure to high
523 temperature continued for longer, additional or stronger effects would be observed. Longer studies
524 with narrower temperature intervals between treatments would improve our understanding of the
525 effects of increasing temperatures on ants' bioturbation and nest architecture. Moreover, more
526 research is also needed to determine how global warming will impact ant bioturbation in different
527 ecosystems (e.g., in the tropics, in different soil types, in presence of plants) and if this could in turn
528 affect other ecological processes such as soil organic matter or water dynamics in soils.

530 REFERENCES

- 531 Abril, S., Oliveras, J., & Gómez, C. (2008). Effect of temperature on the oviposition rate of
532 Argentine ant queens (*Linepithema humile* Mayr) under monogynous and polygynous
533 experimental conditions. *Journal of Insect Physiology*, 54(1), 265–272.
534 <https://doi.org/10.1016/j.jinsphys.2007.09.009>
- 535 Adler, F. R., & Gordon, D. M. (2003). Foraging Ranges in Ants. *The American Naturalist*, 162(5),
536 529–543. <https://doi.org/10.1086/378856>
- 537 Andrew, N. R., Hart, R. A., Jung, M. P., Hemmings, Z., & Terblanche, J. S. (2013). Can temperate
538 insects take the heat? A case study of the physiological and behavioural responses in a
539 common ant, *Iridomyrmex purpureus* (Formicidae), with potential climate change. *Journal of*
540 *Insect Physiology*, 59(9), 870–880. <https://doi.org/10.1016/j.jinsphys.2013.06.003>
- 541 Asano, E., & Cassill, D. L. (2012). Modeling temperature-mediated fluctuation in colony size in the
542 fire ant, *Solenopsis invicta*. *Journal of Theoretical Biology*, 305, 70–77.
543 <https://doi.org/10.1016/j.jtbi.2012.03.011>
- 544 Atkinson, D. (1994). Temperature and Organism Size—A Biological Law for Ectotherms?
545 *Advances in Ecological Research*, 25(C), 1–58. [https://doi.org/10.1016/S0065-](https://doi.org/10.1016/S0065-2504(08)60212-3)
546 [2504\(08\)60212-3](https://doi.org/10.1016/S0065-2504(08)60212-3)
- 547 Benckiser, G. (2010). Ants and sustainable agriculture. A review. *Agronomy for Sustainable*
548 *Development*, 30(2), 191–199. <https://doi.org/10.1051/agro/2009026>
- 549 Blomqvist, M. M., Olff, H., Blaauw, M. B., Bongers, T., & Van Der Putten, W. H. (2000).
550 Interactions between above- and belowground biota: importance for small-scale vegetation
551 mosaics in a grassland ecosystem. *Oikos*, 90(3), 582–598. [https://doi.org/10.1034/j.1600-](https://doi.org/10.1034/j.1600-0706.2000.900316.x)
552 [0706.2000.900316.x](https://doi.org/10.1034/j.1600-0706.2000.900316.x)

- 553 Bollazzi, M., & Roces, F. (2010). The Thermoregulatory Function of Thatched Nests in the South
554 American Grass-Cutting Ant, *Acromyrmex heyeri*. *Journal of Insect Science*, *10*(137), 1–17.
555 <https://doi.org/10.1673/031.010.13701>
- 556 Booth, S., Kurtz, B., Heer, M. I., Mooney, S. J., & Sturrock, C. J. (2020). Tracking wireworm
557 burrowing behaviour in soil over time using 3D X- ray computed tomography. *Pest*
558 *Management Science*, *76*(8), 2653–2662. <https://doi.org/10.1002/ps.5808>
- 559 Bottinelli, N., Jouquet, P., Capowiez, Y., Podwojewski, P., Grimaldi, M., & Peng, X. (2015). Why
560 is the influence of soil macrofauna on soil structure only considered by soil ecologists? *Soil*
561 *and Tillage Research*, *146*(PA), 118–124. <https://doi.org/10.1016/j.still.2014.01.007>
- 562 Cammeraat, E. L. H., & Risch, A. C. (2008). The impact of ants on mineral soil properties and
563 processes at different spatial scales. *Journal of Applied Entomology*, *132*(4), 285–294.
564 <https://doi.org/10.1111/j.1439-0418.2008.01281.x>
- 565 Cerdà, A., & Jurgensen, M. F. (2008). The influence of ants on soil and water losses from an orange
566 orchard in eastern Spain. *Journal of Applied Entomology*, *132*(4), 306–314.
567 <https://doi.org/10.1111/j.1439-0418.2008.01267.x>
- 568 Cheik, S., Jouquet, P., Maeght, J. L., Capowiez, Y., Tran, T. M., & Bottinelli, N. (2021). X- ray
569 tomography analysis of soil biopores structure under wetting and drying cycles. *European*
570 *Journal of Soil Science*. <https://doi.org/10.1111/ejss.13119>
- 571 Coenen-Stass, D., Schaarschmidt, B., & Lamprecht, I. (1980). Temperature Distribution and
572 Calorimetric Determination of Heat Production in the Nest of the Wood Ant, *Formica*
573 *Polycтена* (Hymenoptera, Formicidae). *Ecology*, *61*(2), 238–244.
574 <https://doi.org/10.2307/1935180>
- 575 Cronin, A. L., Molet, M., Doums, C., Monnin, T., & Peeters, C. (2013). Recurrent Evolution of
576 Dependent Colony Foundation Across Eusocial Insects. *Annual Review of Entomology*, *58*(1),

577 37–55. <https://doi.org/10.1146/annurev-ento-120811-153643>

578 Czechowski, W., Radchenko, A., Czechowska, W., & Vepsäläinen, K. (2012). *The ants of Poland*
579 *with reference to the myrmecofauna of Europe*. Natura Optima dux Foundation.

580 Czerwinski, Z., Jakub-zyk, H., & Petal, J. (1971). Influence of ant hills on the meadow soils.
581 *Pedobiologia*.

582 Dauber, J., & Wolters, V. (2005). Colonization of temperate grassland by ants. *Basic and Applied*
583 *Ecology*, 6(1), 83–91. <https://doi.org/10.1016/j.baae.2004.09.011>

584 Diamond, S. E., Sorger, D. M., Hulcr, J., Pelini, S. L., Toro, I. Del, Hirsch, C., Oberg, E., & Dunn,
585 R. R. (2012). Who likes it hot? A global analysis of the climatic, ecological, and evolutionary
586 determinants of warming tolerance in ants. *Global Change Biology*, 18(2), 448–456.
587 <https://doi.org/10.1111/J.1365-2486.2011.02542.X>

588 Farji-Brener, A. G., & Werenkraut, V. (2017). The effects of ant nests on soil fertility and plant
589 performance: a meta-analysis. *Journal of Animal Ecology*, 86(4), 866–877.
590 <https://doi.org/10.1111/1365-2656.12672>

591 Finér, L., Jurgensen, M. F., Domisch, T., Kilpeläinen, J., Neuvonen, S., Punttila, P., Risch, A. C.,
592 Ohashi, M., & Niemelä, P. (2013). The Role of Wood Ants (*Formica rufa* group) in Carbon
593 and Nutrient Dynamics of a Boreal Norway Spruce Forest Ecosystem. *Ecosystems*, 16(2),
594 196–208. <https://doi.org/10.1007/s10021-012-9608-1>

595 Frouz, J., & Jilková, V. (2008). The effect of ants on soil properties and processes (Hymenoptera :
596 Formicidae). *Myrmecological News*, 11(August), 191–199.

597 Gerard, B. M. (1977). Factors affecting earthworms in pastures. *Journal of Animal Ecology*, 36(1),
598 235–252. <https://doi.org/10.2307/3024>

599 Holec, M., & Frouz, J. (2006). The effect of two ant species *Lasius niger* and *Lasius flavus* on soil
600 properties in two contrasting habitats. *European Journal of Soil Biology*, 42(SUPPL. 1),

601 S213–S217. <https://doi.org/10.1016/j.ejsobi.2006.07.033>

602 Hulbert, A., Ballantyne, F., & Powell, S. (2008). Shaking a leg and hot to trot: the effects of body
603 size and temperature on running speed in ants. *Ecological Entomology*, 33, 144–154.
604 <https://doi.org/10.1111/j.1365-2311.2007.00962.x>

605 Jenkins, C. N., Sanders, N. J., Andersen, A. N., Arnan, X., Brühl, C. A., Cerda, X., Ellison, A. M.,
606 Fisher, B. L., Fitzpatrick, M. C., Gotelli, N. J., Gove, A. D., Guénard, B., Lattke, J. E.,
607 Lessard, J. P., Mcglynn, T. P., Menke, S. B., Parr, C. L., Philpott, S. M., Vasconcelos, H. L.,
608 ... Dunn, R. R. (2011). Global diversity in light of climate change: The case of ants. *Diversity
609 and Distributions*, 17(4), 652–662. <https://doi.org/10.1111/j.1472-4642.2011.00770.x>

610 Jørgensen, L. B., Malte, H., Ørsted, M., Klahn, N. A., & Overgaard, J. (2021). A unifying model to
611 estimate thermal tolerance limits in ectotherms across static, dynamic and fluctuating
612 exposures to thermal stress. *Scientific Reports*, 11(1), 12840. [https://doi.org/10.1038/s41598-
613 021-92004-6](https://doi.org/10.1038/s41598-021-92004-6)

614 Jørgensen, L. B., Ørsted, M., Malte, H., Wang, T., & Overgaard, J. (2022). Extreme escalation of
615 heat failure rates in ectotherms with global warming. *Nature*, 611(7934), 93–98.
616 <https://doi.org/10.1038/s41586-022-05334-4>

617 Jouquet, P., Dauber, J., Lagerlöf, J., Lavelle, P., & Lepage, M. (2006). Soil invertebrates as
618 ecosystem engineers: Intended and accidental effects on soil and feedback loops. *Applied Soil
619 E*, 32, 153–164. <https://doi.org/10.1016/j.apsoil.2005.07.004>

620 Kadochová, Š., & Frouz, J. (2013). Thermoregulation strategies in ants in comparison to other
621 social insects, with a focus on red wood ants (*Formica rufa* group). *F1000Research*, 2.
622 <https://doi.org/10.12688/f1000research.2-280.v2>

623 Kaspari, M. (2005). Global energy gradients and size in colonial organisms: Worker mass and
624 worker number in ant colonies. *Proceedings of the National Academy of Sciences of the*

625 *United States of America*, 102(14), 5079–5083. <https://doi.org/10.1073/pnas.0407827102>

626 Kassambara, A. (2021). *rstatix: Pipe-Friendly Framework for Basic Statistical Tests*. R package
627 *version 0.7.0*. <https://cran.r-project.org/package=rstatix>

628 Kassambara, A., & Mundt, F. (2020). *Factoextra: Extract and Visualize the Results of Multivariate*
629 *Data Analyses*. R Package Version 1.0.7. <https://cran.r-project.org/package=factoextra>

630 Kleineidam, C., Ernst, R., & Roces, F. (2001). Wind-induced ventilation of the giant nests of the
631 leaf-cutting ant *Atta vollenweideri*. *Naturwissenschaften*, 88(7), 301–305.
632 <https://doi.org/10.1007/S001140100235>

633 Korb, J., & Linsenmair, K. E. (1998). The effects of temperature on the architecture and distribution
634 of *Macrotermes bellicosus* (isoptera, macrotermitinae) mounds in different habitats of a West
635 African Guinea savanna. *Insectes Sociaux*, 45(1), 51–65.
636 <https://doi.org/10.1007/s000400050068>

637 Lavelle, P., Bignell, D., Lepage, M., Wolters, V., Roger, P., Ineson, P., Heal, O. W., & Dhillon, S.
638 (1997). Soil function in a changing world: The role of invertebrate ecosystem engineers.
639 *European Journal of Soil Biology*, 33(4), 159–193.

640 Lei, Y., Jaleel, W., Faisal Shahzad, M., Ali, S., Azad, R., Muhammad Ikram, R., Ali, H., Ghramh,
641 H. A., Ali Khan, K., Qiu, X., He, Y., & LYU, L. (2021). Effect of constant and fluctuating
642 temperature on the circadian foraging rhythm of the red imported fire ant, *Solenopsis invicta*
643 Buren (Hymenoptera: Formicidae). *Saudi Journal of Biological Sciences*, 28(1), 64–72.
644 <https://doi.org/10.1016/j.sjbs.2020.08.032>

645 Li, T., Shao, M., & Jia, Y. (2017). Effects of activities of ants (*Camponotus japonicus*) on soil
646 moisture cannot be neglected in the northern Loess Plateau. *Agriculture, Ecosystems &*
647 *Environment*, 239, 182–187. <https://doi.org/10.1016/j.agee.2017.01.024>

648 Lutterschmidt, W. I., & Hutchison, V. H. (1997). The critical thermal maximum: History and

649 critique. *Canadian Journal of Zoology*, 75(10), 1561–1574. <https://doi.org/10.1139/z97-783>

650 Meysman, F. J. R., Middelburg, J. J., & Heip, C. H. R. (2006). Bioturbation: a fresh look at
651 Darwin's last idea. *Trends in Ecology & Evolution*, 21(12), 688–695.
652 <https://doi.org/10.1016/j.tree.2006.08.002>

653 Mikheyev, A. S., & Tschinkel, W. R. (2004). Nest architecture of the ant *Formica pallidefulva*:
654 structure, costs and rules of excavation. *Insectes Sociaux*, 51(1), 30–36.
655 <https://doi.org/10.1007/s00040-003-0703-3>

656 Minter, N. J., Franks, N. R., & Robson Brown, K. A. (2012). Morphogenesis of an extended
657 phenotype: four-dimensional ant nest architecture. *Journal of The Royal Society Interface*,
658 9(68), 586–595. <https://doi.org/10.1098/rsif.2011.0377>

659 Monaenkova, D., Gravish, N., Rodriguez, G., Kutner, R., Goodisman, M. A. D., & Goldman, D. I.
660 (2015). Behavioral and mechanical determinants of collective subsurface nest excavation.
661 *Journal of Experimental Biology*, 218(9), 1295–1305. <https://doi.org/10.1242/jeb.113795>

662 Moreira, A. A., Forti, L. C., Andrade, A. P. P., Boaretto, M. A. C., & Lopes, J. F. S. (2004). Nest
663 architecture of *Atta laevigata* (F. Smith, 1858) (Hymenoptera: Formicidae). *Studies on*
664 *Neotropical Fauna and Environment*, 39(2), 109–116.
665 <https://doi.org/10.1080/01650520412331333756>

666 Nascimento, G., Câmara, T., & Arnan, X. (2022). Critical thermal limits in ants and their
667 implications under climate change. *Biological Reviews*, 97(4), 1287–1305.
668 <https://doi.org/10.1111/brv.12843>

669 Nobua-Behrmann, Beatriz E. Lopez de Casenave, Javier Milesi, F. A., & Farji-Brener, A. (2017).
670 Coexisting in harsh environments: temperature-based foraging patterns of two desert leafcutter
671 ants (Hymenoptera: Formicidae: Attini). *Myrmecological News*, 25(October), 41–49.

672 Parr, C. L., & Bishop, T. R. (2022). The response of ants to climate change. *Global Change*

673 *Biology, January*, 1–18. <https://doi.org/10.1111/gcb.16140>

674 Paton, T. R., Humphreys, G. S., & Mitchell, P. B. (1995). *Soils. A new global view* (First edit). UCL
675 Press.

676 Penick, C. A., Diamond, S. E., Sanders, N. J., & Dunn, R. R. (2017). Beyond thermal limits:
677 comprehensive metrics of performance identify key axes of thermal adaptation in ants.
678 *Functional Ecology*, *31*(5), 1091–1100. <https://doi.org/10.1111/1365-2435.12818>

679 Penick, C. A., & Tschinkel, W. R. (2008). Thermoregulatory brood transport in the fire ant,
680 *Solenopsis invicta*. *Insectes Sociaux*, *55*(2), 176–182. [https://doi.org/10.1007/s00040-008-](https://doi.org/10.1007/s00040-008-0987-4)
681 [0987-4](https://doi.org/10.1007/s00040-008-0987-4)

682 Perel, T. S. (1977). Differences in Lumbricid Organization Connected with Ecological Properties.
683 *Ecological Bulletins*, *25*, 56–63.

684 Perna, A., Jost, C., Couturier, E., Valverde, S., Douady, S., & Theraulaz, G. (2008). The structure of
685 gallery networks in the nests of termite *Cubitermes* spp. revealed by X-ray tomography.
686 *Naturwissenschaften*, *95*(9), 877–884. <https://doi.org/10.1007/S00114-008-0388-6>

687 Pham, Q. V., Nguyen, T. T., Lam, D. H., Capowiez, Y., Nguyen, A. D., Jouquet, P., Tran, T. M., &
688 Bottinelli, N. (2023). Using morpho-anatomical traits to predict the effect of earthworms on
689 soil water infiltration. *Geoderma*, *429*, 116245.
690 <https://doi.org/10.1016/j.geoderma.2022.116245>

691 Pinter-Wollman, N. (2015). Nest architecture shapes the collective behaviour of harvester ants.
692 *Biology Letters*, *11*(10). <https://doi.org/10.1098/rsbl.2015.0695>

693 Porter, S. D. (1988). Impact of temperature on colony growth and developmental rates of the ant,
694 *Solenopsis invicta*. *Journal of Insect Physiology*, *34*(12), 1127–1133.
695 [https://doi.org/10.1016/0022-1910\(88\)90215-6](https://doi.org/10.1016/0022-1910(88)90215-6)

696 Porter, S., & Tschinkel, W. (1993). Fire ant thermal preferences: behavioral control of growth and

697 metabolism. *Behavioral Ecology and Sociobiology*, 32(5), 321–329.
698 <https://doi.org/10.1007/bf00183787>

699 Rasse, P., & Deneubourg, J. L. (2001). Dynamics of Nest Excavation and Nest Size Regulation of
700 *Lasius niger* (Hymenoptera: Formicidae). *Journal of Insect Behavior*, 14(433–449).
701 <https://doi.org/10.1023/a:1011163804217>

702 Richards, P. J. (2009). Aphaenogaster ants as bioturbators: Impacts on soil and slope processes.
703 *Earth-Science Reviews*, 96(1–2), 92–106. <https://doi.org/10.1016/j.earscirev.2009.06.004>

704 Römer, D., Halboth, F., Bollazzi, M., & Roces, F. (2018). Underground nest building: the effect of
705 CO₂ on digging rates, soil transport and choice of a digging site in leaf-cutting ants. *Insectes*
706 *Sociaux*, 65(2), 305–313. <https://doi.org/10.1007/s00040-018-0615-x>

707 Römer, D., & Roces, F. (2014). Nest Enlargement in Leaf-Cutting Ants: Relocated Brood and
708 Fungus Trigger the Excavation of New Chambers. *PLoS ONE*, 9(5), e97872.
709 <https://doi.org/10.1371/journal.pone.0097872>

710 Römer, D., & Roces, F. (2015). Available space, symbiotic fungus and colony brood influence
711 excavation and lead to the adjustment of nest enlargement in leaf-cutting ants. *Insectes*
712 *Sociaux*, 62(4), 401–413. <https://doi.org/10.1007/s00040-015-0419-1>

713 Sankovitz, M., & Purcell, J. (2021). Ant nest architecture is shaped by local adaptation and plastic
714 response to temperature. *Scientific Reports 2021 11:1*, 11(1), 1–10.
715 <https://doi.org/10.1038/s41598-021-02491-w>

716 Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S.,
717 Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K.,
718 Tomancak, P., & Cardona, A. (2012). Fiji: an open-source platform for biological-image
719 analysis. *Nature Methods*, 9(7), 676–682. <https://doi.org/10.1038/nmeth.2019>

720 Seifert, B. (2018). *The Ants of Central and North Europe*. Lutra-Verlag.

721 Sousa, K. K. A., Camargo, R. S., Caldato, N., Farias, A. P., Matos, C. A. O., Zanuncio, J. C.,
722 Santos, I. C. L., & Forti, L. C. (2021). Carbon dioxide levels in initial nests of the leaf-cutting
723 ant *Atta sexdens* (Hymenoptera: Formicidae). *Scientific Reports*, *11*(1), 20562.
724 <https://doi.org/10.1038/s41598-021-00099-8>

725 Toffin, E., Di Paolo, D., Campo, A., Detrain, C., & Deneubourg, J. L. (2009). Shape transition
726 during nest digging in ants. *Proceedings of the National Academy of Sciences of the United*
727 *States of America*, *106*(44), 18616–18620. <https://doi.org/10.1073/pnas.0902685106>

728 Tschinkel, W. R. (2003). Subterranean ant nests: trace fossils past and future? *Palaeogeography,*
729 *Palaeoclimatology, Palaeoecology*, *192*(1–4), 321–333. [https://doi.org/10.1016/S0031-](https://doi.org/10.1016/S0031-0182(02)00690-9)
730 [0182\(02\)00690-9](https://doi.org/10.1016/S0031-0182(02)00690-9)

731 Tschinkel, W. R. (2004). The nest architecture of the Florida harvester ant, *Pogonomymex badius*.
732 *Journal of Insect Science*, *4*(1). <https://doi.org/10.1093/jis/4.1.21>

733 Tschinkel, W. R. (2010). Methods for Casting Subterranean Ant Nests. *Journal of Insect Science*,
734 *10*(88), 1–17. <https://doi.org/10.1673/031.010.8801>

735 Tschinkel, W. R. (2021). *Ant architecture. The wonder, beauty, and science of underground nests*.
736 Princeton University Press.

737 Tschinkel, W. R., & Seal, J. N. (2016). Bioturbation by the Fungus-Gardening Ant, *Trachymymex*
738 *septentrionalis*. *PLOS ONE*, *11*(7), e0158920. <https://doi.org/10.1371/journal.pone.0158920>

739 Verberk, W. C. E. P., Atkinson, D., Hoefnagel, K. N., Hirst, A. G., Horne, C. R., & Siepel, H.
740 (2021). Shrinking body sizes in response to warming: explanations for the temperature–size
741 rule with special emphasis on the role of oxygen. *Biological Reviews*, *96*(1), 247–268.
742 <https://doi.org/10.1111/brv.12653>

743 Verza, S. S., Gervásio, R. C. R. G., Alves e Silva, O. M., Gomes, M. O., Souza, S. A., & Mussury,
744 R. M. (2020). Nest structure engineering of the leaf-cutting ant, *Acromymex landolti*, in the

745 semiarid Caatinga biome. *Insectes Sociaux*, 67(1), 147–153. <https://doi.org/10.1007/s00040->
746 019-00738-4

747 Vicente- Serrano, S. M., McVicar, T. R., Miralles, D. G., Yang, Y., & Tomas- Burguera, M.
748 (2020). Unraveling the influence of atmospheric evaporative demand on drought and its
749 response to climate change. *WIREs Climate Change*, 11(2). <https://doi.org/10.1002/wcc.632>

750 Viles, H. A., Goudie, A. S., & Goudie, A. M. (2021). Ants as geomorphological agents: A global
751 assessment. *Earth-Science Reviews*, 213, 103469.
752 <https://doi.org/10.1016/j.earscirev.2020.103469>

753 Villani, M. G., & Wright, R. J. (1990). Environmental influences on soil macroarthropod behavior
754 in agricultural systems. *Annual Review of Entomology*, 35(1), 249–269.
755 <https://doi.org/10.1146/annurev.en.35.010190.001341>

756 Vogt, J. T., Wallet, B., & Coy, S. (2008). Dynamic thermal structure of imported fire ant mounds.
757 *Journal of Insect Science*, 8. <https://doi.org/10.1673/031.008.3101>

758 Weber, N. A. (1938). The biology of the fungus-growing ants. Part 4. Additional new forms.
759 *Revista de Entomologia*, 9(1–2), 154–206.

760 Weil, R. R., & Brady, N. C. (2017). *The nature and properties of soils* (15th editi). Pearson
761 Education.

762 Wilkinson, M. T., Richards, P. J., & Humphreys, G. S. (2009). Breaking ground: Pedological,
763 geological, and ecological implications of soil bioturbation. In *Earth-Science Reviews* (Vol.
764 97, Issues 1–4, pp. 257–272). Elsevier. <https://doi.org/10.1016/j.earscirev.2009.09.005>

765 Woodell, S. R. J., & King, T. J. (1991). The influence of mound-building ants on British lowland
766 vegetation. In C. R. Huxley & D. F. Cutler (Eds.), *Ant-plant interactions* (pp. 521–535).
767 Oxford Univ. Press.

768

769

770

771

772

773

774

775

776

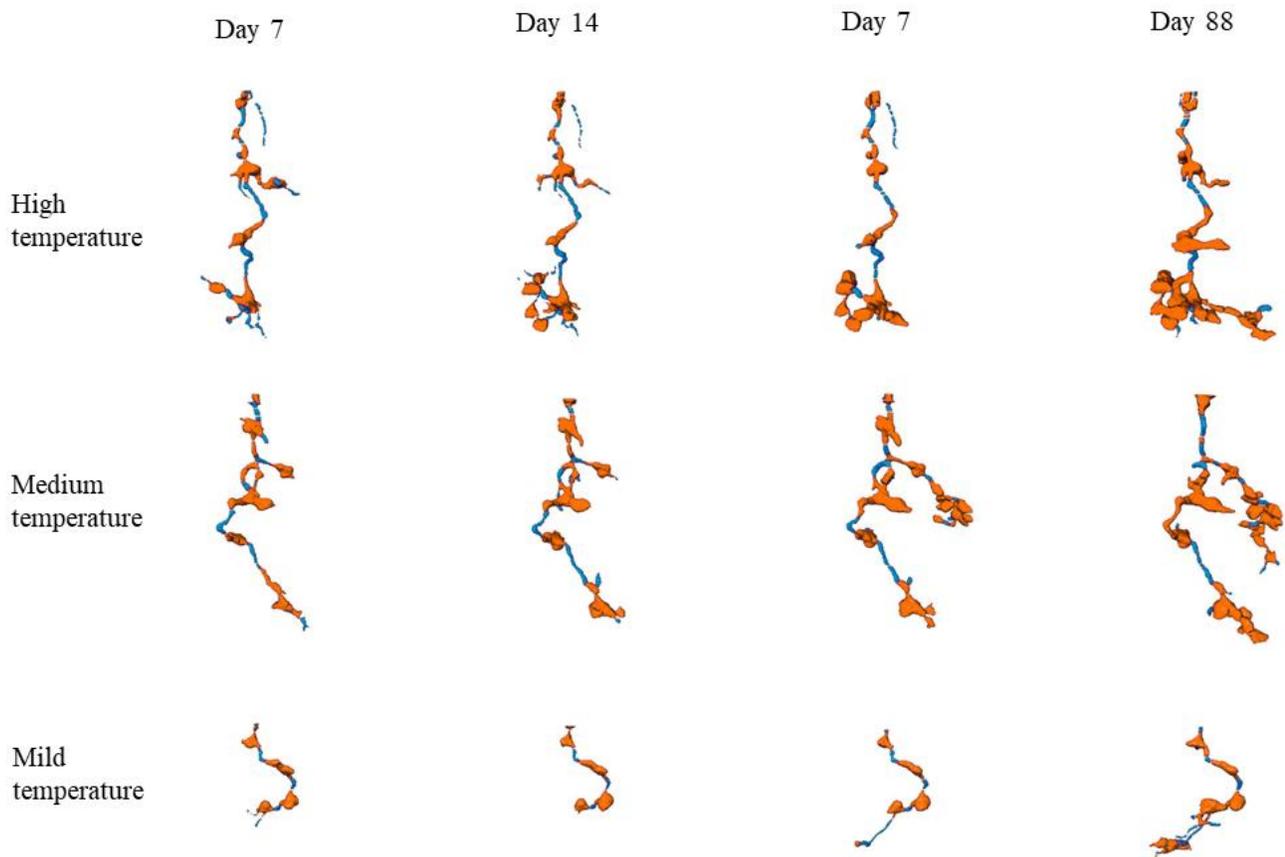


Fig 1. Examples of three nests illustrating the effects of treatment and time on their structure. Chambers (orange) and tunnels (blue) can be distinguished.

777

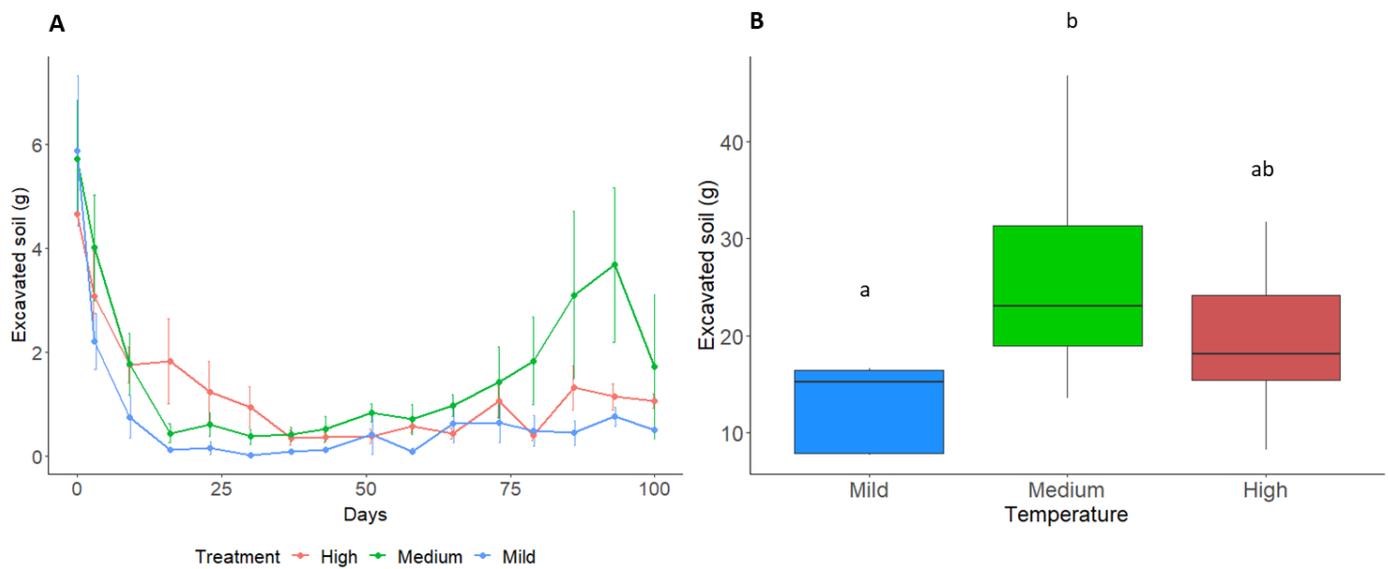


Fig 2. Amount of soil excavated (g) at each time point during the whole experiment (A) and total amount of soil excavated during the experiment (B). Bars in (A) indicate SEM.
778

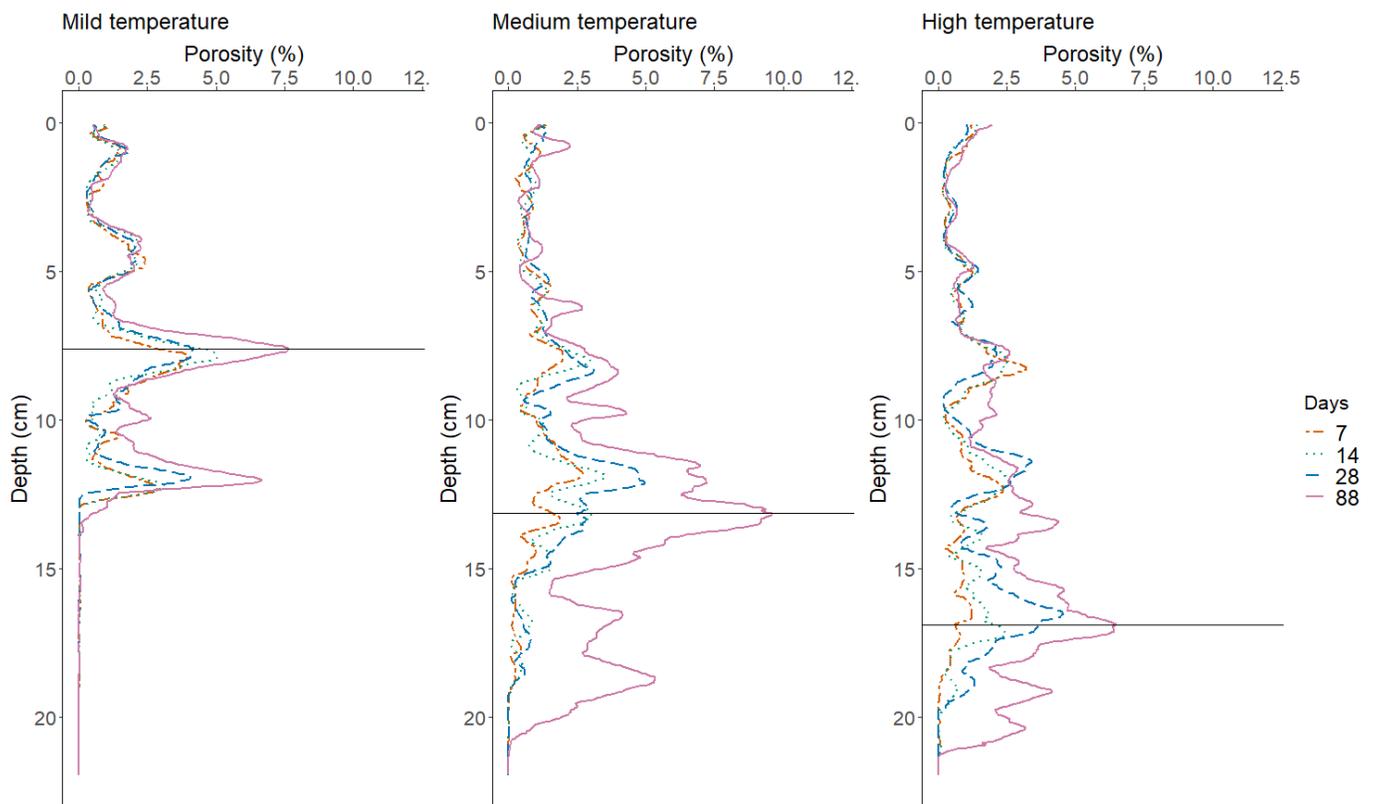


Fig 3. Mean porosity profiles (percentage of the empty volume compared to soil volume) per treatment. Colours and line types correspond to dates. Black horizontal lines indicate the point of maximal porosity on day 88.

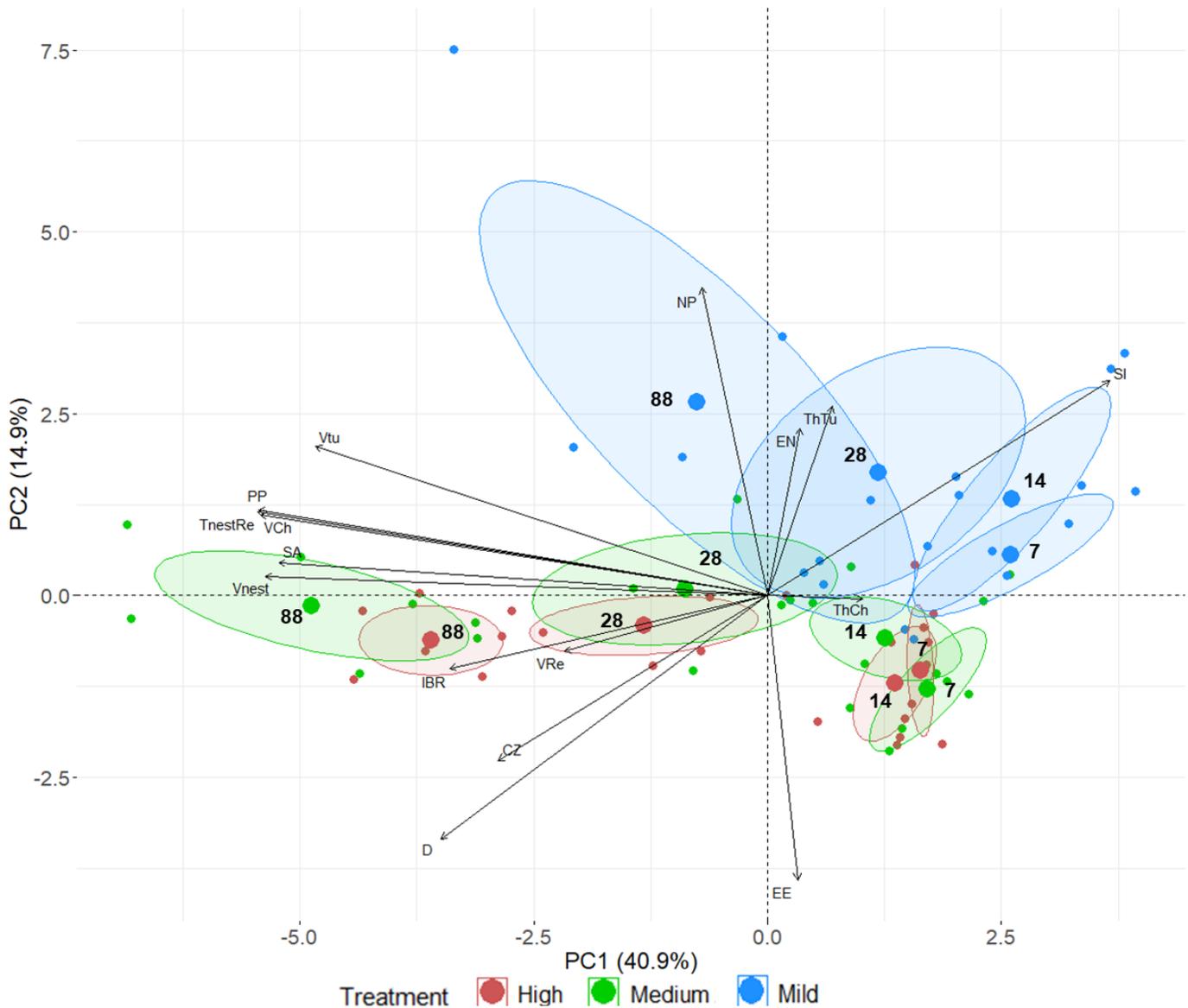


Fig 4. Biplot showing the principal component analysis (PCA) from variables describing the nest architectural features. Variables are sphericity index (SI), largest inscribed ball radius (IBR), ellipse elevation (EE), number of pores (NP), surface area (SA), Euler's number (EN), centroid Z (CZ), maximal nest depth (D), tunnel thickness (Th_{Tu}), chamber thickness (Th_{Ch}), nest volume (V_{nest}), tunnel volume (V_{Tu}), chamber volume (V_{Ch}), refilled chambers volume (V_{Re}), nest volume + refilled chambers volume (T_{nestRe}) and porosity percentage (PP). All volumes are expressed in percentage of volumes compared to total column volume. Numbers correspond to the day of tomography. Ellipses correspond to the confidence interval (95%).

779

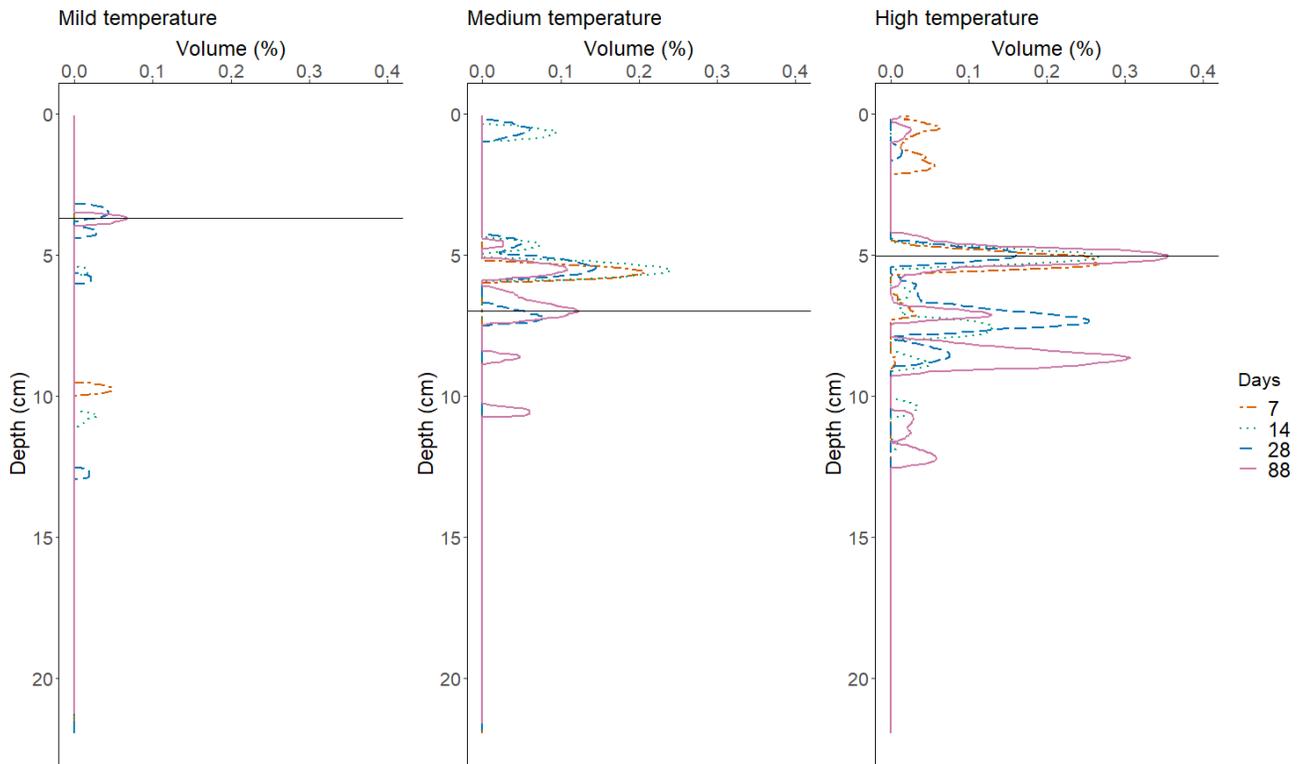


Fig 5. Mean volume profiles of refilled galleries only (percentage of V_{Re} compared to soil volume) per treatment. Colors and line types correspond to dates. Black horizontal lines indicate the point of maximal refilled volume on day 88.

780