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RESEARCH ARTICLE

Experimental evidence that increased surface temperature affects bioturbation by ants

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Abstract

1. Ants are important bioturbators that actively produce biopores and move soil particles. They could be particularly affected by global warming as they are ectotherms. Nevertheless, they can indirectly regulate their temperature, through changes in their circadian cycles and the architecture of their nests (e.g. digging deep nests or using insulating materials). Nest architecture has been considered an expanded functional trait of ant colonies and thus sensitive to environmental changes such as increasing temperatures. This work aimed to study the nest architecture of ants as a functional trait and its effects on soil bioturbation. We hypothesized that, when exposed to increased surface temperatures, ants would increase their excavation activities, build deeper nests and alter the layout of chambers to maintain their preferred temperature and humidity, thus enhancing soil porosity.
2. We allowed 17 young *Lasius niger* ant colonies to excavate nests in soil columns exposed to three surface temperatures (mild, $n=5$; medium, $n=6$; and high, $n=6$) for 100 days. We measured the amount of soil excavated weekly and took X-ray scans of the soil column on Days 7, 14, 28, and 88 to characterize the three-dimensional structure of the nests (depth, shape, volume of chambers and tunnels). We then collected the colonies and measured their growth during the experiment, and the size and weight of workers.
3. Ants reacted to surface temperature. Colonies exposed to medium and high temperatures excavated larger and deeper nests than those exposed to mild temperature. Nests excavated under high and medium temperatures had the same maximal depth, but chambers were located deeper in the former, which were further characterized by the refiling of some of the upper chambers. Colonies grew well in all treatments, although less under mild temperature. They produced normal-sized workers despite differences in surface temperature. Overall, these results suggest that ants exposed to higher temperatures live in deeper chambers.

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

KEYWORDS
ant nest architecture, bioturbation, global warming, high temperature, *Lasius niger*, X-ray tomography

1 | INTRODUCTION

Global warming may particularly affect ectotherms including ants, since they largely depend on external temperatures to regulate their own body temperature (Jørgensen et al., 2022). For instance, increasing temperatures accelerate ant development (Porter & Tschinkel, 1993), yielding smaller adults (Verberk et al., 2021), and increase their metabolism, that is their movements become faster (Hulbert et al., 2008) and their food and oxygen consumption increase (Coenen-Stass et al., 1980). Ants can thermoregulate to some extent, but this is an energy-consuming process that depends on many factors such as population size, moisture and thermal conductivity of the material, and nest size (Coenen-Stass et al., 1980; Kadochová & Frouz, 2013).

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

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

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

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

An example is the study carried on by Sankovitz and Purcell (2021) where *Formica podzolica* colony fragments collected at two different elevations were reared in a two-dimensional set-up under two temperature regimes in a full factorial experiment, and differences between nests were analysed. Nest architecture differed between the two temperature treatments, with maximal complexity matching the original temperature of the colonies (i.e. ants from higher sites built more complex nests under cool temperatures, whereas ants from lower sites had the opposite response). These results are interesting yet should be treated with caution for the artificial approach of the experimentation (i.e. only a small number of workers without brood nor the queen, two-dimensional set-up, and no temperature gradient in the soil).

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

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

2 | MATERIALS AND METHODS

2.1 | Study species

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

2.2 | Collection of colonies and rearing

We collected 51 fertilized *L. niger* queens immediately after their mating flight in Paris in July 2021. This species uses claustral colony foundation to found new colonies. After the mating flight the queen excavates a small nest and starts rearing the first generation of workers using her metabolic reserves only, without ever leaving the safety of the nest. We set up the queens to found colonies in the laboratory, in a windowless climatized room, by keeping ants in glass test tubes (10 cm length × 0.7 cm diam.) with a water reserve held by wet cotton. Once the queens had produced cocoons, 18 queens were randomly selected for the experiment. The growth of their colonies was boosted by providing each of them with the cocoons produced by two other queens. Note that this procedure is not unnatural as *L. niger* colonies raid neighbouring colonies to steal and adopt their brood as a mean to increase colony growth (e.g. Pollock & Rissing, 1989; Sommer & Hölldobler, 1995). Workers started hatching after 3 months. Colonies were then removed from the tubes and installed in 12.5 cm length × 12.5 cm width × 5.5 cm height clear plastic boxes. They were fed with a mix of equal parts of honey and apple puree, fresh mealworms, and a balanced protein gel twice a week, with water provided ad libitum by a test tube filled with water and plugged with cotton. The experiment started 7 months later (February 2022). At that time, colonies contained in average 106 ± 27 (mean ± SD) workers, the queen and brood.

2.3 | Experimental design

Twenty-one polyvinyl chloride (PVC) tubes (30 cm height × 20 cm diameter) had the bottom closed with a nylon cloth (mesh size = 150 μm) to retain soil but allow water to pass through. They were filled to a height of 24 cm with air-dried soil previously sampled in the topsoil layer (0–20 cm) of the park of the French National Research Institute for Sustainable Development (IRD) campus in Bondy (7.5 km Northeast of Paris), France. The soil had a neutral pH ($pH_{H20} = 7.2$) and was sandy (70% sand, 15% silt, and 15% clay) with 4.5% organic carbon. It was sieved at 2 mm to discard large size particles and roots, and then manually compacted to reach the bulk density found in the field ($1.08\,g\,cm^{-3}$), stepwise in 3 cm layers to reach 24 cm height. The soil column was afterward watered to reach 70% of the field capacity ($0.14\,g\,H_2O\,g^{-1}$ soil) following Weil and Brady (2017). This level of humidity is considered standard for experiments where soils are incubated, as it provides enough moisture to avoid water stress but not too much to promote anoxic bacteria development (see e.g. Bompadre et al., 2014; Zhou et al., 2023).

To avoid border effects affecting the shape of the excavated nest, e.g. tunnels following the side of the PVC tubes, a plastic lid with a central opening was placed on the soil surface. The side of the lid was glued to the side of the PVC tubes to force ants to dig through the lid central opening only, i.e. in the centre of the soil surface. The lid had numerous perforations (10 mm diameter each) totalling ¼ of its surface, to allow watering of soil and gaseous exchanges between the soil and the atmosphere. The lid was underlined by a nylon fabric that prevented ants from digging through the perforations. A short plastic tube connected the central opening of the lid to a tall plastic petri dish (8.5 cm diameter × 5 cm height) whose bottom had been drilled. The petri dish allowed providing food and water to the colony as well as collecting the excavated soil for weighing (Figure S1).

The 18 colonies were randomly assigned to high (T_{high}), medium (T_{medium}), and mild temperature (T_{mild}) treatments ($n=6$ for each treatment). The surface of the soil columns was heated using 40W UV lamps placed 20 or 40cm above the soil surface (T_{high} and T_{medium}, respectively) or fluorescent lights 50cm above the soil surface (T_{mild}). Soil temperature was monitored in two columns per treatment using two iButton temperature sensors on the surface and at ~10cm depth. To avoid any effect of the sensors on the nest structure, they were placed on the edge of the column. None of the ant nests reached an iButton during the experiment. This protocol was successful in producing three treatments with significantly different temperatures. Specifically, daytime average temperatures (i.e. while the lamps were on) on the soil surface were 22.2 ± 1.1, 35.9 ± 5.8 and 47.9 ± 10.7°C for T_{mild}, T_{medium} and T_{high} treatments, respectively (mean ± SD, $F_{2,7497}=83$, $p<0.001$, T_{mild} vs. T_{medium}, $p<0.001$, T_{mild} vs. T_{high}, $p<0.001$, T_{medium} vs. T_{high}, $p<0.001$), and night-time average temperatures (when the lamps were off) were 21.5 ± 1.1, 24.8 ± 5.4 and 28.4 ± 11.1°C ($F_{2,7527}=584.5$, $p<0.001$, 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, this difference markedly declined in the soil, with a mid-depth daytime temperature of 22.2 ± 1.1, 25.5 ± 2.1, and 26.2 ± 2.4°C for T_{mild}, T_{medium}, and T_{high} treatments, respectively ($F_{2,2469}=567.4$, $p<0.001$, T_{mild} vs. T_{medium}, $p<0.001$, T_{mild} vs. T_{high}, $p<0.001$, T_{medium} vs. T_{high}, $p<0.001$), and night-time temperature of 21.8 ± 1.0, 23.8 ± 2.1 and 24.6 ± 2.4°C ($F_{2,2487}=270.1$, $p<0.001$, T_{mild} vs. T_{medium}, $p<0.001$, T_{mild} vs. T_{high}, $p<0.001$, T_{medium} vs. T_{high}, $p<0.001$). Because one colony died during the experiment, the number of replicates was $n=5$ for T_{mild}, and $n=6$ for T_{medium}, and T_{high}. The impact of ants on water evaporation was also monitored using soil columns without ants as control ($n=1$ per treatment). All colonies were kept with a light/dark 12h/12h schedule for 100days.

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

2.4 | Soil bioturbation by ants

To assess the underground excavation activity, the amount of soil excavated by ants and deposited on the ground was collected. This assessment, may underestimate the underground activity as it has been reported that ants refill chambers and tunnels according to their space necessities taking the soil previously deposited out of the nest (Römer & Roces, 2015). However, such remodelling of the nest in our experiment may be revealed by the tomography if refilled spaces differ in density from the surrounding soil. The excavated soil was collected weekly, dried at 80°C for 48h and weighted. Soil columns were also weekly weighed for measuring water loss and watered to maintain soil moisture at 70% of the field capacity to allow the development of ants, without promoting the development of anoxic bacteria. The amount of evaporated water (i.e. the added water to keep 70% field capacity), was calculated as follows:

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

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

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

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

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

During the image processing, we noticed a third level of grey on some images (Figure S5), intermediate between those corresponding to the soil (higher density) and the nests (density equal to zero). It represented structures less dense than the soil and herein suspected to correspond to refilled parts of the nests i.e. denoting nest

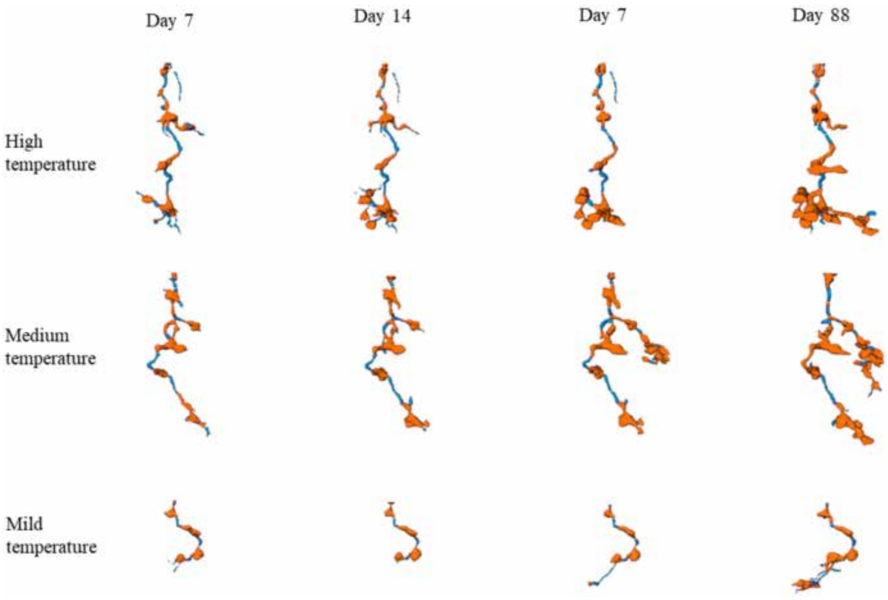

FIGURE 1 Examples of three nests illustrating the effects of treatment and time on their structure. Chambers (orange) and tunnels (blue) can be distinguished.

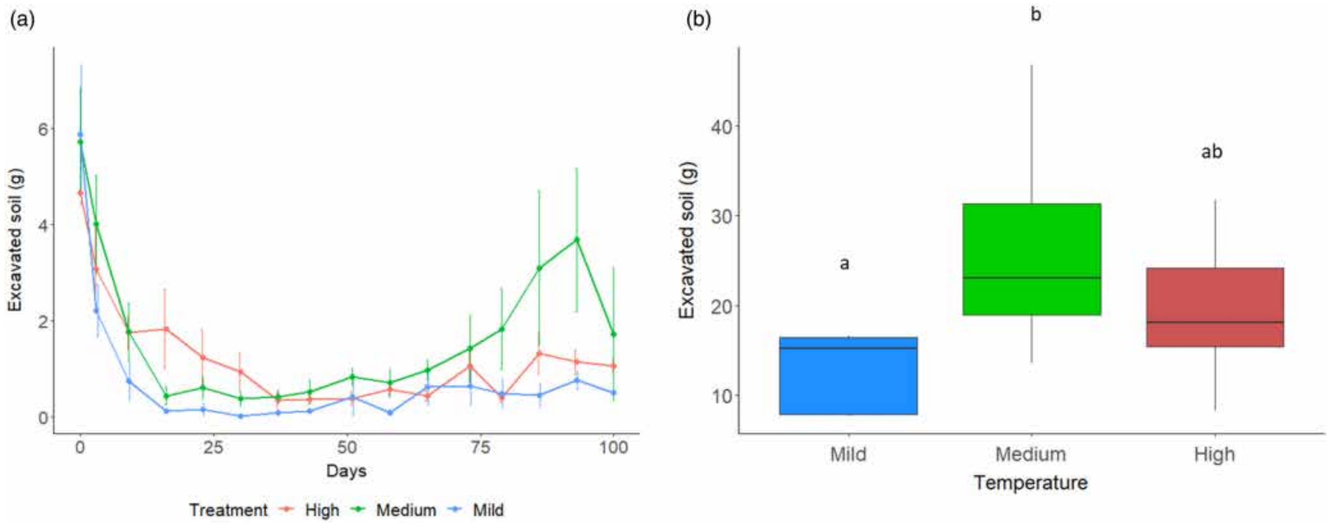

FIGURE 2 Amount of soil excavated (g) at each time point during the whole experiment (A) and total amount of soil excavated during the experiment (B). Bars in (A) indicate SEM. "a" and "b" show statistically significant differences ($p < 0.05$).

remodelling. Prior to segmentation, a 3D non-local means filter of three pixels was applied to reduce noise and scatter. Avizo manual thresholding (110, 160) and a manual 3D ball opening were used to ensure the desired objects were segmented (Figures S2–S5). Volume was measured with the Volume Fraction tool on ImageJ. All volumes (i.e. nests, chambers, tunnels and refilled chambers) were transformed into percentages (structure volume/soil volume × 100).

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

2.5 | Growth of colonies

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

2.6 | Statistical analysis

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

3 | RESULTS

3.1 | Soil bioturbation

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

From the beginning of the experiment, ants produced nests whose maximum depth did not change with time ($F_{3,64} = 2.56$, $p > 0.05$; Figure 3; Figures S2-S4). No nest reached the bottom of the soil column, in any of the treatments. However, differences in temperature led to differences in nest 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 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} vs. T_{high}, $p < 0.001$, T_{medium} vs. T_{high}, $p < 0.05$).

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

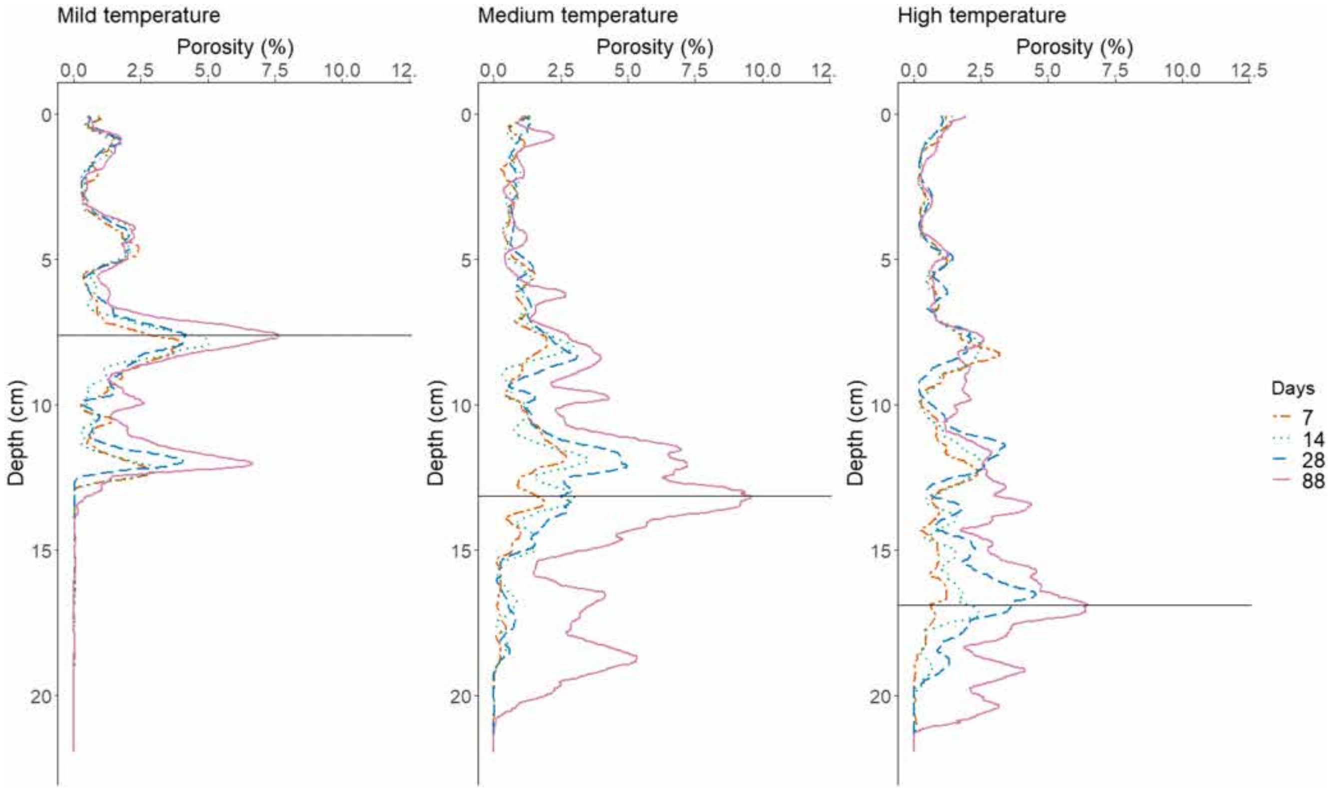

FIGURE 3 Mean porosity profiles (percentage of the empty volume compared to soil volume) per treatment. Colours and line types correspond to dates. Black horizontal lines indicate the point of maximal porosity on Day 88.

The principal component analysis (PCA) of the variables describing nest architecture (Figure 4, see also Tables S1 and S2) showed that the first axis described 40.9% of the total variance and corresponded mainly to a gradient of size, with smaller nests towards higher values and bigger nests towards lower values. This gradient also corresponds to nests development, with younger nests towards higher values and older nests towards lower values. The second axis described 14.9% of the whole variance and corresponded to a shape and position gradient. T_{medium} and T_{high} were better described by size related variables (i.e. volume of nests, volume of chambers, volume of tunnels, surface area (number of pixels corresponding to the external boundary of the 3D nest)) than by shape or position related variables (i.e. number of pores, sphericity index). Conversely, T_{mild} was better explained by shape than by size related variables. Nests excavated under T_{mild} differed from those under T_{medium} and T_{high}. Nests under T_{medium} and T_{high} were similar at Days 7, 14 and 28, but less so at Day 88 where the overlap in characteristics was lower. Within each treatment, nests differed little between Days 7 and 14, and increasingly differed from Day 28 onwards, with nests becoming deeper. This temporal change was less evident under T_{mild} than under T_{medium} and T_{high}, with nest characteristics overlapping largely over Days under T_{mild}, but not under T_{medium} and T_{high}.

Nest volume (V_{nest}) was affected by the interaction between the time and the temperature treatments ($F_{3,24}=3.28$, $p<0.001$).

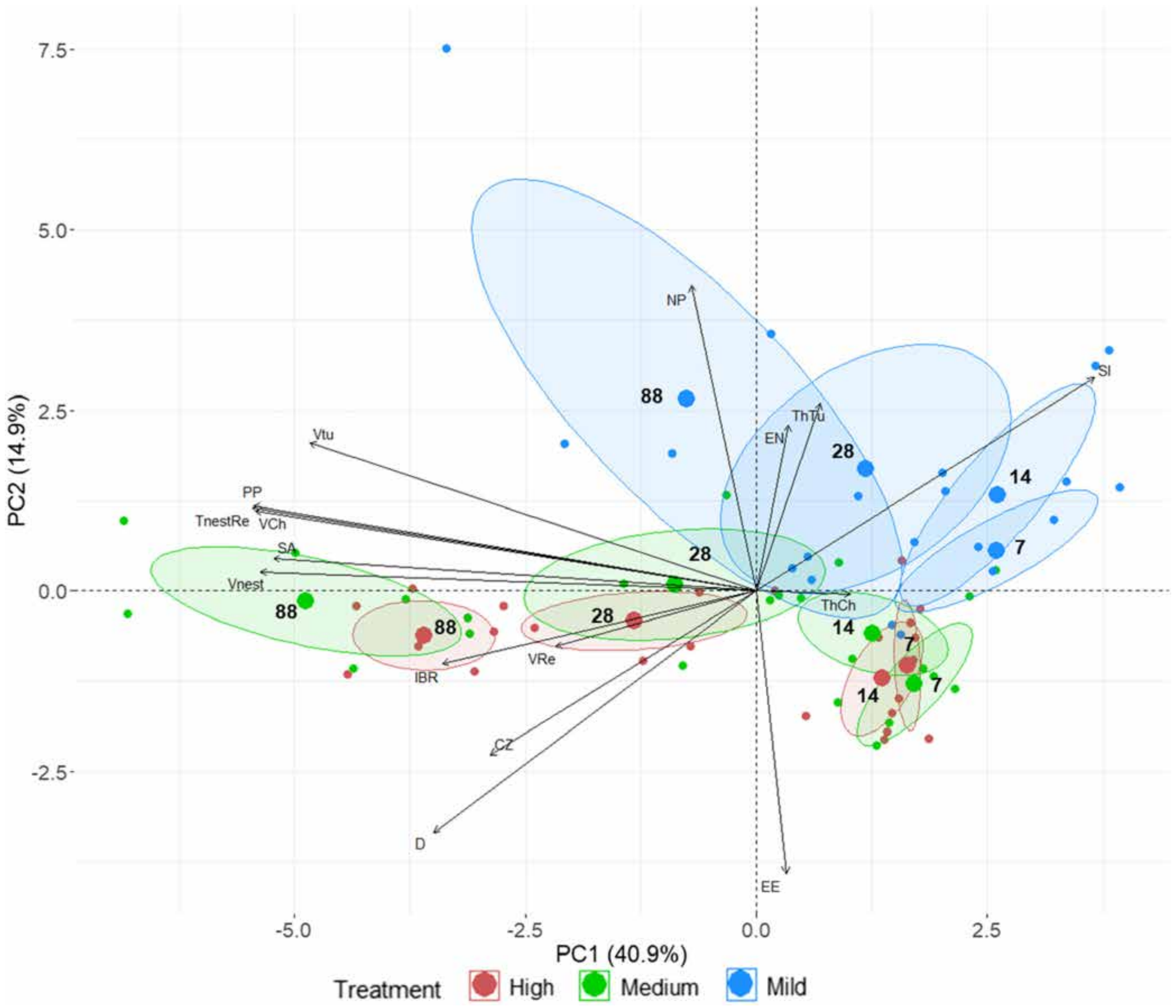

FIGURE 4 Biplot showing the principal component analysis (PCA) from variables describing the nest architectural features. Variables are sphericity index (SI), largest inscribed ball radius (IBR), ellipse elevation (EE), number of pores (NP), surface area (SA), Euler's number (EN), centroid Z (CZ), maximal 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%).

There was no difference among treatments until Day 88 when the nests at T_{mild} were smaller than those at T_{medium} ($F_{2,14} = 5.20$, $p < 0.05$, Figure S6). Nests of colonies at T_{high} showed intermediate volumes compared to the other two treatments. Chambers volume (V_{Ch}) showed the same trend as the nest volume, that is it was affected by the interaction between the time and temperature ($F_{3,24} = 3.67$, $p < 0.001$, Figure S6). Only on Day 88, chambers in T_{medium} were larger than T_{mild} whereas T_{high} had intermediate volumes ($F_{2,14} = 5.71$, $p < 0.05$, Figure S6). Tunnels volume (V_{Tu}) increased over time ($F_{1,26} = 0.26$, $p < 0.001$), without influence of the temperature ($F_{2,14} = 0.26$, $p > 0.05$, Figure S6).

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

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

3.2 | Water evaporation

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

3.3 | Development of the colonies

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

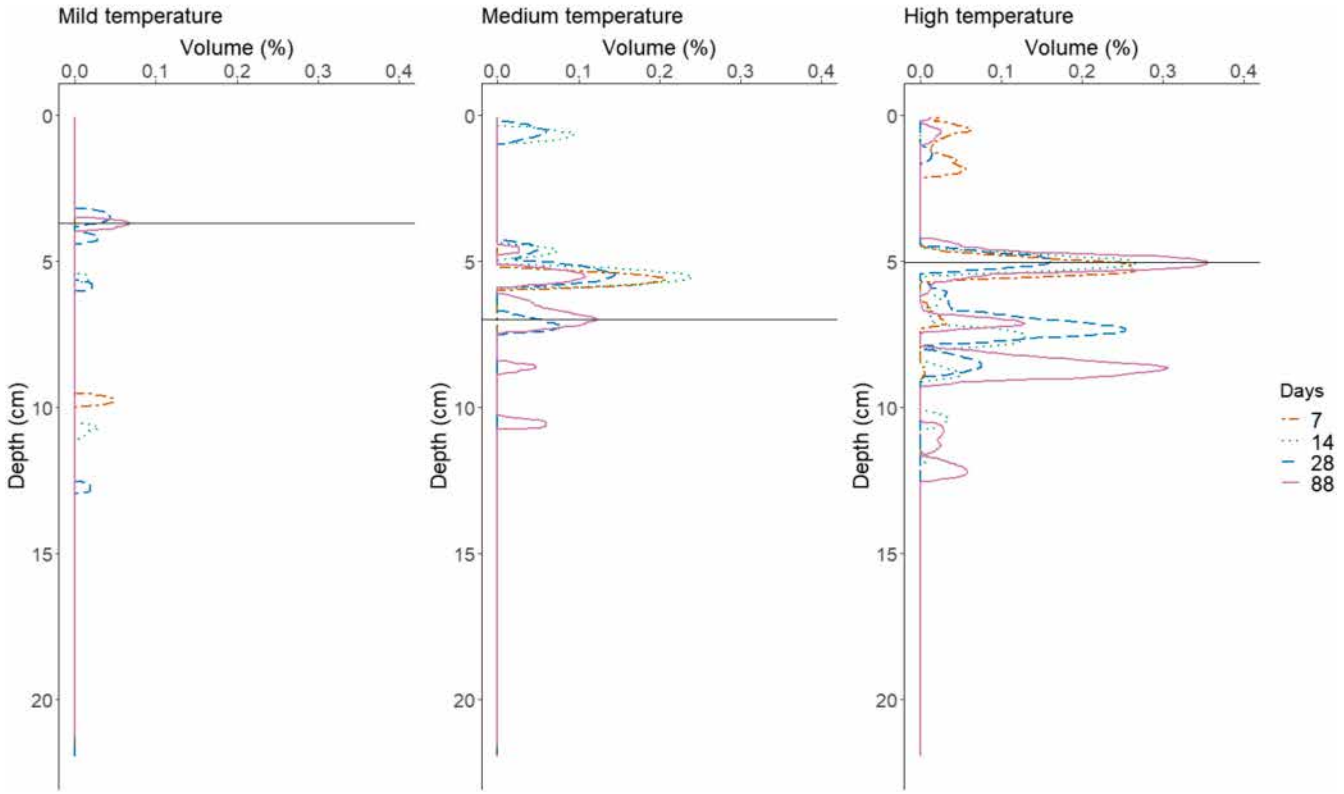

FIGURE 5 Mean volume profiles of refilled galleries only (percentage of V_{Re} compared to soil volume) per treatment. Colours and line types correspond to dates. Black horizontal lines indicate the point of maximal refilled volume on Day 88.

4 | DISCUSSION

4.1 | Soil bioturbation by ants

Ant activity is known to increase with the temperature in the field, that is during spring and summer (Andrew et al., 2013; Nobua-Behrmann et al., 2017). Although this general trend was confirmed by our experiment, we also found that soil excavation did not increase proportionally to the increase in temperature (i.e. no difference between T_{medium} and T_{high}, Figure 2). However, ants seem to be located proportionally deeper in the nests with increasing temperatures (Figure 3). We assume that the modification of the nest architecture provided suitable conditions of temperature and humidity, so that the ants did not need to excavate further to survive and develop. Also, the average daytime surface temperature on T_{high} (47.9°C) was above their critical thermal maximum (CT_{max}, i.e. the temperature at which the individuals no longer control their locomotion (Lutterschmidt & Hutchison, 1997)). At this temperature, ants could be at risk of thermal stress if the duration of exposure was too long or if they were close to their point of desiccation. The CT_{max} of *L. niger* measured through a stepwise method in our laboratory was 47.5°C, hence workers could be active outside, for example for foraging and rejecting excavated soil, but not for too long or solely at night when the temperatures decreased (Lei et al., 2021).

Soil excavation by *L. niger* occurred very rapidly in our experiment. Nests had already reached their maximal depths at the time of the first X-ray scan at Day 7, and they were later enlarged by excavating chambers. Excavation in all treatments occurred with a phase of intense digging (between Days 1 and 28) followed by a phase of lower activity (between Days 28 and 88, Figure 2A). These results are comparable to the findings of Rasse and Deneubourg (2001) and Toffin et al. (2009), who described an initial phase of maximal and fast digging activity (amplification phase), followed by a second phase (saturation phase) when the activity ceases almost completely. However, our results show that ants also respond to increased temperatures by modifying their excavation process, since ants exposed to moderately increased temperatures (T_{medium}) intensified their excavation after being almost inactive (around Day 50, Figure 2A) while the others kept their digging activity low. We could assume that the temperature stimulated the oviposition of the queen, as previously described for other species like *Solenopsis invicta* (Abril et al., 2008; Asano & Cassill, 2012). As expected, we also found that ants exposed to room temperature produced the most superficial nests among the three treatments. Ants exposed to medium and high temperatures built nests of similar maximal depth. However, chambers were distributed differently along the depth of the nest. They were located deeper under high temperatures than under medium temperatures (Figure 3), hinting that the higher the temperature on the surface, the deeper the ants were located. In ant species living belowground, queens, workers and brood are primarily located in intermediate and lower chambers inside the nest, while upper chambers are empty, used for stocking or seasonally used for warming up cocoons (Kadochová & Frouz, 2013; Mikheyev & Tschinkel, 2004). As such, ants likely continually reshape their nests by refilling the less crowded upper chambers, leaving the bottom, more populous part of the nest, untouched. In our study, we showed that changes in surface temperature could significantly influence the dynamics of the nest chambers. Indeed, we found that ants exposed to higher temperatures responded by filling back some of their most shallow galleries (Figure 5). Although these structures were small (~2% of the total nest volume), they were significantly larger for T_{medium} and T_{high}, in comparison with T_{mild}. The underground movement of soil material carried by ants has been previously reported by Tschinkel and Seal (2016) who showed that 17% of the soil excavated by the gardening ant, *Trachymyrmex septentrionalis* was deposited underground. Also, Römer and Roces (2014) reported that in a 2D setup, workers of *Acromyrmex lundii* adjust the size of chambers and tunnels depending on the number of inhabiting workers and the presence of brood and fungus by either digging or refilling spaces using pellets of soil previously removed from the nest.

Despite changes in soil porosity, no difference in soil water evaporation could be measured between the columns with ants and those without ants in all three treatments. These results did not confirm other previously reported findings that suggested that ants decrease (Li et al., 2017) or increase (Blomqvist et al., 2000; Woodell & King, 1991) water evaporation from soil. Our results could be explained by the specific conditions of our experiment. Nests had a single small opening, and the total porosity due to nests was very low (less than 0.5% of the column volume). Also, our soil columns were only 24 cm deep, and the differences in soil water evaporation among treatments (Figure S8) most likely steamed from the increased temperatures, which intensifies the atmospheric evaporative demand (i.e. drying, or evaporating power of the atmosphere) (Vicente-Serrano et al., 2020), rather than from the ants' burrowing activities. Soil humidity is an important factor in nest excavation. Ants burrowing activity depends on the grain size and level of soil moisture (i.e. ants tend not to dig when the soil is fully wet and when it is completely dry (Monaenkova et al., 2015)). Our results show that ants respond to warming by modifying nest architecture, presumably setting up chambers at the depth providing adequate temperature for brood development. We kept humidity constant in our experiment; however, soil humidity may also be affected by global changes so that ants may have to compromise between nest architecture optimizing one factor or the other.

4.2 | Influence of changes in surface temperature on colony development

This study dealt with the influence of an increase in temperature and ant colony development. Colonies grew under the three temperature treatments, which suggests that the nests and food provided adequate conditions for colony development. However, colonies reared under room temperature (T_{mild}, 22.2°C during daytime) produced fewer workers than those reared under higher temperatures (35.9 and 47.9°C). This may be due to room temperature being lower than the natural temperature since *L. niger* typically occurs in open environments where the sun heats the soil, often above this temperature. Colonies produced normal-sized workers under the three temperatures (3.5–5.0 mm length (Seifert, 2018)), despite ant development being sensitive to temperature (i.e. high temperatures promote maturity at smaller size in ectotherms; Atkinson, 1994; Verberk et al., 2021). Given that nest architecture was affected by soil surface temperature, one may hypothesize that ants modified nest architecture so that it provided an adequate abiotic environment (temperature, humidity) to allow for the development of brood into normal-sized workers. Indeed, ant nests provide protection against predators but also against environmental hazards (Porter, 1988; Porter & Tschinkel, 1993), and it is well known that ants place brood of various developmental stages at varied nest depths to provide it with optimal environment (Penick & Tschinkel, 2008). This modification of the nest requires additional soil excavation and is energetically costly. Therefore, species with shallow nests (i.e. more exposed to increased surface temperature) or with small colony size or with foundation of new colonies by a solitary queen (i.e. less capable of supporting the cost of extra excavation) may be more affected by increased temperature than species with deep nests, large populations or foundation of new colonies by colony fission (Cronin et al., 2013). For instance, queens founding new colonies solitarily could excavate deeper nests to provide optimal conditions to their brood, but this extra energetic expenditure would presumably decrease the number of workers they would produce, hence negatively affecting the incipient colony survival and growth. Alternatively, they could not adjust nest depth and their brood would develop at a higher temperature than normal and presumably yield smaller workers with lower foraging efficiency and lifespan, again presumably negatively affecting the incipient colony survival and growth. In comparison, queens founding new colonies by colony fission would be less affected as the workers accompanying the queen carry out nest excavation.

In our study, increased temperatures seem to allow the growth of the colonies. Overall, the only negative effect that we could observe in ants exposed to high temperatures (T_{medium} and T_{high}) was the reduction of worker body mass compared to those reared under room temperature (T_{mild}, Figure S10). This may result from increased metabolic costs during worker development in warm environments, as previously reported in ants (Kaspari, 2005). This could decrease workers' longevity and foraging efficiency, for instance by lowering their competitiveness against heavier normal-sized workers of the same species. Alternatively, it may be that workers reared under T_{mild} were heavier than those exposed to high and medium temperatures because the brood grew slower (as colonies grew less) and workers stored food resources as fat bodies.

4.3 | Global warming and its consequences on nest and colony

Our study, as well as several others (Jenkins et al., 2011; Nascimento et al., 2022; Parr & Bishop, 2022; Verberk et al., 2021), support the idea that ants would be affected by the augmentation of global temperatures. At first glance, ants successfully mitigated the effect of high temperatures by excavating deeper nests as colonies grew well and produced normal-sized workers under high temperatures. However, our experiment focused on nest architecture and did not include the effects of temperature on worker longevity or foraging (directs effects such as heat stress and desiccation, indirect effects such as food availability and competition). In addition, it lasted 100 days and longer exposure to high temperature may reveal accumulated effects, which are important when evaluating thermal risk (Jørgensen et al., 2021).

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

Carbon dioxide is expected to rise in the atmosphere as a consequence of climate change. Its effects on ants are not well understood yet (Parr & Bishop, 2022) and we did not measure it in our experiment. Nevertheless, it could affect nest architecture. Ants cope with levels of CO_2 through ventilation systems and moving the brood to shallower chambers where the levels are lower. For example, the leafcutter ants *A. lundii* prefers to excavate soils with lower content of CO_2, which could explain why they excavate superficial chambers probably to suit the needs of the fungus inside and brood the nest (Römer et al., 2018). Nevertheless, these effects seem to depend on the size of the colony, as experiments with fewer individuals of *Formica pallidefulva* did not show any difference on excavation (Mikheyev & Tschinkel, 2004). We show that *L. niger* ants respond to heat by adjusting nest architecture in the laboratory, but global warming is a complex phenomenon with multiple effects (heat, humidity, gases, etc.) that need to be investigated in several ant species to see how they will respond to these changes.

5 | CONCLUSIONS

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

AUTHOR CONTRIBUTIONS

Fátima García Ibarra, Pascal Jouquet and Thibaud Monnin conceived the ideas, designed the methodology and led the writing of the manuscript. Fátima García Ibarra and Angélique Bultelle carried out the experiment. Fátima García Ibarra and Nicolas Bottinelli analysed the CT Scan images. Fátima García Ibarra analysed the data. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

We have no conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1: Experimental setup with the three temperature treatments: T_{high} ($n=6$), T_{medium} ($n=6$) and T_{mild} ($n=5$).

Figure S2: Nest reconstructions of all nests under T_{high}, showing chambers and tunnels in grey and refilled chambers in blue.

Figure S3: Nest reconstructions of all nests under T_{medium}, showing chambers and tunnels in grey and refilled chambers in blue.

Figure S4: Nest reconstructions of all nests under T_{mild}, showing chambers and tunnels in grey and refilled chambers in blue.

Figure S5: Example of refilled chambers.

Figure S6: Total, chambers, and tunnels volume (percentage compared with the total column volume).

Figure S7: Volume of refilled galleries, measured as the percentage of the total volume of the earth column.

Figure S8: Water evaporation of colonies.

Figure S9: Maximal depth of galleries.

Figure S10: Growth, size, and weight of ant workers at the end of the experiment.

Table S1: Eigenvalues of Principal Components Analysis describing nest architecture.

Table S2: Contribution of variables to the first two dimensions (principal components) of PCA.

Table S3: Number of workers at the start and end of the experiment.

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