Population and community responses of soil organisms after heat extremes

Inaugural dissertation

of the Faculty of Science,

University of Bern

presented by

Gerard Martínez De León

from Tortosa (Spain)

Supervisors of the doctoral thesis:

Prof. Dr. Madhav P. Thakur

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CONTENTS

SUMMARY

In the context of contemporary climate change, heat extremes are becoming more frequent and severe. Heat extremes can cause immediate ecological impacts, known as resistance, but populations and communities can recover beyond the extreme event, when thermal conditions return to normal. However, the underlying mechanisms behind various types of recovery after heat extremes remain largely unexplored, and importantly, loosely linked to mechanisms conferring resistance. This thesis examines the significance of various factors influencing ecological resistance and recovery, mainly with the use of experimental approaches. We tested these ideas using populations and communities of soil-dwelling Collembola, one of the most abundant and functionally important groups of invertebrates in terrestrial ecosystems. In the first chapter, we assessed how thermal effects on life-history traits affected population resistance and recovery after an extreme heat event, using populations of four Collembola species in monocultures. In the second chapter, we explored how various levels of population density at the onset of extreme heat events could determine their subsequent population responses. In the third chapter, we examined how natural soil communities from two distinct elevations respond to extreme heat events occurring at different seasons. In the fourth chapter, we synthesized the main mechanisms buffering the immediate biological impacts of heat extremes as well as their associated costs, and proposed a temporally-explicit conceptual framework describing the links between short- and long-term population or community responses to heat extremes.

Our results demonstrate that various kinds of relationships between resistance and recovery to heat extremes can be explained by the thermal sensitivity of vital rates, which varies among species and across different spatiotemporal contexts. In the first and second chapters, we show that higher thermal sensitivity of fecundity compared to survival can cause negative recovery despite negligible effects on resistance, especially in growing populations of cold-adapted species. Then, in the third chapter, we show that lowland communities are more vulnerable to heat extremes than high elevation communities, mainly in spring and summer seasons. While Collembola responded strongly in these contexts, we found that fungi remained generally stable to heat extremes, with notable exceptions in the case of fungal pathogens and

saprotrophs (increased and reduced abundances, respectively). In addition, our findings from the second chapter suggest that density-dependent recovery processes are negligible in small populations. Yet, in populations closer to carrying capacity, compensatory effects seem to facilitate recovery after heat extremes, as revealed from the responses of the lowland natural communities of the third chapter. This finding suggests that recruitment could counteract previous heat-induced mortality, owing to a relaxation of competitive pressure in shrunk populations. Finally, we propose that several mechanisms that immediately dampen the biological impacts of heat extremes, such as behavioral thermoregulation or the production of heat shock proteins, can have lagged costs known as 'ecological debts', that constrain the recovery of populations and communities in the long term. These ecological debts could accumulate as heat extremes become more severe and frequent, emphasizing the importance of the linkages between short- and long-term ecological responses to heat extremes. In conclusion, we show that recovery processes are fundamental to fully capture the whole range of ecological responses, including lagged effects. We demonstrate that ecological responses to heat extremes are strongly influenced by thermal effects on vital rates, as well as by various spatiotemporal contexts. These findings can contribute to enhance our mechanistic understanding of ecological responses to climate change, and accordingly devise policies and management strategies to halt climate-driven biodiversity declines.

GENERAL INTRODUCTION

Climate extremes are the new norm in the Anthropocene

Human activities can leave a large footprint on the environment, which has grown vastly since the start of the Anthropocene in the late eighteenth century (Crutzen 2002; Sage 2020; Steffen *et al.* 2015). The ever-growing exploitation and consumption of resources has rapidly altered the environmental conditions under which many species have evolved and adapted (Pimm *et al.* 2014), but also many others have perished (McKinney & Lockwood 1999). The various anthropogenic drivers of alteration, embraced within the umbrella concept of "global change", include completely novel kinds of disturbances (e.g., pollution by synthetic pesticides) and disturbance regimes (e.g., altered precipitation) (Turner & Seidl 2023), posing unprecedented threats to biodiversity (Sage 2020).

One of the main global change drivers contributing to the current biodiversity decline is climate change, caused by the accumulation of greenhouse gases in the atmosphere. The higher concentrations of greenhouse gases increase the Earth's net radiative balance, leading to the gradual rise in global average temperatures seen in the past decades (IPCC 2023). In this context of warming, climate extremes, such as heat extremes and droughts, are becoming more frequent and intense (IPCC 2023; Ummenhofer & Meehl 2017). Climate extremes can generate conditions that exceed the physiological limits of many organisms (Smith 2011), driving abundance declines and species extinctions (Smith *et al.* 2023; Thakur *et al.* 2022). Yet, compared to the well-known biological effects of gradual climate warming (i.e., phenological shifts, changes in distribution ranges, and shrinking body sizes; Harvey *et al.* 2022; Parmesan 2006; Sheridan & Bickford 2011), our current understanding of the biological impacts of climate extremes lags considerably behind (Harvey *et al.* 2020; Jentsch *et al.* 2007). This is a surprising fact considering the known potential of climate extremes to trigger large-scale ecological impacts (Harris *et al.* 2018). Indeed, this knowledge gap hinders our ability to predict and mechanistically depict ecological dynamics in the context of global change (Thakur *et al.* 2022), and further precludes management recommendations and policies aiming to halt climate-driven biodiversity declines (Harvey *et al.* 2022).

Ecological stability and underlying mechanisms

Climate extremes represent novel disturbance regimes that exceed the historical range of climatic variability, which explains their potential to cause abrupt ecological impacts (Turner & Seidl 2023). The ecological impacts of climate extremes unfold over time, during and after the extreme events. Short-term impacts revealed during or immediately after climate extremes are known as resistance responses. For instance, at the population level, survival and dispersal rates can react as soon as tolerance thresholds are surpassed (e.g., heat-induced mortality; Jørgensen *et al.* 2022), leading to abundance declines during extreme events. Nonetheless, given that climate extremes are pulse disturbances, they allow for physiological repair and population regrowth under the more tolerable conditions met at the end of an extreme event (Smith 2011). Such long-term effects after extreme events have stopped are known as recovery responses. Collectively, resistance and recovery responses represent different facets of ecological stability, that is, the ability of an ecological system (i.e., population, community, or ecosystem) to maintain its attributes (e.g., vital rates, network properties, biomass production) in the face of perturbations (Donohue *et al.* 2016; Hillebrand *et al.* 2018; Van Meerbeek *et al.* 2021). When ecological systems are severely perturbed, they might display very slow recovery and even lead to irreversible state changes, denoting low stability (Holling 1973). Investigating patterns of stability, as well as their underlying mechanistic drivers, are fundamental tasks if we are to portrait a more complete picture of the biotic responses to climate extremes (e.g., Hillebrand & Kunze 2020; Isbell *et al.* 2015; Pennekamp *et al.* 2018). We note that, in equilibrium systems, ecological stability is approximately equivalent to the concept of 'resilience' sensu Holling (1973) (Ingrisch & Bahn 2018; Van Meerbeek *et al.* 2021). However, there is much confusion regarding the term 'resilience', as it is also used to refer to the rate of recovery (Pimm 1984; Van Meerbeek *et al.* 2021). For this reason, we avoid using the term 'resilience' in this dissertation, and instead we adopt the more well-delimited concept 'ecological stability'.

The kinds of mechanisms mediating ecological stability in the face of climate extremes are likely to vary according to the level of biological organization (Harvey *et al.* 2020). For instance, functional traits are expected to mediate species-level responses to climate extremes (e.g., Thakur *et al.* 2022), while the seasonal timing could be determinant when considering

population and community-level effects (e.g., De Boeck *et al.* 2011). Ultimately, these mechanisms need to be linked to fitness/demographic effects, which will then determine the subsequent ecological dynamics. For example, the seasonal timing has direct implications on the life stages exposed to the extreme event (Cinto Mejía & Wetzel 2023), which often have different vulnerabilities according to their physiological tolerances or the microclimates they inhabit (Kemppinen *et al.* 2024; Kingsolver *et al.* 2011; Kingsolver & Buckley 2020). Therefore, those factors expected to alter fitness more intensely deserve particular attention in the search for mechanistic-based explanations of ecological responses to climate extremes.

Due to the inherent rare and sudden occurrence of climate extremes, inferring their ecological impacts by means of observations is particularly challenging (Altwegg *et al.* 2017). Luckily, experimental approaches provide unique opportunities to quantify and to mechanistically assess drivers of ecological stability with climate extremes (Jentsch *et al.* 2007). Experiments can yield valuable insights into the processes involved at different levels of biological organization, for instance, by manipulating functional traits, population structure, or species interactions. Alternatively, factors related to the extreme event (e.g., type of disturbance, intensity, frequency, spatial and temporal extent) could also be controlled using experimental approaches, providing useful information on how various kinds of climate extremes influence their accompanying ecological impacts. Thus, exploring the main mechanisms driving variation in biotic responses to climate extremes by means of experiments represents a robust and valuable approach to ecological research.

Heat extremes and associated stress responses

The common defining feature of climate extremes is that they are discrete events of climatic origin acting at daily to seasonal scale. However, the various types of climate extremes differ substantially in the kind of abiotic stressor that they represent. For instance, flooding involves physical disturbance and oxygen depletion, which can impact benthic organisms, but it can also favor the recovery of early-colonizing species (McMullen *et al.* 2017). By contrast, droughts pose risks for organisms that are sensitive to high solute concentrations, while promoting those that

maximize water uptake from distant locations (e.g., deep-rooting plants and cord-forming fungi; Guhr *et al.* 2015). The two examples above illustrate the need to individually consider the unique aspects of each type of climate extreme if we are to mechanistically infer their ecological impacts, given that they involve completely different stressors and associated biotic responses.

Among the various types of climate extremes, heat extremes are expected to increase markedly at the global scale in terms of frequency and severity (Buckley & Huey 2016; Fischer *et al.* 2021; IPCC 2021). This is because gradual climate warming shifts the baseline temperatures upon which existing thermal variability takes place, thereby fueling the occurrence of heat extremes (IPCC 2021). This contrasts with other kinds of climate extremes, whose trends are more uncertain or only predicted to increase regionally, such as droughts or storms (IPCC 2021). Heat extremes can affect virtually all kinds of biota because the homeostatic state of organisms is disrupted at very high temperatures (Ørsted *et al.* 2022), owing to the impairment of protein structure, dysfunction of cellular membranes, rising metabolic costs and associated oxygen demands (González-Tokman *et al.* 2020; Verberk *et al.* 2021; Williams *et al.* 2016). As a result, heat extremes can act as a prevailing selective force driving thermal adaptations at the organismal level (Buckley & Kingsolver 2021), and also shaping species composition (Sorte *et al.* 2010) as well as interactions at the community level (Polazzo *et al.* 2023; Thakur *et al.* 2021). However, despite the ecological significance and the rising prevalence of heat extremes, there are important knowledge gaps regarding the drivers of variation that influence their associated ecological effects. More specifically, mechanisms promoting recovery after heat extremes have been poorly examined so far, and the relationships between resistance and recovery are relatively unknown in the context of heat extremes. Without solving these issues, it is challenging to infer the contribution of different factors influencing population and community responses during and after heat extremes. Determining the role of these factors has major significance for fundamental and applied purposes in the fields of ecological stability and climate change ecology.

Soil ecosystems in a changing world

Soils provide habitat for vast numbers of organisms, ranging from microbes to small mammals, and deliver key ecosystem functions such as carbon storage and decomposition, supporting aboveground productivity (Bardgett & Van Der Putten 2014). Given that soil organisms have high genetic and functional diversity (Anthony *et al.* 2023; Potapov *et al.* 2022) and occur in almost all habitats (Decaëns 2010), they could deliver valuable and generalizable insights for the study of biotic responses to heat extremes. Besides, there is wide interest in depicting how soil organisms respond to climate-related disturbances, because impacts on their populations and communities are likely to disrupt the functions that they provide (Bardgett & Van Der Putten 2014). In addition, soils are tractable systems to experimentally test the effects of climate extremes, given that fully functioning populations or communities can be established at a small scale, while retaining their essential habitat properties (e.g., physical structure; Erktan *et al.* 2020). These are highly appropriate features to infer the results from manipulative experiments to real-world systems.

Despite the fundamental role of soil organisms, there are still important knowledge gaps concerning their general functioning and diversity patterns (Decaëns 2010). These gaps represent both challenges and opportunities for the use of soil organisms in global change ecological research. On one side, interpreting climate-driven ecological responses requires robust knowledge on the natural history of the system examined. This is especially challenging in the soil, where the difficulties for direct observation have hampered the empirical demonstration of general ecological patterns (Thakur *et al.* 2020) and the quantification of trophic interactions (Potapov *et al.* 2022). On the flip side, using soil systems in global change research provides unique opportunities for novel findings. For instance, it has been shown that belowground phenological shifts in plants are generally less marked compared to aboveground ones (Liu *et al.* 2022), suggesting mismatches between aboveground and belowground responses to climate warming (Berg *et al.* 2010; Thakur 2020), even within the same organism. This illustrates how global change studies can deliver new insights on the structure and functioning of soil ecosystems, providing fundamental knowledge into how soil organisms cope with changing environments. Consequently, we put forward that soil organisms can be highly suitable systems

for climate-oriented ecological research, given their high diversity, tractability, and prospects for novel and stimulating discoveries.

Scope of the thesis

The main aim of this doctoral dissertation is to examine the role of various mechanisms driving population and community responses of soil organisms to heat extremes. To enhance the generality and representativeness of our findings, we explored different aspects of ecological stability in the context of heat extremes, by investigating factors acting on various levels of biological organization (i.e., from physiological to community levels), as well as by examining both intrinsic (i.e., related to the features of biological systems) and extrinsic factors (i.e., related to the abiotic environment). We focused on those factors that were expected to predominantly drive ecological responses to heat extremes, based on their fitness/demographic consequences, or their capacity to shape the structure and function of communities. The order of the different chapters is based on the complexity of the system examined, starting from simple but highly mechanistic experiments to more complex studies aimed at representing real-world conditions.

Among soil organisms, we use one of the most abundant and ubiquitous classes of soil invertebrates as our main model system: Collembola (Arthopoda: Hexapoda). These invertebrates are closely related to insects, and act as microbivores that inhabit different depths along the soil profile. Indeed, distinct Collembola species can coexist mainly by partitioning their vertical distribution in the soil (Potapov *et al.* 2016), as well as by diversifying their main food sources (e.g., fungi, soil detritus, algae; Potapov *et al.* 2021). Collembola are highly sensitive to changes in environmental temperature and moisture (Martin *et al.* 2024; Thakur *et al.* 2023), making them a suitable model system for investigating responses to heat extremes. In the experiments covered in this doctoral dissertation, we employ laboratory cultures of various Collembola species, as well as field-collected communities containing Collembola with their predators and natural resources (e.g., fungi). The climatic responses of these other trophic groups interacting with Collembola are assessed at times, to explore how they can mediate collembolan responses to heat extremes.

Overview of the thesis

In *Chapter 1,* we examine how variation in population responses to extreme heat events may be driven by the effects of temperature on life-history traits. For this purpose, we employ four closely-related Collembola species distributed along a latitudinal gradient, as we suspect that their distinct geographic distributions could underlie variation in their thermal performances, consequently affecting their population responses to the same extreme heat event. We first measure the thermal reaction norms of traits related to their life-history (survival, reproduction), and then use this trait-based approach to inform on a subsequent population-level experiment in soil microcosms.

In *Chapter 2*, we investigate the importance of the density of individuals, a fundamental feature determined at the population level, in influencing the recovery of populations exposed to an extreme heat event. In this case, we use two litter-living Collembola species that differ in their mode of reproduction (sexual or parthenogenetic reproduction), and manipulate the density of their populations at the onset of the extreme event. As in *Chapter 1*, we assess the resistance and recovery of populations, with the aim to determine how recruitment post-extreme event could help rescue populations differently depending on their initial densities.

In *Chapter 3*, our goal is to explore how the spatiotemporal context of natural communities could affect their responses to an extreme heat event. We thus obtain soil communities from different elevations across three seasons (spring, summer and autumn), and expose them to an extreme heat event in the laboratory. The extreme event is based on statistical extremity, and is therefore adjusted to the elevation and season in which the samples were collected. In addition, we assess the responses of two trophic groups, Collembola and fungi, and explore how their associations might shift when exposed to extreme heat.

Finally, in *Chapter 4*, we synthesize and extend on the knowledge gathered from the previous chapters, to lay out a conceptual framework describing population and community responses to heat extremes. We bridge existing concepts from the fields of thermal biology and ecological stability, and propose that ecological responses to heat extremes are determined along three stages: exposure, resistance and recovery. One key objective of this chapter is to

focus on how lagged effects induced by heat extremes –known as 'ecological debts' – are generated and accumulated at the three response stages, inducing impacts that may be overlooked without an explicit consideration of temporal dynamics.

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Chapter 1

Population resistance and recovery after an extreme heat event are explained by thermal effects on life-history traits

Population resistance and recovery after an extreme heat event are explained by thermal effects on life-history traits

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Abstract

Extreme heat events lower the fitness of organisms by inducing physiological stress and increasing metabolic costs. Yet, little is known about the role of life-history traits in elucidating population responses to extreme heat events. Here, we used a trait-based approach to understand population resistance and recovery using four closely related species of soil-dwelling Collembola. We measured thermal reaction norms of life-history traits (survival and reproductive traits) and used this information to identify ecological mechanisms linked to population responses after an extreme heat event (i.e., one week at 26-30 °C, representing +10 °C above ambient conditions). Furthermore, we investigated potential shifts in the body size distribution of recovering populations to better understand if extreme heat events can restructure body size spectra within populations. While resistance remained unaltered across species in our study, the recovery response of the most heat-sensitive species (*Protaphorura pseudovanderdrifti*, predominantly a boreal species) was strongly affected by the extreme heat event (-54% population change compared to ambient conditions). Given that the fecundity (linked to recovery) of *P. pseudovanderdrifti* was more sensitive to heat than their survival (linked to resistance), we detected a decoupling between population resistance and recovery to an extreme heat event in this species. In addition, the detrimental effects of heat on fecundity were largely responsible for a drop in the proportion of small-sized (juvenile) individuals in the recovering populations of *P. pseudovanderdrifti*. Thermally insensitive resistance and recovery in the other three species (*P. armata*, *P. fimata*, *P. tricampata*; predominantly temperate species) can be explained by their high survival and fecundity at warmer temperatures. We highlight that life-history trait responses to warming can help explain population resistance and recovery after extreme heat events.

Keywords: body size, climate change, fecundity, functional traits, survival

Introduction

Climate extremes are getting more frequent as a result of anthropogenic global warming, with detrimental consequences for biodiversity (Meehl & Tebaldi 2004; Christidis *et al.* 2015; Buckley & Huey 2016; IPCC 2021). Extreme heat events, in particular, compromise the fitness of organisms as a result of severe physiological stress (Harvey, Heinen, Gols, & Thakur, 2020; Ma, Ma, & Pincebourde, 2020; Thakur, Risch, & Van der Putten, 2022) and increased metabolic costs (Gillooly *et al.* 2001; Dillon *et al.* 2010). Ectothermic animals are especially vulnerable to extreme heat events as their body temperatures get pushed towards their upper critical thermal limits (Deutsch *et al.* 2008; Buckley & Huey 2016), potentially triggering large ecological responses (Harris *et al.* 2018). These responses are composed both by the magnitude of the impact on a species, reflected in an initial reduction of its population size (also known as resistance) and the ability of that species to return to a reference state - such as the population size in absence of disturbance (also known as recovery; Hillebrand et al., 2018). While recent syntheses have shown that several species usually return close to their reference population levels after pulse disturbances, including extreme heat events, we still know little about when and how do species recover (Hillebrand & Kunze 2020; Neilson *et al.* 2020), and whether population resistance can predict recovery (Isbell *et al.* 2015; Capdevila *et al.* 2022; Thakur *et al.* 2022). Establishing links between resistance and recovery can provide a comprehensive picture of how organisms respond to climate extremes, as even if populations may not show an immediate response (i.e., greater resistance), there could be a cost in the longer run, which would be reflected only through studying their recovery over a period of time (Harris et al., 2018; Ma et al., 2020). To this end, we here aim to apply trait-based approaches to better understand population resistance and recovery of various species exposed to extreme heat events (Neilson *et al.* 2020; Thakur 2020).

Among the suite of traits potentially affected by temperature, life-history traits deserve particular attention since they could directly relate to population level responses (McLean *et al.* 2016; Sinclair *et al.* 2016; Capdevila *et al.* 2022). These comprise all traits influencing the schedule of the life cycle of an individual ranging from reproduction, growth and maturation to survival. During extreme heat events, physiological responses to warming can trigger shifts in life-history traits that, in turn, might scale up to higher levels of ecological organization (Harvey *et al.* 2020).

For instance, regular periods of extreme or moderate warming induce small effects on mortality that accumulate over time, thereby triggering population crashes even below critical temperatures (Rezende *et al.* 2020). Negative impacts at the population level may also emerge from declines in the reproductive output of surviving individuals (Harvey et al., 2020; Ma, Rudolf, & Ma, 2015), as a result of trade-offs between stress tolerance or avoidance and reproduction (Klockmann *et al.* 2017; Walsh *et al.* 2019). Predicting population level responses to extreme heat events may therefore require the consideration of warming impacts on reproductive traits, given that the thermal ranges of these traits are narrower than those of survival (Ma et al., 2020; Rezende & Bozinovic, 2019; Walsh et al., 2019). Indeed, heat-induced shifts in reproductive strategies can arise as a result of altering the number of offspring in each reproductive event (i.e., brood/clutch size), a delay or altered frequency of reproductive events (e.g. skipping reproduction), or simultaneously affecting both (Forsman 2001). Such effects of heat on reproductive traits will only translate into changes in the population size after a time period necessary for recruitment, which underscores the need to consider this time lag in assessing population recovery (Neilson *et al.* 2020).

In addition to life-history traits, relationships between population size and trait variation within populations in changing environments can be further linked to functional traits like body size. Warming can trigger changes in the relative abundances of distinct life stages in a population when thermal tolerance differs across stages (Ohlberger 2013), which can be linked to variation in their body sizes (Franken *et al.* 2018; Peralta-Maraver & Rezende 2020). Across species, it has been shown that smaller organisms could be more tolerant to short periods of extreme heat (e.g. hours or days), whereas larger organisms can endure longer periods of moderate warming (e.g. weeks; Peralta-Maraver & Rezende, 2020). Population size structures might also shift in response to warming as a result of altered life-history traits (Gårdmark & Huss 2020). For instance, extreme heat events could induce lowered proportions of small-sized (juvenile) individuals in a population because of detrimental impacts of heat on fecundity. As opposed to the general pattern of body size reductions at higher temperatures (Gardner *et al.* 2011; Sheridan & Bickford 2011), this mechanism could actually produce greater average body sizes in the short-term response to

warming, making it further challenging to predict how heat events affect population size structures.

Population responses to extreme heat events might also be shaped by their biotic environments (Stoks *et al.* 2017; Boukal *et al.* 2019). One of such biotic environments is predation, which itself is temperature-sensitive and a key determinant of prey population dynamics (Rall *et al.* 2010; Brose *et al.* 2012; Thakur 2020). Predation can shape prey responses to heat events in various ways, which obscures predictions of resistance and recovery purely on prey traits. For instance, because trophic interactions are often strong when prey are smaller than their predators (Brose *et al.* 2006; Rall *et al.* 2012), prey population recovery after extreme heat might be constrained if their body sizes put them at risk of predation, regardless of their thermal sensitivity (Thakur 2020). However, predators could also be more vulnerable to extreme heat than their prey as they often have higher metabolic demands and could easily reach the risk of starvation if prey availability at higher temperatures is low (Fussmann *et al.* 2014; Huey & Kingsolver 2019). Altogether, how predation can modulate trait-based predictions of prey population responses to extreme heat events remains unclear.

Here, by using two complementary experiments, we aim to apply information obtained from life-history responses to warming to predict population level responses to an extreme heat event. We first measured thermal reaction norms (i.e., phenotypic changes as a function of temperature) of life-history (survival and reproductive traits) and physiological traits (lipid concentration and dry body mass) for one week in four closely related species of Collembola (from the genus *Protaphorura*). Collembola are among the largest groups of terrestrial invertebrates (in terms of density and diversity) living in soils (Potapov *et al.* 2023). This first experiment allows us to build testable hypotheses to predict population level responses to an extreme heat event (simulating a one-week heat wave) in our second experiment, using the same four study species together with a predator in soil microcosms. We expect that the distinct climatic niches of our four study species (details in Methods section) will underlie differences in their thermal performance, which will then affect their resistance and recovery after the extreme heat event. Specifically, we predict that the population resistance response will reflect thermal effects on survival, whereas the population recovery response will depend on thermal effects on

fecundity (i.e., reproductive output). Given that warming induces higher metabolic rates and may therefore reduce organisms' energy reserves (e.g., storage lipids; Meehan, Turnbull, Sinclair, & Lindo, 2022), we predict that the lipid concentration of collembolans will decline at higher temperatures, thereafter limiting their population's ability to recover after the extreme heat event. Moreover, since changes in life-history traits affect particular life stages and could subsequently alter the population size structure, we expect that the body size distribution (as a measure of functional trait in our study) will shift in response to extreme heat. More precisely, we predict that changes in the body size distribution in the resistance response will depict size-specific effects on survival, as a result of distinct thermal tolerances across body sizes (e.g., a lower proportion of small-sized individuals will indicate lower survival of juveniles compared to other size stages). In turn, the body size distribution in the recovery response will depict thermal effects on fecundity (e.g. a lower proportion of small-sized individuals will indicate negative impacts on fecundity during the week of extreme heat). Finally, we hypothesize that predation will constrain prey recovery, particularly for prey of smaller size (e.g., juvenile individuals) as they might be more susceptible to predation. Our study offers potential links among life-history, population size and functional trait responses to extreme heat events to help improve our predictions on climate change effects on population resistance and recovery.

Materials and methods

Study species

We used four species of the genus *Protaphorura* (Collembola: Onychiuridae): *P. armata, P. fimata, P. pseudovanderdrifti and P. tricampata* (Supporting Information). These are sexually reproducing, euedaphic invertebrates (i.e., permanently living in the soil) commonly found in various habitats across Europe (Gisin 1960; Fjellberg 1998). These four closely related species differ markedly in their geographic distributions, which in turn reflect their distinct climatic niches (Supporting Information). Specifically, *P. armata* is typically a warm-temperate species; *P. fimata* and *P. tricampata* are cold-temperate species; and *P. pseudovanderdrifti* is found in boreal and artic regions at high latitudes (Gisin 1960; Fjellberg 1998). These species further differ in body

size (potentially related to predation risk as well as to thermal tolerance; Franken et al., 2018; Rall et al., 2012), with *P. fimata* (mean ± SD body length at the start of the life-history experiment: 1829 ± 233 µm; *N* = 180) and *P. pseudovanderdrifti* (1698 ± 198 µm; *N* = 180) being larger species than *P. tricampata* (1487 ± 158 µm; *N* = 181) and *P. armata* (1373 ± 166 µm; *N* = 181). Hence, using these four closely related species not only offers a conservative experimental design due to their evolutionary relatedness, but their ecological differences further help to capture important organismal variation to offer some level of generality to understand what underlies the relationship between population resistance and recovery. Therefore, we aimed to capture variation in their population responses when each of the four species are exposed to the same extreme heat event, and link these population responses to their distinct thermal performances.

The origin of the animals that initiated the cultures as well as their local climatic conditions are provided in Table 1 in Xie et al. (2023). All species were reared for several generations (since the time of their collection in the field) in incubators at 20 °C and fed with dry yeast before we used them for the experiments (Supporting Information). We therefore adopted 20 °C as the ambient temperature in our experiments. To recreate typically dark conditions experienced by soil-dwelling collembolans, as those used in our study (Hopkin 1997), we kept all cultures and experiments under constant darkness. Additionally, we measured egg development time (i.e., time from egg laying to hatching) of all four species at the ambient temperature (20 °C) of our experiments (Supporting Information). Egg development time at 20 °C was shorter in *P. fimata* (estimated mean ± SE: 12.7 ± 0.7 days; *N* = 96), followed by *P. armata* (13.9 ± 0.6 days; *N* = 145) and *P. pseudovanderdrifti* (14.8 ± 0.7 days; *N* = 35), and was longest in *P. tricampata* (15.8 ± 0.5 days; *N* = 55). Based on our own observations as well as developmental times from other Collembola species (Siepel 1994), we estimate that the generation time of the study species at 20 °C is around 4-5 weeks.

Experiment 1: Life-history responses to warming

We established experimental units by adding 20 adult individuals into 60-mm Petri dishes with a moist substrate of plaster of Paris and activated charcoal (9:1), of which we measured the body

length of ten individuals at 5X (Keyence VHX 970F with high performance camera VHX 7020, Keyence Japan). Petri dishes were then exposed to three different temperatures separately: 20 °C, 25 °C and 30 °C. This temperature range spans +10 °C from the control conditions, which aims to simulate an extreme heat event as predicted in temperate regions for the next 100 years (CH2018 2018; IPCC 2021). Furthermore, the highest experimental temperature is considerably above the warmest soil temperatures experienced by the four species in their collection sites (Lembrechts *et al.* 2022; Xie *et al.* 2023), demonstrating the thermal extremity of our treatments. We established a total of 72 experimental units: 4 Collembola species x 3 temperature regimes x 6 replicates. Every day for the entire duration of the experiment (i.e., one week), we counted the number of dead animals (i.e., individuals not responding to a tactile stimulus), clutches (i.e., discrete groups made of at least three eggs; further details in Supporting Information), and the number of eggs in each clutch. All dead individuals and eggs were removed daily, and food (dry yeast) was provided *ad libitum* and replaced once during the experimental period to prevent any excessive fungal growth. At the end of the experiment, all living animals (12-20 individuals per plate) were collected and stored in the freezer at -20 °C for ten days, before the lipid analysis. Lipid concentration, an indicator for stored energy reserves, was determined with the gravimetric method, adapted from Williams, Thomas, MacMillan, Marshall, & Sinclair (2011). After being dried at 60 °C, animals were transferred to glass vials with 1 mL of analytical chloroform. The chloroform containing dissolved lipids was withdrawn daily and replaced three times to ensure the complete extraction of lipids. Later, the animals were dried again in the oven and weighed to obtain lipid free dry mass, which was used as a measure of body mass at the end of the experiment. The lipid concentration was then calculated as the weight difference between total dry body mass (with lipids) and lipid free dry mass, and afterwards divided by total dry body mass. All weight measurements were determined to the nearest 0.001 mg (Mettler Toledo XP6, Switzerland).

Experiment 2: Population response to extreme heat event

We established soil microcosms with monocultures of the same four *Protaphorura* species used above in the life-history experiment, and this time further with a generalist predatory mite, *Stratiolaelaps scimitus* (formerly known as *Hypoaspis miles*), which is known to prey on Collembola (Koehler 1999), even those of similar or greater size as the predator itself (Thakur *et al.* 2017, 2018). Predatory mites were purchased (Andermatt Biocontrol Suisse AG) and acclimated at 20 °C during ten days before they were added to the microcosms.

Soil microcosms consisted of polypropylene pots (height: 7.5 cm and diameter: 8 cm) filled with 100 g of commercial soil (3:1 mixture of garden soil and sand; pH = 6.8, C: N ratio = 10.1, organic matter = 10.5%) and 500 mg of hay litter on the surface to provide habitat structure and resources for the soil animals (Klironomos & Kendrick 1995; Kalinkat *et al.* 2013). Both substrates were sterilized at 121 °C (autoclaved), and hay litter was then dried at 50 °C for 72 h. To further promote fungal colonization, 20 mg of baker's yeast were added on top of the substrates (the same yeast used in culturing of the four Collembola species), and then incubated for five days at 20 °C. After this period, we added 20 adult Collembola individuals of similar body size (based on the measurement of body size for experiment 1) in every microcosm, and those with the predation treatment received six individuals of *Stratiolaelaps scimitus* a week later to allow some time for collembolan (prey) populations to establish. To prevent animal escape from the microcosms, we installed a 90 µm mesh at the bottom and a 5 cm high plastic fence (from the top of the microcosm; Supporting Information) coated with olive oil around the upper edge of the pots. The experimental populations were thus established and incubated at control conditions from week 1 until week 5, exposed to an extreme heat event on week 6, and were allowed to recover at control conditions from week 7 until week 12, i.e., until the end of the experiment. The length of the recovery period was chosen according to the estimated generation time of the study species at 20 °C (details in section 2.1), as suggested by Neilson et al. (2020). Even though the generation times might differ to some extent across species and temperature regimes (Siepel 1994), our five-week recovery period encompasses at least one full generation time for all four species, and thus manages to capture possible effects of the extreme heat event on their reproduction and recruitment. Further, to add more realism to our temperature regimes

in the population dynamics experiment, we adopted a diel temperature cycle (8h:16h, constant darkness) set at 16-20 °C for control conditions, and 26-30 °C for the extreme heat treatment. The extreme heat event (+10 °C above control conditions) was chosen to simulate a heat wave scenario as described for the life-history experiment (see previous section). Air temperature and relative humidity were monitored at 30-minute intervals throughout the experiment, and soil temperature at 5 cm depth was recorded twice every day during the extreme heat phase to capture the night (measurement at 08.00-10.00 h) and day (measurement at 14.00-18.00 h) soil temperatures. The realised air temperatures during extreme heat were +9.4 °C compared to control conditions (mean ± SD, extreme heat: 27.5 ± 2.2 °C; control: 18.1 ± 2.2 °C), while in the soil, this difference reached +8.4 °C (extreme heat: 25.4 ± 1.5 °C; control: 17.0 ± 1.4 °C; Supporting Information). As a result of the buffering effect of the soil, the temperature conditions experienced by the animals during extreme heat closely resemble those of the 25 °C regime from the life-history experiment.

We measured Collembola densities and body size at three time points: before the extreme heat event (harvest 1, week 5), after the extreme heat event (harvest 2, week 6), and at the end of the experiment (harvest 3, week 12). All treatment combinations and harvests were replicated five times except for the first harvest of *P. pseudovanderdrifti*, which had only four replicates because of the low numbers of animals of this species in our stock cultures. This resulted into a total of 236 experimental units: 4 Collembola species treatments x 2 temperature treatments x 2 predation treatments x 3 harvests x 5 replicates. In each harvest, soil animals were sampled using heat extraction with gradual heating from 25 °C up to 55 °C for 7 days following the Macfayden extraction method (Macfadyen 1961). All animals were collected in glycol water solution (1:1) and later transferred to 70% ethanol. Counts of Collembola and predatory mites as well as body size measurements of Collembola were performed under the stereomicroscope (Keyence VHX 970F with high performance camera VHX 7020, Keyence Japan). For each sample, we measured body length from 20% of the animals at 20X, with a minimum number of 20 individuals when available. We took measurements at random (i.e., adults and juveniles indistinctively) with the aim to detect shifts in the body size distribution triggered by the

experimental treatments. This yielded a total of 9,480 body size measurements from 42,039 collected Collembola individuals.

Statistical analyses

In the life-history experiment, we tested the effect of temperature, species and their interaction on survival by means of Cox proportional hazards (R package *survival*, v. 3.2-13; Therneau, 2021). Given that mortality was negligible at 20 °C, we restricted this analysis to the 25 °C and 30 °C temperature regimes. Furthermore, we fitted linear regressions to investigate the effect of the experimental treatments on reproduction (i.e., fecundity, clutch size and egg laying frequency) and physiological variables (i.e., lipid concentration and dry body mass). We did not include the 30 °C treatment in the linear models including reproduction-related variables since this temperature regime induced a complete infertility in nearly all study species (see 3.1 for details in the Results section). Fecundity (i.e., total number of eggs laid over the study period) was analysed with zero-inflated negative binomial models to account for overdispersed and zero-inflated counts (R package *glmmTMB*, v.1.1.2.3; Brooks et al., 2017). Clutch size (i.e., number of eggs per clutch) and egg laying frequency (i.e., number of clutches per day) were tested using generalized linear mixed models with negative binomial and Poisson distribution, respectively. In these mixed models, sample ID was added as a random intercept to account for repeated observations during the experimental period. For the physiological variables, we used linear models to analyse lipid concentration, as well as generalized linear models for lipid free dry body mass (R package *lme4,* v.1.1-26; Bates, Mächler, Bolker, & Walker, 2015). The Gamma distribution was adopted for the latter, given that this variable can only take positive values (Zuur *et al.* 2009), and because linear models with Gaussian distribution provided higher AIC values and linearity assumptions (e.g., homogeneity of variance) were not met. An overview of all models fitted for the life-history experiment and their structure is provided in Supporting Information.

In the population response experiment, Collembola densities were examined using generalized linear models with negative binomial distribution (R package *MASS*, v.7.3-54; Venables & Ripley, 2002). Fixed effects were species, extreme heat, predation, and the

interaction between species and extreme heat. Interactions with predation lacked statistical support and always resulted in higher AIC values, so they were not retained in the final models (Supporting Information). In order to examine population responses to extreme heat, we decomposed population responses into two components: resistance and recovery (e.g., Hillebrand et al., 2018). First, we fitted separate models for each sampling time: baseline (harvest 1), resistance (i.e., harvest 2, at the end of the extreme heat) and recovery (i.e., harvest 3, after five weeks, equivalent to the estimated species generation time). We then obtained Cohen's *d* standardised effect sizes with 95% confidence intervals (CI) using the function *eff_size* (R package *emmeans*, v.1.7.0; Lenth, 2021). Cohen's *d* effect sizes were calculated using differences of estimates between control and extreme heat treatments, divided by the pooled standard deviation (Lenth 2024). This method allows to compare means between treatments while adjusting for differences in scale among species (Koricheva *et al.* 2013). Therefore, Cohen's *d* immediately after the week of extreme heat (i.e., harvest 2) is considered as resistance in our study, and Cohen's *d* obtained five weeks after extreme heat (i.e., harvest 3) is a measure of recovery. In addition, we visually explored relationships between population responses to extreme heat by plotting standardised effect sizes of resistance against recovery across the four study species. Finally, we examined the effects of the experimental treatments on the body size distribution with quantile regressions (R package *lqmm*, v.1.5.6; Geraci, 2014), using sample ID as a random intercept to account for non-independent measurements from individuals of the same population. This method allows to estimate conditional quantiles of the response distribution (Cade & Noon 2003) enabling us to explore how our treatments might have particular effects across the body size distribution within populations. More specifically, we assessed the response on the 0.1, 0.5 and 0.9 quantiles; which represent small (juveniles), medium (young adults) and large-sized (old adults) individuals of the population (Jørgensen *et al.* 2008). Linearity assumptions for all linear models from both life-history and population response experiments were tested and visually inspected with the DHARMa package (v. 0.4.1; Hartig, 2021). We performed post-hoc tests to obtain all p-values, using the function *emmeans* from the package *emmeans* (Lenth 2024). All statistical analyses were carried out in R statistical software (v.4.0.2; R Core Team, 2020).

Results

Life-history responses to warming

After exposing our study species for one week to one of the three temperature regimes (20 °C, 25 °C, 30 °C), we found that warming affected life-history traits differently across the four species. Survival declined strongly in *Protaphorura pseudovanderdrifti* from 25 °C to 30 °C, with no individuals remaining alive after five days of exposure at 30 °C. We detected effects on survival of a moderate magnitude in *P. tricampata* (hazard ratio ± SE: 8.40 ± 5.36; *P* < 0.001), while no differences in survival between 25 °C and 30 °C were found in *P. armata* and *P. fimata* (Fig. 1).
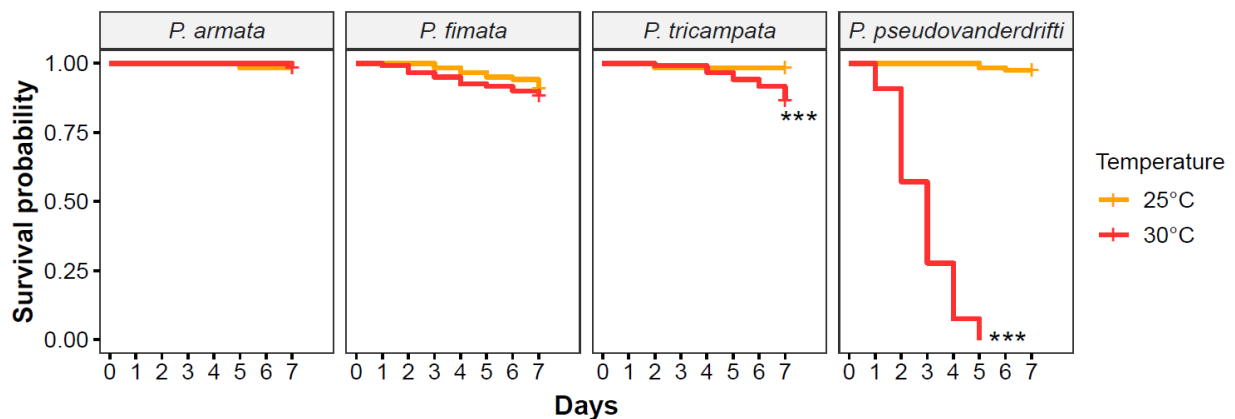

Fig. 1. Survival probability as a function of temperature across four study species of *Protaphorura* in the life-history experiment. Survival curves were plotted with the package *survminer* (v. 0.4.9; Kassambara, Kosinski, & Biecek, 2021). Species are displayed by ranking of heat tolerance, from higher (left side) to lower tolerance (right side), based on their survival. We present the same order of species in all following figures. Symbols show significant differences between temperature treatments: n.s. *P* > 0.05, ***P* < 0.001.

In contrast, the 30 °C treatment drastically affected reproduction in all species as barely any eggs were found in this temperature regime, thereby causing a complete infertility across study species (Fig. 2). Focusing on the response of fecundity from 20 °C to 25 °C (Fig. 2), this again depended on the species considered: egg production in *P. pseudovanderdrifti* dropped by 71.9% as a result of a lower frequency of reproductive events, whereas *P. armata* produced 76.6% more

eggs at the higher temperature because of an increase in clutch size (Supporting Information). Clutch size declined in *P. tricampata* at 25 °C (Supporting Information), but this did not cause any detectable change in the fecundity of this species (Fig. 2). All reproductive traits remained unaltered in *P. fimata* between 20 °C and 25 °C (Supporting Information). Regarding the physiological traits, the lipid concentration in living animals at the end of the one-week study period did not differ across temperature regimes in any of the species, but lipid free dry body mass declined in all species from 25 °C to 30 °C (Supporting Information). Overall, our results suggest that *P. pseudovanderdrifti* is the most heat-sensitive species among the four Collembola species due to its lowest survival at 30 °C and fecundity at 25 °C, whereas the other three Collembola only ceased their reproduction at 30 °C with little effects on their survival. All detailed model outputs are provided in Supporting Information.
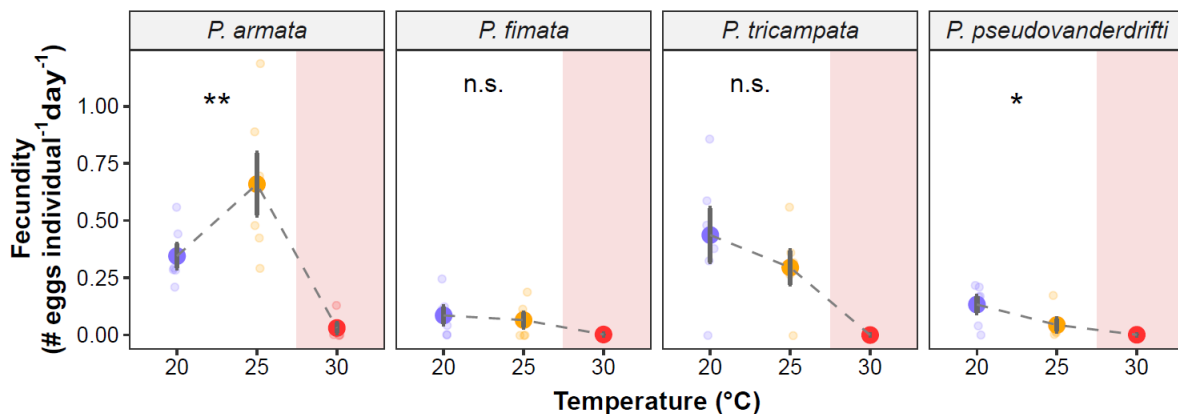

Fig. 2. Fecundity as a function of temperature across four study species of *Protaphorura* in the life-history experiment. Solid points represent means, dark solid bars represent standard errors, and faded points are raw data. The red faded area indicates that the 30 °C temperature regime was not included in the models because this treatment induced nearly complete infertility across four species. Symbols show significant differences between temperature treatments: n.s. *P* > 0.05, *P* < 0.05, **P* < 0.01.

Population response to an extreme heat event

   Population growth was generally slower at the start of the experiment (from H0 to H1), but later increased by the end of the experiment (from H2 to H3), especially in *Protaphorura armata* and *P. fimata* (Fig. 3). One week of an extreme heat event did not affect population resistance responses in any of the four study species (Fig. 3-4, Supporting Information). Later, after a recovery period of one generation time, we detected an incomplete recovery in the most heat-sensitive species, *Protaphorura pseudovanderdrifti*, showing a -54% population change in the extreme heat treatment compared to the control (Fig. 3, Supporting Information).
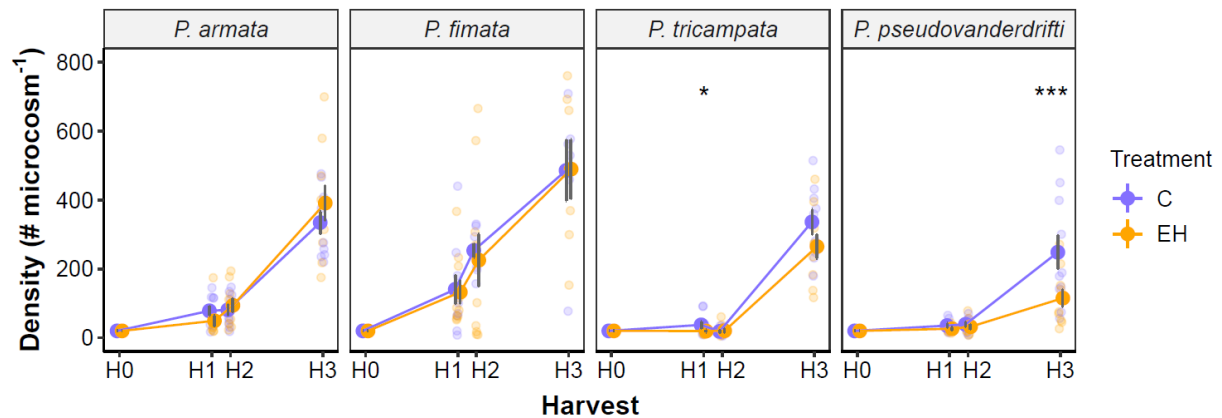

Fig. 3. Temporal dynamics of the densities across four study species of *Protaphorura* in the population response experiment. "Harvest" specifies the different time points in which Collembola densities were assessed during the experiment: H0: start of the experiment; H1: baseline; H2: resistance; H3: recovery. Solid points represent means, dark bars represent standard errors, and faded points are raw data. Note that raw data points for densities above 800 individuals are not displayed for visualisation purposes. Colours indicate different experimental treatments: blue: control (C); orange: extreme heat (EH; +10 °C on ambient temperature). Stars show significant differences between treatments: *$P < 0.05$, ***$P < 0.001$.

We did not detect any significant impacts of extreme heat on the recovery responses of the other three species (Fig. 4, Supporting Information). Note that even in the species with the strongest warming-driven decline in fecundity (*P. pseudovanderdrifti*), population growth was positive from H2 to H3 in the extreme heat treatment, but with a much shallower population increase compared to the control treatment.
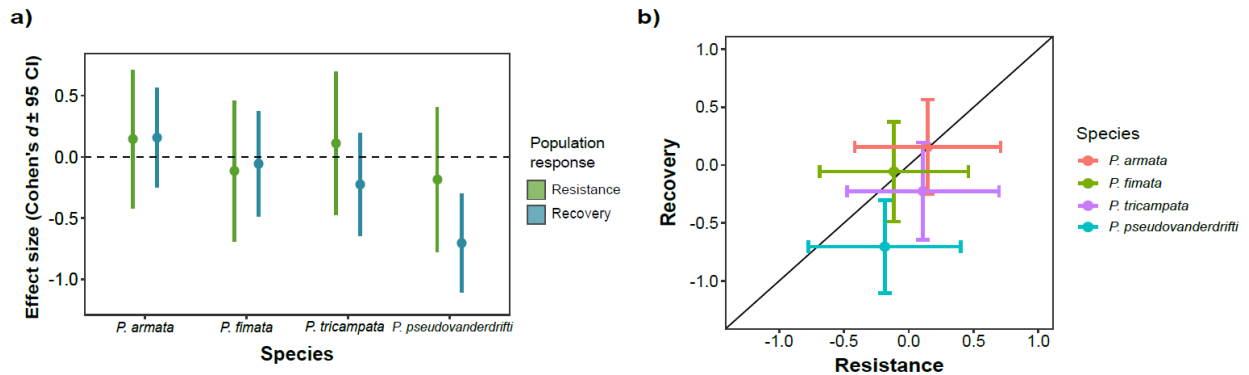

Fig. 4. (a) Standardised effect sizes (Cohen's *d*) with 95% confidence intervals (CI) showing the population responses (resistance and resilience) to a one-week extreme heat event in the population response experiment. Confidence intervals crossing zero values (dotted line) are statistically indifferent to zero. Negative values indicate a population decline in response to extreme heat, compared to the control treatment. Colours indicate different population responses: green: resistance; turquoise: recovery. (b) Resistance against recovery response, using standardised effect sizes with 95% CI. The solid diagonal line shows the 1:1 relationship between resistance and recovery. Collembola species are indicated with different colours: red: *P. armata;* green: *P. fimata;* purple: *P. tricampata;* blue: *P. pseudovanderdrifti*.

The body size distribution within *P. pseudovanderdrifti* population was also not affected during its resistance response, but it shifted during the recovery response (Fig. 5). More specifically, the individuals at the lower end of the distribution (quantile 0.1) were larger in size in the extreme heat treatment within *P. pseudovanderdrifti*, while the sizes at the median (quantile 0.5) and higher end of the distribution (quantile 0.9) were little affected (Fig. 5,

Supporting Information). The body size distribution did not change for any of the other three Collembola species during both resistance and recovery phase.
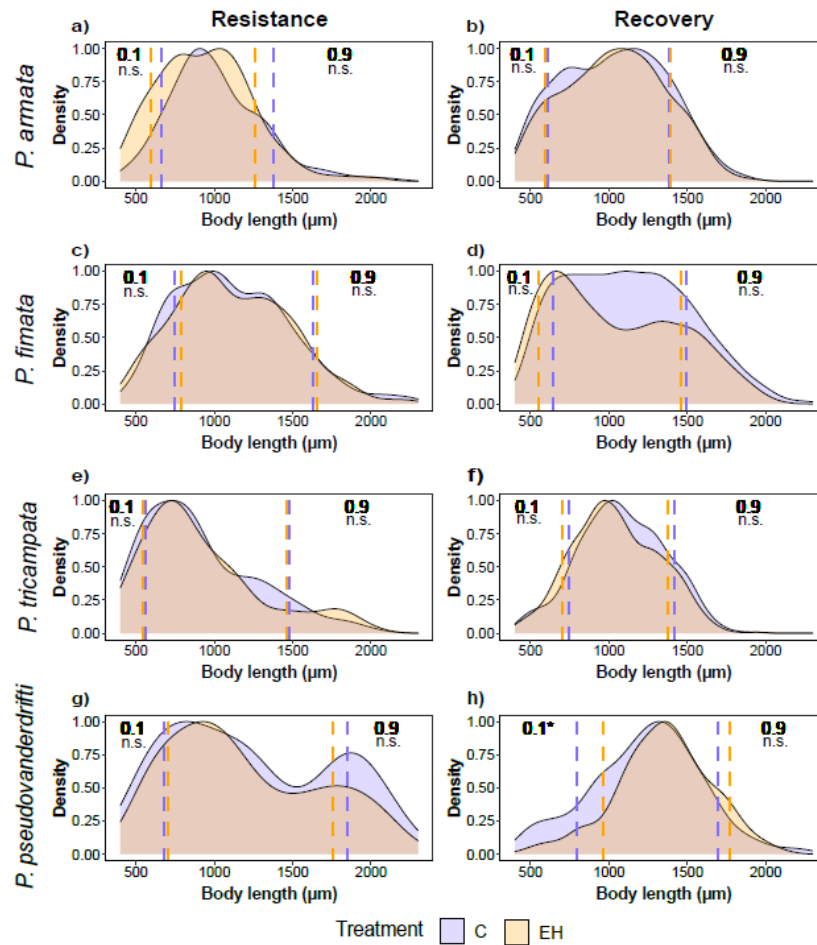

Fig. 5. Pooled body size distribution of the four *Protaphorura* species during the resistance phase (a, c, e, g; left column) and the recovery phase (b, d, f, h; right column) of the population response experiment. The number of body size measurements in each species is provided for the resistance and recovery responses, respectively: *P. armata* (N = 487 and N = 1407), *P. fimata* (N = 977 and N = 2402), *P. tricampata* (N = 245 and N = 1142) and *P. pseudovanderdrifti* (N = 336 and N = 841). Dashed lines display the estimates of quantile regressions at the 0.1 and 0.9 quantiles with sample ID as a random intercept. Colours indicate different experimental treatments: blue: control (C); orange: extreme heat (EH). Symbols show significant differences between temperature treatments: n.s. P > 0.05, *P < 0.05.

Predation did not have any detectable effect on Collembola densities but altered their body size distribution at the baseline harvest, by reducing the number of the largest individuals in the populations for all Collembola species (quantile 0.9; Supporting Information). Nonetheless, the effect of predation faded out in subsequent harvests, as predatory mites did not manage to reproduce and persist until the end of the study period (Supporting Information). All model outputs are provided in Supporting Information.

Discussion

Our results from two complementary experiments highlight that population responses (i.e., resistance and recovery) to an extreme heat event can to some extent be explained by depicting how life-history traits, namely survival and fecundity, respond to warming. We found that although population resistance was not affected across four species, the recovery response was negatively affected in the most heat-sensitive species. In contrast, the other three species did not have any detectable recovery response to extreme heat, implying a coupling between their population resistance and recovery after an extreme heat event. The decoupling between population resistance and recovery responses in one of the species (*Protaphorura pseudovanderdrifti*) could be linked to thermal effects on its fecundity, which also affected the body size distribution of its recovering populations. More specifically, the detrimental effects of heat on fecundity were most likely responsible for a decline in the number of small-sized (juvenile) individuals within recovering populations of *P. pseudovanderdrifti*. Taken together, our study provides a novel insight by demonstrating trait-based explanations (both life-history and functional traits) for how temperature extremes could impact populations during their recovery.

We found that reproductive traits were consistently more heat-sensitive than survival. Indeed, the highest temperature regime of 30 °C induced a substantial mortality in *P. pseudovanderdrifti*, whereas in the other three species, there were minor or no effects on their survival, which could have contributed to negligible resistance responses to extreme heat. On the contrary, the 30 °C temperature regime caused a near complete infertility across four species, while all species managed to lay eggs at 25 °C. Interestingly, quantitative differences in fecundity

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across species at 25 °C might underlie distinct adaptations to the thermal conditions in their distribution ranges. The species that showed the lowest survival at 30 °C, *P. pseudovanderdrifti*, was also the only one that reduced egg production (~70%) from 20 °C to 25 °C, whereas the change in fecundity in the species *P. armata* was of similar magnitude but in the opposite direction (Fig. 2). Remarkably, warming affected fecundity in these two species by altering their reproductive strategies in different ways: egg laying frequency declined in *P. pseudovanderdrifti*, whereas clutch size increased in *P. armata* (Supporting Information). In the case of *P. tricampata* and *P. fimata*, our results suggest that their thermal optimum for fecundity might lie between 20 °C and 25 °C, which would explain the apparent lack of fecundity responses in these two species. It has been shown that acute thermal limits vary substantially across the four Collembola species given the variation in their geographic range, and are related to the warmest soil temperatures found in their natural habitats (Xie *et al.* 2023). Our results also confirm that variation in thermal performance of ectotherms could depend on their latitudinal origin (Sunday *et al.* 2011; Sengupta *et al.* 2017), as species collected from temperate regions (*P. armata, P. fimata, P. tricampata*) maintained a more constant performance across experimental temperatures than boreal species (*P. pseudovanderdrifti*). It is worth noting that dry body mass declined only at 30 °C across species, which indicates that animals were experiencing significant heat stress at this temperature, hindering their growth and development (Mallard *et al.* 2020). Although collembolans attained lower body masses at 30 °C, their lipid concentration remained constant across species in all temperature regimes. We speculate that the organisms' energy reserves in terms of lipid concentration were possibly maintained as a result of down regulating their metabolism (e.g., reduced physiological and/or behavioural activities) at 30 °C to avoid further heat stress (Ehnes *et al.* 2011) or, alternatively, that lipid concentration may be responsive to warming only over longer exposure times (e.g., Meehan et al., 2022). Altogether, these findings allowed us to predict that *P. pseudovanderdrifti* would show the lowest population resistance due to high mortality, and the lowest recovery after an extreme heat event due to cessation of reproduction- hence a coupling between its population resistance and recovery. In contrast, we expected that the other three species would display a decoupling between resistance (high survival) and recovery (low reproduction) after the week of extreme heat event.

Agreeing with our expectations based on the results from the life-history experiment, we found that the most heat-sensitive species, *P. pseudovanderdrifti*, was also showing the lowest population recovery after an extreme heat event (+10 °C above ambient conditions). However, no effects on population resistance were detected in *P. pseudovanderdrifti* nor in other three species. Such strong resistance to extreme heat can potentially be explained by the buffering effect of the soil in the moist conditions of our experiment, that produced conditions on average 2 °C cooler and with more dampened thermal fluctuations than in the air (Lembrechts *et al.* 2022). Indeed, the average temperatures reached in the soil during the week of extreme heat remained just above 25 °C, and the population responses nearly mirrored our results of the life-history experiment at this temperature regime. For instance, survival in *P. pseudovanderdrifti* was hardly affected but fecundity dropped by 72% at 25 °C, which can be linked to the strong resistance (demonstrating high survival) and a 54% population decline at recovery (demonstrating the legacy of thermal impacts on fecundity). By contrast, a 77% increase in fecundity in *P. armata* at 25 °C was surprisingly not reflected in the population recovery response, which did not differ from control conditions. It is possible that such warming-driven increase in fecundity came at the cost of compromising egg viability, although this merits further investigation. Alternatively, differences in daily temperature fluctuations between the life-history experiment (constant temperature) and the population experiment (daily fluctuation of 4 °C) could further explain quantitative discrepancies between the both, given that temperature cycles provide greater population growth, as shown by Liefting et al. (2017) in the collembolan *Orchesella cincta*. One limitation from the temperature buffering in the soil at the 30 ºC treatment in the population experiment is that it prevents comparison between the corresponding population responses and life-history responses at 30 ºC. Nevertheless, we interpret that the coupling between population resistance and recovery in *P. armata*, *P. fimata* and *P. tricampata* (i.e., strong resistance and recovery) can be explained by the high survival and lack of thermal impact on fecundity for these species in the temperature conditions of our experiment. Our findings are consistent with theoretical and empirical evidence showing lower thermal tolerances at higher levels of organisation (Rezende & Bozinovic 2019; Bozinovic *et al.*

2020), since recovery (mostly affected by fecundity) was more heat-sensitive than resistance (mostly affected by individual survival), particularly in *P. pseudovanderdrifti.*

Our results show that the extreme heat event triggered shifts in the population body size distribution in *P. pseudovanderdrifti* that were detectable only during the recovery period, mirroring with the responses of recovering populations. By measuring the body size from a large set of individuals (min. 20% of the population) and through the use of quantile regression, we provide a mechanistic link between warming effects on life-history traits and population level responses. An extreme heat event reduced the number of small-sized individuals (juveniles) in the population of the most heat-sensitive species (*P. pseudovanderdrifti*) during the recovery response, while no such body size-specific shifts were found during the resistance phase. In this species, reproduction was disrupted during the week of extreme heat, thus the individuals that should have recruited in the population failed to do so during the recovery period. Importantly, the body size distribution of the other three species remained unaltered where a strong coupling between resistance and recovery was also observed in response to extreme heat. As opposed to our findings, Lindo (2015) and Holmstrup et al., (2018) showed that positive effects of warming on fecundity contributed to a greater representation of smaller invertebrate species in natural soil communities, therefore causing an overall decline in body size at the community level. The occurrence of heat-tolerant species in these communities might thus explain the discrepancy with our results related to the shifts in the body size distribution of *P. pseudovanderdrifti*.

Predation had a negligible role on the population responses of their prey to the extreme heat event in our study. We argue that although predators initially removed prey individuals, as detected by shifts in the body size distribution, their top-down control became weak as prey populations outpaced predators by several fold. In fact, predators also did not manage to effectively reproduce perhaps due to the excretion of deleterious defence substances by prey species in our study system (Jensen *et al.* 2019).

We conclude that distinct effects of warming on life-history traits can scale up to population level responses after pulse disturbances like extreme heat events, and can be effectively linked through shifts in the body size distribution of recovering populations. Our findings related to the

(de)coupling between population resistance and recovery responses to an extreme heat event have important implications for a better understanding of both the short and long-term responses of species exposed to climate extremes. Without an appropriate consideration of recovery periods tailored according to the species generation time, one might risk overlooking the shifts in life-history traits, such as fecundity, that subsequently could shape population recovery responses. We demonstrated this even with one generation time of our study species, which essentially resembles a shorter recovery period. Our study is a step towards establishing relationships between thermal effects on life-history traits and population responses to climate warming, and we call for future studies exploring these relationships by incorporating trait-based approaches (e.g., life-history and functional traits) to predict both population- and community-level resistance and recovery in a changing world.

Acknowledgements

Data availability

The complete dataset and R script used in this study are available in the Figshare repository: https://doi.org/10.6084/m9.figshare.19494665.v4.

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Supporting Information

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Table S12	Output of quantile regression for the quantile 0.5 of the body size distribution.
Table S13	Output of quantile regression for the quantile 0.9 of the body size distribution.
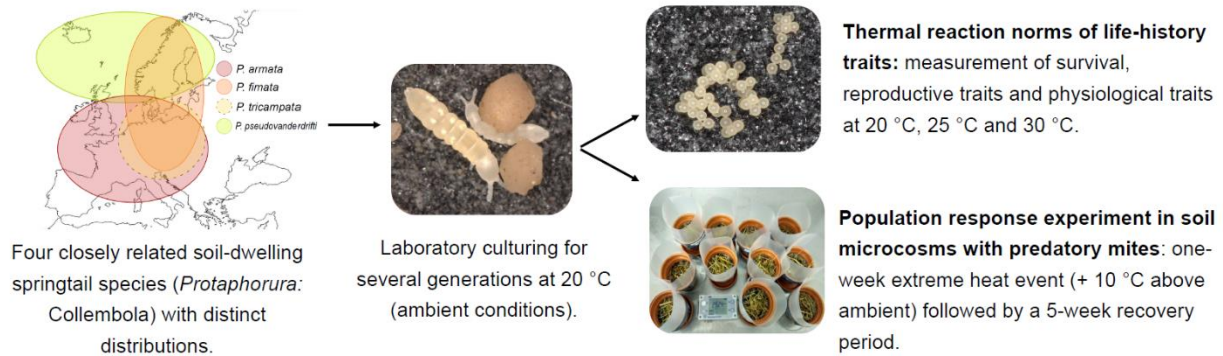
Fig. S1. Schematic representation of the experimental design. We used four Collembola species (genus *Protaphorura*) with different geographic distributions (left image) that were cultured in the laboratory for several years at 20 °C (middle image). We then established two experiments: experiment 1 tested life-history responses to warming in Petri dishes (upper right picture, showing egg clutches on the substrate), and experiment 2 tested population responses to extreme heat events in soil microcosms (lower right picture). The simplified geographic distributions of the different Collembola species shown on the left image were based on Gisin (1960) and Fjellberg (1998).

a)

b)

P. armata

P. fimata

c)

d)

P. pseudovanderdrifti

P. tricampata

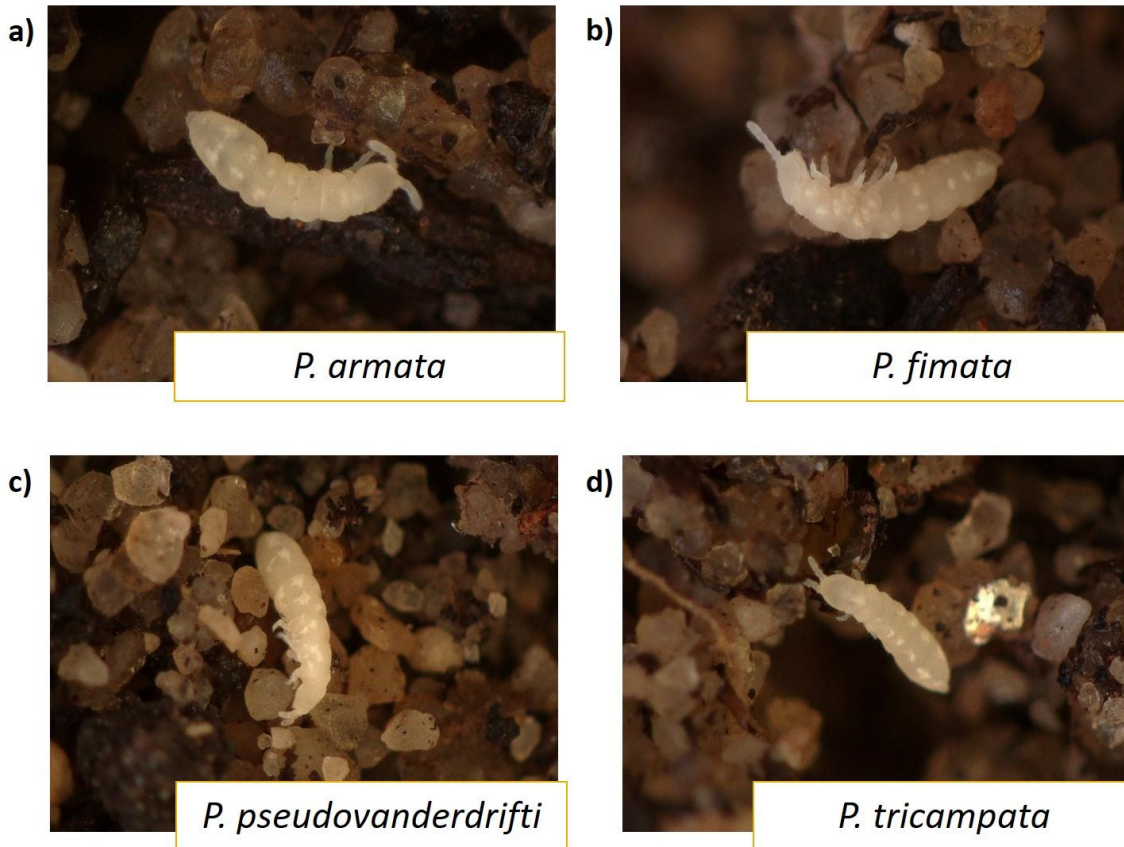**Fig. S2.** Pictures from the four *Protaphorura* study species taken under the microscope at 30X magnification (Keyence VHX 970F with high performance camera VHX 7020, Keyence Japan): a) *P. armata*, b) *P. fimata*, c) *P. pseudovanderdrifti*, d) *P. tricampata*. The images were cropped and magnified for visualisation purposes, so the relative differences in body sizes across species are not reflected in these pictures.

Table S1. Output of linear mixed-effect model used to estimate egg development time across four study species of *Protaphorura* at 20 °C. This data was obtained from additional experimental trials, in which we added twelve adult Collembola into 90-mm Petri dishes with a moist substrate of plaster of Paris and charcoal (replicated five times), and monitored them daily until at least one clutch of eggs was laid. At this point, adult Collembola were removed, and all eggs were tracked daily until hatching. We then noted egg development time (i.e. days from egg laying until hatching) for every hatching individual (N = 331). In the models, plate ID was treated as a random intercept in order to account for the lack of independence of animals hatching in the same plate (likely coming from the same clutch). Estimates and standard errors (SE) are provided.

Egg development time (days)		
Species	Estimate	SE
P. armata	13.910	0.619
P. fimata	12.728	0.710
P. tricampata	15.816	0.515
P. pseudovanderdrifti	14.803	0.654
Random effects (Plate ID)	1.464	

Fig. S3. Egg laying frequency as a function of temperature across four study species of *Protaphorura*. Clutches were considered as groups made of at least three eggs, but the results remain qualitatively the same when the minimum number taken to define a clutch ranges from two to four eggs. Solid points represent means, dark solid bars represent standard errors, and faded points are raw data. Note that the 30 °C treatment is not displayed since it induced nearly complete infertility in all study species. Symbols show significant differences between temperature treatments: n.s. $P > 0.05$, *$P < 0.05$.

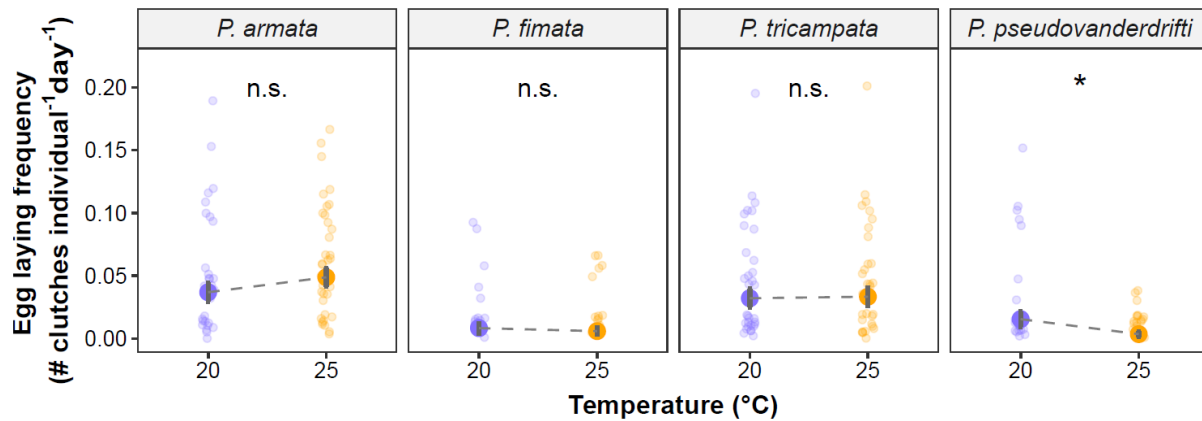**Fig. S4.** Clutch size as a function of temperature across four study species of *Protaphorura*. Clutches were considered as groups made of at least three eggs, but the results remain qualitatively the same when the minimum number taken to define a clutch ranges from two to four eggs. Solid points represent means, dark solid bars represent standard errors, and faded points are raw data. Note that the 30 °C treatment is not displayed since it induced nearly complete infertility in all study species. Symbols show significant differences between temperature treatments: n.s. $P > 0.05$, **$P < 0.01$.
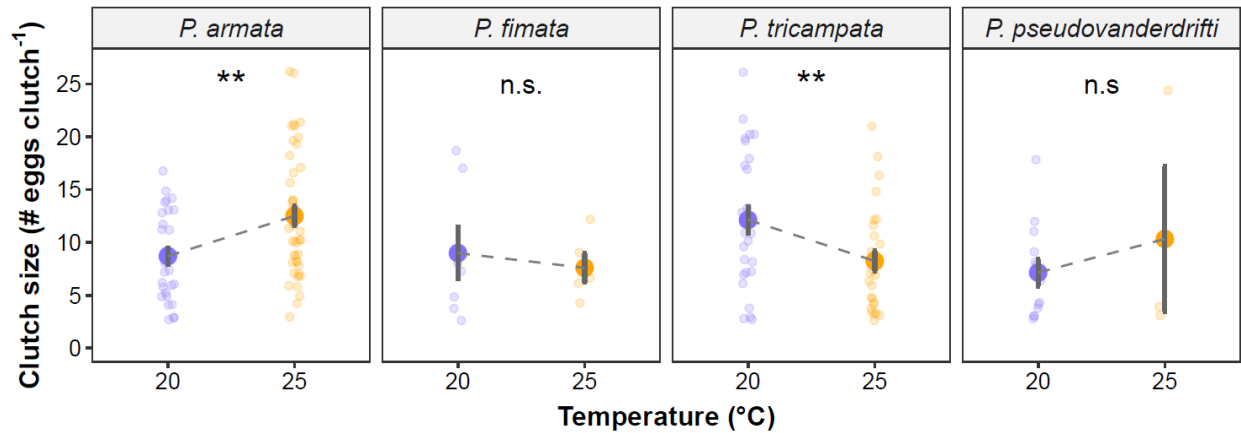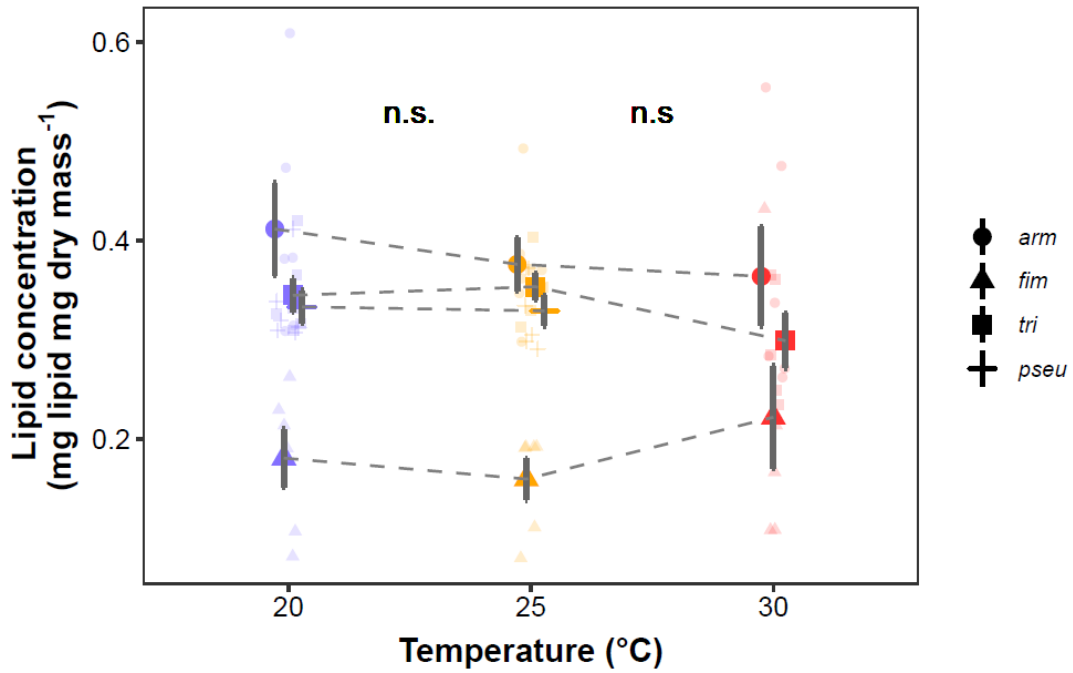
Fig. S5. Lipid concentration as a function of temperature across four study species of *Protaphorura*. Abbreviations in the legend stand for the species names: *arm: P. armata*, *fim: P. fimata, tri: P. tricampata, pseu: P. pseudovanderdrifti.* Solid points represent means, dark solid bars represent standard errors, and faded points raw data. Note that this variable was measured in animals surviving at the end of the one-week experiment, hence the lack of data points for *P. pseudovanderdrifti* at 30 °C. Symbols show significant differences between temperature treatments: n.s. *P* > 0.05.
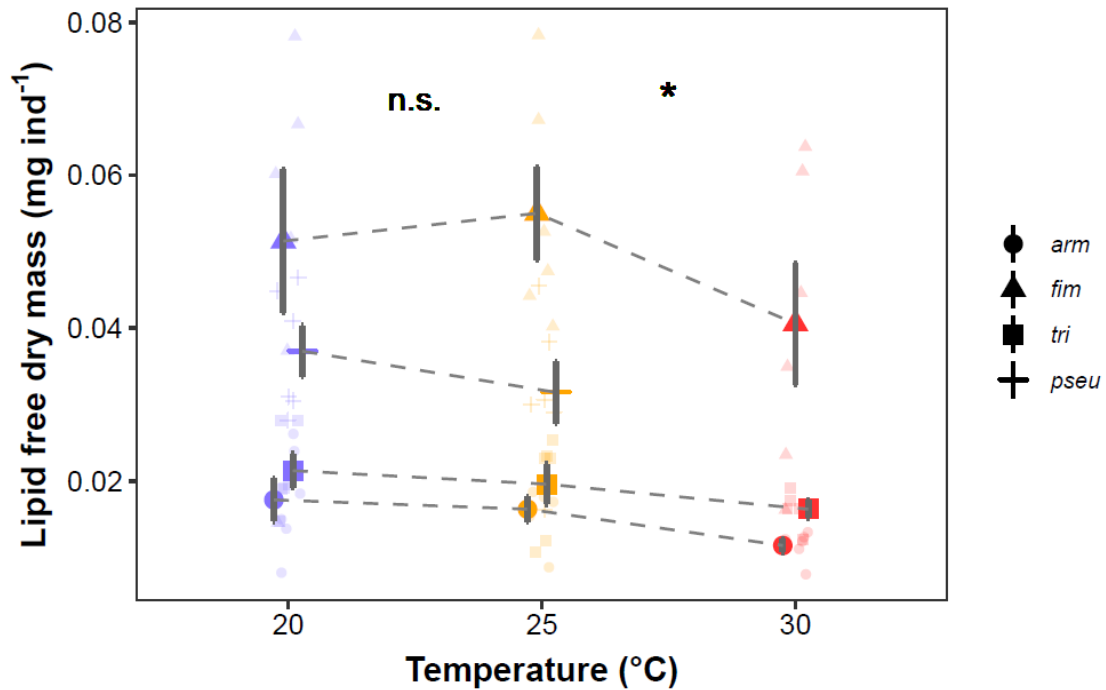
Fig. S6. Lipid free dry body mass as a function of temperature across four study species of *Protaphorura*. Abbreviations in the legend stand for the species names: *arm: P. armata*, *fim: P. fimata, tri: P. tricampata, pseu: P. pseudovanderdrifti.* Solid points represent means, dark solid bars represent standard errors, and faded points raw data. Note that this variable was measured in animals surviving at the end of the one-week experiment, hence the lack of data points for *P. pseudovanderdrifti* at 30 °C. Symbols show significant differences between temperature treatments: n.s. $P > 0.05$, *$P < 0.05$.

Table S2. Overview of the statistical models used in the life-history experiment. We provide the model type and structure for each of the response variables (traits), as well as the name of the table where the model output is shown. Abbreviations: GLM: Generalized Linear Model; GLMM: Generalized Linear Mixed Model.

Trait	Model type	Model structure	Output table
Survival	Cox proportional hazards	species x temperature	Table S3
Fecundity	GLM zero-inflated negative binomial	species x temperature	Table S4
Egg laying frequency	GLMM Poisson	species x temperature + (1 | plate)	Table S5
Clutch size	GLMM negative binomial	species x temperature + (1 | plate)	Table S6
Lipid concentration	Linear model	species + temperature	Table S7
Lipid free dry body mass	GLM Gamma	species + temperature	Table S7

Table S3. Output of the Cox proportional hazards analysis used to evaluate the effect of the experimental treatments on survival after one week across four study species of *Protaphorura*. The treatment combination "*P. armata,* 25 °C" was arbitrarily taken as the baseline hazard (hazard ratio = 1). The hazards for all remaining treatment combinations are thus proportional to this reference level. Observations were clustered by sample ID. Hazard ratios (i.e., exponential of the estimate), estimates, standard errors (SE) and p-values (*P*) of the contrasts between temperature treatments for a given species are provided. Significant p-values (*P* < 0.05) are highlighted in bold.

		Hazards			
Species	Temperature	Hazard ratio	Estimate	SE	*P*
P. armata	25 °C	1.000	0.000	0.000	0.993
	30 °C	0.993	-0.007	0.821	
P. fimata	25 °C	5.700	1.740	0.815	0.721
	30 °C	7.525	2.018	0.786	
P. tricampata	25 °C	1.007	0.007	0.827	**< 0.001**
	30 °C	8.399	2.128	0.638	
P. pseudovanderdrifti	25 °C	1.504	0.408	0.860	**< 0.001**
	30 °C	444.440	6.097	0.623	

Table S4. Output of the zero-inflated negative binomial model used to evaluate the effect of the experimental treatments on the fecundity across four study species of *Protaphorura*. The species *P. fimata* was an important source of zero-inflation, so it was included in the binomial model. Estimates, standard errors (SE) and p-values (*P*) of the contrasts between temperature treatments for a given species are provided. Significant p-values ($P < 0.05$) are highlighted in bold.

	Fecundity (log-scale)			
Species	Temperature	Estimate	SE	*P*
P. armata	20 °C	3.903	0.160	**0.007**
	25 °C	4.472	0.122	
P. fimata	20 °C	2.932	0.308	0.812
	25 °C	3.040	0.337	
P. tricampata	20 °C	4.291	0.146	0.126
	25 °C	3.939	0.172	
P. pseudovanderdrifti	20 °C	3.155	0.250	**0.010**
	25 °C	1.828	0.429	

Table S5. Output of the Poisson mixed-effects model used to evaluate the effect of the experimental treatments on the egg laying frequency across four study species of *Protaphorura*. Reproductive events were considered as such for clutches made of at least three eggs, but the results remain qualitatively the same when the minimum number taken to define a clutch ranges from two to four eggs. Sample ID was added as a random intercept to account for repeated observations. Estimates, standard errors (SE) and p-values (*P*) of the contrasts between temperature treatments for a given species are provided. Significant p-values (*P* < 0.05) are highlighted in bold.

	Egg laying frequency (log-scale)			
Species	Temperature	Estimate	SE	*P*
P. armata	20 °C	-0.315	0.192	0.273
	25 °C	-0.036	0.171	
P. fimata	20 °C	-1.803	0.384	0.570
	25 °C	-2.140	0.453	
P. tricampata	20 °C	-0.454	0.205	0.897
	25 °C	-0.418	0.201	
P. pseudovanderdrifti	20 °C	-1.184	0.286	**0.023**
	25 °C	-2.651	0.581	

Table S6. Output of the negative binomial mixed-effects model used to evaluate the effect of the experimental treatments on the clutch size across four study species of *Protaphorura*. Clutches were considered groups made of at least three eggs, but the results remain qualitatively the same when the minimum number taken to define a clutch ranges from two to four eggs. Sample ID was added as a random intercept to account for repeated observations. Estimates, standard errors (SE) and p-values (*P*) of the contrasts between temperature treatments for a given species are provided. Significant p-values (*P* < 0.05) are highlighted in bold.

	Clutch size (log-scale)			
Species	Temperature	Estimate	SE	*P*
P. armata	20 °C	2.194	0.097	**0.006**
	25 °C	2.527	0.070	
P. fimata	20 °C	2.164	0.207	0.835
	25 °C	2.097	0.249	
P. tricampata	20 °C	2.479	0.089	**0.009**
	25 °C	2.114	0.106	
P. pseudovanderdrifti	20 °C	1.983	0.164	0.659
	25 °C	2.144	0.327	

Table S7. Output of the models used to evaluate the effect of the experimental treatments on the physiological variables across four study species of *Protaphorura*. Note that these variables were measured in animals surviving at the end of the one-week experiment, hence the lack of data points for *P. pseudovanderdrifti* at 30 °C. We employed linear models to analyse lipid concentration, as well as generalized linear models with Gamma distribution for lipid free dry body mass (both *N* = 65). Given that interactions species by temperature lacked statistical support, only the additive effect of temperature across the four study species was tested. Estimates, standard errors (SE) and adjusted p-values (*P*) of the contrasts between temperature treatments are provided. Significant p-values (*P* < 0.05) are highlighted in bold.

Physiological variables				
Response variable	Temperature	Estimate	SE	*P*
Lipid concentration	20 °C	0.318	0.016	
(mg lipids mg dry mass^{-1})	25 °C	0.305	0.016	
	30 °C	0.303	0.020	
Lipid free dry body mass (mg individual^{-1}, log-scale)	20 °C	-3.538	0.062	
	25 °C	-3.600	0.062	
	30 °C	-3.864	0.078	

Contrasts

Lipid concentration	20 °C vs. 25 °C	0.013	0.022	0.827
(mg lipids mg dry mass^{-1})	20 °C vs. 30 °C	0.014	0.025	0.838
	25 °C vs. 30 °C	0.001	0.025	0.999
Lipid free dry body mass (mg individual^{-1}, log-scale)	20 °C vs. 25 °C	0.062	0.087	0.757
	20 °C vs. 30 °C	0.326	0.099	**0.003**
	25 °C vs. 30 °C	0.264	0.099	**0.021**

Fig. S7. Picture of the soil microcosms employed in the population response experiment after five weeks of incubation. Note that the microcosms were briefly put close together for the purpose of the picture, but then again they were set apart from each other during the experiment.
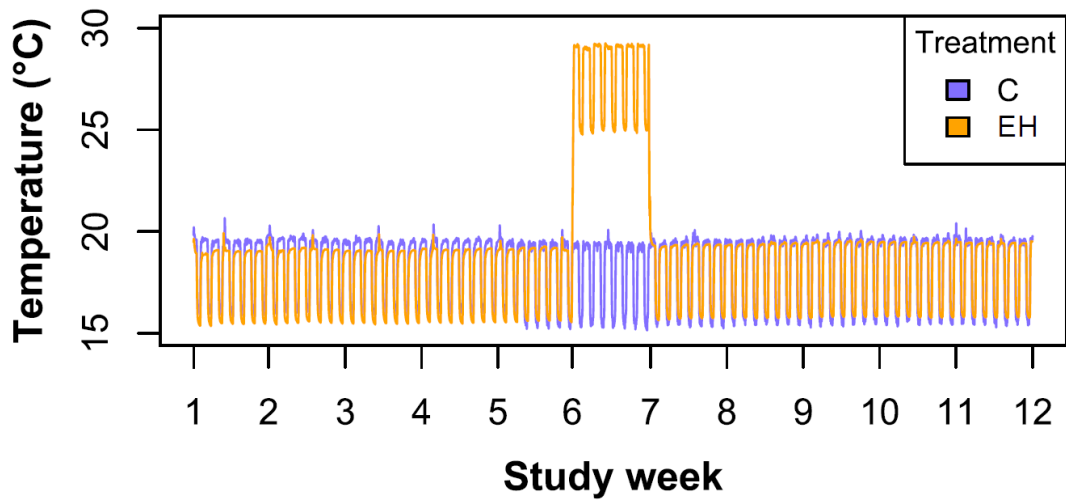
Fig. S8. Air temperatures in the climate rooms throughout the study period, monitored at 30-minute intervals. Colours indicate different experimental treatments: blue: control (C); orange: extreme heat (EH). Note the +10 °C temperature increase in the extreme heat treatment starting at week 6 until week 7.
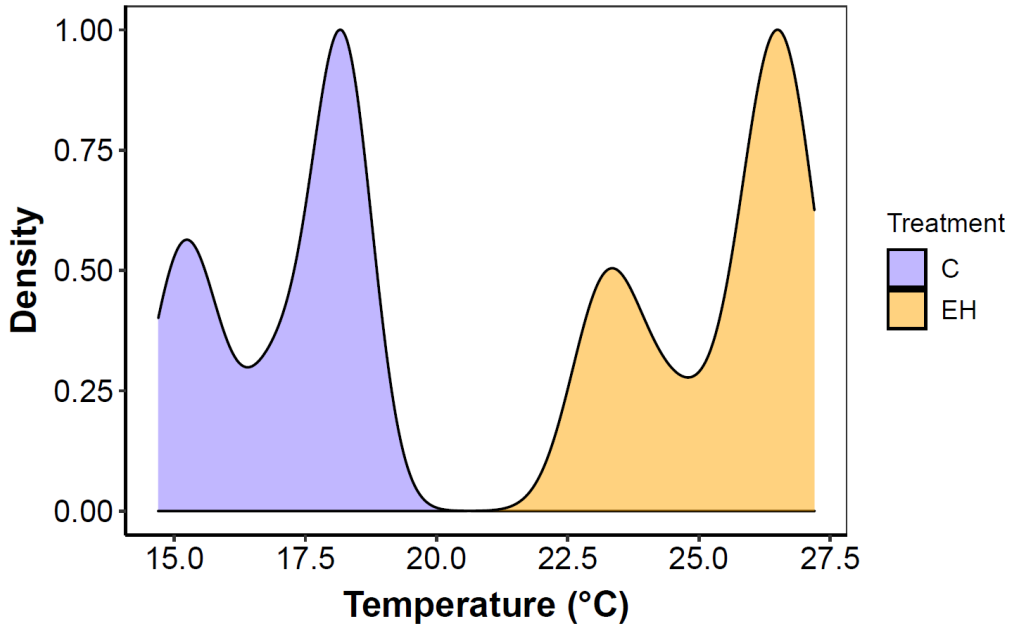
Fig. S9. Density plot displaying soil temperatures at 5 cm depth during the extreme heat event (study week 6). Colours indicate different experimental treatments: blue: control (C); orange: extreme heat (EH). Note that the different density peaks within experimental treatments reflect measurements taken to capture the night (measurement at 08.00-10.00h) and day (measurement at 14.00-18.00h) soil temperatures. We took five measurement replicates at every time point ($N = 12$) in each experimental treatment, giving a total of 120 data points of soil temperature.
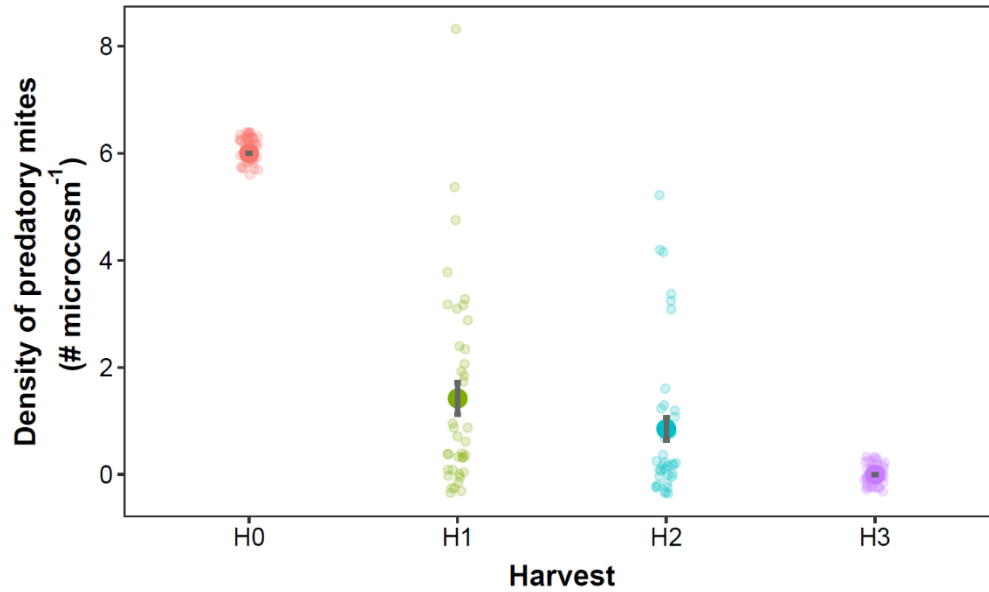
Fig. S10. Temporal dynamics of the densities of predatory mites (*Stratiolaelaps scimitus*) in the microcosms. "Harvest" specifies the different time points in which mite densities were assessed during the experiment: H0: start of the experiment; H1: baseline; H2: resistance; H3: recovery. Colours indicate different harvests. Solid points represent means, dark bars represent standard errors, and faded points are raw data. Note that the statistical significance of the differences in predatory mite densities across harvests was not assessed.

Table S8. List of fitted negative binomial models ranked by AIC used to evaluate the effect of the experimental treatments on densities across four study species of *Protaphorura*. Two harvests are shown: resistance (harvest 2, after extreme heat) and recovery (harvest 3, after one generation time from extreme heat). The model structure of all fitted models is provided. Interactions between species and temperature were included in all models, since testing different responses to temperature across species was a main question of the present study. Note that temperature treatments did not differ by the baseline harvest (harvest 1), so selecting models based on AIC might be misleading in this particular case.

Harvest	Model structure	AIC	Δ AIC
Resistance (harvest 2)	Species x Temperature + Predation	799.8	0
	Species x Temperature + Predation x Temperature	800.9	1.1
	Species x Temperature + Species x Predation	805.4	5.6
	Species x Temperature x Predation	810.9	11.1
Recovery (harvest 3)	Species x Temperature + Predation	1024.2	0
	Species x Temperature + Species x Predation	1026.1	1.9
	Species x Temperature + Predation x Temperature	1026.2	2.0
	Species x Temperature x Predation	1032.2	8.0

Table S9. Output of the negative binomial model used to evaluate the effect of the experimental treatments on densities across four study species of *Protaphorura* at each harvest: baseline (harvest 1, before extreme heat), resistance (harvest 2, after extreme heat) and recovery (harvest 3, after one generation time from extreme heat). Estimates, standard errors (SE) and p-values (*P*) of the contrasts between treatments for a given species are provided. Significant p-values (*P* < 0.05) are highlighted in bold. C: Control temperature, EH: Extreme heat, NP: No predator, P: Predator.

	Species	Treatment	Estimate	SE	*P*
	Collembola densities (log-scale)				
Baseline (harvest 1)	*P. armata*	C	4.360	0.201	0.110
		EH	3.903	0.203	
	P. fimata	C	4.945	0.200	0.825
		EH	4.882	0.200	
	P. tricampata	C	3.619	0.205	**0.027**
		EH	2.971	0.211	
	P. pseudovanderdrifti	C	3.567	0.229	0.362
		EH	3.270	0.232	
	Across species	NP	3.930	0.105	0.892
		P	3.950	0.105	
Resistance (harvest 2)	*P. armata*	C	4.388	0.223	0.611
		EH	4.548	0.222	
	P. fimata	C	5.543	0.233	0.697
		EH	5.418	0.221	
	P. tricampata	C	2.906	0.232	0.710
		EH	3.028	0.230	
	P. pseudovanderdrifti	C	3.662	0.238	0.538
		EH	3.460	0.227	
	Across species	NP	4.175	0.116	0.486
		P	4.063	0.113	
Recovery (harvest 3)	*P. armata*	C	5.797	0.160	0.450
		EH	5.968	0.160	
	P. fimata	C	6.566	0.168	0.799
		EH	6.506	0.168	
	P. tricampata	C	5.806	0.169	0.295
		EH	5.562	0.160	
	P. pseudovanderdrifti	C	5.512	0.153	**< 0.001**
		EH	4.748	0.162	
	Across species	NP	5.873	0.080	0.258
		P	5.743	0.082	

Table S10. Standardised effect sizes (Cohen's *d*) and 95% confidence intervals, resulting from the differences between the estimates of the control and extreme heat treatments shown in Table S9. Results are provided for the four *Protaphorura* study species in each population response (resistance and recovery). Note that values close to zero indicate no differences between treatments, and negative values indicate a population decline in response to extreme heat. Confidence intervals not overlapping zero are highlighted in bold.

		Standardised effect sizes (Cohen's *d*)		
	Species	Effect size	SE	95% Confidence Intervals
Resistance (harvest 2)	*P. armata*	0.146	0.287	-0.416, 0.708
	P. fimata	-0.114	0.293	-0.687, 0.460
	P. tricampata	0.111	0.298	-0.474, 0.695
	P. pseudovanderdrifti	-0.185	0.300	-0.772, 0.403
Recovery (harvest 3)	*P. armata*	0.157	0.208	-0.251, 0.566
	P. fimata	-0.056	0.219	-0.486, 0.374
	P. tricampata	-0.225	0.214	-0.645, 0.195
	P. pseudovanderdrifti	-0.704	0.205	**-1.105, -0.302**

Table S11. Output of quantile regression for the quantile 0.1 of the body size distribution (small individuals) from the four *Protaphorura* study species. Sample ID was used as a random intercept to account for clustered observations. Estimates, bootstrapped standard errors (SE) and p-values (*P)* of the contrasts between treatments for a given species are provided. Significant p-values (*P* < 0.05) are highlighted in bold. Note that body size data was centred and scaled (mean = 0, standard deviation = 1) to facilitate the estimation of model parameters. C: Control temperature, EH: Extreme heat, NP: No predator, P: Predator.

	Species	Treatment	Estimate	SE	*P*
		Quantile 0.1 of the body size distribution (centred and scaled)			
Baseline (harvest 1)	*P. armata*	C	-1.071	0.455	
		EH	-1.057	0.446	0.879
	P. fimata	C	-0.968	0.451	
		EH	-0.921	0.462	0.717
	P. tricampata	C	-1.163	0.462	
		EH	-1.169	0.473	0.941
	P. pseudovanderdrifti	C	-0.890	0.475	
		EH	-0.924	0.488	0.752
	Across species	NP	-0.986	0.467	0.134
		P	-1.055	0.450	
Resistance (harvest 2)	*P. armata*	C	-1.038	0.434	0.059
		EH	-1.221	0.429	
	P. fimata	C	-0.846	0.447	0.673
		EH	-0.743	0.439	
	P. tricampata	C	-1.297	0.477	0.685
		EH	-1.350	0.480	
	P. pseudovanderdrifti	C	-1.132	0.615	0.830
		EH	-1.074	0.471	
	Across species	NP	-1.119	0.462	0.410
		P	-1.056	0.449	
Recovery (harvest 3)	*P. armata*	C	-1.323	0.282	0.717
		EH	-1.379	0.299	
	P. fimata	C	-1.234	0.287	0.337
		EH	-1.482	0.352	
	P. tricampata	C	-0.952	0.291	0.495
		EH	-1.062	0.305	
	P. pseudovanderdrifti	C	-0.814	0.300	**0.022**
		EH	-0.357	0.275	
	Across species	NP	-1.050	0.285	0.702
		P	-1.101	0.253	

Table S12. Output of quantile regression for the quantile 0.5 of the body size distribution (medium-sized individuals) from the four *Protaphorura* study species. Sample ID was used as a random intercept to account for clustered observations. Estimates, bootstrapped standard errors (SE) and p-values (*P)* of the contrasts between treatments for a given species are provided. Significant p-values (*P* < 0.05) are highlighted in bold. Note that body size data was centred and scaled (mean = 0, standard deviation = 1) to facilitate the estimation of model parameters. C: Control temperature, EH: Extreme heat, NP: No predator, P: Predator.

	Species	Treatment	Estimate	SE	*P*
			Quantile 0.5 of the body size distribution (centred and scaled)		
Baseline (harvest 1)	*P. armata*	C	-0.335	0.145	
		EH	-0.459	0.144	0.458
	P. fimata	C	0.022	0.250	
		EH	-0.390	0.323	0.134
	P. tricampata	C	-0.359	0.157	
		EH	-0.331	0.237	0.896
	P. pseudovanderdrifti	C	0.447	0.206	
		EH	0.505	0.326	0.849
	Across species	NP	0.009	0.144	0.080
		P	-0.234	0.160	
Resistance (harvest 2)	*P. armata*	C	-0.291	0.095	0.086
		EH	-0.461	0.110	
	P. fimata	C	-0.103	0.142	0.735
		EH	-0.214	0.374	
	P. tricampata	C	-0.681	0.158	0.690
		EH	-0.747	0.170	
	P. pseudovanderdrifti	C	0.353	0.134	0.184
		EH	0.079	0.184	
	Across species	NP	-0.320	0.097	0.326
		P	-0.196	0.128	
Recovery (harvest 3)	*P. armata*	C	-0.077	0.189	0.395
		EH	-0.228	0.191	
	P. fimata	C	0.801	0.419	0.108
		EH	0.155	0.427	
	P. tricampata	C	-0.222	0.208	0.426
		EH	-0.401	0.241	
	P. pseudovanderdrifti	C	0.580	0.149	0.138
		EH	0.828	0.170	
	Across species	NP	0.129	0.167	0.614
		P	0.230	0.241	

Table S13. Output of quantile regression for the quantile 0.9 of the body size distribution (large individuals) from the four *Protaphorura* study species. Sample ID was used as a random intercept to account for clustered observations. Estimates, bootstrapped standard errors (SE) and p-values (*P)* of the contrasts between treatments for a given species are provided. Significant p-values (*P* < 0.05) are highlighted in bold. Note that body size data was centred and scaled (mean = 0, standard deviation = 1) to facilitate the estimation of model parameters. C: Control temperature, EH: Extreme heat, NP: No predator, P: Predator.

	Species	Treatment	Estimate	SE	*P*
			Quantile 0.9 of the body size distribution (centred and scaled)		
Baseline (harvest 1)	*P. armata*	C	0.907	0.196	
		EH	0.903	0.170	0.985
	P. fimata	C	1.244	0.323	
		EH	1.098	0.310	0.662
	P. tricampata	C	1.247	0.145	
		EH	1.249	0.218	0.993
	P. pseudovanderdrifti	C	2.072	0.169	
		EH	2.185	0.218	0.640
	Across species	NP	1.694	0.153	**0.001**
		P	1.032	0.146	
Resistance (harvest 2)	*P. armata*	C	0.745	0.296	0.162
		EH	0.448	0.373	
	P. fimata	C	1.367	0.366	0.879
		EH	1.430	0.627	
	P. tricampata	C	0.979	0.356	0.924
		EH	0.955	0.441	
	P. pseudovanderdrifti	C	2.064	0.424	0.280
		EH	1.818	0.463	
	Across species	NP	1.321	0.353	0.426
		P	1.131	0.417	
Recovery (harvest 3)	*P. armata*	C	0.787	0.351	0.890
		EH	0.812	0.387	
	P. fimata	C	1.100	0.461	0.772
		EH	1.011	0.602	
	P. tricampata	C	0.889	0.357	0.562
		EH	0.778	0.381	
	P. pseudovanderdrifti	C	1.645	0.411	0.199
		EH	1.854	0.412	
	Across species	NP	0.965	0.350	0.214
		P	1.254	0.445	

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Gisin, H. 1960*. Collembolenfauna Europas*. Museum D'Histoire Naturelle Genève.

Chapter 2

Density-independent population recovery after an extreme heat event in Collembola

Density-independent population recovery after an extreme heat event in Collembola

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Abstract

Population responses to climate extremes can vary with population size, often referred to as density-dependent responses. For instance, small populations could recover more slowly than larger populations due to positive density-dependence (Allee effects). Yet, we know little about density-dependent population responses in soil invertebrates, such as Collembola, to climate extremes. Here, using various densities of two Collembola species differing in their mode of reproduction (*Folsomia candida*: parthenogenetic; *Proisotoma minuta*: sexually reproducing), we examine their population resistance and recovery responses after an extreme heat event. We found that minor effects on resistance after the extreme heat event were followed by strong negative responses in *F. candida* (deviation of -90% population sizes relative to controls). However, these effects were independent of the initial population density of both species. Our findings suggest that the responses of soil invertebrate populations during and after extreme heat events may not be influenced by positive density-dependence.

Climate extremes, such as extreme heat events, can shrink populations and affect their dynamics over long periods (Harris *et al.* 2018). Populations may indeed recover after climate extremes have stopped, but predicting recovery trajectories remains challenging (Thakur *et al.* 2022). For instance, populations can encounter extreme heat events at very different densities, for instance, due to seasonal changes in abundance, variation in habitat quality, or prior exposure to other pulse disturbances. Yet, despite the potential role of population density in determining population responses after stress (McMullen *et al.* 2017), its importance is still relatively unknown in the context of climate extremes (Thakur *et al.* 2022). At very low densities, population growth following extreme events could be constrained as a result of positive density-dependence (Allee effects; Courchamp et al., 1999), underpinned by mechanisms such as genetic drift (Luque *et al.* 2016; Willi & Hoffmann 2009) or demographic stochasticity (Melbourne & Hastings 2008). Negative consequences of low population sizes could be further exacerbated if the effects of heat-induced physiological costs, such as loss of fecundity (Walsh *et al.* 2019), persist beyond the extreme event (Sales *et al.* 2021; Xie *et al.* 2023), increasing the likelihood of population extinction before reproduction is restored (Melbourne & Hastings 2008). In turn, larger populations could recover more quickly after extreme events due to greater trait variation (e.g., heat tolerance; Franken et al., 2018) and higher genetic diversity (Reusch *et al.* 2005), which increases the likelihood of including more tolerant and/or fast-growing genotypes within the population.

Here, we investigate density-dependence of population recovery using two species of Collembola, *Folsomia candida* Willem and *Proisotoma minuta* Tullberg (family Isotomidae), after an extreme heat event in experimental populations by manipulating initial densities. Given that collembolans are among the most abundant groups of terrestrial animals (Potapov *et al.* 2023; Rusek 1998), unravelling their density-dependent responses can help obtain general insights about the responses of ectotherms to heat extremes. Using a range of initial population densities whereby intraspecific competition should be minimal (i.e., several times smaller than the expected carrying capacity; Fig. 1a), we predicted that higher initial densities would bring populations closer to control conditions after an extreme heat event, indicative of stronger Allee effects in populations exposed to extreme heat. That is, we expect a steeper positive relationship

between initial density and density at recovery in populations exposed to extreme heat, compared to control treatments (Fig. 1b) (Muir *et al.* 2024). Moreover, we expected reduced population recovery (primarily influenced by fecundity in closed populations) compared to resistance (mainly affected by survival) after the extreme heat event (Hillebrand & Kunze 2020; Martínez-De León *et al.* 2024b), assuming that fecundity is generally a more heat-sensitive trait than survival (Bozinovic *et al.* 2020; van Heerwaarden & Sgrò 2021). Finally, we predicted that recovery in the parthenogenetic *Folsomia candida* would be less affected by initial density than in the sexually reproducing *Proisotoma minuta.* This is because at low densities, mate limitation should only constrain population growth in species with sexual reproduction (Courchamp *et al.* 2008), and because parthenogenetic reproduction is a strategy associated with rapid recolonization after disturbance (Siepel 1994),

We set up soil microcosms with monocultures of the two Collembola species, following Martínez-De León et al., (2024b). Briefly, microcosms made of fenced and bottom-meshed polypropylene pots (height: 7.5 cm and diameter: 8 cm) were filled with 100 g of autoclaved commercial soil and 500 mg of hay litter on the surface. After incubating the soils for five days at 20 °C, we added collembolans at different initial densities: 10, 20, 30, 40, or 50 adult individuals. The individuals were previously raised in batch cultures in incubators set at 20 °C, and fed with dry yeast before the experiments (details of the cultures in Marty et al., (2022)). The experimental populations were incubated at control conditions for one week, exposed to an extreme heat event for ten days, and allowed to recover at control conditions for five additional weeks until the end of the experiment (Fig. 1a). The recovery period of five weeks encompasses at least one generation time of both species at 20 °C (Martínez-De León *et al.* 2024a), thereby allowing for recruitment after the extreme event. We adopted a diel temperature cycle (8h:16h, constant darkness) set at 16-20 °C for control conditions and 26-30 °C for the extreme heat treatment. The extreme heat event (+10 °C above control conditions) was chosen based on temperatures known to impact egg viability, development and fecundity in both species (Mallard *et al.* 2020; Martínez-De León *et al.* 2024a). Due to thermal buffering in the soil, actual recorded soil temperatures at 5 cm depth were 25.4 ± 1.5 °C for the extreme heat treatment and 17.0 ± 1.4 °C for the control (further details of temperature measurements in Martínez-De León et al., (2024b)).

We assessed Collembola densities at three destructive harvests: before the extreme heat event (harvest 1, week 2 of the experiment), after the extreme heat event (harvest 2, week 3-4), and at the end of the experiment (harvest 3, week 9). All treatment combinations and harvests were replicated six times, resulting in a total of 360 experimental units: 5 densities x 2 temperature treatments x 2 Collembola species x 3 harvests x 6 replicates. In each harvest, collembolans were collected in glycol water solution (1:1) using heat extraction with gradual heating from 25 °C up to 55 °C for 7 days (Macfadyen 1961), and later stored in 70% ethanol.

Collembola densities were analysed using generalized linear models (GLM) with negative binomial distribution (R package *MASS*, v.7.3-60; Venables and Ripley, 2002). Fixed effects were initial density, extreme heat, species, and their three-way interaction. We decomposed population responses to extreme heat into their resistance and recovery components (e.g., Hillebrand et al., 2018), by fitting separate models for each response stage: baseline (harvest 1), resistance (i.e., harvest 2, at the end of the extreme heat) and recovery (i.e., harvest 3, five weeks after the extreme heat event). We modelled initial density as a continuous variable, and tested whether the intercepts and slopes of the regression lines describing the relationship between population density (at a given harvest) and initial density differed between control and extreme heat treatments, using the function *emtrends* (package *emmeans*, v.1.10.0; Lenth, 2024). In addition, we calculated Cohen's *d* standardised effect sizes at three densities (10, 30 and 50 individuals), using the functions *emmeans* and *eff_size*. Cohen's *d* effect sizes were calculated from the estimated differences between control and extreme heat treatments (obtained from the GLMs), divided by the pooled standard deviation (Lenth 2024). Finally, we visually compared relationships between population responses to extreme heat in both species by plotting standardised effect sizes of resistance against recovery. We note that the sign of resistance and recovery responses refers to deviations of population sizes (positive or negative) in the extreme heat treatment relative to the controls, not to the sign of population growth rates. Linearity assumptions of all the GLMs were tested and visually inspected with the DHARMa package (v. 0.4.6; Hartig, 2022). All statistical analyses were carried out in R (v.4.3.2; R Core Team, 2024).

We detected weak negative resistance responses in populations of *Folsomia candida* (difference in the intercepts of control vs. extreme heat ± SE (log scale): -0.788 ± 0.454; *P* = 0.082),

followed by more drastic negative recovery responses (-2.336 ± 0.396; $P < 0.001$; Fig. 1b; Table S1), with deviations from control populations of -90% (across densities). However, these effects were independent of their initial density (difference in the slopes of control vs. extreme heat ± SE; resistance: 0.009 ± 0.013; $P = 0.504$; recovery: 0.011 ± 0.012; $P = 0.325$; Fig. 1b). In other words, even if high initial densities (i.e., 50 individuals) in the extreme heat treatment yielded greater final population sizes at recovery (Fig. 1a), the relative difference compared to the control treatment was the same as in the low initial densities (i.e., 10 individuals).
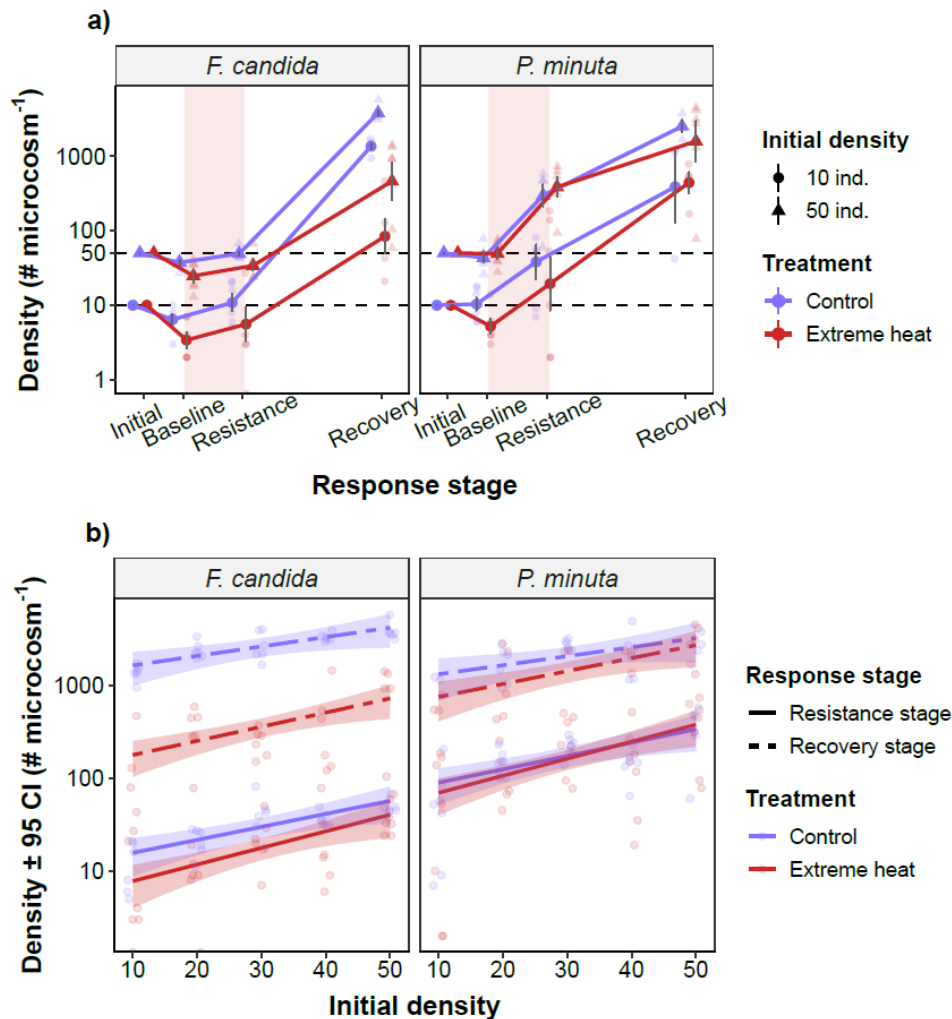

Fig. 1. (a) Temporal dynamics of Collembola densities (log-transformed) over the course of the experiment in the control (blue) and extreme heat treatments (red). Populations of *Folsomia candida* (left panel) and *Proisotoma minuta* (right panel) were established at different initial densities (10, 20, 30, 40, or 50 individuals), and then examined at three time points (response

stages): baseline (week 2; harvest 1; before the extreme heat event), resistance (week 3-4; harvest 2; after the extreme heat event) and recovery (week 9; harvest 3; five weeks after the extreme heat event). Solid points represent means, dark bars represent standard errors, and faded points are raw data. Only the lowest and highest values of initial densities are displayed: 10 individuals (circles) and 50 individuals (triangles). The red faded area indicates the period whereby the extreme heat event (+10 °C above control conditions) was applied. (b) Predicted density of Collembola populations (± 95% confidence intervals; CI) at the resistance (solid lines) and recovery (dashed lines) response stages, using initial density as a continuous variable in the models. Faded points represent raw data.

Populations of *Proisotoma minuta* were generally not affected by extreme heat, as their resistance and recovery responses were negligible (intercepts of control vs. extreme heat ± SE; resistance: -0.342 ± 0.414; P = 0.410; recovery -0.658 ± 0.460; P = 0.152; Fig. 1b; Table S1), and these effects were as well independent of the initial density (slopes of control vs. extreme heat; resistance: 0.009 ± 0.013; P = 0.465; recovery: 0.010 ± 0.014; P = 0.477; Fig. 1b). Overall, these findings were consistent when examining standardised effect sizes individually at different density levels (Fig. 2a), further illustrating that *F. candida* had much stronger negative recovery responses than *P. minuta*, with negligible resistance responses in both species (Fig. 2b).

Our findings suggest that the density of populations at the onset of an extreme heat event does not affect their recovery, as opposed to our initial hypothesis. We speculate that more intense extreme heat events could induce greater impacts on resistance and push low-density populations to even smaller sizes, thereby limiting their recovery and even leading to local extinction. Our results further highlight that population recovery can be more heat-sensitive than resistance, particularly in *Folsomia candida*, supporting previous findings in other Collembola species (Martínez-De León *et al.* 2024b)*.* This pattern is likely explained by the negative impacts of heat on reproduction (Walsh *et al.* 2019) that can persist even after the extreme heat event is over (both in *Proisotoma minuta* and *Folsomia candida*: Gremion et al., Unpublished data; Sales et al., 2021), and propagate at the population level after a time period necessary for recruitment

(Martínez-De León *et al.* 2024b). Furthermore, our findings corroborate that recovery after extreme heat is not affected by initial density in the parthenogenetic species *F. candida*, but given the lack of responsiveness of *P. minuta*, we could not determine whether density-dependent recovery plays a more influential role in sexually reproducing species, for example due to mate limitation.
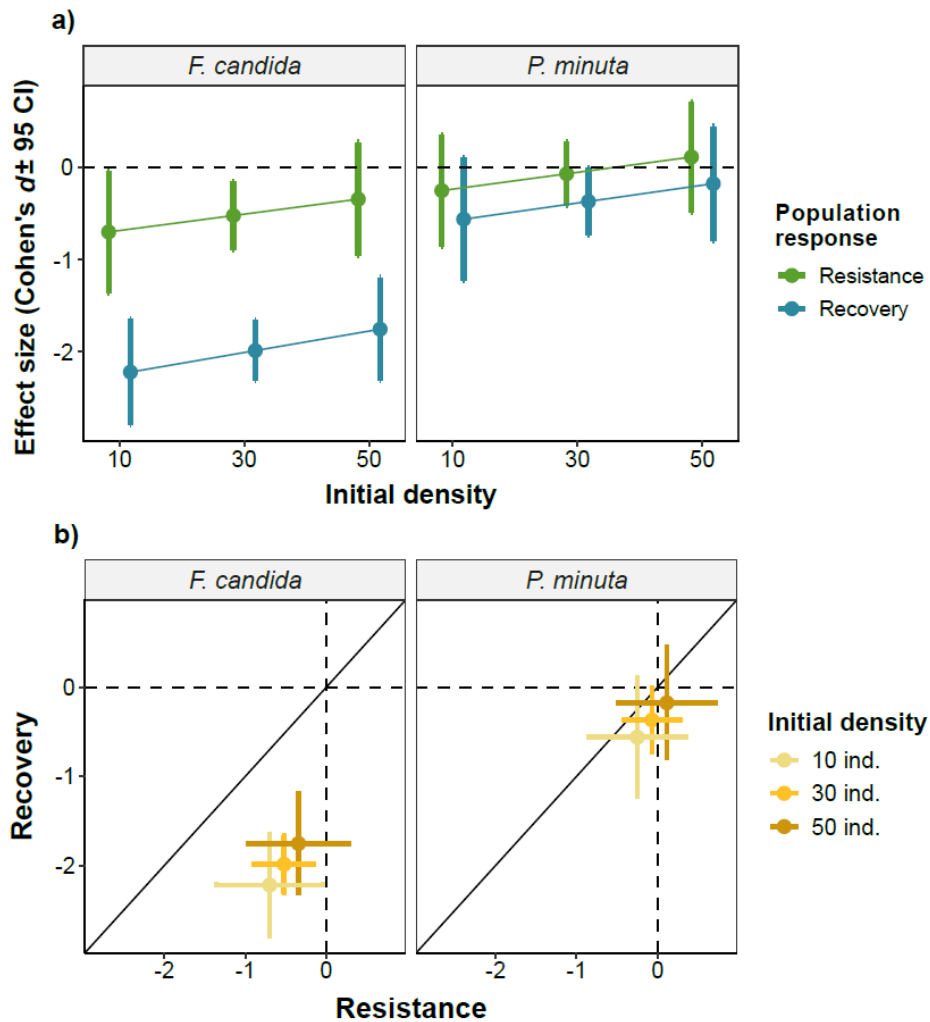

Fig. 2. (a) Standardised effect sizes (Cohen's *d*) with 95% confidence intervals (CI) representing the population responses (resistance and recovery) to the extreme heat event. Confidence intervals crossing zero values (dotted line) are statistically indifferent to zero. Negative values indicate a population decline in response to extreme heat, compared to the control treatment. Colours indicate different population responses: green: resistance; turquoise: recovery. (b) Resistance against recovery response, using standardised effect sizes with 95% CI. The solid

diagonal line shows the 1:1 relationship between resistance and recovery, and the dotted lines depict neutral resistance (vertical line) and recovery (horizontal line) responses. Colours indicate different initial densities: light yellow: 10 individuals; gold: 30 individuals; brown: 50 individuals. Only three densities are shown for visual clarity (across all densities, the results remain the same, e.g., Fig. 1b).

In conclusion, we showed that the population recovery of two Collembola species after an extreme heat event was independent of their initial densities, at least in the range of densities and temperature regimes used in our study. We propose that the role of initial density in population recovery should be further explored, especially considering population sizes approaching extinction. Furthermore, additional factors expected to affect density-dependent responses in populations after heat extremes could be examined, such as genetic and trait variation within populations (Luque *et al.* 2016; Willi & Hoffmann 2009), the functional response of predators (Gascoigne & Lipcius 2004), or the strength of intra- (Mallard *et al.* 2020) and interspecific competition (Muir *et al.* 2024).

Acknowledgements

Data availability

The complete dataset and R script used in this study are available in the Figshare repository: https://figshare.com/s/f48feac9c4edab771cc7.

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Supporting Information

Table S1. Output of the negative binomial GLM used to evaluate the effect of the experimental treatments (extreme heat and initial density) on the populations of *Folsomia candida* (intercept of the models) and *Proisotoma minuta* at each response stage: baseline (harvest 1, before extreme heat), resistance (harvest 2, after extreme heat) and recovery (harvest 3, five weeks after the end of extreme heat). Estimates, standard errors (SE), p-values (*P*), and the explained variance of the model (Zuur et al., 2009) are provided. Significant p-values (*P* < 0.05) are highlighted in bold.

	Parameter	Estimate	SE	*P*	Explained deviance (%)
Baseline (harvest 1)	(Intercept)	1.771	8.999	**< 0.001**	
	P. minuta	0.423	1.577	0.115	
	Initial density	0.039	7.045	**< 0.001**	
	Extreme heat	-0.071	-0.253	0.800	
	P. minuta x Initial density	0.001	0.147	0.883	65.11
	P. minuta x Extreme heat	-0.244	-0.631	0.528	
	Initial density x Extreme heat	-0.002	-0.240	0.811	
	P. minuta x Initial density x Extreme heat	0.004	0.361	0.718	
Resistance (harvest 2)	(Intercept)	2.429	0.306	**< 0.001**	
	P. minuta	1.730	0.423	**< 0.001**	
	Initial density	0.032	0.009	**< 0.001**	
	Extreme heat	-0.788	0.454	0.082	
	P. minuta x Initial density	0.001	0.013	0.946	67.94
	P. minuta x Extreme heat	0.447	0.615	0.467	
	Initial density x Extreme heat	0.009	0.013	0.504	
	P. minuta x Initial density x Extreme heat	0.000	0.018	0.991	
Recovery (harvest 3)	(Intercept)	7.160	0.272	**< 0.001**	
	P. minuta	-0.209	0.432	0.629	
	Initial density	0.023	0.008	**0.004**	
	Extreme heat	-2.336	0.396	**< 0.001**	
	P. minuta x Initial density	-0.001	0.013	0.936	58.03
	P. minuta x Extreme heat	1.678	0.606	**0.006**	
	Initial density x Extreme heat	0.012	0.012	0.325	
	P. minuta x Initial density x Extreme heat	-0.002	0.018	0.912	

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Chapter 3

Greater vulnerability of lowland communities to extreme heat events across seasons

Martinez-De León, G., Formenti, L., Salamon, J-A., & Thakur, M.P. Greater vulnerability of lowland communities to extreme heat events across seasons. In preparation.

Greater vulnerability of lowland communities to extreme heat events across seasons

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Abstract

Ecological responses to climate extremes vary drastically in different spatiotemporal contexts. The seasonal timing could be a major factor influencing community responses, but its importance is likely to vary across geographic gradients, such as at different elevations. Here, we tested how soil communities at high- and low-elevation sites respond to extreme heat events at different seasons (spring, summer and autumn). We simulated one-week heat events based on site-specific climatic history in a laboratory experiment using field-collected soil cores, and measured the resistance and recovery of two major groups of soil biota: Collembola and fungi. We found that collembolan communities from low elevations were most sensitive to extreme heat in spring and summer, with complete recovery only occurring in spring. Although fungal communities generally remained stable, pathogens increased and saprotrophs declined following extreme heat. We found increased connectance of positive associations between Collembola and fungi in lowland sites, suggesting that biotic effects may promote stability in recovering communities after heat extremes. Our findings highlight that extreme heat events can restructure ecological communities in lowlands, followed by seasonal-dependent recovery.

Keywords: Collembola, fungi, heat extremes, phenology, soil, recovery, resistance, stability

Introduction

Contemporary climate change is causing more frequent and severe extreme heat events, with significant ecological impacts (Harris *et al.* 2018; IPCC 2023; Ummenhofer & Meehl 2017). For instance, extreme heat can push organisms beyond their adaptive capacities, exceeding physiological thermal optima and causing drastic declines in their performance (Ma *et al.* 2020; Williams *et al.* 2016). Short-term vulnerability to extreme heat (i.e., resistance during and immediately after the event) is determined by the magnitude of thermal change experienced by an organism (i.e., exposure) and the fitness response to that amount of thermal change (i.e., sensitivity) (Buckley & Kingsolver 2021; Martínez-De León & Thakur 2024; Williams *et al.* 2008) . For example, thermal vulnerability varies across latitudinal gradients, with tropical and mid-latitude ectotherms being more susceptible to elevated temperatures. This increased vulnerability arises because, despite having similar heat tolerances to organisms from higher latitudes (Sunday *et al.* 2019), tropical and mid-latitude ectotherms experience temperatures closer to their thermal limits (Deutsch *et al.* 2008; Kingsolver *et al.* 2013). When scaling up from organismal to population and community levels, additional factors can influence thermal vulnerability (Louthan *et al.* 2021), such as the seasonal timing of heat events (Cinto Mejía & Wetzel 2023; Jentsch *et al.* 2007).

The ecological significance of the timing of extreme events depends on the degree to which heat-sensitive life-history processes (e.g., juvenile survival (Ma *et al.* 2018), reproduction (Walsh *et al.* 2019)) are exposed. Consequently, the impact of extreme heat will be greater when it coincides with key phenological periods (Cinto Mejía & Wetzel 2023; Forrest & Miller-Rushing 2010), influencing long-term ecological dynamics such as population recovery (Martínez-De León *et al.* 2024; Martínez-De León & Thakur 2024). For example, during reproductive periods, heat-induced impacts on adult survival can be compensated by enhanced recruitment (Cinto Mejía & Wetzel 2023), but such impacts may persist in the long term if additional breeding attempts are no longer possible (Pilakouta *et al.* 2023) (e.g., late in the reproductive period) or if recruitment is compromised (e.g., owing to reduced juvenile viability). Given that thermal vulnerability and phenology vary across geographic gradients (Louthan *et al.* 2021; Roslin *et al.* 2021), the ecological consequences of extreme heat events could differ depending on both the geographical

context and the seasonal timing. Yet, these important spatial and temporal ecological dimensions (i.e., geography and seasonal timing) have rarely been considered in comparative studies of thermal vulnerability, despite their potential to interactively influence short- and long-term ecological responses to extreme heat events.

Elevational gradients provide unique opportunities to examine variation in ecological responses to temperature changes (Sundqvist *et al.* 2013), including extreme heat events. Local climatic conditions vary radically across elevations as a result of temperature lapse rates (Körner 2007), and, in many temperate environments, due to orographic precipitation (Hodkinson 2005). These abiotic factors are key drivers of phenology at the site scale (Forrest & Miller-Rushing 2010), and thereby generate variation in phenological patterns across elevations (Hodkinson 2005). For instance, in temperate ecosystems, organisms living at high elevation sites have typically short activity periods condensed around the summer months (Forrest & Miller-Rushing 2010; Hodkinson 2005). In turn, organisms inhabiting low elevation sites have generally longer activity periods, only interrupted in dry summers and in the winter months. These distinct phenological patterns may underlie distinct periods of high thermal vulnerability and, therefore, the seasonal timing of extreme heat events is expected to exert distinct impacts across elevations. For example, at low elevations, very hot conditions can be attained in summer months, which are likely to cause significant impacts on survival (Buckley *et al.* 2021). However, low-elevation organisms may be able to evade the harsh conditions imposed by extreme heat if biological activities are reduced, owing to seasonal escape or induced diapause (Kefford *et al.* 2022; Sgrò *et al.* 2016). At higher elevations, summer represents a crucial period for the reproduction and recruitment of many species, but these processes could be compromised if temperatures attained during extreme heat events exceed the thermal limits of fertility or embryo viability (van Heerwaarden & Sgrò 2021; Walsh *et al.* 2019).

Within a given community, there is enormous variation across different taxa in their life-histories and thermal responsiveness (Berg *et al.* 2010; Franken *et al.* 2018). In belowground or soil communities, fungi are key drivers of ecosystem functioning (Delgado-Baquerizo *et al.* 2020b) and represent important resources for many invertebrate consumers, especially for microbivores such as Collembola (Pollierer & Scheu 2021; Potapov *et al.* 2016). Fungi form the base of the slow

energy channel in soil food webs (Moore & Hunt 1988; Thakur & Geisen 2019), and as such, fungal communities are often highly resistant to climate extremes (e.g., droughts) (de Vries *et al.* 2018), despite their slow recovery (de Vries *et al.* 2012). Given the overall stability of fungal communities to climate extremes, they might become readily available resources for recovering populations of invertebrate consumers like Collembola, thereby promoting overall food web stability (Bardgett & Caruso 2020). However, increasing severity of climate extremes (e.g., drought) (Cordero *et al.* 2023) could affect fungal responses in the long term, constraining the recovery of invertebrate consumers. In addition, climate-driven shifts in fungal communities could also result in increased dominance of fungal species that represent poor-quality resources (because of e.g., low palatability or nutritional value) (Sanders *et al.* 2024) or even pathogens (Delgado-Baquerizo *et al.* 2020a), further limiting the recovery of soil Collembola. The structure of association networks between Collembola and fungi can therefore yield additional insights into their responses to extreme heat events. Specifically, a higher prevalence of positive Collembola-fungal associations in recovering communities after extreme heat (i.e., more connectance, indicating more generalized associations) (Blüthgen *et al.* 2008; Petchey *et al.* 2010) could be expected, given an increased dependence of Collembola on their fungal resources to sustain their abundances. Correspondingly, negative Collembola-fungal associations could also become more prevalent during the recovery after extreme heat, indicating climate-driven increases in poor fungal resources and/or animal pathogens.

Here, we investigated how belowground communities respond to extreme heat events, using intact soil cores collected from temperate grasslands at two different elevations (spanning ~1000 m of altitude difference) and across three seasons (spring, summer, autumn) (Fig. 1). We exposed these field-collected soil cores to one-week extreme heat events in controlled laboratory conditions, and tracked the responses of two trophic levels (Collembola and fungi) at the end of extreme heat (i.e., resistance response) and after a five-week recovery period (i.e., recovery response) -representing the generation time of several Collembola species. We examined how the extreme heat events altered total abundances, species abundances (using joint species distribution models), diversity indices (by means of Hill numbers), and bipartite association networks of Collembola and fungi. Our hypotheses are (1) that heat events reaching higher

temperatures (e.g., low elevation sites in summer) will induce more negative responses, given that the thermal safety margins of organisms are narrower (i.e. closer to their thermal limits) and metabolic costs are greater at high absolute temperatures (Deutsch *et al.* 2008; Dillon *et al.* 2010). Moreover, (2) negative resistance responses (mainly caused by increased mortality in closed populations) are likely to be followed by negative recovery responses (largely determined by recruitment after the extreme event), particularly in the case of cold-adapted taxa (e.g., due to lower heat tolerances or reduced performance at high temperatures) (Martínez-De León *et al.* 2024). We further predict (3) stronger responses in Collembola species living deeper in the soil, given their greater sensitivity to thermal variation (van Dooremalen *et al.* 2013; Thakur *et al.* 2023). We finally hypothesize (4) that the structural properties of the association networks between Collembola and fungi will shift with extreme heat in the recovery response, resulting in increased connectance of positive (Petchey *et al.* 2010) and/or negative (Sanders *et al.* 2024) associations.
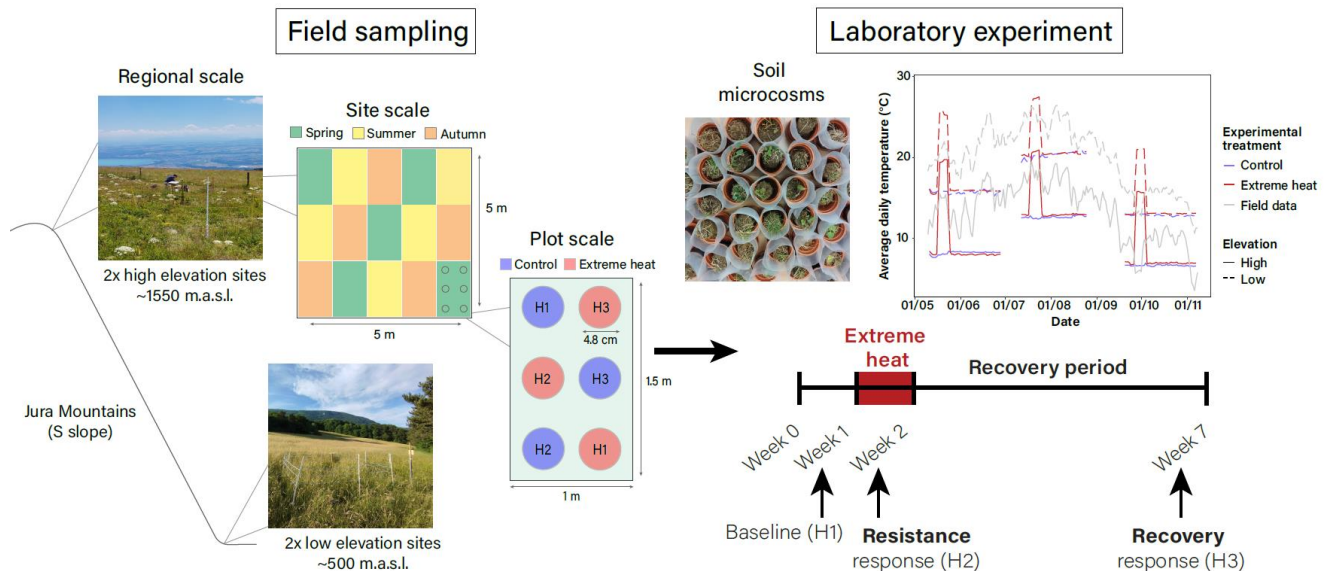

Fig. 1. Scheme of the experimental design of the study. We used a split-plot sampling design (left side of the figure), whereby samples (intact soil cores) were taken from two regional-scale blocks, each containing one high- and one low-elevation site. Sites were defined as a delineated 5 x 5 m area representative of the dry grasslands of the study region. Within sites and seasons (i.e., spring, summer, autumn), six soil cores were obtained from each of five 1 m x 1.5 m plots.

The sampling locations of data-level predictors (temperature regimes and harvests) were randomized within each plot, whereas the sampling locations of plot-level predictors (seasons) were kept constant in all sites to avoid the sampling from adjacent plots in the same season. The pictures displayed in the figure were taken in the summer season from high (above: Chasseron) and low (below: Onnens) elevation sites (Fig. S1). The colors of the plots (site scale) denote different sampling seasons: spring (green), summer (yellow) and autumn (orange). The circles shown at the plot scale represent the soil cores used as microcosms in the laboratory experiment (right side of the figure), which were allocated to one of two temperature treatments (control: blue; extreme heat: red) and one of three harvests (H1: baseline or harvest 1; H2: resistance or harvest 2; H3: recovery or harvest 3). All harvests were destructive, meaning experimental replications were true for each harvest. The size of the soil cores relative to the plot is enhanced for visualization purposes. Average daily soil temperatures (depth 3-5 cm) measured over the course of the laboratory experiments are shown, together with the temperatures recorded in the field sites during the same period (6 May – 9 November 2022). Mean temperatures from the two sites at the same elevation are displayed as grey lines; site-specific temperature values are provided in Fig. S3.

Methods

Field sites and experimental design

The study area was located in the Swiss Jura Mountains, consisting of two blocks (regions) located ca. 40 km apart (Fig. S1). Each block had two sites at different elevations: low (ca. 500 m.a.s.l.) and high elevation (ca. 1550 m.a.s.l.) (Fig. 1.; Fig. S1). The climate in the study area is temperate continental, with low elevations characterized by average yearly temperatures of 10.7 °C (monthly average of the coldest and warmest month: 1.8 °C and 20.1 °C, respectively) and 956 mm of annual precipitation (based on the weather station at 485 m.a.s.l.; Table S3). At high elevations, the average yearly temperature is 4.3 °C (monthly average of the coldest and warmest months: −2.8 °C and 12.1 °C, respectively) with 1396 mm of annual precipitation (based on the weather station at 1594 m.a.s.l.; Table S3). All sites were located in extensively managed dry

meadows representative of the study area, on south-facing slopes and with no recent soil disturbances (Table S1). We monitored soil temperatures (at 5 cm depth) at 30-min intervals throughout the duration of the study (6 May – 9 November 2022) using data loggers (HOBO Pendant® MX, Onset Computer Corporation, USA), and retrieved mean, minimum, and maximum daily temperatures at each of the study sites (Fig. S3).

Our experimental units were intact soil cores (diameter 4.8 cm, depth 5.5 cm; Vienna Scientific Instruments, Austria) obtained in 2022 at three different seasons: spring (6-9 May), summer (4-7 July) and autumn (13-16 September). We used a split-plot experimental design (Quinn & Keough 2002), composed by three grouping factors (block, site and plot), as well as predictors at the site level (elevation), at the plot level (season), and at the sample level (temperature regime and harvest (Schielzeth & Nakagawa 2013)) (Fig. 1). Within each site and season, we sampled five plots of 1.5 m x 1 m. We collected six soil cores from each plot, and randomly allocated them to the experimental treatments: one of the two temperature treatments (control conditions vs. extreme heat; details in Temperature treatments), and one of the three destructive harvests (details in Data collection). We therefore established a total of 360 experimental units: 2 elevations x 2 sites (nested within elevation) x 3 seasons x 5 plots (nested within season) x 2 temperature treatments x 3 harvests. With this sampling design, we aimed to capture large-scale variation in the composition of soil communities from different sites, hence enhancing the generality of our study, while minimizing small-scale variation by sampling all experimental treatment combinations within the same plot (Fig. 1).

Before all soil cores were sampled, we cut the vegetation at 5 cm from the ground level to avoid overcrowding when soil cores were later incubated in the laboratory. Immediately after collecting the soil cores, we stored them in polypropylene pots (height: 7.5 cm and diameter: 8 cm) with a 90 µm mesh at the bottom and a 5 cm high plastic fence (from the top of the pot), to minimize the escape of invertebrates from the pots while allowing for vegetation growth. The pots containing intact soil cores (hereafter referred as microcosms) were transported to the laboratory on the same day of field sampling, weighed, and allocated to lit incubators set at their respective temperature regimes (details in the next section; Table S3). The gravimetric soil water content at the time of sampling was determined by drying five additional soil samples at 70 °C

during at 48h (Table S2; Fig. S2). We maintained the same water content as in the time of sampling during the entire duration of the experiment (except in the extreme heat treatment during the week of the heat event; details in the following section), by weighing each microcosm every third day and adjusting evaporative losses with deionized water. In order to avoid keeping exceedingly dry soil conditions during the experiments, we made sure that the sampling of soil cores took place shortly after the occurrence of precipitation events in the field sites (> 5 mm during the previous week). Additionally, we took three soil cores across seasons to determine soil pH (Table S2), and one soil core to monitor soil temperature in the incubators over the course of the experiments (collected at random locations within plots).

Temperature treatments

Ambient (control) temperatures in the incubators were set to simulate the average climatic conditions in the field sites, and were therefore adjusted to the corresponding elevation and season of the samples. We retrieved climatic data of the reference period 2015-2020 from two representative weather stations (one for each elevation, Table S3). This time reference was chosen due to the increasing frequency of heat waves in the region, especially in recent years (CH2018 2018). Ambient conditions were defined as the mean average daily temperatures of the two months that our microcosms were incubated in the laboratory. For example, samples collected in spring were exposed to the average temperature conditions of May and June as the ambient temperature in our lab experiment for the entire experimental duration of this season. To simulate heat events that were statistically extreme in all elevations and seasons (CH2018 2018; IPCC 2023), we calculated the 99th percentile of average daily temperature across the reference period (Jentsch *et al.* 2007), and applied this temperature during seven consecutive days (Fig. 1). All ambient and extreme heat temperature values for each season and site are provided in Table S3. We additionally tested how our experimental extreme heat events compared to naturally occurring heat extremes in the field sites during the study period (details in Table S7).

To imitate typically dry conditions encountered during extreme heat events, microcosms allocated to the extreme heat treatment did not receive any water inputs during the week of the heat event, and water losses were compensated only at the start of the recovery phase (soil water content data shown in Fig. S2). All temperature regimes adopted a diel light and temperature cycle (8h night/ 16h day), with a 6 °C-amplitude between night and day (Table S3). Air temperature and humidity, light intensity and soil temperature (depth 3-5 cm; Fig. 1) were monitored in the incubators (SANYO MIR-253, Japan) at 30-min intervals (HOBO® MX Multi-Channel, Onset Computer Corporation, USA).

Data collection

After field sampling, all soil microcosms were acclimated for one week in the incubators at ambient temperatures. We collected data of soil-living communities of microarthropods (Collembola) and fungi across three harvests for each season. Each microcosm was accordingly allocated to one of three harvests: harvest 1 (week 2 after field sampling, before the extreme heat event), harvest 2 (week 3, immediately after the extreme heat event), and harvest 3 (week 8, after a five-week recovery period following the extreme heat event). At each harvest, we took a scoop of moist soil from the bottom of each microcosm (mean weight (g) ± SD: 8.55 ± 0.44) and stored it at -20 °C until extraction of fungal DNA (March-May 2023). Next, we extracted all microarthropods from the soil cores with gradual heating from 25 °C up to 55 °C for 7 days following the Macfayden extraction method (Macfadyen 1961). All animals were collected in glycol water solution (1:1) and later transferred to 70% ethanol.

Collembolans were sorted and identified to species level (details in Table S4). We retrieved information on the vertical stratification of Collembola species to explore how this trait mediates species responses to extreme heat. We assigned each species to one of three categories depending on their adaptations to occupy different depths of the soil profile: epedaphic (surface-living), hemiedaphic (living in litter and upper soil layers) and euedaphic (permanently living in the soil). The abundances and vertical stratification of all Collembola species are listed in Table S4.

Fungal ITS metabarcoding

Fungal DNA was extracted from 250 mg of bulk fresh soil using the Qiagen DNAeasy PowerSoil Pro Kit, following the manufacturer's instructions. We then carried out PCR-amplification targeting the primers 'TCCGTAGGTGAACCTGC' (forward) and 'GCATATCAATAAGCGGAGGA' (reverse), followed by amplicon sequencing of the full ITS region (ITS1-ITS2) with PacBio Sequel II instrument (Pacific Biosciences, USA). Libraries were loaded into three SMRTcells, each including five blanks and five controls (listed in Table S5). PCR and amplicon sequencing were conducted at the Next Generation Sequencing Platform of the University of Bern. Processing of the HiFi reads was performed with the pb-16S-nf pipeline (https://github.com/PacificBiosciences/HiFi-16S-workflow), which makes use of QIIME2 (Bolyen *et al.* 2019) and DADA2 (Callahan *et al.* 2016). Briefly, after demultiplexing, low-quality reads (<Q20) were discarded, primers trimmed (mean read length after processing: 670 bp), and denoised ASVs were obtained. Next, singletons and ASVs with less than five reads were filtered out, and taxonomical assignment with VSEARCH was performed using the UNITE QIIME release 9 (Abarenkov *et al.* 2023). We then merged the data from the different sequencing runs and retained only fungal ASVs agglomerated at the species level (R package phyloseq v. 1.48.0) (McMurdie & Holmes 2013). We also obtained the main trophic strategy of each fungal species (i.e., saprotroph, symbiotroph, pathogenic) using the package FUNGuildR v. 0.2.0.9000 (Nguyen *et al.* 2016). We selected the first annotated trophic strategy for those taxa with mixed trophic modes, and we only retained the trophic strategies assigned with "probable" and "highly probable" confidence (following Nguyen *et al.* (2016)), treating the remaining as "unassigned".

Data analyses: total abundances and diversity indices

All analyses were performed in R version 4.4.0 (R Core Team 2024). We tested how the effects of extreme heat on belowground communities were modulated by elevation and season, using the following three-way interaction model:

Eq. 1. *Response variable ~ Elevation x Season x Temperature treatment + (1 | Site)*

Where site (N = 4) was treated as a random factor in all models to control for non-independence among experimental units at each site (Schielzeth & Nakagawa 2013). All models were fitted separately for each experimental harvest: harvest 1 or baseline (H1), harvest 2 or resistance response (H2), and harvest 3 or recovery response (H3; Fig. 1). Linear models with univariate response variables were fitted with the R package glmmTMB v.1.1.9 (Brooks *et al.* 2017). Linearity assumptions (i.e., normality of residuals, overdispersion, zero-inflation, homogeneity of variance) were verified with the package DHARMa v.0.4.6 (Hartig 2022). We obtained marginal means and contrasts between control and extreme heat treatments using the emmeans package v.1.10.1 (Lenth 2024), and calculated conditional and marginal R^2 of the linear models (Nakagawa & Schielzeth 2013) with the r.squaredGLMM function from the package MuMIn v.1.47.5 (Bartón 2023).

Total Collembola abundances were analyzed with generalized linear mixed-effects models (GLMM) with negative binomial distribution (Eq. 1). We also employed negative binomial GLMMs to analyze the total number of reads for different groups of fungi according to their trophic strategy (saprotrophs, pathogens, symbionts and unassigned fungi), including the log-transformed number of reads as a covariate to control for variation in sequencing depth across samples (Leite & Kuramae 2020; Tedersoo *et al.* 2022). The diversity of Collembola and fungi was assessed by means of diversity profiles, obtained across three values of Hill numbers (order q): q = 0 (species richness), q = 1 (Shannon-Hill) and q = 2 (Simpson-Hill). The diversity profiles describe how the different diversity metrics change along a gradient of leverage of species' rarity, with lower values of q emphasizing the contribution of rare species, while higher values of q heighten the contribution of more common species (Roswell *et al.* 2021). We computed diversity estimates using coverage-based rarefaction and extrapolation to equalize samples (coverage value of 0.90 for Collembola, and 0.98 for fungi) with the iNEXT package v.3.0.1 (Chao *et al.* 2014; Hsieh *et al.* 2022). The resulting point estimates of diversity were tested using linear mixed models (Eq. 1) with Gaussian distribution. Before calculating the diversity indices, we applied an abundance cut-off to restrict the diversity analysis to samples with at least ten individuals (only needed for Collembola).

Data analyses: species abundances and association networks

Species abundances were evaluated using joint species distribution models (jSDMs) (Ovaskainen *et al.* 2017; Warton *et al.* 2015) within the Hierarchical Modelling of Species Communities framework (package Hmsc v.3.0-13) (Tikhonov *et al.* 2022), assuming default prior distributions (Ovaskainen & Abrego 2020). The ecological interpretation of the parameters estimated with the jSDMs is shown in Table 1. Block (*N* = 2) was added as a random effect in all fitted jSDMs to account for variation in species occurrences driven by their large-scale geographic distributions. We adopted a prevalence threshold of 25% to discard rare taxa (i.e., species occurring in less than 30 out of the 120 experimental units sampled at each harvest), which may provide low statistical power due to the scarcity of data (e.g., Burg *et al.* (2024)). In all jSDMs, we performed variance partitioning to extract the proportion of total variance explained by the experimental treatment (extreme heat), the natural variables (elevation and season), and the random effects (site and block). We build three sets of models with different groups of response variables: 1) the Collembola model, measuring responses of Collembola communities; 2) the fungal model, assessing responses of fungal communities; and 3) the Collembola-fungal models, examining associations between Collembola and fungi (details below). First, in the Collembola model, we used the log-normal Poisson distribution (analogous to negative binomial distribution) (Ovaskainen & Abrego 2020). We further modelled the influence of the species' traits on their abundance responses, by including the species' vertical stratification as a factor variable with three levels (epedaphic, hemiedaphic, and euedaphic). Second, in the fungal model, we accounted for zero-inflation, as typically encountered in sequencing data, by constructing a hurdle model that consisted of two parts: presence-absence (modelled with probit regression), and abundance conditional on presence (linear regression with normal distribution, using log-transformed and scaled counts). We further controlled for variation in sequencing depth by including the log-transformed number of reads as a covariate (Leite & Kuramae 2020; Tedersoo *et al.* 2022). We additionally included the fungal species' trophic strategy in the models as a factor variable with four levels (saprotrophs, symbionts, pathogens, and unsassigned), to examine how this trait can mediate fungal occurrence and abundance responses. The explanatory power of the jSDMs was evaluated by means of pseudo-R^2 (Collembola model), Tjur R^2 (presence-absence part

of the fungal model) and R^2 (abundance part of the fungal model) (Ovaskainen & Abrego 2020). MCMC convergence for all estimated parameters was assessed in terms of potential scale reduction factors (Table S6) (Gelman & Rubin 1992). All jSDMs were fitted with four chains of 250 samples each, yielding 1000 posterior samples in total. The thinning intervals and the number of samples used as burn-in were adjusted for the different models according to the amount required to achieve adequate model convergence (Table S6) (Collembola model: thinning 1,000 and burn-in 125,000; fungal models: thinning 300 and burn-in 37,500; Collembola-fungi association models: thinning 150 and burn-in 18,750).

Table 1. Ecological interpretation of the parameters from the joint species distribution models (jSDMs) used in our study. We test the effects of season, elevation, treatment, and their three-way interactions, on Collembola and fungal species abundances. In the schematic visualization, green and orange lines represent positive and negative parameter estimates, respectively, while grey lines represent estimates that lack statistical support (i.e., blank fields in Fig. 3).

Parameter	Ecological interpretation	Schematic visualization
Intercept	Species abundances in the treatment combination set as the intercept: spring, at high elevation, in the control treatment.	
Summer	Shifts in phenological abundance from spring to summer (relative to the intercept).	
Autumn	Shifts in phenological abundance from spring to autumn (relative to the intercept).	

Low elevation	Shifts in altitudinal-related abundance from high to low elevation (relative to the intercept).	
Summer x Low elevation Autumn x Low elevation	Given the phenological abundance shifts as described above, it shows whether this effect is modulated by elevation (in control treatment).	
EH (extreme heat; including all the interactions involved)	Effect of the extreme heat event, compared to their corresponding reference level in the control treatment.	

The third set of jSDMs (Collembola-fungal models) allowed us to estimate associations between Collembola and fungi, followed by the analysis of network properties (i.e., connectance) to summarize these associations at the network level. We focused this analysis on the recovery response to gain more robust and ecologically meaningful insights into the role of biotic effects in mediating responses to extreme heat. Resistance responses are primarily driven by abiotic effects of extreme heat on species' abundances, while recovery responses can be further influenced by biotic effects, such as associations with other species. This is because heat-driven changes in the abundance of one species (e.g., fungi) may take time to affect the abundance of a

second species (e.g., Collembola). We assume that our measurement of recovery (i.e., five weeks after the end of the extreme heat events) can generally capture such a time lag in disturbance effects across the two trophic levels (Jackson *et al.* 2021). For this analysis, we created separate subsets from the full dataset for each elevation, season and experimental treatment, resulting in twelve subsets, each containing ten replicate samples. We applied a prevalence threshold of 25% within each subset (i.e., discarding species occurring in fewer than three samples) for all Collembola and fungal species, as previously described. Due to the very low prevalence of Collembola species in summer at low elevation, we could not determine associations in this case. Next, we built the jSDMs using fungal species abundances as response variables (only abundances conditional on presence), while treating Collembola species abundances (log-transformed and scaled) as explanatory variables. We then retained the associations between Collembola and fungi with 95% posterior probability. These associations can be indicative of bottom-up regulation through feeding (positive associations) or repulsion (negative associations), but they should be interpreted with care, as they may also capture the signal of joint responses to unmeasured abiotic variables (Blanchet *et al.* 2020; Ovaskainen *et al.* 2017). Besides, the mismatch in the spatial scales at which Collembola and fungi were measured (see Data collection section) may lessen the statistical signal of their associations (Blanchet *et al.* 2020), particularly due to small-scale variation in fungal abundances within the soil cores (Erktan *et al.* 2020), although experimental replication partly accounts for this issue.

After fitting the jSDMs, we examined how the connectance of association networks (i.e., the ratio of the number of realized associations to the number of potential associations; May 1972) differed between control and extreme heat treatments, considering positive and negative associations separately. We used the resulting associations from the Collembola-fungi jSDMs, and visualized them using the igraph package v.2.0.2 (Csárdi *et al.* 2024). We then calculated the observed differences in network connectance between the experimental treatments, and generated null models to test how the observed differences diverged from random expectations. To do this, we produced 1000 permutations of each association matrix using the r2dtable algorithm (implemented in the package vegan v.2.6-4) (Oksanen *et al.* 2022), as this method keeps the matrix dimensions and marginal totals constant while allowing for variation in the

number of non-zero elements (i.e., number of Collembola-fungi associations), and hence connectance (Dormann *et al.* 2009). We then calculated differences in connectance between the random networks from control and extreme heat treatments, and compared these to the observed differences. To do so, we computed z-scores (Eq. 2), and obtained the corresponding p-values using two-tailed tests of population proportion.

$$\text{Eq. 2 } z = \frac{Observed\ connectance\ difference - Mean\ null\ connectance\ differences}{SD\ null\ connectance\ differences}$$

Results

Collembola communities: total abundance and diversity responses

Total Collembola abundances, diversity and species abundances were affected by extreme heat at low elevation in spring and summer, while the effects in autumn and at high elevation (across seasons) were negligible. At low elevation sites, Collembola abundances dropped in spring (-69%) and summer (-77%) at the resistance response. Remarkably, total Collembola abundances at low elevation recovered completely in spring, but significant deviations from control treatments persisted by the end of the recovery period in summer (-76%; Fig. 2, Table S8). Diversity metrics mirrored the responses of total Collembola abundances in spring at low elevation (i.e., negative resistance in all diversity metrics, e.g., -49% Shannon-Hill; followed by complete recovery), but not in summer, since diversity metrics were not affected by extreme heat in this case (Fig. S4). Negative recovery responses of Shannon-Hill and Simpson-Hill diversity were also detected at high elevation in autumn, although the magnitude of such responses was less notable (-23% Shannon Hill and -26% Simpson-Hill compared to control treatment; Fig. S4).
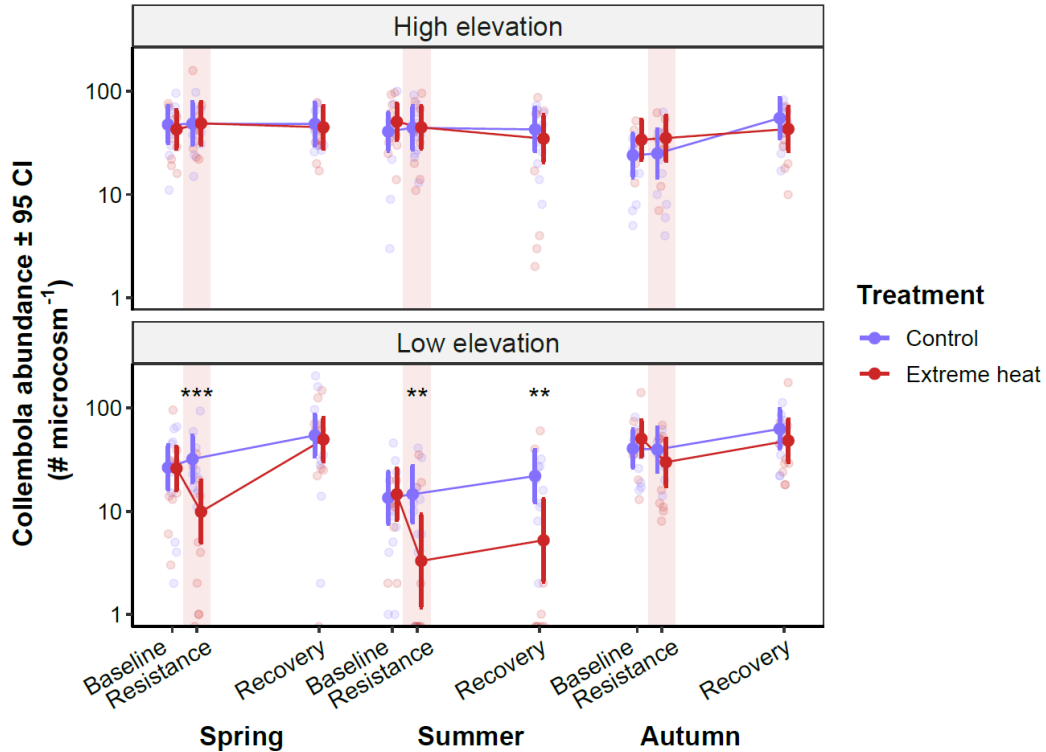
Fig. 2. Responses of total Collembola abundances to experimental extreme heat events across elevations and at different seasons. Estimated marginal means (± 95 confidence intervals) of Collembola abundance (log-transformed) are shown over the course of the experiments in spring, summer and autumn. The labels on the x-axis specify the different time points in which Collembola densities were assessed during the experiment (i.e., harvests): baseline (harvest 1); resistance (harvest 2); recovery (harvest 3). The faded red areas represent the one-week extreme heat events. Colours indicate different experimental temperature treatments: blue: control; red: extreme heat. Asterisks show significant differences between treatments at each harvest: **$P <$ 0.01, ***$P <$ 0.001. Full model outputs are provided in Table S8.

Collembola communities: responses of species abundances

Out of the nine Collembola species included in the analysis of species abundances (see Methods for the inclusion criteria), eight species showed negative resistance responses in spring at our low elevation sites (Fig. 3a). Later, most of them attained a complete recovery, except for *Protaphorura pseudovanderdrifti* and *Lepidocyrtus cyaneus* (Fig. 3b). Even though these species

occurred at both elevations, they were significantly lesser abundant at low elevation sites (Fig. 3; Fig. S5). The mean proportion of raw variance in species abundances explained by extreme heat increased from the baseline (pseudo-$R^2 = 0.06$) to the resistance response (pseudo-$R^2 = 0.10$), and was then maintained at recovery (pseudo-$R^2 = 0.09$; Fig. 3). Finally, the vertical stratification of Collembola species did not explain changes in species abundances driven by extreme heat (Fig. S5).
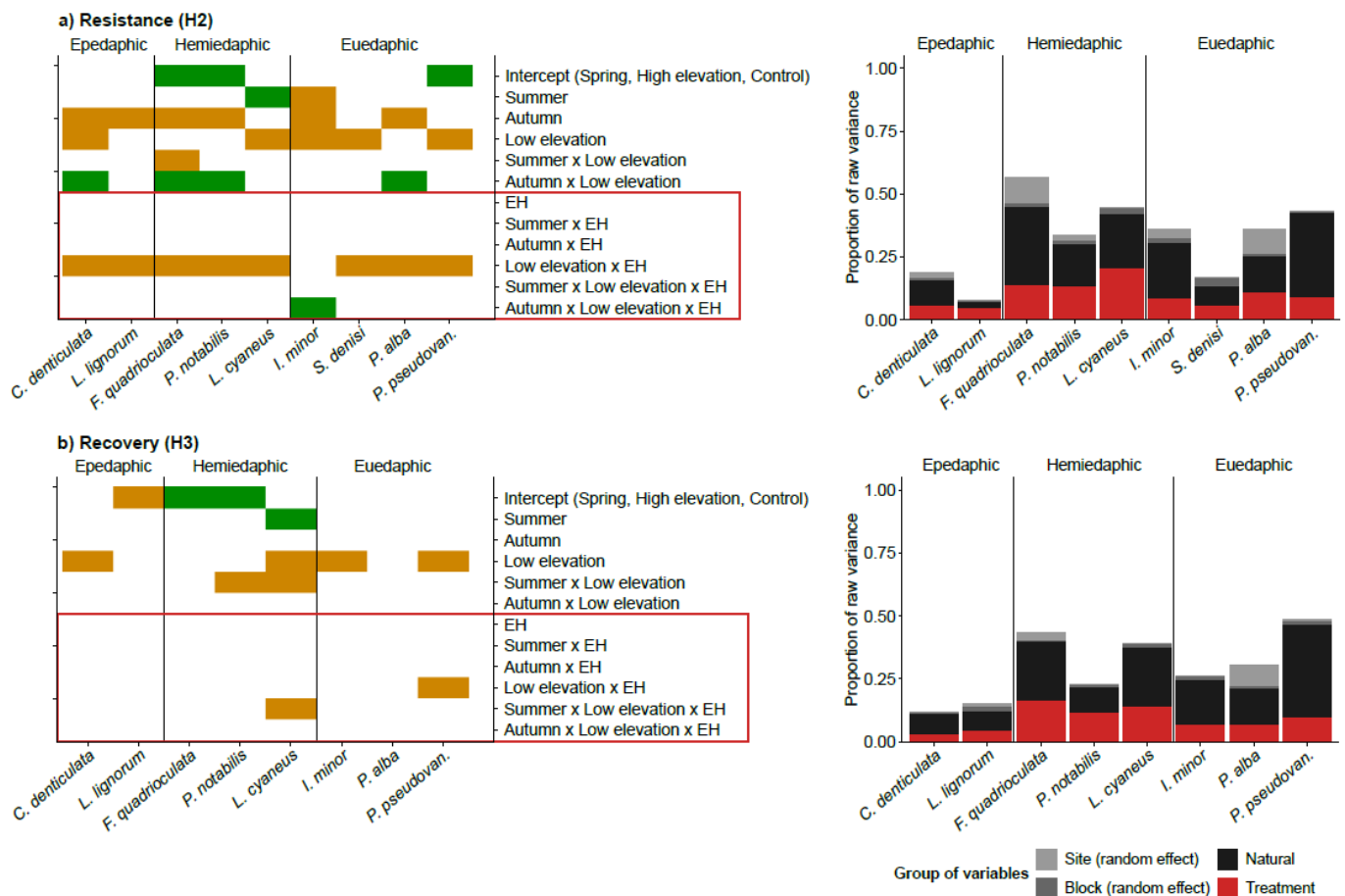

Fig. 3. Output of the joint species distribution models (jSDMs) fitted to investigate the responses of Collembola species abundances. We tested the effects of season, elevation, treatment, and their three-way interactions, in the resistance (a; harvest 2: H2; panels above) and the recovery response (b; harvest 3: H3; panels below). The results from the baseline response are provided in Fig. S5. Estimates from the beta parameters (left panels) show the

responses of species abundances (x-axis) to each of the model parameters (y-axis). Green and orange colors indicate positive and negative responses with 95% posterior probability, respectively, while blank spaces denote responses that lacked statistical support (should, therefore, be interpreted as neutral response). Species abundances at the intercept (spring, high elevation, control treatment) denote more abundant species in green, less abundant species in orange, and blank spaces indicating intermediate abundances (Table 1). Parameters enclosed within the red area represent species responses to the experimental treatment (extreme heat: EH; see Table 1 for an ecological interpretation of the model parameters). The proportion of raw explained variance (right panels) is provided for different groups of variables: random effects (site and block), natural variables (season and elevation), and treatment (containing the variance explained by all parameters influenced by extreme heat, shown within the red area of the left panels). Collembola species are ordered according to their vertical stratification across the soil profile: epedaphic (surface-living), hemi-edaphic (living in litter and shallow soil layers), and euedaphic (permanently living in the soil).

Fungal communities

Fungal communities generally remained stable in response to the extreme heat events across elevations and seasons, as extreme heat did not alter either fungal diversity (Fig. S7) or, in general terms, the occurrences and abundances of fungal species (Figs. S8-10). However, different fungal trophic groups exposed to extreme heat shifted in the recovery response in some cases: total saprotroph reads declined in autumn (-34%) (Fig. 4a) and non-significantly in spring and in summer at low elevation (Table S9), whereas pathogen reads increased markedly in summer at low elevation (+129%) (Fig. 4b; Table S10). Besides, total reads of unassigned fungi increased (+28%), while those of symbiotic fungi declined (-61%) in autumn at low elevation (Fig. S11). The occurrences of several pathogens exposed to extreme heat were higher at recovery (mainly in spring at low elevation, and in summer at high elevation), but not their species abundances (Fig. S10).
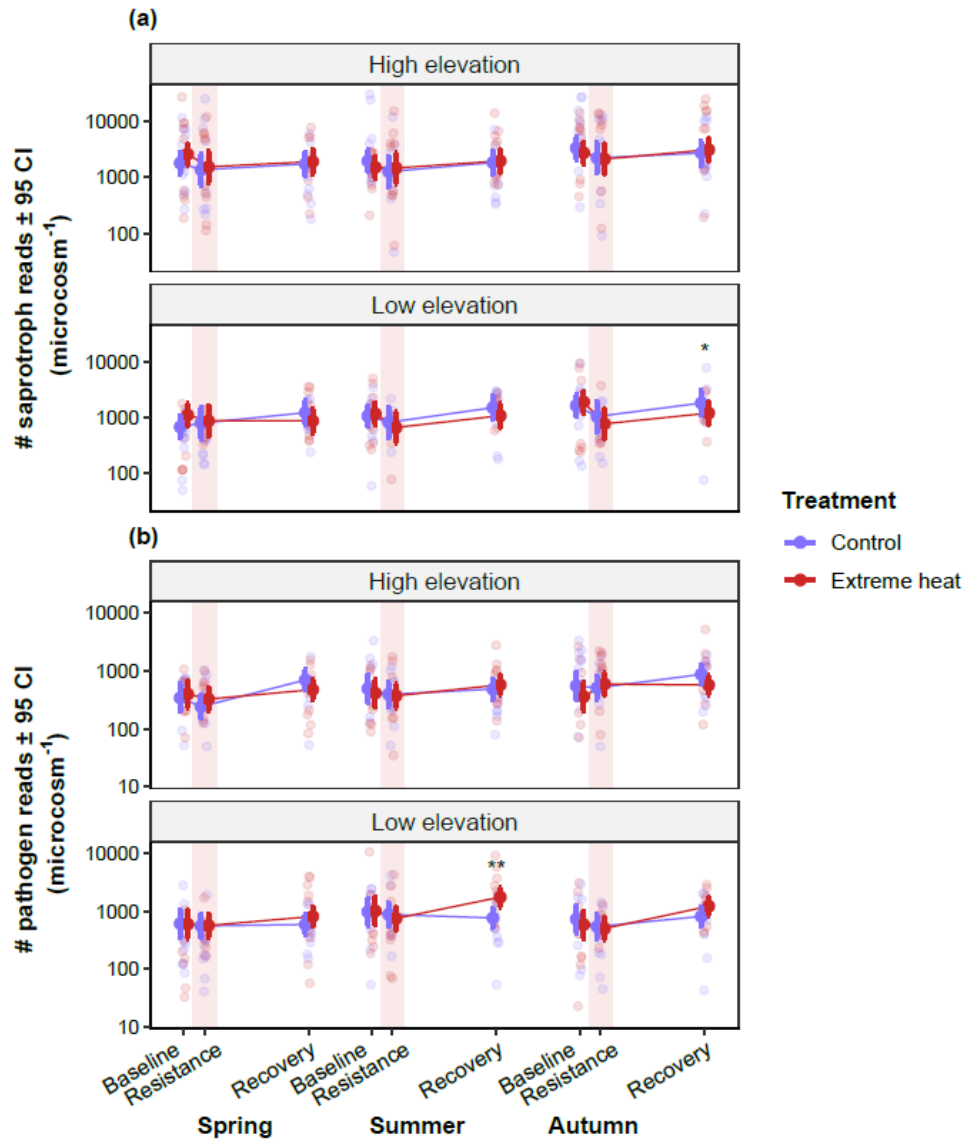
Fig. 4. Responses of saprotrophic and pathogenic fungi to experimental extreme heat events across elevations and at different seasons. Estimated marginal means (± 95 confidence intervals) of the number of reads (log-transformed) of saprotrophs (a; upper panel) and pathogenic fungi (b; lower panel) over the course of the experiments in spring, summer and autumn. The labels on the x-axis specify the different time points in which fungal metabarcoding reads were assessed during the experiment (i.e., harvests): baseline (harvest 1); resistance (harvest 2); recovery (harvest 3). The faded red areas represent the one-week extreme heat events. Colours indicate different experimental temperature treatments: blue: control; red: extreme heat. Stars show

significant differences between treatments at each harvest: *$P < 0.05$, **$P < 0.01$. Full model outputs are provided in Tables S8-S9.
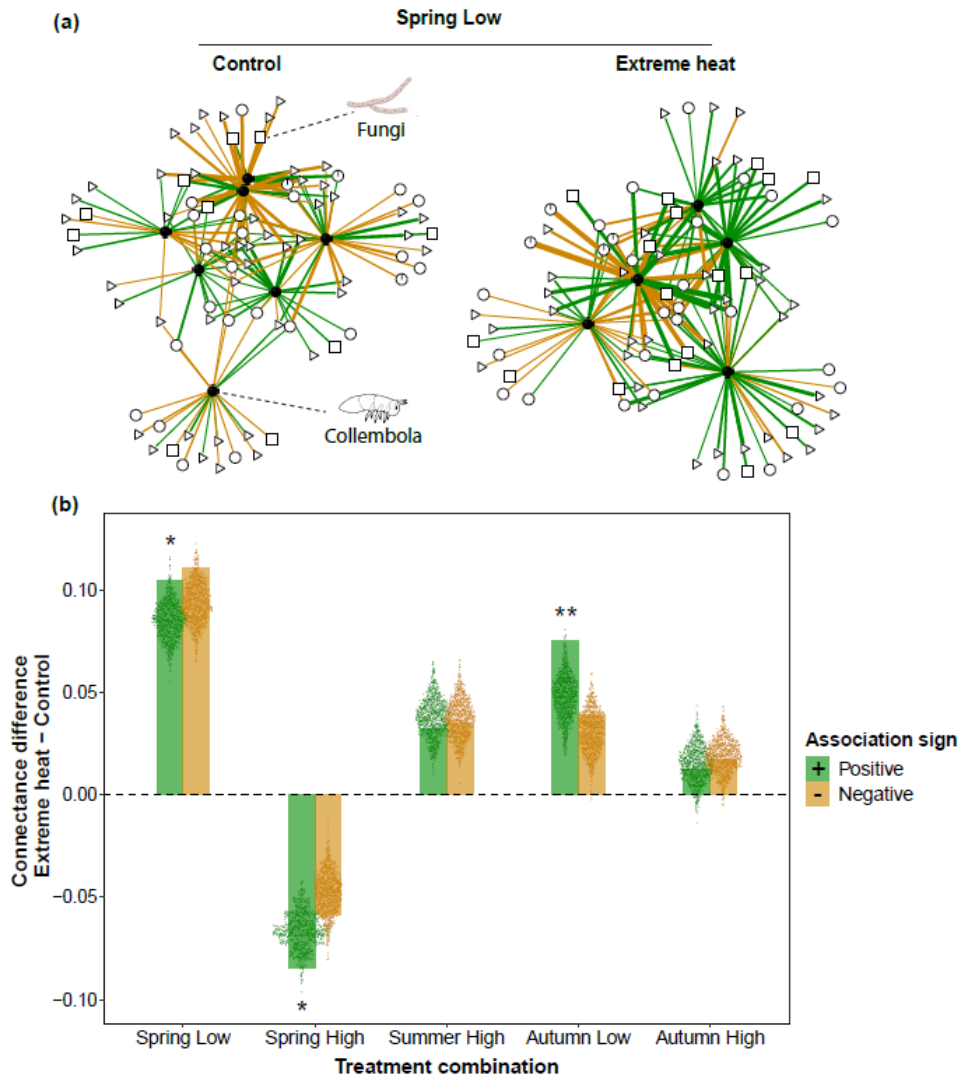

Fig. 5. Collembola-fungal association networks and connectance at the recovery response. (a) Comparison of Collembola-fungal association networks between control and extreme heat treatments. An example is shown from the association networks from spring at low elevation. Positive associations are displayed with green colors and negative associations are shown with orange colors. Black and white signs denote Collembola and fungal species, respectively. Different sign shapes represent various fungal trophic groups: saprotrophs (square), pathogens

(circle), symbionts (pie), and unassigned fungi (triangle). (b) The differences in connectance between extreme heat and control treatments were calculated, and tested against those differences obtained from null models. The height of the barplot shows the observed connectance, while the points display the connectance differences from the null models. Positive values indicate higher connectance in extreme heat treatments, whereas negative values denote higher connectance in control treatments. Stars show significant greater connectance differences between treatments compared to random expectations: *$P < 0.05$, **$P < 0.01$.

Collembola-fungal association networks at the recovery response

The connectance of Collembola-fungal association networks at recovery was altered in communities exposed to extreme heat, but only so for positive associations (Fig. 5). Compared to random expectations from null models, low elevation networks exposed to extreme heat had higher connectance of positive associations, both in spring and autumn (Fig. 5). By contrast, high elevation networks in spring had reduced connectance when experiencing extreme heat (Fig. 5).

Discussion

We found that belowground communities responded differently to experimental extreme heat events across elevations and seasons, as well as depending on the trophic group. Collembola communities were especially vulnerable to extreme heat events at low elevations, mainly in spring and summer. Fungal communities were in general stable to extreme heat events, with some marked exceptions for fungal saprotroph and pathogen species. Our results further revealed that extreme heat altered the connectance of Collembola-fungal positive associations during their recovery phase, with increased connectance at low elevations (spring and autumn) and decreased connectance at high elevations (spring).

Extreme heat events caused stronger ecological effects on low elevation communities

Low elevation belowground communities were disproportionally impacted by extreme heat compared to those at high elevation, especially in the case of Collembola. This finding supports known geographic patterns of organismal thermal vulnerability across latitudinal gradients (Louthan *et al.* 2021), demonstrating that organisms currently experiencing warm conditions or occasional hot periods (e.g., at low elevations) are prone to greater physiological and metabolic costs with further warming (Deutsch *et al.* 2008; Dillon *et al.* 2010; Kingsolver *et al.* 2013). In turn, organisms at high elevations have wider thermal safety limits because their heat tolerances remain constant across elevations (Sunday *et al.* 2014). The reason might be a lack of local adaptation in widely-distributed temperate species (Sunday *et al.* 2019; Tüzün & Stoks 2018), or alternatively, that high heat tolerances help to cope with radiation-driven thermal extremes occurring at higher elevations (Baudier *et al.* 2018; Buckley *et al.* 2013). Even though the abundances of Collembola at higher elevations remained unaltered by extreme heat, some typical highland species were impacted when they also occurred at lower elevations. For example, *Protaphorura pseudovanderdrifti* showed negative resistance and recovery responses to spring heat events*,* and *Lepidocyrtus cyaneus* had negative recovery in summer. Such negative recovery responses are likely explained by the deleterious impacts of heat on fecundity, as previously showed in laboratory populations of *P. pseudovanderdrifti* (Martínez-De León *et al.* 2024). These findings suggest that the elevational ranges of typical high-elevation species could shrink in response to extreme heat events, especially as warm-adapted species may recover better and therefore exclude other species closer to their thermal niche limits (Moore *et al.* 2023). Importantly, heat extremes of similar severity to those simulated in our experiment are already taking place occasionally (Table S7), underscoring the relevance of our findings for natural communities in the face of present-day and future heat extremes. One limitation of our results is that greater responsiveness in certain communities may have been explained by the lack of possibilities to behaviorally thermoregulate by moving deeper in the soil (Holmstrup & Bayley 2013; Sunday *et al.* 2014), given our soil cores. However, this limitation should not alter qualitatively our main insight, that is, that soil communities are more vulnerable to extreme heat events at lower elevations, especially for species at the edge of their thermal niches.

We also found that fungal communities remained generally unaltered in response to the experimental heat events. Given that soil fungi utilize nutrients relatively slowly, they represent the slow energy channel within soil microbial communities, which could help them to buffer pulse disturbances and confer them enhanced resistance to climate extremes (Bardgett & Caruso 2020). Indeed, it has been previously shown that soil fungal communities are generally robust to extreme heat and drought (Bei *et al.* 2023; de Vries *et al.* 2018), partly because water and nutrients can be redistributed from different parts of the fungal mycelium (Guhr *et al.* 2015). It is thus possible that fungal species are more responsive to heat exposure over longer timescales (seasonal to annual scales) (Sanders *et al.* 2024), when the effects of water scarcity and reduced resource availability (e.g., soil organic matter) may affect the composition of fungal communities (Pec *et al.* 2021). Nonetheless, certain trophic groups from low elevation fungal communities (i.e., saprotrophs and pathogens) responded clearly to the extreme heat events, mainly in the recovery response. In particular, saprotrophic fungi declined in response to extreme heat mainly in autumn, and similar non-significant trends were observed in spring and summer (Fig. 4a; Table S9). These findings are consistent with their global distribution patterns, as saprotrophs are more abundant in cold and wet regions with high soil carbon content (Feng *et al.* 2022). In contrast, fungal pathogens became much more abundant with extreme heat in summer (Fig. 4b; Table S10), partly because of increased occurrences of pathogen species (Fig. S10), corroborating previous findings showing that hotter conditions promote fungal pathogens at the global scale (Delgado-Baquerizo *et al.* 2020a).

Seasonal-dependent effects of extreme heat on low elevation communities

Extreme heat events had distinct effects on low elevation Collembola communities depending on whether they occurred in spring or summer. In these seasons, extreme heat generally affected collembolan survival, as revealed by their negative resistance responses. Remarkably, this was followed by a complete recovery of the abundances of most species in spring, indicating that their recruitment managed to compensate for the previous heat-induced mortality. Those individuals that survived the heat event may have benefited from reduced competition, allowing

for a higher fecundity and/or enhanced juvenile viability during the recovery period. By contrast, recovery remained incomplete in the summer season. However, we suspect that most species used a strategy of seasonal escape (Kefford *et al.* 2022), which implies that recruitment was possibly delayed until the end of a summer diapause period (Masaki 1980; Testerink 1983). The influence of pathogens could additionally explain the limited recovery of Collembola in summer, given that pathogenic fungi became more abundant in heat-exposed soils (Fig. 4), and were therefore more likely to infect Collembola hosts (Anslan *et al.* 2018). However, this possibility remains unclear, given that Collembola can exhibit high tolerance to various entomopathogenic fungi found in soils (Dromph & Vestergaard 2002).

In autumn, resistance and recovery responses to extreme heat events were negligible across elevations. As opposed to spring and summer, ecological responses to extreme heat in autumn are likely delayed for a much longer period than the recovery phase used in our study. Many species enter a period of reduced activity or complete dormancy before the onset of winter (Testerink 1983), especially at high elevations. During this period, non-feeding individuals need to endure metabolic costs that can become even greater during extreme heat events, leading to reduced survival after the winter diapause (Nielsen *et al.* 2022). It is thus plausible that our recovery responses could not capture the deleterious effects of autumn extreme heat events, which would require the measurement of post-winter or multiyear effects in controlled experiments (e.g., Cope *et al.* (2023)).

Moreover, we found that the vertical stratification of Collembola did not mediate their responses to the extreme heat events (Fig. S5), contrary to our hypothesis, since most species reacted similarly regardless of the soil depth that they inhabit. We speculate that the more mobile epedaphic species were less well represented in our soil samples (Table S4), blurring the potential variation among vertical stratification groups in their responses to extreme heat. Indeed, epedaphic species are expected to be much more tolerant to drier and hotter conditions (Ferrín *et al.* 2023), as opposed to the more sensitive euedaphic species (van Dooremalen *et al.* 2013; Thakur *et al.* 2023).

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Collembola-fungal associations

We show that the connectance of positive associations between Collembola and fungi increased in the extreme heat treatments at the recovery response at low elevation, namely in spring and autumn. We caution that these associations capture the statistical signature of the relationships between collembolan and fungal abundances, but not observed feeding interactions (as in e.g., Anslan *et al.* (2018)). Yet, given the dependence of Collembola on fungal abundances, the observed shifts in the structure of association networks can have plausible implications for the stability of soil communities. We suggest that the recovery of lowland Collembola communities after extreme heat might have been favored by feeding on a wider range of fungal resources (i.e., higher generality), or alternatively, by having their abundances more heavily regulated by the occurrence of their preferred fungal species (i.e., stronger bottom-up effects). Both processes would result in higher connectance of positive associations (Petchey *et al.* 2010) and are contingent on the high stability displayed by fungal communities. In line with our findings, a study using freshwater mesocosms exposed to experimental heatwaves showed that link-weighted connectance increased in recovering food webs, which was in turn associated to the recovery of total community biomass (Polazzo *et al.* 2023). Unlike the heat-exposed networks at low elevations, we observed reduced connectance of positive associations at high elevations in spring, contrary to our expectations. While this pattern suggests that the influence of heat events on the structure of ecological networks varies across geographic (Pellissier *et al.* 2018) and seasonal contexts (Yin & Rudolf 2024), the underlying processes are less well defined, especially as the abundance responses of Collembola and fungi at high elevations were negligible. We speculate that warming-driven increases in predation rates could have strengthened top-down control on Collembola abundances (Lang *et al.* 2014; Vucic-Pestic *et al.* 2011), thereby weakening the Collembola-fungal associations. In the case of negative associations, we did not detect any effects of extreme heat on their connectance. This suggests that possible heat-induced shifts in deleterious fungal species (e.g., poor resources or animal pathogens) were not severe enough to drive abundance changes in recovering Collembola communities. Overall, the results obtained from the analysis of Collembola-fungal association networks allowed us to generate

complementary insights into their responses to extreme heat events, suggesting a role of biotic effects in mediating the recovery of consumer communities after heat-induced declines.

Conclusions

As extreme heat events become more frequent and severe due to contemporary climate change, examining how distinct ecological settings differ in their degree of vulnerability has direct implications for our broad understanding of climate change effects on biodiversity. The findings from our comparative experiment, testing the impacts of extreme heat events in distinct spatiotemporal contexts (i.e., different elevations and seasons), corroborate that lowland communities are disproportionally sensitive to extreme heat, with stronger effects on invertebrate consumers (Collembola) than on their microbial resources (fungi). Notably, Collembola communities managed to recover in spring but not in summer, which emphasizes the importance of phenological processes in determining recovery after pulse disturbances like heat extremes. Despite the general stability of fungal communities, heat-induced shifts in the relative abundances of certain trophic groups could have cascading effects on other ecological processes (e.g., infection prevalence, decomposition of organic matter), especially if these changes prevail over longer timescales. Our study illustrates how depicting resistance and recovery to heat extremes in different spatiotemporal contexts and across trophic groups can contribute to draw a more complete picture of ecological stability in a changing world.

Data and code availability statement

The complete dataset and R scripts used in this study are available in the Figshare repository: https://figshare.com/s/6e97dcd9e93c64ff6b60.

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Supporting Information

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Table S1. Description of the field sites. All plots were located in extensively managed dry meadows (i.e. one hay cut per year occurring not before July 1st and/or low-intensity grazing, no inputs of fertilizer or irrigation), with no recent soil disturbances.

Location	Chasseral	Le Landeron	Chasseron	Onnens
Block	North	North	South	South
Elevation	High (1558 m)	Low (481 m)	High (1565 m)	Low (540 m)
Coordinates	47°07'43" N 7°02'52" E	47°03'39" N 7°03'49" E	46°50'58" N 6°32'18? E	46°50'49" N 6°41'07" E
Aspect	170° (S)	210° (SSW)	190° (S)	140° (SE)
Slope	6%	21%	10%	5%
Mowing (frequency, period)	Annually; August-September	Biannually; July-August	No mowing	Annually; July-August
Grazing (type, period)	Not grazed	Not grazed	Cow grazing in the past years, currently not grazed	Sheep grazing, October-November
Dominant plant species	*Carex nigra*, *Agrostis capillaris*, *Dactylis glomerata*	*Securigera varia*, *Bromus erectus*, *Carex* sp.	*Carex montana*, *Sanguisorba officinalis*, *Agrostis capillaris*	*Bromus erectus*, *Trisetum flavescens*, *Salvia pratensis*

Table S2. Description of soil physicochemical parameters at the time of field sampling (i.e., not exposed to subsequent incubation in the laboratory) across the three studied seasons (spring, summer, autumn). For soil pH, we measured $N = 3$ per site and across seasons. For bulk density and gravimetric water content, we measured $N = 5$ per site and season combination.

Site (block and elevation)	Season	Soil pH	Bulk density (g cm^{-3})	Gravimetric water content (%)
Chasseral (north high)	Spring	5.54 ± 0.67	0.60 ± 0.15	44.91 ± 3.94
	Summer		0.69 ± 0.11	36.10 ± 2.37
	Autumn		0.80 ± 0.20	36.70 ± 2.60
Le Landeron (north low)	Spring	7.91 ± 0.05	0.84 ± 0.13	24.47 ± 3.90
	Summer		0.95 ± 0.21	24.51 ± 1.93
	Autumn		0.89 ± 0.14	22.44 ± 1.07
Chasseron (south high)	Spring	5.10 ± 0.21	0.72 ± 0.12	44.00 ± 3.09
	Summer		0.68 ± 0.13	30.35 ± 2.86
	Autumn		0.61 ± 0.13	28.90 ± 4.55
Onnens (south low)	Spring	5.98 ± 0.25	1.19 ± 0.15	25.56 ± 1.30
	Summer		1.27 ± 0.05	15.29 ± 1.10
	Autumn		1.29 ± 0.16	20.35 ± 1.38

Table S3. Description of the experimental temperature regimes. Climatic data representative of high elevations was obtained from the weather station in Chasseral (47°07'54"N 7°03'16"E; 1596 m.a.s.l.), whereas for low elevation, we acquired data from the weather station in Neuchâtel (47°00'00"N 6°57'12"E; 485 m.a.s.l.). We retrieved air temperatures recorded at 2 m aboveground from the period 2015-2020 (source: Meteoswiss). Control temperatures were set as the average daily temperature over the reference period for every combination of elevation and season. To recreate extreme heat events for each elevation and season, we adopted the 99th percentile of daily temperatures across the reference period for spring (May-June), summer (July-August) and autumn (Spring-October). For both control and extreme heat temperature regimes, we included a diel light and temperature cycle (8h night/ 16h day), with a 6 °C-amplitude between night and day. C: Control temperature, EH: Extreme heat.

Elevation	Season	Temperature treatment	Average daily temperature (°C)	Daytime temperature (°C)	Nighttime temperature (°C)
High	Spring	C	8.8	10.8	4.8
		EH	20.5	22.5	16.5
	Summer	C	13.5	15.5	9.5
		EH	21.7	23.7	17.7
	Autumn	C	7.3	9.3	3.3
		EH	16.2	18.2	12.2
Low	Spring	C	16.5	18.5	12.5
		EH	26.6	28.6	22.6
	Summer	C	21.2	23.2	17.2
		EH	28.0	30.0	24.0
	Autumn	C	13.6	15.6	9.6
		EH	21.7	23.7	17.7

Table S4. Total Collembola species abundances (*N* = 360) and vertical stratification of the species across the soil profile: epedaphic (surface-living), hemiedaphic (living in litter and upper soil layers) and euedaphic (permanently living in the soil). The sources for the identification of Collembola species were Dunger & Schlitt (2011); Fjellberg (1998, 2007); Gisin (1960); Hopkin (2007); Thibaud *et al.* (2004). The vertical stratification of each Collembola species was extracted mainly from Gisin (1943), as well as Chauvat *et al.* (2014); Ferlian *et al.* (2015); Leinaas & Bleken (1983); Urbášek & Rusek (1994). The abundances of immature individuals that could not be assigned to a particular species are displayed at the bottom of the table.

Collembola species	Family	Vertical stratification	Total abundance
Folsomia quadrioculata	Isotomidae	Hemiedaphic	3502
Parisotoma notabilis	Isotomidae	Hemiedaphic	2867
Isotoma viridis	Isotomidae	Hemiedaphic	918
Isotomiella minor	Isotomidae	Euedaphic	890
Protaphorura pseudovanderdrifti	Onychiuridae	Euedaphic	863
Lepidocyrtus cyaneus	Entomobryidae	Hemiedaphic	820
Pseudosinella alba	Entomobryidae	Euedaphic	750
Lepidocyrtus lignorum	Entomobryidae	Epedaphic	461
Ceratophysella denticulata	Hypogastruridae	Epedaphic	351
Stenaphorura denisi	Tullbergiidae	Euedaphic	288
Sminthurinus signatus	Katiannidae	Hemiedaphic	251
Choreutinula inermis	Hypogastruridae	-	116
Sminthurinus aureus	Katiannidae	Epedaphic	48
Sphaeridia pumilis	Sminthurididae	Hemiedaphic	41
Neanura muscorum	Neanuridae	Hemiedaphic	20
Sminthurus viridis	Sminthurididae	Epedaphic	15
Orchesella flavescens	Orchesellidae	Epedaphic	5
Entomobrya multifasciata	Entomobryidae	Epedaphic	4
Pogonognathellus flavescens	Tomoceridae	Hemiedaphic	2
Heteromurus nitidus	Orchesellidae	Euedaphic	1

Immature individuals

Isotomidae			397
Hypogastruridae			191
Entomobryidae			132
Symphypleona			7

Table S5. List of the controls incorporated in the amplicon sequencing pipeline.

Type of control	Description	Reference
Blank	Buffers from the extraction kit; added at the extraction phase	https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/microbial-dna/dneasy-powersoil-pro-kit
Negative	Elution buffer: buffer used to dilute samples, primers and in MasterMix	https://www.pacb.com/wp-content/uploads/Procedure-Checklist-%E2%80%93-Amplification-of-Full-Length-16S-Gene-with-Barcoded-Primers-for-Multiplexed-SMRTbell-Library-Preparation-and-Sequencing.pdf
Negative	MasterMix	https://www.pacb.com/wp-content/uploads/Procedure-Checklist-%E2%80%93-Amplification-of-Full-Length-16S-Gene-with-Barcoded-Primers-for-Multiplexed-SMRTbell-Library-Preparation-and-Sequencing.pdf
Positive	ATCC MSA-1010	https://www.atcc.org/products/msa-1010
Positive	ZymoBIOMICS Microbial Community Standard	https://zymoresearch.eu/products/zymobiomics-microbial-community-dna-standard-ii-log-distribution

Table S6. Potential scale reduction factors for the parameters estimated in the joint species distribution models.

Model	Collembola					
Harvest	Baseline		Resistance		Recovery	
Parameter	Beta	Gamma	Beta	Gamma	Beta	Gamma
Min.	1.00	1.00	1.00	1.00	1.00	1.00
1st Qu.	1.00	1.00	1.00	1.00	1.00	1.00
Median	1.00	1.00	1.00	1.00	1.00	1.00
Mean	1.00	1.00	1.00	1.00	1.00	1.00
3rd Qu.	1.00	1.01	1.00	1.00	1.00	1.00
Max.	1.01	1.01	1.01	1.01	1.01	1.01
Model	Fungi (presence-absence)					
Harvest	Baseline		Resistance		Recovery	
Parameter	Beta	Gamma	Beta	Gamma	Beta	Gamma
Min.	1.00	1.00	1.00	1.00	1.00	1.00
1st Qu.	1.00	1.00	1.00	1.00	1.00	1.00
Median	1.00	1.00	1.00	1.00	1.00	1.00
Mean	1.00	1.00	1.00	1.00	1.00	1.00
3rd Qu.	1.00	1.00	1.00	1.00	1.00	1.00
Max.	1.02	1.01	1.02	1.01	1.01	1.01
Model	Fungi (abundance conditional on presence)					
Harvest	Baseline		Resistance		Recovery	
Parameter	Beta	Gamma	Beta	Gamma	Beta	Gamma
Min.	1.00	1.00	1.00	1.00	1.00	1.00
1st Qu.	1.00	1.00	1.00	1.00	1.00	1.00
Median	1.00	1.00	1.00	1.00	1.00	1.00
Mean	1.00	1.00	1.00	1.00	1.00	1.00
3rd Qu.	1.00	1.00	1.00	1.00	1.00	1.00
Max.	1.02	1.01	1.02	1.01	1.01	1.01

Model	Collembola-fungal models (only recovery)				
Parameter	Beta				
Treatment	Low spring control	High spring control	High summer control	Low autumn control	High autumn control
Min.	1.00	1.00	1.00	1.00	1.00
1st Qu.	1.00	1.00	1.00	1.00	1.00
Median	1.00	1.00	1.00	1.00	1.00
Mean	1.00	1.00	1.00	1.00	1.00
3rd Qu.	1.00	1.00	1.00	1.00	1.00
Max.	1.02	1.03	1.03	1.02	1.02

Table S6. (continuation)

Model	Collembola-fungal models (only recovery)				
Parameter	Beta				
Treatment	Low spring extreme heat	High spring extreme heat	High summer extreme heat	Low autumn extreme heat	High autumn extreme heat
Min.	1.00	1.00	1.00	1.00	1.00
1st Qu.	1.00	1.00	1.00	1.00	1.00
Median	1.00	1.00	1.00	1.00	1.00
Mean	1.00	1.00	1.00	1.00	1.00
3rd Qu.	1.00	1.00	1.00	1.00	1.00
Max.	1.04	1.03	1.02	1.02	1.02

Table S7. Output of model used to compare the average daily soil temperature (measured at 3-5 cm depth) in the extreme heat events simulated in the lab, against the hottest days recorded in the field sites during the study period (N = 6 days, per each elevation and season combination). This analysis was conducted to evaluate the severity of our experimental treatments compared to the natural variability of heat extremes in the field sites. We fitted a linear mixed effect model with the R package nlme v.3.1-163 (Pinheiro *et al.* 2023), accounting for heterogeneity of residuals by taking the origin of the data (field or lab) as an offset term, due to the greater variance of the data collected from the field compared to the temperature data from the lab experiments. We note that the year in which the study took place (2022) was one of the warmest on record in the area, exceeding the norm of monthly mean temperature of May-October by 2.3-2.5 °C on average (relative to the 1990-2010 reference period; source Meteoswiss).

			Average daily soil temperature (°C)			
Elevation	Season	Origin of data	Estimate	SE	P	Marginal/ Conditional R^2
High	Spring	Lab	19.60	0.33	0.060	
		Field	18.02	0.24		
	Summer	Lab	20.75	0.33	0.087	
		Field	19.44	0.26		
	Autumn	Lab	15.72	0.33	**0.011**	
		Field	11.87	0.26		
						0.993 / 0.998
Low	Spring	Lab	25.47	0.33	0.962	
		Field	25.49	0.24		
	Summer	Lab	27.23	0.33	0.322	
		Field	26.69	0.26		
	Autumn	Lab	21.03	0.33	**0.010**	
		Field	16.82	0.26		

Table S8. Output of the generalized linear mixed-effects model with negative binomial distribution used to evaluate the effect of the temperature treatments, modulated by elevation and season, on total Collembola abundances. Separate models were fit for each experimental harvest: baseline (harvest 1, before extreme heat), resistance (harvest 2, at the end of extreme heat) and recovery (harvest 3, five weeks after the end of extreme heat). Estimates, standard errors (SE), p-values (*P*) of the contrasts between temperature treatments, marginal and conditional R^2 (trigamma estimate) are provided. Significant p-values ($P < 0.05$) are highlighted in bold. Abbreviations of temperature treatment levels: C: Control temperature, EH: Extreme heat.

	Elevation	Season	Temperature treatment	Estimate	SE	*P*	Marginal/ Conditional R^2
			Total Collembola abundances (log-scale)				
Baseline (harvest 1)	High	Spring	C	3.86	0.21	0.651	0.263/0.305
			EH	3.76	0.21		
		Summer	C	3.71	0.22	0.356	
			EH	3.93	0.20		
		Autumn	C	3.18	0.25	0.218	
			EH	3.53	0.23		
	Low	Spring	C	3.28	0.25	0.949	
			EH	3.26	0.25		
		Summer	C	2.60	0.30	0.827	
			EH	2.68	0.29		
		Autumn	C	3.70	0.22	0.356	
			EH	3.92	0.20		
Resistance (harvest 2)	High	Spring	C	3.88	0.24	0.969	0.512/0.566
			EH	3.89	0.24		
		Summer	C	3.79	0.24	0.965	
			EH	3.80	0.24		
		Autumn	C	3.22	0.27	0.208	
			EH	3.56	0.25		
	Low	Spring	C	3.47	0.26	**<0.001**	
			EH	2.29	0.35		
		Summer	C	2.68	0.32	**0.007**	
			EH	1.19	0.52		
		Autumn	C	3.68	0.25	0.277	
			EH	3.40	0.27		

Table S8. (continuation)

	Elevation	Season	Temperature treatment	Estimate	SE	*P*	Marginal/ Conditional R²
			Total Collembola abundances (log-scale)				
Recovery (harvest 3)	High	Spring	C	3.88	0.24	0.787	0.356/0.387
			EH	3.80	0.24		
		Summer	C	3.76	0.25	0.503	
			EH	3.55	0.26		
		Autumn	C	4.01	0.23	0.378	
			EH	3.77	0.24		
	Low	Spring	C	4.00	0.23	0.747	
			EH	3.91	0.24		
		Summer	C	3.09	0.29	**0.005**	
			EH	1.65	0.47		
		Autumn	C	4.14	0.22	0.323	
			EH	3.88	0.24		

Table S9. Output of the generalized linear mixed-effects model with negative binomial distribution used to evaluate the effect of the temperature treatments, modulated by elevation and season, on the total number of metabarcoding reads of saprotrophic fungi. Separate models were fit for each experimental harvest: baseline (harvest 1, before extreme heat), resistance (harvest 2, at the end of extreme heat) and recovery (harvest 3, five weeks after the end of extreme heat). Estimates, standard errors (SE), p-values (*P*) of the contrasts between temperature treatments, marginal and conditional R^2 (trigamma estimate) are provided. Significant p-values (*P* < 0.05) are highlighted in bold. Abbreviations of temperature treatment levels: C: Control temperature, EH: Extreme heat.

	Elevation	Season	Temperature treatment	Estimate	SE	*P*	Marginal/ Conditional R^2
			Number of reads of saprotrophic fungi (log-scale)				
Baseline (harvest 1)	High	Spring	C	7.503	0.226	0.167	
			EH	7.870	0.225		
		Summer	C	7.586	0.226	0.319	
			EH	7.322	0.225		
		Autumn	C	8.120	0.232	0.471	
			EH	7.925	0.226		0.804/0.818
	Low	Spring	C	6.525	0.231	0.068	
			EH	7.013	0.239		
		Summer	C	6.974	0.225	0.730	
			EH	7.066	0.225		
		Autumn	C	7.401	0.225	0.562	
			EH	7.555	0.225		
Resistance (harvest 2)	High	Spring	C	7.223	0.322	0.581	
			EH	7.347	0.322		
		Summer	C	7.148	0.322	0.503	
			EH	7.297	0.322		
		Autumn	C	7.712	0.322	0.843	
			EH	7.667	0.323		0.720/0.820
	Low	Spring	C	6.680	0.327	0.680	
			EH	6.775	0.322		
		Summer	C	6.710	0.322	0.351	
			EH	6.502	0.322		
		Autumn	C	6.953	0.325	0.195	
			EH	6.652	0.322		

Table S9. (continuation)

	Elevation	Season	Temperature treatment	Estimate	SE	P	Marginal/ Conditional R^2
Recovery (harvest 3)	High	Spring	C	7.465	0.250	0.616	0.742/0.817
			EH	7.564	0.249		
		Summer	C	7.524	0.250	0.735	
			EH	7.591	0.249		
		Autumn	C	7.917	0.248	0.490	
			EH	8.054	0.252		
	Low	Spring	C	7.112	0.250	0.092	
			EH	6.775	0.249		
		Summer	C	7.320	0.249	0.089	
			EH	6.986	0.249		
		Autumn	C	7.511	0.253	**0.038**	
			EH	7.094	0.249		

Number of reads of saprotrophic fungi (log-scale)

Table S10. Output of the generalized linear mixed-effects model with negative binomial distribution used to evaluate the effect of the temperature treatments, modulated by elevation and season, on the total number of metabarcoding reads of pathogenic fungi. Separate models were fit for each experimental harvest: baseline (harvest 1, before extreme heat), resistance (harvest 2, at the end of extreme heat) and recovery (harvest 3, five weeks after the end of extreme heat). Estimates, standard errors (SE), p-values (*P*) of the contrasts between temperature treatments, marginal and conditional R^2 (trigamma estimate) are provided. Significant p-values (*P* < 0.05) are highlighted in bold. Abbreviations of temperature treatment levels: C: Control temperature, EH: Extreme heat.

			Number of reads of pathogenic fungi (log-scale)				
	Elevation	Season	Temperature treatment	Estimate	SE	*P*	Marginal/ Conditional R^2
Baseline (harvest 1)	High	Spring	C	5.845	0.277	0.606	0.649/0.689
			EH	5.997	0.278		
		Summer	C	6.211	0.277	0.544	
			EH	6.034	0.276		
		Autumn	C	6.327	0.280	0.136	
			EH	5.892	0.279		
	Low	Spring	C	6.417	0.280	0.945	
			EH	6.396	0.287		
		Summer	C	6.887	0.277	0.906	
			EH	6.922	0.276		
		Autumn	C	6.602	0.277	0.449	
			EH	6.377	0.280		
Resistance (harvest 2)	High	Spring	C	5.497	0.235	0.272	0.643/0.671
			EH	5.795	0.235		
		Summer	C	5.983	0.234	0.837	
			EH	5.928	0.234		
		Autumn	C	6.241	0.233	0.571	
			EH	6.394	0.236		
	Low	Spring	C	6.326	0.239	0.952	
			EH	6.342	0.235		
		Summer	C	6.781	0.235	0.560	
			EH	6.625	0.240		
		Autumn	C	6.301	0.234	0.774	
			EH	6.224	0.233		

Table S10. (continuation)

	Elevation	Season	Temperature treatment	Estimate	SE	P	Marginal/ Conditional R^2
				Number of reads of pathogenic fungi (log-scale)			
Recovery (harvest 3)	High	Spring	C	6.557	0.199	0.151	0.656/0.659
			EH	6.172	0.193		
		Summer	C	6.195	0.198	0.541	
			EH	6.365	0.204		
		Autumn	C	6.782	0.193	0.114	
			EH	6.353	0.197		
	Low	Spring	C	6.383	0.194	0.241	
			EH	6.703	0.198		
		Summer	C	6.642	0.196	**0.002**	
			EH	7.471	0.196		
		Autumn	C	6.704	0.193	0.126	
			EH	7.110	0.193		
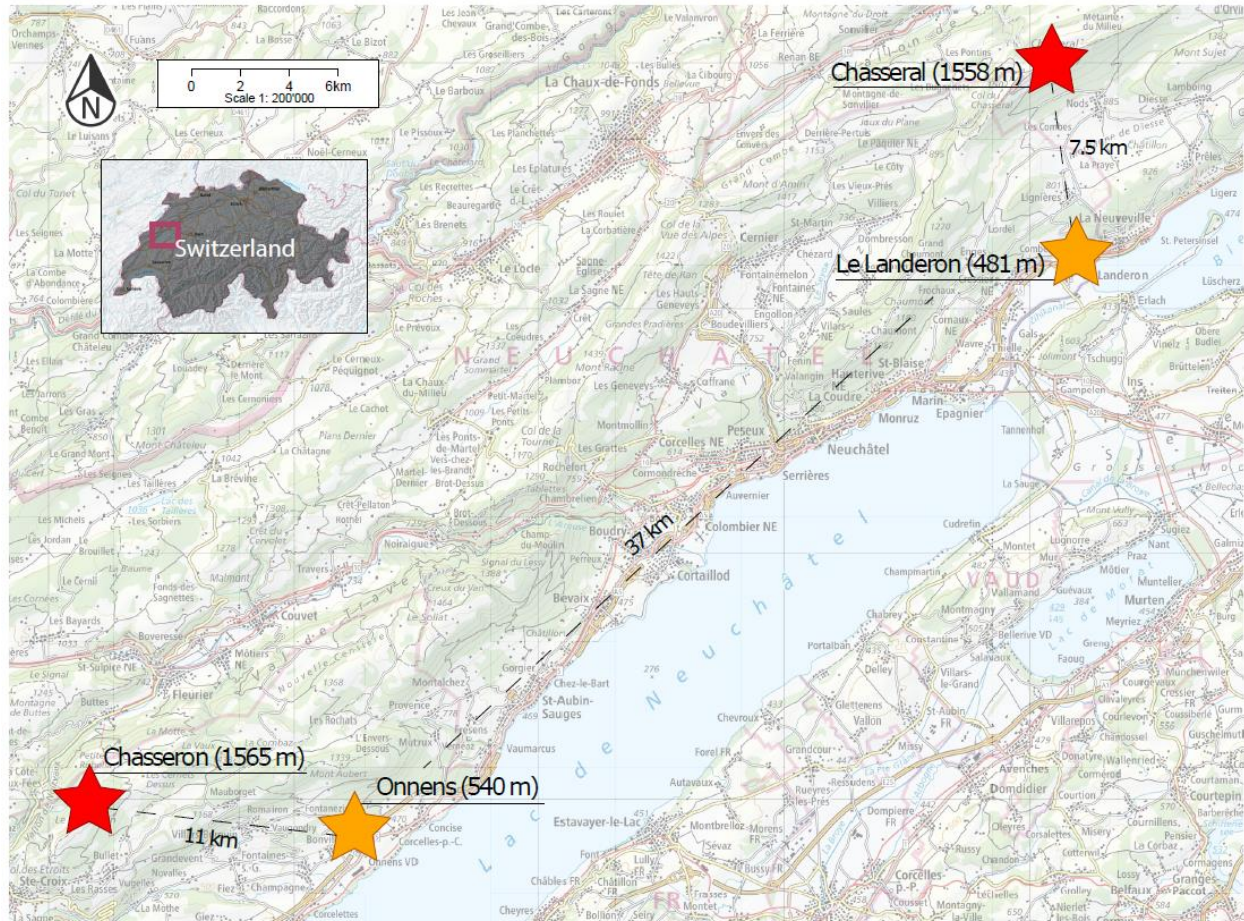
Fig. S1. Map of the study area showing the geographic position and elevation of the sampling sites, indicated with star signs. The shortest distance between sites of different blocks (north: Chasseral and Le Landeron; south: Chasseron and Onnens), as well as the distance between sites of the same block, are provided. Stars' colors indicate sites at different elevations: red: high elevation; orange: low elevation. Map adapted from https://map.geo.admin.ch.
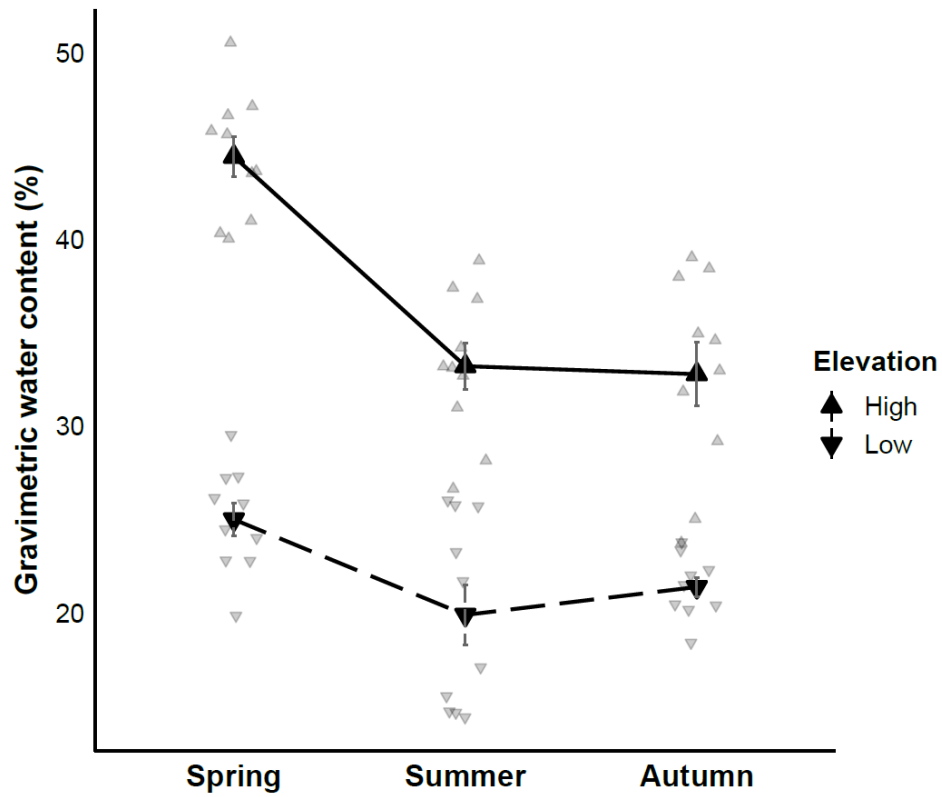
Fig. S2. Gravimetric soil water content, measured immediately after field sampling. Solid black points represent means, grey bars represent standard errors, and faded points are raw data (*N* = 10 per each elevation and season combination).
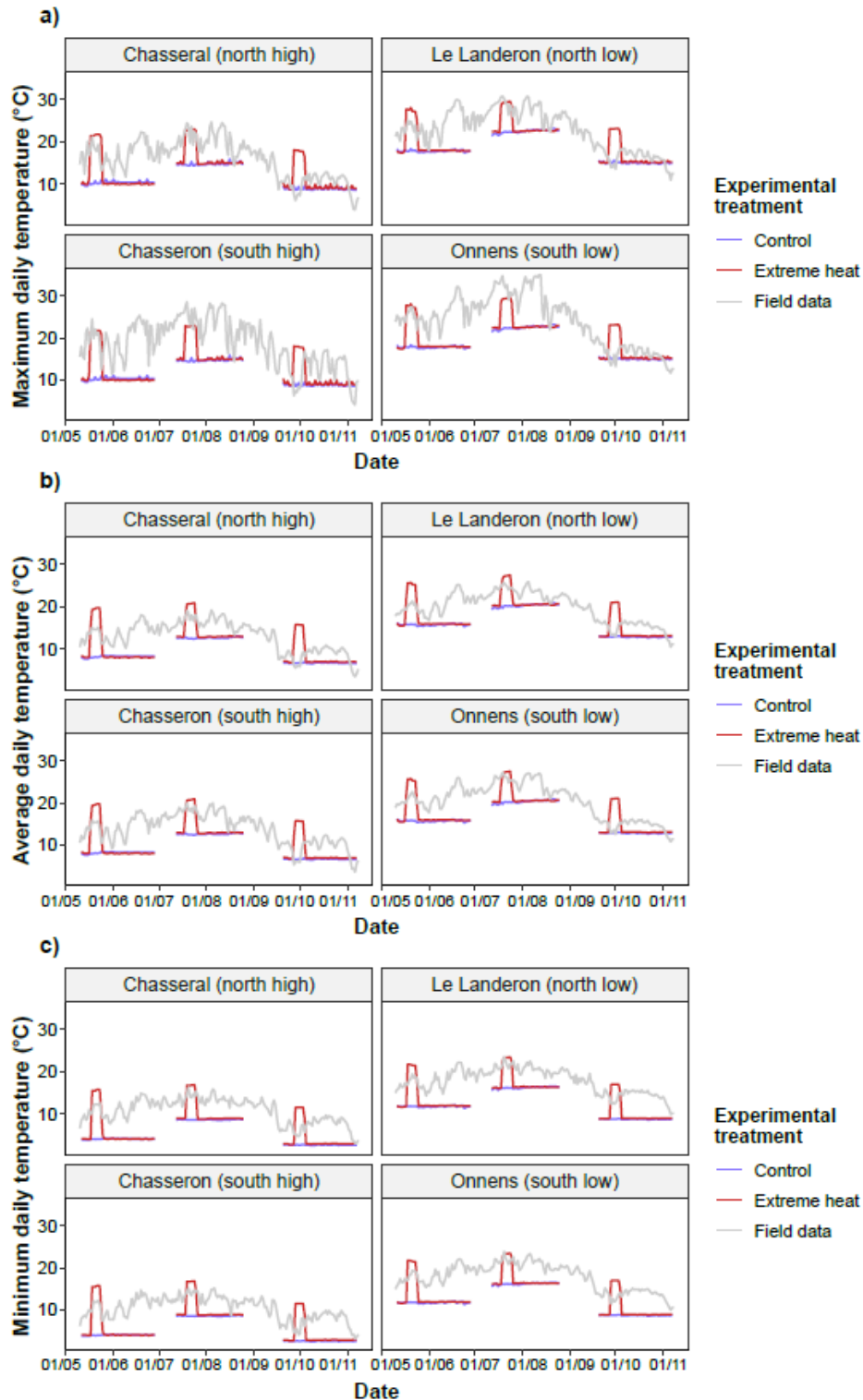
Fig. S3. Site-specific maximum (a), average (b) and minimum (c) daily soil temperatures at 5 cm depth, together with the daytime (a), average (b), and nighttime temperatures (c) recorded during the lab experiment, for both control (blue lines) and extreme heat treatments (red lines).
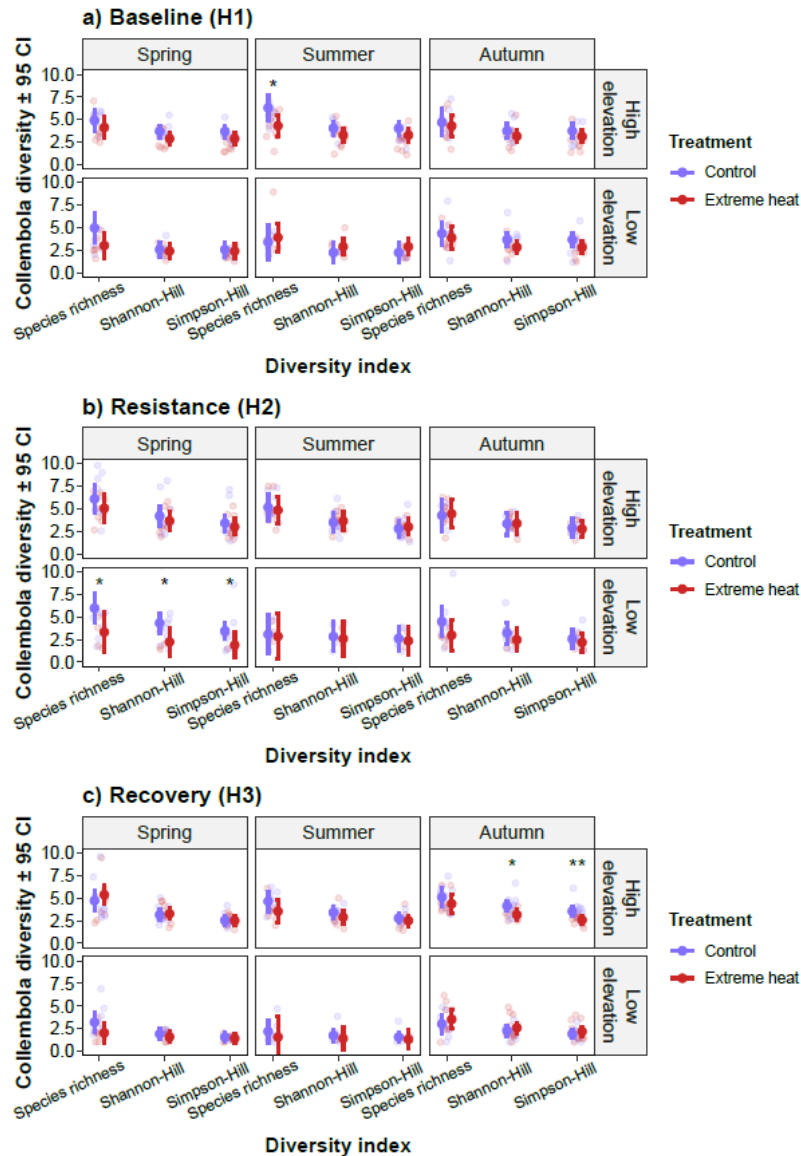
Fig. S4. Estimated marginal means (± 95 confidence intervals) of diversity profiles of Collembola communities, showing three indices calculated from various values of Hill number exponents (q): $q = 0$ (species richness), $q = 1$ (Shannon-Hill), $q = 2$ (Simpson-Hill). Lower values of the q exponent provide diversity estimates that give more leverage to rare species (e.g., species richness), while higher values give more leverage to dominant species (Roswell *et al.* 2021). Diversity profiles are shown for each experimental harvest separately: baseline or harvest 1 (H1; $N = 97$), resistance or harvest 2 (H2; $N = 91$), and recovery or harvest 3 (H3; $N = 103$). Colours indicate different experimental temperature treatments: blue: control; red: extreme heat. Stars show significant differences between treatments at each harvest: *$P < 0.05$, **$P < 0.01$.

Fig. S5. Output of the joint species distribution models (jSDMs) fitted to investigate the responses of Collembola species abundances to season, elevation, treatment, and their three-way interactions, in the baseline response (i.e., harvest 1: H1; before the onset of the extreme heat events). Estimates from the beta parameters (left panels) show the responses of species abundances (x-axis) to each of the model parameters (y-axis). Green and orange colors indicate positive and responses with 95% posterior probability, respectively, while blank spaces denote responses that lacked statistical support. Species abundances at the intercept (spring, high elevation, control treatment) denote more abundant species in green, less abundant species in orange, and blank spaces indicating intermediate abundances. Parameters enclosed within the red area represent species responses to the experimental treatment (extreme heat: EH; see Table S7 for an ecological interpretation of the model parameters). The proportion of raw explained variance (right panels) is provided for different groups of variables: random effects (site and block), natural variables (season and elevation), and treatment (containing the variance explained by all parameters influenced by extreme heat, shown within the red area of the right panels). Collembola species are ordered according to their vertical stratification across the soil profile: epedaphic (surface-living), hemi-edaphic (living in litter and shallow soil layers), and euedaphic (permanently living in the soil).
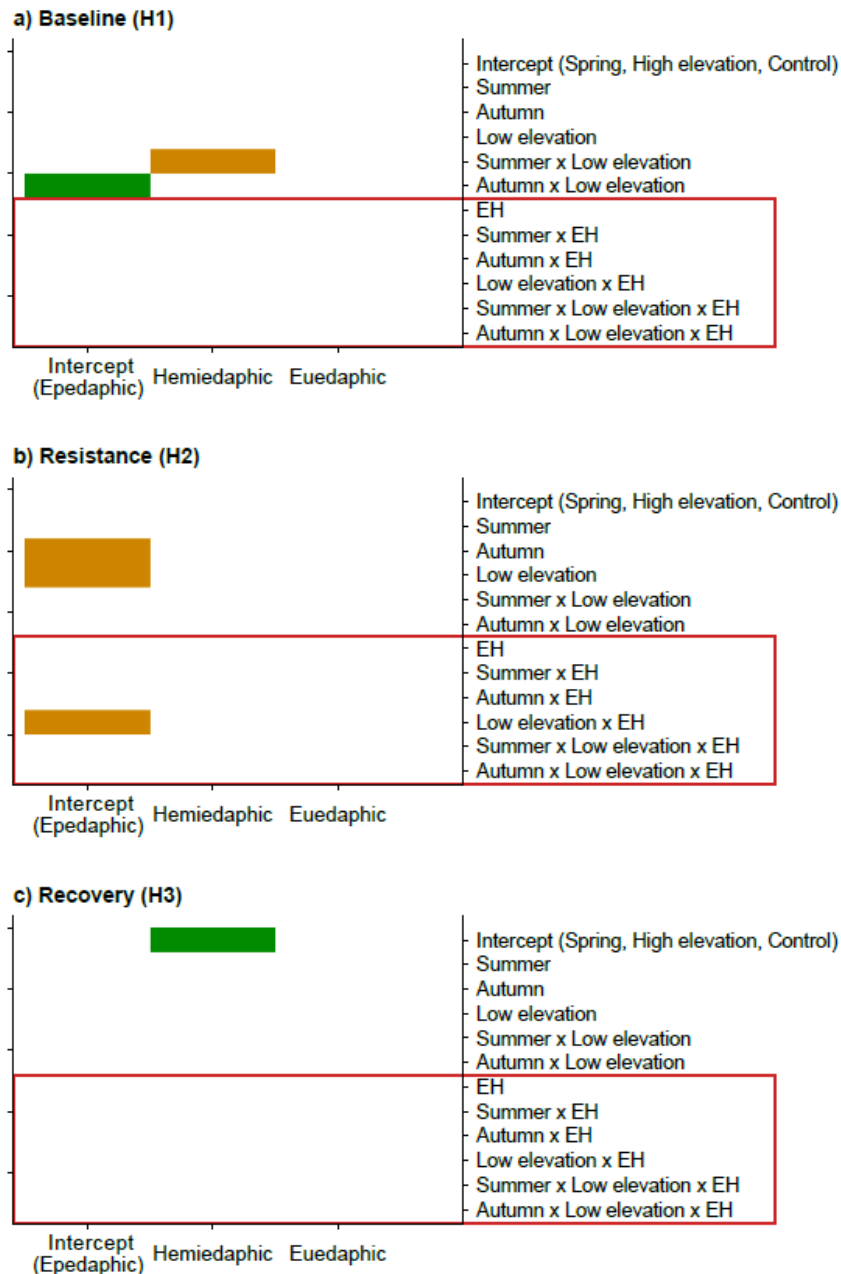
Fig. S6. Output of the joint species distribution models (jSDMs) fitted to investigate the responses of Collembola species abundances to season, elevation, treatment, and their three-way interactions, in the baseline (H1), resistance (H2) and recovery responses (H3). Estimates from the gamma parameters show whether species traits (i.e., vertical stratification; x-axis) mediate species abundance responses to each of the model parameters (y-axis). Three types of the vertical stratification of Collembola across the soil profile were investigated: epedaphic (surface-living), hemi-edaphic (living in litter and shallow soil layers), and euedaphic (permanently living

in the soil). Green and orange colors indicate positive and responses with 95% posterior probability, respectively, while blank spaces denote responses that lacked statistical support. Parameter estimates at the intercepts (x-axis: epedaphic Collembola; y-axis: spring, high elevation, control treatment) denote higher overall abundances in green, lower overall abundances in orange, and blank spaces indicating intermediate abundances. The variation in species abundances explained by their vertical stratification (R_T^2; Ovaskainen *et al.* 2017) amounts to: 0.15 (baseline), 0.35 (resistance), 0.43 (recovery). Parameters enclosed within the red area represent species responses to the experimental treatment (extreme heat: EH; see Table S7 for an ecological interpretation of the model parameters).
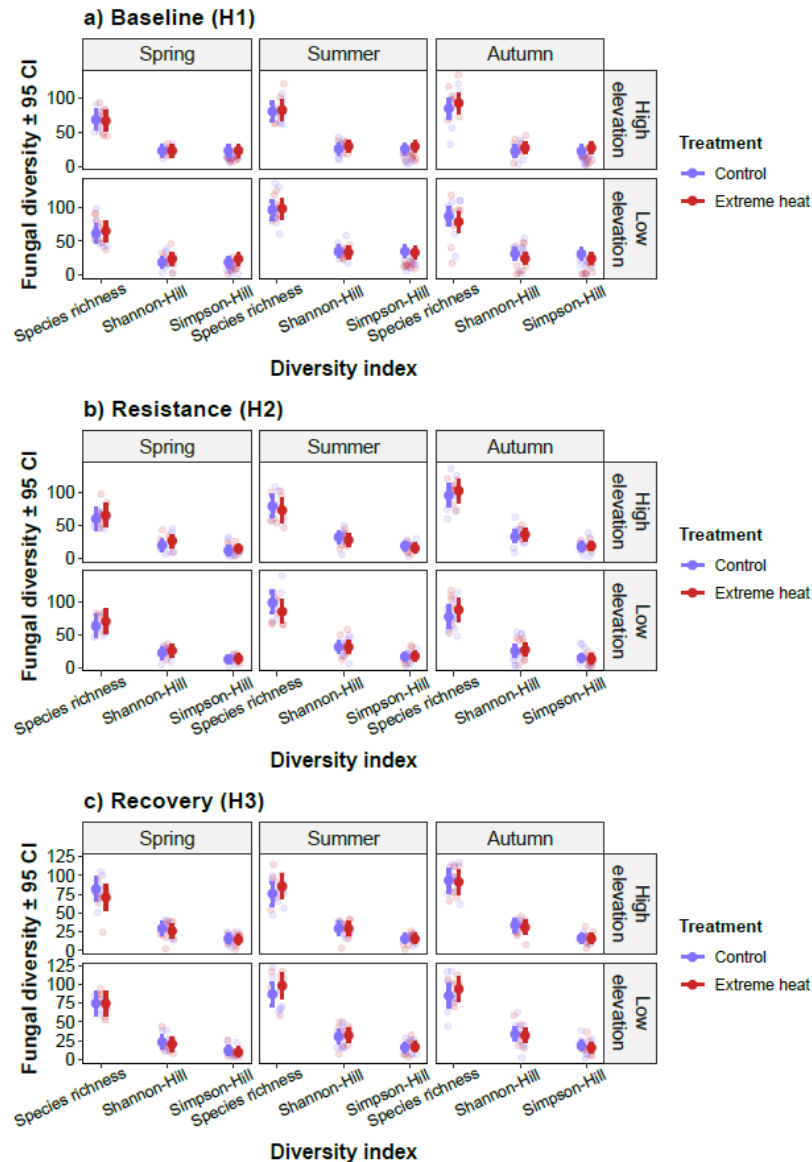
Fig. S7. Estimated marginal means (± 95 confidence intervals) of diversity profiles of fungal communities, showing three indices calculated from various values of Hill number exponents (*q*): *q* = 0 (species richness), *q* = 1 (Shannon-Hill), *q* = 2 (Simpson-Hill). Lower values of the *q* exponent provide diversity estimates that give more leverage to rare species (e.g., species richness), while higher values give more leverage to dominant species (Roswell *et al.* 2021). Diversity profiles are shown for each experimental harvest separately: baseline or harvest 1 (H1; *N* = 120), resistance or harvest 2 (H2; *N* = 120), and recovery or harvest 3 (H3; *N* = 120). Colours indicate different experimental temperature treatments: blue: control; red: extreme heat. The extreme heat treatment did not have significant effects on fungal diversity in any case.

Fig. S8. Output of the joint species distribution models (jSDMs) fitted to investigate the responses of fungal species occurrences (panels above) and abundances (panels below) to season, elevation, treatment, and their three-way interactions, in the baseline response (i.e., harvest 1: H1; before the onset of the extreme heat events). Estimates from the beta parameters (left panels) show the responses of species abundances (x-axis) to each of the model parameters (y-axis). Green and orange colors indicate positive and responses with 95% posterior probability, respectively, while blank spaces denote responses that lacked statistical support. Species abundances at the intercept (spring, high elevation, control treatment) denote more abundant species in green, less abundant species in orange, and blank spaces indicating intermediate abundances. Parameters enclosed within the red area represent species responses to the experimental treatment (extreme heat: EH; see Table S7 for an ecological interpretation of the model parameters). The proportion of raw explained variance (right panels) is provided for different groups of variables: random effects (site and block), natural variables (season and elevation), and treatment (containing the variance explained by all parameters influenced by extreme heat, shown within the red area of the right panels). Fungal species are ordered according to their main trophic modes: pathogens, saprotrophs, symbionts, and unassigned fungi.
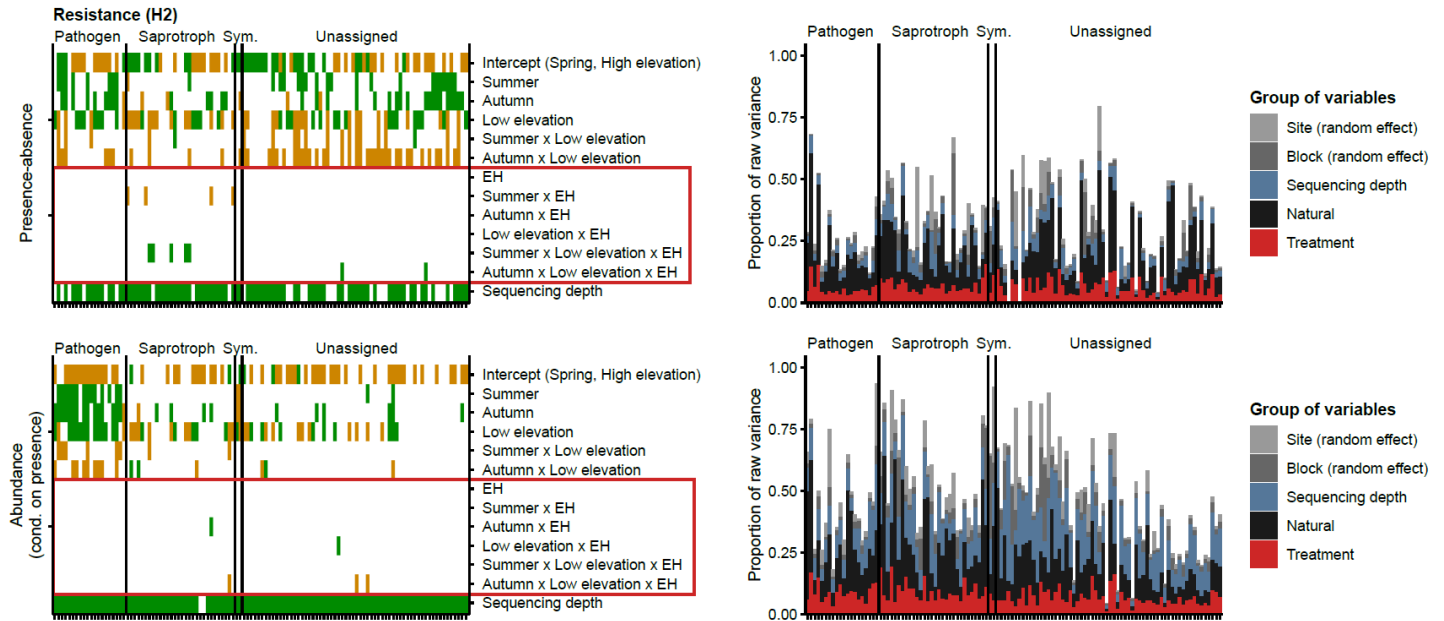
Fig. S9. Output of the joint species distribution models (jSDMs) fitted to investigate the responses of fungal species occurrences (panels above) and abundances (panels below) to season, elevation, treatment, and their three-way interactions, in the resistance response (i.e., harvest 2: H2; after the extreme heat events). Estimates from the beta parameters (left panels) show the responses of species abundances (x-axis) to each of the model parameters (y-axis). Green and orange colors indicate positive and responses with 95% posterior probability, respectively, while blank spaces denote responses that lacked statistical support. Species abundances at the intercept (spring, high elevation, control treatment) denote more abundant species in green, less abundant species in orange, and blank spaces indicating intermediate abundances. Parameters enclosed within the red area represent species responses to the experimental treatment (extreme heat: EH; see Table S7 for an ecological interpretation of the model parameters). The proportion of raw explained variance (right panels) is provided for different groups of variables: random effects (site and block), natural variables (season and elevation), and treatment (containing the variance explained by all parameters influenced by extreme heat, shown within the red area of the right panels). Fungal species are ordered according to their main trophic modes: pathogens, saprotrophs, symbionts, and unassigned fungi.
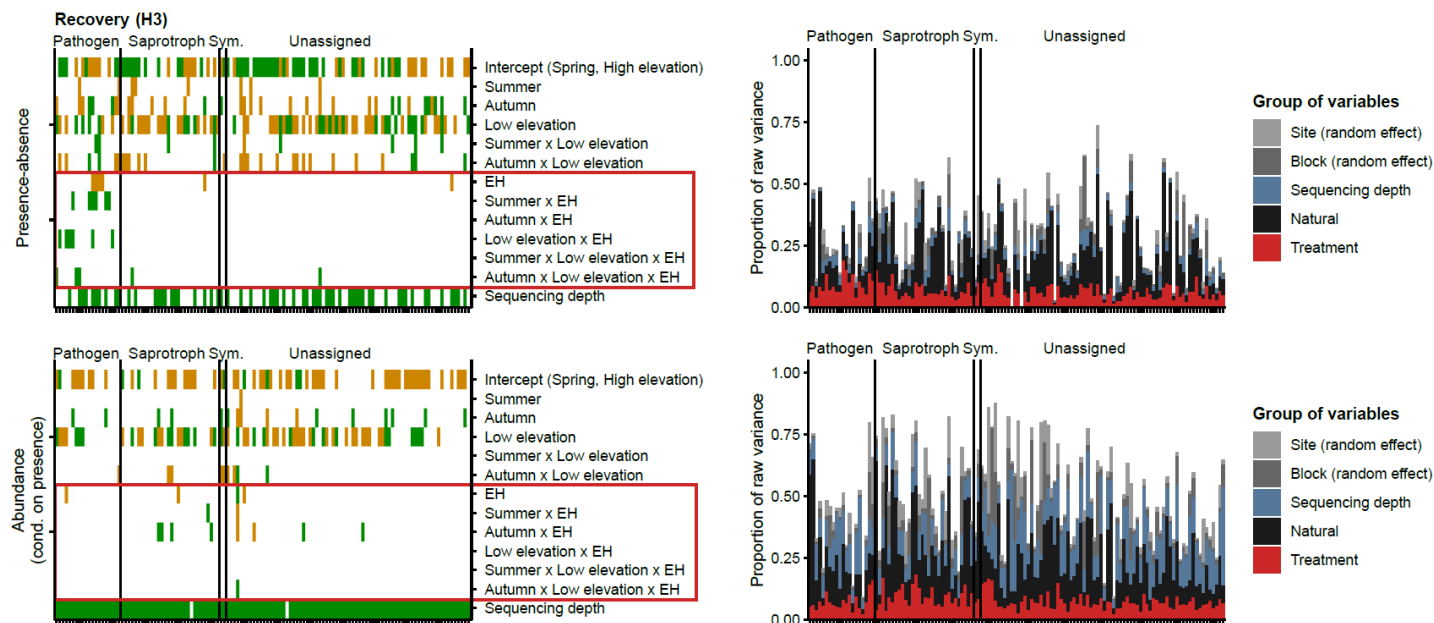
Fig. S10. Output of the joint species distribution models (jSDMs) fitted to investigate the responses of fungal species occurrences (panels above) and abundances (panels below) to season, elevation, treatment, and their three-way interactions, in the recovery response (i.e., harvest 3: H3; five weeks after the end of the extreme heat events). Estimates from the beta parameters (left panels) show the responses of species abundances (x-axis) to each of the model parameters (y-axis). Green and orange colors indicate positive and responses with 95% posterior probability, respectively, while blank spaces denote responses that lacked statistical support. Species abundances at the intercept (spring, high elevation, control treatment) denote more abundant species in green, less abundant species in orange, and blank spaces indicating intermediate abundances. Parameters enclosed within the red area represent species responses to the experimental treatment (extreme heat: EH; see Table S7 for an ecological interpretation of the model parameters). The proportion of raw explained variance (right panels) is provided for different groups of variables: random effects (site and block), natural variables (season and elevation), and treatment (containing the variance explained by all parameters influenced by extreme heat, shown within the red area of the right panels). Fungal species are ordered according to their main trophic modes: pathogens, saprotrophs, symbionts, and unassigned fungi.
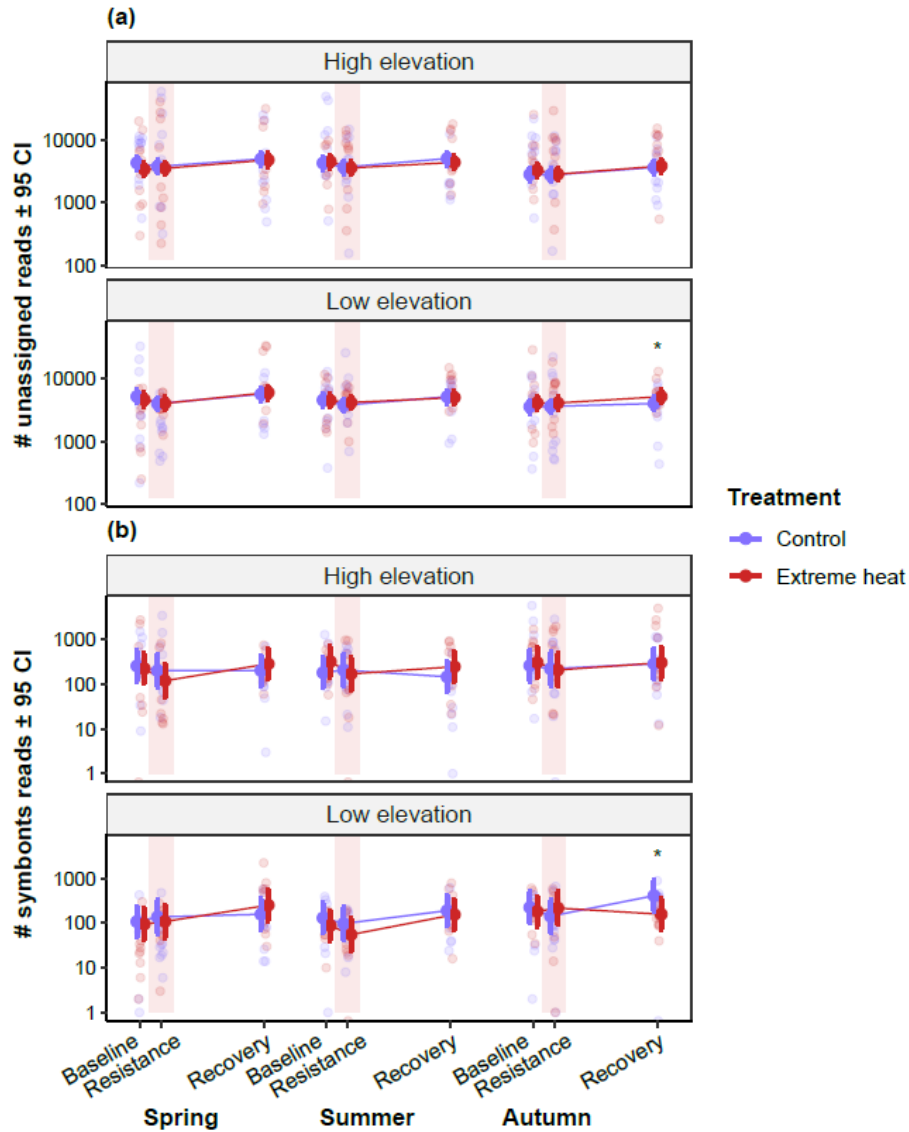
Fig. S11. Estimated marginal means (± 95 confidence intervals) of the number of reads (log-transformed) of unassigned (a; upper panel) and symbiotic fungi (b; lower panel) over the course of the experiments in spring, summer and autumn. The labels on the x-axis specify the different time points in which fungal metabarcoding reads were assessed during the experiment (i.e., harvests): baseline (harvest 1); resistance (harvest 2); recovery (harvest 3). The faded red areas represent the one-week extreme heat events. Colours indicate different experimental temperature treatments: blue: control; red: extreme heat. Stars show significant differences between treatments at each harvest: *$P < 0.05$.

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Chapter 4

Ecological debts induced by heat extremes

Ecological debts induced by heat extremes

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Abstract

Heat extremes have become the new norm in the Anthropocene. Their potential to trigger major ecological responses is widely acknowledged, but their unprecedented severity hinders our ability to predict the magnitude of such responses, both during and after extreme heat events. To address this challenge, we propose a conceptual framework inspired by core concepts of ecological stability and thermal biology to depict how responses of populations and communities accumulate at three response stages (exposure, resistance and recovery). Biological mechanisms mitigating responses at a given stage incur associated costs that only become apparent at other response stages, known as 'ecological debts'. We outline several scenarios for how ecological responses associate with debts to better understand biodiversity changes caused by heat extremes.

Heat extremes as a major stress in a warming world

Anthropogenic climate change is paving the way for more frequent and severe climatic extreme events (Buckley & Huey 2016b; Seneviratne *et al.* 2021). The observed ecological impacts of recent climate extremes demonstrate their consequences at large spatial scales, such as forest diebacks and coral bleaching (Harris *et al.* 2018; Smith *et al.* 2023; Ummenhofer & Meehl 2017). In fact, many present day-climate extremes, including heat extremes (see Glossary), have been unprecedented compared to those in recent evolutionary history in terms of intensity and frequency (Fischer *et al.* 2021; Seneviratne *et al.* 2021). The predicted rate of increase of heat extremes in the next 100 years is much steeper than that of gradual climate change (Seneviratne *et al.* 2021), and their extremity is expected to cause stronger ecological effects than by the rise in mean temperatures (Jørgensen *et al.* 2022; Murali *et al.* 2023). Therefore, understanding how natural systems will respond to novel heat extremes represents a pressing issue for both fundamental and applied ecological research (Jentsch *et al.* 2007; Turner & Seidl 2023).

Our current knowledge of species responses to heat extremes mainly comes from studies on thermal sensitivity (Buckley & Kingsolver 2021) and vulnerability (Clusella-Trullas *et al.* 2021), which generally point out to greater risks of tropical and mid-latitude organisms to warming than high-latitude ones (Deutsch *et al.* 2008; Dillon *et al.* 2010; Kingsolver *et al.* 2013). While these studies have yielded important insights into how organismal fitness responds to warming, as well as which species will shift their ranges or get extinct, we still lack frameworks that consider both short- and long-term responses of populations and communities to heat extremes (Donohue *et al.* 2016; Johnstone *et al.* 2016; Thakur *et al.* 2022). Moreover, understanding how short-term responses feedback to long-term responses and how these relationships help predict population and community stability against heat extremes is crucial for advancing climate change ecology (Louthan *et al.* 2021; Pinsky *et al.* 2022).

Short-term responses, such as reduction in population sizes, can indeed persist for the long term (e.g., beyond the end of an extreme heat event) due to shifts in genetic diversity (Gurgel *et al.* 2020; Reusch *et al.* 2005), biotic interactions (Suryan *et al.* 2021; Thakur *et al.* 2021) and functional traits (Martínez-De León *et al.* 2024; Thakur *et al.* 2022). The immediate impacts of

heat extremes can be dampened by biological mechanisms emerging at the (sub-)organismal level (Table 1), but the derived costs and consequences of such mechanisms for population and community-level responses have only received little attention so far. Here, we review mechanisms operating in the short term that subsequently propagate into population and community dynamics in the long term in response to heat extremes. For this purpose, we integrate key concepts emerging from the fields of thermal biology, related to how organisms cope with different thermal environments, and ecological stability, related to how populations and communities respond to pulse disturbances (Hillebrand *et al.* 2018; Ingrisch & Bahn 2018). We extend from previous frameworks on organismal responses to heat extremes (Buckley & Kingsolver 2021; Williams *et al.* 2008), by depicting how population and community responses to heat extremes (collectively referred to as 'ecological responses') unfold over time. To this end, we suggest that many biological processes and mechanisms buffering immediate responses to heat extremes at the organismal level incur significant costs, but these costs only become apparent in the longer term at population or community levels (Essl *et al.* 2015; Johnstone *et al.* 2016)- we refer to such costs as 'ecological debts' (Box 1; Figure 1; Table 1). Ecological debts can, therefore, alter how thermal responses scale up across levels of organization (Rezende & Bozinovic 2019), and how immediate responses are linked to long-term responses to heat extremes (Hillebrand & Kunze 2020).
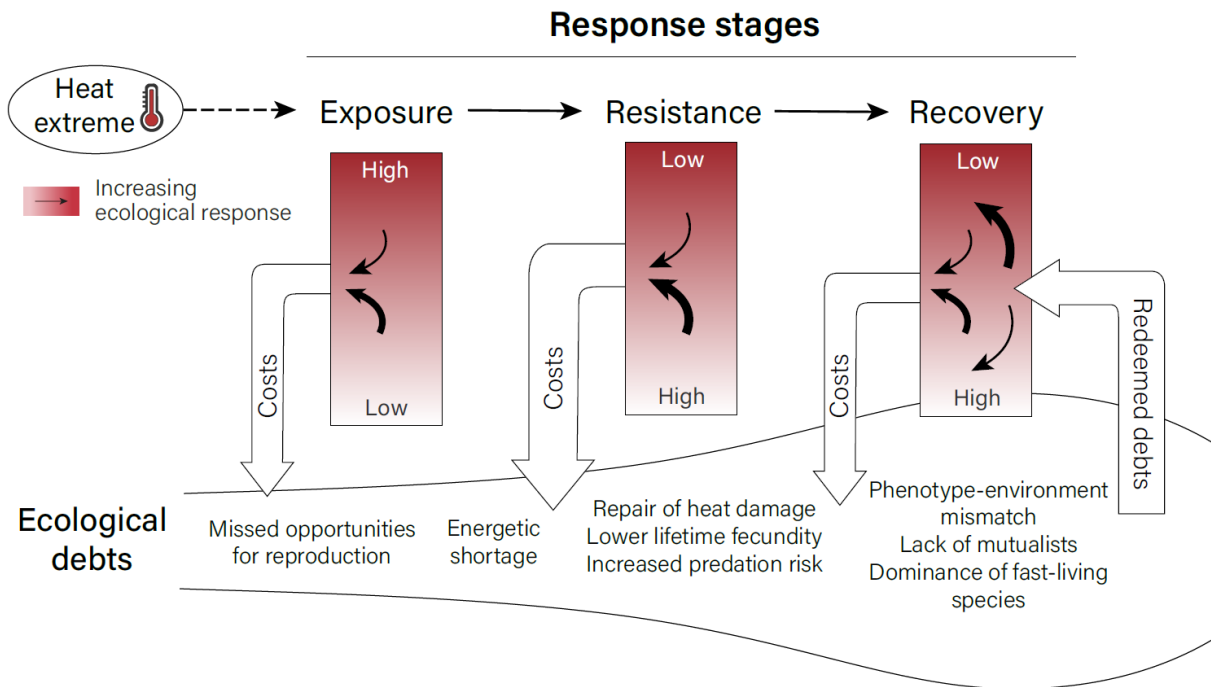
Figure 1. Accumulation of ecological debts along the three stages of the response to heat extremes. Following the onset of a heat extreme (circle), ecological responses unfold over the exposure, resistance and recovery stages. At each response stage, organisms employ mechanisms to immediately reduce heat-induced impacts, but these mechanisms inccur delayed costs accumulating as ecological debts (also see Table 1). These are mainly redeemed during recovery, causing larger ecological responses than expected at this stage in the absence of debts. Note that mechanisms favoring recovery also have debts that become apparent when facing a subsequent heat extreme or another disturbance.

Ecological responses to heat extremes unravel over time: exposure, resistance and recovery

Ecological responses to heat extremes develop over time in three stages: exposure, resistance and recovery (Figure 1). The three stages are defined based on how body temperatures of ectotherms oscillate during and after heat extremes, underscoring the main mechanisms involved at each stage (e.g., thermoregulation in the exposure stage; Table 1) as well as their

associated costs. We stress that such mechanisms are likely to overlap across stages, reflecting the non-independence of each response stage in determining ecological debts (Figure 1, Table 1). For instance, species frequently exposed to potentially deleterious temperatures (high exposure) often display high heat tolerance and, therefore, high resistance to heat extremes (Vives-Ingla *et al.* 2023). Likewise, different organismal traits involved in population resistance and recovery might be subjected to trade-offs and, as a consequence, high resistance is likely to come at the expense of reduced ability to recover (Capdevila *et al.* 2022). Despite the fact that these three stages form the continuum of ecological responses, decomposing them helps understand the underlying mechanisms and processes through which ecological debts arise in populations or communities (Table 1).

Table 1. Summary of the biological mechanisms and processes modulating the magnitude of ecological responses to heat extremes at different stages of the response (exposure, resistance, recovery), as well as their associated costs/ecological debts. The biological scale of mechanisms and processes are indicated as follows: I, individual; P, population; C, community.

Response stages	Biological mechanisms and processes	Costs/debts
Exposure	Plastic adjustments • Activity patterns: diel narrowing, diapause, seasonal escape (I, P) • Movement: tracking of buffered habitats, postural changes (I, P) • Physiological: evapotranspiration (I) Habitat features • Thermal buffering • Thermal heterogeneity	• Acquisition trade-offs (e.g., between foraging and thermoregulation) (Sears & Angilletta 2015) • Higher metabolic costs (Nielsen *et al.* 2022) and altered habitat features after seasonal escape (Bokhorst *et al.* 2012) • Missed opportunities for reproduction (Cinto Mejía & Wetzel 2023)

Resistance	Thermal performance
	• Plastic changes in the thermal performance curve and energy balance (I)
	    ○ Reversible acclimation
	    ○ Developmental plasticity
	    ○ Hardening
	• Protective mechanisms against heat damage (I)
	• Altered demographic structure (P)
	• Altered resource availability/interacting stressors (I, P)
	• Biotic interactions (e.g., enemy release, lower mutualist performance) (P, C)
			• Allocation trade-offs (between production of heat shock proteins and life-history traits, e.g., growth, fecundity) (Kingsolver & Woods 2016; Zizzari & Ellers 2014)
			• Long-term reproductive damage (Walsh *et al.* 2019)
			• Demographic bottlenecks (Lindmark *et al.* 2019)
			• Mismatches phenotype-environment caused by previous acclimation (Vinton *et al.* 2022)
			• Reduced protection against additional biotic or abiotic pressures (Cope *et al.* 2023; Diez *et al.* 2012)

Recovery	Redeemed debts (legacies) • Physiological repair of heat damage (I) • Altered fecundity and development (I) • Altered resource availability (I, P) • Biotic interactions (e.g., hindered species re-establishment – "community closure") (P, C)	Compensation and rescue • Life-history strategy (fast vs. slow) (P) • Dispersal (immigration and emigration) (P) • Genetic/species diversity (P, C)	• Incomplete repair of physiological heat damage (Ma *et al.* 2018; Williams *et al.* 2016) • Increased dominance of fast-living species in communities (Harvey *et al.* 2020) • Loss of habitat-forming species and ecosystem engineers (Hesketh & Harley 2023)

Exposure

At the onset of a heat extreme, organisms experience the occurrence of unsuitable external temperatures, and consequently initiate several mechanisms to avoid conforming to such conditions. We refer to this response stage as exposure, including all mechanisms at the individual level used to adjust body temperatures in relation to habitat or operative temperatures (thermoregulation). At the population level, such mechanisms can result in a lower number of active individuals experiencing an extreme event. Since exposure to heat extremes differs across environments and temporal scales (e.g., more variable occurrence of extremes at higher latitude sites, especially at daily to weekly time scales; (Kingsolver & Buckley 2017)), mechanisms mitigating exposure are expected to have greater fitness consequences in more thermally variable environments. External physical features can significantly modify exposure at the habitat level, affecting the thermal conditions that any population or community will actually experience (Kemppinen *et al.* 2024; Woods *et al.* 2015) and thus determining costs at the latter response stages (Table 1). For instance, thermal buffering occurs when heat transmission is reduced in a given habitat, generally by blocking solar radiation and thereby keeping cooler temperatures with narrower fluctuations (e.g., under the cover of plants or sessile invertebrates; (Frenne *et al.* 2019; Hesketh & Harley 2023; Vives-Ingla *et al.* 2023)).

Activity changes resulting in reduced exposure can be entirely induced by the heat extremes, such as diapause (González-Tokman *et al.* 2020; Sgrò *et al.* 2016), or can act upon constituent activity patterns, such as diel narrowing and seasonal escape (Kefford *et al.* 2022). In fact, seasonal escape is shaped throughout a species' evolutionary history to avoid harsh conditions predictably occurring at weekly to seasonal timescales, for instance, by means of aestivation. As a result, ecological responses after heat extremes could remain small when these events occur during periods of seasonal escape, with relatively little biological activity (Buckley & Huey 2016a; Cinto Mejía & Wetzel 2023). Yet, heat extremes could impact inactive individuals via increased metabolic costs (Nielsen *et al.* 2022) and altered habitat features (Bokhorst *et al.* 2012), causing ecological debts when biological activity is resumed (post extreme heat event) after the seasonal escape. For example, heat extremes accelerate snowmelt in periods when deleterious freeze-thaw cycles occur frequently (e.g., early in the growing season), thereby

exposing organisms living in the soil to harmful temperature fluctuations due to the loss of insulating snow cover (Bokhorst *et al.* 2012). Mobile organisms can move to habitat patches with buffered thermal conditions, or can alter their orientation, mainly to reduce incoming solar radiation (Buckley & Kingsolver 2021; Woods *et al.* 2015). The occurrence of distinct thermal environments in space and time (i.e., thermal heterogeneity; (Woods *et al.* 2015)) is key to allow for tracking of suitable thermal conditions, which could essentially lower costs at other response stages (Sears & Angilletta 2015). On the contrary, if the activation of mechanisms reducing exposure becomes exceedingly costly, ecological debts are likely to accumulate and become apparent as legacies at later stages (Box 1; Figure 1; Table 1). For instance, tracking thermally favorable habitats or adopting diel narrowing comes at the expense of lower resource acquisition, leading to high costs of thermoregulation in landscapes with low thermal heterogeneity (Sears & Angilletta 2015). The associated energy expenditure could lead to greater detrimental effects in subsequent response stages (e.g., resistance), as energetic shortage leads to reduced thermal tolerance (Huey & Kingsolver 2019; Litchman & Thomas 2023).

Box 1. Inferring ecological debts induced by heat extremes.

Inferring ecological responses to heat extremes requires a thorough understanding of the mechanisms involved and ecological debts. However, empirical measures of debts are rare, risking the underestimation of ecological responses when these debts are not taken into account. Theoretically, redeemed ecological debts could be detected as alterations in biological rates or processes (e.g., reduced vital rates, altered connectance in interaction networks) that cannot be explained by, apparently favorable, current environmental or biotic factors (e.g., temperature or competition) and should be attributed to previous exposure to a heat extreme. Ecological debts caused during early response stages (e.g., exposure) can be challenging to measure empirically during a heat extreme event, given that the direct costs of heat can overshadow these accumulated debts. However, ecological debts can be measured more easily in the recovery stage, which is actually the stage where debts are most likely to be redeemed. Shortly after the end of a heat extreme, a measure affecting the response from heat-exposed organisms (e.g.,

population size) can be compared against naïve organisms at similar density (orange lines in Figure I), mainly by means of controlled experiments, modelling approaches, or well-replicated observational studies. It must be noted that ecological debts are usually the expression at higher organizational levels of heat-induced costs, but these costs often arise at lower levels (e.g., physiological). For instance, heat-induced damage and repair of the reproductive physiological or morphological machinery can be measured already during a heat extreme (e.g., Sales *et al.* (2021)), but its effects on reproductive output, which is often the measure of ecological relevance (e.g., fitness), cannot be quantified until a time period necessary for gamete production, mating and embryonic development (Walsh *et al.* 2019). It is therefore essential to strengthen the scaling of physiological impacts of heat extremes to more relevant ecological scales, using metrics linked to energy or performance (Box 2).
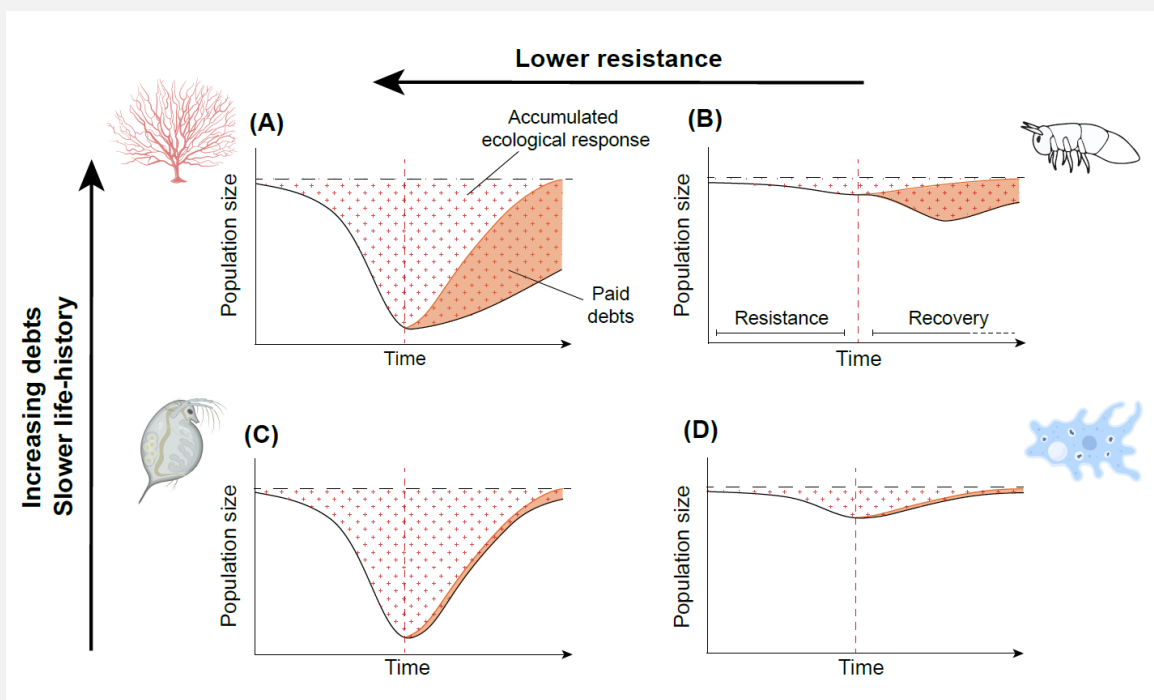

Figure I. Scenarios of ecological responses and debts to heat extremes, using population size as a response variable. A heat extreme takes place over time (horizontal axis) until its end (vertical red dotted line). The accumulated ecological response is represented as the spotted area bounded between the baseline population sizes (i.e., not exposed to the heat extreme; horizontal dotted lines) and the temporal dynamics of the ecological response (black curves). The fractions

of ecological responses caused by paid debts are displayed as orange filled areas. The expected population recovery trajectories in absence of ecological debts (i.e., only accounting for demographic compensation and rescue after the extreme event) are shown with orange curves. Focal organisms from studies reporting comparable responses after heat extremes are represented next to each scenario, together with their generation times relative to the other organisms shown: (A) coral, slow-living (Gómez-Gras *et al.* 2021): low resistance, high debt; (B) springtail, slow-living (Martínez-De León *et al.* 2024): high resistance, high debt; (C) cladoceran, fast-living (Vad *et al.* 2023): low resistance, low debt; (D) predatory protist, fast-living (Thakur *et al.* 2021): high resistance, low debt. Note that ecological debts were not quantified explicitly in these studies, but were suggested as drivers of observed legacies after heat extremes. Images drawn with Biorender.com.

Resistance

Organismal performance in ectotherms is directly linked to body temperature, typically described by a unimodal asymmetric relationship known as thermal performance curve (Sinclair *et al.* 2016). Thermal performance curves are widely used to assess organismal and population responses to warming (Huey & Berrigan 2001), including the immediate effects of heat extremes at a given body temperature (Box 2). In the resistance stage, a widespread strategy to buffer potential heat-induced impacts is the adjustment of key attributes of thermal performance curves (i.e., optimum, breadth, limits, area under the curve), mainly through developmental plasticity, reversible acclimation and hardening (Buckley & Kingsolver 2021; González-Tokman *et al.* 2020; Seebacher *et al.* 2015; Sgrò *et al.* 2016). However, under sustained heat stress, the mechanisms that actively modulate physiological functions may fail to maintain an optimal fitness of individuals, or may even be maladaptive. In such stress response stages, shifts in morphological and life-history traits are more likely (Cerini *et al.* 2023), potentially leading to large ecological debts (Figure 1; Table 1). For example, excessive heat-induced impairment of physiological functions can lead to declines in life-history traits (i.e., growth, survival, reproduction, development), at temperatures beyond their respective thermal optima

(González-Tokman *et al.* 2020; Ma *et al.* 2020; Smith *et al.* 2023; Williams *et al.* 2016). Physiological heat damage can thus cause large ecological debts due to allocation trade-offs, such as by diverting energy and resources to protective mechanisms that would be otherwise allocated to performance traits (e.g., production of heat shock proteins at the expense of reduced growth; (Kingsolver & Woods 2016)). Additionally, ecological debts accumulate when developmental plasticity at higher temperatures produces phenotype-environment mismatches once climatic conditions return to normal, causing reduced performance during recovery (Vinton *et al.* 2022). For example, reduction in body sizes as a result of warmer conditions during development (a pattern known as the temperature-size rule; (Atkinson 1994; Verberk *et al.* 2021)) could incur long-term costs when temperatures return to normal after a heat extreme, since smaller organisms often have lower fecundity and higher predation risks (Kingsolver & Huey 2008). This phenomenon could be particularly significant for small body-sized organisms with developmental times matching the temporal scales of heat extremes (e.g., several days to weeks).

At the population level, resistance responses to heat extremes further depend on the demographic structure (Ohlberger 2013). This is because different life- or size-stages have distinct selective pressures on their thermal tolerances (e.g., Sales *et al.* (2021)), given that each stage is characterized by specific life-history processes (e.g., hatching, development, mating; (Harvey *et al.* 2020)) and particular microhabitat requirements (Kingsolver *et al.* 2011). Consequently, large resistance responses are more likely when heat extremes have disproportionate deleterious effects on thermally sensitive life-stages (Cinto Mejía & Wetzel 2023; Ma *et al.* 2018). The resulting altered demographic structure could represent an ecological debt itself if, for example, dominant life-stages after heat extremes constrain population growth through demographic bottlenecks (Lindmark *et al.* 2019). In addition, co-occurring stressors (e.g., heat extremes combined with drought) and low resource availability could bring ecological responses to heat extremes beyond critical thresholds, often as a result of interactive responses (Hector *et al.* 2021; Jackson *et al.* 2021; Litchman & Thomas 2023; Rozen-Rechels *et al.* 2019), leading to local extinction of populations.

Indirect effects from biotic interactions during the resistance stage can further impact populations (Stoks *et al.* 2017). For example, ectothermic predators are considered particularly prone to reducing their population sizes and even becoming locally extinct during heat extremes, given that metabolic costs increase more steeply at higher temperatures than their feeding rates (Fussmann *et al.* 2014; Lindmark *et al.* 2019). This could release prey species from predation after heat extremes, likely favoring population explosions in heavily top-down controlled communities (Harvey *et al.* 2020). Heat extremes can also provide windows of opportunity for the establishment of range-expanding species towards higher latitudes or elevations (Diez *et al.* 2012). This has been shown experimentally in communities composed with several native and a single range-expanding *Drosophila* species, where heat waves inhibited fecundity of the native species while promoting fecundity of the range-expanding species, thereby facilitating the establishment of the latter (Chen & Lewis 2023). A recent global meta-analysis further supports the synergies between heat extremes and range-expanding species, showing that heat extremes have stronger impacts on native than on non-native species, particularly in freshwater systems (Gu *et al.* 2023). Negative consequences mediated by biotic interactions are expected as well for species dependent on heat-sensitive mutualists, such as many insect-pollinated plants or habitat-forming marine species (Hesketh & Harley 2023; Smith *et al.* 2023).

Recovery

Ecological responses determined after an extreme heat event, when body temperatures return to normal following environmental temperatures, belong to the recovery stage. While ecological responses can persist in the long term after the occurrence of heat extremes, mechanisms underlying recovery remain poorly understood, and more importantly, loosely linked to mechanisms buffering immediate responses to heat extremes (Thakur *et al.* 2022). Essentially, heat-induced ecological debts accumulated in earlier stages are often redeemed and manifested as legacies at the recovery stage, when (abiotic) stressful conditions are reduced (Figure 1; Table 1). Ecological debts affecting long-term fecundity may be common in the face of heat extremes, given that reproductive thermal limits are often much lower than those of survival, causing

legacies at the population level after a time period necessary for recruitment (Box 1 & 2) (Bozinovic *et al.* 2020; van Heerwaarden & Sgrò 2021; Walsh *et al.* 2019). The overlap between key life-history processes and the timing of heat extremes is critical for recovery responses (Cinto Mejía & Wetzel 2023), given that skipped reproductive events due to heat extremes can represent missed opportunities in seasonal environments (McLean *et al.* 2016). For instance, a heat extreme can dramatically reduce reproductive success in beetles during mating while inducing minor effects shortly before or after mating (Pilakouta *et al.* 2023). Interestingly, physiological recovery of reproductive traits after heat stress may be decoupled from heat tolerance (Xie *et al.* 2023), suggesting weak relationships between population resistance and recovery to heat extremes.

Debts caused by biotic interactions are expected to affect recovery in populations and communities in compound ways, requiring a thorough understanding of the trait responses and life histories of the species involved. Among the possible factors at play, we put emphasis on the timing of heat extremes relative of the phenology of the interacting species (Cope *et al.* 2023), as well as on the differences in thermal tolerance and recruitment among species (Harvey *et al.* 2020; Ma *et al.* 2020; Thakur *et al.* 2022). Furthermore, at the community level, compositional recovery after pulse disturbances often mediates functional recovery (Hillebrand *et al.* 2018; Hillebrand & Kunze 2020), but this relationship could be altered in the context of heat extremes because of temperature effects on metabolism. For instance, in an outdoor experiment with freshwater communities exposed to experimental heat waves (Polazzo *et al.* 2023), complete functional recovery (biomass production) was observed despite low compositional recovery, possibly due to warming-induced increases of metabolic rates. Invasive species, owing to their high propagule pressure and fast resource acquisition, could also recover better than native species after heat extremes (Diez *et al.* 2012; Gu *et al.* 2023), potentially leading to long-term changes in community composition (Sorte *et al.* 2010). Assessing whether such compositional changes are transient or persistent, especially with the expected increase in the frequency of heat extremes, warrants further research. In addition, altered resources after heat extremes can slow down the rate of recovery, such as when long-living predators experience delayed scarcity

of short-living prey because of time lags in the propagation of disturbance effects across trophic levels (Essl *et al.* 2015; Jackson *et al.* 2021; Suryan *et al.* 2021).

Mechanisms involving compensation and rescue, mostly at the population level, depend more strongly on the intrinsic features of species than on previous heat-induced debts, and can largely explain differences in recovery responses among species (Capdevila *et al.* 2020). Life-history strategies explain how long-lived and low-reproductive animal species often display high resistance but low recovery after pulse disturbances, whereas the opposite is found in short-lived and highly-reproductive species (Capdevila *et al.* 2022; Neilson *et al.* 2020). Dispersal (i.e., immigration and emigration) is critical in accelerating recovery after heat extremes (De Boer *et al.* 2014; Harvey *et al.* 2022); for instance, large responses may persist over time in heavily fragmented and dispersal-limited landscapes (Pinsky *et al.* 2022). A high degree of thermal heterogeneity, denoting spatial asynchrony in thermal exposure, can promote the arrival of heat tolerant and fast-colonizing species in focal patches (Sorte *et al.* 2010), thus fueling community and ecosystem recovery (Loreau *et al.* 2003). However, in spite of their population's dispersal abilities from source habitats, locally extinct species may fail to reestablish in the recovery phase if the biotic environment has shifted during heat extremes. For example, rotifers returning to heat-exposed microcosms after becoming extinct did not manage to re-establish ("community closure"), or either resulted in greatly divergent trajectories of community reassembly during recovery (Seifert *et al.* 2015).

Box 2. Can TPCs inform about ecological debts?

Thermal performance curves (TPCs) are the gold standard for assessing organismal responses to temperature changes (Huey & Berrigan 2001; Rezende & Bozinovic 2019; Sinclair *et al.* 2016), and have been widely applied to assess the vulnerability of ectotherms to climate warming (Deutsch *et al.* 2008; Kingsolver *et al.* 2013). We argue that the use of TPCs can be extended to infer how ecological debts emerging from physiological and organismal processes propagate into population and community levels. TPCs are commonly measured in individuals previously acclimated to benign thermal conditions, but performance can also change substantially as a

result of thermal history (Dowd *et al.* 2015; Sinclair *et al.* 2016). It is well-known that previous exposure to acute heat (hardening) or chronic warming (acclimation) can provide enhanced tolerance/performance to a subsequent heat exposure (Seebacher *et al.* 2015), but the costs associated to such mechanisms (summarized in Table 1) have received far less attention in the context of TPCs. The production of heat shock proteins and other energy-demanding mechanisms is expected to reduce performance traits such as growth, as shown in theoretical models (Kingsolver & Woods 2016). Therefore, heat-induced costs on performance traits (e.g., fecundity, growth) could be characterized based on TPCs (Figure IA) to inform about ecological debts redeemed at the recovery phase (Figure IB), as well as the resulting performance when facing a subsequent heat extreme (vertical dotted lines in Figure IA). Importantly, TPCs could be additionally described as a function of time, either the time of heat exposure (to illustrate how ecological debts may amplify with the buildup of heat stress; (Ørsted *et al.* 2022)) or the time of recovery (to depict how ecological debts persist or dampen over time when temperatures return to normal). For instance, TPCs could be measured at different time steps along the recovery of individuals previously exposed to a heat extreme, to track their deviation in terms of relevant performance metrics from individuals exposed to control temperatures, such as fecundity at the resistance or recovery temperatures (Figure I). However, TPCs have major assumptions and limitations (Sinclair *et al.* 2016), for example, related to the incorporation of realistic variability in thermal regimes (Dowd *et al.* 2015; Kingsolver & Buckley 2017) or the substantial variation in TPCs depending on the trait and life stage under examination, among other methodological aspects (Hoffmann *et al.* 2023). Therefore, the application of TPCs to estimate ecological debts is also subjected to these known limitations, and studies should be carefully designed by considering these caveats. Experimental studies measuring thermal effects across levels of organization (e.g., from organismal to population levels; (Bozinovic *et al.* 2020)), using species with well-known life-histories, can prove highly valuable to assess how ecological debts arise and translate into altered population (Martínez-De León *et al.* 2024) and community dynamics. Such integrative approaches can be further accompanied with simulations and mechanistic models to obtain more accurate estimates of ecological debts in natural populations and communities (analogous to the methodologies proposed to assess extinction debts; (Figueiredo *et al.* 2019)).
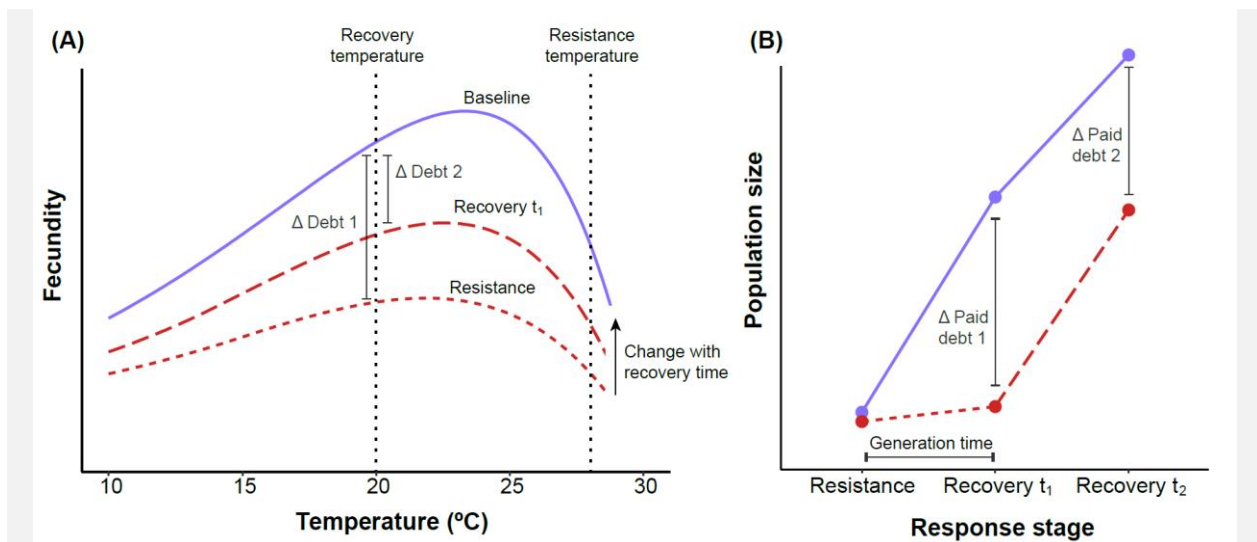
Figure I. Physiological costs induced by heat extremes decrease organismal performance (e.g., thermal performance curves (TPC) of fecundity, left side-panel A), and these effects later scale up to alter population dynamics in the form of paid ecological debts (right side-panel B). TPCs of fecundity and population dynamics are displayed at different response stages: resistance, and recovery at two time intervals (t_1 and t_2). The displayed TPCs and population dynamics are based on Martínez-De León *et al.* (2024), where heat exposure (dotted red lines) strongly reduced fecundity (but not survival) in a boreal springtail species, resulting in divergent population growth trajectories from the baseline (blue lines) during recovery. Heat-induced costs affect the TPC most strongly at the resistance stage (left side-panel A), which can slowly converge back into the TPC of individuals not exposed to the heat extreme (baseline) as ecological debts are paid over the recovery period (upwards-facing arrow). Ecological debts originating from heat-induced declines in fecundity translate into reduced population growth (right side-panel B) after a time interval necessary for recruitment, such as a period equivalent to the species' generation time. For example, fecundity debts generated at recovery t_1 are paid at the population level at recovery t_2. Note that emergent properties at the population level (e.g., density-dependent vital rates) and life-history trade-offs (e.g., reduced fecundity but increased offspring viability) can blur how organismal effects propagate to population levels, and should be taken into account to accurately use TPCs in the context of ecological debts.

Sequential heat extremes

Recovery mechanisms reduce ecological responses (thus lowering ecological debts) to a single extreme heat event. This is true when populations and communities are not immediately exposed to another sequential extreme event, including heat extremes. However, increasing frequency of heat extremes can substantially reduce a population's potential to shrink its ecological debt from the previous heat extreme event (Figueiredo *et al.* 2019; Ma *et al.* 2018). Accumulated ecological debts from the previous heat extreme could even lead to local extinction or migration of species particularly when they are exposed to another heat extreme event (or a stress with similar severity, such as extreme drought). As such, the buildup of small effects of heat stress on survival (e.g., accumulation of ecological debts) can cause population crashes following multiple heat extremes (Rezende *et al.* 2020). This could override the benefits of priming effects, which are mechanisms providing enhanced performance to sequential stressors, mostly over an individual's lifespan (Zhou & Wang 2023). At the community level, priming effects can also occur through increased dominance of heat-resistant or fast-growing genotypes after heat extremes, such as in the case of the Great Barrier Reef, where coral reefs surviving a single extreme heat event were more resistant to the exposure of another heat event in the following year (Hughes *et al.* 2019b). In general, we suggest that the accumulation of ecological debts will make responses to heat extremes more likely to be amplified, rather than buffered, with the occurrence of sequential events (Box 2). In the above example, recruitment in the coral reefs was severely compromised after the two consecutive heat extremes due to earlier adult mortality, perhaps hindering a complete recovery of coral populations prior to subsequent heat extremes (Hughes *et al.* 2019a). Indeed, considering how the frequency of sequential events relates to the species' generation times is key to predict these consequences (Jackson *et al.* 2021), as short-lived species are more expected to recover completely before the onset of a subsequent heat extreme (Neilson *et al.* 2020; Suryan *et al.* 2021; Box 1, Figure I).

Concluding remarks and future directions

Ecological responses to heat extremes are determined by both distinct and overlapping mechanisms across different response stages, mainly to overcome thermal stress. We advocate that a more temporally-explicit view of ecological responses to heat extremes could yield insights into biodiversity conservation in a world with increasing extreme climatic events. By considering how exposure, resistance and recovery affect the dynamics of populations and communities as proposed in our conceptual framework (Fig. 1), we can better identify vulnerable species and ecosystems, and accordingly devise management and mitigation strategies against the impacts of heat extremes on ecological systems. For example, restoration programs can enhance the availability of cooler microhabitats by promoting ecosystem engineers, thereby reducing ecological impacts already at early response stages (Kemppinen *et al.* 2024; Thakur *et al.* 2020). Faster recovery could be promoted by enabling the flow of individuals across thermally heterogeneous landscapes, enhancing access to decimated resources (e.g., natural vegetation cover, water availability) and preventing the establishment of invasive species after heat extremes (Diez *et al.* 2012). Implementing such strategies can potentially shorten the time frames for reducing ecological debt, especially as the intervals between heat extremes are shrinking (Seneviratne *et al.* 2021). Yet, to achieve this, we need to overcome current knowledge gaps for our understanding of responses to heat extremes (see Outstanding questions). For instance, mechanisms promoting recovery after heat extremes are largely understudied (e.g., rates of repair of physiological heat damage; (Ørsted *et al.* 2022)), despite their strong contribution to the overall ecological response. We recommend that measurements assessing recovery should capture lagged effects on heat-sensitive biological processes otherwise overlooked (e.g., dispersal, recruitment) (Harvey *et al.* 2022; Martínez-De León *et al.* 2024), requiring careful consideration of the life-history (e.g., generation time) of the study species (Neilson *et al.* 2020). In addition, mechanisms modulating responses at earlier response stages often have associated debts, but we still ignore the importance of these debts in driving long-term ecological responses. Notably, a greater focus on evolutionary changes promoting recovery after heat extremes is needed, and should build up on our current understanding of how gradual warming and extremes drive the evolution of thermal performance and tolerance (e.g., Buckley & Huey (2016a); van Heerwaarden & Sgrò (2021); Hoffmann *et al.* (2023); Williams *et al.* (2016)). Possible trade-offs

in the evolutionary potential of mechanisms conferring resistance and recovery could be explored, as well as how these relationships differ with heat extremes of varying severity. Finally, even though sequential heat extremes are more likely to occur as climate gets warmer, we are only starting to depict their potential impacts in relation to single extreme heat events. Our ability to anticipate and act upon the ecological consequences of heat extremes will only improve by acknowledging the response continuum during and after heat extremes, and mechanisms that underlie variation in responses that contribute to ecological debts. Our conceptual framework is a step towards achieving this, while we also maintain that its application is more likely to be successful in experimental studies, and more likely exclusive to ectotherms. Integrative approaches combining experiments and theory can help to infer ecological debts in real world settings, a necessary step towards understanding biodiversity dynamics in response to climate extremes.

Outstanding questions

- How can variation in ecological responses to heat extremes be predicted?
- What are the relative contributions of different mechanisms (across different ecological scales) at the various stages of the response to heat extremes?
- Can heat extremes induce evolutionary changes that reduce ecological responses? If so, are there trade-offs in evolutionary changes of mechanisms involved at different response stages? For instance, could the evolution of higher heat tolerance (i.e., related to resistance) come at the expense of reduced repair of heat damage and/or compensatory mechanisms (i.e., related to recovery)?
- Can we identify environmental settings where biotic interactions are particularly important in determining ecological responses to heat extremes?
- How do ecological debts change with more severe heat extremes and with the occurrence of sequential extreme events?
- How will heat extremes trigger gradual ecological responses over yearly to decadal time scales due to the accumulation of ecological debts?

Glossary

- **Acclimation:** plastic phenotypic changes (e.g., physiological, morphological) that can help to anticipate and provide improved performance (e.g., higher survival or reproductive output) to an ongoing or future exposure to heat extremes. However, acclimation can sometimes be maladaptive, for instance when thermal conditions change across life stages.

- **Aestivation**: phase of reduced metabolic activity, usually spent in thermal refugia, to minimize exposure to seasonal periods of deleterious hot and dry conditions.

- **Biological mechanisms**: in the context of heat extremes, active biological responses emerging mainly at the level of organismal traits, with the aim to buffer heat-induced ecological impacts.

- **Biological processes:** in the context of heat extremes, passive biological responses, for instance as a result of thermodynamics (i.e., increased biological rates at higher temperatures) or heat-induced damage.

- **Ecological debts:** delayed costs resulting from the activation of biological mechanisms buffering impacts of heat extremes at earlier response stages (akin to the concept of "resilience debt", whereby a preconditioned state of the system incurs effects that are only apparent after the system is disturbed; (Johnstone *et al.* 2016)).

- **Ecological stability:** study of the dynamics and attributes of biological systems in response to disturbances.

- **Exposure:** response stage where organisms perceive and avoid conforming to environmental temperatures that potentially cause performance declines.

- **Demographic bottleneck:** in the context of heat extremes, constrained population growth due to a higher (heat-induced) proportion of less energetically efficient life stages.

- **Heat extreme:** periods of extremely high environmental temperatures at daily to weekly time scales, defined in statistical terms (e.g., several consecutive days with temperatures above the 90th percentile for a reference period) or as absolute temperatures (e.g., related to biologically relevant thresholds), such as CLIMDEX indices (e.g., Buckley & Huey (2016b)).

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- **Legacies:** redeemed ecological debts.

- **Ecological response:** accumulated deviation of a given biological feature (e.g., population size, community composition) from baseline conditions, induced from exposure to a heat extreme (also known as "perturbation").

- **Recovery:** response stage where body temperatures return back to normal after a heat extreme, but ecological responses remain detectable and may even continue to accumulate.

- **Resistance:** response stage where environmental temperatures induce changes in body temperatures, with concomitant and immediate effects on organismal performance.

- **Thermal performance curve:** unimodal relationship between body temperatures and (performance-related) traits, typically displaying an asymmetric shape with a steeper performance drop at high temperatures.

- **Thermal sensitivity:** physiological or fitness response to a given amount of thermal change.

- **Thermal vulnerability:** measure of how close key attributes of thermal performance curves (e.g., optimum, upper thermal limits) are to environmental temperatures that organisms experience. Thermal safety margin (i.e., difference between a trait's thermal optimum and environmental/operative temperature) is one of the main indices of thermal vulnerability.

- **Thermoregulation:** organismal responses via activity patterns, movement and physiology, in order to keep body temperatures within a temperature range providing optimal performance.

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GENERAL DISCUSSION

Throughout their evolutionary history, species have adapted to the typical range of variability of climate extremes in their habitats. Consequently, they have evolved specific physiological tolerances and morphological traits, adjusted the timing of life-history processes and activity patterns, and exhibited various degrees of phenotypic plasticity. However, these strategies may not be sufficient to cope with a higher prevalence and severity of heat extremes, driven by the current rise in global average temperatures. These novel disturbance regimes are expected to alter abundance dynamics in many species and, as a result, trigger shifts in biotic interactions and ecosystem functioning. In this dissertation, we have conducted various experiments using soil organisms to enhance our mechanistic understanding of population and community responses to heat extremes, and utilized the findings of these studies to integrate them into a conceptual framework, that can broadly be applied to understand ecological responses of ectotherms to heat extremes.

Revisiting the main findings

In *Chapter 1,* we illustrate how among-species variation in population responses to an extreme heat event is driven by the distinct thermal sensitivities of life-history traits. More specifically, we showed that population resistance mirrored thermal effects on survival (i.e., negligible effects when exposed at the experimental temperatures), while population recovery reflected thermal effects on fecundity. The most striking response was illustrated by the boreal species*, Protaphorura pseudovanderdrifti,* with a decline in population size of -54% compared to control populations. Remarkably, the relationship between fecundity and population recovery was confirmed by means of shifts in the body size distributions, which were characterized by a lower proportion of small-sized individuals (i.e., juveniles) in *P. pseudovanderdrifti*. These findings provide a trait-based explanation linking life-history and population responses to extreme heat events.

Next, in *Chapter 2*, we show that density dependence, focusing on Allee effects, played a minor role in determining population recovery after heat extremes. We demonstrated this using two Collembola species that differed in their mode of reproduction (parthenogenetic or sexually reproducing), a functional trait expected to influence variation in density-dependent responses to extreme heat. In addition, we confirm the pattern previously shown in *Chapter 1*, that small resistance responses can be followed by more drastic negative recovery after extreme heat events. This finding confirms that, at least in growing populations of invertebrates, heat extremes can cause long-lasting deleterious effects on their abundances, likely due to the impacts of heat on fecundity.

In *Chapter 3*, we demonstrate that communities at lower elevations are more vulnerable to extreme heat events across seasons, especially in spring and summer. This was especially the case for Collembola communities, which showed marked declines in total abundances in spring and summer at the resistance response, but managed to recover to control levels in spring. Fungal communities remained stable shortly after extreme heat events, but after a recovery period of five weeks, the abundances of pathogens increased greatly in summer, while those of saprotrophs declined in autumn at lower elevations. In addition, the connectance of association networks between Collembola and fungi shifted at recovery mainly at low elevations, denoting increased connectance, and hence, more generalistic associations. These results support that extreme heat events have stronger ecological impacts at lower elevations, and these impacts are contingent on the trophic group and the seasonal timing, with implications for community stability.

In *Chapter 4*, building up from the findings of our previous experiments, we synthesize existing knowledge on ecological responses to heat extremes more generally in ectotherms, suggesting several factors that may alter stability after heat extremes in the long run –known as ecological debts. These ecological debts will become larger as heat extremes get more severe in the near future, given that biological mechanisms buffering immediate heat-induced impacts will have greater associated costs. These costs are more easily redeemed during the recovery after heat extremes, and they could accumulate with the occurrence of sequential heat extremes. We provide a summary of the main mechanisms buffering ecological responses to heat extremes and

their associated debts, highlighting the potential importance of debts in determining long-term ecological dynamics after heat extremes.

Ecological stability meets extreme heat: relationships between resistance and recovery

One of our main goals in this thesis was to examine the relationships between different components of stability, namely resistance and recovery, in the context of heat extremes. These relationships provide important insights to infer how ecological systems react in the face of disturbances (Hillebrand & Kunze 2020; McMullen *et al.* 2017), and also underlie fundamental biological processes and strategies to cope with environmental variation (e.g., life-history strategies; Capdevila *et al.* 2022). In this thesis, we observed four main qualitative patterns describing the relationships between resistance and recovery to heat extremes, with a focus on population and community abundances: (1) full stability: negligible effects on resistance or recovery (*Chapters 1, 2 and 3*); (2) debts: negligible effects on resistance, followed by negative recovery (*Chapters 1 and 2*); (3) compensatory: negative resistance, followed by complete recovery (*Chapter 3*); and (4) full instability: negative resistance, followed by negative recovery (*Chapter 3*) (Fig. 1). We underscore that these patterns form a continuum, but separating them in discrete categories allows us to inspect the mechanisms responsible for each pattern in more detail.
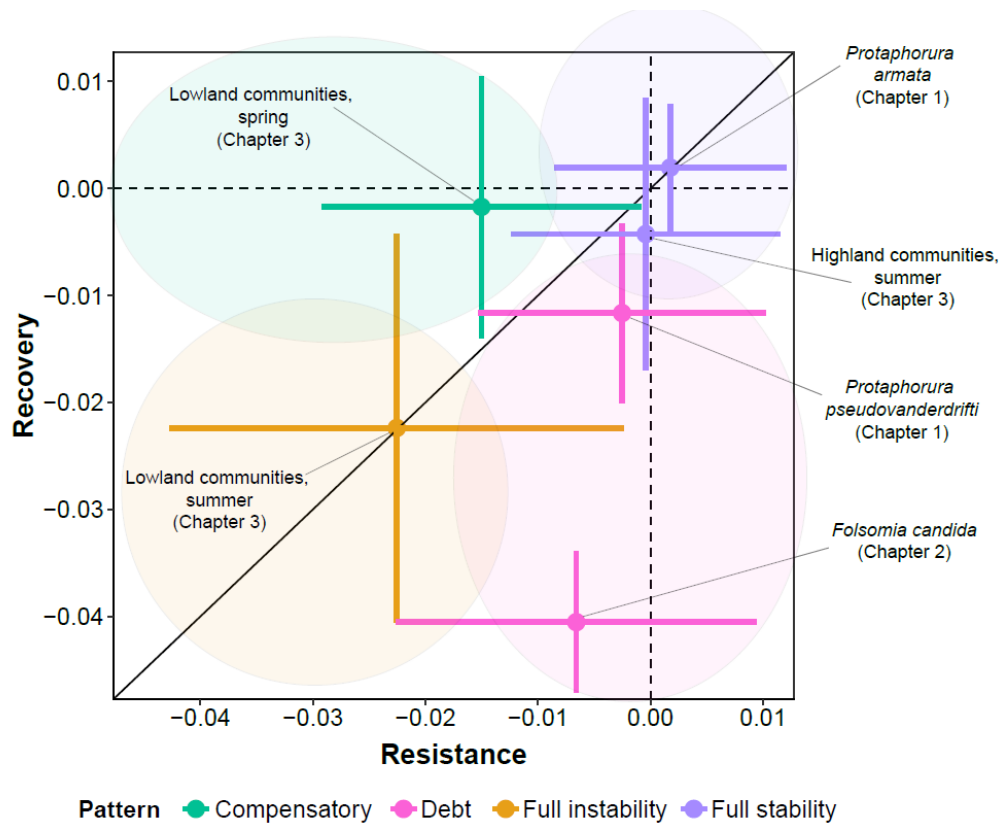
Fig. 1. Synthesis of the main patterns revealed in this thesis on the relationship between stability components (resistance and recovery) in response to heat extremes. Resistance and recovery are estimated by means of standardized effect sizes (Cohen's d ± 95 CI), representing differences between extreme heat and control treatments. To obtain effect sizes, we gathered the designated data from the different chapters and fitted a generalized linear mixed model with the R package glmmTMB (Brooks *et al.* 2017), followed by the calculation of effect sizes using the package emmeans (Lenth 2024). The raw data were species abundances (*Chapters 1 and 2*) and total community abundances of Collembola (*Chapter 3*). We included the identity of the study (chapters) as a random effect. The regions in the parameter space belonging to the various qualitative patterns are displayed with different colors: compensatory (green), debt (pink), full instability (orange), full stability (violet). The diagonal line shows the 1:1 relationship between resistance and recovery, while the dotted lines denote null resistance or recovery responses (i.e., no differences between extreme heat and control treatments).

The pattern representing full stability (Pattern 1) arises when the combined effects of heat on survival and reproductive rates are negligible or cancel out completely (Coblentz *et al.* 2024). This was the case when heat extremes did not pose significant stress, mostly in organisms adapted to cope with more severe thermal regimes than the ones simulated in our experiments (e.g., high elevation communities in *Chapter 3*). The pattern displaying debts (Pattern 2), as thoroughly discussed in *Chapter 4*, initially denotes apparent stability (i.e., negligible resistance responses), but later reveals negative recovery responses, beyond the end of an extreme heat event. This pattern was mainly observed in growing populations of species with heat-sensitive reproduction, such as *Protaphorura pseudovanderdrifti* (*Chapter 1*) and *Folsomia candida* (Chapter 2). In these populations, growth rates depend heavily on reproduction and subsequent recruitment (i.e., the incorporation of newborn individuals into the population, determined by fecundity and juvenile survival). Hence, the impacts of heat extremes on reproduction can substantially slow down the growth of populations that are weakly constrained by competition.

In contrast, the pattern displaying compensatory responses after heat extremes (Pattern 3) might emerge when heat extremes occur shortly before the reproductive peak and/or in populations experiencing strong competition. In these situations, heat-induced mortality (causing negative resistance responses) can relax the constrains on population growth rates imposed by intra- or interspecific competition, possibly favoring enhanced recruitment at the recovery phase that compensates the previous mortality during the heat event. However, less tolerant or low-reproductive species might still suffer negative recovery, as seen in the case of wild populations of *P. pseudovanderdrifti* in *Chapter 3*. This example belongs to the pattern of full instability (Pattern 4), in which sensitive species suffer high mortality and hindered recruitment. It is also possible, as we speculate in the case of lowland communities in summer from *Chapter 3*, that subsequent recruitment after heat extremes is limited by phenological processes. For instance, recruitment after a heat event might not take place until the next reproductive period (e.g., on the following season or year), which means that measurements of recovery should take into account the specific life-history characteristics (including phenology) of the system under investigation. One limitation of our interpretation is that we consider that these patterns are primarily driven by thermal effects on species' vital rates (as illustrated in

Chapter 1). However, the effects of interacting species (e.g., predators) or other factors driving species abundances (e.g., resource availability) could play a substantial role in determining relationships between stability components in populations and communities.

From physiology to communities: strengthening the scaling of heat-induced impacts across levels of complexity

One of the key insights of this dissertation is that ecological responses to heat extremes measured at high levels of biological organization, such as communities, require an integrative understanding of how these responses emerge at the level of physiological processes (Rezende & Bozinovic 2019) and subsequently propagate to traits, population processes, and finally to species abundances and interactions (*Chapter 4*). This is because heat exposure primarily disrupts the physiological machinery of organisms (González-Tokman *et al.* 2020; Ørsted *et al.* 2022), with consequences that scale up to higher levels of organization (van Moorsel *et al.* 2023; Rezende & Bozinovic 2019). A complete mechanistic understanding of these processes might be only possible in well-studied model systems (e.g. *Drosophila*; Bozinovic *et al.* 2020), but we underscore that a high degree of generality requires the assessment of a wide range of ecological systems. In our experiments, we demonstrated how major insights into ecological responses to heat extremes can be gathered in less well-studied groups of organisms, such as Collembola. Our approach was to concentrate on those factors expected to influence fitness components/vital rates more directly, such as life-history traits (*Chapter 1*) or the geographic and seasonal context (*Chapter 3*). In addition, we have built up from classic research topics in climate change ecology, such as thermal vulnerability, to consider other components less frequently assessed but highly relevant in ecological systems, such as long-term dynamics (e.g., recovery; *all Chapters*) and emerging properties at higher levels of organization (e.g., density-dependence; *Chapter 2*). However, in all cases, we put substantial effort to infer ecological responses to heat extremes with a strong physiological grounding (*Chapter 4*). For instance, we show how the response to heat extremes of the species *Protaphorura pseudovanderdrifti* within natural communities (*Chapter 3*) could be understood thanks to the measurement of thermal effects on life-history

traits using laboratory populations (*Chapter 1*). This example illustrates how physiological measurements can be routinely integrated in long-term observational studies and field experiments to inform on the mechanistic basis of ecological responses to climate extremes.

Implications for soil ecosystems in a world with extreme events

The degree of responsiveness of soil organisms to climatic disturbances can have direct consequences on the functions that they provide (e.g., decomposition of organic matter; Peguero *et al.* 2019; Thakur *et al.* 2018) and could cause mismatches between aboveground and belowground processes (Berg *et al.* 2010; Thakur 2020). In this dissertation, we shed light on the possible consequences of heat extremes for soil ecosystems. For instance, as shown in *Chapter 3*, the stability of fungal resources exposed to heat extremes could promote the recovery of their invertebrate consumers (e.g., Collembola). Collembola are mainly primary consumers and therefore represent intermediate trophic levels (Potapov *et al.* 2022), involved in population feedbacks with their resources and predators. Across the different chapters, we provide evidence on how the resistance and recovery of Collembola differs greatly among species (*Chapters 1 and 2*) and depending on the spatiotemporal context (*Chapter 3*). More specifically, we show that high-latitude Collembola species are more heat-sensitive (*Chapter 1*), whereas thermal vulnerability to heat extremes (i.e., contingent on sensitivity and exposure; *Chapter 4*) is greater in lowland Collembola communities (*Chapter 3*). Collectively, these findings suggest that Collembola populations and communities could experience significant short-term declines after heat extremes, but long-term recovery might be possible in some contexts (e.g., heat extremes in spring; *Chapter 3*). One limitation of our experiments is that the importance of soil predators could not be assessed in detail, despite their role in in mediating top-down responses during and after heat extremes (introduced in *Chapter 1*). In fact, top predators in soil ecosystems, such as mites, centipedes or spiders, can affect the climatic responses of lower trophic levels through changes in their abundances or feeding rates, as demonstrated with Collembola and predatory mites (Meehan & Lindo 2023; Thakur *et al.* 2017), with concomitant effects on ecosystem functioning (Lang *et al.* 2014). In addition, top predators might be more susceptible to increased

energetic costs and therefore to local extinction (Fussmann *et al.* 2014; Petchey *et al.* 1999; Vucic-Pestic *et al.* 2011). However, the high degree of omnivory and the various types of energy channels in soil ecosystems could confer them enhanced stability when confronted to heat extremes (Moore & Hunt 1988; Thakur 2020). Integrative studies of whole belowground food webs can yield important insights in this direction, and these could be supported by mechanistic studies focused on one or few trophic levels, such as those included in this dissertation.

Future research prospects

Given the broad scope of topics covered in this thesis, our work represents only a small subset of the potential factors influencing ecological responses to heat extremes. I suggest several prospective research avenues to achieve a more integrative understanding of stability and climate change ecology:

- **Identifying the main mechanisms promoting recovery in populations and communities after pulse disturbances, including heat extremes**. This requires the adoption of a more temporally-explicit view in climate change ecology, which is a field largely based on studies describing single snapshots of ecological responses to warming. From our viewpoint, this research avenue can offer major insights into how population and communities will change their abundances, composition and functioning with the predicted increased occurrence of climate extremes.

- **Evaluating the main sources of variation on the relationship between resistance and recovery after heat extremes**. Due to evident time and logistic constrains, only few factors influencing ecological stability to heat extremes were considered in this dissertation. Other variables that deserve special attention are related to the features of the extreme events (e.g., exposure duration, intensity), demographic processes (e.g., population structure, dispersal), habitat structure (e.g., thermal heterogeneity, habitat

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size and connectivity), or species interactions (e.g., food web complexity, prevalence of disease and parasites).

- **Linking our results obtained from controlled experiments to other complementary approaches, such as mechanistic models and observational studies**. This would enhance our predictive ability and enable more robust inference on the impacts of naturally-occurring heat extremes. For instance, the information obtained from the life-history responses in *Chapter 1* could be used to feed demographic models projecting the long-term dynamics of populations exposed to heat extremes, and these results could be then validated with additional experiments or observational studies in wild populations.

- **Investigating interactions with other global change drivers, as well as sequential heat extremes.** Given that multiple global change factors affect natural systems simultaneously, it is crucial to pinpoint which combinations of factors deserve particular attention given their potential to induce threshold responses (e.g., species extinctions) via additive or synergistic effects. Besides, sequential heat extremes could be driving gradual changes in species abundances, requiring a thorough understanding of cumulative effects (e.g., ecological debts; *Chapter 4*).

- **Bridging the application of our findings to enhance biodiversity conservation in a more extreme world.** Our findings highlight the importance of recovery processes in determining ecological stability in the face of heat extremes. Consequently, a greater focus on conservation policies and management actions aiming to promote recovery after climate extremes could have positive consequences to mitigate the impacts of climate-related disturbances on biodiversity. Among the possible actions (some of which are discussed in *Chapter 4*), we suggest enhancing the availability of cooler microhabitats acting as sources of propagules to enable recolonization after heat extremes, or preventing the establishment of fast-growing and heat-tolerant invasive species following extreme events.

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Declaration of consent

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