Ant resistance to temperature increase : a comparative approach to the effects of precipitation, morphological traits and bioturbation activity in soils

Fátima Abigail García Ibarra

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Résistance des fourmis à l'augmentation de température : approche comparative des effets de la précipitation, des traits morphologiques et de l'activité de bioturbation dans les sols

Par Fátima Abigail García Ibarra

Thèse de doctorat en Sciences écologiques et agronomiques

Dirigée par Pascal Jouquet et Thibaud Monnin

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Devant un jury composé de :

Elena Angulo Aguado	Professeure associée (CSIC, Séville, Espagne)	Rapporteuse
Serge Aron	Professeur (Université Libre de Bruxelles)	Rapporteur
Apolline Auclerc	Maîtresse de conférences (Université de Lorraine)	Examinatrice
Xavier Arnan Viadiu	Professeur associé (Université de Pernambuco, Brésil)	Examinateur
Sandrine Meylan	Professeure (Sorbonne Université)	Présidente du jury
Pascal Jouquet	Directeur de recherche (IRD)	Co-Directeur
Thibaud Monnin	Directeur de recherche (CNRS)	Co-Directeur

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General introduction

I.1 Climate change

I.1.1 Consequences on temperatures and precipitation regimes

Climate change is probably one of the most concerning and researched topics nowadays. While alterations in climate have been observed prior to the pre-industrial era, and there exists evidence of human impact on climate even before this period (Koch et al., 2019), it is the industrial revolution that serves as the catalyst for the myriad climate modifications confronting us today. According to the Intergovernmental Panel on Climate Change (IPCC, 2021), climate change resulting from human influence is causing detrimental effects on agriculture and crop production, a decline in fisheries and aquaculture yields, and is linked to shifts in species distribution ranges, seasonal timing, and the structure of terrestrial, freshwater, and oceanic ecosystems. Furthermore, phenomena such as extreme hot or cold events, upper ocean acidification, increased heavy precipitations and floods, glacier retreat, rising sea levels, and ultimately drought are increasingly attributed to human influence.

Climate change has consequences on practically all components of climate, such as temperature (of air, soils, and water), precipitation, sea-levels, among others. Nonetheless, the most studied and representative ones, probably due to their impact on living beings and environment, are surface and air temperature and changes in precipitations. Global warming (i.e., augmentation of the mean temperature of land and ocean surface) is expected to continue increasing due to accumulation of greenhouse gases (mainly CO_2) and to reach 1.5 °C above the preindustrial times in the near term (2021-2040), even under the very low emissions scenario (SSP1-1.9) (IPCC, 2021). Temperatures in the temperate environments are expected to increase more than in the tropics. The consequences of shifts in climatic patterns are plenty, but some of the most important are those associated to extreme events. The variability of water cycle (monsoon precipitations and very wet and very dry events and seasons) is expected to intensify, and heatwaves and droughts are projected to become more frequent (IPCC, 2021). Additionally, the projected rise in the intensity of extreme precipitation is expected to increase the frequency and magnitude of pluvial floods due to a surpass on the capacities of artificial and natural drainage systems.

I.1.2 Effects on ecosystem functioning

One of the most important consequences of climate change is biodiversity loss. It has been predicted that for 2100, climate change will be the second most important cause of biodiversity loss in terrestrial ecosystems, only preceded by land use change (IPCC, 2021). Nevertheless, the modifications of the components of climate are expected to change biodiversity from the individuals to the biomes (Fig. 1, Parmesan, 2006).

At the individual level, climate change is expected to trigger directional selection (i.e., natural selection that promotes traits differing from the current average in a specific direction, such as those smaller than the current average), and rapid migration, which may lead to the decrease in genetic diversity of populations and affect the ecosystem functioning and resilience (Botkin et al., 2007). Moreover, the effects on populations can modify virtually all interactions at the community level (Walther, 2010). Basically, it has been suggested that the impact of climate change on one species could impact direct or indirectly all the species that interact with it. For instance, Koh et al. (2004) reported that 6300 species of a total of 9650 species studied, pollinators and parasites included, could disappear as a result of the extinction of species associated with them, and thus can be considered “co-endangered”. Also, climate change is behind numerous synchronic mismatches that lead to alterations in plant-pollinator, prey/predators, host/parasites and mutualist relationships to mention a few (Lafferty, 2009; Yang & Rudolf, 2010; Walther, 2010; Rafferty & Ives, 2011).

I.1.3 Effects on susceptible organisms (invertebrates)

Even though it is largely accepted that climate change will and already affects practically all species on Earth (IPCC, 2021), its consequences depend on the specific susceptibilities, phenotypic plasticity, adaptive capacity and migration potential as well as the components of climate change specific to each region (Sala et al., 2000; Dunn et al., 2007; Bellard et al., 2012).

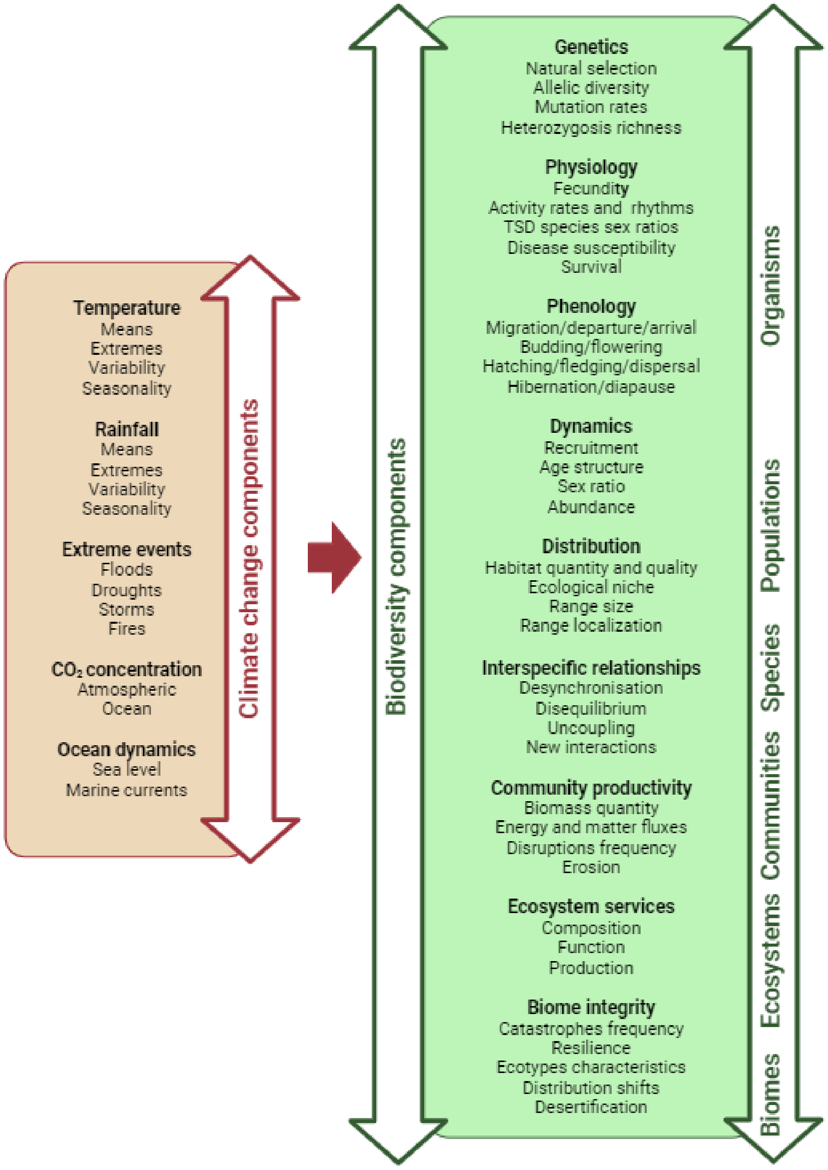


Fig.1. Summary of predicted effects of climate change on biodiversity. Shifts in temperature, rainfall, and oceanic dynamics, coupled with an increase in extreme climatic events and concentrations of CO2, are anticipated to impact biodiversity across all organizational levels. This influence extends to genetic processes like natural selection and mutation rates, alterations in species phenology (e.g., migration, flowering, fruiting, hibernation), changes in species distribution, modifications in ecosystem services, and ultimately, consequences at the biome level such as desertification. TDS stands for Temperature-Dependent Sex determination. From Bellard et al., 2012. A full summary can be found in Parmesan, 2006.

The capacity to acclimate to rising temperatures is crucial in identifying organisms most susceptible to climate change, especially warming. Janzen (1967) proposed that thermal specialists, characterized by limited acclimation capacity, are more prevalent in the tropics where daily and seasonal temperature variations are lower compared to colder environments. However, it is inaccurate to assume uniform sensitivity to temperature increases across all organisms within an ecosystem (Jenkins et al., 2011)

Invertebrates, being ectothermic, face increased vulnerability to climate change as their metabolism is directly influenced by environmental temperature and humidity. Despite this vulnerability, invertebrates play a vital role as they constitute over 80% of eukaryotic species on the planet (Brusca & Brusca, 2002) and significantly impact virtually all ecosystem services (Prather et al., 2013).

Invertebrates are not different than other organisms as the consequences of increasing temperatures affect them at all scales (Fig, 1). At the individual level, increasing temperatures can modify the emergence, accelerate it (e.g., in mayflies (Sardiña et al., 2017), cicadas (Sato & Sato, 2015), and butterflies (Roy et al., 2015)), or delay it (e.g., ground beetles (Pozsgai & Littlewood, 2014), and butterflies (Karlsson, 2014)). These modifications on early development have consequences in adults, for example an increasing number of adults in overwintering (in moths, Ouyang et al., 2016), or delaying or advancing reproduction according to the environmental conditions (in grasshoppers, Buckley et al., 2015). Even more, it has been reported that longer and warmer growing seasons increase insect voltinism (i.e., number of generations per annum, (Forrest, 2016)). These changes in individuals translate in shifts in the population’s distribution (Thomas et al., 2001), phenology, and genetics (Thomas et al., 2001; Parmesan & Yohe, 2003; Parmesan, 2006). Moreover, populations of invertebrates can also migrate to higher elevations or latitudes to find more suitable temperatures (Ramalho et al., 2023), and their homogeneity can be increased (Taylor et al., 2004; Maes et al., 2010).

All the modifications in the functioning of invertebrates have consequences on the ecosystem services they provide (i.e, supporting services provisioning services, regulating services, and cultural services, (Prather et al., 2013). Some examples are primary production resulting from relationships between invertebrates and plants, decomposition by annelids, nematodes and arthropods, nutrient cycling and hydrologic flux by burrowing organisms, and habitat formation and modification by social insects (Fig.2). Climate change has already been associated with the decrease in pollination by insects, especially butterflies (Forrest, 2016) and bees (Decourtye et al., 2019), seed dispersal (Donoso et al., 2022), and decomposition (Figueroa et al., 2021) and the increase of pests insects (Volney & Fleming, 2000; Rojas et al., 2010). However, all the services could be compromised if invertebrates do not counterbalance the effects of climate change.

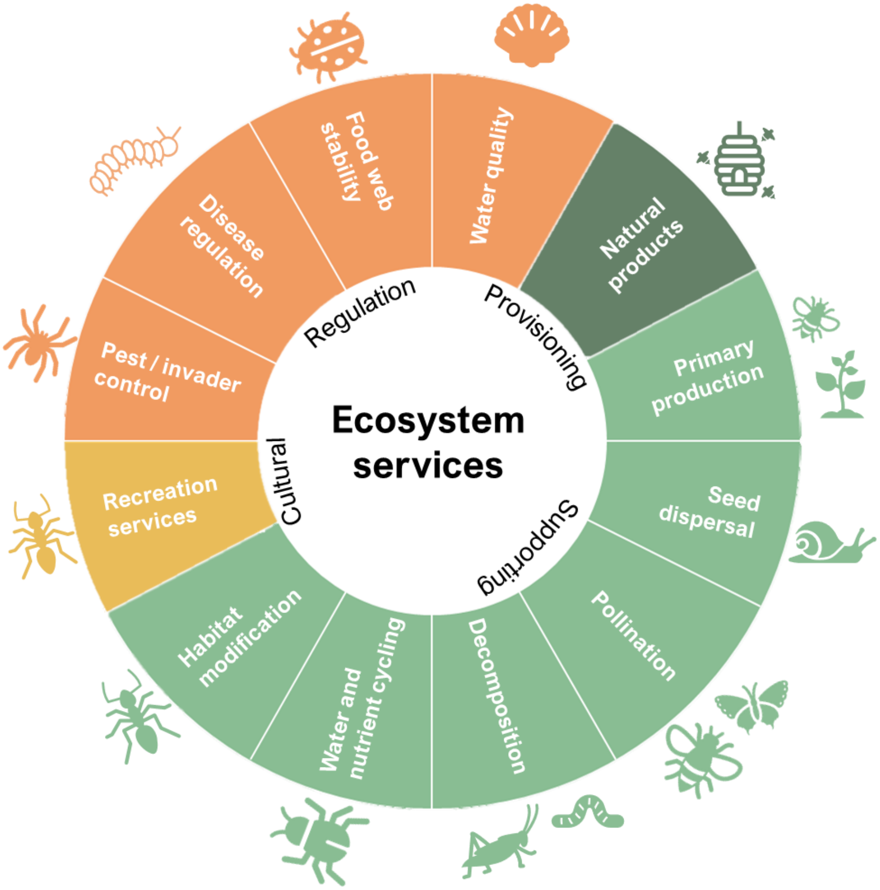


Fig. 2. Ecosystem services affected by invertebrates. Classification and examples from (Prather et al., 2013).

Invertebrates respond to climate change in varied ways. For instance, by carrying genetic mediated changes in photoperiod (in the pitcher mosquito (Bradshaw & Holzapfel, 2001), and the date of egg hatching (on the winter moth (van Asch et al., 2013 in Johnson). Also, some invertebrates counter climate change by melanising (the shell of snails, Cameron & Cook, 2013) or by changing their thermal-performance curves (in butterflies, Higgins et al., 2014). Moreover, some invertebrates can shift their distributions towards the poles when temperatures increase (Parmesan et al., 1999; Sunday et al., 2012). Nonetheless, it is generally accepted that the responses of invertebrates to climate change will be multifactorial and highly complex (Buckley & Kingsolver, 2012; Carnicer et al., 2017). To fully understand the consequences of climate change on invertebrates, it is necessary to explain how they affect ecosystem functioning.

I.2 Soil invertebrates

I.2.1 Ecological importance

It has been estimated that soils host up to 59% of the Earth's species, ranging from bacteria to mammals (Anthony et al., 2023). This biodiversity is likely the result of a scale-dependent physical and chemical heterogeneity, varied microclimatic characteristics, and phenologies of organisms that promote, create and maintain numerous niches (Tiedje et al., 2001; Ettema & Wardle, 2002). Invertebrates are a key part of soil functioning by carrying out some ecosystem service. Some of these services directly benefit human populations with processes like soil formation, nutrient cycling, and primary production (Lavelle et al., 2006). Other services of the soil like those related to the dynamics of organic matter and physical properties of the soil itself, also contribute to essential regulation services, and to the regulation of below- and aboveground biodiversity. These include climate regulation through the control of gas fluxes and carbon sequestration, flood control, and detoxification (Lavelle et al., 2006). The myriad of organisms residing in soils are responsible for carrying out these ecosystem services.

Soil organisms have been categorized based on body width (Swift et al., 1979; Wall et al., 2001) into three groups: microfauna (<0.1 mm), which includes acari, protozoa, nematodes, bacteria, viruses, rotifers, and fungi; mesofauna (0.1 – 2 mm); and macrofauna (>2 mm), encompassing invertebrates like Diptera, Coleoptera, earthworms, termites, ants, molluscs, and small vertebrates such as rodents and reptiles. While this classification does not imply specific functions or ecological relationships, it remains widely used due to its simplicity and practicality in field studies.

Many other classifications for soil organisms have been proposed (for example Yeates et al., 1993 for nematodes; Luxton, 1972 and Siepel & Ruiter-Dijkman, 1993 for mites; Faber, 1991 for fungi; Bouché, 1977 for earthworms; Rückamp et al., 2010 for termites; and Folgarait, 1998 for ants). These classifications have been based on characteristics such as food regime and source, size and morphology, burrowing activities, associated microorganisms, and even biogeography and behavioural dominance (Briones, 2014). However, it is the classification proposed by Lavelle et al. (1993) that is most useful for this thesis. Such classification is part of a hierarchical model that aimed to describe the importance of physical, chemical, and biological factors as determinants of soil processes. The model organises the soil organisms into four broad functional groups: microorganisms (i.e., responsible for the majority of chemical transformations essential for organic matter cycling and chemical fertility), micropredators (i.e., contribute to micro foodwebs and expedite the mineralization of organic matter), litter transformers (i.e., construct organic structures facilitating the incubation of microorganisms and play a role in the organization of soil humification), and ecosystem engineers (i.e., engage in digging and physically modifying the soil, influencing the availability of resources for other organisms). The latter are particularly important for this thesis as ants are one of the main groups included in it.

I.2.2 Soil bioturbators and engineers

As mentioned above, the ecosystem engineers (sensu Jones et al., 1994) provide, maintain, and even create habitats for organisms other than themselves. They do so either via their own physical structures, i.e., living, and dead tissues (autogenic engineers) or by physically transforming living and non-living materials (allogenic engineers). In the soil, plant roots, earthworms, termites, other soil-dwelling invertebrates, and small rodents, are some examples of ecosystem engineers, that physically change biotic or abiotic materials.

Bioturbation is the physical displacement, modification, mixing and layering of sediments by organisms. This activity, described by Darwin (1881), is performed by many kinds of organisms including plants, and burrowing vertebrates and invertebrates (Meysman et al., 2006), and it is key for mixing and turnover of material, but also movements of soils downslope on sloping surfaces (Richards et al., 2011). Much of the bioturbation activity in terrestrial ecosystems is carried out by soil invertebrates (although other invertebrates (e.g., crabs) and mammals (e.g., rats and moles) can significantly alter soil structure and functioning in some specific situations). This activity alters the heterogeneity of soil composition and texture due to the sometimes differential movement of different grain sizes (De Bruyn & Conacher, 1994). Also, invertebrates create macropores that affect water infiltration and runoff (i.e., the process when a portion of water on a surface does not infiltrates nor accumulates but runs downslope instead, Hillel, 2005).

Among the terrestrial invertebrates with bioturbation activity, earthworms, termites, and ants are the most important. Earthworms are probably the best-known bioturbators, as they are abundant and active, and create tunnels that, depending on the soil type can collapse or retain their form thanks to the mucilage they excrete while digging (Shipitalo & Protz, 1988). It has been estimated that earthworms may move between 5.4×10^{-4} to 0.01 m^3 of soil per m^2 every year (Mitchell, 1988), leaving macropores that increase soil porosity by 3 to 10-fold depending on the particular conditions (Edwards & Bohlen, 1996). Furthermore, earthworms ingest soil and by doing so they alter the biochemical composition and particle size (they help to break down the particles), and they create casts (i.e., faeces created with the soil and bound together by mucus, bacteria, and plants and other fibres present in their guts), which seem to accelerate the mineralization of soils and increase turnover of organic matter and increase the transfer of N and C into the soils aggregates (Bhadauria & Saxena, 2010).

Termites excavate underground galleries that create a complex network of macropores increasing with this the hydraulic conductivity of soils. It has been estimated that termites move from 1.3×10^{-5} to 4.1×10^{-4} m^3 of soil per m^2 per year (Whitford, 2000). Also, they sometimes create above-ground mounds that can persist on the landscape during decades (De Bruyn & Conacher, 1990) and constitute patches of biodiversity and fertility at the landscape scales. Termites are macrodetritivores that decompose organic matter even during dry seasons (Veldhuis et al., 2017) and create soil sheeting above the organic materials they consume to protect themselves from predators and desiccation (Jouquet et al., 2022). Also, some species of termites grow a fungus inside their nest and by doing so, they create fertility patches and participate in nutrient cycling (Van Thuyne & Verrecchia, 2021; Muon et al., 2023).

I.2.3 Ants as bioturbators

Ants are the most important soil-turners due to how diverse, abundant and widely spread they are in terrestrial ecosystem (Folgarait, 1998; Frouz & Jilková, 2008). They are also one of the most diverse groups of insects with more than 14 100 named species (Bolton, 2023) and an up to 25 000 estimated species and thus account for a large portion of total animal biomass (Hölldobler & Wilson, 1990; Ward, 2014). In constructing and maintaining their nests, ants mobilise large amounts of soil from deep to superficial layers (around 4.5×10^{-6} to 1.8×10^{-3} m^3 of soil per m^2 per year (Whitford, 2000).

The tunnelling activity of soil-dwelling ants contributes to a reduction in bulk density, thereby enhancing soil aeration and water permeability (Eldridge, 1993). However, the impact on water infiltration is a more intricate process. Ant nests not only augment macroporosity, but also influence the organic matter content within specific chambers or outside the nest (Hölldobler & Wilson, 1990; Folgarait, 1998). This alteration in the physical and chemical properties of the surrounding soils and vegetation results in improved soil fertility (Farji-Brener & Werenkraut, 2017). Nevertheless, the enhanced organic matter content renders the soil more repellent to water in arid conditions, leading to increased infiltration in humid environments but decreased infiltration in dry environments (Cammeraat et al., 2002). The changes in physicochemical properties of soils due to ants burrowing activities have in turn consequences in the ecosystem. For instance, they influence nutrient cycling and fosters microbial activity (Viles et al., 2021), improve soil fertility and increase plant productivity (Farji-Brener & Werenkraut, 2017).

Additionally, ant activities affect other organisms inhabiting the soils in intricate and environment-dependant ways (Fig.3). The positive effects of ants on soil properties directly improve conditions for plants, and by improving the conditions for microbial decomposers, ants affect indirectly the plants too. Some soil conditions can be beneficial for decomposers, but ants can regulate the abundance by directly consuming them. Also, ants can indirectly modify soil properties by affecting plants. For instance, ants can benefit plants by directly or indirectly regulating predators and herbivores. Seed harvesting or seed collector ants can either predate (negative effect for plants) or disperse (positive effect) seeds. Ants can protect honeydew-producing insects, causing a detrimental impact on plants. However, while tending to these insects, ants can predate more other sap sucking insects and disturb herbivores, leading to a decrease in plant herbivory so that the overall effects of ants is unclear. These direct and indirect relationships are dully detailed in Del Toro et al., (2012) and Wills & Landis, (2018).

I.3 Effects of climate change on ants

As mentioned above, species living in mesic environments are expected to resist to higher temperatures yet to be more susceptible to warming than species from colder environments, because they have narrower resistances (Janzen, 1967). Ants are no exception to this rule, hence, species living in tropical and mesic, low elevation biomes are the most susceptible to temperature augmentations (Jenkins et al., 2011; Diamond et al., 2012). Moreover, ants like all ectothermic organisms are particularly sensitive to warming (Jørgensen et al., 2022).

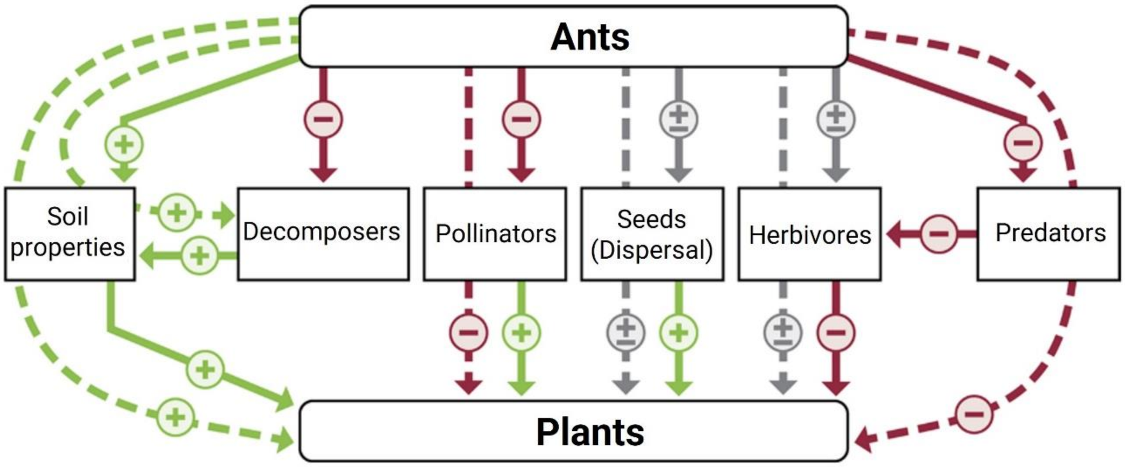


Fig. 3. Interactions between ants, soil properties and other organisms (decomposers, pollinators, seeds, herbivores and predators)(Wills & Landis, 2018). Lines represent direct (solid) and indirect (dashed) impacts of ants. Interactions are coloured in red when negative, green when positive, and grey when they are species specific. From Wills & Landis, 2018).

At the colony level, ants respond to increasing temperatures by making deeper nests to suit the thermal needs of their brood (Bollazzi et al., 2008; Tschinkel, 2015a) or under stones (Dean & Turner, 1991). Moreover, they can change the location of the brood during the day or the season seeking the right temperatures for its development (Penick & Tschinkel, 2008). Although the nests of ants are perceived as sessile, (Feener & Linghtom, 1991), many species (for instance, some *Aphaenogaster* spp., and *Pogonomyrmex* spp.) relocate their nests during the development of the colony (Smallwood, 1982; McGlynn, 2011). Furthermore, ants are expected to use relocation to overcome rising temperatures by relocating the nest as the first measure (Ord, 2023). However, not all species have the same capacity of relocating throughout their habitat, as it is energetically expensive (Franks et al., 2003), implies the loss of workers, food stored (Tschinkel, 2014), and foraging time (Brown, 1999), and ultimately because it increases the risks of predation (Bonte et al., 2012) and desiccation.

As global temperatures rise, many ant species may experience shifts in their geographical ranges (Diamond et al., 2012). Some may adapt by migrating to higher altitudes or latitudes in search of more suitable climates (Sankovitz & Purcell, 2021). However, the ability of ant species to successfully relocate and establish new colonies in these altered environments varies, potentially leading to changes in community composition and ecosystem dynamics. One of the consequences of the different adaptation potentials in ants are the invasions, as some species with high capacity of thermal adaptability (for instance *Linepithema* humile, Roura-Pascual et al., 2004) expand their geographical ranges with important effects on the environment. Invasive ants are expected to continue having a global distribution under climate change, but the geographical spread and effects on community levels will most likely be influenced by how the existing ant communities adapt to local environmental conditions (Lach, 2021).

Temperature fluctuations associated with climate change also have profound implications for ant physiology and life history traits. As climates warm, the phenology of ant colonies may shift, affecting critical events in their life cycles such as mating flights, nest construction, and foraging patterns. For instance, changes in foraging behaviour and nest construction patterns may influence nutrient distribution in the soil, potentially impacting plant growth and the broader soil ecosystem (Folgarait, 1998). These changes in phenology can cascade through ecosystems, influencing the timing of interactions with other species and potentially disrupting established ecological relationships.

The availability of resources crucial for ant survival, such as food and nesting materials, is also being influenced by climate change. Changes in precipitation patterns and temperature can impact the abundance and distribution of plants and arthropods that ants depend on for sustenance (Prather et al., 2013; Forrest, 2016). Extreme weather events, like intense storms or prolonged droughts, may directly impact ant colonies by damaging nests, disrupting foraging routes, and affecting food storage (Schowalter, 2011). Such disruptions can have cascading effects on ant-dependent ecosystems, potentially altering the dynamics of plant-animal interactions and community structures (Harvey et al., 2020).

Adaptation and resilience strategies employed by ant colonies in the face of climate change are complex and species-specific. Some species may exhibit behavioural plasticity (Cerdá, Retana, & Manzaneda, 1998; Cerdá, 2001), adjusting their foraging strategies or nesting behaviours in response to changing environmental conditions. Others may face challenges in adapting to rapidly shifting climates, especially in the presence of other stressors such as habitat loss and pollution (Sala et al., 2000; Prather et al., 2013).

I.4 Functional traits

In ecology, one way to analyse the effects of the environmental conditions (for example, of climate change) or one organism on others is through the analysis of the functional traits. These are defined by Violle and colleagues (2014) as a feature either morphological, physiological, phenological or behavioural measurable at the individual level that impacts directly or indirectly their fitness, via its effects on growth, reproduction and survival (these three characteristics are also defined as performance). It is important to notice that although most measurable traits can eventually have an effect on the organism’s performance, for a trait to be considered a functional one, it needs to be directly linked to ecosystem processes (Mlambo, 2014; de Bello et al., 2021).

Functional traits depend on the type of organisms and are as diverse as the organisms themselves. They are useful in addressing a variety of ecological questions from how individuals, populations, and communities respond to environmental changes; how the processes of community assembly shape biodiversity patterns; and how traits affect ecosystem functioning and services (Mcgill et al., 2006; de Bello et al., 2021). In this regard, Moretti and others (2017) proposed five categories of functional traits for terrestrial invertebrates: i) morphology (e.g., body size, eye morphology, colour); ii) feeding (i.e., feeding guild, ingestion rate, biting force); iii) life history (e.g., ontogeny, life span, age at maturity, voltinism); iv) physiology (e.g., resting metabolic rate, desiccation resistance, temperature tolerance); and v) behaviour (e.g., activity time, aggregation, dispersal mode, locomotion speed). The selection of the category or the traits used depends on the nature of the question, the scale, and the resources available.

Thermal tolerance is crucial in defining the consequences of climate change in ectothermic organisms. One of the most used traits to evaluate it is the Critical Thermal Tolerance (CT_{max}), which is defined as the temperature at which the locomotion is lost (Lutterschmidt & Hutchison, 1997). Although there is a considerable debate related to the methodological, physiological and ecological definitions of CT_{max} (Jørgensen et al., 2021; Ørsted et al., 2022), it is accepted as a functional trait when used as an extreme value to limit the activity or the microhabitats in ectotherms (Kearney et al., 2021). CT_{max} will be explained in more detail in Chapters 1 and 2 of this thesis.

I.5.1 Functional traits in ants

In ants, the study of functional traits needs to be assessed at both individual and colony level as they live in complex societies where some individuals perform the somatic functions (workers), and others perform the sexual functions (queens and males). The most studied traits in ants -and other arthropods- are certainly the morphological ones, as several features have been related to different ecological functions and are somewhat easy to measure on individuals (for a general summary see for example Schofield et al., 2016; Drager et al., 2023). For instance, ant’s head width and length have been related to predatory strategies as wider heads can support bigger mandibles for larger prey (Sarty et al., 2006). Longer scape (i.e., more distal section of the antennae) facilitate following the trails of pheromones (Weiser & Kaspari, 2006). Higher pilosity in ants has been related to higher tolerances to dehydration and mechanoreception (Wittlinger et al., 2007). Polymorphism, which is visible at the colony level, allows a higher level of specialisation since castes perform different tasks (Wilson, 1953).

Not all functional traits are morphological. For example, species where colonies have several nests (polydomy) have a competitive advantage for central place foraging compared to species where colonies have a single nest (monodomy, (McGlynn, 2011). Also, the type of colony foundation is also a functional trait. Foundation by a winged queen (independent colony foundation) allows a long-distance dispersal compared to foundation by an apterous queen (dependant colony foundation), but at the cost of a higher mortality as winged queens found solitarily (ant workers are apterous) while apterous queens are assisted by workers (Cronin et al., 2013).

Due to the fact that ants are such a diversified and ecologically important group of organisms, a global data base of their functional traits was developed by Parr et al., (2017). This pivotal work made evident that some morphological patterns emerge among subfamilies and strong variations exist when comparing biomes and continents, which contribute to the study of complex biogeographic patterns. Although less frequently, other authors have used life-story traits and behavioural traits to measure ant's reactions to environmental gradients. One of these studies found that the variations in functional traits in a community of ants was better explained by the vegetation productivity and type than by the climate (Arnan et al., 2014). However, temperature and precipitation seasonality influenced some functional responses, which suggests that functional traits may regulate the responses of ants to the upcoming climate change.

For this thesis, some morphological traits (i.e., length of the hind leg, interocular distance, and eye position) were particularly important, so the methodology to evaluate them and their relevance on ants' biology will be discussed in the following chapters.

I.6 Objectives and general organisation of the thesis

This thesis aimed to contribute to the studies on how high temperatures and modified precipitations, two major actors in climate change, influences the activity of ants, and their impact on ecosystems as bioturbators and engineers. To do so, I approached the issue from three levels of organisation: the individual, the colony, and the community, exploring on each one of them crucial features for ants' development.

The first axis of my work, which corresponds to Chapters 1 and 2, was an analysis of how selected morphological functional traits of foraging ants relate to their resistance to surface temperatures, for which I used the Critical Thermal maximum (CT_{max}) as proxy. On Chapter 1, I explored these relationships between functional traits at the individual level of four communities along a gradient of humidity and temperature in semi-arid, Mediterranean, and temperate environments. The main objective is to determine which morphological traits best explain their heat resistance and whether they are the same for polymorphic and monomorphic species as the former are more specialised than the latter. I also focus on comparing the communities under the hypothesis that ants in mesic environments will be more resistant to temperature. Moreover, I investigate the extent to which the environment determines the thermal resistance and if it is more important than morphology and mono/polymorphism.

In Chapter 2, I explore the same traits than in the previous chapter but this time for a community of Mediterranean ants. As for the first chapter, the goal is to find the most important morphological traits for heat resistance, but this time using finer statistics at individual level. The first hypothesis was that hind leg length would be important but other traits must also be used when assessing the resistance to heat. The second hypothesis was that the same traits would be important for mono and polymorphic species (e.g. leg length), and that larger workers would be more resistant because polymorphism is a difference in body size (with or without changes in body shapes), which is one of the important functional traits for thermal resistance.

The second axis (Chapter 3) looks at the effects of surface temperature on colony development and explores the possibility to use nest architecture as a functional trait, a characteristic poorly explored compared to other functional traits. To assess this question, I experimented with colonies of a widespread ant in temperate environments and exposed them to three different surface temperatures. I evaluated the bioturbation activity and the development of the colony as well as the modification of the nest architecture. The main hypothesis was that the depth and architecture of the nests would be proportional to the temperature and the number of workers in the colony.

Lastly, the third axis, which corresponds to Chapter 4, aimed to investigate the effects of temperature and humidity modification on a community in temperate climate. To achieve this, I used greenhouses to create different conditions for ants to develop and followed the richness and abundance of ant species in the community for two years. Also, I evaluated the bioturbation activity of ants by measuring the soil infiltration. I hypothesised that the shift in these abiotic conditions will alter the composition of ant community and thus their burrowing activity and their effect as ecosystem engineers.

Chapter 1

Critical Thermal Limits of ants: does morphology matter more than habitat?

This Chapter will be a paper, which is currently been revised by all the authors and will be soon submitted to Insect Science.

Critical Thermal Limits of ants: does morphology matter more than habitat?

Fátima García Ibarra, Pascal Jouquet, Angélique Bultelle, Pierre Fédérici, Mohamed Taieb Labiadh, Xim Cerdá, Thibaud Monnin

Abstract

Global climate change is expected to affect biodiversity at all its levels and in all environments. Ants impact numerous ecological processes in practically all terrestrial environments. Hence it is important to fully understand what determines their resistance to environmental hazards, and especially to the increase in temperatures resulting from climate change. This study aimed to evaluate the accuracy of morphological traits to explain heat resistance of ants across different environments considering their genus and worker polymorphism. To do so, we sampled 49 species of ants in four sites (one semi-arid, two Mediterranean and one temperate) and used the dynamic method (i.e., exposure to a gradually increasing temperature) to evaluate the Critical Thermal maximum (CT_{max}) of forager workers exclusively. We then dissected a subset of these ants to measure several morphological traits. We found that the ants in southernmost sites were on average more heat resistant than those in cooler environments. However, the range of heat resistance (i.e., difference between the CT_{max} of the least and most heat resistant species in each site) was similar across latitude. The resistance of *Cataglyphis* spp., a highly thermophilic genus, could be explained by measuring the hind leg only, while the interocular distance was also important for non-*Cataglyphis* ants. Within polymorphic species, larger workers were more heat resistant than smaller ones, supporting the idea that worker polymorphism may be advantageous in warm habitats. Species collected at two sites did not differ in heat resistance across sites. Overall, these results highlight the importance of morphological traits and

phylogeny in heat resistance, but also suggest that strategies of adaptation to heat other than heat resistance also occur.

Keywords

Critical Thermal Limit, CT_{max}, ants, morphology, functional traits, semi-arid climate, Mediterranean climate, temperate climate

Introduction

Climatic and land use changes have already caused shifts in the structure, diversity and phenology of numerous species in virtually every ecosystem on the planet (IPCC, 2021). The rise in temperature is one of the most stressful abiotic factors affecting the development of organisms, particularly ectotherms that rely on environmental temperatures to regulate their body temperature (Jørgensen et al., 2022). Ants are present in nearly all terrestrial habitats (Hölldobler & Wilson, 1990; Parker & Kronauer, 2021). Their contributions are considered key to many processes such as soil bioturbation, nutrient cycling, pest control, seed dispersal, and community regulation (Del Toro et al., 2012). Accordingly, changes in ant populations assemblage and diversity due to climate change are expected to trigger important modifications in ecosystems across the globe (Parr & Bishop, 2022).

Global warming is expected to induce a higher increase in temperatures at higher than lower latitudes (IPCC, 2021). Ants in temperate environments may thus be exposed to higher increases in temperature in the coming decades than tropical ants. However, temperate ants may be relatively more resistant to temperature variations (i.e., may have a higher response plasticity) because they are naturally exposed to larger seasonal variations (Janzen, 1967; Kaspari et al., 2015; Bujan, et al., 2020). Indeed, ants in tropical and mesic environments may be more vulnerable to changes in temperatures considering that they live closer to their upper physiological thermal tolerance (Diamond et al., 2012). These species-level effects of temperatures could translate to community levels, as it has been found that the thermal breadth of ant communities (i.e. the combination of the thermal niches of the ant species making up each community) is wider in the North than in the South of Western Europe (i.e., in colder and more seasonal regions vs. warmer and less seasonal regions, respectively, Arnan et al., 2015). Hence, ant communities from relatively colder and seasonal regions may be more resilient to warming than ant communities from warmer and less seasonal regions.

Heat tolerance in ants is determined by both constitutive factors (e.g., morphology, phylogeny, Drager et al., 2023) and inducible factors (e.g., production of heat shock proteins following exposure to high temperatures (Evgen'ev et al., 2007; Ślipiński et al., 2015), change in behaviour such as phenological plasticity (Andrew et al., 2013; Roeder et al., 2022), modifications in nest architecture (Perez et al., 2021; García Ibarra et al., 2023)). Constitutive and inducible factors can be considered as functional traits, i.e., features measurable on individuals that impact directly or indirectly their fitness (Violle et al., 2014). Multiple morphological characteristics have been identified as functional traits in ants (Weber, 1938; Kaspari & Weiser, 1999; Davidson et al., 2004; Weiser & Kaspari, 2006; Sarty et al., 2006; Parr et al., 2017b). However, the length of the hind leg and the body size are probably the two traits most widely used to assess thermal resistance (Clémencet et al., 2010; Sommer & Wehner, 2012; Oms et al., 2017). Other things being equal, ants with relatively longer legs are expected to be more resistant than ants with shorter legs, for they can move faster (i.e. minimise the duration of heat exposure during foraging and increase the convective cooling, Hulbert et al., 2008; Sommer & Wehner, 2012) and raise their body away from the heat radiating from the soil surface (Kaspari & Weiser, 1999; Farji-Brener et al., 2004). A larger body is also expected to be positively related to resistance to temperature variation as larger bodies lose and gain heat more slowly than smaller ones (Willmer & Unwin, 1981; Nascimento et al., 2022).

Studies of polymorphic species have shown that, within the same species, larger workers are more heat resistant than smaller ones (for example, *Eciton burchellii* (Baudier & O'Donnell, 2018), *Cataglyphis velox* (Cerdá et al., 1997), *C. piliscapa* (Clémencet et al., 2010), *Messor barbarus, M. bouvieri* and *M. capitatus* (Arnan et al., 2022)). Moreover, worker polymorphism seems to be an advantageous trait in dry ecosystems. This characteristic enables a more efficient utilization of resources and the continuation of foraging activities during the hot hours of the day, as larger foragers exhibit greater resistance to heat and desiccation (Clémencet et al., 2010; Baudier et al., 2015; La Richelière et al., 2022), and thus, can spend more time outside the nests while small workers perform other activities inside the nest. Even though polymorphism is more frequent in hot and dry than in cool environments (La Richelière et al., 2022), there are also highly resistant monomorphic species in these habitats. It has been suggested that thermal resistance in polymorphic ants is highly related to morphology (i.e. larger body, longer legs), while monomorphic species have developed other adaptations, such as an elevated body water content, the expression of proteins involved in the reduction of reactive oxygen species (which cause cellular damage under thermal stress, Perez et al., 2023), lower cuticular permeability and metabolic rate compared to same-sized less resistant ants (Cerda & Retana, 2000). Also, mechanisms like the morphological ability to raise the abdomen vertically, hence away from the hot soil, to lower the risk of overheating of vital organs have been reported in monomorphic thermoresistant ants (Cerdá, 2001). However, differences in heat resistance between monomorphic and polymorphic species have been studied mostly in ants inhabiting dry environments, especially the highly thermophilic genus *Cataglyphis*. Ants in this genus are also known to present heat-shock proteins (although not exclusively). These molecules repair cell damage and prevent the denaturation (i.e., the disruption of the three-dimensional structures) of other proteins (Evgen'ev et al., 2007; Ślipiński et al., 2015). In *Cataglyphis* ants, these proteins can be synthesized even before the exposure to high temperatures, which contribute to their thermoresistance (Gehring & Wehner, 1995). Studies are needed to compare monomorphic and polymorphic ants in other environments.

Ants' individual performance (e.g., the ability to move, grow, survive, reproduce) increases with temperature, until the point where the temperature is too high to allow normal body functioning. Important increases or decreases in the temperature to which ants are exposed provoke a decrease in their performance and metabolism, which in turn can lead to shifts in their diversity (Bestelmeyer, 2000; Jenkins et al., 2011; Zakharov & Zakharov, 2014). Determining the resilience of ant communities to global warming is very arduous. It requires characterising the susceptibility of species composing these communities to heat, the projected heat to which they will likely be exposed, whether species may behaviourally escape this heat (e.g. by adjusting their circannual activity rhythm) and at which cost. Therefore, several indices have been used to describe the tolerance of ants to extreme heat (Roeder et al., 2021). The one most commonly used is the Critical Thermal maximum (CT_{max}, see for example Diamond et al., 2012; Bujan et al., 2020). CT_{max} is a physiological trait defined as the maximal temperature at which locomotion ceases or muscular spasms occur. At this temperature, ants can no longer escape heat and may die (Lutterschmidt & Hutchison, 1997).

This study aimed to compared the resistance to heat of several ant species from semi-arid, Mediterranean and temperate environment using the measure of their CTmax values. We do this by measuring their CT_{max}. We considered worker polymorphism and determined the relationships between resistance to heat and morphological traits. We hypothesized that: i) species from temperate habitats are less resistant on average than species from mesic and semi-arid habitats (i.e. have lower CT_{max}); ii) mesic and semi-arid species vary less in heat resistance than temperate species (i.e. have narrower ranges of CT_{max}) because heat is a stronger selective force in mesic and semi-arid habitats; iii) thermal resistance can be explained by simple morphological traits, such as the length of the legs, regardless of the latitude; and iv) the relationship between functional traits and the resistance to heat are the same in monomorphic and polymorphic species, but large workers in the latter are expected to be more heat resistant than smaller workers.

Material and methods

Study locations and measure of CT_{max}

We sampled 49 species of ants between October 2021 and August 2022 in four sites corresponding to semi-arid (Dar Dhaoui, 18 species), Mediterranean (Doñana, 11 species, and Argelès-sur-Mer, 14 species), and temperate (Ile-de-France, 13 species) climates (Table 1). The number of colonies sampled for each species depended on their occurrence (ranging from 1 to 7, Table 1), and they were located at least 10 m apart. For each colony, we sampled workers which were foraging on the ground. Using a vacuum, we gently collected 30 foragers per colony for monomorphic species, and 15 foragers per caste per colony for polymorphic species. In total, we used workers from 164 colonies. We placed the ants in 16.1×15×9 cm plastic boxes containing a wet cotton ball and immediately transported them to the lab for analysis. We measured ant’s CT_{max} within 12h after collection to avoid acclimatisation to laboratory conditions.

Table 1. Sampled ant species per site and climate. “MAP” stands for Mean Annual Precipitation. Ant species were either monomorphic (M) or polymorphic (P). For each species, the table gives the number of colonies and of workers sampled.

Climate and site description	Ant species	Morphology (Monomorphic or Polymorphic)	Number of colonies / number of workers sampled
Semi-arid Biotope: Desert Dar Dhaoui experimental range in Médenine, Tunisia: 33°17′41″N, 10°46′57″E. MAP ≈ 200mm y^{-1} (Akrimi et al., 1993). June 2022	*Camponotus barbaricus*	P	4 / 152
	Camponotus oasium	P	4 / 149
	Cataglyphis albicans	M	3 / 65
	Cataglyphis bombycina	P	1 / 31
	Cataglyphis rubra	P	6 / 178
	Cataglyphis savignyi	P	4 / 120
	Lepisiota frauenfeldi	M	1 / 30
	Messor aegyptiacus	P	4 / 179
	Messor arenarius	P	4 / 180
	Messor medioruber	P	2 / 90
	Messor picturatus	P	2 / 92
	Monomorium salomonis	M	6 / 180
	Monomorium sommieri	M	3 / 90
	Monomorium subopacum	M	3 / 90
	Pheidole pallidula	P	1 / 32
	Plagiolepis maura	M	1 / 32
	Tapinoma simrothi	P	3 / 91
	Tetramorium sericeiventre	M	3 / 9
Mediterranean Biotopes: freshwater marshes and shrubland Doñana National Park, Sevilla, Spain: 37°01′12″N, 6°26′24″W. MAP ≈ 549mm y^{-1} (ICTS Doñana Biological Reserve, 2023). October 2021	*Aphaenogaster senilis*	M	3 / 90
	Cataglyphis tartessica	M	3 / 90
	Crematogaster scutellaris	M	3 / 90
	Formica rufibarbis	M	3 / 90
	Lasius grandis	M	1 / 30
	Linepithema humile	M	4 / 120
	Messor marocanus	P	7 / 317
	Myrmica aloba	M	3 / 90
	Oxyopomyrmex saulcyi	M	1 / 30
	Tapinoma erraticum	M	1 / 30
	Tapinoma nigerrimum	P	3 / 90
Mediterranean Biotopes: Coastal sand-dunes and sand beaches Argelès-sur-Mer, France: 42°34'7"N, 3°2'46"E. MAP ≈ 642mm y^{-1} (Climate-data.org, 2021).	*Aphaenogaster senilis*	M	3 / 90
	Camponotus vagus	P	3 / 88
	Cataglyphis piliscapa	P	3 / 90
	Crematogaster scutellaris	M	3 / 90
	Formica rufa	M	3 / 90
	Lasius cinereus	M	3 / 90
	Messor barbarus	P	3 / 135
	Messor bouvieri	P	3 / 135
	Pheidole pallidula	P	3 / 90
	Plagiolepis pygmaea	M	3 / 30

	Plagiolepis pyrenaica	M	3 / 30
April 2022	*Tapinoma erraticum*	M	2 / 60
	Tapinoma nigerrimum	P	3 / 90
	Tetramorium caespitum	M	3 / 90
Temperate Biotope: temperate deciduous forest Ile-de-France: Vincennes Forest: 48°50'32"N, 2°27'18"E. Melun Forest: 48°29'35.016"N, 2°39'6.3648"E. Rambouillet Forest: 48°38'56"N, 1°52'57"E. **MAP ≈ 720 mm y^{-1} (Climate-data.org, 2021).** March to August 2022	*Dolichoderus quadripunctatus*	M	1 / 30
	Formica fusca	M	3 / 90
	Formica rufa	M	3 / 90
	Lasius brunneus	M	3 / 85
	Lasius emarginatus	M	3 / 89
	Lasius flavus	M	3 / 90
	Lasius niger	M	4 / 120
	Myrmecina graminicola	M	3 / 90
	Myrmica ruginodis	M	4 / 120
	Myrmica scabrinodis	M	2 / 60
	Myrmica specioides	M	1 / 29
	Temnothorax nylanderi	M	3 / 90
	Tetramorium caespitum	M	3 / 90

We measured CT_{max} using the dynamic method (reviewed by Roeder et al., 2021). Ants were placed on a hot plate that was gradually heated (Fig. S1), starting from room temperature and up to 70°C. We used a high precision (110°C max ± 0.1°C) hot plate (model PZ28-1, Gestigkeit, Germany). To be able to monitor the ants' locomotion and to prevent them from escaping from the hot plate, we placed 5.2 cm diameter × 6 cm height plastic tubes open at both ends and covered with liquid polytetrafluoroethylene (Fluon® PTFE) directly on the hot plate, as shown in Fig. S2. A maximum of three ants were placed in the same tube at once. In some species, workers occasionally climbed on top of one another, in which case we gently pushed them back on the plate with entomological tweezers. The CT_{max} of each ant was recorded as the temperature at which each individual lost locomotor coordination (i.e., stopped walking or spasm occurred). After reaching its CT_{max}, each ant was placed in a vial containing 70% ethanol for morphological measurements.

Morphological traits

We dissected a subset of the ants tested for CT_{max} (i.e., 5 ants per colony in monomorphic species or 5 ants per worker caste per colony for polymorphic species) and measured a set of morphological traits with functional significance according to Schofield et al. (2016). Specifically, we measured maximal head width, maximal head length, clypeus length, leg length (of each of the three pairs of legs), and Weber's length as indicative of body size (Table 2). To reduce the unevenness of measures due to differences in the angle of body parts, those were independently glued to a 12×12 cm plastic box using double-faced tape, then photographed with a Leica EZ4 W stereomicroscope. Measures were carried out with ImageJ software version 1.53s (Schindelin et al., 2012).

Table 2. Selected morphological traits and their functional significance. Leg length was measured for the three pairs of legs, as the sum of tibia length and femur length (only right side). Adding these two measures is biologically justified and they are highly correlated (>0.97) for all pairs of legs.

Character	Trait measured	Functional significance
Head size	Head width (max); Head length (clypeus-occiput)	Head size may vary allometrically with body size, head size may also be linked to predatory strategies, with wider heads allowing for more powerful mandible muscles and larger mandibles hence larger prey and larger seeds (Kaspari & Weiser, 1999; Sarty et al., 2006).
Clypeus	Clypeus length	Modified clypeus is more common in species that rely on liquid food (Eisner, 1957; Davidson et al., 2004)
Eyes	Interocular distance; Eye position ((head width – interocular distance) / head length)	Visual predators have more dorsally positioned eyes (higher interocular distance, Fowler et al., 1991).
Leg length, for each pairs of legs	Sum of Tibia length and femur length.	Leg length is related to thermoregulatory strategy (Sommer & Wehner, 2012) and increases locomotion speed in simple habitats; in complex habitats shorter legs allow exploitation of crevices and manoeuvrability (Sarty et al., 2006; Gibb & Parr, 2010).
Mesosoma	Mesosoma (Weber's length)	Indicative of overall body size and often linked to thermoresistance (Willmer & Unwin, 1981), and resource use (Kaspari and Weiser 1999)

Data analysis

For each worker caste and colony, we calculated the median CT_{max} (i.e., it can be interpreted as the LD50, Camacho et al., 2023) based on all workers measured for CT_{max}, and the mean of each morphological trait (there was no extreme value) based on the subsample measured for morphology, resulting in 251 samples. Hereafter, we expressed temperatures as median and interquartile range (i.e., median (IQR)). Data were analysed using R (version 4.0.0) software. We analysed the differences among sites in terms of median CT_{max} (i.e., median of CT_{max} of all species of each site, with Kruskal-Wallis and Wilcoxon pairwise comparisons) and range of CT_{max} (i.e., difference between lowest and highest CT_{max} of each site, with a linear model and an Anova test). To compare the differences in CT_{max} between castes in polymorphic species, we used Kruskal-Wallis and Wilcoxon pairwise comparison. To assess which morphological traits explained CT_{max} the best, we first verified Pearson's correlation (stats package R Core Team, 2023) to avoid collinearity among variables. Only variables with a correlation < 0.9 were conserved (Fig. S3). Then, we analysed the influence of these traits on the CT_{max} by performing a series of generalised linear models assuming a gamma distribution (i.e., CT_{max} has positive continuous values only) and selected those with the lowest AIC.

To assess the influence of the preselected morphological traits on CT_{max} in all our sampled species, we constructed a series of models considering different interactions. The first model included all species. The second comprised only the species from *Cataglyphis* genus, which were separated from the other species because of their much higher resistance to heat (Boulay et al., 2017), and because they are conspicuous in semi-arid zones in the Paleartic (Amor & Ortega, 2014). We thus constructed a third model with all non-*Cataglyphis* species, for comparison. To evaluate the effects of worker polymorphism on CT_{max}, we constructed two additional models, one for non-*Cataglyphis* monomorphic species and one for non-*Cataglyphis* polymorphic species (hereafter mono- and polymorphic species, respectively). Homogeneity, normality, and independence of residuals were analysed through validation plots (plot function, R Core Team, 2023).

To assess the differences in intraspecific variation of CT_{max} between monomorphic and polymorphic species, we calculated the coefficient of variation (*cv= (standard deviation / mean)* × *100*) for each species, in each sampled site, and performed a Wilcoxon-Mann-Whitney test (stats package, R Core Team, 2023) to compare differences between groups. In addition, for species sampled in more than one site we tested for difference in heat resistance between sites using linear mixed effect models considering the colonies as a random effect (lmer4 package, R Core Team, 2023). For polymorphic species sampled in more than one site, we compared the same worker caste across sites (i.e., minors vs minors, majors vs majors).

Results

Heat resistance (CT$_{max}$)

Within species, workers had similar CT$_{max}$ (Fig. S4 to S7) and there was no noticeable difference between colonies. In addition, there was no difference in the intraspecific variation of CT$_{max}$ between monomorphic species (*cv* = 2.59, IQR=1.421%) and polymorphic species (*cv* = 3.0, IQR= 1.13%, Wilcoxon test: W=196, p>0.05). This allowed determining the CT$_{max}$ of each species, computed as the mean of the CT$_{max}$ of the *n* colonies sampled for the species, which are themselves the median of the CT$_{max}$ of the workers of each colony. CT$_{max}$ differed markedly between species, ranging from 41.1°C for *Myrmecina graminicola* (from temperate environment) up to 65.4°C for *C. savignyi* (from semi-arid environment), i.e. a difference of 24.3°C (Fig. 1).

The six species of *Cataglyphis* were the most resistant to heat. Excluding *Cataglyphis* spp, the genus with the highest CT$_{max}$ was *Aphaenogaster* (56.2, (55.5, 57.3) °C) and the one with the lowest was *Myrmecina* (40.5 (40.4, 41.1) °C). These genera were represented by one monomorphic species each. CT$_{max}$ per genus are shown on Fig. S8. For polymorphic ants, the most resistant genus was *Camponotus* (52.9 (52.3, 53.4) °C) and the least resistant was *Pheidole* (46.5 (45.8, 47.1) °C).

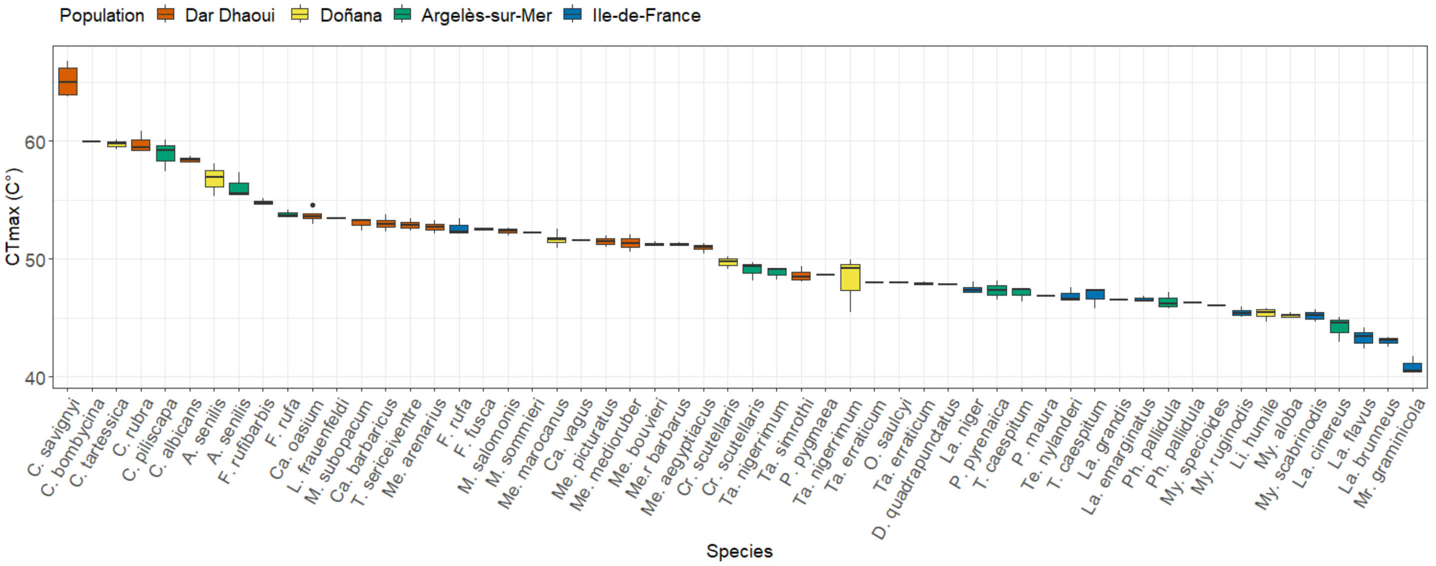


Fig 1. Heat resistance of species per site. Boxplots show the median and interquartile of CT$_{max}$. For polymorphic species, all worker sizes were combined. Corresponding data is available in Table S1.

17 species were polymorphic. The CT$_{max}$ significantly differed between worker castes in 15 of these species. It was higher for the larger castes in 35 out of 41 comparisons (i.e. large workers were more resistant than medium workers in 10 of 12 cases, than small workers in 15 of 17 cases, and medium workers were more resistant than small workers in 10 of 12 cases, Fig. 2, Table S2).

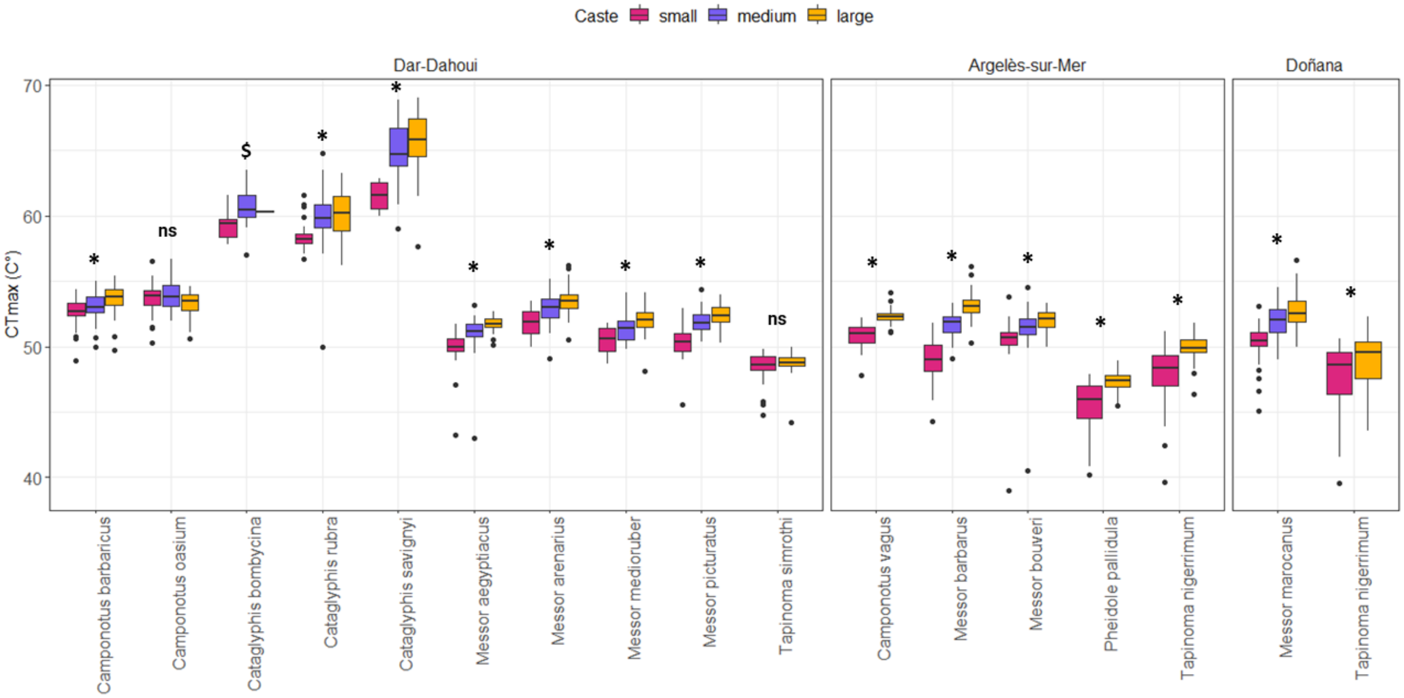


Fig. 2: CT$_{max}$ of worker castes in polymorphic species. The plot shows the CT$_{max}$ of small, medium, and large workers of polymorphic species found across sites. All values were pooled per caste regardless the colony. Results of Kruskal-Wallis and Wilcoxon pairwise tests are shown in Table S2. * means that the larger caste is statistically significantly more resistant than the medium one and medium caste is more resistant than small one, $ shows comparisons where the larger caste is less resistant than the smaller caste(s), ns shows no differences between castes..

Seven species were encountered at two sites. Six species showed no difference in CT$_{max}$ between sites (Fig. 3, Table S3). *Formica rufa* was significantly more heat resistant in its southernmost site but the difference is small (Argelès-sur-Mer: 53.8 (53.0, 54.6) °C *vs.* Ile-de-France: 52.6 (51.6, 53.5) °C, t= -2.935, $p<0.05$).

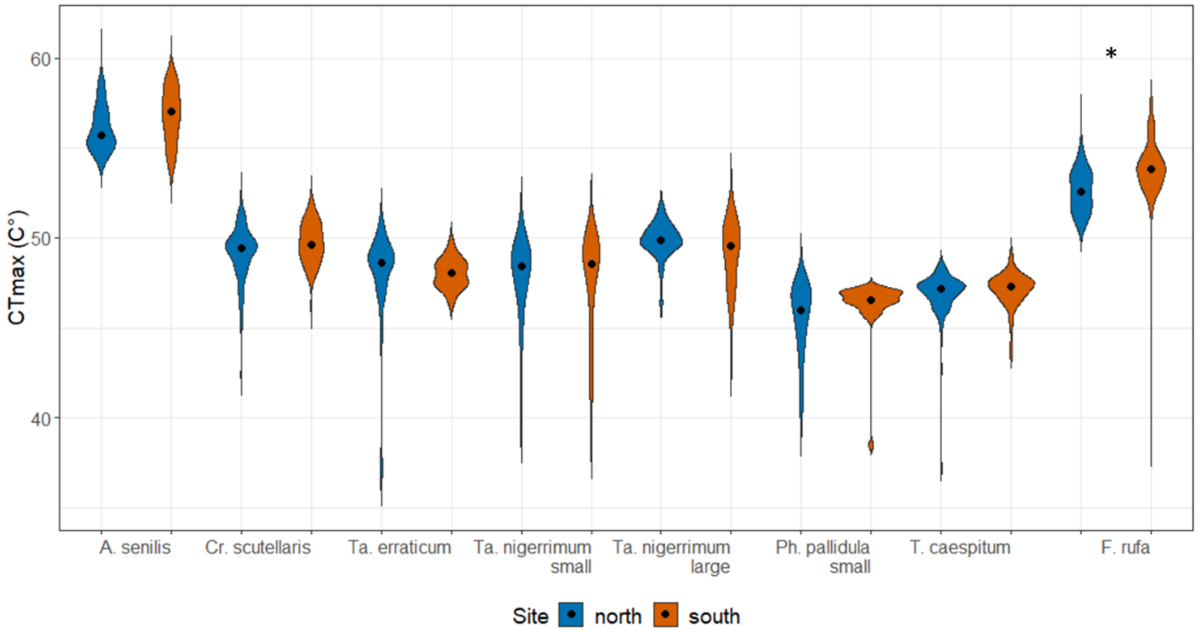


Fig. 3. CT_{max} of species sampled in two different sites. The plot shows the IQR of CT_{max} in southern and northern sites. Black dot shows median value. Comparisons are shown in Table S3. * shows a statistically significant difference of resistance between sites.

Habitat influence on CT_{max}

Combining the CT_{max} of the species for each site allowed us to determine a community-level distribution of heat resistance and to show that it varied across latitudes (Fig. 4). While many species had a similar CT_{max} (around 48-52°C) across the four sites, the temperate region was characterised by a large peak of species with a low CT_{max} (46.3°C or less) while the southernmost site was characterised by two peaks of species with high CT_{max} (52.8 °C or more). Consequently, temperate species had lower CT_{max} on average than species from the three warmer sites ($\chi^2 = 55.717$, df=2, $p<0.001$, Île-de-France vs each of the three other sites: $p<0.001$), and Mediterranean species were less heat resistant than species from Dar Dhaoui ($\chi^2 = 55.683$, df=2, Dar Dhaoui vs each Mediterranean site: $p<0.001$, Fig. 4). However, while median heat resistance increased at lower latitude, the range in heat resistance (i.e. the difference between the highest and lowest CT_{max} in each site) differed little between sites (anova test $F_{3/52} = 8.418$, $p<0.001$). Argelès-sur-Mer had a narrower range (25.4 °C) than Dar-Dahoui (30.5 °C, $p = 0.001$) and Ile-de-France (26.2 °C, $p < 0.001$), which did not differ from one another, whereas Doñana had the same range (24.5 °C) than the other sites ($p > 0.05$ in all cases).

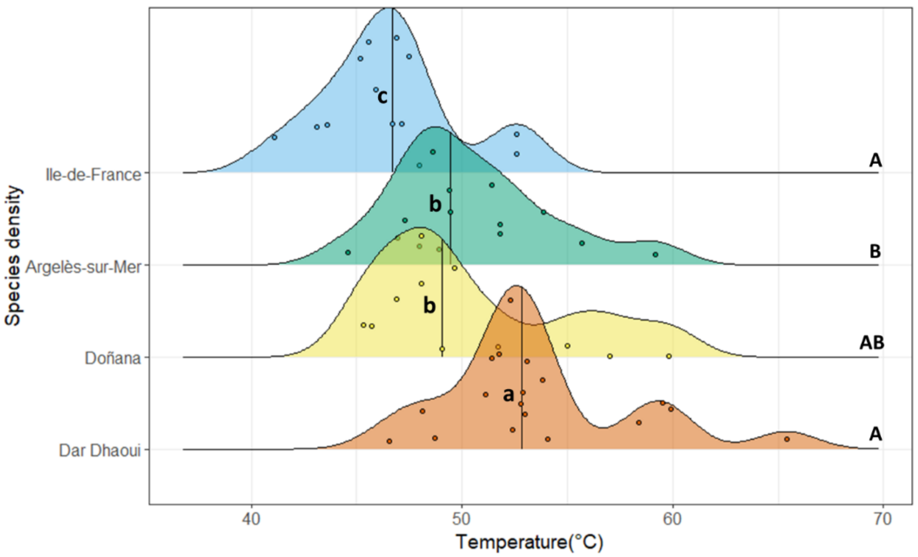


Fig. 4. Medians and ranges of CT_{max} across sites. The Kernel density plots were computed using the median CT_{max} of each species, which are shown as points. Vertical lines show the median CT_{max} of each site. Data available in Table S1. Lower case letters show differences of medians (Kruskal-Wallis test) and capital letters differences between ranges (Anova test).

Selected morphological traits and GLMs

The most independent morphological traits were interocular distance, eye position and hind leg length (Table 3, for all traits, see Fig. S3). CT_{max} correlated with hind leg length and interocular distance, which are correlated to one another ($r^2 = 0.73$, Fig. S3), but not with eye position (Table 3).

Table 3. Pearson's correlation coefficient between the morphological traits retained ($r^2 < 0.9$) and the CT_{max}. Repeated values have been removed.

	Interocular distance	**Hind leg length**	**Eye position**	**CT_{max}**
Interocular Distance	1.00			
Hind leg length	0.73 (*p* < 0.001)	1.00		
Eye position	-0.20 (*p*= 0.001)	0.17 (*p*= 0.006)	1.00	
CT_{max}	0.42 (*p* < 0.001)	0.70 (*p* < 0.001)	0.10 (*p*= 0.127)	1.00

Results from the GLM models are shown in Table 4. The best model for all species included the three selected morphological traits and their first-order interactions, as well as the sampling sites and the genus. The genus was particularly important since the model explained much less variance when this variable was removed. When considering *Cataglyphis* species only, the model that fitted best the variance of CT_{max} included hind leg length, site and species. When considering non-*Cataglyphis* species only, the best model included the full interaction among the three morphological traits, site, and genus. The CT_{max} of monomorphic species were best explained by the three morphological traits, site, and genus. For polymorphic species, the best model included the full interaction among the three morphological traits, site, genus, and worker caste. Overall, site and genus (or species) was always included in the best models, but site always had a negligible effect whereas genus (or species) always had an important effect.

Table 4. GLMs for resistance to temperature. Only the best four models per category are displayed and the selected ones appear in bold (lowest AIC). * denote a full interaction of variables (i.e. effects of each variable and effect of their interaction) and × denote simple interactions (i.e. no effect of the variables but effect of their interaction).

	Model	**AIC**	**R^2**
All species	Hind leg + eye position + interocular + hind leg × eye position + hind leg × interocular + eye position × interocular + site + genus	794.15	0.95
	Hind leg + eye position + interocular + hind leg × eye position + hind leg × interocular + eye position × interocular + genus	**797.32**	**0.95**
	Hind leg + eye position + interocular + hind leg × eye position + hind leg × interocular + eye position × interocular + site	1236.60	0.66
	Hind leg + eye position + interocular + hind leg × eye position + hind leg × interocular + eye position × interocular	1275.90	0.60
Cataglyphis spp	Hind leg + site + species	86.67	0.91
	Hind leg + species	**86.67**	**0.91**
	Hind leg + site	95.38	0.86
	Hind leg	94.46	0.84
Non *Cataglyphis spp.*	Hind leg * eye position * interocular + site + genus	704.30	0.90
	Hind leg * eye position * interocular + genus	**706.52**	**0.90**
	Hind leg * eye position * interocular + site	994.25	0.59
	Hind leg * eye position * interocular	1033.30	0.49
Monomorphic species	Hind leg + eye position + interocular + site + genus	322.59	0.93
	Hind leg + eye position + interocular + genus	**341.29**	**0.92**
	Hind leg + eye position + interocular + site	427.41	0.74
	Hind leg + eye position + interocular	465.31	0.42
Polymorphic species	**Hind leg * eye position * interocular + site + genus + caste**	**331.23**	**0.86**
	Hind leg * eye position * interocular + genus + caste	331.96	0.86
	Hind leg * eye position * interocular + site + genus	332.86	0.86
	Hind leg * eye position * interocular + genus	335.02	0.85

Morphological traits and CT_{max}

The relations between each selected morphological trait and CT_{max} are shown in Fig. 5 for our three groups of species (*Cataglyphis* spp, non-*Cataglyphis* monomorphic and non-*Cataglyphis* polymorphic species). Hind leg length and CT_{max} were positively related for the three groups (Fig. 5). However, while the relationship was the same for monomorphic and polymorphic ants (Anova test, $F_{219,220} = 0.12$, $p>0.05$), the slope and intercept were higher for *Cataglyphis* spp. ($F_{248,249} = 272.02$, $p<0.001$, Fig. 5, see also Table S4). Similar results were observed for the interocular distance: positive relationship with CT_{max}, no differences between monomorphic and polymorphic species ($F_{219,220}=2.07$, $p>0.05$), and difference between these two groups and *Cataglyphis* spp. ($F_{248,249}=338.09$, $p<0.001$) which again had higher slope and intercept (Fig. 5). We found no relationship between eye position and CT_{max} for the three groups of species (Fig. 5, Table S4).

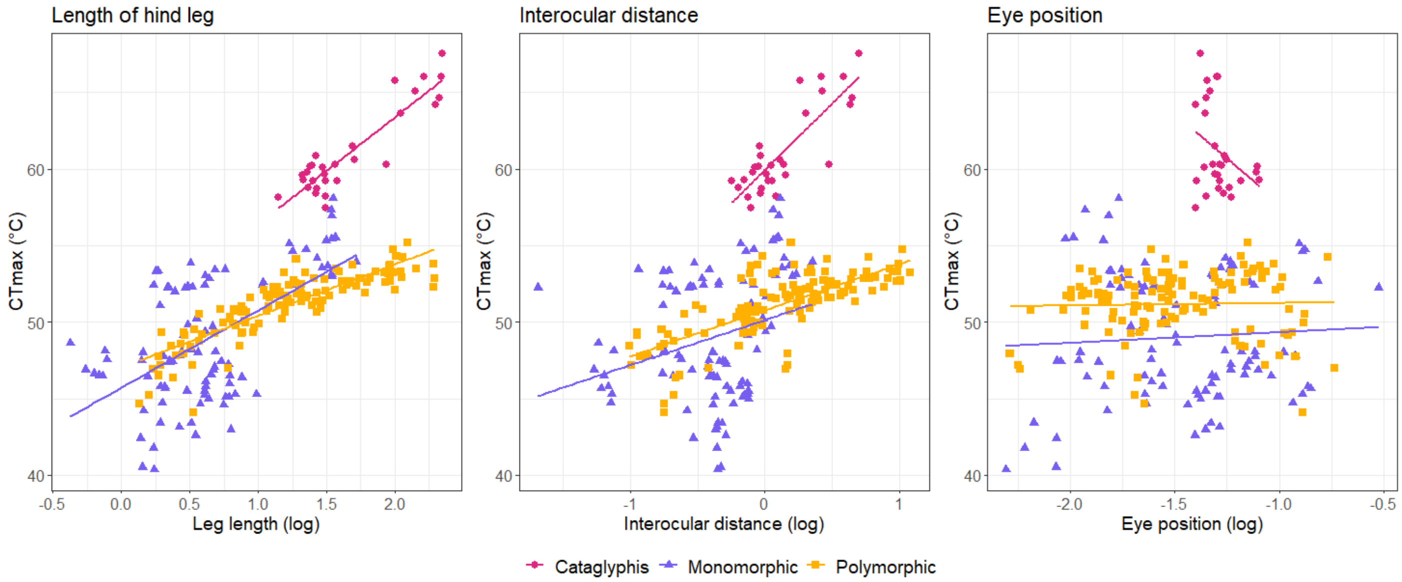


Fig. 5. Relationship (linear regressions) between the three morphological traits and the CT_{max}. Lines correspond to linear models per group calculated with $y \sim x$.

Discussion

Differences of CT_{max} among environments.

The heat resistance of ants has been associated with environmental temperatures, with ants from warmer environments more resistant to high temperatures (Janzen, 1967). Our findings unambiguously support this statement, as the most resistant ant species were observed in southernmost sites, while the least resistant species were found in northernmost sites (Fig. 2, Table S2). It has been suggested that heat resistance is a plastic trait, and ant species may acclimatize to a specific microhabitat to some extent (Baudier et al., 2018). However, our results diverge from these findings. Despite sampling ants during different seasons, species that were present in two sites had the same resistance across sites. The only exception was *F. rufa* from temperate and Mediterranean environments, which were more heat resistance in the Mediterranean. However, although statistically significant the difference between the two sites was low (< 1°C). Arguably, *F. rufa* is a seasonal generalist that is active during several seasons and may be more plastic in heat resistance than many other species, as found in other species (Bujan, et al., 2020b). Consequently, the observed difference in CTmax between the two sites may not necessarily reflect actual variations between colonies but could more likely be attributed to seasonal effects.

Our initial prediction that Mediterranean and semi-arid species would have narrower ranges of CT_{max} was not verified as the observed differences among sites are not latitude-dependant. If anything, we observe the opposite trend that the range of CT_{max} increases at lower latitude (Fig. 4). This could be attributed to the fact that elevated temperatures favour the development of increased heat resistance in certain thermophilic species. However, this may not be as pronounced in other species, prompting them to adapt to heat using strategies different from heat resistance. These findings contrast with other studies associating the range of community resistance with temperature (Huey et al., 2012; Buckley & Kingsolver, 2012). Furthermore, our results differ from previous reports on European ant communities (Arnan et al., 2015), suggesting narrower thermal niches in mesic environments compared to temperate ones, hinting at potentially lower resilience in the former. Nonetheless, our results must be considered with caution as we did not analyse the impacts of significant temperature variations between day and night, particularly important in the semi-arid site (Louw & Seely, 1982). These daily temperature fluctuations have been linked to a broad range of resistance, especially regarding the upper limit (Garcia-Robledo et al., 2018; Calazans et al., 2020). Additionally, our study did not consider phenology, circadian rhythm of activity, or dominance/subordination interactions in the communities, factors known to influence resistance (Cerdá, et. al., 1998). Moreover, we only focused on the upper critical temperature and lack information about the thermal optimum of the studied species, limiting our understanding of their thermal niche.

Morphological traits and phylogeny

In our study, we confirmed that morphological traits can be used as a proxy for understanding the heat resistance of ants, and especially the importance of the length of the legs (Cerdá & Retana, 2000; Sommer & Wehner, 2012). Longer legs allow ants to move faster and reduce the exposure to heat (Hulbert et al., 2008). Also, they keep vital organs in the abdomen farther from the surface of the soil (Wehner & Wehner, 2011), as air temperature is considerably lower when moving even only a few millimetres away from the soil surface (Lembrechts et al., 2020). The relationship between the length of the leg and the resistance to heat is considered particularly important for *Cataglyphis* ants (Boulay et al., 2017), and our findings support the notion that leg length provides sufficient information about heat resistance (Table 4). Although our focus was on morphological traits, it's worth noting that in *Cataglyphis* ants, resistance to heat is also attributed to the presence of heat-shock proteins that protect against cellular damage resulting from exposure to high temperatures (Gehring & Wehner, 1995).

Importantly, our study also confirmed significance of leg length regardless of the ant genus or morphology. For non-*Cataglyphis* ants, interocular distance and eye position also emerged as important contributors to the model. However, since hind leg length was highly correlated to all the morphological traits other than interocular distance and eye position (e.g. $r^2 = 0.94$ with mesosoma and total body length, Fig. S3), it remains unclear which of these traits determine heat resistance. Body size, frequently mentioned in the literature, is associated with larger ants taking longer to heat up and experiencing less water loss due to their lower surface/volume ratios compared to smaller ants (Kühsel et al., 2017). The high correlation among these traits, as they are generally larger in bigger ants, further complicates the disentanglement of individual trait contributions to heat resistance.

We found that interocular distance was positively related to the CT_{max}, regardless of the genus (Table S4). Although the functional relevance of eye position to heat resistance is not evident (Fig. 5), it can serve as a useful proxy if it correlates with other traits associated with heat resistance. Notably, high interocular distances have been linked to the reliance on visual cues for predation (Fowler et al., 1991). The use of visual cues has been reported in desertic ant genera like *Cataglyphis* and *Melophorus* (Cheng et al., 2014; Ronacher, 2020)), as well as in temperate ants like *Formica rufa* (Fernandes et al., 2015)). These ant genera are typically found in open and visually simple environments such as deserts or grasslands (Silva & Brandão, 2010; Gibb & Parr, 2013). In such environments, where soil temperatures are elevated due to sun exposure (Lembrechts et al., 2020), the indirect correlation between interocular distance and heat resistance becomes apparent.

Worker polymorphism

Our results showed that the resistance of monomorphic and polymorphic species was explained by the same morphological traits (i.e., length of hind leg, interocular distance and eye position, Fig. 5). However, in the latter species the relationship between traits is more complex, due to their higher intraspecific variability of morphological traits (i.e., the best model included full interactions between the three morphological traits). We could confirm that in polymorphic species larger workers were more resistant than smaller ones, as previously reported by other authors for different species (Cerdá et al., 1997; Baudier & O'Donnell, 2018). Moreover, a low intraspecific variance in heat resistance was measured, despite the high variance in body size in polymorphic species such as *Pheidole pallidula* or *Camponotus barbaricus*. These findings indicate that, in polymorphic species, the thermal resistance cannot be solely attributed to the individual sizes of body parts. This phenomenon has been previously observed in thermophilic ants (Sommer & Wehner, 2012). However, this may be species-specific, as we found two species whose castes did not show any difference in CT_{max} (*Camponotus oasium* and *Tapinoma simrothi* (Fig. S4-S7, Table S2).

Conclusion

In this study, we confirmed the importance of a limited number of morphological traits for predicting the resistance of ants to heat. Our initial hypothesis that simple morphological traits could explain the heat resistance seemed more accurate when we also took the genera and environment into account. *Cataglyphis* genus's CT_{max} can be explained simply by using the length of hind legs as an indicator. This explains why so many authors have decided to use this trait only. Nevertheless, our study shows that for other genera of ants, other traits may be useful in addition to the length of the hind leg, yet this trait remains the best (i.e., Table S4). Combinations of size and eye-related traits are important when trying to explain heat resistance. Overall, we could distinguish two patterns: one for *Cataglyphis* and one for the other genera (mono and polymorphic combined). Also, we observed higher heat resistances in warmer environments than in more temperate sites, but not a narrower range in heat resistance. This suggests that strategies other than heat resistance allow adaptation to hot environments. These results hint that we should consider other morphological traits than hind leg, or even other aspects of ant biology, as bioturbation activity, behaviour and hierarchical relationships with other species.

Supplementary material

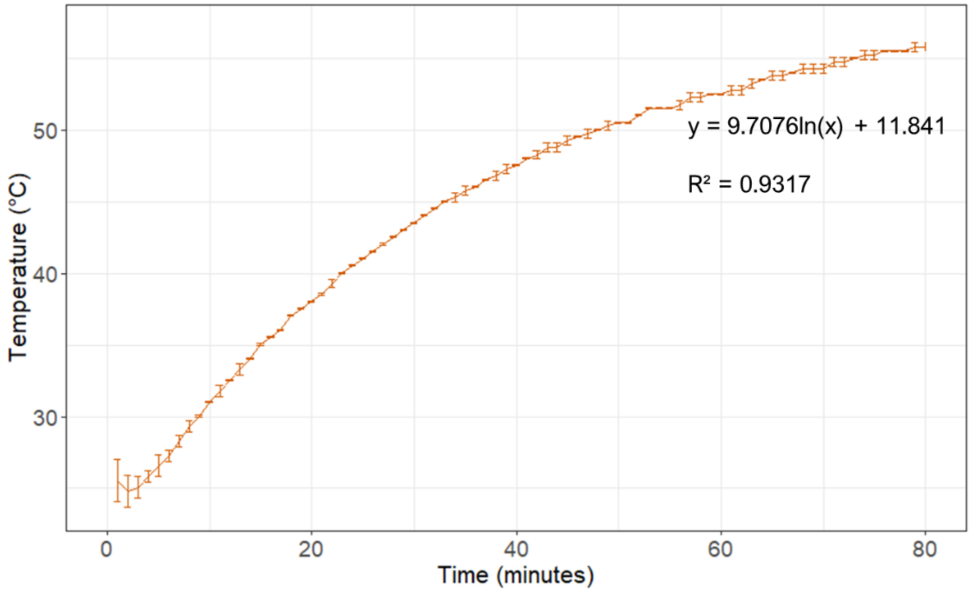


Fig S1. Temperature augmentation during CT$_{max}$ essays (n=3). The plate was set with a maximal temperature of 70°C and constant electric power. The temperature increased relatively linearly at first and then logarithmically, but repeatedly between trials. One could compare our results with those from other studies regardless of the method used (static or dynamic) with the method proposed by Jørgensen LB, Malte H, Ørsted M, Klahn NA, Overgaard J (2021) A unifying model to estimate thermal tolerance limits in ectotherms across static, dynamic, and fluctuating exposures to thermal stress. *Sci Rep* **11**:12840. https://doi.org/10.1038/s41598-021-92004-6.

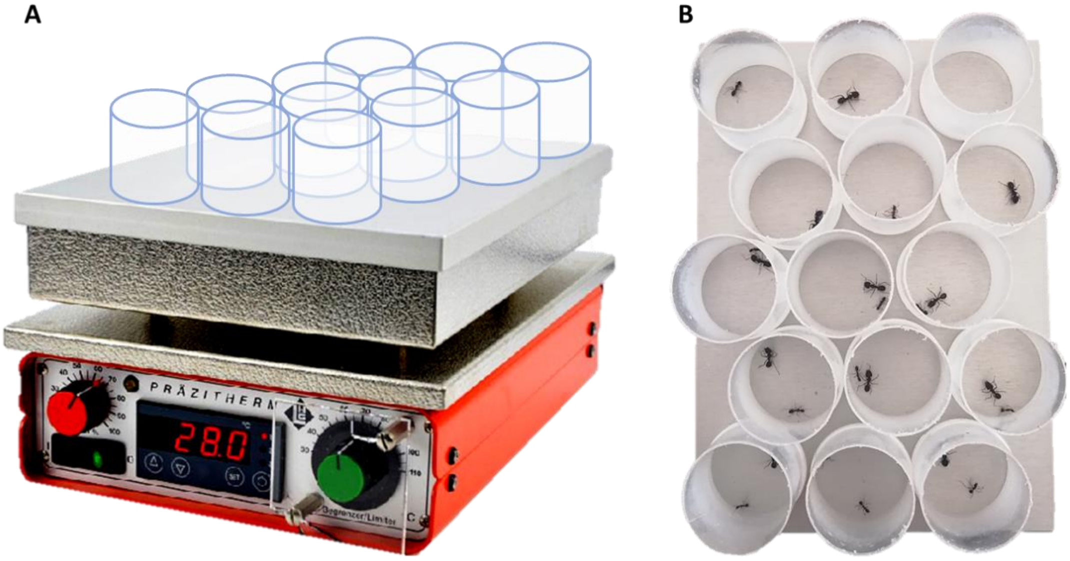
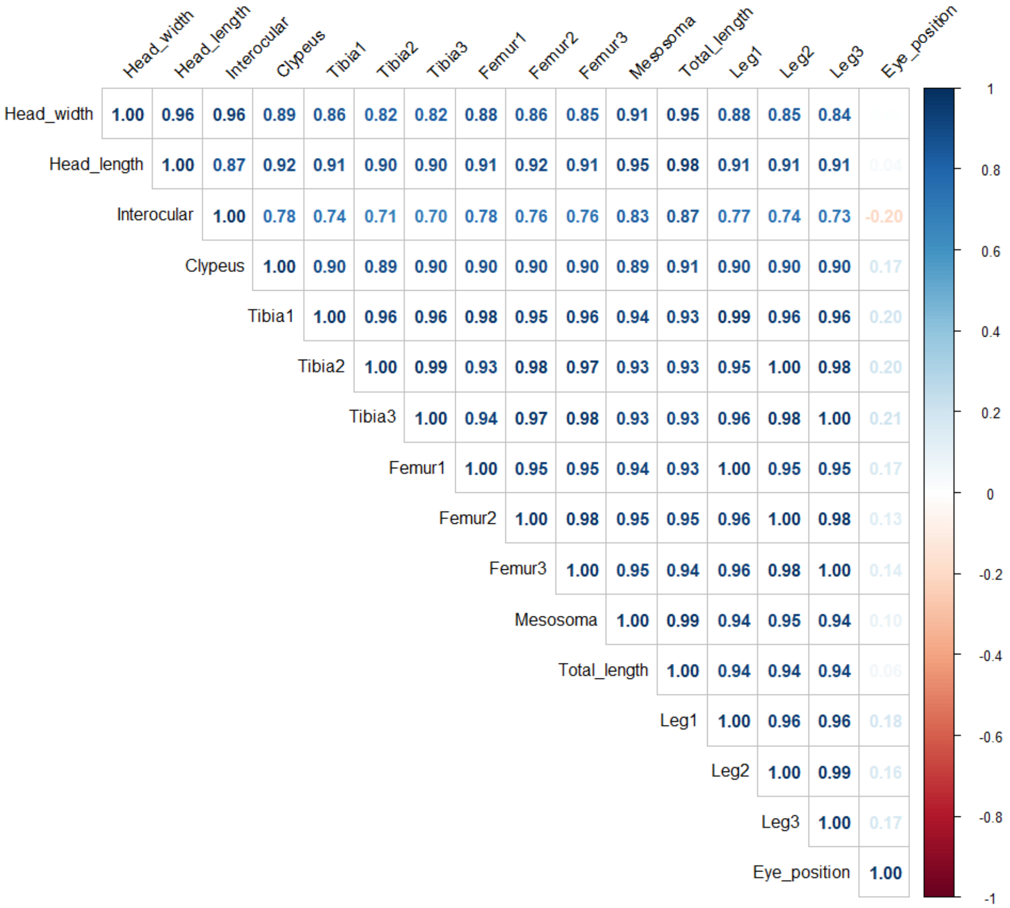


Fig. S2. Setup used to measure CT$_{max}$. (A) shows the hot plate (model PZ28-1, Gestigkeit, Germany) with a sketched disposition of open-bottom and open-top cylinders on its hot surface. (B) is a top view of the plate showing ants in cylinders. Here, each cylinder contains two workers differing in size.



	Head_width	Head_length	Interocular	Clypeus	Tibia1	Tibia2	Tibia3	Femur1	Femur2	Femur3	Mesosoma	Total_length	Leg1	Leg2	Leg3	Eye_position
Head_width	1.00	0.96	0.96	0.89	0.86	0.82	0.82	0.88	0.86	0.85	0.91	0.95	0.88	0.85	0.84	0.01
Head_length		1.00	0.87	0.92	0.91	0.90	0.90	0.91	0.92	0.91	0.95	0.98	0.91	0.91	0.91	0.04
Interocular			1.00	0.78	0.74	0.71	0.70	0.78	0.76	0.76	0.83	0.87	0.77	0.74	0.73	-0.20
Clypeus				1.00	0.90	0.89	0.90	0.90	0.90	0.90	0.89	0.91	0.90	0.90	0.90	0.17
Tibia1					1.00	0.96	0.96	0.98	0.95	0.96	0.94	0.93	0.99	0.96	0.96	0.20
Tibia2						1.00	0.99	0.93	0.98	0.97	0.93	0.93	0.95	1.00	0.98	0.20
Tibia3							1.00	0.94	0.97	0.98	0.93	0.93	0.96	0.98	1.00	0.21
Femur1								1.00	0.95	0.95	0.94	0.93	1.00	0.95	0.95	0.17
Femur2									1.00	0.98	0.95	0.95	0.96	1.00	0.98	0.13
Femur3										1.00	0.95	0.94	0.96	0.98	1.00	0.14
Mesosoma											1.00	0.99	0.94	0.95	0.94	0.10
Total_length												1.00	0.94	0.94	0.94	0.06
Leg1													1.00	0.96	0.96	0.18
Leg2														1.00	0.99	0.16
Leg3															1.00	0.17
Eye_position																1.00

Fig. S3. Correlation matrix of measured morphological traits.

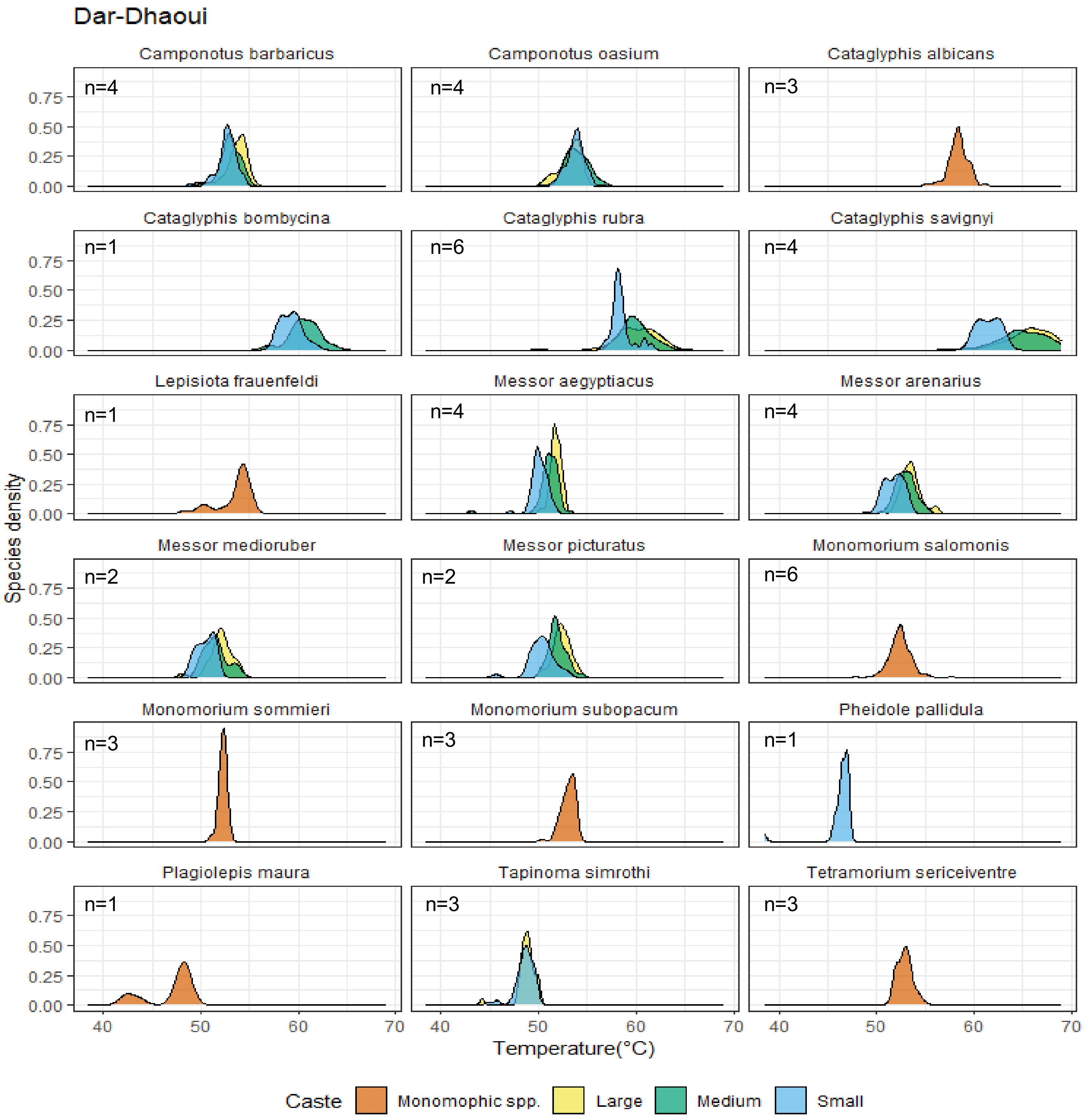


Fig. S4. Temperature resistance of ants sampled in Dar-Dhaoui, Tunisia. Colonies (number given in top left corner of each plot) are pooled by caste.

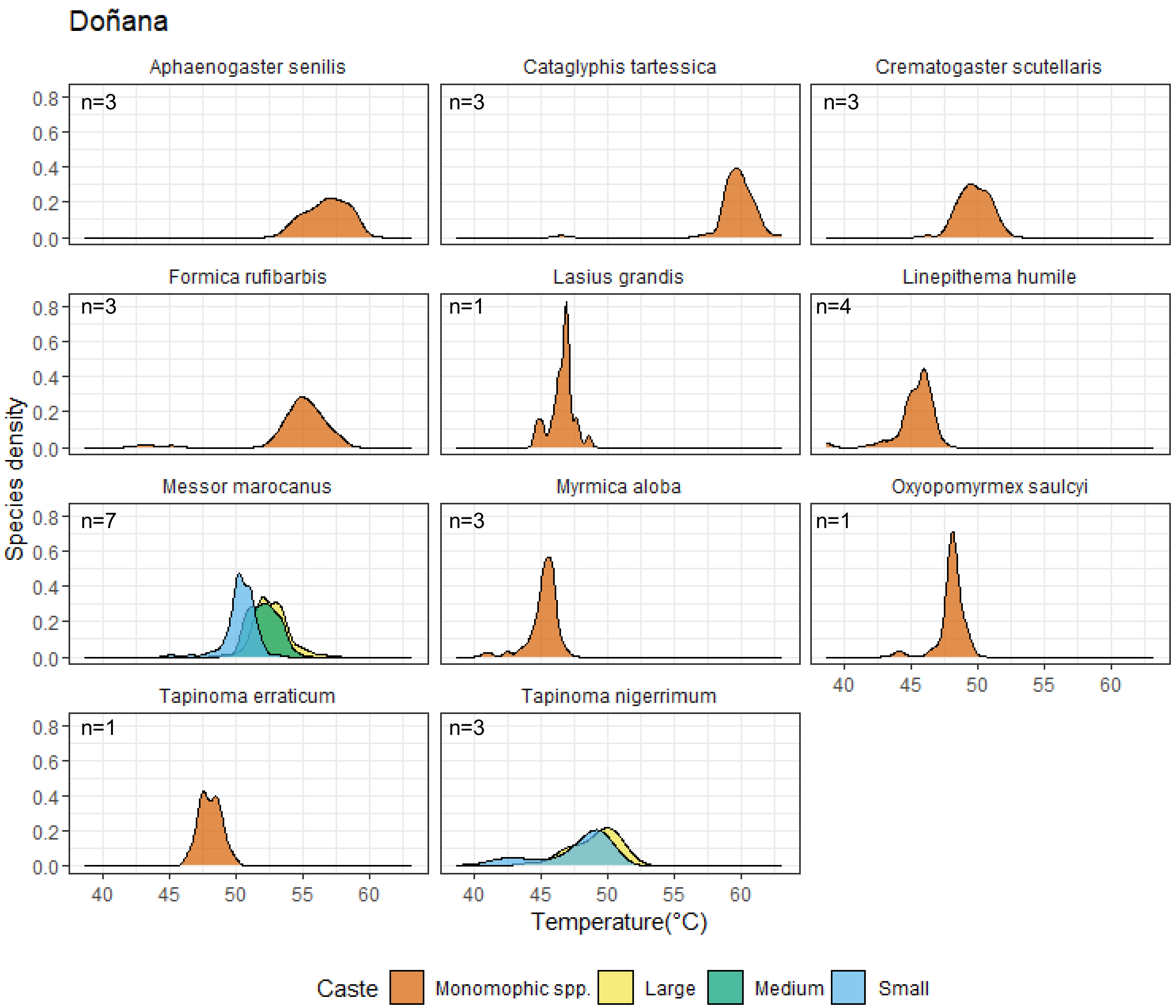


Fig. S5. Temperature resistance of ants sampled in Doñana National Park, Sevilla, Spain. Colonies (number given in top left corner of each plot) are pooled by caste.

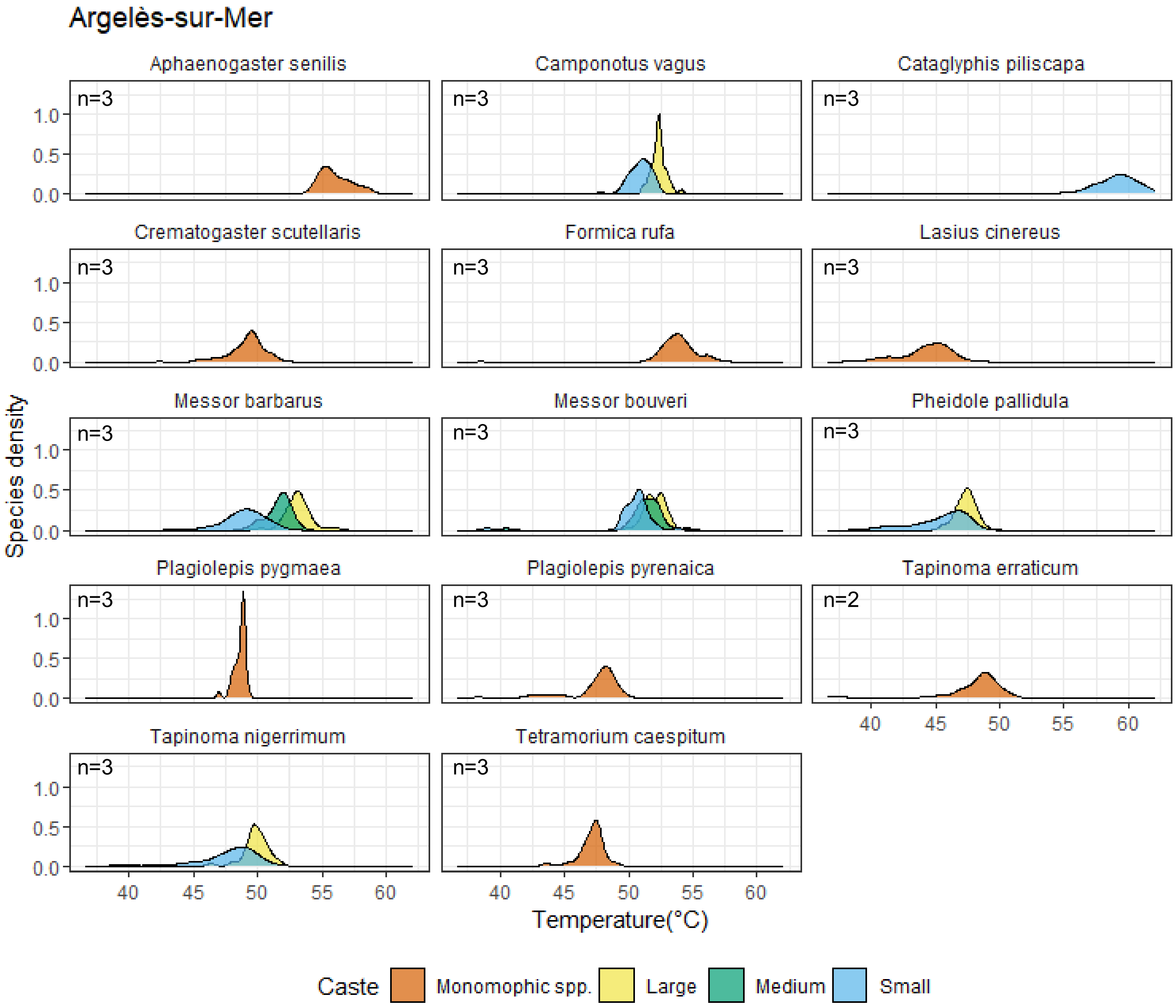


Fig. S6. Temperature resistance of ants sampled in Argelès-sur-Mer, Pyrennées Orientales, France. Colonies (number given in top left corner of each plot) are pooled by caste.

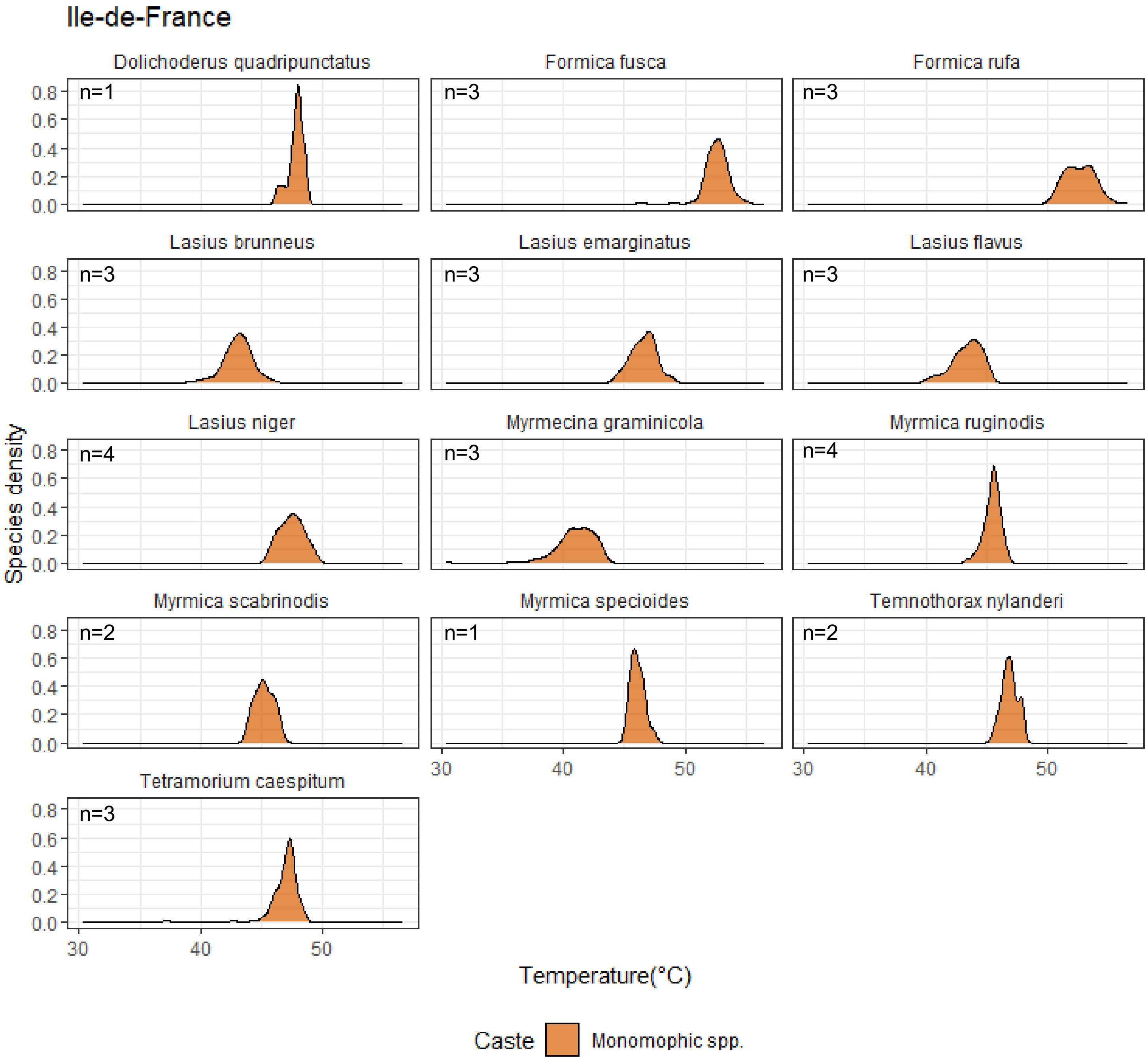


Fig. S7. Temperature resistance of ants sampled in Ile-de-France, France. Colonies (number given in top left corner of each plot) are pooled.

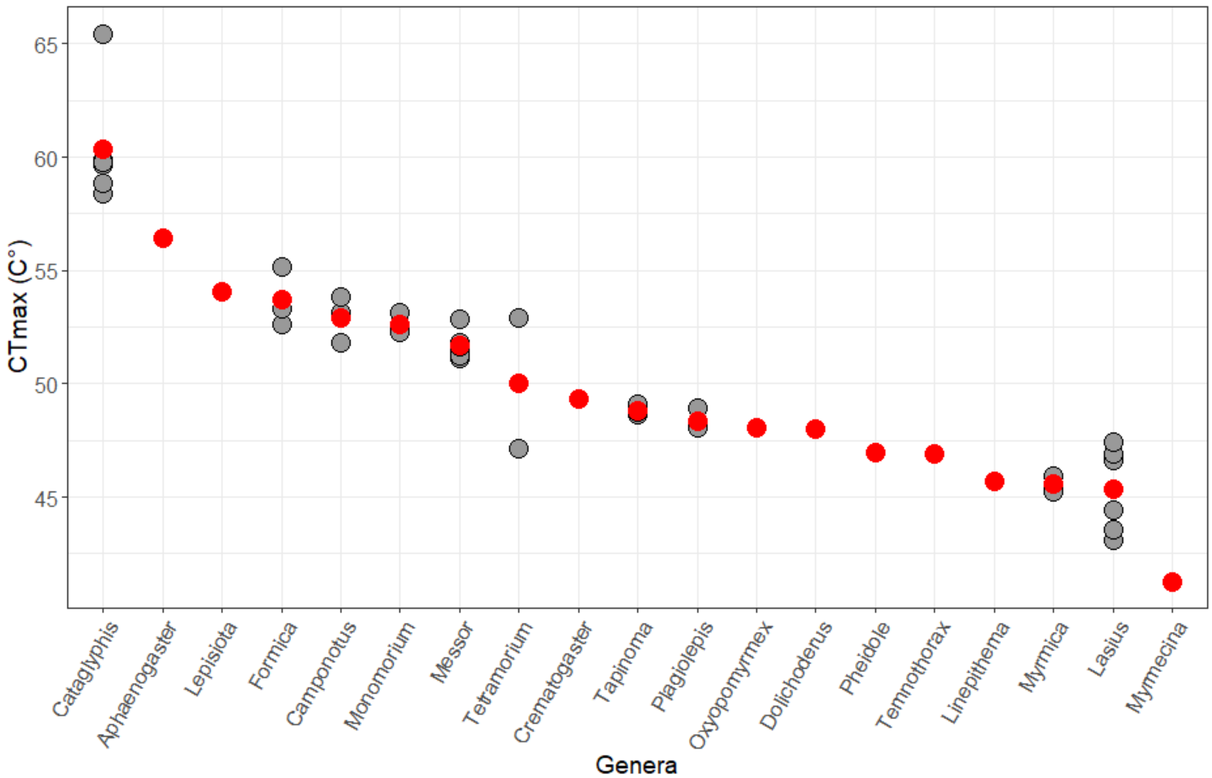


Figure S8. Heat resistance of genera. Gray points correspond to the mean CT_{max} per species (calculated as the mean of the median per colony), regardless the site. Red points correspond to the mean of all species of the same genus.

Table S1. CT$_{max}$ per species for all the environments. The values correspond to the mean of median CT$_{max}$ per colony, regardless the caste.

Site	Species	median	1st Qu.	3rd Qu.
Dar-Dhaoui	*Camponotus barbaricus*	53.00	52.50	53.70
	Camponotus oasium	53.80	53.10	54.30
	Cataglyphis albicans	58.40	57.90	58.90
	Cataglyphis bombycina	59.90	59.10	60.75
	Cataglyphis rubra	59.50	58.60	60.80
	Cataglyphis savignyi	65.40	63.58	67.03
	Lepisiota frauenfeldi	54.05	53.10	54.50
	Messor aegyptiacus	51.10	50.40	51.70
	Messor arenarius	52.80	52.00	53.60
	Messor medioruber	51.40	50.50	52.00
	Messor picturatus	51.75	50.58	52.50
	Monomorium salomonis	52.40	51.80	53.00
	Monomorium sommieri	52.30	52.00	52.58
	Monomorium subopacum	53.10	52.53	53.58
	Pheidole pallidula	46.55	46.38	47.00
	Plagiolepis maura	48.10	46.30	48.43
	Tapinoma simrothi	48.70	48.35	49.25
	Tetramorium sericeiventre	52.90	52.20	53.40
Doñana	*Aphaenogaster senilis*	57.02	55.55	58.04
	Cataglyphis tartessica	59.80	59.05	60.53
	Crematogaster scutellaris	49.65	48.88	50.60
	Formica rufibarbis	55.00	54.25	55.90
	Lasius grandis	46.90	46.40	47.00
	Linepithema humile	45.70	44.90	46.10
	Messor marocanus	51.70	50.60	52.60
	Myrmica aloba	45.30	44.95	45.90
	Oxyopomyrmex saulcyi	48.07	48.07	48.07
	Tapinoma erraticum	48.05	47.50	48.60
	Tapinoma nigerrimum	49.07	47.20	50.06
Argelès-sur-Mer	*Aphaenogaster senilis*	55.70	55.10	56.88
	Camponotus vagus	51.80	50.98	52.30
	Cataglyphis piliscapa	59.15	57.95	60.08
	Crematogaster scutellaris	49.45	48.43	49.90
	Formica rufa	53.85	53.00	54.55
	Lasius cinereus	44.60	43.43	45.60
	Messor barbarus	51.80	49.90	52.70
	Messor bouvieri	51.40	50.80	52.20
	Pheidole pallidula	46.95	45.90	47.50
	Plagiolepis pygmaea	48.90	48.43	48.90

	Plagiolepis pyrenaica	48.00	47.15	48.60
	Tapinoma erraticum	48.60	47.35	49.20
	Tapinoma nigerrimum	49.40	48.03	50.10
	Tetramorium caespitum	47.30	46.73	47.70
Ile-de-France	*Dolichoderus quadripunctatus*	48.00	47.60	48.18
	Formica fusca	52.60	52.03	53.10
	Formica rufa	52.60	51.60	53.50
	Lasius brunneus	43.10	42.30	43.80
	Lasius emarginatus	46.70	45.90	47.30
	Lasius flavus	43.60	42.60	44.28
	Lasius niger	47.50	46.70	48.20
	Myrmecina graminicola	41.10	40.30	42.00
	Myrmica ruginodis	45.55	45.10	45.90
	Myrmica scabrinodis	45.20	44.60	45.83
	Myrmica specioides	45.90	45.70	46.50
	Temnothorax nylanderi	46.90	46.50	47.30
	Tetramorium caespitum	47.15	46.60	47.50

Table S2. Results of Kruskal-Wallis test between castes of polymorphic species by site. The medians of small, medium, and large workers are shown. Letters correspond to a Wilcoxon pairwise comparison between castes of the same species (data not shown). Blue background highlights species with no significant difference between castes, and red labels shows comparisons where the larger caste is not more heat resistant than the smaller caste.

Species	Kruskal-Wallis test	p-value	small	medium	large
Dar Dahoui					
Camponotus barbaricus	X^2= 18.952, df = 2	7.666e-05	52.7^{b}	53.0^{ab}	53.85^{a}
Camponotus oasium	X^2= 5.1509, df = 2	0.07612	53.9	53.85	53.5
Cataglyphis bombycina	X^2= 7.7854, df = 2	0.02039	59.4^{b}	60.5^{a}	60.3^{ab}
Cataglyphis rubra	X^2= 29.581, df = 2	3.771e-07	58.2^{b}	59.8^{a}	60.25^{a}
Cataglyphis savignyi	X^2= 23.552, df = 2	7.688e-06	61.55^{b}	64.7^{a}	65.85^{a}
Messor aegyptiacus	X^2= 94.105, df = 2	< 2.2e-16	50.0^{c}	51.2^{b}	51.7^{a}
Messor arenarius	X^2= 61.977, df = 2	3.483e-14	51.9^{c}	53.0^{b}	53.5^{a}
Messor medioruber	X^2= 28.513, df = 2	6.433e-07	50.6^{c}	51.4^{b}	52.05^{a}
Messor picturatus	X^2= 40.356, df = 2	1.725e-09	50.4^{c}	51.8^{b}	52.35^{a}
Tapinoma simrothi	X^2= 1.0903, df = 1	0.2964	48.6	–	48.8
Argelès-sur-Mer					
Camponotus vagus	X^2= 51.502, df = 1	7.154e-13	51.0^{b}	–	52.3^{a}
Messor barbarus	X^2= 99.023, df = 2	< 2.2e-16	49.0^{c}	51.9^{b}	53.1^{a}
Messor bouvieri	X^2= 46.649, df = 2	7.419e-11	50.7^{c}	51.5^{b}	52.1^{a}
Pheidole pallidula	X^2= 29.231, df = 1	6.424e-08	46.0^{b}	–	47.4^{a}
Tapinoma nigerrimum	X^2= 32.766, df = 1	1.04e-08	48.4^{b}	–	49.9^{a}
Doñana					
Messor marocanus	X^2= 150.65, df = 2	< 2.2e-16	50.46^{c}	52.06^{b}	52.56^{a}
Tapinoma nigerrimum	X^2= 7.0114, df = 1	0.0081	48.57^{b}	–	49.57^{a}

Table S3. CT_{max} (°C) of ants sampled in two different sites. Results of GLMs (*y=temperature ~ site + (1|site*colony)*). CT_{max} values are given in median (IQR). For the polymorphic species, the castes were compared separately. Note that for *Ph. pallidula* only small workers were compared because the large caste was not foraging hence not tested at the two sites.

Species	Caste	Ile-de-France	Argelès-sur-Mer	Doñana	Dar-Dahoui	*t*	p
A. senilis	Monomorphic		55.7 (55.1, 56.9)	57.0 (55.6, 58.0)		0.677	0.54
Cr. scutellaris	Monomorphic		49.4 (48.4, 49.9)	49.7 (48.9, 50.6)		1.098	0.33
Ta. erraticum	Monomorphic		48.6 (47.3, 49.2)	48.0 (47.5, 48.6)		0.245	0.80
Ta. nigerrimum	Small		48.4 (47.0, 49.3)	48.6 (46.4, 49.6)		-0.229	0.83
	Large		49.9 (49.6, 50.5)	49.6 (47.6, 50.4)		-0.727	0.51
Ph. pallidula	Small		46.0 (44.5, 47.0)		46.6 (46.4, 47.0)	0.837	0.51
T. caespitum	Monomorphic	47.2(46.6, 47.5)	47.3 (46.7, 47.7)			-0.388	0.72
F. rufa	Monomorphic	52.6(51.6, 53.5)	53.8 (53.0, 54.6)			-2.935	0.04

Table S4. Parameters estimates for the linear models relating morphological traits and CT_{max}.

Length of hind leg	**Other genera**		***Cataglyphis* spp.**
Intercept	46.37		49.38
β	3.95		7.01
R^2	0.49		0.82
p	<0.001		<0.001
Interocular distance	**Other genera**		***Cataglyphis* spp.**
Intercept	50.54		59.96
β	3.27		8.68
R^2	0.27		0.69
p	<0.001		< 0.001
Eye position	**Monomorphic**	**Polymorphic**	***Cataglyphis* spp.**
Intercept	50.02	51.4	46.08
β	0.69	0.16	-11.69
R^2	-0.007	0.007	0.08
p	> 0.05	> 0.05	> 0.05

Chapter 2

Morphological determinants of Critical Thermal Limits in ants

This is only a preliminary presentation of the study we intend to conduct before submitting to a journal.

Morphological determinants of Critical Thermal Limits in ants

Fátima García Ibarra, Thibaud Monnin, Angélique Bultelle, Pierre Fédérici, Xim Cerdá, Pascal Jouquet

Abstract

The ongoing effects of climate change and alterations in land use have induced notable shifts in the structure, diversity, and phenology of species across global ecosystems (IPCC, 2021). Ectothermic organisms, particularly vulnerable to rising temperatures, are significantly impacted, with ants, ubiquitous in terrestrial habitats, playing crucial roles in ecological processes. The anticipated changes in ant populations due to climate change are expected to have important effects on global ecosystems (Parr & Bishop, 2022).

The study focuses on assessing the heat resistance of ants in a Mediterranean site and explores the relationships between Critical Thermal maximum (CTmax) values and morphological traits at the individual level. Larger ants, with longer legs, are presumed to be more heat-resistant due to increased mobility and better heat dissipation. Polymorphic species, characterized by larger workers, are expected to exhibit higher heat resistance, enhancing resource utilization in dry environments. Conversely, monomorphic species may employ different adaptations, such as increased body water content and foraging during less warm hours, to cope with thermal stress (Cerda & Retana, 2000).

Preliminary results indicate that hind leg length, clypeus height, and interocular distance interact in explaining ant heat resistance, with hind leg length being the most influential trait. The distribution of genera suggests a potential relationship between body size and CTmax, with larger workers in certain genera exhibiting higher heat resistance. Notably, so far, we have not observed an obvious advantage of polymorphism over monomorphism on heat resistance.

While these preliminary findings imply a connection between morphological traits and heat resistance, further analysis, including linear mixed models, is needed to draw

definitive conclusions. Additionally, exploring differences between castes in polymorphic species and comparing relationships across monomorphic and polymorphic species will provide a more comprehensive understanding of ant heat resistance in the studied community.

Keywords

Critical Thermal Limit, CT_{max}, ants, morphology, functional traits, semi-arid climate, Mediterranean climate, temperate climate

Introduction

Climate change and land use alterations have led to shifts in the structure, diversity, and phenology of species across various ecosystems worldwide (IPCC, 2021). Rising temperatures, a major abiotic stressor, particularly impact ectothermic organisms reliant on environmental temperatures for temperature regulation (Jørgensen et al., 2022). Ants, ubiquitous in terrestrial habitats, play crucial roles in soil bioturbation, nutrient cycling, pest control, seed dispersal, and community regulation (Del Toro et al., 2012). Consequently, changes in ant populations due to climate change are expected to bring about significant modifications in global ecosystems (Parr & Bishop, 2022).

Global warming is projected to cause greater temperature increases at higher latitudes (IPCC, 2021), potentially exposing ants in temperate environments to more substantial temperature rises than their tropical counterparts. However, temperate ants might exhibit higher resistance to temperature variations due to their adaptation to larger seasonal fluctuations (Janzen, 1967; Kaspari et al., 2015; Bujan, Roeder, Yanoviak, et al., 2020). Ants in tropical and mesic environments, living closer to their upper physiological thermal tolerance, may be more vulnerable to temperature changes (Diamond et al., 2012). These species-level effects could extend to community levels, with ant communities in colder, more seasonal regions potentially demonstrating greater resilience to warming than those in warmer, less seasonal regions (Arnan et al., 2015).

Ant heat tolerance is influenced by both constitutive factors (e.g., morphology, phylogeny) and inducible factors (e.g., production of heat shock proteins, behavioural changes). Morphological traits such as leg length and body size are commonly used indicators of thermal resistance (Clémencet et al., 2010; Sommer & Wehner, 2012). Larger ants, with longer legs, are expected to be more heat-resistant due to increased mobility and better heat dissipation. Polymorphic species, with larger workers, often exhibit higher heat resistance, facilitating efficient resource utilization in dry environments. Monomorphic species, however, may employ different adaptations such as elevated body water content and the expression of proteins to cope with thermal stress (Cerda & Retana, 2000).

Ants' individual performance, including movement, growth, survival, and reproduction, increases with temperature until a critical point where extreme temperatures adversely affect their metabolism, potentially leading to shifts in diversity (Bestelmeyer, 2000; Jenkins et al., 2011; Zakharov & Zakharov, 2014). Assessing the resilience of ant communities to global warming involves characterizing species' susceptibility to heat, predicting their exposure, and understanding potential behavioural adaptations. Indices like Critical Thermal maximum (CT_{max}) are commonly used to describe ant tolerance to extreme heat. This study focuses on evaluating the heat resistance of ants from a diversity of species from one ant community, by measuring their CT_{max}, and exploring the relationships between CT_{max} and morphological traits at the individual level. We expect simple morphological traits to determine thermal resistance, in particular leg length and body size (Clémencet et al., 2010; García Ibarra et.al., in prep., Sommer & Wehner, 2012; Oms et al., 2017), and differences in the relationship between functional traits and heat resistance between monomorphic and polymorphic species.

Material and methods

Study location and measure of CT_{max}

In April 2022, we sampled workers of 14 ant species at a Mediterranean site in Argelès-sur-Mer, France (42°34'7''N, 3°2'46''E) (Table 1). The site's mean annual precipitation was 642mm (Climate-data.org, 2021). This location consisted of a combination of coastal sand dunes and sandy beaches. We sampled workers from three colonies per species, with the exceptions of *Messor bouvieri*, *Plagiolepis pyrenaica*, *Tapinoma erraticum* (two colonies) and *Plagiolepis pygmaea* (one colony, see Table 1). For each species, colonies were located at least 10 m apart. Using a vacuum, we collected workers that were foraging outside of their colony, on the ground. We sampled 30 foragers per colony for monomorphic species, and 15 foragers per caste per colony for polymorphic species. We placed the ants in 16.1×15×9 cm plastic boxes containing a damp cotton ball and promptly transported them to the lab for analysis. To prevent acclimatization to laboratory conditions, we measured the ants' CT_{max} within 12h after collection.

Table 1. Sampled ant species. Ant species were either monomorphic (M) or polymorphic (P). For each species, the table gives the number of colonies and of workers sampled.

Ant species	**Morphology (Monomorphic or Polymorphic)**	**Number of colonies / number of workers sampled**
Aphaenogaster senilis	M	3 / 90
Camponotus vagus	P	3 / 91
Cataglyphis piliscapa	P	3 / 90
Crematogaster scutellaris	M	3 / 89
Formica rufa	M	3 / 90
Lasius cinereus	M	3 / 90
Messor barbarus	P	3 / 135
Messor bouvieri	P	2 / 89
Pheidole pallidula	P	3 / 90
Plagiolepis pygmaea	M	1 / 30
Plagiolepis pyrenaica	M	2 / 59
Tapinoma erraticum	M	2 / 60
Tapinoma nigerrimum	P	3 / 90
Tetramorium caespitum	M	3 / 90

We measured CT_{max} using the dynamic method (reviewed by Roeder et al., 2021). Ants were placed on a hot plate that was gradually heated, starting from room temperature and up to 70°C. We used a high precision (110°C max ± 0.1°C) hot plate (model PZ28-1, Gestigkeit, Germany). To facilitate the observation of ant locomotion and prevent their escape from the hot plate, we utilized plastic tubes with dimensions of 5.2 cm in diameter and 6 cm in height, open at both ends and coated with liquid polytetrafluoroethylene (Fluon® PTFE). A maximum of three ants were placed in the same tube at once. Each tube accommodated a maximum of three ants simultaneously. In instances where workers of certain species climbed on top of each other, we gently guided them back onto the plate using entomological tweezers. The CT_{max} for each ant was recorded as the temperature at which individual ants lost locomotor coordination, signified by the cessation of walking or the occurrence of spasms. After reaching its CT_{max}, each ant was transferred to a vial containing 70% ethanol for morphological measurements.

Morphological traits

We dissected all the ants and measured a set of morphological traits with functional significance according to Schofield et al. (2016). Specifically, we measured maximal head width, maximal head length, clypeus length, leg length (of each of the three pairs of legs), and Weber's length as indicative of body size (Table 2). To reduce the unevenness of measures due to differences in the angle of body parts, those were independently glued to a 12×12 cm plastic box using double-faced tape, then photographed with a Leica EZ4 W stereomicroscope. Measures were conducted using ImageJ software version 1.53s (Schindelin et al., 2012).

Table 2. Chosen morphological characteristics and their functional implications. The measurement of leg length involved the sum of the length of the tibia and femur (right side only) for each of the three pairs of legs. Adding these two measures is well-founded, and there exists a strong correlation (>0.97) between them for all leg pairs.

Character	Trait measured	Functional significance
Head size	Head width (max); Head length (clypeus-occiput)	Head size may vary allometrically with body size, head size may also be linked to predatory strategies, with wider heads allowing for more powerful mandible muscles and larger mandibles hence larger prey and larger seeds (Kaspari & Weiser, 1999; Sarty et al., 2006).
Clypeus	Clypeus length	Modified clypeus is more common in species that rely on liquid food (Eisner, 1957; Davidson et al., 2004)
Eyes	Interocular distance; Eye position ((head width – interocular distance) / head length)	Visual predators have more dorsally positioned eyes (higher interocular distance, Fowler et al., 1991).
Leg length, for each pairs of legs	Sum of Tibia length and femur length.	Leg length is related to thermoregulatory strategy (Sommer & Wehner, 2012) and increases locomotion speed in simple habitats; in complex habitats shorter legs allow exploitation of crevices and manoeuvrability (Sarty et al., 2006; Gibb & Parr, 2010).
Mesosoma	Mesosoma (Weber's length)	Indicative of overall body size and often linked to thermoresistance (Willmer & Unwin, 1981), and resource use (Kaspari and Weiser 1999)

Data analysis

I hereafter present a preliminary analysis. All data have and will be analysed using R (version 4.0.0) software. Hereafter, temperatures will be expressed as the median and interquartile range (i.e., median (IQR)). The selection of median over mean was based on the fact that it can be interpreted as the LD50, (lethal dose 50, i.e., the temperature at which 50 percent of individuals perish, (Camacho et al., 2023)). To assess which morphological traits explained CT_{max} the best, we first verified Pearson's correlation (stats package R Core Team, 2023) to avoid collinearity among variables. Only variables with a correlation < 0.9 were conserved (Fig. S1). Then, we explored how these four variables explained the CT_{max} using a single linear model considering full interactions between variables (stats package R Core Team, 2023). We also explored how each variable was related to heat resistance using linear regressions.

Preliminary results

The most resistant species was *Cataglyphis piliscapa* (59.15 (58.55, 59.33)°C) and the least resistant one was *Lasius cinereus* (47.70 (43.94, 44.79)°C). Median values for all species are shown in Fig. 1 and Table S1.

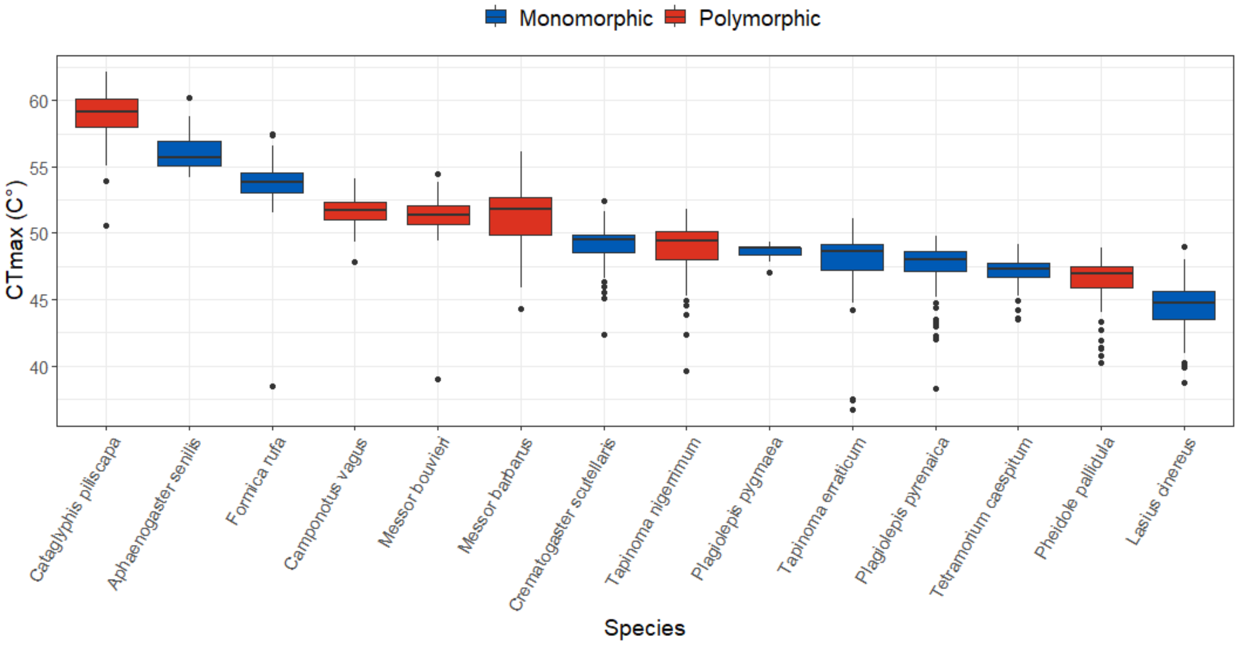


Fig 1. Heat resistance of species per site. Boxplots show the median and interquartile of CT_{max}. For polymorphic species, all worker sizes were combined. Corresponding data is available in Table S1.

The full interaction linear model (temperature ~ hind leg * clypeus * interocular distance * eye position) presented a $R^2 = 0.66$, $p<0.001$. Plotting the interactions of these variables showed the most heat-resistant genera (Fig. 2).

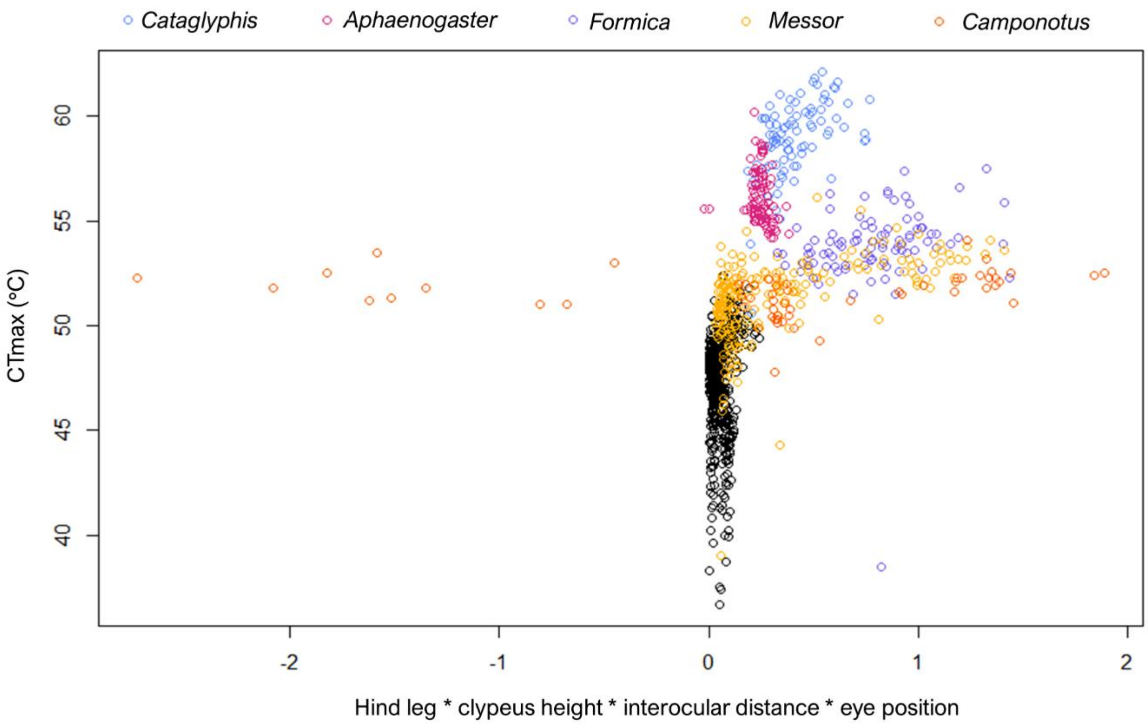


Fig. 2. Interaction of the four selected morphological traits. Coloured dots show the four more heat-resistant genera, black dots denote the remaining genera.

Linear regressions of the selected morphological traits are shown in Fig. 3. The length of hind leg was the trait that explained the CT_{max} the best ($R^2= 0.52$, $p<0.001$), but interocular distance and height of clypeus showed a positive relationship, although not as high. On the other hand, the eye position explained very little the heat resistance ($R^2=0.04$, $p<0.01$).

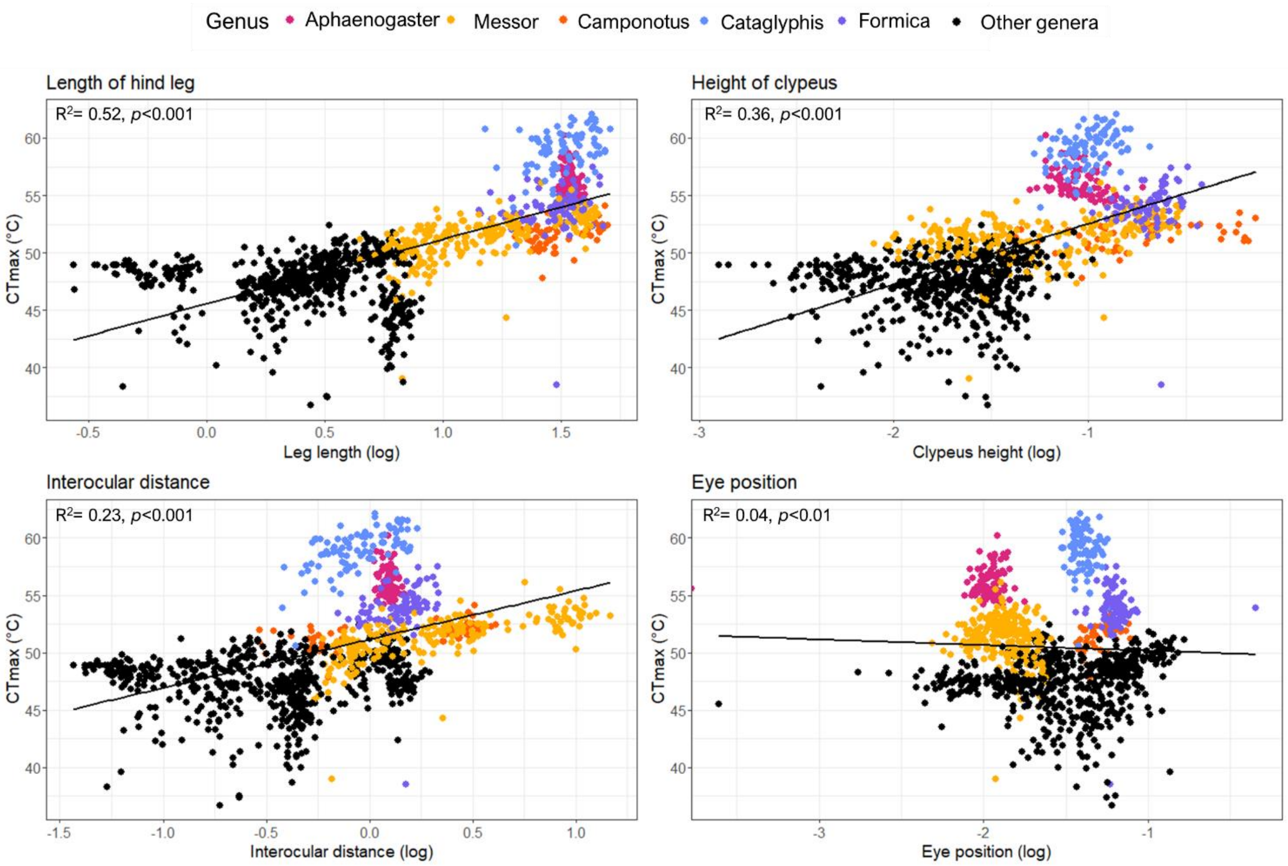


Fig. 3. Relationship (linear regressions) between the four selected morphological traits and the CT_{max}. Lines correspond to linear regressions calculated with $y \sim x$.

Perspectives for this study

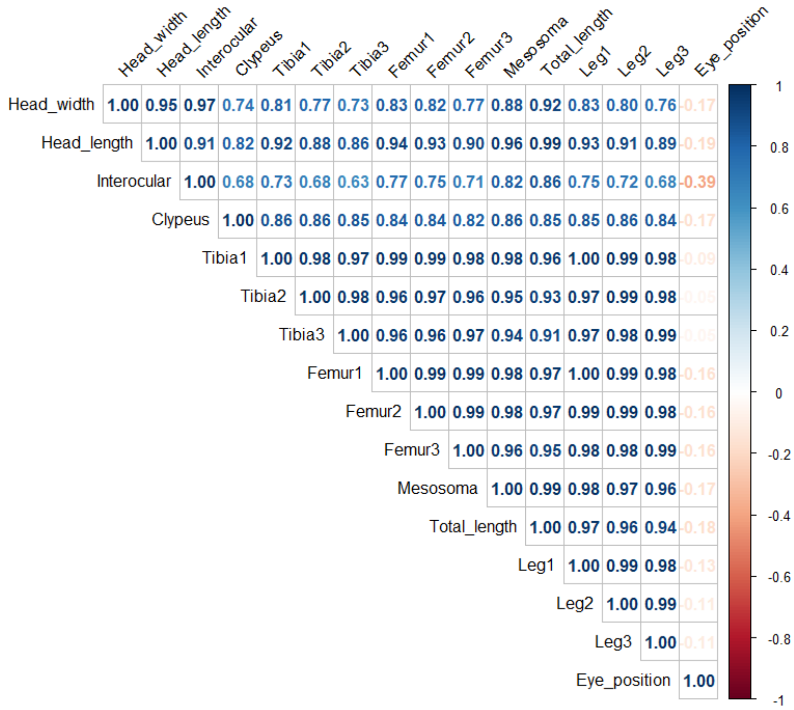
The preliminary results suggest that the resistance of ants in this community could be explained by some kind of interaction between the hind leg, the clypeus height and the interocular distance. However, the position of the eyes does not seem to have much relevance on resistance.

Distribution of genera in Fig. 2 hints on a relationship between body size and CT_{max} as the five genera located on the upper limit of the community distribution have large workers. The same trend exists for each independent trait (Fig.3). It is interesting to notice that three of these genera are polymorphic (*Cataglyphis, Messor* and *Camponotus*) and two are monomorphic (*Aphaenogaster* and *Formica*). It has been reported that polymorphism facilitates a more effective use of resources as species have a wider range of temperatures. This allows them to engage in foraging activities amid the hot hours of the day (larger foragers have increased resistance to heat and desiccation), as well as during less warm hours, allowing them to endure challenging environmental conditions (Clémencet et al., 2010; Baudier et al., 2015; La Richelière et al., 2022). However, these results suggest that the resistance may be more related to the individual body size more than to the range within the species.

These preliminary results suggest that the resistance to heat in this site could be partially explained by morphological traits. Nevertheless, no real conclusions can be made before further analysis of the data.

The next step will be to use linear mixed models to find the best model to explain our data, considering not only morphological traits but also to compare if the relationship is the same in monomorphic and polymorphic species. Also, we plan to analyse in detail de differences between castes in polymorphic species.

Supplementary material



	Head_width	Head_length	Interocular	Clypeus	Tibia1	Tibia2	Tibia3	Femur1	Femur2	Femur3	Mesosoma	Total_length	Leg1	Leg2	Leg3	Eye_position
Head_width	1.00	0.95	0.97	0.74	0.81	0.77	0.73	0.83	0.82	0.77	0.88	0.92	0.83	0.80	0.76	-0.17
Head_length		1.00	0.91	0.82	0.92	0.88	0.86	0.94	0.93	0.90	0.96	0.99	0.93	0.91	0.89	-0.19
Interocular			1.00	0.68	0.73	0.68	0.63	0.77	0.75	0.71	0.82	0.86	0.75	0.72	0.68	-0.39
Clypeus				1.00	0.86	0.86	0.85	0.84	0.84	0.82	0.86	0.85	0.85	0.86	0.84	-0.17
Tibia1					1.00	0.98	0.97	0.99	0.99	0.98	0.98	0.96	1.00	0.99	0.98	-0.09
Tibia2						1.00	0.98	0.96	0.97	0.96	0.95	0.93	0.97	0.99	0.98	-0.05
Tibia3							1.00	0.96	0.96	0.97	0.94	0.91	0.97	0.98	0.99	-0.05
Femur1								1.00	0.99	0.99	0.98	0.97	1.00	0.99	0.98	-0.16
Femur2									1.00	0.99	0.98	0.97	0.99	0.99	0.98	-0.16
Femur3										1.00	0.96	0.95	0.98	0.98	0.99	-0.16
Mesosoma											1.00	0.99	0.98	0.97	0.96	-0.17
Total_length												1.00	0.97	0.96	0.94	-0.18
Leg1													1.00	0.99	0.98	-0.13
Leg2														1.00	0.99	-0.11
Leg3															1.00	-0.11
Eye_position																1.00

Fig. S1. Correlation matrix of measured morphological traits.

Table S1. CTmax per species for all the environments. The values correspond to the mean of median CTmax per colony, regardless the caste.

Species	**Median**	**1st quartile**	**3rd quartile**
Cataglyphis piliscapa	59.15	58.55	59.32
Aphaenogaster senilis	55.70	55.86	56.41
Formica rufa	53.85	53.36	54.22
Camponotus vagus	51.75	51.26	51.82
Messor bouvieri	51.40	50.95	51.66
Messor barbarus	51.80	50.91	51.62
Crematogaster scutellaris	49.50	48.77	49.44
Tapinoma nigerrimum	49.40	48.46	49.30
Plagiolepis pygmaea	48.90	48.43	48.82
Tapinoma erraticum	48.60	47.10	48.62
Plagiolepis pyrenaica	48.00	46.71	47.87
Tetramorium caespitum	47.30	46.89	47.33
Pheidole pallidula	46.95	46.03	46.79
Lasius cinereus	44.70	43.94	44.79

Table 3. Pearson's correlation coefficient between the morphological traits retained ($r^2 < 0.9$) and the CT_{max}. Repeated values have been removed.

	Interocular distance	**Hind leg length**	**Eye position**	**Clypeus**	**CT_{max}**
Interocular Distance	1.00				
Hind leg length	0.68 ($p < 0.001$)	1.00			
Eye position	-0.39 ($p < 0.001$)	0.11 ($p < 0.001$)	1.00		
Clypeus	0.68 ($p < 0.001$)	0.84 ($p < 0.001$)	0.22 ($p < 0.001$)	-1.00	
CT_{max}	0.43 ($p < 0.001$)	0.78 ($p < 0.001$)	-0.06 ($p < 0.05$)	0.58 ($p < 0.001$)	1.00

Chapter 3

Experimental evidence that increased surface temperature affects bioturbation by ants

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Experimental evidence that increased surface temperature affects bioturbation by ants

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

Abstract

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.

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.

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.

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

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, i.e. 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 above mentioned 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, e.g., 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, i.e., the dispersal and reorganization of soil particles and aggregates through the activity of animals (Meysman et al., 2006; Bottinelli et al., 2015).

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 (Paton et al., 1995; Lavelle et al., 1997; Jouquet et al., 2006). Ants are likely to play a key role in the dynamics of nutrients and water in the soils (Cammeraat & Risch, 2008; Benckiser, 2010; Finér et al., 2013; Farji-Brener & Werenkraut, 2017; 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 on their feeding type, the latitude or type or 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 cm 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 2D setup 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 setup, 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 (Perna et al., 2008; Booth et al., 2020; Cheik et al., 2021). 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.

Materials and methods

Study species

L. 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.

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 x 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 three months. Colonies were then removed from the tubes and installed in 12.5 cm length x 12.5 cm width x 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 seven months later (February 2022). At that time, colonies contained in average 106 ± 27 (mean±SD) workers, the queen and brood.

Experimental design

Twenty-one polyvinyl chloride (PVC) tubes (30 cm height x 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 ($0.14\text{g H}_2\text{O g}^{-1}$ soil) following Weil & Brady (2016). 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 for example 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 x 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 (Fig 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 40 W UV lamps placed 20 or 40 cm above the soil surface (T_{high} and T_{medium}, respectively) or fluorescent lights 50 cm 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 ~10 cm 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 100 days.

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.

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:

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

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

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 0.8 mm thick images with a pixel size of 0.45 mm. The X-ray beam was operated at 58 mA and 120 kV. Images (16-bit DICOM format, 512 x 512 pixels) were obtained and subsequently transformed into 8-bit TIFF format and rendered isotropic of 0.45 mm. Images were processed and quantified with ImageJ software version 1.53s (Schindelin et al., 2012) and visualized 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 (Figs 1, S2, S3, 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 to 3.67 mm) and chambers (4.41 to 7.61 mm) were calculated with ImageJ BoneJ plugin.

During the image processing, we noticed a third level of grey on some images (Fig 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 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 (Fig S2 to 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 x100).

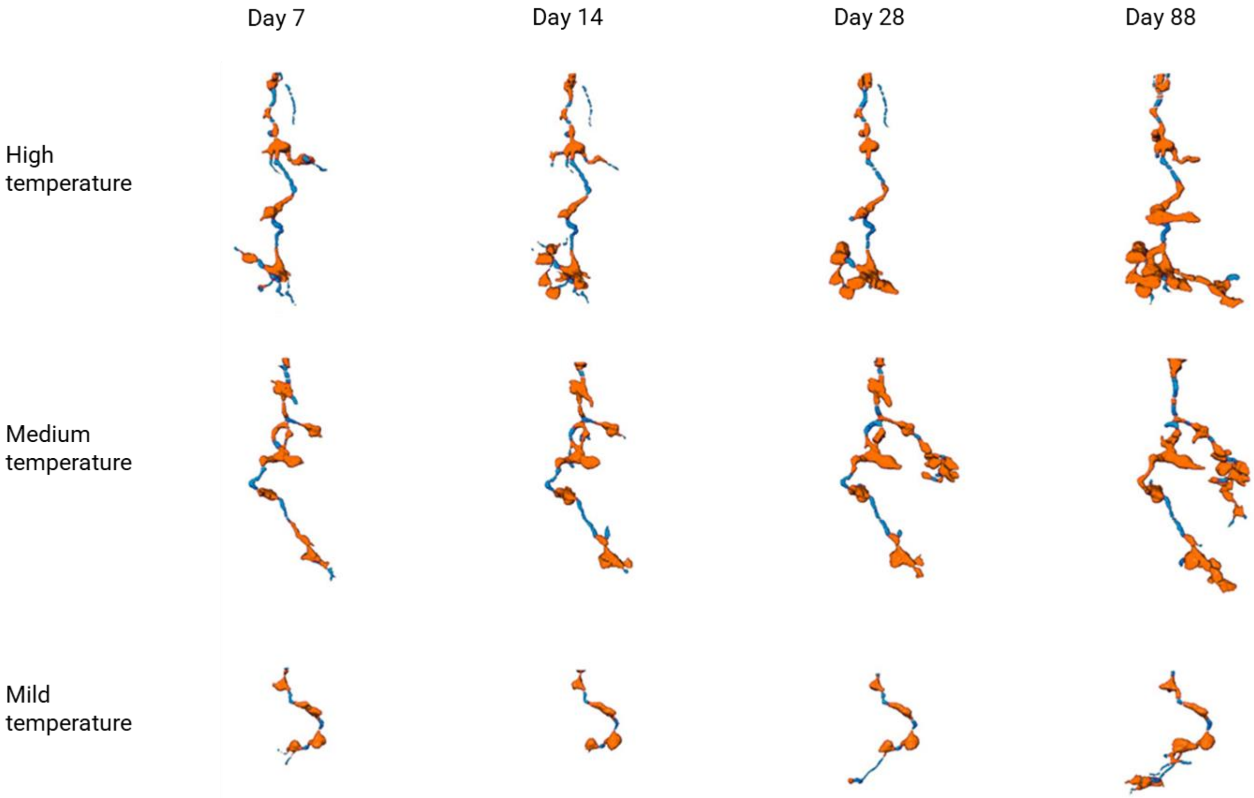

Fig 1. Examples of three nests illustrating the effects of treatment and time on their structure. Chambers (orange) and tunnels (blue) can be distinguished.

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 x 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).

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 hours, dried in a stove at 40°C for 48 hours, and weighed using a Sartorius Cubis microbalance.

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.

Results

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} (Fig 2A).

The total amount of soil excavated (Fig 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, $F_{2,14} = 3.06$, $P < 0.05$).

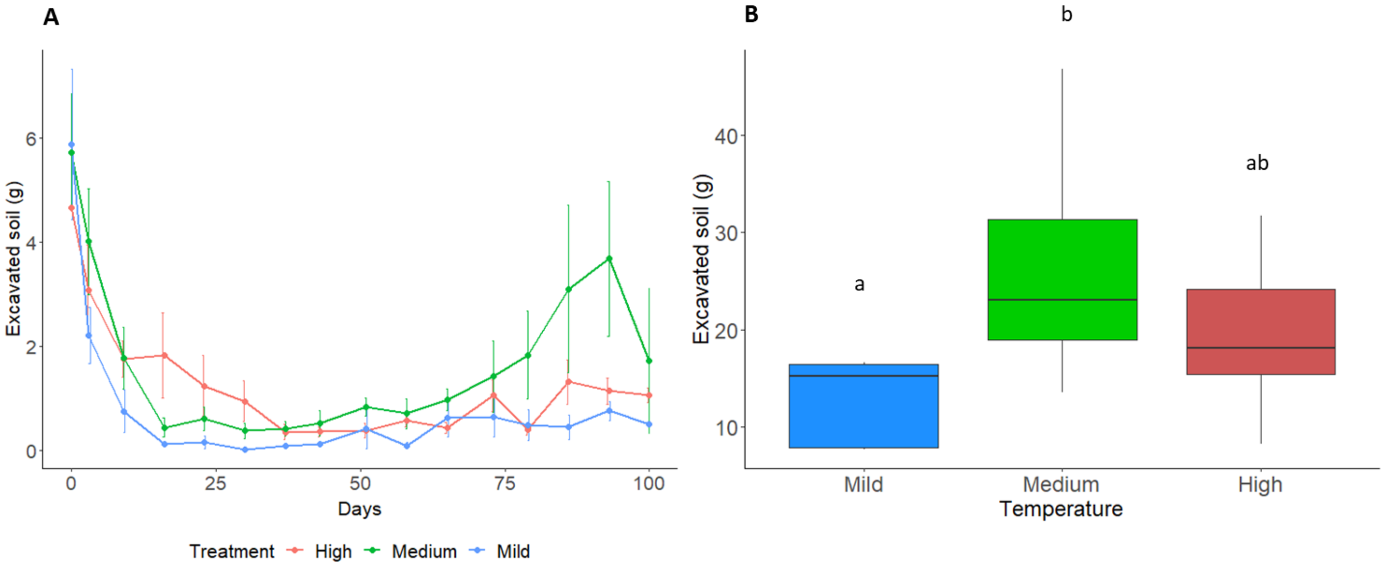

Fig 2. Amount of soil excavated (g) at each time point during the whole experiment (A) and total amount of soil excavated during the experiment (B). Bars in (A) indicate SEM.

From the beginning of the experiment, ants produced nests whose maximum depth did not change with time ($F_{3,64} = 2.56$, $P > 0.05$, Figs 3, S2 to S4). No nest reached the bottom of the soil column, in any of the treatment. 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 (Fig 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 (Fig S9).

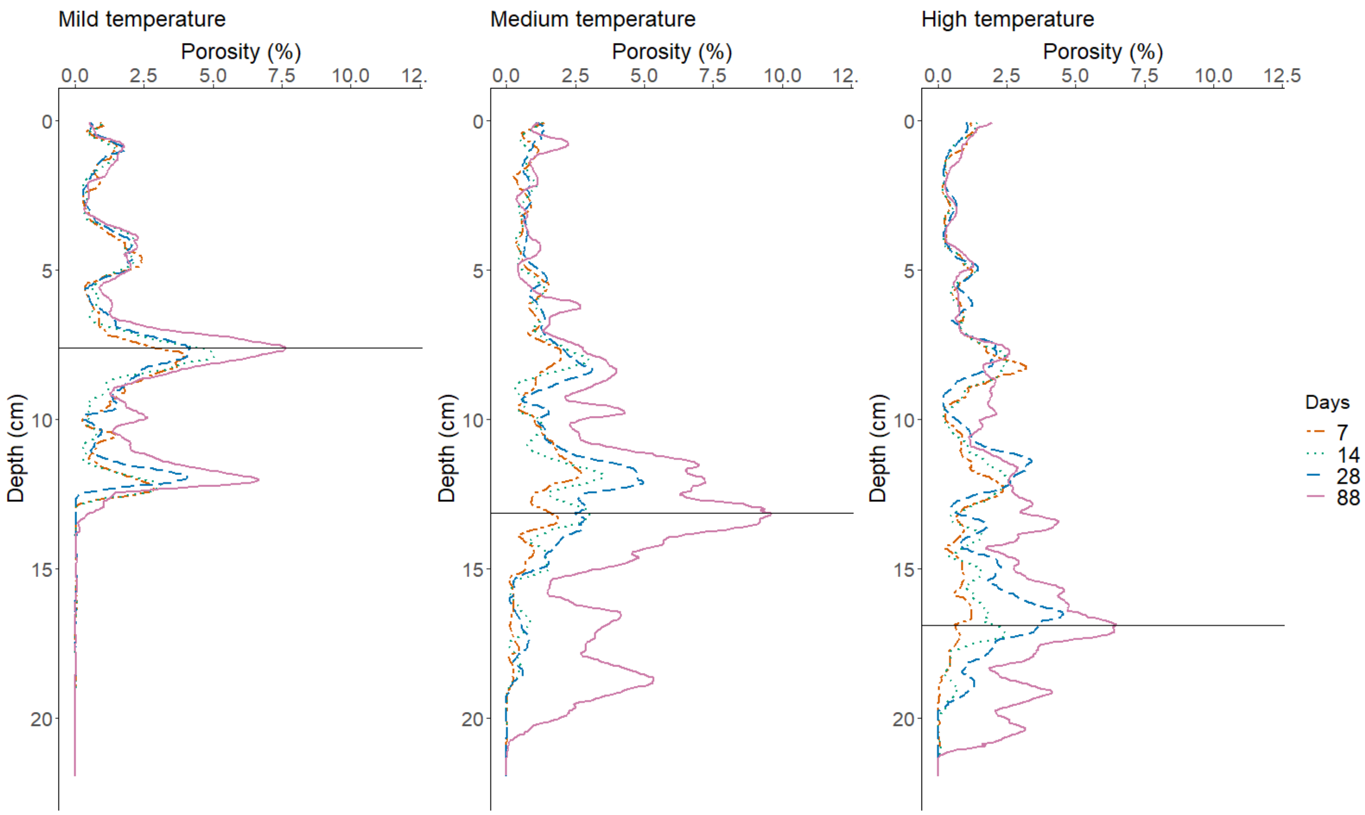

Fig 3. Mean porosity profiles (percentage of the empty volume compared to soil volume) per treatment. Colours and line types correspond to dates. Black horizontal lines indicate the point of maximal porosity on day 88.

The Principal Component Analysis (PCA) of the variables describing nest architecture (Fig 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 toward 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}.

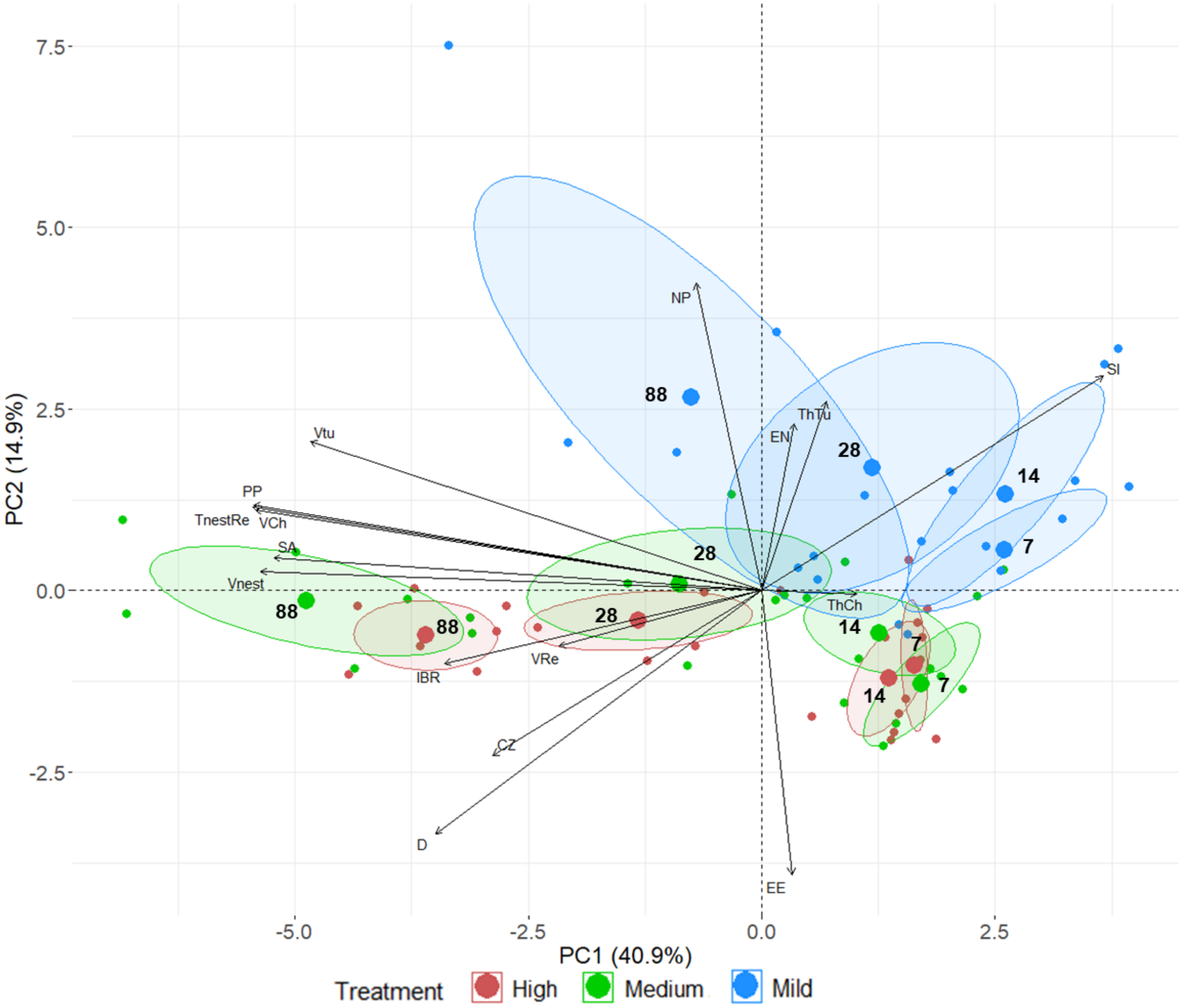

Fig 4. Biplot showing the principal component analysis (PCA) from variables describing the nest architectural features. Variables are sphericity index (SI), largest inscribed ball radius (IBR), ellipse elevation (EE), number of pores (NP), surface area (SA), Euler's number (EN), centroid Z (CZ), maximal 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 intterval (95%).

Nest volume (V_{nest}) was affected by the interaction between the time and the temperature treatments ($F_{3,24} = 3.28$, $P < 0.001$). There was no difference among treatments until day 88 when the nests at T_{mild} were smaller than those at T_{medium} ($F_{2,14} = 5.20$, $P < 0.05$, Fig 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, i.e. it was affected by the interaction between the time and temperature ($F_{3,24} = 3.67$, $P < 0.001$, Fig S6). Only on day 88, chambers in T_{medium} were larger than T_{mild} whereas T_{high} had intermediate volumes ($F_{2,14} = 5.71$, $P < 0.05$, Fig S6). Tunnels volume (V_{Tu}) increased over time ($F_{1,26} = 0.26$, $P < 0.001$), without influence of the temperature ($F_{2,14} = 0.26$, $P > 0.05$, Fig 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 (Fig S7). A similar trend was observed on day 88 but was not statistically significant. The porosity profiles of the refilled chambers (Fig 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.

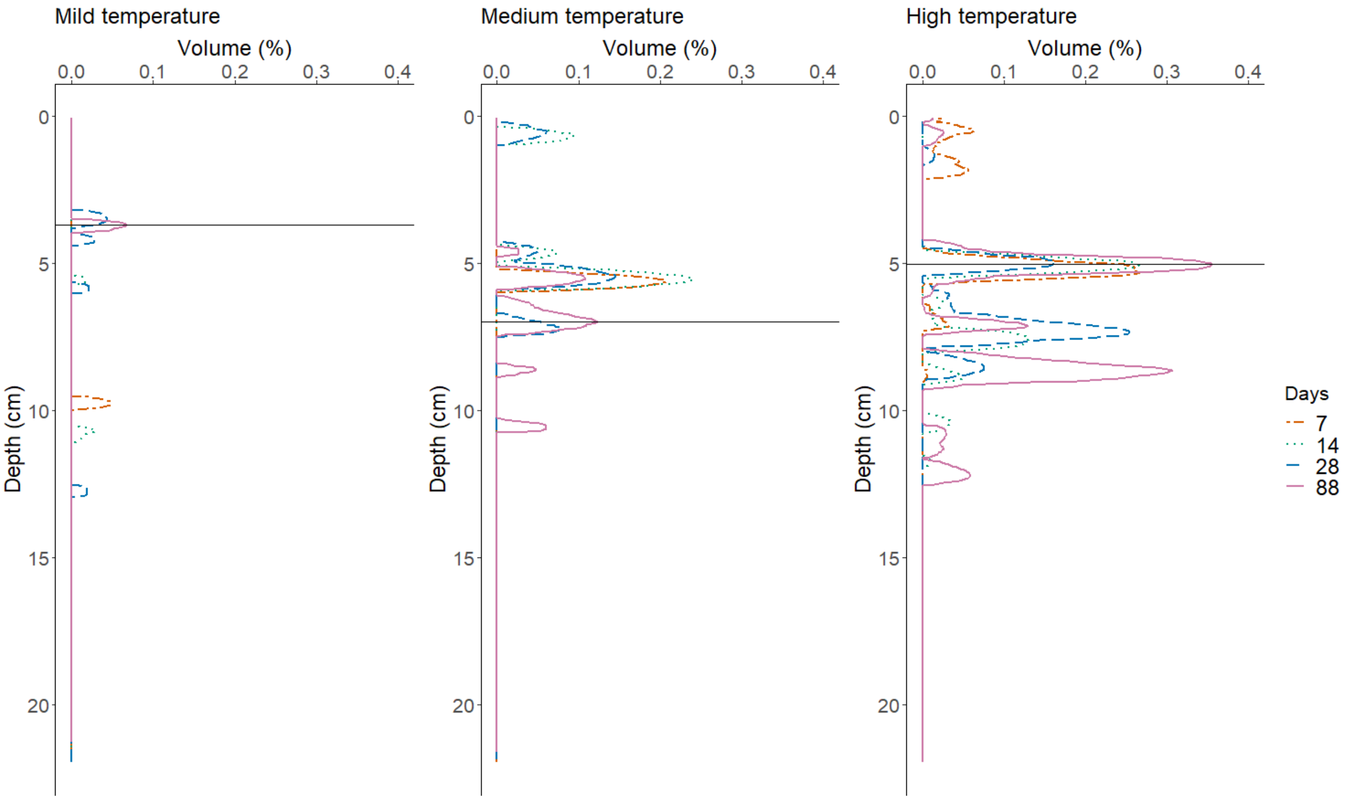

Fig 5. Mean volume profiles of refilled galleries only (percentage of V_{Re} compared to soil volume) per treatment. Colors and line types correspond to dates. Black horizontal lines indicate the point of maximal refilled volume on day 88.

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 amongst treatments at any moment of the experiment ($F_{3,26} = 0.965$, $P > 0.05$).

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} ($F_{2,238} = 3.19$, $P < 0.001$, Fig 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).

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, $F_{2,14} = 10.45$ $P < 0.05$, Fig S9, Table S3). Temperature had no effect on the size of workers (1.67 ± 0.10 mm, mean ± SD, $F_{2,167} = 0.37$, $P > 0.05$, Fig 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, $F_{2,167} = 19.67$, $P < 0.001$, Fig S10).

Discussion

Soil bioturbation by ants

Ant activity is known to increase with the temperature in the field, i.e., 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}, Fig 2). However, ants seem to be located proportionally deeper in the nests with increasing temperatures (Fig 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, *e.g.* 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, Fig 2A). These results are comparable to the findings of Rasse & 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, Fig 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 (Fig 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 (Mikheyev & Tschinkel, 2004; Kadochová & Frouz, 2013). 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 (Fig 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 & 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 (Woodell and King, in 1991; Blomqvist *et al.*, 2000) 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 (Fig S8) most likely steamed from the increased temperatures, that intensify 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 optimising one factor or the other.

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 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 affect 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 colony 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}, Fig 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.

Global warming and its consequences on nest and colony

Our study, as well as several others (Jenkins et al., 2011; Verberk et al., 2021; Parr & Bishop, 2022; Nascimento et al., 2022), 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 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 (Diamond et al., 2012; Andrew et al., 2013). 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.

Conclusion

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.

Supplementary material

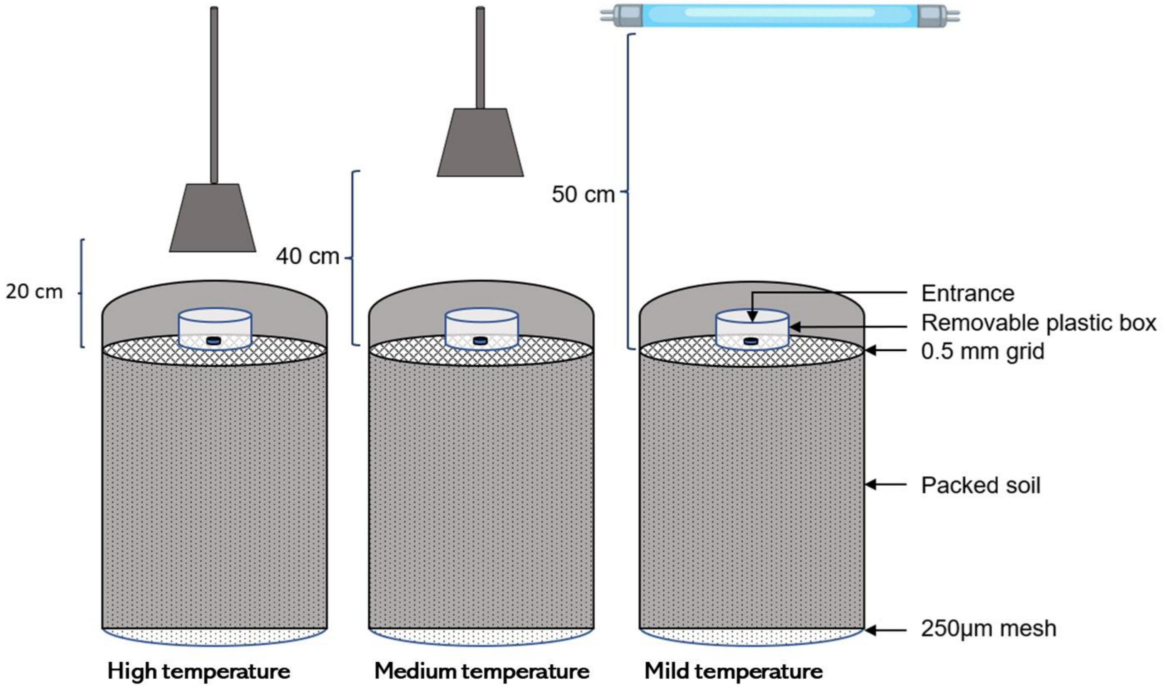

Fig S1. Experimental setup with the three temperature treatments: T_{high} (n = 6), T_{medium} (n = 6) and T_{mild} (n = 5).

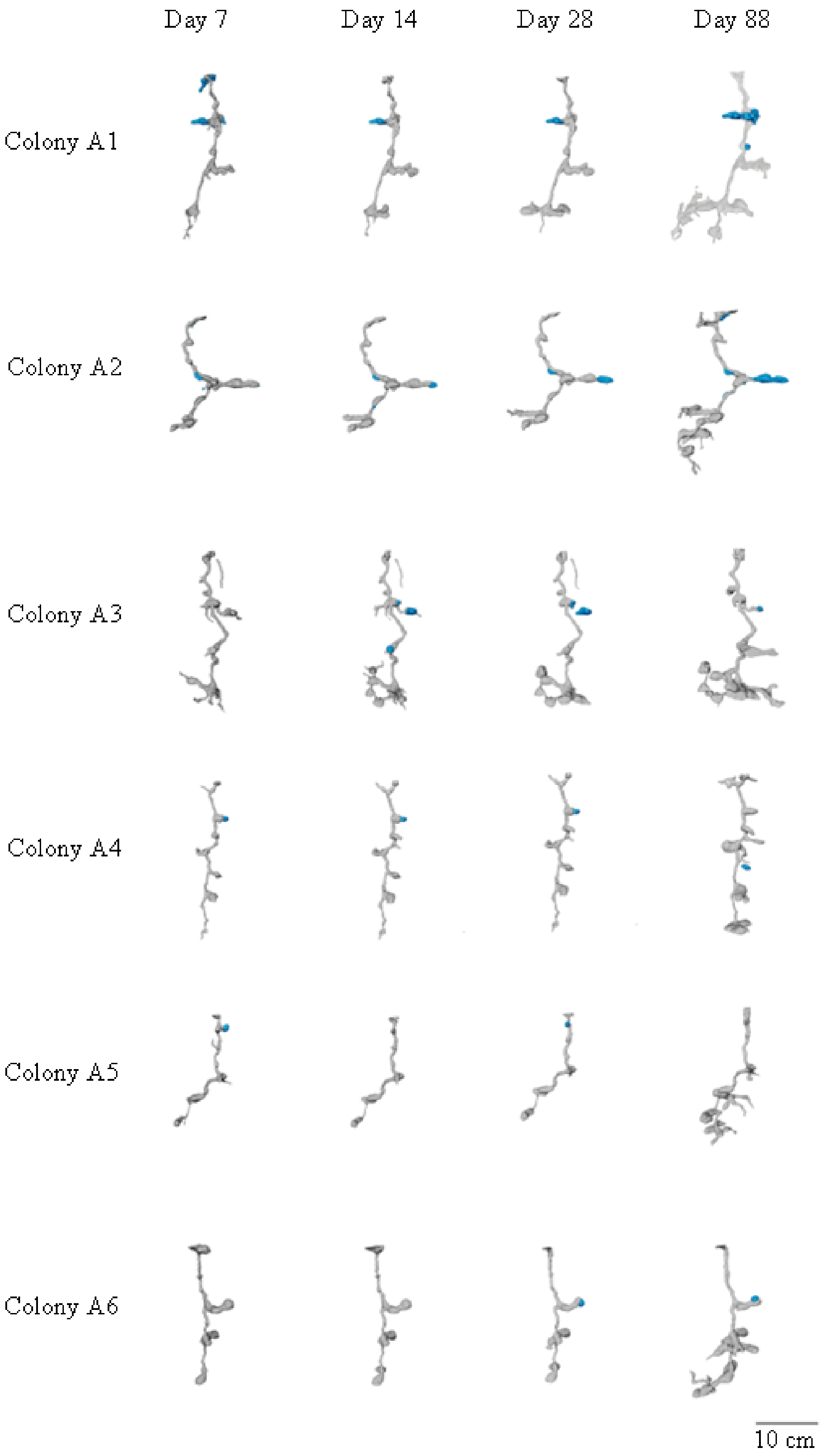

Fig S2. Nest reconstructions of all nests under T_{high}, showing chambers and tunnels in grey and refilled chambers in blue. All nests are shown at the same scale.

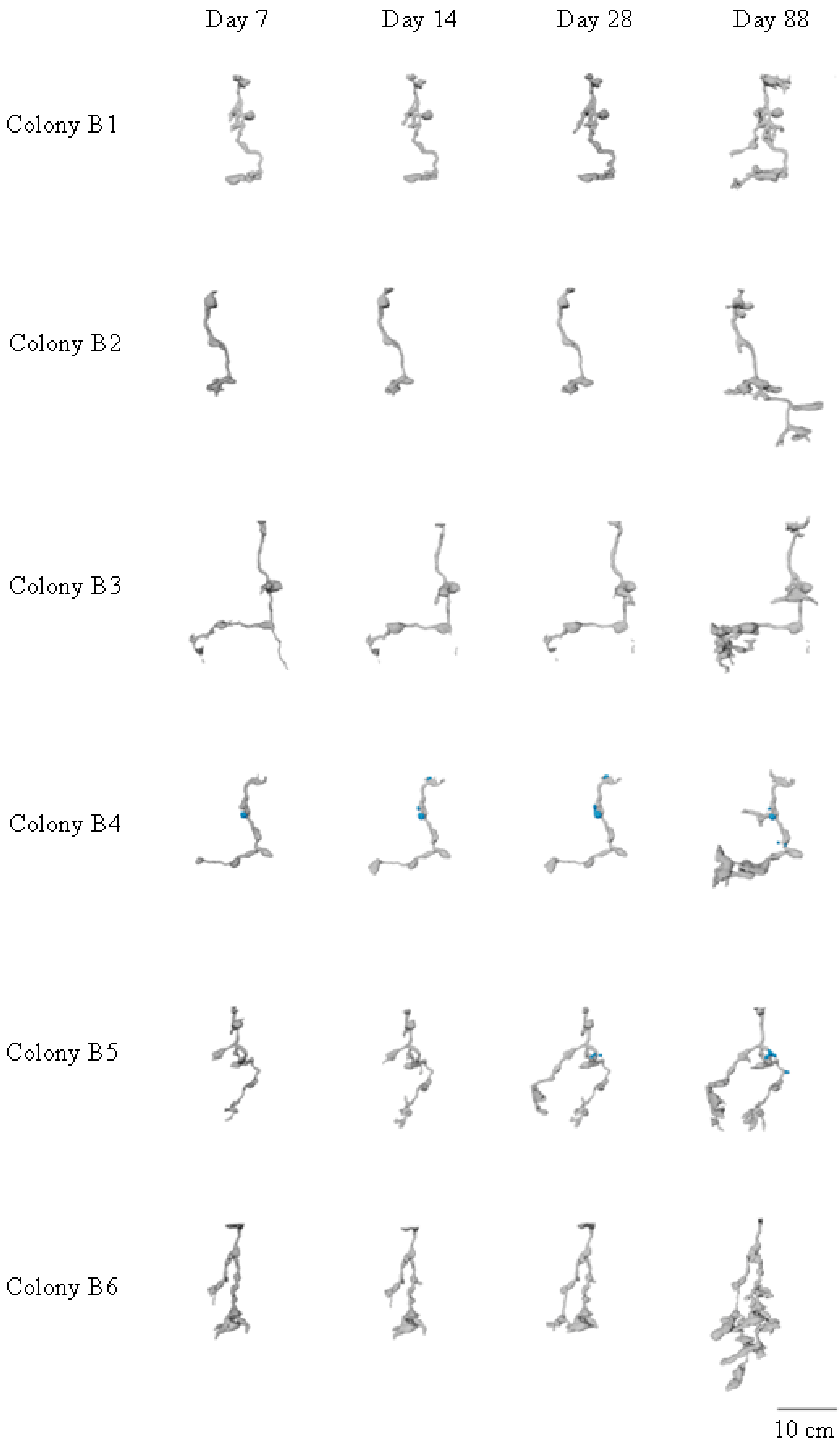

Fig S3. Nest reconstructions of all nests under T_{medium}, showing chambers and tunnels in grey and refilled chambers in blue. All nests are shown at the same scale.

Fig S4. Nest reconstructions of all nests under T_{mild}, showing chambers and tunnels in grey and refilled chambers in blue. All nests are shown at the same scale.

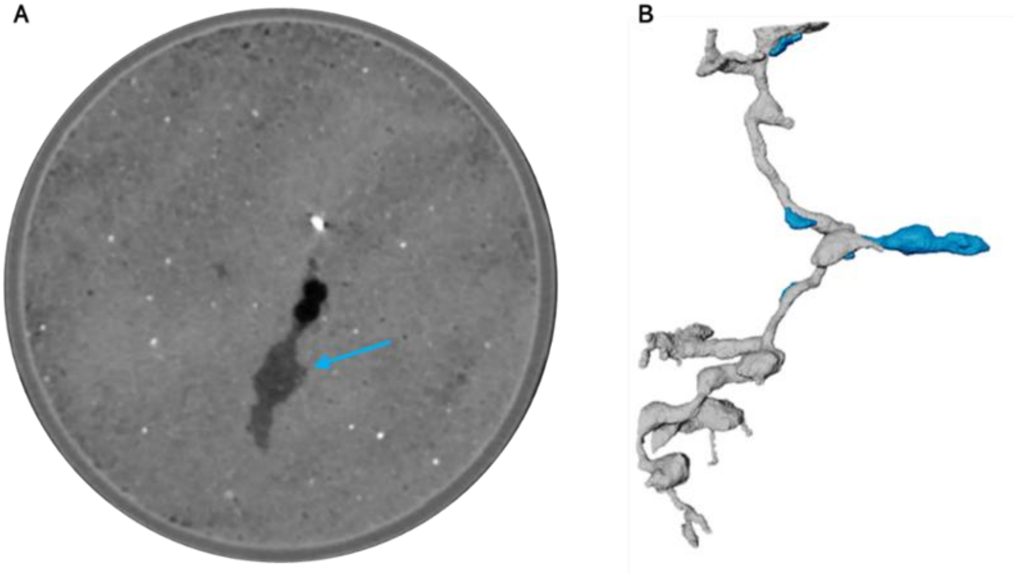

Fig S5. Example of refilled chambers. In the 2D image (A) black areas represent zero density (pore, i.e. galleries), light grey areas correspond to high density (matrix soil) and dark grey areas (blue arrow) represent soil volumes with intermediate densities i.e. refilled galleries. In the 3D image (B), the dark grey areas are represented in blue and show up as refilled galleries next to open galleries.

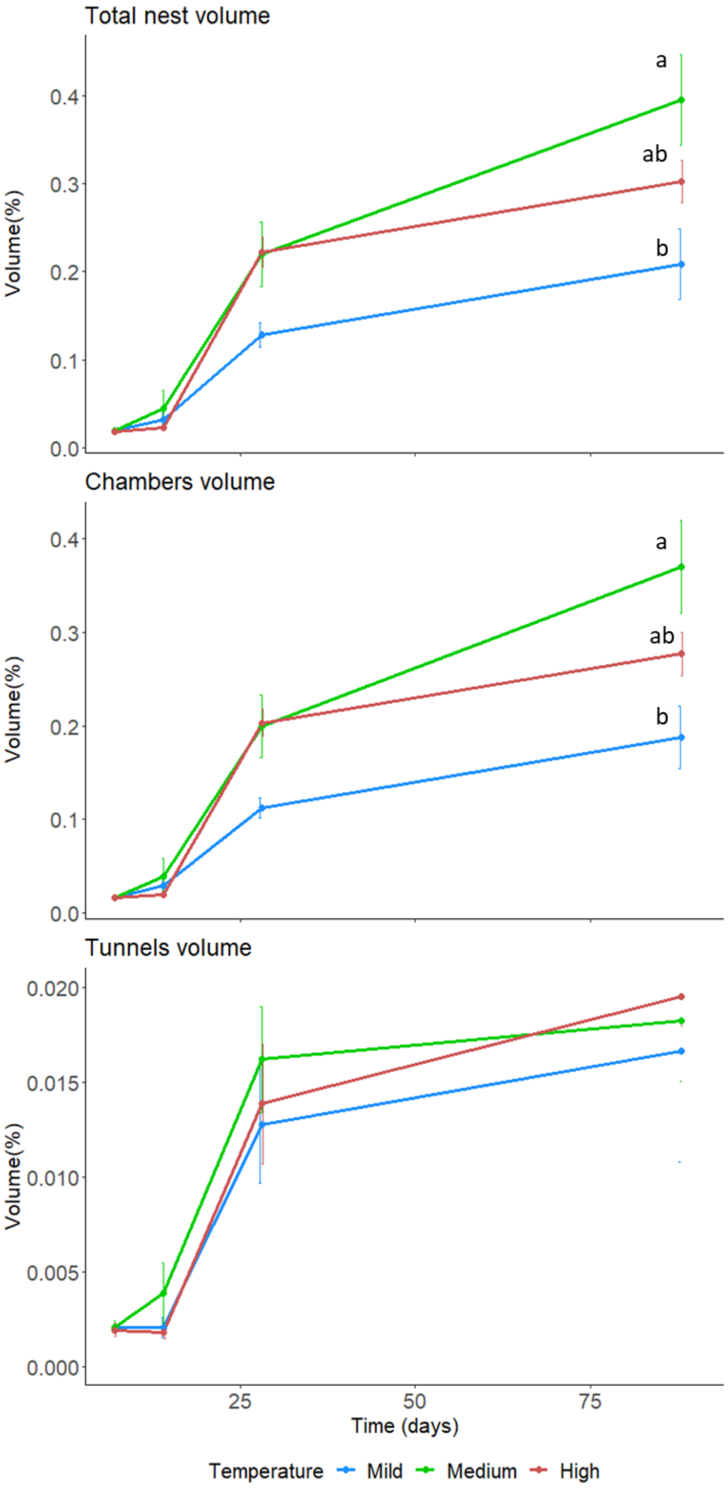

Fig S6. Total, chambers, and tunnels volume (percentage compared with the total column volume). Similar letters show no difference during day 88. The total volume of galleries was 0.37 ± 0.12%, 0.28 ± 0.06 %, and 0.19 ± 0.07%, for T_{medium}, T_{high}, and T_{mild}, respectively ($F_{2,14} = 5.20, P < 0.05$). The volume of chambers was 0.37 ± 0.13%, 0.31 ± 0.06 %, and 0.21 ± 0.09%, for T_{medium}, T_{high}, and T_{mild}, respectively ($F_{2,14} = 5.20, P < 0.05$).

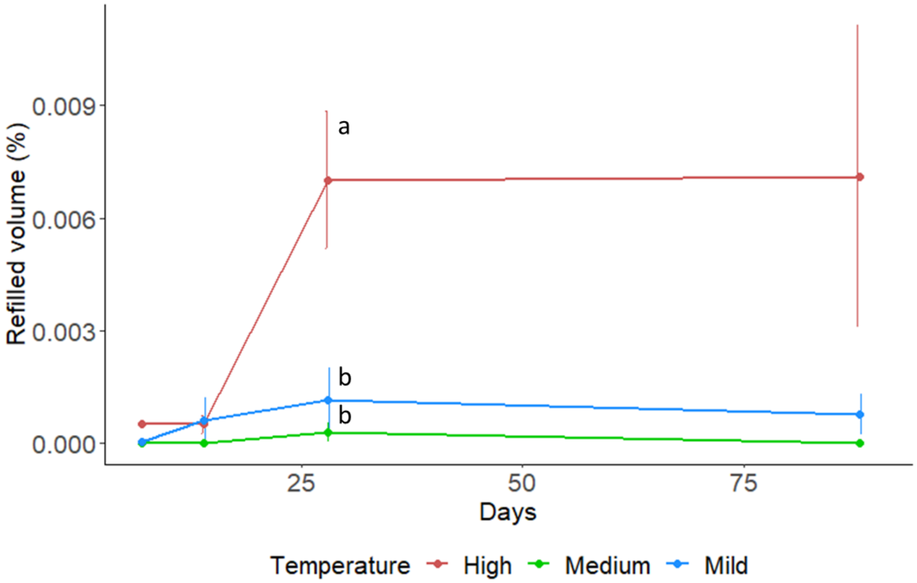

Fig S7. Volume of refilled galleries, measured as the percentage of the total volume of the earth column. Similar letters indicate no differences during day 28 (one-way Anova $F_{2,14}=8.625$, $P<0.05$). Volumes on day 28 were 0.007 ± 0.01%, 0.001±0.002%, and 0% for T_{high}, T_{medium}, and T_{high}, respectively.

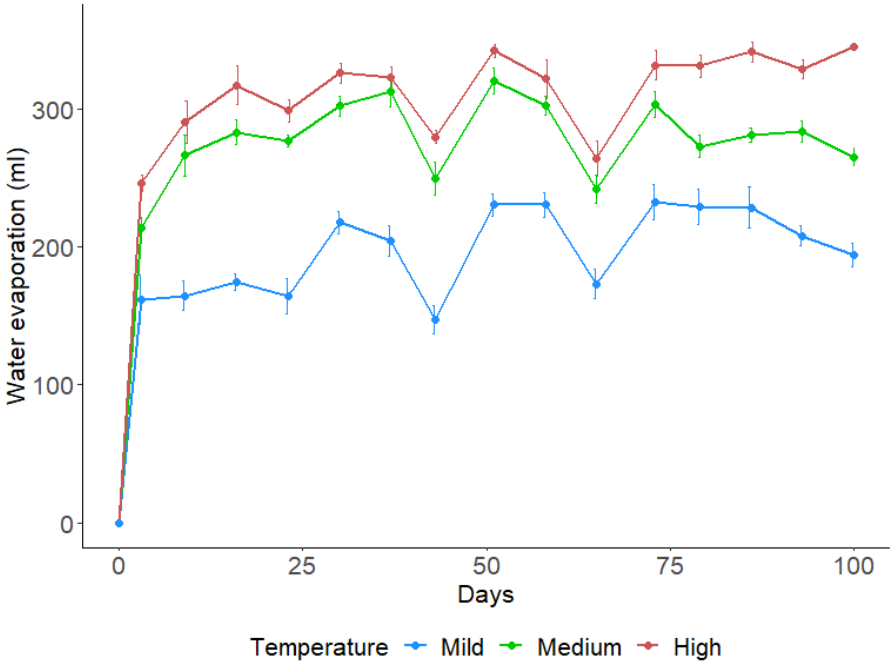

Fig S8. Water evaporation of colonies. Columns of ants exposed to mild temperature had less water evaporation than those exposed to medium and high temperatures during the whole experiment (two-way mixed Anova $F_{28,238}=3.193$, $P<0.001$). Colonies exposed to medium and high temperatures had the same water evaporation from the start until day 79 when the latter had higher evaporation than the former ($P<0.001$). This trend continued until the end of the experiment.

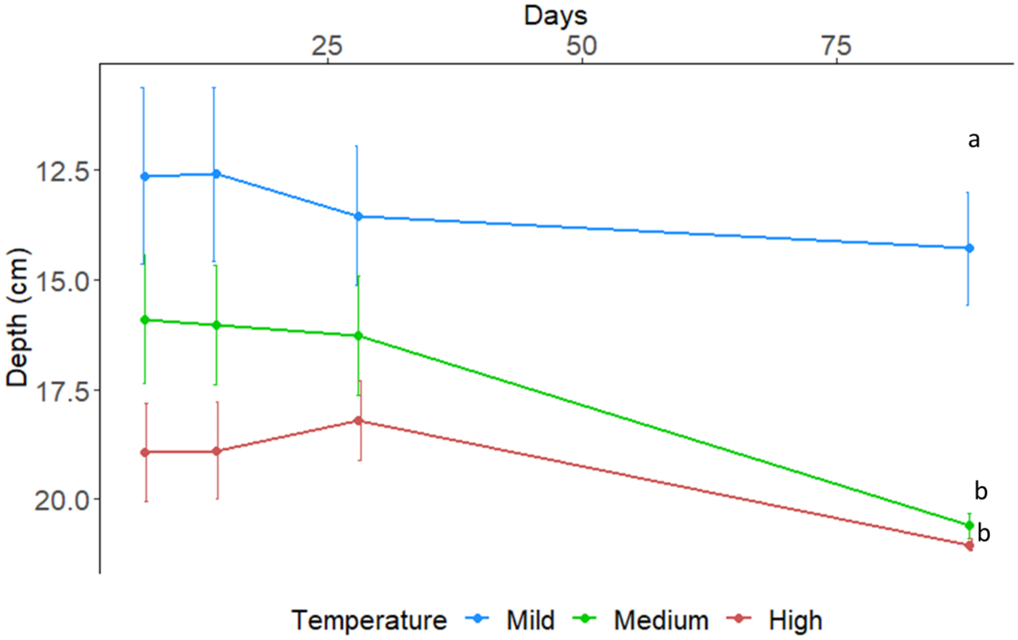

Fig S9. Maximal depth of galleries. No difference was observed among times on any of the treatments (two-way mixed Anova $F_{1,\ 18}=18.099,\ P>0.05$). Similar letters indicate no difference during day 88 ($F_{2,14}=7.99,\ P<0.05$).

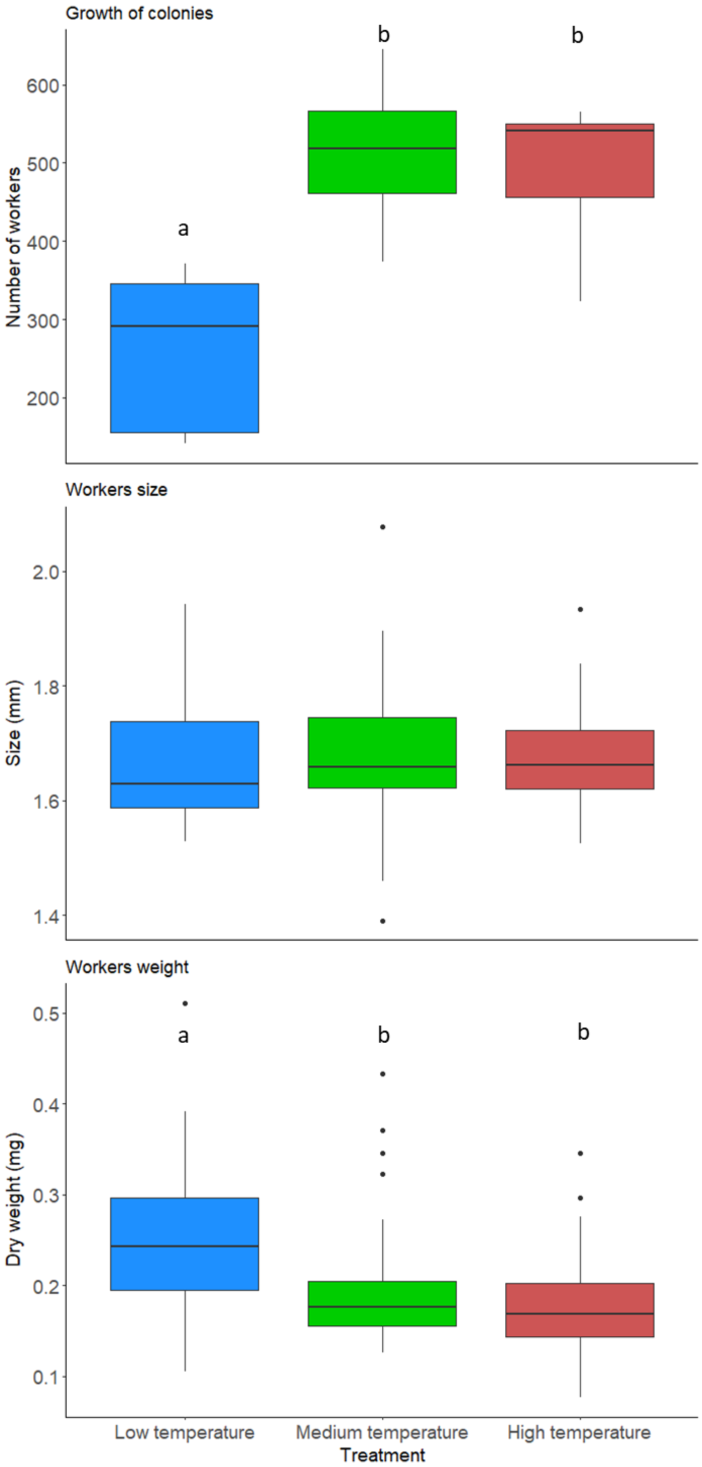

Fig S10. Growth, size, and weight of ant workers at the end of the experiment. All colonies grew over time. However, more workers were produced in colonies exposed to high and medium temperatures compared to those exposed to mild temperature (one-way Anova test, $F_{2,14}=10.45, p<0.05$). The temperature had no effect on the size of ants (one-way Anova test, $F_{2,167}=0.366, P>0.05$), but ants exposed to mild temperatures were significantly heavier than those exposed to both increased temperatures (one-way Anova test, $F_{2,167}=19.67, P<0.001$).

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

	Eigenvalue	Variance (%)	Cumulative variance (%)
Dim.1	6.550379e+00	40.939870862	40.93987
Dim.2	2.382942e+00	14.893388111	55.83326
Dim.3	1.473494e+00	9.209339976	65.04260
Dim.4	1.130755e+00	7.067221376	72.10982
Dim.5	1.028378e+00	6.427359431	78.53718
Dim.6	8.950415e-01	5.594009679	84.13119
Dim.7	6.672976e-01	4.170610260	88.30180
Dim.8	5.850810e-01	3.656756378	91.95856
Dim.9	4.456273e-01	2.785170804	94.74373
Dim.10	3.983940e-01	2.489962594	97.23369
Dim.11	1.991253e-01	1.244533245	98.47822
Dim.12	1.683594e-01	1.052246548	99.53047
Dim.13	6.765930e-02	0.422870643	99.95334
Dim.14	7.445885e-03	0.046536783	99.99988
Dim.15	1.972975e-05	0.000123311	100.00000

Table S2. Contribution of variables to the first two dimensions (principal components) of PCA. See Fig 4 for variable names signification.

	Dim.1	Dim.2
SI	6.07203821	10.849131109
IBR	5.23186281	1.240958144
EE	0.04788105	19.027275755
NP	0.22649898	22.291446384
V_{nest}	13.04366303	0.090322611
SA	12.37983968	0.256162058
EN	0.05509984	6.547565057
CZ	3.76368293	6.406575626
D	5.53657451	13.976643055
Th_{Tu}	0.22075993	8.450826437
Th_{Ch}	0.47151691	0.002149346
V_{Ch}	13.34216467	1.542073140
V_{tu}	10.58179245	5.223803558
PP	13.42074623	1.730398248
V_{Re}	2.12906852	0.729197379
T_{nestRe}	13.47681023	1.635472095

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

Treatment	**Colony**	**Workers at the start**	**Workers at the end**	**Colony growth (W_{end} - W_{start})**	**% of new workers**
High temperature	A1	116	656	540	82.3
	A2	123	552	429	77.7
	A3	98	663	565	85.2
	A4	82	634	552	87.1
	A5	86	629	543	86.3
	A6	127	450	323	71.8
Medium temperature	B1	95	469	374	79.7
	B2	65	523	458	87.6
	B3	93	565	472	83.5
	B4	84	650	566	87.1
	B5	143	788	645	81.9
	B6	127	694	567	81.7
Low temperature	C2	90	231	141	61.0
	C3	135	290	155	53.4
	C4	106	477	371	77.8
	C5	75	421	346	82.2
	C6	162	453	291	64.2

Chapter 4

Do changes in temperature and humidity induce shifts in abundance in a temperate ant community?

This chapter is a preliminary version of still needs more work before being submitted to a journal.

Do changes in temperature and humidity induce shifts in abundance in a temperate ant community?

Fátima García Ibarra, Thibaud Monnin, Marius Surun, Mahina Hittinger, Pascal Jouquet

Abstract

Climate change is inducing global ecological shifts, affecting species diversity and phenology. The observed temperature increase of 1.09°C since 1900, coupled with altered precipitation patterns, has direct consequences on organisms, including phenological changes, biodiversity loss, and distribution shifts. These effects, predicted to escalate with decreased mean precipitation and increased variability, impact ecosystems from individual organisms to entire communities. Ectothermic communities, notably ants, representing approximately 99% of global species, are particularly relevant for climate change studies due to their temperature-dependent physiology. This research, spanning two years in a temperate urban park, manipulated temperature and precipitation in three treatments to understand their impact on a local ant community.

The study successfully created distinct environments, varying soil temperature, air humidity, and other abiotic conditions. Ant abundance and richness were influenced by treatment, season, block, diversity of other invertebrates, and plant biomass. The T+130%P treatment notably differed in ant community composition. Increased ant abundance was associated with higher temperature and precipitation treatments, suggesting climate change's influence on ant communities. A negative correlation between predicted ant richness and plant biomass hinted at potential impacts of vegetation on ant species. Seasonal variations revealed higher ant abundance during spring and summer. The study underscores that abiotic modifications indirectly affect ant communities through altered resource availability and interactions with other invertebrates. It emphasizes the importance of considering microclimates and broader ecological contexts for accurate interpretations, contributing valuable insights into the intricate dynamics of ant communities under changing climatic conditions. Further research is warranted to deepen our understanding of these relationships and their implications.

Keywords

Temperature, humidity, ant abundance, soil invertebrates, vegetal biomass

Introduction

Climate change is causing significant ecological shifts, altering species diversity and phenology worldwide (Pecl et al., 2017; Tilman et al., 2017). Two important consequences of this phenomena are the rise in temperatures and changes in precipitation patterns. Since 1900, average temperatures have increased by 1.09°C. Climate models predict that mean precipitation will decrease, while variability will increase, resulting in extreme weather events (IPCC, 2021). Some of the most direct consequences of these abiotic variations on the species include phenological changes, loss of diversity, shifts in species distribution, and extinction (Williams et al., 2008; Pecl et al., 2017). Furthermore, the effects of global climate change extend across various scales, affecting organisms from individual entities to entire communities (Peñuelas et al., 2013). As a result, the functional consequences of climate change involve not only direct alterations in temperature and precipitation but also arise from feedback loops and cascading effects (Williams et al., 2008). Changes in factors such as competition, predator-prey dynamics, herbivory, associations between hosts and plants, parasitism, and mutualistic interactions can induce shifts at the community level, leading to consequences that are challenging to anticipate (Walther, 2010; Pecl et al., 2017).

While climate change affects nearly all organisms globally, it is especially crucial to investigate the responses of ectothermic communities. Ectotherms, whose body temperature depends on their surrounding environment (Jørgensen et al., 2022), constitute approximately 99% of all species worldwide and represent the majority of global biomass (Hölldobler & Wilson, 1990; Atkinson & Sibly, 1997). Among ectotherms, ants play a significant role, being abundant in various ecosystems and often regulating key ecosystem functions (Hölldobler & Wilson, 1990; Dunn et al., 2007). For instance, ants contribute to seed dispersal (Almeida et al., 2013), act as bioturbators influencing soil dynamics and nutrient cycling (Holec & Frouz, 2006), and engage with other species as predators (Philpott & Armbrecht, 2006) and prey (Pekár, 2004).

Ants have been identified as susceptible to global changes at individual, population, and community levels. At the individual level, responses may manifest as reductions in body size (Kaspari et al., 2015; Roeder et al., 2021), shortened development durations (Penick et al., 2017), and alterations in foraging activities (Cerdá & Retana, 2000). On a population scale, ants adapt to climate change by relocating their nests to sites with more suitable thermal environments (Kadochová & Frouz, 2014). At the community level, the consequences have been varied. It has been reported that hot microclimates produced by logging and habitat conversion in tropics, increase the abundance of ants with high thermal resistance, and reduce that of species with low resistance (Boyle et al., 2021). It has also been described that in temperate grasslands, climate changed produced an overall decrease of ant richness and a homogenization of the community (Gallé, 2017). Another study realized over 7 years in a semi-arid grassland, showed that ants did not benefit directly from the augmentation in precipitation, but indirectly through the increasing of plant productivity (Deguines et al., 2017).

Most of the studies aiming to investigate the effects of climate change on communities of ants focused on modifying either (air or soil) temperature (Diamond et al., 2016; Gallé, 2017) or precipitation (Deguines et al., 2017). Experiments where both parameters are modified are rare. In this work, we manipulated both temperature and humidity to untangle the effects of these factors on a small community of ants in a temperate environment. Our initial hypotheses were: i) that a reduction in climatic stochasticity accompanied by increases in temperature and in precipitation increase the biomass of plants, along with the biomass and/or diversity of soil invertebrates; ii) that the community composition would be different among treatments, responding to a temperature gradient ; iii) that increase in ant activity is positively related to soil hydraulic conductivity; and iv) that ants living in warmer plots would be smaller than those in control plots.

Materials and methods

Experimental design

We carried out a field experiment in the park of the French National Research Institute for Sustainable Development (IRD) campus in Bondy (7.5 km Northeast of Paris), France from June 2021 to May 2023. The soil had a neutral pH (pH_{H20} = 7.2) and was sandy (70% of sand, 15% of silt and 15% of clay) with 4.5% organic C. Vegetation in the park was visibly diverse. We placed 20 plots of 3 x 3 m within an area of approximatively 20 m x 25 m. Groups of four plots were arranged in a square and formed a block. Blocks were separated from one another by several meters, and within each block the plots were separated from one another by at least one meter. The four plots of each block were randomly assigned to the four treatments, i.e. each block repeated the four treatments (Fig. S2B). The four treatments were:

- T+70%P: increased temperature; 70% of decennial rainfall regularly distributed all along the year.
- T+100%P: increased temperature; 100% of decennial rainfall regularly distributed all along the year.
- T+T+130%PP: increased temperature; T+130%P of decennial rainfall regularly distributed all along the year.
- Control: no artificial heating, natural rainfall

Plots of all treatments with increased temperature were passively heated using 3×3m transparent plastic greenhouses fixed 5cm above the surface to allow the free transit of invertebrates among parcels. Monthly precipitation for all treatments was calculated using the average decennial rainfall reported in ClimateData.org (2021) for this area (720 mm $year^{-1}$) for all treatments. The same precipitation was used during the whole year. Irrigation occurred twice a week in the evening during summer to avoid immediate evaporation inside the greenhouses and at noon during winter to avoid freezing. A central irrigation system controlled by Claber 1” solenoid valves was used outside the greenhouses. Inside the greenhouses, a micro drip system was installed below the roof to simulate rainfall.

Monitoring of abiotic factors

We recorded the temperature (°C) and relative humidity (RH%) of soil and air every hour in three plots per experimental treatment (T+70%P, T+100%P and T+130%P) and one control plot using WatchDog 1450 and 1650 probes. Soil and air recordings were carried out at 15 cm depth and 30 cm height, respectively. Rainfall was measured daily with a Campbell Scientific meteorological station with an acquisition central CR300 and bucket rain gauge (mechanical tilting 0.199mm), during the whole experiment.

To evaluate how water moved in each plot, we measured the soil infiltration with a MiniDisk infiltrometer (METER Group) at the end of the experiment. A suction of 0.5 cm was used for all the measurements. We then calculated unsaturated hydraulic conductivity (K/ms) using the method proposed by Zhang (1997).

Vegetation and invertebrates sampling

To estimate the effects of temperature and precipitation changes on vegetation, we carried out an inventory of species after the first and second years (summer 2022 and summer 2023). In 2022, the identification was carried out at the species level for all the plants present in each plot. We estimated the coverage surface of every species in each plot. In 2023, we registered the presence or absence of the previously identified species and classified new species when present. Additionally, we calculated the biomass yearly by harvesting 1 m^2 of vegetation, drying it in a stove at 60°C for 96 hours, and weighting it. Afterward, we trimmed the vegetation in all the plot, dried it and returned to the soil.

We sampled ants and other invertebrates every season using 30mm diameter pitfall traps with 30 mL of ethanol and 1 mL of liquid soap. We placed the traps in the centre of the plot and left them in place for 36 hours. We identified the ants to the species level, while the rest of the invertebrates were identified only at the family level using the European Atlas of Soil Diversity (Jeffery et al., 2010). We dissected 10 ant individuals per plot of the most abundant species during spring and summer 2022 and 2023 and then measured the head width according to Schofield (2016), as it is often used as a good indicator of body size. To ensure that heads were placed perpendicularly to the axis of the camera, we carefully glued them to a 12×12 cm plastic box using double-faced tape, then photographed them with a Discovery

V12 Zeiss stereomicroscope connected to a Zeiss AxioCam ICc 5 camera. Measures were carried out with ImageJ software version 1.53s (Schindelin et al., 2012).

Statistical analysis

To analyse the differences on abiotic parameters (i.e., soil and air temperature and relative humidity, hydraulic conductivity, and rainfall) among treatments, we performed a nested Anova (treatment nested in block, stats package, R Core Team, 2023) for each variable and carried out Tukey test (stats package) when we found differences. To assess the floristic composition of each plot, we purposely disregarded the treatment since vegetation was heterogeneous among plots. We created a dissimilarity matrix using Bray-Curtis index (vegan package, Oksanen et al., 2022) for vegetation cover by species. Then, using Ward's hierarchical clustering method (stats package), we assigned each plot to a group. Also, we calculated the abundance (total number of individuals), richness (number of species), and Shannon's diversity index of the groups of invertebrates other than ants (vegan package).

We determine the effect of the variations in temperature and humidity on ants' abundance and richness using all the biotic parameters mentioned above as indicators. Before analysing the relationships between variables, we verified Spearman's correlation (stats package) between variables to avoid collinearity on the next steps. We conserved variables that were weakly correlated (correlation < 0.7) and excluded those that were highly correlated (≥ 0.7) (Ratner, 2009). To evaluate the abundance, we performed a series of generalised linear mixed models considering a negative binomial distribution (i.e., abundance is a discrete positive variable with high overdispersion), and the variable year as a random effect. We selected the GLMM with the lowest AIC. The quality of the models was tested using the DHARMa package (Hartig, 2022). To analyse ants' richness, we used a 'zero inflated' model with a Poisson distribution (pscl package, Zeileis et al., 2008) as some seasons presented very few species. Once we selected the models, we analysed the predicted results of our models using Tukey HSD post hoc tests (emmeans package, Lenth, 2023) when differences between groups were significant. The block and floristic composition were partially confounded (Fig. S2), thus we created two sets of models considering one or the other variable and, then selected the one with the smallest AIC value.

Lastly, to evaluate the effect of different temperatures and humidities on the communities of ants, we carried out a non-metric multidimensional scaling (NMDS, vegan package) followed by a PERMANOVA test (vegan package) using Euclidean distance, and a pairwise multilevel comparison (vegan package) to evaluate differences between communities when present.

RESULTS

Abiotic parameters

Precipitation recorded on the site of the experiment was 472.1 mm from August 2021 to July 2022, and 706.9 mm from August 2022 to May 2023, with a peak in precipitation in August 2022 (> 150 mm in one day, Fig. 1). Daily average temperatures and relative humidities of soil and air are shown in Fig. 1 and Tables S1 and S2. Specifically, the temperatures among treatments were different on the soil (Anova test $F_{3,4} = 55.13$, $p<0.001$, Fig. 2A) and the air ($F_{3,3} = 7.15$, $p<0.001$, Fig. 2B). Similarly, we observed differences among treatments on relative soil moisture ($F_{3,4} = 77.82$, $p<0.001$, Fig 2C) and air humidity ($F_{3,3} = 264.61$, $p<0.001$, Fig 2D). Table 1 shows the average abiotic conditions by treatment.

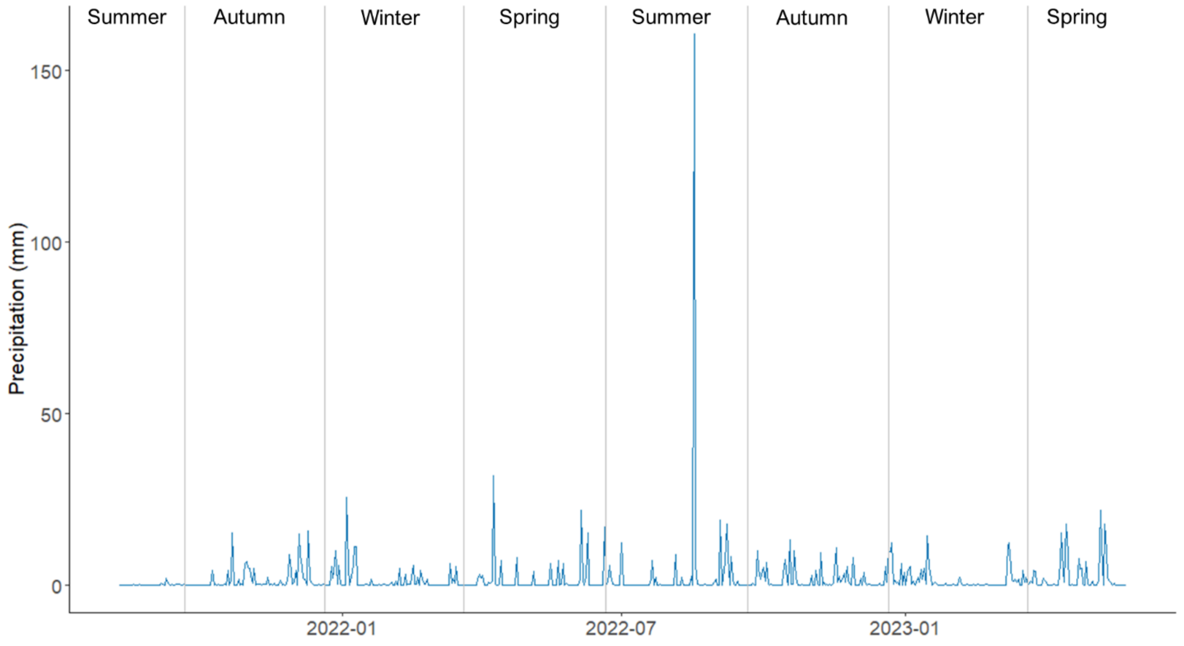

Fig 1. Daily precipitation recorded on the study site, from August 2021 to May 2023. Data were acquired with a Campbell Scientific meteorological station with an acquisition central CR300 and bucket rain gauge (mechanical tilting 0.199mm), during the whole experiment.

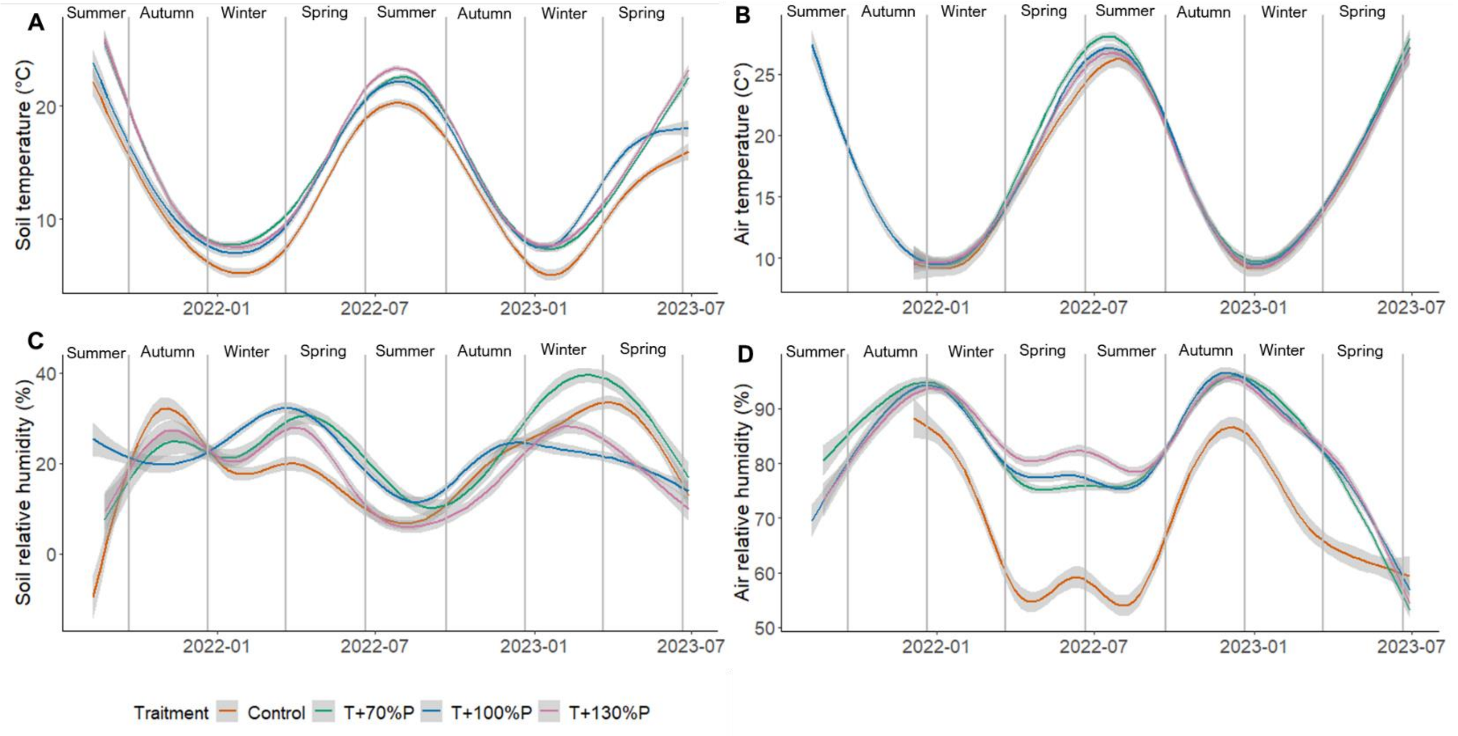

Fig. 2. Abiotic conditions of the treatments T+70%P, T+100%P, T+1+130%P, and control. Lines correspond to daily average temperature (°C) of soil (A) and air (B). Soil moisture (HR%) (C) and air humidity (D). Vertical lines correspond to seasons.

Table 1. Temperature of soil and and soil moisture and air, calculated as the average of all seasons during the whole experiment. Different letters correspond to differences among treatments (nested one-way (block) Anova test).

Season	***Treatment***	***Soil temperature ($\bar{x}$ ± sd, °C)***	***Soil moisture ($\bar{x}$ ± sd, %)***	***Air temperature ($\bar{x}$ ± sd, °C)***	***Air humidity ($\bar{x}$ ± sd, %)***
Summer	Control	19.63 ± 1.63^{c}	8.24±7.88^{b}	24.84±3.26^{a}	57.23±9.37^{c}
	T+70%P	21.95 ± 2.01b^{a}	14.01±10.43^{a}	26.22±3.62^{a}	77.55±6.99^{ab}
	T+100%P	21.33 ± 1.85^{b}	14.73±7.05^{a}	25.46±3.43^{a}	76.76±7.08^{b}
	T+130%P	22.66 ± 2.57^{a}	8.15±6.75^{b}	25.04±3.42^{a}	79.40±8.53^{a}
Autumn	Control	10.59 ± 3.73^{b}	21.45±14.40^{a}	12.81±4.98^{a}	80.68±7.73^{b}
	T+70%P	12.39 ± 4.01^{a}	20.04±12.58^{a}	13.38±4.71^{a}	91.18±5.37^{a}
	T+100%P	11.92 ± 3.93^{a}	20.36±10.54^{a}	13.44±4.45^{a}	90.44±6.80^{a}
	T+130%P	12.29 ± 4.14^{a}	16.59±13.94^{b}	13.16±4.68^{a}	90.24±6.63^{a}

Winter	Control	6.30 ± 1.90^{b}	23.92 ± 14.72^{b}	10.83 ± 3.11^{a}	76.47 ± 11.16^{b}
	T+70%P	8.55 ± 2.29^{a}	29.93 ± 17.04^{a}	11.38 ± 2.96^{a}	89.56 ± 7.86^{a}
	T+100%P	8.66 ± 2.95^{a}	25.97 ± 10.49^{b}	11.28 ± 2.96^{a}	88.65 ± 8.10^{a}
	T+130%P	8.51 ± 2.08^{a}	24.80 ± 12.32^{b}	11.17 ± 2.88^{a}	89.35 ± 7.83^{a}
Spring	Control	13.43 ± 5.22^{b}	20.82 ± 12.11^{cb}	19.79 ± 4.39^{ab}	59.80 ± 8.58^{d}
	T+70%P	16.06 ± 3.58^{a}	28.34 ± 15.41^{a}	21.05 ± 4.82^{a}	72.31 ± 11.18^{c}
	T+100%P	15.83 ± 4.88^{a}	22.58 ± 9.18^{b}	20.40 ± 4.58^{ab}	74.28 ± 10.68^{b}
	T+130%P	16.22 ± 3.80^{a}	19.32 ± 10.91^{c}	20.14 ± 4.41^{b}	76.20 ± 11.61^{a}

Floristic composition and biomass

Plant species identified in the summer 2022 and 2023 (see Tables S3 and S4 for a list of the species) were very similar as only few species (with few individuals) were encountered solely in one year. Hence, we decided to use the classification carried out in 2022, as surface cover provided more precise data. Hierarchical clustering allowed us to identify four vegetation groups (Fig. S1) characterised by the dominant species: group 1) noble yarrow (*Achillea nobilis*), buckhorn plantain (*Plantago lanceolata*), black medick (*Medicago lupulina*) and hairy violet (*Viola hirta*); group 2) buckhorn plantain, black medick, and small grasses; group 3) black knapweed (*Centaurea nigra*), hedge bedstraw (*Gallium mollugo*), black locust (*Robinia pseudoacacia*) and tall grasses; and group 4) noble yarrow, black medick, hedge bedstraw, and tall grasses. We found no effect of the treatment on ($F_{3,9}$= 1.55, p>0.05) or the group of vegetation ($F_{3,9}$= 1.01, p>0.05) on dry biomass, however there was an effect of the year as the dry biomass was higher in 2022 than in 2023 ($F_{1,9}$= 16.14 p<0.001, Fig. S2).

Ant communities

In total, eight species of ants were collected: *Lasius niger, Myrmica scabrinodis, Myrmica schencki, Lasius flavus, Solenopsis fugax, Myrmecina graminicola, Formica cunicularia* and *Hypoponera eduardi*. Partial and total abundances by species treatment, and season are shown in Fig. S3. Ant communities differed among treatments (PERMANOVA test, F=2.14, df=3, p<0.05, Fig. 3A). Specifically, the community of ants in the T+130%P treatment was different from the one in the control (F=5.23, df=1, p<0.05). Furthermore, communities also differed among seasons (F=18.54, df=3, p<0.001, Fig. 3B). They were similar between summer and spring only (F=2.47, df=1, p>0.05). All comparisons between seasons are shown in Table S7.

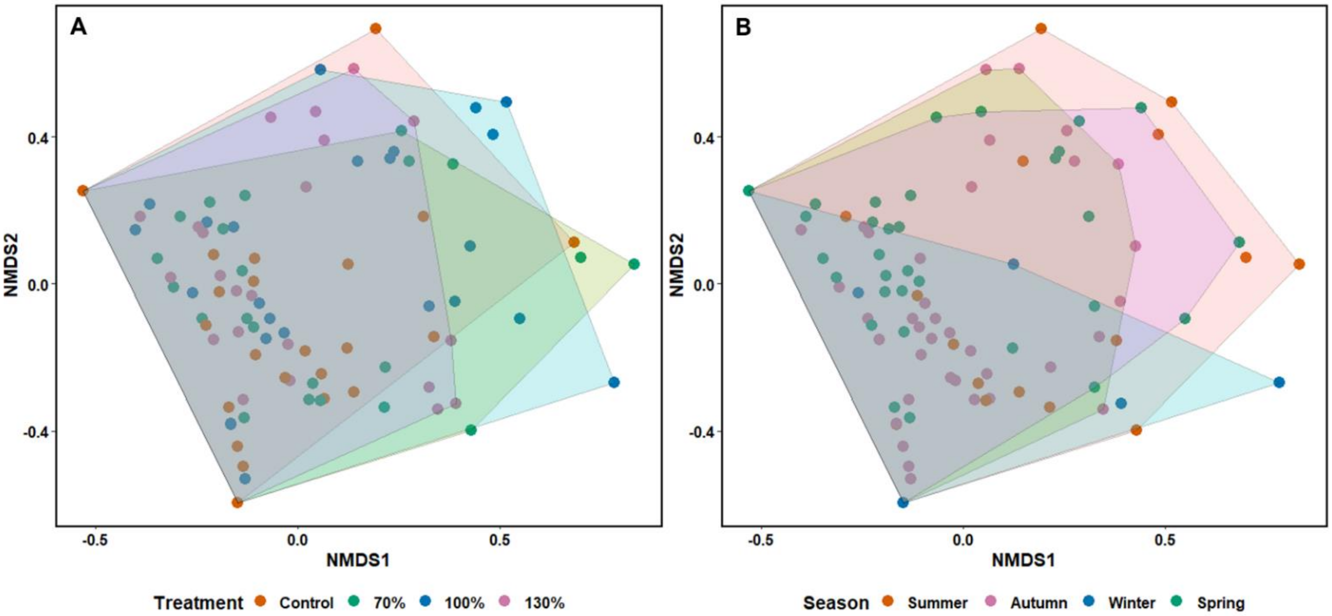

Fig. 3. Communities of ants by treatment (A), and by season (B). Points correspond to coordinates calculated by a NMDS.

Ant size

Since the most abundant ant was *L. niger* (Fig. S4) we measured the size of that specie only, in order to test for an effect of our treatments on worker size. We did so at four moments during the course of the experiment: at the beginning of the experiment in summer of 2021, in summer 2022, in spring 2023 and in May 2023. This last measure was carried out on ants sampled one month after the removal of the greenhouses in April 2023. Owing to the temporal polyethism (i.e, the division of labor observed in numerous eusocial insect colonies where the task of an individual is related to their age, (Fewell, 2019) displayed by ants, these workers had metamorphosed into imago before the removal of the greenhouses.

The head width of *Lasius niger* was different in the T+100%P treatment when comparing the three years of sampling (Kruskal-Wallis X^2 = 47.901, df = 15, p<0.001). Specifically, the ants in this treatment were significantly smaller in 2021 compared to 2022 (p=0.027), 2023 (p=0.027), and the post experiment sampling (p=0.033). No differences were observed across years in any of the other treatments (Fig. S5).

Ants abundance

The best model explaining ant abundance is shown in Table 2 (see Table S5 for a comparison with the 4 best models). This model described 90.2% of the observed variance and showed that the treatment, the season, the block, the diversity of other invertebrates (expressed as Shannon index) and plant biomass significantly influenced the abundance of ants.

Table 2. Analysis of deviance best model describing the abundance of ants

Treatment + season + block + Shannon invertebrates + log (dry biomass) +(1|year)

	X^2	**Df**	**p value**
(Intercept)	8.7032	1	0.003
Treatment	21.1470	3	9.81e-05
Season	256.3746	3	< 2.2e-16
Block	26.5115	3	2.50e-05
Shannon index invertebrates	22.4925	1	2.11e-06
Dry plant biomass (log)	5.1185	1	0.024

The predicted abundance of ants was different between treatments (Fig. 2A). Specifically, the abundance of ants in the control treatment was expected to be lower than the abundance of the T+70%P treatment (Z= -4.468, p<0.0001), the T+100%P treatment (Z= -2.773, p<0.05), and the T+130%P treatment (Z= -3.356, p<0.01). There were no differences between the predicted abundances of the T+70%P, T+100%P and T+130%P treatments. We also found differences on the predicted abundances between seasons (Fig. 2B). The model showed higher predicted abundances during spring (compared to autumn (Z= -6.673, p<0.0001) and winter (Z= -11.693, p<0.0001)), and summer (compared to autumn (Z= -10.220, p<0.0001) and winter (Z= 13.532, p<0.0001)). Moreover, we found that abundance during autumn was higher than during winter (Z=6.900, p<0.0001). The only two seasons with similar abundances were spring and summer (Z=1.566, p>0.05). Also, we found some differences between blocks (Fig. 2C), since the abundance in block 5 was higher than in block 2 (Z= -4.253, p<0.001) and block 4 (Z= -3.979, p<0.001), and block 3 was predicted to be more abundant than block 2 (Z= -2.997, p 0.0229).

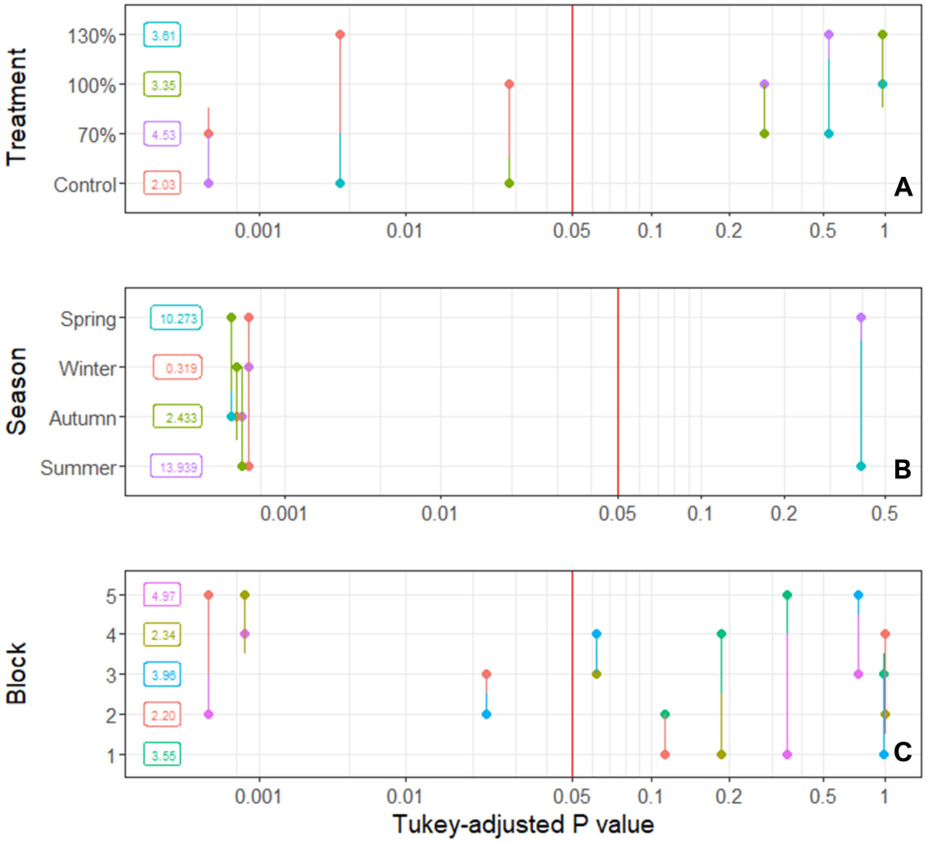

Fig. 2. Differences in mean of predicted ant abundance (number of individuals) between treatments (A), seasons (B), and blocks (C). Data correspond to the output of the best model and not to observed data. All comparisons to the left of the red line show significant differences.

The predicted abundance of ants was also affected by the diversity of other invertebrates. Specifically, it increased with the abundance of other invertebrates, regardless of the treatment, block and season (Fig. 3A). The predicted abundance was lower in control compared to the three treatments (Fig. 3A, Z ratio= -4.468, $p<.0001$ compared to T+70%P; Z ratio = -2.773, $p<0.05$ compared to T+100%P; Z ratio = -3.356, $p<0.01$, compared to T+130%P) and we found no differences between the three treatments. Regarding blocks, ant abundance was highest in block 5 compared to block 2 (Z= -4.249, $p<0.001$) and block 4 (Z= -3.975, $p<0.001$), that were the ones where ants were least abundant. The effects of seasons were lower (lower slopes) than those of treatments or blocks. As expected, ant abundances were higher during summer and spring (i.e., no differences between them, Z= 1.566, $p>0.05$), intermediate during autumn (Z= -6.668, $p<0.001$, compared to spring; Z= 10.213, $p<0.001$, compared to summer), and lowest during winter (Z= -6.668, $p<0.001$, compared to autumn).

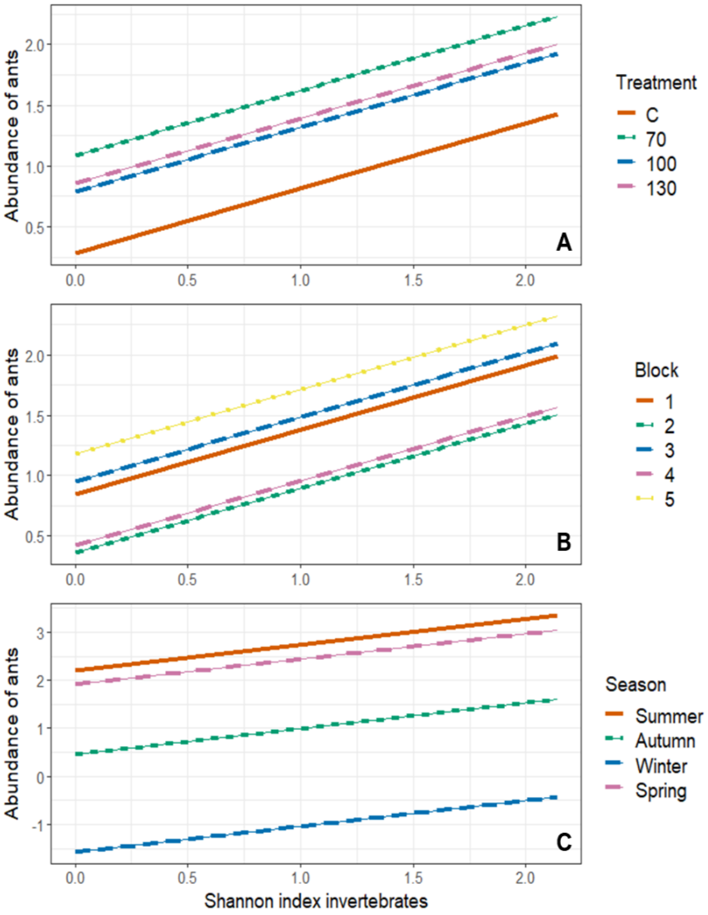

Fig. 3. Predicted ant abundance (number of individuals) as a function of the diversity of invertebrates (Shannon index) between treatments (A), blocks (B), and seasons (C). Data correspond to the output of the best model and not to observed data.

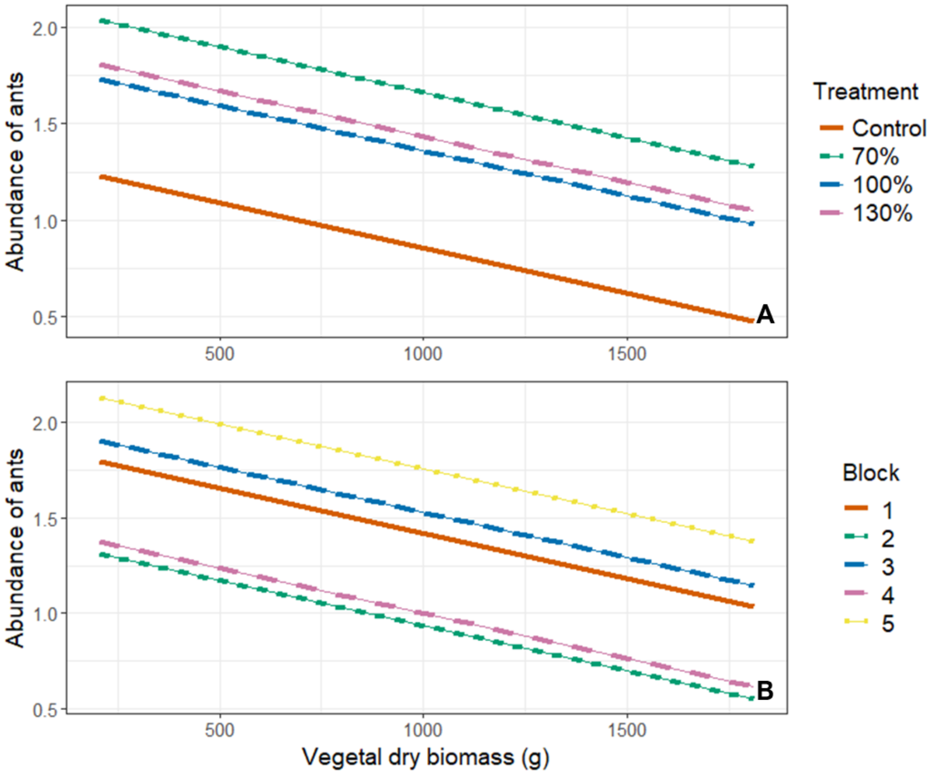

Fig. 4. Predicted ant abundance (number of individuals) as a function of the vegetal biomass (dry, in g) between treatments (A), and blocks (B). Data correspond to the output of the best model and not to observed data.

The vegetal biomass influenced the predicted abundance of ants as well (Fig. 3). In all treatments, ant abundance decreased as plant biomass increased (Fig. 4A). The predicted abundance was lower in control than in the three treatments (T+70%P treatment: Z ratio = -4.468, $p<.0001$; T+100%P treatment: Z ratio= -2.773, $p<0.05$; T+130%P treatment: Z ratio = -3.356, $p<0.01$) and we found no differences between the three treatments. The same trend was observed when comparing blocks (Fig 4B). The abundance was highest in block 5 and lowest in blocks 2 (Z ratio = -4.249, $p<0.001$) and 4 (Z ratio = -3.975, $p<0.001$).

Ant species richness

The zero-inflated models built to describe the influence of shifts in temperature and humidity on ant diversity (Table S6) were less accurate than the GLMMs created for the abundance. Hence, the best model explaining ant richness described only 63.6% of the observed variance, and it included only the richness of other invertebrates and the dry biomass (Table 3).

Table 3. Summary of the model selected to explain the richness of ants.

Invertebrates richness + log (dry biomass) | season

	Z value	**p value**
(Intercept)	-1.443	0.1489
Invertebrates richness	2.358	0.0184
Log (dry biomass)	2.064	0.0390

The richness in ant species as a function of the richness in invertebrates (Fig. 5A) was expected to be positive during all the seasons. However, the slope was higher during the summer, autumn and spring compared to winter (Z ratio= 8.648, p<0.001, Z ratio= 6.775, p<0.001, and Z ratio= -8.648, p<0.001, respectively). This shows that the influence (and presence) of invertebrates was lower during the winter compared to the other seasons. The same trend was observed for the biomass. The number of ant species increased as the vegetal biomass increased, and this relationship was significantly lower during winter than during the other seasons (vs summer: Z ratio= 8.648, p<0.001; vs autumn: Z ratio= 6.775, p<0.001; vs spring: Z ratio= -8.648, p<0.001).

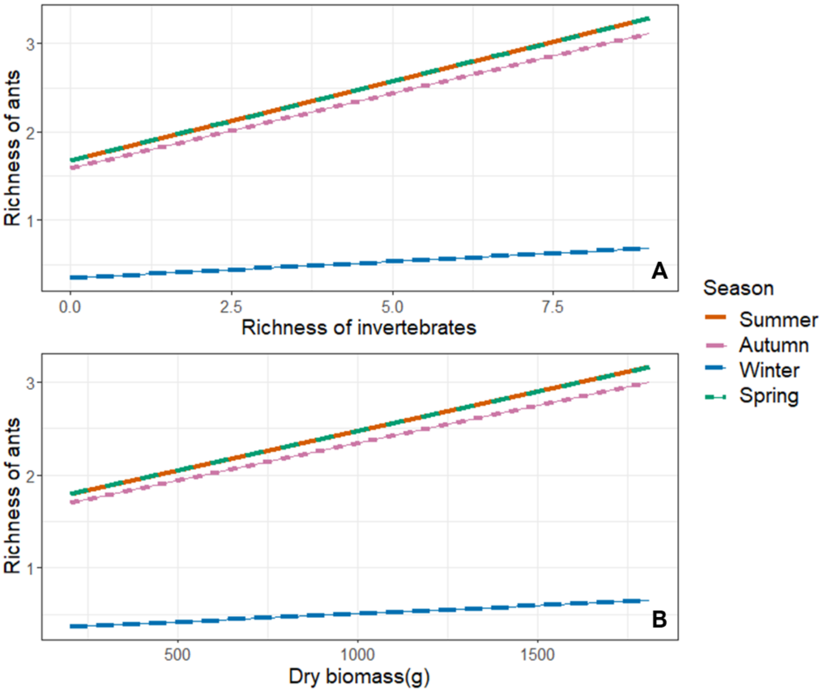

Fig. 5. Predicted ant species richness (number of species) as a function of richness in other invertebrates Family (A), and dry vegetal biomass (B) between seasons. Data correspond to the output of the best model and not to observed data.

Hydraulic conductivity

We found hydraulic conductivities ranging from 1.52×10^{-5} m s^{-1} (T+130%P treatment) and 7.40×10^{5} m s^{-1} (control treatment). We found no differences on the hydraulic conductivity between treatments (data shown in Table S7, $F_{3,4}$= 1.06, p>0.05). Moreover, the block (nested on the treatment) did not influence this property of the soils either ($F_{3,4}$=3.04, p>0.05).

DISCUSSION

The precipitation was lower during those two years than the average with which our treatments were calculated, and a peak of rainfall occurred in August 2022 (Fig. 1).

In all cases, control plots were different than the other treatments (but see air temperature), and there were various combinations of conditions between treatments. We also observed an effect of the block in all these variables (Table S3).

Our set up was successful in creating one control and three treatments that significantly differed in temperature and humidity. The soil temperature in treatments (plots with greenhouses) was around 2°C above the temperature of the control (without greenhouse). It has been suggested that it is the soil temperature rather than the air temperature that defines many ecosystem functions such as decomposition, root growth, soil respiration, which could be directly or indirectly affecting ant communities.

Although we did not measure the effects of these abiotic conditions on ant individuals or nests, our models allowed us to confirm our first hypothesis that modifications in abiotic conditions would change vegetal biomass or other invertebrates' abundance. Indeed, the relationship between biomass and the diversity of other invertebrates, as well as the richness and abundance of ants, has been confirmed.. We could thus argue that the altered abiotic conditions affect indirectly the ants by changing the availability of resources and potentially provoking shifts in the relationships with other invertebrates (i.e. the number of spiders, potential predators of ants, or other invertebrates that could compete with them for the same resources). It is important to highlight that our experiment could have also had effects on other relationships, for instance, birds and small rodents were not able to enter the greenhouses, which provided shelter inside these plots, which could be an important source of bias.

As for the communities of ants, we only found differences between the control and the T+130%P treatment. When we observed the abiotic conditions in these two treatments (Table 1), we see that both the soil temperature and the air humidity are different between these two groups. Therefore, in the treatment T+130%P, we expected more ants and more ant species. Moreover, this treatment is fully overlapped with group 2 of vegetation (Fi. S2). This led us to think that the differences in the communities between these two groups are related to the temperature of the soil that can be more suitable for ants in the T+130%P treatment than in the control, but also to indirect relations with plants and other invertebrates. Analysing the abundances of these other groups of animals and plants could permit to better understand the relationships behind these observations.

An interesting finding in our study is that in our model, the predicted richness in ant species was negatively related to plant biomass, meaning that the more abundant the vegetation, the fewer species are expected to be found. It has been reported that more complex environments, with a denser vegetation, are better suited for smaller ants because they are capable of accessing smaller interstices and crevices, while bigger ants with longer legs (such as *Formica cunicularia*) are more suited to simpler, more open environments. Therefore, according to our models, the augmentation of temperature and humidity could increase the plant biomass, which would increase the abundance of ants but also, the homogeneity of the communities as has been previously reported to happen in long term experiments.

As expected in a temperate environment, the season was a very important factor when analysing ant abundance and ant species richness. Our models confirm that ants are more abundant during spring and summer, which was expected from the beginning of the experiment. Moreover, the community composition was similar in spring and summer and different to autumn and winter. However, the humidity of air was higher in the T+70%P, T+100%P and T+130%P treatments compared to the control (Fig 1D). This did not negatively affect the ants but allowed them to stay active for longer periods, being more abundant in plots with greenhouses in autumn as observed in Fig S4.

We could not confirm our hypothesis that ants in warmer plots would have an impact on the water infiltration as we found no difference between plots. This may be due to the heterogeneity of the soil texture in the park where we carried out the experiment, which is partially confounded with the heterogeneity in plants. More detailed calculations of the soil texture will allow us to better estimate the infiltration. Also, it might have been useful to identify the nests and follow their activities throughout the experiment.

Perspectives for this study

Our study thus far suggests that climate changes are more likely to affect ant communities indirectly through feedback mechanisms, rather than having a direct impact on the performance or development of ant colonies.

The next step will be to test alternative approaches to the statistic analysis to confirm our current results. Also, we will try to test if there is some relationship between a specific species of plants with the total abundance of ants or one species only. Finally, we will analyse each group of invertebrates independently to test the possibility of one group affecting the abundance of ants.

Supplementary material

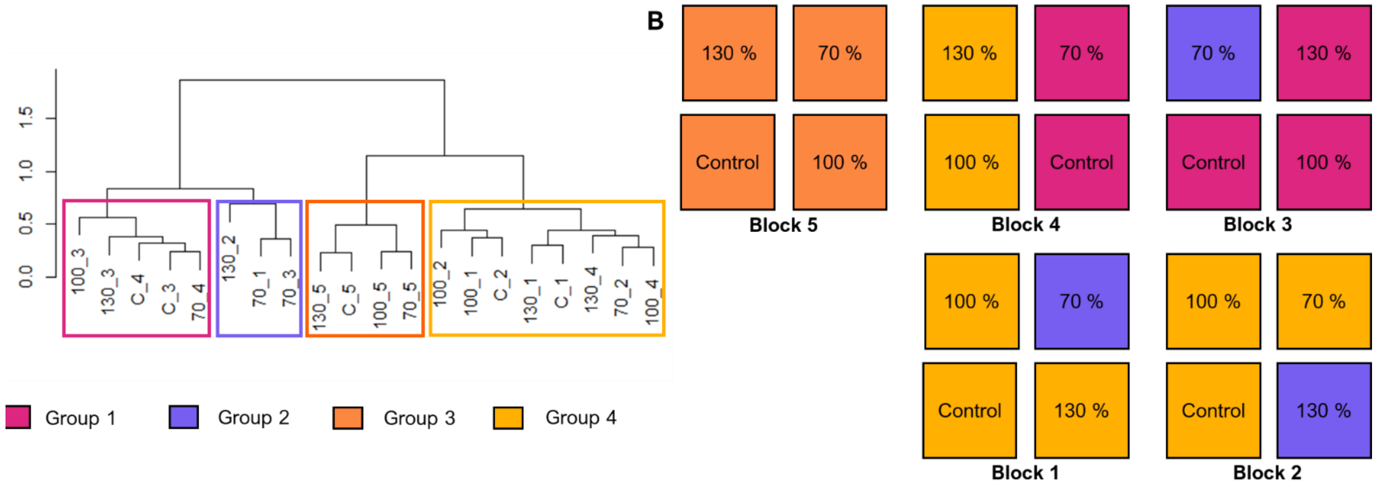

Fig. S1. Relationship between plots and vegetation groups. (A) Hierarchical clustering tree diagram of the 20 plots based on vegetation groups (labels combine treatment name and block number). (B) Layout of plots within blocks, with vegetation groups.

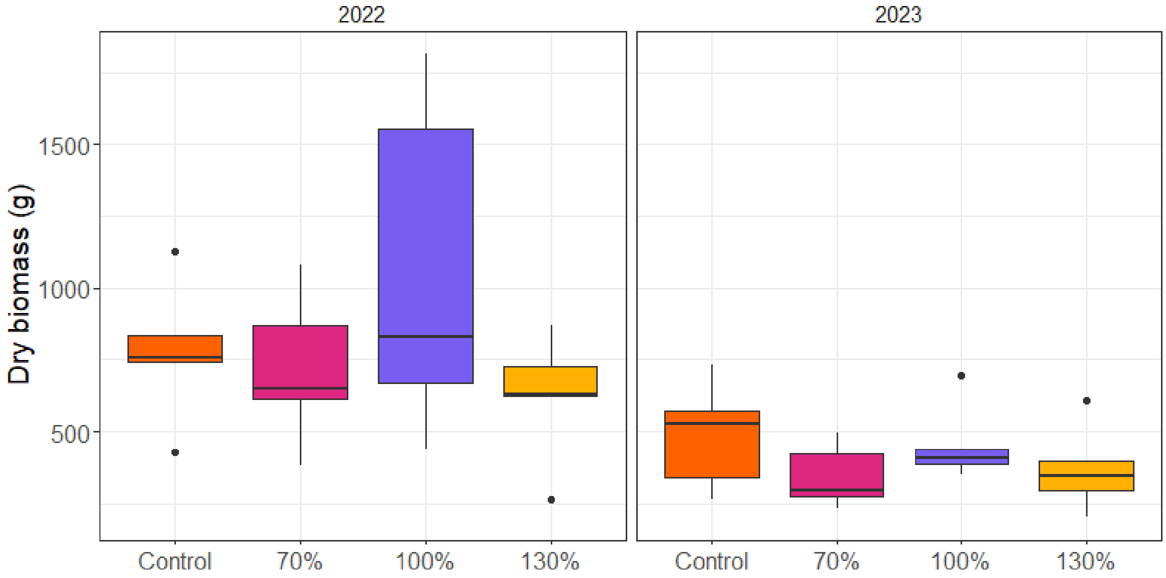

Fig. S2. Dry plant biomass by treatment during 2022 and 2023. No difference between treatments were reported in any year (nested Anova test, $F_{3,9}$= 1.55, p>0.05).

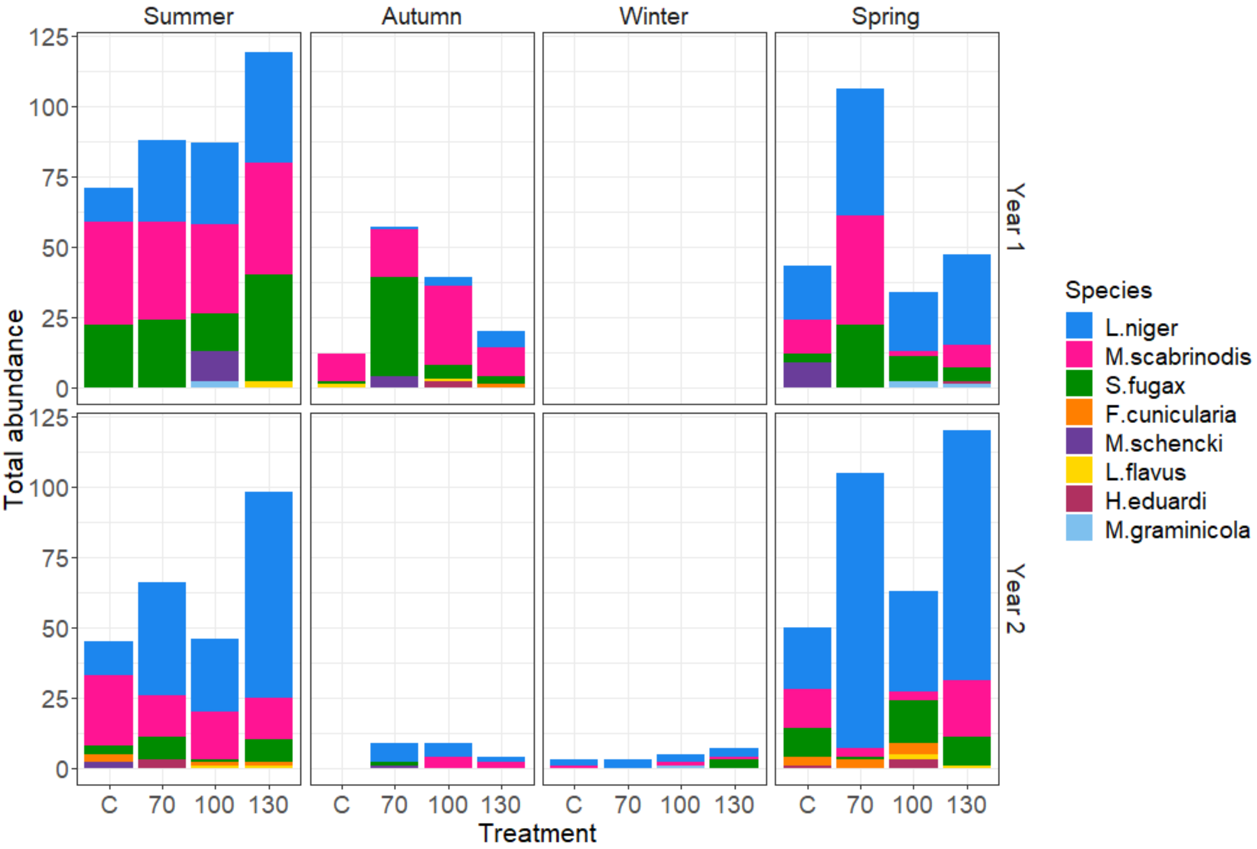

Fig. S3. Abundance of ants per species, treatment and season during year 1 (summer 2021 to spring 2022), and year 2 (summer 2022 to spring 2023). Data correspond to the number of sampled individuals.

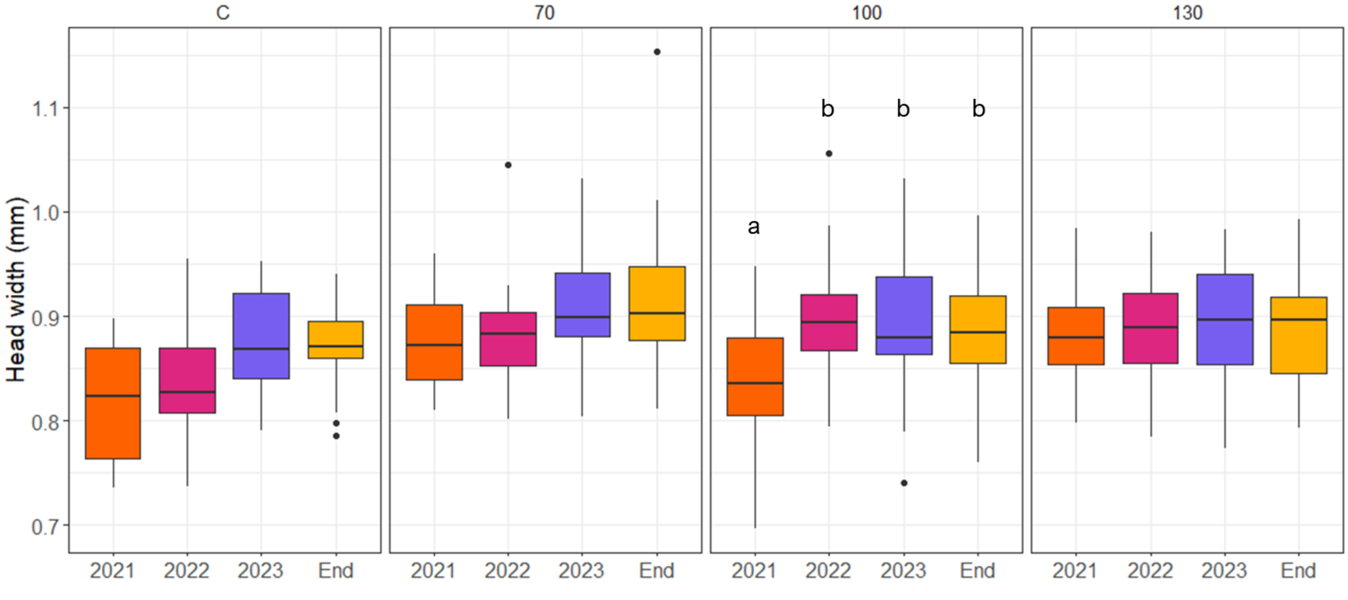

Fig. S4. Head width of *Lasius niger* workers by treatment across years. "End" stands for the post-experiment sampling. Add statistical test, values of statistics and p value. Letters denote significant differences.

Table S1. Temperature and relative humidity of soil and air by plot. Note that only three of the five blocks were equipped with recorders, and that the air recorders failed in two control plots.

Treatment	***Block***	***Soil temperature (x̄ ± sd, °C)***	***Soil humidity (x̄ ± sd, %)***	**Air temperature *(x̄ ± sd, °C)***	**Air humidity *(x̄ ± sd, %)***
Control	2	11.81 ± 6.02	20.84 ± 15.04	-	-
Control	3	12.47 ± 6.09	12.73 ± 11.10	-	-
Control	5	11.95 ± 5.36	24.84 ± 12.28	16.56 ± 6.59	68.43 ± 13.52
70 %	2	14.92 ± 5.39	35.38 ± 14.88	18.70 ± 6.86	83.65 ± 11.97
70 %	3	13.84 ± 6.05	18.97 ± 14.63	17.55 ± 7.45	81.20 ± 13.74
70 %	5	13.55 ± 5.17	17.29 ± 9.80	17.15 ± 6.45	81.88 ± 9.35
100 %	2	15.10 ± 6.04	21.00 ± 10.40	17.98 ± 6.71	81.37 ± 12.17
100 %	3	13.43± 6.24	26.38 ± 10.03	17.38 ± 6.92	81.62 ± 11.82
100 %	5	13.29 ± 4.98	17.68 ± 8.26	17.04 ± 6.28	83.13 ± 9.44
130 %	2	14.82 ± 5.96	22.15 ± 15.66	17.07 ± 6.38	79.47 ± 10.71
130 %	3	13.92 ± 6.15	8.95 ± 5.97	16.89 ± 6.75	86.55 ± 11.03
130 %	5	13.77 ± 5.41	23.18 ± 8.94	16.79 ± 6.26	85.76 ± 10.00

Table S2**.** Temperature and relative humidity of soil and air by block.

Block	***Soil temperature (x̄ ± sd, °C)***	***Soil humidity (x̄ ± sd, %)***	**Air temperature *(x̄ ± sd, °C)***	**Air humidity *(x̄ ± sd, %)***
2	14.18±6.01	25.08±15.44	17.93±6.69	81.49±11.74
3	13.42±6.16	16.85±12.77	17.27±7.05	83.13±12.49
5	13.14±5.28	20.75±10.47	16.88±6.40	79.80±12.64

Table S3. Plant species abundance during summer 2022. Data are expressed on % of surface coverage. Empty species correspond to absence.

	Control					**70%**					**100%**					**130%**				
Species / Block	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Achillea nobilis	23	6	24	33	1		14	15	19	11	9	12	43	15	8	33	2	19	8	1
Arrhenatherum elatius	11	33	15	8	29	8	8	8	8	2	20	29	2	7	1	5	9	5	2	9
Centaurea nigra	2				7	2		5		10	3				24	5		7	1	9
Dactylis glomerata	5	2	2	1	28	7	2	8	2	13	1	7	4		21	5	2	1	2	35
Galium mollugo	30	29		9	27	20	40		5	32	34	6		34	28	25	2	1	30	33
Medicago lupulina	9	11	15	5		27	7	20	20		4	12	14	13	1	5	9	8	10	
Plantago lanceolata	5	4	29	35		11	10	15	25	4	1	4	9	3	1	10	16	25	33	1
Ranunculus bulbosus	1		1			1		2	1			1	1	1		1		2	1	
Cirsium arvense											1					1				
Trifolium repens		5		1	1	9		19						3		10				
Pseudoscleropodium purum	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Trifolium repens	4		10				5		3	2	5	10	2		1		1	19	1	
Convolvulus arvensis			1	1		2		2	1	5	9			3	4				3	1
Geranium dissectum		3	1	1		3	8			8	1	6	14	1	2	1	8	1	1	1
Glechoma herderacea											7									
Poa pratensis	4				3					5	4	5	4		4			4	1	6
Prunella vulgaris						2	3	2	4			1						1	3	
Bellis perennis	1		1	1								1	7					5	1	
Taraxacum officinale	1		1				1	1	1	1		3		1			1		2	
Viola hirta	6		1	1	1			2	1				1					1	1	1
Daucus carota		1		3		3	3		9	1				12			19		1	
Lolium perenne																	1			
Hypochaeris radicata			1					1	1			1						1		
Ophrys apifera													1					1		
Picris hieracioides																		1		
Jacobaea vulgaris								1											1	
Fraxinus excelsior																				1
Quercus robur																				1
Robinia pseudoacacia					1					1					1					
Potentilla reptans		3															27			
Sonchus asper		1																		
Veronica persica										1										

Table S4. Plant species presence during summer 2023. “X” represent presence. Empty spaces correspond to absence.

	Control					**70%**					**100%**					**130%**				
Species / Block	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Achillea nobilis	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Arrhenatherum elatius	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Dactylis glomerata	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Lolium perenne	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Poa pratensis	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Centaurea nigra	X				X	X		X		X	X			X	X	X		X	X	X
Galium mollugo	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X		X
Medicago lupulina	X	X	X				X		X		X	X	X	X	X		X		X	
Plantago lanceolata	X	X	X	X		X		X	X		X	X	X	X	X	X	X	X	X	X
Ranunculus bulbosus	X		X	X	X	X	X	X	X	X		X	X	X		X	X		X	
Cirsium arvense											X									
Trifolium repens	X	X	X	X	X	X	X	X	X		X	X	X	X		X			X	X
Veronica persica		X								X			X			X				
Geranium dissectum	X	X	X	X	X	X		X		X	X	X	X		X	X	X	X		X
Bellis perennis	X		X					X	X			X	X	X				X		
Pseudoscleropodium purum			X	X														X		X
Prunella vulgaris									X									X		
Taraxacum officinale			X	X			X	X	X	X		X	X				X		X	
Viola hirta	X		X	X	X			X	X				X						X	X
Glechoma herderacea	X				X						X									
Daucus carota						X			X	X				X						
Potentilla reptans		X															X			
Lamium maculatum		X															X			
Hypochaeris radicata									X			X								
Crataegus							X	X												
Sonchus asper		X								X										
Ophrys apifera																		X		
Picris hieracioides																				
Prunus sp.																		X		
Cornus sp.																		X		
Leontodon sp.													X					X		
Rosa sp.					X														X	
Convolvulus arvensis						X			X	X	X			X	X				X	X
Hypericum sp.																				X
Robinia pseudoacacia					X					X										

0

Table S5. GLMMs for ant abundance. Models are shown from best (lowest AIC) to worst (higher AIC).

Model	AIC	Condicional R^2	Marginal R^2
Treatment + season + block + invertebrates' Shannon + log (dry biomass) + (1|year)	838.9	0.902	0.88
Treatment + season + block + invertebrates' abundance + invertebrates' Shannon + log (dry biomass) + (1 | year)	840.2	0.903	0.88
Treatment + season + block + invertebrates' Shannon + (1 |Year)	842	0.9	0.881
Season + block + invertebrates' Shannon + log (dry biomass) + (1|Year)	851.1	0.887	0.868

Our models considered the year as a random effect, hence the parentheses. Marginal R^2 describe the proportion of variance explained by the fixed factors alone. Conditional R^2 express the proportion of variance explained by both fixed and random effects.

Table S6. Zero-inflated models for ant richness. Models are shown from best (lowest AIC) to worst (higher AIC). In our zero-inflated models, the variable season was considered the source of zeroes

Model	AIC	R^2	R^2 adj.
Invertebrates' richness + log (dry biomass) | Season	461.42	0.642	0.636
Treatment + Invertebrates' richness + log (dry biomass) | Season	466.28	0.647	0.635
Treatment + Invertebrates' richness + log (dry biomass) | Season	466.28	0.647	0.635
Block + Invertebrates' richness + log (dry biomass) | Season	466.90	0.651	0.636
Treatment + Plant group + Invertebrates' richness + log (dry biomass) Season	471.84	0.657	0.636

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Table S7. Pairwise multilevel comparison (post-hoc of a PERMANOVA test) between communities of ants by season.

Pairs	Df	F. model	R^2	p value	p value adj.
Summer/autumn	1	17.06	0.179	0.001	0.006
Summer/winter	1	48.72	0.332	0.001	0.006
Summer/spring	1	2.47	0.031	0.074	0.444
Autumn/winter	1	9.86	0.091	0.001	0.006
Autumn/spring	1	15.37	0.165	0.001	0.006
Winter/ spring	1	32.45	0.249	0.001	0.006

Table S8. Mean and sd of hydraulic conductivity (m/s) by treatment.

Treatment	n	K ($\bar{x}$)	K (sd)
Control	15	7.40e-05	1.56e-04
70	15	3.21e-05	2.67e-05
100	15	2.76e-05	1.596e-05
130	15	1.52e-05	1.94e-05

General discussion

Ants play a crucial role in ecosystems as one of the most significant groups of invertebrates worldwide. Extensive research has been conducted to understand how ants respond to climate change. However, identifying the specific factors that determine their resistance is challenging because of the diverse approaches used and the ecological levels at which they are evaluated. This challenge is further exacerbated for species living in hot and dry environments, as these climates will change to other climates not yet present in the current biomes.(Jenkins et al., 2011). Therefore, this PhD project aimed to study the effects of climate change on ants using an integrative approach where different aspects of ants' biology were studied.

In Chapter 1, I explored the resistance to high temperatures as well, using the colony as the unit of focus and evaluating different environments. Focusing on the colony level is relevant as social insects are often considered to function as superorganisms (Hölldobler & Wilson, 2009). I found that the resistance of ant species was consistent with the environmental temperature. Specifically, it was highest in the semi-arid habitat, intermediate in the two Mediterranean habitats, and lowest in the temperate habitat. Despite this, the breadth of the resistance (i.e. the difference between the most and least resistant species within each habitat) was not different among environments. Also, the heat resistance and its relationship with morphological traits seemed species-dependant. The length of the legs was adequate to account for the resistance of the thermophilic genus *Cataglyphis*, while other genera were explicable through other, potentially more intricate combinations of traits. Nevertheless, the former exhibited greater resistance than anticipated based solely on morphological traits. This can be attributed to additional factors influencing their resistance, such as the synthesis of heat-shock proteins and behavioural patterns.

In Chapter 2, I explored the relationship between morphological traits and temperature resistance of ants at the individual level. The preliminary results indicated a strong correlation among all morphological traits, especially the length of the hind leg, clypeus, the distance between the eyes, and the eye position. Species with higher temperature resistance also tend to be larger, confirming the findings in Chapter 1. However, ants of the species *Cataglyphis cursor*, considered thermophilic, do not appear to show any distinct morphological relationship compared to other species.

The aim of Chapter 3 was to evaluate how temperature on the soil surface would affect the construction of the nest and development of the colony. I found that the burrowing activity increased with high surface temperatures, that is ants dug deeper, presumably to provide adequate temperature and humidity for the development of the brood. Additionally, ants exposed to the highest temperature stopped rejecting the excavated soil outside the nest and stored it in previously excavated top chambers, i.e. refilled them. This confirms the plasticity of the nest architecture, in responses to soil temperature and over time.

Finally, in Chapter 4, I explored the effects of modified temperature and precipitation on the abundance, richness, and community composition of ants in a temperate environment. I found that the abundance of ants increased with temperature and that they seemed to stay active for a longer time when temperatures were higher. Overall, it seems that modifying the abiotic conditions affects ants indirectly by changing vegetation and other invertebrates' abundance.

The approach to evaluate thermal resistance

Many of the studies trying to evaluate how climate change affects organisms, focus on measuring their thermal tolerance, which has been described as the capacity of a cell or organism to resist heat stress that would typically be fatal (Norris & Hightower, 2000).

Thermal resistance on ectotherms is often estimated by directly measuring CT_{max}. Indeed, it is considered that species with high heat resistance have higher CT_{max}. Nevertheless, from a methodological standpoint, the accuracy of CT_{max} measurements is highly dependent upon the specific conditions under which it is determined. Various methodological factors contribute to the variability in measurements, including the choice between static (constant temperature) and dynamic (ramping temperature) methods, the heating rate applied (dynamic method), the time of day during experimentation, the duration of the assay (static method), and even the ecological relevance of the experiments

(Terblanche et al., 2007, 2011; Rezende et al., 2014). To address some of these methodological discrepancies, a mathematical model has been developed (Jørgensen et al., 2021). This model aims to standardise the different methods and facilitate meaningful comparisons. Unlike previous approaches that focused solely on duration or temperature, the proposed method suggests calculating and comparing damage as a function of both temperature and exposure duration. However, it is important to note that relying only on CT_{max} as a measure (even a standardised one) of thermal resistance, may introduce potential biases, as other influential factors remain unanalysed.

Analysing solely CT_{max} of a species (or any other metrics) to estimate thermal resistance, may lead to inaccuracies, as the temperature changes can influence a broad spectrum of factors affecting their fitness (Penick et al., 2017). Other studies have approached the assessment of ectotherm thermal tolerance by analysing CT_{max} jointly with heat-shock proteins (Gehring & Wehner, 1995; Lighton & Turner, 2004), colony survival and growth (Penick et al., 2017), dominance-subordinance relationships (Garcia-Robledo et al., 2018), phenology (Roy et al., 2015; Forrest, 2016), behaviour (Youngsteadt et al., 2023). The results of these studies hint at how complex the study of thermal resistance is. Equally intricate are the effects of climate change (specifically temperature augmentation and shifts in precipitation) on ant biology. By assessing the effects of climate change through the functional traits, the bioturbation activity and the community composition, this thesis confirmed this statement. Fig. 1 summarises the relationships found during the development of this work.

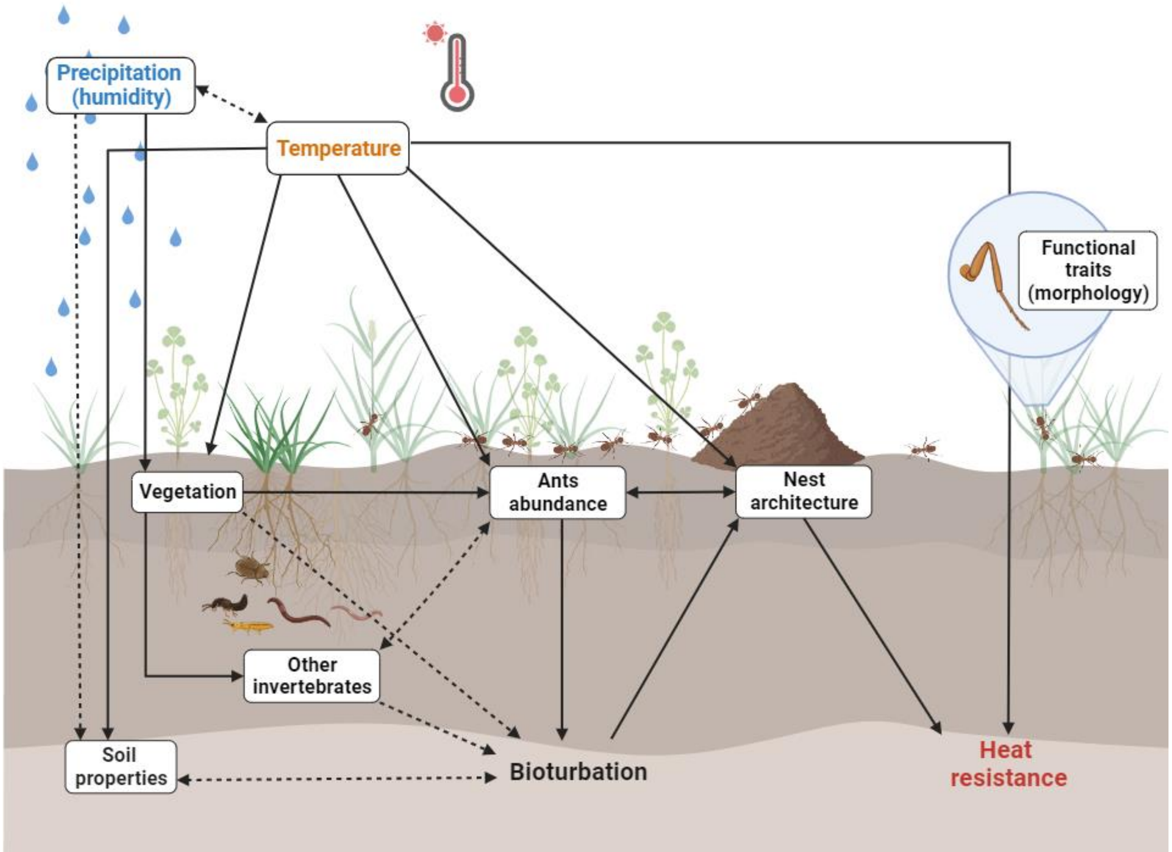

Fig. 1. Summary of the effects of temperature and precipitation on ants' resistance to heat and bioturbation activity. Solid arrows denote effects evaluated in this project; dashed arrows correspond to relationships that we could notice but did not study directly.

Direct effects of temperature on ant biology

Ant development and abundance

Higher temperature exerts a direct impact on ant abundance by accelerating their development (Porter & Tschinkel, 1993) and by enlarging their season of annual activity. We observed an increase in ant abundance at higher temperatures at both the nest level (Chapter 3) and the community level (Chapter 4). While I did not directly monitor brood development, my results likely result, at least in part, from the direct effects of temperature on individual ants. Moreover, increased temperatures can alter the duration of their active period throughout the year. This modification may involve an earlier onset of activity or a prolonged activity period later in the year (Hölldobler & Wilson, 1990), a phenomenon I documented in my investigation of a temperate ant community, and this yields a higher annual growth of ant colonies hence a higher abundance of ants.

Nest architecture

As discussed more in-depth in Chapter 2, the temperature also influences the bioturbation activity of ants. They dynamically rearrange the architecture of their nest in order to provide the adequate temperature for the development of their brood (Penick & Tschinkel, 2008), and this bioturbation activity may affect local soil properties (e.g., transport of soil particles on the soil surface, increased soil permeability to water) which may affect other organisms (e.g. promote growth of bacteria, fungi, plant roots) (Dauber et al., 2008)

The architecture of the nest has been considered plastic for a long time (Jones et al., 1994). Moreover, ants, as well as termites have been described as "extended phenotype engineers" (Jouquet et al., 2006), meaning organisms that construct structures or effects directly impacting the fitness of individuals, or in the case of social insects, entire colonies (Dawkins, 1982). Through the construction of biogenic structures, "extended ecosystem engineers" respond to their own requirements. In the case of ants and termites, the construction of nests and mounds aims to keep the temperature and humidity for the development of the colony and to protect themselves from predators (Turner, 2000). This concept is opposed to the so-known "accidental engineers" whose structures have no direct positive effect on themselves (Jones et al., 1994). Some geophagous earthworms, for instance, move through the soil to find optimal conditions of temperature and humidity and forage for food. The structures they create can affect soil properties such as infiltration and compaction, but the earthworms do not live in such structures. Thus, the casts produced are more a by-product of the earthworm activity.

An example of ants as "extended phenotype engineers" is found in leaf-cutter *Atta* ants which excavate massive nests, and mobilize tons of soil (Nascimento et al., 2024). These ants not only adjust the volume of the nest to accommodate the number of workers but also build specialized chambers for their fungal gardens. These chambers are enlarged as the fungus grows until they reach their maximum size, at which point the fungus is transferred to new chambers (Römer & Roces, 2014). The disposal of waste varies by species, with some ants discarding it on the surface and others storing it in specialized chambers. In species where the fungus waste is disposed of outside the nest, the chambers either remain empty or are replenished with soil (Schaefer et al., 2021). In species where the waste remains within the nest, it is isolated from the fungus garden to prevent the proliferation of pathogens (Farias et al., 2020).

This thesis supports the concept of ants as "extended phenotype engineers” and the one of ant nest as extended phenotype. However, the construction of the nest has not been traditionally considered a functional trait. Yet, the results presented here underscore its functional character. This study reveals that nest architecture is a trait measurable at the super-organismal level (i.e., considering the individual as the colony), directly impacting its performance. Although the effects of nest architecture were specifically tested on abundance and survival, the relationship between them constitutes a feedback loop. The architecture of the nest facilitates colony development, and more populous colonies, in turn, construct larger nests (Buhl et al., 2005; Toffin et al., 2009).

As thoroughly discussed in Chapter 3, in ants, the construction of the nest, has direct positive effects on the colony. This includes facilitating access to optimal temperatures for brood development (Penick & Tschinkel, 2008), maintaining stable temperatures within the colony (Bollazzi & Roces, 2010), and even enhancing solar radiation when necessary (Blomqvist et al., 2000; MacMahon et al., 2000). The experiment presented in Chapter 3 demonstrates that ants modify the architecture of their nests in response to the need to cope with increased surface temperatures. Furthermore, the colonies appeared to exhibit a positive response, as they were more populous when exposed to higher temperatures. Some authors have suggested that not only the size but also the complexity of nests is modified with temperature (Sankovitz & Purcell, 2021). Our results did not show the same tendency. However, we were constrained to use small colonies. Larger colonies would have required larger, hence heavier, earth columns and thus complex logistics to transport and manipulate them in the CT scan. It is plausible that larger colonies would have produced more complex nest architecture.

Ant morphology

Temperature is also an important selective pressure for ant morphology. Specifically, higher temperatures due to climate change seem to affect communities by either reducing the occurrence of larger species or selecting against small or large body size (Gibb et al., 2018). This association is tied to the functional aspects of morphological traits, emphasizing the close connection between temperature and functional traits.

In this thesis, I affirmed that certain morphological functional traits enhance resistance to high temperatures (Sommer & Wehner, 2012). Legs, notably hind legs, likely play a pivotal role as a morphological functional trait in assessing resistance in ants. They enable ants to run faster and keep vital organs away from the hot soil surface (Sarty et al., 2006; Gibb & Parr, 2013). Chapter 1 proposed that hind leg length is a reliable measure when evaluating resistance in thermophilic ants (i.e., *Cataglyphis*). However, when studying non-thermophilic ants, other morphological traits should also be considered, serving as proxies (though not necessarily functional traits), for their heat resistance. Furthermore, these morphological traits may serve as valuable indicators to assess the potential responses of species to rising temperatures, especially in cases where selection pressure has not influenced hind leg length or where other adaptations assist in coping with increasing temperatures.

Ant morphology is linked to their bioturbation activity. However, since it is also associated with abiotic conditions such as soil grain size and moisture, this relationship will be further discussed in the next section.

Indirect effects of temperature on ant biology

In addition to the direct effects discussed above, temperature affects ants indirectly in numerous ways. First, it is important to consider that temperature is not an isolated climatic property, but closely related to, for example, precipitation. Overall, increased temperatures due to climate change cause an intensification of precipitation extremes, although it variates according to the region of the world (O'Gorman, 2015). Because temperatures are so closely related to precipitation, some of the consequences of the former in organisms are partially confounded with the effects of the latter.

Interactions bioturbation-soil properties

Temperature and humidity also influence soil properties. Expectedly, I found that modifying the precipitation and temperature of the air (Chapter 4) also augmented temperature and moisture of soils. It has been suggested that microclimatic conditions are more important than macroclimatic ones when it comes to soil organisms (Andrew et al., 2013; Duffy et al., 2015; Lembrechts et al., 2020). Soil moisture is particularly important for ants inhabiting them. Excessive humidity or dryness may be lethal to brood (on a species specific and brood stage specific basis), and moisture also conditions the burrowing activity along with grain size, density, and mandible size (Espinoza & Santamarina, 2010), thereby influencing ant bioturbation activities.

The bioturbation activity of ants depends on the size (of the colony and the individuals). Larger ants with highly populous colonies generally excavate substantial amounts of soil, contributing importantly to soil bioturbation. Examples include *Camponotus punctulatus* (Folgarait, 1998), *Atta sexdens* (as mentioned in Gonçalves, 1942) and *Formica exsectoides* (Phillips, 1999). In the Chapter 3 of this thesis, it was demonstrated that more populous colonies were associated with increased porosity and enhanced turnover of the soil layer, a phenomenon previously observed in other ant species such as *Pogonomyrmex badius* (Tschinkel, 2015a).

From a morphological standpoint, larger ants build larger nests out of necessity for more space, made possible by their larger mandibles, which allow them to handle larger grain sizes (Hooper-Bùi et al., 2002; Espinoza et al., 2010). Consequently, the growth and size of ant colonies can be influenced by the type and accessibility of materials used for nest construction (Hansell, 1987). For instance, the density of small-sized ants has been linked to the presence of very fine sand grains (Costa et al., 2010; Costa-Milanez et al., 2014), while larger ants have been associated to coarser soils (Cardoso et al., 2021). Soil granulometry is associated to water retention, with sandy soils retaining less water compared to loamy or clay soils that have higher water retention capacity (Bruand & Tessier, 2000). Some studies suggest that the presence of a certain size of ants in a particular soil is also related to their susceptibility to desiccation (Johnson, 2000). Larger ants with higher desiccation resistance tend to be found in xeric environments with limited water accessibility, while smaller-bodied species, less resistant to desiccation, tend to thrive in mesic environments with moister soils (Feener & Linghtom, 1991; Kühsel et al., 2017; Whyte et al., 2023).

Desiccation resistance is multifactorial. It is determined by body size but also by the cuticular hydrocarbon composition. Indeed, cuticular hydrocarbons are molecules present on the surface of the cuticle of arthropods and their primary function is to prevent water loss. It has been reported that ants with low resistance to desiccation present shorter-chained hydrocarbons compared to those in ants that are highly resistant to desiccation. Short hydrocarbons are related to high cuticular permeability, and thus, low efficacity in preventing desiccation (Blomquist & Ginzel, 2021). Although the composition of ant cuticle was not analysed in this thesis it is considered a physiological trait related to temperature resistance as well.

The ability of ants to nest in one type of environment or another also depends on their plasticity. For instance, *Cataglyphis piliscapa* (formerly *C. cursor*) nests in both the seaside and vineyards in a Mediterranean environment with distinct soils and moisture conditions (Lenoir et al., 1988). Nest structure remains similar in both sites (i.e., a single vertical entrance leading to a gallery), but nests are shallower in the seaside due to restrictions imposed by the subjacent water table and colonies are less populous than in the vineyards (Clémencet & Doums, 2007). The seaside environment may provide less resources for colony growth, but it is also possible that the shallower nests limit colony size.

Bioturbation by ants, mediated by soil and air temperatures, can modify soil properties such as compaction, bulk density and water infiltration as described for other soil organisms like termites, beetles, millipedes and earthworms (Bottinelli et al., 2015). As mentioned before, in this thesis, I showed the increased porosity in soils exposed to high temperatures, however, when analysing the bioturbation at a bigger scale, the effects were not the same (Chapter 4). The abundance of ants was affected by the increased temperatures, but the hydraulic conductivity was not. These results suggest that ants, at least the species we used for this experiment (*Lasius niger*) have a localised positive effect on porosity and infiltration. However, these effects are likely negligible at larger scale.

Interactions soil-plant-ant

In the Chapter 4 of this thesis, it was shown that varied conditions of increased temperature, humidity and soil moisture increased plant biomass. Previous studies have noted that the productivity of certain plants tends to rise with higher temperatures (Sykes, 2009), and processes like leafing, flowering, and fruiting come earlier under warmer conditions. Although this study did not follow plant phenology itself, any change in vegetation can arguably have indirect effects on ants and other organisms' development as a result of increased vegetation biomass or a decoupling of the trophic food web (Visser & Both, 2005).

Furthermore, the excavation of ants indirectly benefits plants through the increment of microbial communities, and the release of mineral nutrients such as ammonium and nitrate (Czerwinski et al., 1971), along with the organic matter (Folgarait, 1998). These alterations in soil properties can boost fertility and induce changes in plant morphology and performance (Woodell & King, 1991). Additionally, such shifts can initiate cascading effects throughout the trophic hierarchy. Changes in plant communities, for example, may lead to variations in the abundance and diversity of herbivores, influencing the prey base for ant species that depend on them. Moreover, alterations in vegetation can impact the foraging behaviour and nesting sites of ants, thereby influencing their interactions with other trophic levels. These modifications in trophic food webs hold implications for ecosystem stability, nutrient cycling, and the overall biodiversity of an area. Understanding the intricate relationships between shifts in vegetation and the responses of ant communities is crucial for comprehending broader ecological patterns and for devising effective conservation and management strategies.

A comparative approach

It is now clear that assessing thermal resistance in ants is a complex task, even more so when we try to discern patters across environments. The conventional approach is to use CT_{max} as proxy of thermal resistance, and it has been documented that ants from warm habitats have a higher CT_{max} than ants from cool habitats (Fig. 2A, Chapter 1). While I could confirm this statement (i.e., the highest CT_{max} were found in the semi-arid environment and the lowest ones in the temperate environment), my results showed that the heat resistance is much more complex than what CT_{max} can explain. Thus, the results obtained at different levels of organisation allow me to propose a comparative approach including other aspects of ant biology as crucial factors in their resistance. This approach, illustrated on Figure 2B comprises three major components: i) CT_{max} and the morphological adaptations associated to it (e.g., length of the hind leg, total body length); ii) bioturbation activity; and iii) the habitat's resilience to temperature changes. I am conscious that other aspects of ant biology such as behaviour, acclimation, dispersal capacity, and phenotypic plasticity, play important roles in ant resistance, but they are not included in the approach as they were not evaluated during this project.

In my approach, I consider that heat resistance increases with the habitat temperature. I represent CTmax, and the associated morphology (Fig. 2B, red arrow) as a trait that can increase unlimitedly, however, any of those traits can behave in this way.

In this thesis, I experimentally assessed the bioturbation and nest architecture of one species of temperate environment (*Lasius niger*). Nonetheless, these results could be generalised to other species of soil-dwelling ants in temperate environments (Fig. 2B, green arrows). While I could not assess the effects of increasing temperatures on bioturbation in species from warmer environments, we may argue that ant bioturbation is relatively more important in warmer and drier environments than in temperate ones.

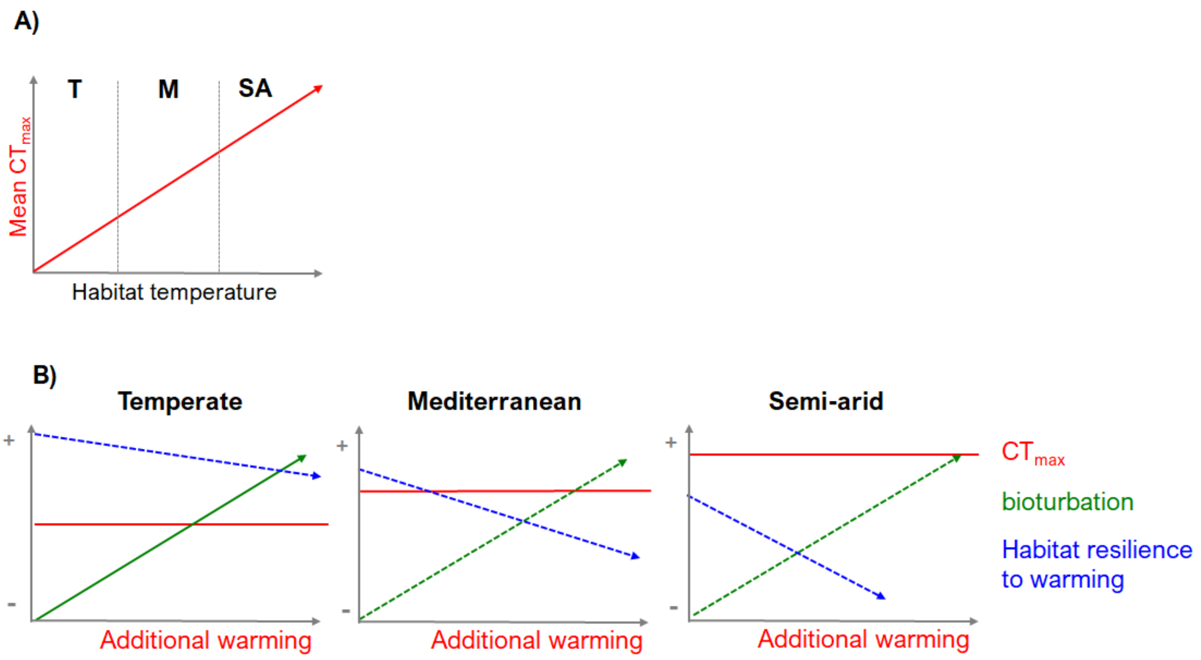

Fig. 2. Theoretical approaches to the study of thermal resistance. Solid arrows show relationships that we measured directly, and dashed arrows denote interactions that we did not measure but can infer.
A corresponds to a simple approach where resistance is measured directly by CT_{max}. Mean CT_{max} of ants increases with temperature regardless of the habitat. Ants inhabiting temperate environments have a lower mean CT_{max} than those in Mediterranean environments. The latter are, in turn, less resistant than those in semi-arid environment.
B corresponds to the approach proposed in this thesis. It shows the short-term effects of warming on the three environments studied. Mean CT_{max} remains somewhat constant but depends on the environmental temperature. Thus, heat resistance is a function of bioturbation and habitat resilience. Bioturbation increases when temperature does regardless of the environment. The resilience to increasing temperatures is likely inverse to the temperature in habitats, because temperate environments offer more ecological niches, while conditions in semi-arid environments are already challenging for most organisms.
Note that these curves and relationships do not correspond to any real data but aim to illustrate trends observed through this and other studies.

Ants, along with termites, seem to replace earthworms as ecosystem engineers (de Bruyn & Conacher, 1990; Jones et al., 1994; Lavelle et al., 1997) in dry warm environments. The highest earthworm biomass is observed in temperate regions such as the Eurasian steppes (Phillips et al., 2019). Consequently, reports indicate that in cold-temperate ecosystems, earthworms play a more substantial role in bioturbation compared to ants (Tschinkel, 2015b; Taylor et al., 2019). However, in dry environments the role of ants in bioturbation surpasses that of earthworms, when the latter are present. For instance, the fungus-growing ant *Trachmyrmex septentionalis* in Florida, deposits 0.5–1.5 t ha^{-1} yr^{-1} on the ground surface

(Tschinkel, 2015b). Funnel ants *Aphaenogaster longiceps* near Sydney, excavate 8.41 Mg ha^{-1} y^{-1} (Humphreys, 1981). Furthermore, *Atta* leaf-cutting ants are well-known for creating big and long-lasting nests in neotropical America. For instance, *A. vollenweideri* nests have been reported to reach depths up to 8m (Gallardo, 1916), with a single colony moving around three tons of soil per year (Farji-Brener, 1992). *Atta* ants significant impact soil fertility, turnover, bulk density, and infiltration (for a full review see Nascimento et al., 2024). These findings lead me to believe that with rising temperatures, ant bioturbation will increase out of the necessity of ants to resist temperatures or due to migration of species that have already an important bioturbation activity.

In the search of how ants would react to the upcoming augmentation in temperature, I noticed that the resources available for ants to adapt to new abiotic conditions are not the same. Ants are exposed to different temperature ranges according to the environment they live in, hence they are under different selective pressures which have determined how resistant they are. Under this premise, the climatic variability hypothesis (Stevens, 1989), states that species in more variable climates should have broader thermal tolerance, which augments the thermal complementarity and thus makes these communities more resilient to temperature changes. In contrast, environments with less variable seasonal temperatures present narrower breadths, which may translate into more susceptible communities. This hypothesis has been supported by numerous studies on endotherms and ectotherms (Addo-Bediako et al., 2000; Khaliq et al., 2014; Arnan et al., 2015). However, this thesis did not test this as we did not measure the temperature niche breath but only its upper limit (CT_{max}) (Chapter 2). Testing this hypothesis would have required determining the CT_{max} and CT_{min} of all ant species in every sampled site.

As climate change accelerates, the niches available for ants in various environments are undergoing profound shifts, influencing the distribution and dynamics of ant communities. In warmer climates, such as tropical regions, rising temperatures may favour heat-tolerant ant species as competitors will likely be more severely impacted by warming, i.e. warming may alter the composition of communities and potentially favour thermophilic species (Wehner & Wehner, 2011). However, these regions also face increased challenges due to more frequent and intense extreme weather events, impacting ant foraging, nesting, and overall community structures (Diamond et al., 2012; Perez & Aron, 2020). The intricate balance of interactions among ant species, plants, and other organisms may be disrupted, leading to cascading effects on ecosystem functioning.

Conversely, in colder environments, such as subarctic and Arctic regions, where ants are not as abundant (Addo-Bediako et al., 2000), climate change may provide new opportunities for colonization. As temperatures rise, previously inhospitable habitats become more suitable for certain ant species, potentially leading to the expansion of their geographic range and the establishment of new ant communities (Parr & Bishop, 2022). This phenomenon can introduce novel ecological dynamics and competition with native species, affecting the delicate equilibrium of these cold-adapted ecosystems. Moreover, alterations in precipitation patterns, snow cover duration, and vegetation composition further contribute to the restructuring of ant niches in these polar and subpolar environments (Niittynen et al., 2020).

In temperate zones, which experience more moderate temperature variations, the impacts of climate change on ant niches are nuanced (Diamond et al., 2012). Shifts in precipitation patterns, the frequency of extreme weather events, and altered seasonal cycles can influence the availability of resources and affect ant foraging strategies (Parr & Bishop, 2022). As ecosystems respond to changing conditions, ant species with greater adaptability and flexibility in resource utilization may have a competitive advantage, potentially leading to shifts in community structure (Sunday et al., 2012).

Overall, the response of ant communities to climate change is complex and multifaceted, highlighting the need for comprehensive ecological studies to understand the intricate interactions within these crucial insect societies in the face of global environmental shifts. This doctoral thesis contributes to the already complex and highly circumstance-dependent knowledge of ants' thermal biology by suggesting providing more data for the already existing studies and by suggesting a new method for the assessment of ant bioturbation.

Possible perspectives derived from this work include the study of the modification of the nest in other soil-dwelling species in different environments using X-ray tomography images. Also, disentangling the relationships between ants, plants, and other invertebrates to understand how the whole community would react to increasing temperatures and modified precipitations. Finally, extending the proposed approach to other types of biomes. Evaluating bioturbation and morphology of ants inhabiting colder and arid environments would be ideal, but exploring communities in warm and humid environments would likely allow us to find new trends that we have not considered so far.

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Autres activités

Encadrement

2021 – 2023 Co-encadrement avec Thibaud Monnin et Pascal Jouquet de 3 stages de licence, 2 stages de Master 1 et 1 stage de M2

Congrès

2023 Présentation orale intitulée « Effect of surface temperature on *Lasius niger* nest architecture and excavation activity ». Scientific days of the Institute of Ecology and Environmental Sciences of Paris, France.9-10 Février.

2023 Présentation orale intitulée « Effet de la température de surface sur l'architecture du nid de *Lasius niger* et l'activité d'excavation ». Colloque de la Section Française de l'Union Internationale pour l'Etude des Insectes Sociaux. Toulouse, France. 28-30 août.

Vulgarisation

2021 Présentation orale KiFéKoa intitulé "Elles courent, elles courent les fourmis" à l'IRD Délégation Île-de-France. 5 mai

2021 Organisation du stand « Le monde des fourmis » lors de la fête de la science. Sorbonne Université, Paris. octobre

2022 Co-animation de la visite de collégiens du dispositif ACTE à l'IRD Délégation Île-de-France- 18 février.

2023 Participation dans la Journée Internationale des droits des femmes à l'IRD Délégation Île-de-France. 17 mars.

2023 Entretien pour la publication de l'article « Les indics du climat » par Amanda Schrepf. Science et Vie, Hors Série Entomologie 308, juillet 2023.

Résumé

Le changement climatique est l'un des phénomènes les plus pertinents pour les écosystèmes aujourd'hui. Bien que ce soit un phénomène complexe qui affecte pratiquement tous les aspects du climat, l'augmentation de la température se distingue comme l'un des aspects les plus importants en raison de son impact direct sur tous les niveaux d'organisation des organismes. Bien que pratiquement toutes les formes de vie subissent déjà les conséquences de cette augmentation de la température, les invertébrés, en particulier les insectes tels que les fourmis, sont particulièrement susceptibles. Étant donné qu'ils ne peuvent pas réguler directement leur température corporelle, ils dépendent de la température ambiante pour réguler leur métabolisme.

Les fourmis, l'un des groupes les plus importants d'insectes, sont présentes dans presque tous les environnements terrestres, occupant diverses niches écologiques et jouant un rôle fondamental dans la génération de services écosystémiques. Comprendre comment l'augmentation des températures mondiales affecte les populations de fourmis est crucial pour comprendre les stratégies qu'elles utilisent pour faire face au changement climatique.

L'une des activités les plus significatives des fourmis est la bioturbation, qui implique la mobilisation des particules du sol et la modification de ses propriétés. Cette activité confère aux fourmis le rôle d'ingénieures de l'écosystème, car leurs actions modifient, maintiennent et même génèrent des habitats tant pour elles-mêmes que pour d'autres organismes.

Bien que l'approche classique pour analyser la résistance à la température chez les fourmis se base sur la température critique maximale (CT_{max}), cette méthode se concentre uniquement sur les adaptations physiologiques sans tenir compte d'autres facteurs importants dans la détermination de la résistance. Cette thèse avait par objectif analyser la réponse des fourmis à l'augmentation de la température dans différents environnements, en observant des aspects cruciaux de leur biologie à plusieurs niveaux d'organisation.

Au niveau de l'organisme, j'ai exploré la relation entre la CT_{max} et certains traits morphologiques fonctionnels dans des populations de divers environnements. J'ai étudié si le polymorphisme conférait un avantage en termes de résistance à la température. À l'échelle de la colonie, je me suis intéressé aux effets de différentes températures en surface et leurs

impacts sur l'architecture des nids souterrains et le développement de la colonie dans une espèce d'environnement tempéré. Enfin, au niveau de la communauté, j'ai étudié la manière dont différentes conditions abiotiques, y compris l'humidité, modifient l'abondance, la richesse et la structure d'une communauté de fourmis dans un environnement tempéré, et comment cela se répercute sur leur activité de bioturbation.

Cette approche intégrale a permis de développer un modèle d'analyse de la résistance à la température qui, autant que je le sache, n'a pas été proposé auparavant. Dans ce modèle, il est proposé que la résistance à la température, évaluée par la CT_{max} et/ou des traits morphologiques en tant qu'indicateurs, dépend de la capacité bioturbatrice et de la résilience de l'habitat. Selon ce modèle, les fourmis pourront maintenir leur résistance actuelle en augmentant leur activité bioturbatrice, en adaptant les nids aux températures lorsque l'environnement le permet. Cependant, cette capacité est limitée par la résilience du milieu, car on s'attend à ce que les environnements tempérés offrent davantage de niches potentielles pour les fourmis, tandis que dans les environnements xériques, les niches pourraient être encore plus rares qu'actuellement.

Abstract

Climate change is one of the most relevant phenomena for biodiversity today. Although it is a complex phenomenon that affects practically all aspects of the climate, the increase in temperature stands out as one of the most important aspects due to its direct impact on all levels of organism organization. Despite virtually all forms of life already experiencing the consequences of this temperature increase, invertebrates, especially insects such as ants, are particularly susceptible. Since they cannot directly regulate their body temperature, they depend on ambient temperature to regulate their metabolism.

Ants, being one of the most important groups of insects, are present in almost all terrestrial environments, occupying various ecological niches and playing a fundamental role in generating ecosystem services. Understanding how the increase in global temperatures affects ant populations is crucial to comprehend the strategies they use to cope with climate change.

One of the most significant activities of ants is bioturbation, involving the mobilization of soil particles and the modification of its properties. This activity gives ants the role of ecosystem engineers, as their actions modify, maintain, and even generate habitats for themselves and other organisms.

Although the classical approach to analysing temperature resistance in ants relies on the analysis of the Critical Thermal Maximum (CT_{max}), this method focuses solely on physiological adaptations without considering other important factors in determining resistance. This thesis aimed to analyse the response of ants to temperature increase in different environments, observing crucial aspects of their biology at various levels of organization.

At the organism level, I explored the relationship between CT_{max} and some morphological functional traits in populations from diverse environments. I investigated whether polymorphism conferred any advantage in terms of temperature resistance. At the colony scale, I examined the effects of different temperatures on the surface and their impacts on the architecture of underground nests and colony development in a species from a temperate environment. Finally, at the community level, I focused on how abiotic conditions,

including humidity, modify the abundance, richness, and structure of an ant community in a temperate environment, and how this affects their bioturbation activity.

This comprehensive approach allowed for the development of a model for analysing temperature resistance that, to the best of my knowledge, has not been proposed before. In this model, I suggest that temperature resistance, assessed by CTmax and/or morphological traits as indicators, depends on bioturbation capacity of ants and habitat resilience. According to this model, ants may maintain their current resistance by increasing their bioturbation activity, adapting nests to temperatures when the environment allows it. However, this capacity is limited by habitat resilience, as temperate environments are expected to offer more potential niches for ants, while in xeric environments, niches could be even scarcer than they are currently under increased temperatures.