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NEW PERSPECTIVES OF SOIL INSECT COMMUNITY ECOLOGY IN THE ANTHROPOCENE

ASSESSING THE IMPACT OF GLOBAL CHANGE ACROSS DIFFERENT CLIMATE REGIONS

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CENTRE DE RECERCA ECOLÒGICA I APLICACIONS FORESTALS · UNIVERSITAT AUTÒNOMA DE BARCELONA

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Dedicatòria

Dedico el meu treball de tesi als meus pares, Miguel-Angel Ferrín i Gemma Guardiola, pel seu suport i afecte incondicionals. A lxs mevxs estimadxs amiguxs i companyxs, Júlia Diumenjó, Vit Marin, Laia "Tranks" Roldan, inter alia, que m'omplen d'alegria als moments més foscos i als dies més brillants. I a la Coco, l'amor indiscutible de la meva vida i la meva millor amiga, que mai deixa el meu costat i el meu cor. Qualsevol esforç a la vida no té sentit sense la millor companyia i, malgrat totes les dificultats, he trobat la meva *manada*.

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que posen la seva vida en perill per salvar els sense-veu. A l'ALF, l'ELF, T.K(F.C), els santuaris i totes les individualitats furibundes. Fins que cada gàbia estigui buida. Alliberament sense compromís.

I finalment, al flux sempre present de la mort que omple alegrement aquestes pàgines de vida!

Dedication

I dedicate my dissertation work to my parents, Miguel-Angel Ferrín and Gemma Guardiola, for their unconditional support and affection. To my dearest friends and partners, Júlia Diumenjó, Vit Marin, Laia "Tranks" Roldan, inter alia, who fill me with joy through the darkest times and the brightest days. And to Coco, the undisputed love of my life and my best friend, who never leaves my side and my heart. Any endeavor in life becomes meaningless without the best companionship, and despite all hardships I've found my pack.

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In the hopes that this work may in some way contribute to the defense of Nature, this is also wholeheartedly dedicated to the warriors, fighters, rioters, propagandists and activists who put their lives in the line to save the voiceless. To the ALF, the ELF, T.K(F.C),

the sanctuaries, and all raging individualities. Until every cage is empty. Uncompromised liberation.

And finally, to the ever present flux of death filling joyously these pages with blossoming life!

"And what is the use of an argument
that leaves people unmoved?"

Paul Feyerabend

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Positionality statement

The author of this dissertation completed the undergraduate degree, MSc and thesis at institutions in formerly colonizer nations in the so-called Global North. Being aware of multiple shapings of race, gender and class, among others, I acknowledge the difficulty of writing about tropical ecology with the author having no cultural or political connections to Indigenous perspectives. I posit myself against neo-colonial science, while admitting the colonial privilege allowing us to do research in French Guiana. This is not a fix to centuries of violence, but rather a contribution to an ongoing discussion (Baker et al., 2019).

General Introduction

Soil insects constitute an extraordinarily rich fraction of soil fauna, with impressive taxonomic and functional diversity, offering great potential for research. However, our understanding of insect ecology is still in its infancy, with most scholars neglecting their response to global change. The current characterization of global change emphasizes the worldwide danger of biodiversity loss rates, climate change, P and N cycle alterations, and novel entities' deposition. Soil insect communities are susceptible to all four drivers, yet little research and no consensus exist on their impacts. Moreover, our knowledge gap extends to the possible interactions between drivers and the responses of insect hotspots such as tropical rainforests and the Mediterranean basin. Recent technological advances, particularly in DNA metabarcoding, are aiding researchers in data acquisition, expanding the niche of soil insect ecology to new reaches. It is thus a fruitful moment to develop new perspectives on soil insect community ecology.

In this dissertation, we study the response of soil insect communities to global change (N and P addition, warming, drought, and novel entities) across different ecosystems (tropical rainforests, the Mediterranean basin, and subarctic shrublands). We will utilize various identification tools currently available (expert-based taxonomic identification, bulk DNA metabarcoding, and eDNA).

By focusing on such a broad subject, we aim to contribute significantly to soil insect ecology while highlighting the knowledge gaps in this discipline.

What do we talk about when we talk about soil insect communities

In this dissertation, we use the term "insect(s)" as a synonym of the subphylum Hexapoda, including species from Class Insecta and Class Entognatha, but we refer to them all as insects for simplicity. Soil insects, therefore, correspond to hexapods with at least one life stage in soils, whether ground-dwelling or living deep inside the soil matrix. Following on from this, soil insect communities frame our definition of soil insects into an assembly of co-occurring species within the soil environment.

Of the 2.13 million described animal species, at least 70% are insects. Species estimates suggest a total of 5.5 million Insecta on Earth, of which 80% remain unknown (IUCN, 2023; Mora et al., 2011; Stork, 2018). This richness is unevenly distributed in space, with most insect hotspots found in tropical ecosystems and the Mediterranean basin (Basset et al., 2012; Fonseca, 2009). This extensive diversity includes various life history strategies, comprising many guilds including carnivores, herbivores, microbivores, and detritivores. Different ecological guilds necessitate a diversity of forms referred to as functional traits: any morphological, physiological, behavioral, or phenological feature measurable at an individual level with impacts on species fitness (McGill et al., 2006; Violle et al., 2014). Combined, this outstanding taxonomic and functional diversity is of great scientific interest (Berg et al., 2010; Haddad et al., 2008).

However, the role of soil insects is still poorly understood and their future uncertain (David, 2014; Montgomery et al., 2019). Despite the efforts of entomologists and ecologists to describe and study soil insects, most arthropod groups remain ostracized, most regions unexplored and most species unidentified. Moreover, current efforts must consider the contemporary ecological context of the planet.

Contextualizing insect ecology in a changing world

The global change paradigm is commonly synthesized into nine planetary boundaries to be respected to stay within Holocene-like conditions (Rockström et al., 2009). Unsurprisingly, five out of the nine boundaries have already been crossed: biodiversity loss rates, P and N cycle alterations, climate change, novel entities' deposition, and land system changes (Persson et al., 2022; Steffen et al., 2015). This dissertation focuses on the first four, which have worldwide distribution regardless of ecosystem type.

Biodiversity loss

Arthropod biodiversity loss constitutes "a quiet extinction" with no reliable global trends (Eisenhauer et al., 2019; Guerra et al., 2021; Montgomery et al., 2019). Of all IUCN-documented population trends, 67% of monitored invertebrate populations show a mean abundance decline of 45%, with 33% of Insecta populations experiencing some degree of decline (Dirzo et al., 2014). Unfortunately, measurements of insect biodiversity loss are uncommon, and the state of most other taxa remains unknown (IUCN, 2023).

Nutrient imbalance

Human-made nutrient imbalance is of great concern for ecosystem conservation (Peñuelas et al., 2013). N and P fertilization and fossil fuels spread macronutrients from northern latitudes to the tropics with N:P ratios increasing southwards (Foley et al., 2011; Galloway et al., 2004; Lamarque et al., 2010; Sardans et al., 2012). These imbalances pose a great risk to the well-being of plants and animals (Elser et al., 1996; Sterner & Elser, 2002). Further research is necessary to assess the impact of N and P availability in arthropods. However, most studies focus on macronutrients and do not pay attention to micronutrient availability. Previous investigations already show significant interactions between macronutrients and elements such as calcium, magnesium, and potassium (Kaspari, 2021; Ouimet et al., 1996; Prather et al., 2020). Therefore, studies must also explore

micronutrients and their potential role as drivers of soil insect community richness, abundance, and composition.

Climate change

Climate change is also a strong driver of worldwide biodiversity patterns (Blowes et al., 2019). Current estimates suggest a global temperature increase of 1°C, combined with shifts in drought and flooding regimes, with a major risk of diversity loss and ecosystem disruption from the tropics and Mediterranean basin outwards (IPCC, 2023). However, there is no consensus on whether soil insect communities respond to temperature increases (Blankinship et al., 2011; Holmstrup et al., 2018; Peng et al., 2022). Our capacity to forecast such responses relies on limited knowledge of the responses of soil insect species and community assemblies, leading to little to no general principles.

Novel entities

Novel entities have recently been recognized as a significant obstacle to biodiversity conservation (Moe et al., 2013; Rillig et al., 2019). The term "novel entities" refers to new xenobiotic molecules of anthropogenic origin with a high potential for unwanted geophysical and biological effects (Steffen et al., 2015). Plastics and antibiotics are some of the most abundant and bioavailable contaminants in the natural environment, yet their fate in soils is still mostly a mystery (Horton et al., 2017; Jensen et al., 2003; Rillig, 2012). Our concern for the well-being of life exposed to novel entities constitutes a crucial gap in our knowledge of the ecology of global change that we must urgently address (Sigmund et al., 2023).

Interactions among drivers

In addition to the individual impact of surpassing these planetary boundaries, we must consider the interactions among them. Single-factor experiments and observational studies allow for nuanced mechanistic interpretations, while multifactorial experiments can

uncover emergent and non-intuitive properties capable of endangering species and ecosystems (Darling & Côté, 2008; Paine et al., 1998; Rillig et al., 2019). Extensive knowledge is strongly advised, but acquiring large amounts of data was previously a major problem in the study of arthropods. Fortunately, current technical advances make data more readily available (Altenburger et al., 2013).

Technical considerations

Soil insect ecology faces many technical difficulties. Montgomery et al. (2019) highlight the challenges, pointing out the paucity of baseline data, incomplete taxonomies, and data gaps, many of which arise from research biases. Geographical biases favor the northern hemisphere, with two major spatial clusters of knowledge: the United States and Europe, while most hotspots are in the tropics with low taxonomic completeness (e.g., South America: 48%) (Rocha-Ortega et al., 2021). Psychological or cultural biases favor focusing on lepidopterans and coleopterans, while other major groups such as Diptera or Hemiptera remain understudied. These research biases can be partially solved through thorough worldwide samplings. But customary expertise-based species identification is highly time-consuming and costly, further hampering data acquisition. Fortunately, new molecular approaches to species identification can help us describe more reliable and comprehensive communities.

State-of-the-art molecular techniques like metabarcoding have recently facilitated worldwide insect identification, where ground insects are molecularly characterized to delimit species-level taxa or operational taxonomic units (OTUs) (Brandon-Mong et al., 2015; Cristescu, 2014; Ficetola & Taberlet, 2023). Alternatively, environmental DNA (eDNA) can be easily extracted from soil samples and still allows for a detailed community description (Taberlet et al., 2018; Zinger et al., 2016). However, such PCR-based methods do not come risk-free and may not provide reliable quantitative estimates of the original relative abundances (Lamb et al. 2019). Research exploring the benefits and learning how to circumvent the limitations

of metabarcoding is essential to ensure its efficient usage and to attain trustworthy species assemblages.

Beyond data acquisition, research focusing on insects has a hard time explaining the variance observed in community richness, abundance, and composition. Consequently, it is not surprising to see researchers claim ecological stochasticity (i.e., drift) as the dominant factor driving insect communities (Peguero et al., 2022; Zinger et al., 2019). However, such conclusions can be seen as a hopeless setback. Our ability to discern between drift and other processes—such as species selection and dispersal—relies on our ability to find the environmental predictors maximizing the explained variance. We must keep trying to find the environmental factors best explaining hexapod assembly before contemplating drift.

Aims

As we have seen, the current state of soil insect community ecology can be described in terms of knowledge gaps rather than unifying principles. Despite the efforts of highly dedicated scholars, soil insect ecological research is still in its dawn, suggesting a necessary rise in academia to shed light on the many folded issues driving today's soil insect communities. Moreover, worldwide emergencies call for the assessment of the ongoing global change as our maximum priority. For this, mechanistic understandings of soil insect ecology must be paired with studies assessing more realistic scenarios focusing on multiple rather than single drivers or locations. Fortunately, technical advances such as DNA metabarcoding can help us attain these new insights into the otherwise alien world of soil insects. In the present dissertation, we aim to produce new perspectives on soil insect ecology by focusing on the impact of global change across multiple ecosystems and utilizing an array of different identification tools.

To address this general objective, we set the following specific objectives:

1. To assess the relative impact of N and P availability on soil insect community assemblies in tropical hotspots (Chapters I and II).

2. To understand the impact of N deposition on soil insect communities in subarctic regions (Chapter III).

3. To describe the response of subarctic soil insect communities to soil warming (Chapter IV).

4. To assess the response of soil insect communities to warming and drought in continental Europe (Chapter V).

5. To explore the response of soil insects to xenobiotic particles and whether it is exacerbated by warming (Chapter VI).

Structure of the thesis

This dissertation comprises six chapters presented as research articles. Half of the chapters have already been published in peer-reviewed international journals. The other three are ready for submission.

We focus on global warming and nutrient allocation in some of the main climatic regions of the world. Initially, we examine human-borne nutrient imbalances. In Chapters 1, we explore the response of soil hexapods to macro- and micronutrient availability in the tropical rainforests of French Guiana, and in Chapter 2 we corroborate our initial observations with N and P fertilization experiments in the same area. Chapter 3 focuses on the response of soil hexapods to N addition in subarctic grasslands, offering an interesting contrast. In Chapter 4, we analyze the response of soil hexapods to a natural geothermal gradient in the same subarctic grasslands. Chapter 5 examines the impact of local experimental conditions of drought and warming throughout continental Europe. Finally,

Chapter 6 explores the interaction between xenobiotics and warming on soil insects through a nanoplastics and antibiotic addition lab experiment. Depending on the study's requirements, we will employ classic expert-based taxonomic identification, bulk DNA metabarcoding, or cutting-edge eDNA metabarcoding to characterize soil insect communities thoroughly.

The general discussions critically examine the main results of the thesis while considering future directions, and the conclusion enumerates the primary findings.

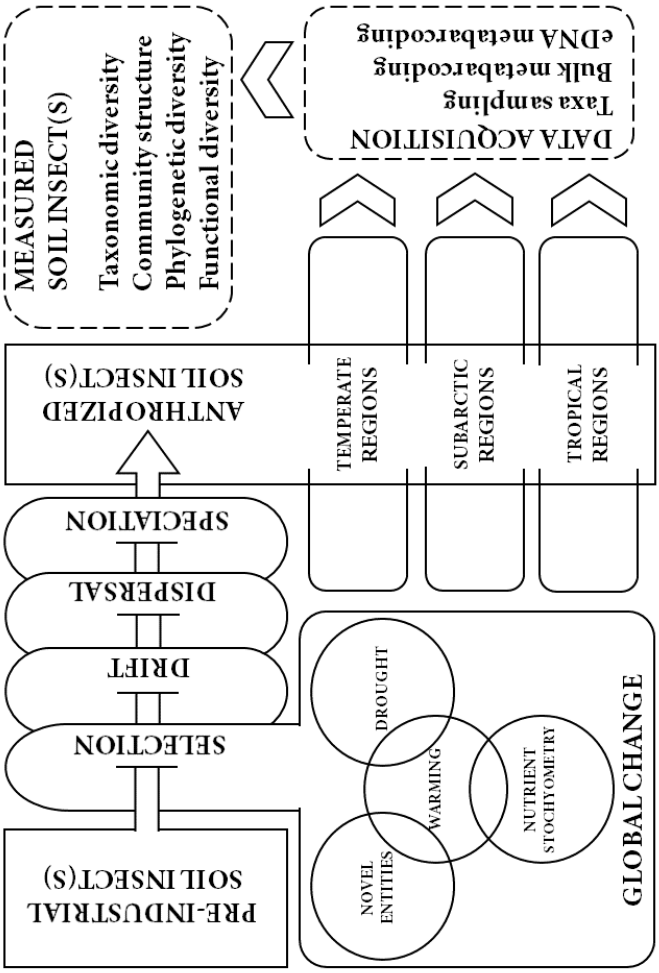

Figure A. Conceptual infographic summarizing the thematic introduction to the dissertation.

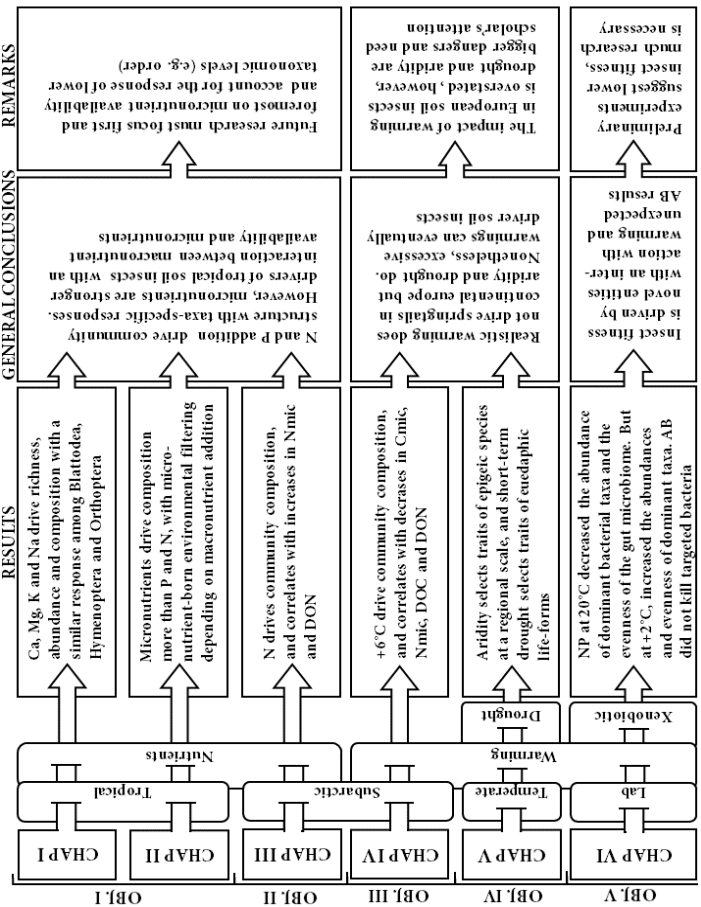

Figure B. Conceptual infographic summarizing the results and conclusions of the dissertation. AB = antibiotic, Ca = Calcium, Cmic = microbial Carbon, DOC = Dissolved Organic Carbon, DON = Dissolved Organic Nitrogen, K = Potassium, Mg = Magnesium, N = Nitrogen, Na = Sodium, Nmic = microbial N, NP = nanoplastics, P = Phosphorous.

Chapter I

Micronutrients are drivers of abundance, richness and composition of soil insect communities in tropical rainforests

Abstract

Communities of soil insects in tropical rainforests are among the richest and most complex, but the mechanisms structuring them remain mostly unknown. Identifying whether nutrient availability plays a relevant role in the assembly of these communities poses several challenges due to the diverse nutritional requirements of insects. We investigated the importance of nutrient availability in accounting for the abundance, richness and composition of soil-insect communities in two tropical rainforests. We sampled soil insects from the litter layer at 72 one square-meter sampling points across three topographic levels at two sites in French Guiana, counted all specimens and characterized each assemblage using DNA metabarcoding. We then determined the importance of nutrient availability by measuring 19 nutrient concentrations and ratios from pools of litter and topsoil. We collected 18,000 specimens from 17 different orders. We found an average density of 262 individuals per m^2. We identified 2634 operational taxonomic units (OTUs) from class Hexapoda, with densities up to 60 OTUs per m^2 and an estimated OTU turnover of 0.98 per m2. Despite this extraordinary diversity and spatial heterogeneity, the concentrations of sodium (Na), potassium (K), magnesium (Mg) and calcium (Ca) were positively correlated with either the abundance or the richness of the communities. The concentrations of soil Na and K alone explained 19 % and 23% of the variation in the abundance

and richness of all hexapods, respectively. We found similar relationships when analyzing the data separately for Blattodea, Coleoptera, Hymenoptera and Orthoptera, the most abundant insect orders with the most OTUs. These micronutrients were also important predictors of the composition of the assemblages. We did not find effects for nitrogen or phosphorus, and only the concentration of carbon in the litter strongly influenced the compositions of the hymenopteran and orthopteran communities. This lack of effect indicated that the availability of macronutrients was not a relevant driver of the communities in tropical rainforests as it is for plants and microbes, in contrast to micronutrients. Our results demonstrated that the availability of micronutrients played a large role in species selection during the assembly of the soil-insect communities in these tropical rainforests. The high unexplained variance, however, suggests that additional neutral and niche deterministic processes, such as stochastic population drift and biotic interactions, likely play complementary roles in structuring insect communities in the soils of tropical rainforests.

1. Introduction

Insects and other arthropods are the most diversified lineages of metazoans on Earth. With an estimated richness of at least five million species, insects represent 70% of all known species of terrestrial animals (Mora et al., 2011; Stork, 2018). This high diversity is unevenly distributed worldwide. Tropical regions harbor more species of insects per hectare than any other biome on Earth (Basset et al., 2012). The current concern over the impacts of global change on the biosphere has led to extensive research on tropical ecology, but studies have disproportionately focused on aboveground communities, neglecting soil fauna, particularly invertebrates. Overlooking "the quiet extinction" of ground-dwelling insects (Eisenhauer et al., 2019) not only poses multiple problems for their conservation, but also compromises the functioning of entire ecosystems. Indeed, soil insects are crucial for decomposing organic matter and cycling nutrients, and tropical forests are no exception (Handa et al. 2014, Seibold et al. 2021, Zanne et al. 2022). The lack of specialized taxonomic knowledge has severely limited our understanding of insect communities in the tropics (Basset et al. 2022). Even simple questions about the most basic community features, such as how many species and individuals they contain, consequently remain either unknown or uncertain at best. The recent advent of DNA metabarcoding has helped us to finally identify the richness

and composition of these highly diverse communities (Basset et al. 2022). Clarifying the drivers controlling the assemblages of soil-insect communities and the abundance, richness and distribution of their species is therefore key for anticipating their responses and designing sound conservation actions.

Biological communities are assembled by multiple drivers varying in the spatiotemporal scales at which they operate. These structuring forces can be synthesized into four fundamental processes: the movement of organisms across space (dispersion), stochastic shifts in the abundance of species (drift), deterministic differences in fitness between individuals of different species due to biotic and abiotic factors (selection), and the evolution of adaptations ultimately leading to species diversification over long periods (speciation) (Vellend, 2017). Dispersal, drift and selection are thought to be the main drivers of community structure and composition at smaller spatiotemporal scales. For terrestrial arthropods, variable dispersal capacity has been suggested to be relevant at a regional or continental level (Gómez-Rodríguez & Baselga, 2018; Soininen et al., 2007), although no clear evidence has been found in tropical forests at smaller spatial scales (Novotny et al., 2007; Peguero et al., 2022). Similarly, finding general abiotic or biotic factors strong enough to determine the composition of insect communities in tropical forests has been difficult. The few attempts to characterize these highly diverse communities have therefore found high levels of unexplained compositional variability across scales, prompting interpretations suggesting that stochastic processes, such as ecological drift, may play a dominant role during assembly (Peguero et al., 2022; Zinger et al., 2018). Nutrient availability has also been recurrently identified as a prevalent abiotic driver of the assembly of tree communities in tropical forests (Peguero et al. 2023). Soil microbes such as fungi and bacteria also have nutrient signatures behind the structure and composition of their communities (Peguero et al., 2022). Identifying whether nutrient availability is among the main environmental filters driving the selection of species within assemblages of soil insects in the tropics poses several challenges due to the diverse nutritional requirements of insects.

Global gradients of available water and solar energy find their highest values in the tropics associated with relatively low climatic variability (Meentemeyer, 1978). Tropical soils are in late developmental stages, with scarce nutrient inputs coupled with a high output from plants and insects, despite these favorable conditions (Peguero et al., 2021; Schlesinger, 2021; Wright et al., 2018). Extensive research has thus found that the availabilities of macronutrients such as carbon, nitrogen and phosphorus (C, N and P, respectively) in tropical soils can limit the decomposition of leaf litter and consequently the structure of soil food webs (Kaspari et al., 2007; Sayer et al., 2010). Recent evidence also suggests that micronutrients can amplify the impact of macronutrients and may also be capable of driving species assembly, not only in plants, but also in microbial and animal communities (Kaspari, 2021; Santiago et al., 2012; Van Langenhove et al., 2021). Nutrient availability in tropical forests is heterogeneously distributed due to landscape-scale variation in forest topography. Topographic features such as slope and ruggedness modulate the hydrological regimen, thereby regulating the weathering, mineralogy and texture of soil (Weintraub et al. 2015), influencing the retention and leaching of nutrients from hilltops to valleys and creating high small-scale variability in ecological constraints via shifts in nutrient availability and stoichiometry (Chadwick & Asner, 2016; Porder et al., 2005). Insects belong to all trophic levels and have complex nutritional needs that vary across taxonomic orders. For example, studies have reported that increases in N availability can favor aphid fitness and decrease the richness of grasshoppers (Hendriks et al., 2013; Sudderth et al., 2005), and P addition can lower overall ant diversity but may favor predatory ants if combined with C, N and P (Bujan et al., 2016; Jacquemin et al., 2012). General conclusions about soil-insect communities as a whole can consequently be elusive. Identifying specific responses at finer taxonomic levels thus becomes crucial for discerning potential discrepancies among groups.

We investigated whether macro- and micronutrients were relevant environmental filters for assemblages of soil insects in tropical rainforests. We sampled complete communities of soil insects from the organic horizon across three topographic levels in two

tropical rainforests in French Guiana. We counted and sorted all specimens by order and classified them into operational taxonomic units (OTUs) using DNA metabarcoding. We also determined the concentration of 10 macro- and micronutrients from both litter and soil pools. We specifically evaluated: (1) whether the availabilities of macro- and micronutrients influenced the abundance, richness and composition of the communities of soil insects, and (2) if the impact of nutrient availability could be due to the dominant insect orders.

2. Material and methods

2.1. Study sites

This study was conducted in two tropical rainforests in French Guiana at the research stations of Paracou (05°16'38'N, 52°55'38'W) and the Nouragues (04°04'53'N, 52°41'13'W). Both sites share the same climate, with a wet season from December to June and a dry season from August to November. Mean annual precipitation and temperature are similar at Paracou and the Nouragues (3102 mm and 25.7 °C vs. 3000 mm and 25.2 °C, respectively). The Guiana Shield is characterized by hydromorphic soils and having one of the lowest concentrations of soil nutrients in tropical South America (Hammond, 2005; Sabatier et al., 1997). The bedrock is Precambrian schist at Paracou and Caribbean granite and gneiss at the Nouragues. Acrisols dominate at both sites, with higher sand concentration and more extractable soil N and P at the bottom of hills, and more clayey minerals and oxides toward the tops, where total nutrient and micronutrient concentrations are highest (Van Langenhove et al., 2021). We established 12 plots of 0.25 ha at each site stratified by three topographic positions to account for spatial heterogeneity: at the top of hills, on the slope and at the bottom (i.e. with four plot replicates per topographic level). We set a central 20-m quadrat in each plot where we marked and geolocated three evenly spaced sampling

points around which we carried out our measurements. This design thus contained a total of 72 sampling points (2 sites × 3 topographic positions × 4 replicate plots per position × 3 sampling points in each plot).

2.2. Nutrient variables

We compiled data for 19 variables describing the total nutrient concentrations and ratios in the soil and litter compartments (Table S1). We collected three randomized soil cores (4 cm in diameter and 15 cm deep) at each sampling point. These three cores were combined as a single composite sample, forming one sample per sampling point. Litter samples for nutrient analysis were collected at each sampling point within a randomly placed 20-cm square of PVC. We then determined the concentrations of macro and micronutrients (carbon (C), N, P, calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na)) in the litter and soil for each sampling point using an Agilent 7500 inductively coupled plasma mass spectrometer (ICP-MS Agilent 7500; Thermo Fisher Scientific, Germany) and an iCAP 6300 Duo inductively coupled plasma optical emission spectrometer (Thermo Fisher Scientific, Germany) (see Urbina et al. (2021) for further methodological details). The concentration of available P in the soil was determined using Olsen and Bray methods (Bray, 1929; Olsen, 1954). We also derived the C:N, C:P and N:P ratios due to their relevance to nutrient cycling. Finally, nutrient data at the sampling and plot levels were summed up at a plot level.

2.3. Taxa sampling

We sampled the communities of soil hexapods at the end of the dry season in 2015. The hexapod communities were sampled from the litter surrounding three sampling points per plot. We collected all litter inside four randomly placed 0.25-m^2 PVC frames and sifted it through a Winkler bag with a 0.7-cm mesh, manually catching all escaping macrofauna. The sifted litter was hung in Moczarsky traps

for 48 h and was then carefully inspected in white plastic trays, and any remaining specimens were captured using entomological aspirators. The bulk communities were stored in 70% ethanol. Before proceeding with the molecular analyses, we classified and counted all specimens into broad taxonomic levels using stereomicroscopes. These samples included specimens of all terrestrial arthropods, but we focused only on specimens belonging to Hexapoda (i.e. classes Insecta and Entognatha) for our molecular analyses. We will, however, refer to all of them as insects for simplicity.

2.4. Molecular analyses

The communities of soil insects were characterized to OTUs using DNA metabarcoding as in Peguero et al. (2022). See Supplementary Materials Section S1: Molecular analyses, for detailed information of the laboratory procedures. The resulting OTU table had a total of 2634 OTUs and 14 thousand reads. We built matrices of the metabarcoded communities at the plot level, aggregating the data of the sampling points in each plot, thus leading to 24 communities.

2.5. Data analyses

We partitioned total β-diversity (Sørensen index) into its turnover (Simpson index) for each sampling scale using the betapart package (Baselga & Orme, 2012). We used β-partitioning to quantify the compositional dissimilarities between sampling points and sites irrespective of the particular environmental conditions. Site and topography did not drive richness and abundances (with the sole exception of Hemiptera's abundances between sites, see Table S2 and S3) and as a result we did not set site as random effect term. Variation in abundance and richness was assessed using general linear models (GLMs) (Bates et al., 2014; Kuznetsova et al., 2017) to detect differences across environmental factors. Normal distribution was selected over other alternatives in all models based on Shapiro–Wilk tests of normality, and we assessed the model fit to the data using the

Akaike information criterion (AIC). Due to a high read variability between samples (Figure 1a) and to avoid the potential impact of laboratory artefacts, richness estimates were based on rarefied communities following the effective number of species at hill number q = 0 (Hsieh and Chao, 2024; Chao et al., 2014). Community matrices were standardized to presence and absence (1 and 0, respectively) to avoid potential discrepancies between DNA sequence reads and the actual abundance of each OTU. Variables were selected using variance inflation factors (VIFs) due to multicollinearity, and the significance of constraining variables was assessed using permutation tests for CCA with marginal effects of terms and 999 permutations. Compositional dissimilarities were analyzed with canonical correlation analysis (CCA) to focus on species composition rather than absolute abundances and to account for potential unimodal responses to nutrients (Ter Braak and Smilauer, 1998). We ultimately selected the dominant orders (i.e. those with >100 OTUs in total) and repeated all analyses for Blattodea, Coleoptera, Hemiptera, Hymenoptera and Orthoptera. All nutrient variables were standardized to z-scores (zero mean and unit variance) before modeling to remove the original measurement units and scales, thus easing the interpretation and helping to address the multicollinearity among covariates (Borcard, 2011). All data handling, visualization and statistical analyses were carried out using R v4.0.6 (R Core Team, 2020).

3. Results

3.1. Abundance and richness of the soil-insect communities

We collected 18070 specimens belonging to 17 orders, with an average density of 262 individuals per m^2. We identified a total of 2634 OTUs from class Hexapoda (1532 in the Nouragues and 1394 in Paracou, Figure S1a and 1c for rarefied estimates), with an estimated OTU turnover of 0.98 per m^2 (Figure 1Sb), indicating the large spatial heterogeneity of species composition in the soils of the tropical

rainforests. We found densities of up to 60 OTUs per m^2 (Figure 1d). Hymenoptera was the most abundant order accounting for 30.2% of specimens collected and 19.7% of all OTU richness, followed by Blattodea with 6.8% of individuals and the highest richness at 28.5% OTUs, Coleoptera (2.6% individuals and 12.6% OTUs), Hemiptera (1.5% individuals and 6.2% OTUs) and Orthoptera (0.2% individuals and 10.9% OTUs) (see Figure 1b and 1d for mean rarefied richness and abundances as well as order-level composition per m2 by orders). The remaining richness was due to orders with totals of <100 OTUs: Entomobryomorpha, Trichoptera, Neuroptera, Diptera, Dermaptera, Psocoptera, Lepidoptera, Thysanoptera, Diplura, Poduromorpha, Embioptera and Mantodea, the from highest to the lowest number of OTUs identified. Despite the large number of OTUs, the curves of species accumulation in Figure 1a importantly had no asymptote, suggesting undersampling, so more species may have thrived at the sampling points than those we were able to characterize, increasing OTU turnover even more. Our ability to assign proper scientific names within each order was limited by the availability of curated DNA barcode libraries used for blasting (see Supplementary Materials, Section S1). Given this severe limitation of accumulated taxonomic knowledge, the majority of taxonomic assignments belonged to Blattodea and Hymenoptera. Termites dominated Blattodea, with most OTUs from the Termitidae family (e.g. *Cylindrotermes*, *Humutermes* and *Nasutitermes*), and ants dominated Hymenoptera, with most OTUs within the genera *Crematogaster*, *Dolichoderus*, *Hypoponera* and *Pheidole*.

3.2. Relationships between abundance, richness and the concentrations of soil nutrients

Micronutrients such as Na, Mg and Ca influenced the abundances of the soil insects. The concentration of soil Na had the largest effect on all hexapods and on the abundances of Blattodea and Hymenoptera and marginally of Coleoptera, three of the five dominant orders

(Figure 2 and Table 1). The concentration of soil Mg was correlated positively with the abundance of Blattodea, and the concentration of Ca had a marginally positive effect on the abundance of Orthoptera. In contrast to these micronutrients, the concentrations of macronutrients such as C, N and P only had modest or marginally significant effects.

The number of OTUs of the communities was significantly higher where the concentration of K in the soil was also higher (Figure 2 and Table 1). In addition to K, Blattodea richness was positively correlated with the Ca concentration in the litter, while Hymenoptera richness significantly correlated with soil concentrations of Na ($P = 0.02$ and 0.005, respectively). In contrast to Hymenoptera, Orthoptera richness decreased with litter Mg and litter Na while increasing with litter Ca and K. ($P = 0.002$, 0.004, 0.02 and 0.005, respectively). The richness of Coleoptera and Hemiptera did not correlate with the nutrient variables tested.

3.3. Importance of the concentrations of soil nutrients to community composition

The composition of all hexapod communities was significantly affected by the concentrations of several micronutrients in the litter pool, such as Ca, K, Mg and Na (Table 2 and Figure 3a). Coleoptera composition was affected by the concentrations of litter Ca and soil K and marginally by the concentration of litter Na (Figure 3b). Hymenoptera composition was affected by the concentrations of Ca, K and Mg (Figure 3c). Orthoptera composition was affected by the concentrations of litter C and Ca and Na ($P = 0.002$, 0.01 and 0.007, respectively; Figure 2d). The concentration of C in the litter was the only concentration affecting the assemblages of Hymenoptera and Orthoptera ($P < 0.01$ in both cases), and the C:N ratio marginally affected the assemblage of Coleoptera ($P < 0.09$). The compositions of the Blattodea and Hemiptera communities were not driven by environmental variables.

Table 1. Relationships between community richness, abundance and the concentrations of soil nutrients in the *Hexapoda* and the main orders in *Insecta*.

	Taxon	Nutrient	Estimate	*F*	*P*	Adjusted R^2
Abundance	Hexapoda (df = 21)	Soil Na	146 ± 59.1	6.16	0.02 *	0.19
	Blattodea (df = 20)	Litter Mg	50.2 ± 22.06	5.19	0.03 *	0.16
		Soil Na	51.7 ± 19.41	7.10	0.01 **	0.22
	Coleoptera (df = 21)	Soil Na	5.84 ± 2.94	1.98	0.06 •	0.11
	Hemiptera (df = 20)	Litter C	-4.74 ± 2.61	3.29	0.08 •	0.09
		Soil C	-5.29 ± 2.17	5.93	0.02 *	0.19
		Litter K	-4.22 ± 2.30	3.36	0.08 •	0.10
		Soil Mg	4.39 ± 2.25	3.80	0.05 •	0.11
		Soil N	-4.68 ± 2.28	4.20	0.05 •	0.13
	Hymenoptera (df = 21)	Soil C	-68.6 ± 37.8	3.27	0.08 •	0.09
		Soil Na	81.5 ± 36.6	4.94	0.03 *	0.15
		Soil P	-70 ± 37.7	3.44	0.07 •	0.10
	Orthoptera (df = 13)	Soil C	1.10 ± 0.60	3.30	0.09 •	0.14
		Litter Ca	1.29 ± 0.63	4.08	0.06 •	0.18
Richness	Hexapoda (df = 21)	Litter Ca	14.1 ± 7.43	3.61	0.07 •	0.10
		Litter K	13.3 ± 7.50	3.16	0.08 •	0.08
		Soil K	17.2 ± 7.12	5.83	0.02 *	0.18
		Soil Na	13.0 ± 7.53	2.99	0.09 •	0.08
		Soil P	-14.8 ± 7.37	4.04	0.05 •	0.12
	Blattodea (df = 19)	Litter Ca	18.3 ± 7.50	5.98	0.02 *	0.19
		Litter K	18.3 ± 7.50	5.98	0.02 *	0.19
		Soil K	16.2 ± 7.75	4.40	0.04 *	0.14
	Coleoptera (df = 19)	Litter Ca	5.26 ± 2.80	3.53	0.07 •	0.11
	Hymenoptera (df = 21)	Soil K	9.54 ± 3.09	9.50	0.005 **	0.27
		Soil Mg	6.30 ± 3.46	3.31	0.08 •	0.09
		Soil Na	9.55 ± 3.09	9.53	0.005 **	0.27
	Orthoptera (df = 18)	Litter Ca	9.77 ± 3.89	6.28	0.02 *	0.21
		Litter K	9.44 ± 3.94	5.74	0.02 *	0.19
		Litter Mg	-12.3 ± 3.45	12.8	0.002 **	0.38
		Litter Na	-11.6 ± 3.59	10.5	0.004 **	0.33

*Note: Results from generalized linear mixed models, with the abundance (number of specimens per plot) richness (rarefied number of OTUs per plot) of each community as response variables modeled against soil nutrient concentrations as single covariates. All nutrient variables were standardized to z-scores (zero mean and unit variance) due to the different units and scales and to ease the interpretation and comparability of different model outputs. Mean estimates of effect are followed by their standard errors. • , * and ** denote P < 0.1, P < 0.05 and P < 0.01, respectively.*

Table 2. Relationships between community composition and the concentrations of soil nutrients in the subphylum *Hexapoda* and the main orders in class *Insecta*.

Taxa	Environmental variable	χ^2	*F*	*P*
Hexapoda (df = 15)	Litter C	0.87	0.98	0.73
	Soil C:N ratio	0.89	1.00	0.23
	Litter Ca	0.91	1.02	0.05 •
	Litter K	0.90	1.02	0.02 *
	Litter Mg	0.92	1.03	0.01 *
	Litter Na	0.90	1.02	0.02 *
Coleoptera (df = 13)	Litter C	0.91	1.01	0.32
	Soil C:N ratio	0.93	1.03	0.09 •
	Litter Ca	0.95	1.05	0.02 *
	Litter K	0.90	1.00	0.26
	Soil K	0.95	1.04	0.03 *
	Soil Mg	0.87	0.96	0.65
	Litter Na	0.93	1.03	0.06 •
	Soil Na	0.90	0.99	0.36
Hymenoptera (df = 13)	Litter C	0.89	1.09	0.009 **
	Soil C:N ratio	0.84	1.03	0.38
	Litter Ca	0.87	1.07	0.042 *
	Litter K	0.92	1.12	0.001 **
	Litter Mg	0.93	1.13	0.001 **
	Litter Na	0.85	1.04	0.113
Orthoptera (df = 17)	Litter C	0.94	1.12	0.002 **
	Soil C:N ratio	0.84	1.00	0.42
	Litter Ca	0.92	1.10	0.019 *
	Litter Na	0.93	1.11	0.007 **

*Note: Results from models of canonical correlation analyses (CCAs) models for the marginal effects of terms and 999 permutations, based on the first CCA axis with P < 0.01. All nutrient variables were standardized to z-scores (zero mean and unit variance) due to the different units and scales, and were selected based on variance inflation factors. •, * and ** denote P < 0.1, P < 0.05 and P < 0.01, respectively.*

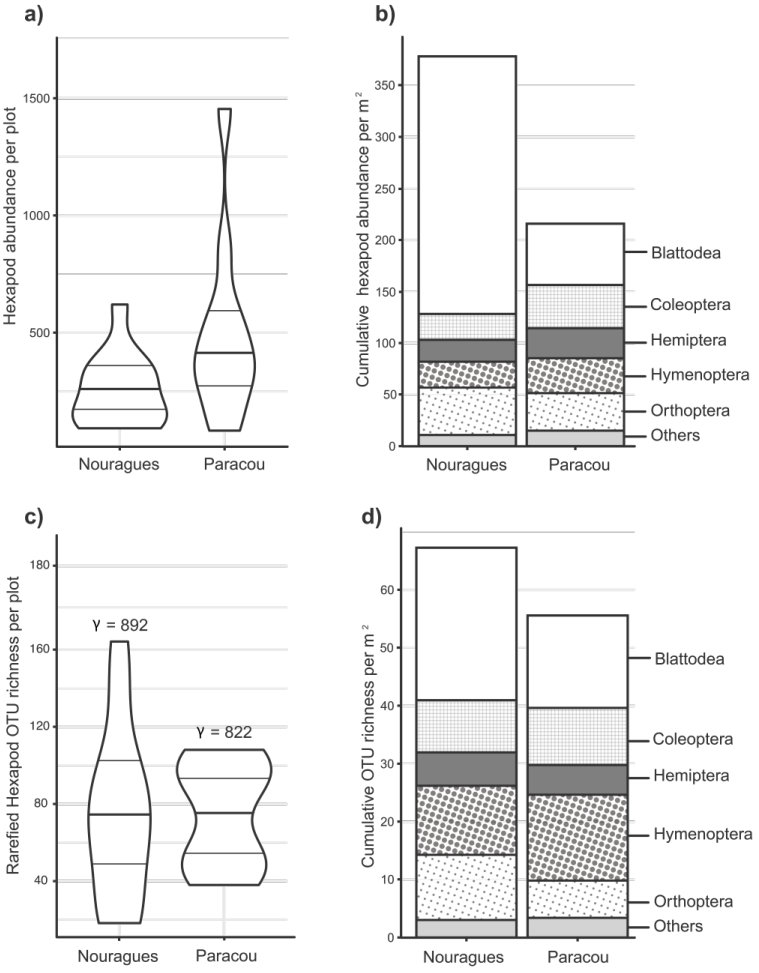

Figure 1. (a) Distribution of the abundance (number of specimens) of Hexapoda per plot. (b) Total abundance (number of specimens) per m2 by order. (c) Distribution of richness (number of OTUs) per plot. (d) Total species richness per m2 by order. γ indicates the total pool of OTUs identified at each site (γ-diversity).

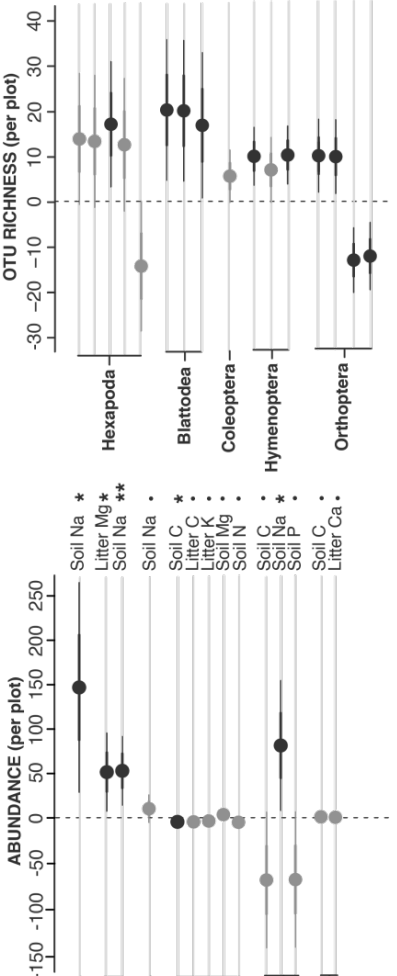

Figure 2. Coefficient plots showing regression estimates and confidence intervals for relationships between community abundance and richness with soil nutrients. Results come from generalized linear mixed models, with abundances (number of specimens per plot) and richness (number of rarefied OTUs per plot) of each community as response variable modelled against soil nutrient concentration as single covariates (see Table 1 for complete outputs). All nutrient variables were standardized to z-scores (zero mean and unit variance) due to the different units and scales, and to ease interpretation and comparability of different model outputs. Grey coefficient plots indicate non-significant regressions ($P \geq 0.05$), black coefficient plots indicate significant regressions ($P < 0.05$). Abbreviations are: carbon (C), calcium (Ca), potassium (K), magnesium (Mg), nitrogen (N), sodium (Na) and phosphorus (P). •, * and ** denote $P < 0.1$, $P < 0.05$ and $P < 0.01$, respectively.

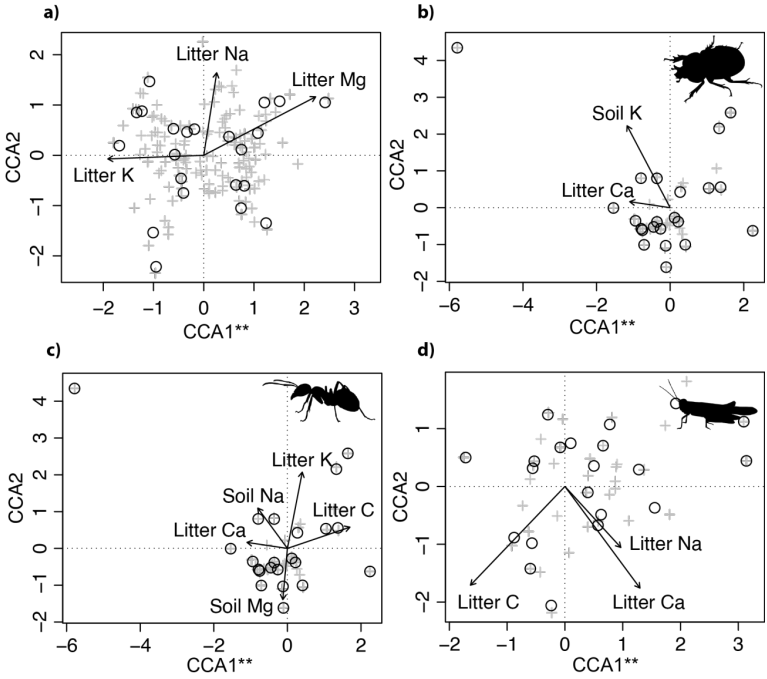

Figure 3. Canonical correspondence analyses of (a) soil hexapod communities, (b) Coleoptera, (c) Hymenoptera and (d) Orthoptera, with standardized nutrient concentrations in the litter or soil pools as environmental predictors. Only significant variables are shown. Significance of the CCA1 axes shown as ** ($P < 0.01$). The crosses and circles indicate samples and plots, respectively.

4. Discussion

Our results suggest that the availability of micronutrients is a multivariate environmental feature that plays a relevant role in species selection during the assembly of the communities of soil insects in the tropical rainforests. The concentrations of essential micronutrients in the soil or litter pool explained a considerable amount of the variation of fundamental properties of our studied communities. The availabilities of Na, K, and to a lower extent of Mg and Ca, were correlated with the abundance, richness and composition of the communities, with varying degrees of strength among the dominant orders. In contrast to micronutrients, the soil or litter concentrations of macronutrients such as C, N and P, typically the main limiting elements in tropical rainforests, were not as important drivers of the soil insects in the communities as they are for plants and microbes (Peguero et al. 2022, Peguero et al. 2023, Vallicrosa et al. 2023). These findings agree with long-term experiments that adding micronutrients, instead of N or P, elicited the largest responses of soil invertebrates in tropical rainforests (Kaspari et al. 2017). Ecological stoichiometry deals with the balance of multiple chemical substances in ecological interactions and processes (Sterner & Elser 2002). This theory has been built upon the observed variation and strong constraints imposed by C:N:P ratios on organisms and ecosystems. Our results, however, highlight the need of extending this theoretical framework to include the entire elementome of species, thereby including the multidimensionality of the stoichiometric or biogeochemical niche of species (Gonzalez et al. 2017, Peñuelas et al. 2019, Kaspari 2021). This need is particularly crucial for understanding the ecology of species and communities of soil fauna, due to the importance of several micronutrients in their elementomes beyond C, N and P (Zhang et al. 2022, Warnke et al. 2023).

The metabolism of all animals depends on the proper functioning of the several kinds of ion pumps in the membranes of their cells. Na-K pumping activity accounts for 25-75% of the entire ATPase energetic budget of an animal cell (Frausto da Silva & Williams

2001). The density of the ground-dwelling Hexapoda in our communities increased with the availability of soil Na, and the two dominant insect orders, Blattodea and Hymenoptera, responded the same. The higher Na concentration in the animal tissue than the leaf litter suggests that Na may be a strongly limiting nutrient for soil fauna (Kaspari 2021). There are several studies documenting the close relationship between Na availability and ant activity in tropical rainforests (Arcila Hernández et al., 2012; Kaspari et al., 2008), and while Na is highly mobile, it is also known to enhance litter decomposition and accumulates in fungal fruiting bodies, thus attracting insect microbivores and fungivores (Kaspari et al., 2014; Schowalter, 2006). Likewise, the concentration of K was also higher in ants than the surrounding litter in a tropical rainforest, although the tissue-litter difference was smaller than for Na (Kaspari 2021). The correlation between K availability and the richness of OTUs in the soil is consistent with K also being a limiting nutrient for soil-insect communities.

Mg and Ca are also important elements for osmoregulation, excretion and both nervous and motor systems. Na and K are highly soluble, so their continuous fluxing across membranes make them prone to be lost (Kaspari 2021). The concentration of Ca was correlated with Blattodea and Orthoptera richness, and the concentration of Mg was correlated with Orthoptera richness, and the abundances of Blattodea. Both elements influenced the compositions of the Hymenoptera, Coleoptera and Orthoptera communities. In addition to muscle contraction, Ca may be particularly important for soil faunal detritivores such as oribatid mites, isopods and diplopods, whose exoskeletons are stiffened by Ca phosphates and carbonates and other mineral depositions that increase defensive capacity against predators (Warnke et al., 2023; Neues et al. 2011; Zhang et al. 2022). Cuticle biomineralization in not common within Insecta, but recent findings suggest that Ca and Mg may play a role in the sclerotization of the cuticle, particularly of the pupae in holometabolan groups (Rong et al., 2019; Yamamoto and Fujiwara, 2023).

Our results, however, contrast with other studies that have found a more modest contribution of the nutrient environment on the assembly of soil-insect communities (Peguero et al. 2022, Zinger et al. 2018). This discrepancy may likely be due to the aggregation of several abiotic variables (e.g. nutrient Euclidean distances in Peguero et al. (2022)) to describe the environmental variability among sites, instead of directly using the concentrations of particular elements in the soil, which may mask the importance of nutrient variables with low concentrations and variability across space. The putative environmental filtering exerted by micronutrient availability, however, only explained a portion of abundance, richness and compositional variability. The high amount of unexplained variance suggests that additional neutral or niche processes, such as stochastic population drift and biotic interactions, may also play a role in structuring insect communities in the soils of tropical rainforests (Peguero et al. 2022, Zinger et al. 2019). In fact, the negative correlations between the concentrations of Na and Mg in the litter with the richness of Orthoptera could be associated with the opposite relationships with Hymenoptera abundance and richness with Na, because many ant species are predators of crickets and grasshoppers and many other ground-dwelling arthropods (Agosti et al. 2002). Distinguishing between the importance of antagonistic biotic interactions, such as predation, competition and neutral processes such as stochastic population drift and dispersal limitation, and the purely abiotic sorting of species, would require a different approach. This approach should probably combine experimental methods with a focus on narrower taxonomic groups such as order-level analyses.

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Chapter II

Micronutrients drive tropical soil insect communities but Phosphorus and Nitrogen fertilization do not

Abstract

Human-borne inputs of N and P are at the core the global nutrient imbalance, endangering ecosystems' functioning and life itself. However, the response of soil arthropods is poorly understood and there is no consensus on whether micro or macronutrients are the main drivers of soil community assemblages. Tropical forests allow us to study the response of nutrients on highly weathered soils, where previous studies show N and P limitation, as well as micronutrient limitation. We assessed the relative importance of micro and macronutrients in order to elucidate the main nutrients driving tropical nutrient imbalances. We did an N, P and NP field fertilization experiment in two French Guiana tropical rainforests (the Nouragues and Paracou) to study the response of metabarcoded soil insect communities to macronutrients, while also assessing the impact of micronutrient availability (calcium (Ca), potassium (K), magnesium (Mg) and sodium (Na)). We assessed the importance of these nutrients through their impact on the abundance, richness, and composition of soil insect communities using linear models, canonical correlation analyses and analyses of variance. Our macronutrient field fertilizations did not drive hexapod abundance, richness or community composition. And in contrast, Ca, Mg and Na increased hexapod abundance, Ca, K and Mg increased OTU richness and Ca and Na drove community composition. Our results argue against the widespread assumption

that macronutrient imbalances drive ground-dwelling insects, and we suggest that micronutrients need much more attention to properly assess the impact of nutrient imbalances and excessive fertilization in soils.

1. Introduction

The global nutrient imbalance compromises the ecological stability of the biosphere and it is of great concern due to the potential consequences for human well-being (Peñuelas et al., 2013). The current inputs of nitrogen (N) and phosphorus (P) fertilization increase unremittingly, with N:P ratio increasing since pre-industrial times from the northern latitudes southwards to the tropical regions (Galloway et al., 2004; Foley et al., 2011; Lamarque et al., 2010; Peñuelas et al., 2012). These stoichiometric imbalances between macronutrients jeopardize the performance of plants and animal (Elser et al., 1996; Sterner & Elser, 2002). However, most studies have been consistently neglecting the response of arthropods despite their impressive diversity, total biomass and ecological functions (Eisenhauer et al., 2019; Guerra et al., 2021; Mora et al., 2011; Stork, 2018). Unlike most organisms, tropical insects are not commonly driven by macronutrients, and in contrast, previous studies suggest that micronutrients explain a considerable amount of the variation of insect community properties (Chapter 1; Kaspari et al., 2017; Peguero et al., 2022; Vallicrosa et al., 2023). Unfortunately, research is still scarce (Kaspari, 2021), and the relative impact of human-borne macronutrient imbalance and natural micronutrient availability on tropical soil insect assemblages is currently unknown.

Previous research shows inconsistent responses reporting that N and P addition can either favor or hinder insect fitness. Prior studies on P addition suggest increased litter decomposition, arthropod density and diversity (Kaspari et al., 2007; McGlynn et al. 2007; Sayer et al., 2010). And studies on N addition suggest control over primary production, and abundance loss in herbivore invertebrates (Elser et al., 2007; Kaspari & Yanoviak, 2009; Kaspari et al., 2017; LeBauer & Treseder, 2008; Wright et al., 2018). Moreover, N and P can be co-limiting, with P availability depending on N availability to ensure phosphatase activity (Marklein & Houlton, 2012) and accelerate the P cycle (Deng et al., 2017; Li et al., 2016). However, despite the constraints exerted by macro-nutrient availability, the relative impact of micro-nutrients can be larger than that of P and N.

The ecological stoichiometry of micronutrients offers valuable knowledge focusing on the balance of chemical elements in ecological processes (Sterner & Elser 2002). Calcium (Ca), potassium (K), magnesium (Mg) and sodium (Na) cations are fluxing micro-nutrients with important functions on osmoregulation as well as nervous and motor systems (Kaspari, 2021). Na-K pumping accounts for 25-75% of the entire ATPase energetic budget of animal cells (Frausto da Silva & Williams, 2001), with higher Na con-centration in insects' tissues than litter (Cromack et al., 1977). Competing cations like Ca and Mg can be as limiting as macronutrients and catalyzers of N and P availability (Han et al., 2019; Neues et al. 2011; Warnke et al., 2023; Zhang et al. 2022). And in addition to muscle contraction, recent findings also suggest that Ca and Mg may play a role in the sclerotization of the cuticle, particularly of the pupae in holometabolic groups (Rong et al., 2019; Yamamoto and Fujiwara, 2023).

The relative impact of macronutrient fertilization and micronutrient availability is thus up to debate, and tropical forests are the epitome of nutrient limitation offering an in-credible context for experimental research. Tropical soils are in late developmental stages with scarce nutrient input coupled with a high output from plants and insects (Peguero et al., 2021; Schlesinger, 2021; Wright et al.,

2018). As a result, extensive research has shown that macronutrients in tropical soils limit litter decomposition and the structure of brown food webs (Kaspari et al., 2007; Sayer et al., 2010). However, recent studies focusing on the tropical rainforests of French Guiana describe a remarkable case study where both macro- and micronutrients have been recognized as important limiting factors. Previous research show P and N limitation in the soil of two tropical rainforests of French Guiana (Vallicrosa et al., 2022; Van Langenhove et al., 2020), while Ferrín et al.'s (Chapter 1) points at micronutrient limitation on soil insects by Ca, K, Mg and Na controlling insect's abundances, richness and composition more than macronutrients.

In this paper, we assessed the relative importance of macronutrient addition and micro-nutrient availability driving tropical soil insect community structure. We did a nitrogen, phosphorus and nitrogen-phosphorus addition field experiment in two primary tropical rainforests of French Guiana (the Nouragues and Paracou) to study the response of 72 soil insect communities to macronutrients in spatially standardized plots. We counted and sorted all specimens by order and classified them into operational taxonomic units (OTUs) using DNA metabarcoding. And we assessed the impact of micronutrient availability by accounting for seven micronutrient variables including litter and soil magnesium, potassium, sodium and litter calcium. Ultimately, we hypothesize that: (i) the impact over insect abundance, richness and community composition will be larger under NP addition compared to the other fertilization treatments due to nutrient co-limitation, and (ii) micronutrients will be the main drivers of insect communities despite macronu-trient addition.

2. Material and methods

2.1. Study sites

This study was conducted in two primary tropical rainforests in French Guiana in the research stations of the Nouragues (04°04'53'N, 52°41'13'W) and Paracou (05°16'38'N, 52°55'38'W). Both sites share the same tropical climate, with a wet season from December to June and a dry season from August to November. Mean annual precipitation and temperature are similar at both sites (the Nouragues: 3000 mm and 25.2 °C; Paracou: 3102 mm and 25.7 °C). The Guiana Shield is characterized by having poor weathered and eroded soil nutrient content, with hydromorphic soils (Hammond, 2005; Sabatier et al., 1997). The bedrock is Caribbean granite and gneiss at the Nouragues and Precambrian schist at Paracou. Acrisols dominate both sites, with higher sand content at hill-bottoms, and more clayey minerals and oxides toward the tops. Additionally, Paracou soils are loamy sand to sandy loam, while soils at the Nouragues contain more clay and span from sandy loam to silty clay.

2.2. Experimental conditions

The nutrient addition experiment was set on October 2016. In Paracou and the Nouragues we stablished three blocks of four 50 × 50 m plots (Courtois et al., 2018). Each block matched a different topographic level: hill-top, slope and bottom; and within each block we set a plot for each treatment: control, N, NP and P. In addition, we set a central 20 m quadrat in each plot where we marked and geolocated three evenly spaced sampling points around which we did our measurements. This design thus contained a total of 72 sampling points (2 sites × 3 topographic positions × 4 treatments × 3 topographic positions × 3 sampling points in each plot). Fertilizer was applied twice per year: half at the peak of the dry season (October-November) and half in the driest period amidst the wet season

(March). For the N treatment we deposited 125 kg N $ha^{-1} \times year^{-1}$ as urea (CH_4N_2O), for the P treatment we deposited 50 kg P $ha^{-1} \times year^{-1}$ as triple superphosphate ($Ca(H_2PO_4)_2$), and NP treatment had both. The triple superphosphate contains a 15% Ca. These amounts were set to allow comparisons with other fertilization experiments (Wright et al. 2011).

2.3. Micronutrient variables

We compiled data for seven variables describing the concentration of calcium, magnesium, potassium and sodium in the litter and soil (soil Ca not available; see table S1). We collected three randomized soil cores (8 cm in diameter and 15 cm in length) at each sampling point down to a 15 cm depth. These three cores were combined as a single composite sample, forming one sample per sampling point. Litter samples for nutrient analysis were collected at each sampling point within a randomly placed 20 cm square of PVC. We then determined the concentrations of micronutrients in the litter and soil for each sampling point using inductively coupled plasma mass spectrometry (ICP-MS Agilent 7500; Thermo Fisher Scientific, Germany) and also by inductively coupled plasma optical emission spectrometry (iCAP 6300 Duo; Thermo Fisher Scientific, Germany) (see Urbina et al., 2021 for further methodological details). Finally, nutrient data at a sampling level was combined at a plot level.

2.4. Taxa sampling

We sampled the communities of soil hexapods at the end of the dry season in 2019. The communities of soil hexapods were sampled from the litter surrounding the three sampling points per plot. We collected all litter inside four randomly placed 0.25-m^2 PVC frames and sifted it through a 0.7 cm mesh Winkler bag, manually catching all escaping macrofauna. The sifted litter was hung in Moczarsky traps for 48 h and then carefully inspected for any remaining specimens, which were collected with entomological aspirators. The bulk

soil hexapod communities were stored in 70% ethanol. These samples included specimens from Class Insecta and Class Entognatha (i.e. subphylum Hexapoda) but we refer to them all as insects for simplicity. Before molecular analyses we counted the abundances of each taxonomic order in the laboratory.

2.5. Molecular analyses

The communities of soil insects were molecularly characterized to delimit molecular operational taxonomic units (OTUs) using DNA metabarcoding. The resulting OTU table had a total of 11981 insect OTUs and 27 thousand reads. We finally built matrices of the metabarcoded communities at plot level aggregating the data of the sampling points in each plot, leading to 24 insect communities. See Supplementary Materials Section S1: Molecular analyses, for detailed information of the procedures.

2.6. Data analyses

Site and topography did not drive richness and abundance (Table S2), and as a result we did not set them as random effect terms. We used general linear models (GLMs) to assess whether micronutrient availability changed with treatments. To take into account the spatial heterogeneity in nutrient distribution potentially linked to the position of each plot. Normal distribution was selected over Poisson in all models after checking with Shapiro–Wilk test of normality and checking model fit with Akaike information criterion (AIC). Due to a high read variability between samples (Figure 1a) and to avoid the potential impact of laboratory artefacts, richness estimates were based on rarefied plot-level communities following the effective number of species at hill number $q = 0$ (Hsieh and Chao, 2024; Chao et al., 2014). For abundance, we did not use the metabarcoding reads but lab countings at order level. Insect community richness and abundances were assessed by means of GLMs to detect differences across environmental factors. Compositional dissimilarities

with micronutrients were analyzed with canonical correlation analysis (CCA), community matrices were standardized to presence – absence (1 and 0, respectively). Compositional dissimilarities were analyzed with CCA to focus on species composition rather than absolute abundances and to account for potential unimodal responses to nutrients (Ter Braak and Smilauer, 1998). Variable selection were done using variance inflation factors (VIFs) due to severe multi-collinearity (Borcard, 2011), and the significance of constraining variables were assessed via analysis of variance (ANOVA) tests with stepwise procedure for final variable selection. Compositional dissimilarities with macronutrients were analyzed with permutational ANOVA (PERMANOVA). Nutrient variables were standardized separately for compositional analyses using z-scores, z-score transformation allows us to minimize the effects of using variables with different units. All data handling, visualization and statistical analyses were carried out using R v4.0.6 (R Core Team, 2020).

3. Results

3.1. Taxonomic diversity

We identified 11981 soil insect OTUs classified into 19 different orders, adding up to 11219 specimens. OTUs density at control samples was the highest with 226 taxa per m2 and 184 counted individuals, followed by P addition with 193 taxa per m^2 and 153 counted individuals, N addition with 183 taxa per m^2 and 221 counted individuals, and NP addition with 173 taxa per m^2 and 202 counted individuals (Figure 1b). Hymenoptera was the most dominant order covering 61% of all abundance and 25% of all OTUs, followed by Collembola (9.6% of abundance and 9% of OTUs), Coleoptera (5.2% of abundance and 15% of OTUs), Hemiptera (2.7% of abundance and 5.7% of OTUs), Blattodea (0.5% of abundance and 9.1% of OTUs) and Orthoptera (0.5% of abundance and 15.7% of OTUs) (see Figure 2d for mean richness and abundances per m^2). The remaining richness was composed of groups with less than 5% of total OTU

richness, in descending order of richness: Thysanoptera, Embioptera, Dermaptera, Diptera, Lepidoptera, Trichoptera, Psocoptera, Diplura, Neuroptera and Archaeognatha. Despite the large amount of OTU, plot-level species accumulation curves in Figure S1 show no asymptote at all, suggesting under-sampling in the field and/or low PCR replicates, sequencing depth, etc. Therefore, more species might thrive in the sampling points than those we were able to characterize, probably increasing OTU richness.

3.2. Response of soil insects to nutrient availability

Fertilization treatments did not impact the natural concentrations of micronutrients in litter and soil (Figure S3). Hexapod abundance increases with litter Ca, soil Na and litter and soil Mg (p-values of 0.008, 0.02, 0.003 and 0.001; respectively, Table 1 and Figure 2). Soil insect OTU richness increases with litter Ca, Mg and K (p-values of 0.03, 0.008 and 0.03; respectively, Table 1 and Figure 2). And Community composition is driven by litter Ca and Mg (p-values of 0.003 and 0.01; respectively, Table 2 and Figure 2). As for the macronutrient fertilization experiment, neither treatment drives hexapod abundance, OTU richness or community composition (Table 2).

4. Discussion

There is an open debate on whether human-borne macronutrient imbalances drive ground-dwelling insect communities more than micronutrient availability (Chapter 1; Kaspari, 2021). Our results suggest that micronutrient availability in litter and soil drive tropical ground-dwelling insect communities through abundance, richness and community composition. The availability of litter Ca increased hexapod abundance, OTU richness and correlated with community composition. Similarly, Mg increased hexapod abundance and OTU richness, and Na increased hexapod abundance and drove community composition. But K availability had a lower impact over ground-dwelling insects, increasing OTU richness but nothing else.

Table 1. Response of the soil hexapod community to micronutrients

Hexapod abundance						
Soil layer	Nutrient	Estimate	*F*	*P*	R^2	Df
Litter	Ca	176 ± 61.2	8.28	0.008 **	0.24	21
	K	502 ± 265	3.57	0.07 ·	0.10	
	Mg	971 ± 291	11.1	0.003 **	0.31	
	Na	0.12 ± 0.06	3.31	0.08 ·	0.09	
Soil	Mg	1.17 ± 0.31	14.2	0.001 **	0.37	
	Na	0.65 ± 0.27	5.61	0.02 *	0.17	
OTU richness						
Soil layer	Nutrient	Estimate	*F*	*P*	R^2	Df
Litter	Ca	52.6 ± 23.5	5.01	0.03 *	0.14	22
	Mg	459 ± 158	8.39	0.008 **	0.24	
	K	189 ± 85	4.87	0.03 *	0.14	
Community composition						
Soil layer	Nutrient	X^2	*F*	*P*		Df
Litter	Ca	0.89	1.03	0.003 **		19
	Mg	0.84	0.98	0.78		
	Na	0.88	1.02	0.01 *		
Soil	Na	0.86	1.00	0.12		

*Notes: Results for abundance and OTU richness are general linear models, with community abundance and richness as the response variable modeled against micronutrient variables. Richness is rarefied by Hill number q = 0. Results for community composition are based on marginal CCA anova, with community composition as the response variable modeled against z-score transformed micronutrient variables. Estimates of models are followed by their standard errors. Abbreviations are: calcium (Ca), magnesium (Mg) and sodium (Na). ** and *** denote P < 0.01 and P < 0.001, respectively.*

Table 2. Response of soil hexapod communities to treatments

Hexapod abundance					
Treatment	Estimate	*t*	*P*	R^2	Df
N	-179 ± 190	0.94	0.35	0.00	19
N + P	63.6 ± 181	0. 35	0.73		
P	-0.83 ± 181	0.00	0.99		
OTU richness					
Treatment	Estimate	*t*	*P*	R^2	Df
N	-72.5 ±64.4	-1.12	0.27	0.00	20
N + P	- 82.3 ± 64.4	-1.27	0.21		
P	-46.7 ± 64.4	-0.72	0.47		
Community composition					
Variable	Sum of squares	*F*	*P*	R^2	Df
Treatment	1.47	0.99	0.47	0.13	23

Notes: Results for abundance and OTU richness are general linear models, with community abundance and richness as the response variable modeled against micronutrient variables. Richness is rarefied by Hill number q = 0. Results for community composition are based on marginal CCA anova, with community composition as the response variable modeled against z-score transformed micronutrient variables. Estimates of models are followed by their standard errors. Abbreviations are: nitrogen (N) and phosphorus (P).

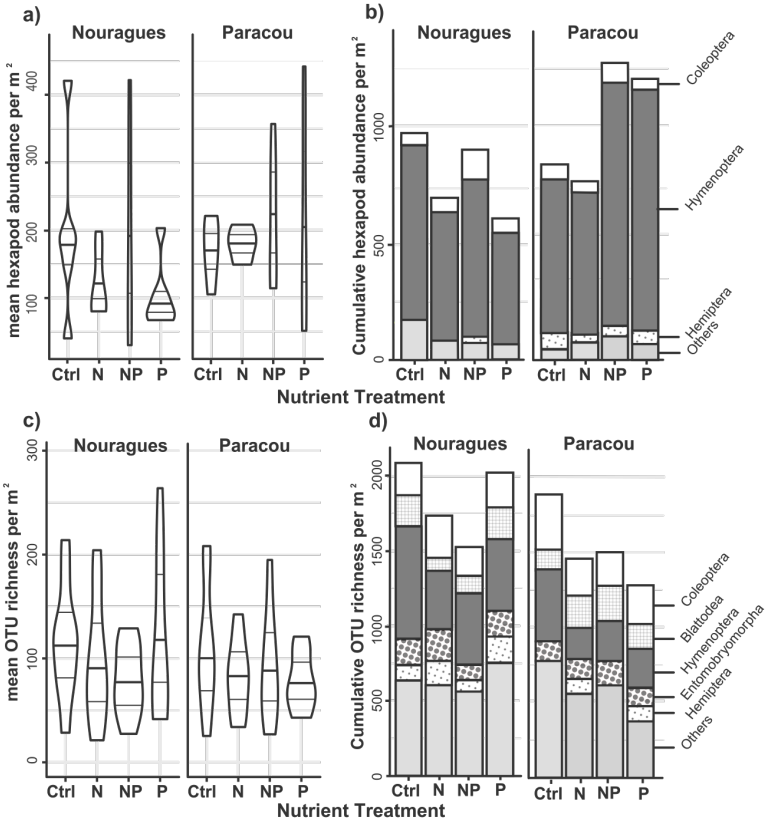

Figure 1. Description of hexapod diversity in Paracou and the Nouragues for all four treatments (Ctrl: control, N: nitrogen, NP: nitrogen-phosphorus, P: phosphorus). (a) Violin plots describing mean hexapod abundance per sample (1m2) , (b) stacked bar plots for mean abundance by order at a sample level, (c) violin plots describing mean rarefied OTU richness per sample and (d) stacked bar plots for mean rarefied OTU richness per sample. Horizontal lines inside each violin from lowest to highest denote the first, second and third quartile. Violins were trimmed to adjust to existing data. Non-dominant orders are gathered in "Others".

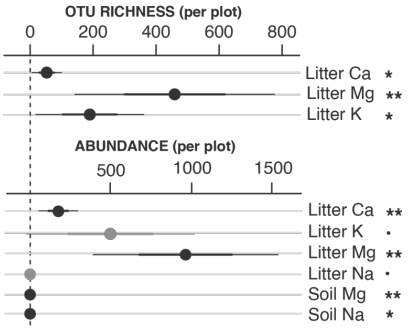

Figure 2. Coefficient plots showing regression estimates and confidence intervals for relationships between community abundance and richness with nutrients. Results come from general linear models, with abundance (number of specimens per plot) and richness (number of rarefied OTUs per plot) of each community as response variable modelled against soil nutrient concentration as single covariates (see Table 1 for complete outputs). Grey coefficient plots indicate non-significant regressions ($P \geq 0.05$), black coefficient plots indicate significant regressions ($P < 0.05$). Abbreviations are: calcium (Ca), potassium (K), magnesium (Mg), and sodium (Na). •, * and ** denote $P < 0.1$, $P < 0.05$ and $P < 0.01$, respectively.

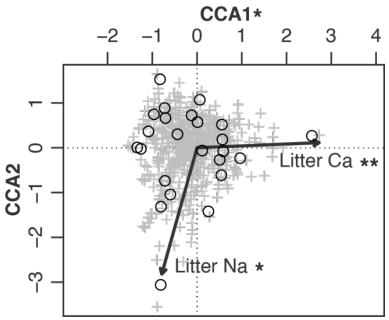

Figure 2. Canonical correspondence analyses of soil hexapod communities with soil nutrient concentrations of litter or soil pools as environmental predictors. Only significant variables are shown (see Table 1 for complete outputs). Nutrient variables were standardized to z-scores (zero mean and unit variance) due to the different units and scales and to ease interpretation. Significance of CCA1 axes shown as * ($P < 0.05$). Crosses indicate samples and circles indicate plots.

In contrast, our field fertilization experiment suggests that imbalances in N and P do not exert any control over tropical ground insect communities whatsoever. Our findings argue against the widespread observation suggesting macronutrient limitation in tropical rainforests, driving other organisms such as plants and microbes (Elser et al., 1996; Sterner & Elser, 2002). We do, however, corroborate previous observations pointing at micronutrient imbalances as an important driver of ground-dwelling tropical insect communities (Chapter 1; Kaspari et al., 2017). As a result, we suggest that the N and P imbalance promoted by global change is not a concerning driver of tropical ground insects, and instead we highlight the relative impact of micronutrients such as Ca, K, Mg and Na.

Our results after the field experiment fall in line with previous observations on Paracou and the Nouragues, corroborating that N and P are not important drivers of tropical ground-dwelling insects. Previous research on the same locations before fertilization describes nutrient-driven community assemblies in trees, bacteria and fungi (Peguero et al., 2022). And Vallicrosa et al.'s (2023) research after fertilization describes N and P colimitation in plant communities. However, unlike plants and microorganisms, pre-fertilization insect community assemblies were not affected by nutrients (Peguero et al., 2022), and N and P availability had no impact over insect abundance, richness and community composition (Chapter 1). Moreover, since our fertilization treatments did not modify the concentrations of micronutrients (Table S3), we argue that the impact of macronutrients is not mediated by micronutrients. And despite previous research describing spatial autocorrelation (Peguero et al., 2022; Van Langenhove et al., 2021), we did not find dissimilarities between sites or topographic levels (Table S2 and S3). Such a lack of differences could stem from the fertilization experiment homogenizing Paracou and the Nouragues enough to become statistically similar in terms of hexapods abundance and OTU richness. However, Ferrin et al. (Chapter 1)'s results prior to fertilization did not find such spatial dissimilarities either.

As we expected, our results suggest that micronutrients explain more variance than macronutrient addition in abundance, richness and composition (Table 1). When focusing on the impact of specific nutrients, Ferrín et al. (Chapter 1) found micronutrient control over hexapods abundance, richness and composition. After applying fertilizers, we describe a similar response to micronutrients. However, the particular impact of Ca, K, Mg and Na differ from those observed before fertilization. Ferrín et al. (Chapter 1) results suggest no impact from Ca and a stronger impact of K over hexapod richness and community composition, while in contrast, we report a strong impact from Ca increasing hexapod abundance, richness and controlling community composition with a comparatively low response to K availability. As we mentioned previously, dissimilarity between pre- and post-fertilization findings are not driven by site, topography or treatment. However, dissimilarities between samples might also arise due to methodological differences regarding metabarcoding performance, with previous research suggesting half and double our rarefied OTU richness and double the hexapod abundance (Chapter 1; Peguero et al., 2022). Moreover, Ferrin et al. (Chapter 1) analyses at the order-level show that Blattodea richness and abundance increase with K. Since we found lower Blattodea abundance and OTU richness in our hexapod communities, the impact of K over our postfertilization findings might have been lower than previously reported. Likewise, prefertilization research also reports that Hymenoptera richness and abundance increases with K availability, yet despite our postfertilization experiment finding a larger hymenopteran richness and abundance we did not find any noticeable impact of K over hexapods. Such contradiction might come from biotic interactions and demographic stochasticity (e.i. drift) taking place during the three years of fertilization. (Peguero et al. 2022, Zinger et al. 2018).

Our study proves that human-made tropical macronutrient imbalances do not drive ground-dwelling hexapods. Nonetheless such disagreements are attested by previous observational reports on hexapods' response to macronutrients in the same locations

(Chapter 1; Peguero et al., 2022). In contrast, micronutrients such as Ca, K, Mg and Na control tropical soil insect communities' richness, abundance and composition; corroborating past accounts describing micronutrient limitation. However, the relative impact of Ca, K, Mg and Na remains inconsistent across research before and after fertilization (Chapter 1). We suggest that stochastic processes and unknown biotic interactions during the field experiment are behind these dissimilarities. Ecological drift is an important process determining the fate of ground insects with most variances remaining unexplained in all our models (Peguero et al. 2022, Zinger et al. 2018). A better understanding of the impact of micronutrients and hexapod stoichiometry is thus necessary to unravel the mechanisms relating micronutrients to ground-dwelling insects. Moreover, we suggest that the impact of macronutrient fertilization on tropical rainforests has been overstated and future research most focus on micronutrient availability. We encourage researchers to critically assess the relative impact of micronutrients in over-fertilized soils, and explore other understudied ecosystems prone to stoichiometric imbalances.

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Chapter III

Responses of soil hexapod communities to increasing Nitrogen in a subarctic grassland*

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Abstract

The warming of boreal ecosystems accelerates decomposition and increases nitrogen (N) availability. The impact of increased N on subarctic soil fauna communities, however, remains poorly understood. We investigated the response of soil hexapods to a N addition experiment in a subarctic grassland. We characterized the soil hexapod communities using environmental DNA metabarcoding and analyzed the levels of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), microbial carbon (Cmic), and microbial nitrogen (Nmic). N addition increased DON and Nmic, while DOC and Cmic pools remained unchanged. Furthermore, N addition caused shifts in soil hexapod community compositional diversity between control and N plots in herbivore and microbivore taxa. The levels of DON and Nmic strongly correlated with these shifts, explaining 54% and 45% of the compositional variability, respectively. This study demonstrates a clear link between N availability and shifts in soil hexapod communities, associated to changes in microbial and dissolved N pools in subarctic grasslands.

Main text

The subarctic region harbors the largest pool of terrestrial carbon (C) on Earth (Scharlemann et al., 2014). Increasing temperatures in these ecosystems are expected to accelerate the decomposition of soil organic matter (SOM) eventually leading to a higher availability of nitrogen (N) due to the higher N mineralization rates of soil microbes (Marañón-Jiménez et al., 2018; Walker et al., 2018). Yet, the impacts of this potential increase in N availability on the communities of soil fauna in subarctic ecosystems remain largely unknown. This is of particular importance due to our increasing appreciation of the interactions between microbes and soil fauna, which typically accelerate SOM decomposition (Handa et al. 2014), and how these relationships may be mediated by overall nutrient availability at an ecosystem level (Peguero et al. 2019).

Arthropod-mediated decomposition is a prominent example of our knowledge gap regarding the impact of N addition. Soil hexapods facilitate decomposition and mineralization via litter fragmentation and habitat transformation (Bardgett & van der Putten, 2014; Filser, 2002), but little is known about their response to shifts in N availability. Most detritivore hexapods are adapted to low-N diets, and microbial N (Nmic) is their main path for N assimilation (Douglas, 2009). Consequently, N availabity may drive a bottom-up control on microbivore soil hexapods mediated by microbial communities

(i.e. food resources), ultimately regulating hexapods' abundance, diversity and activity (Chahartaghi et al. 2005; Fiedler et al. 2007; Filser, 2002; Hyodo et al. 2011; Traugott et al. 2008). However, to our knowledge no previous research has assessed the validity of this trophic cascade involving the response of hexapod communities to shifts in nutrient availability and microbe-derived C and N, which may be particularly sensitive at high-latitude ecosystems.

To better understand the impact of changes in carbon (C) and nitrogen (N) availability on soil hexapod communities in subarctic ecosystems, we conducted a N fertilization experiment (+150 kg N ha^{-1}) in a natural grassland in Iceland. We characterized soil hexapod communities by means of environmental DNA (eDNA) metabarcoding, and we analyzed the levels of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), microbial carbon (Cmic), and microbial nitrogen (Nmic). Additionally, we investigated whether these environmental variables could account for the compositional dissimilarities observed among hexapod communities. We hypothesized that N addition would affect the structure and compositional diversity of soil microbivore hexapod communities through indirect effects arising from alterations in microbial C and N.

We conducted this study at the ForHot research site in Iceland (Sigurdsson et al., 2016) from August 2017 to June 2018 (64°0'N, 21°11'W) (see the supplementary materials for further information on the sampling site). Since 2014, the experimental plots had received annual treatments of 150 kg N ha^{-1}, applied in three doses as NH_4-NO_3. We collected soil cores using an auger to a depth of 10 cm from five replicate plots (2 x 2 m) per N level (control vs. N addition) in four seasons (2 treatments x 5 replicates x 4 seasonal samplings; N = 40) (see Table S1 for the nil impact of seasonality over arthropod communities). We characterized the soil hexapod communities using molecular Operational Taxonomic Units (mOTUs) obtained through eDNA metabarcoding of the 16S mitochondrial rDNA region (see supplementary materials for detailed protocol information). We quantified dissolved organic C (DOC) and dissolved organic N in all soil samples in 1 M KCl extracts. We determined

soil Cmic and Nmic using the chloroform-fumigation extraction method, followed by 1 M KCl extraction. We analyzed all extracts for DOC and DON concentrations using a TC/TN-Analyzer (Shimadzu, TOC-VCPH/CPNTNM-1 analyzer). The units for all environmental variables are concentrations in parts per million (ppm). We conducted data handling, visualization, and statistical analyses using R v4.0.6 (R Core Team, 2020) (see the supplementary materials for further details on the statistical analyses).

Principal component analysis (PCA) showed that DON and Nmic were the most significant environmental variables distinguishing the control and N treatment groups (Figure 1), with non-overlapping confidence ellipses in the environmental PCAs between the treatments. General linear models (GLMs) confirmed that N addition increased DON and Nmic, while reduced the microbial C:N ratio ($P < 0.01$, < 0.05 and < 0.05, respectively; Table 1). The number of eDNA reads and mOTU richness did not differ between plots with or without N addition ($P = 0.84$ and 0.57, respectively; Figure S1 in the supplementary materials). However, we identified significant differences between the soil hexapod communities in the control and the N plots ($P < 0.01$, explained variance 15%; Figure 2) based on a sparse partial least squares discriminant analysis (sPLS-DA). A higher score in the first variate of the sPLS-DA indicated a greater compositional dissimilarity with the control plots. The compositional dissimilarities driven by N addition were primarily influenced by certain species, notably the collembolans *Protaphorura armata*, *Sminthurinus bimaculatus*, and *Megalothorax minimus*, as well as the plant hopper *Javesella obscurella* (Figure 3). In contrast, the collembolan *Pogonognathellus flavescens*, the rove beetle P*hilhygra debilis*, and the crane fly *Tipula cockerelliana* stood out as the more distinct taxa under control conditions (Figure 3). The first sPLS-DA variate was then subjected to GLMs against the set of environmental variables to assess to what extent these predictors could account for compositional variability. The values of this first variate positively correlated with the amount of Nmic and soil DON ($P < 0.05$ and < 0.01, respectively) and also marginally correlated with the amount

of DOC ($P = 0.06$; Table 2). Thus, this indicated that the higher the amount of Nmic, DON and to a lower extent of DOC, the greater was the compositional dissimilarity of the soil hexapod communities between the control and the N plots.

Nitrogen addition had a clear impact on the compositional diversity of soil hexapod communities in our studied grassland in the subarctic, which correlated with the increased level of N both in the soil solution and the microbial pool (Table 2). The extreme variations in N requirements among trophic levels and across phylogenetic lineages preclude generalizations of the effect of N for the whole subphylum Hexapoda (Fagan et al., 2002). Previous research has shown that increased N concentrations in the soil can benefit the fitness of insect herbivores via lower C:N ratio in plant tissues (Mattson 1980; Zechmeister-Boltenstern et al., 2015). For instance, the increase of the phloem-sucking *J. obscurella* after our N-addition experiment may underscore the end of the N limitation (Firn et al., 2019; Gargallo-Garriga et al., 2021). Similarly, lower C:N ratio in leaf-litter may favor microbial biomass and ultimately detritivore populations (Table 1) (Gargallo-Garriga et al., 2021). Therefore, it comes as no surprise that –since *S. bimaculatus* can be classified as an epigeic primary decomposer feeding on recently fallen litter, and *P. armata* and *M. minimus* are euedaphic secondary decomposers (Chahartagi et al., 2005; Potapov et al., 2016)– detritivore collembolan species prefer N rich soils.

Interestingly, certain taxa thrived in conditions of lower N availability. A notable example is *T. cockerelliana*, whose larvae demonstrated a competitive advantage when nitrogen was scarce due to their ability to address nitrogen deficiency through microbial nitrogenase activity (Kostina et al., 2020). Additionally, the microbivore collembolan *P. flavescens* also flourished in lower N concentrations, supporting a previous study that suggested enhanced fitness for this species in environments with reduced microbial nitrogen (Chagnon et al., 2001). Regrettably, there are no published studies that shed light on the preference of the rove beetle *P. debilis* for N-poor soils.

In contrast to N, microbial C did not influence the compositional diversity of soil hexapod communities and only the increase of C in the soil solution appeared to have a minor role (Table 2 and Figure 2). The breadth of the isotopic signature of detritivores in soil meso and macrofauna is narrower for C than for N (Korobushkin et al., 2014; Potapov et al., 2016). This suggests a relatively small variation of C sources in soil detritivores that could eventually result in lower sensitivity to variations of this resource in the soil environment. Yet, the experimental addition of N barely affected the pools of C in the soil solution and in the microbial communities. Nonetheless, N addition might influence nutrient availability beyond C pools. For instance, previous research show how a large N supply favors phosphatase synthesis and increases P availability increasing plant productivity and ultimately modifying soil quality (Deng et al., 2016; Marklein & Houlton, 2012). Overall, this study provides a clear linkage between the availability of N and shifts in the compositional diversity of soil hexapod communities, which are related with changes in the microbial and dissolved N pools in subarctic grasslands.

Further research is needed to elucidate the specific mechanisms underlying these responses, with a closer examination of the trophic interactions between subarctic hexapods and soil microorganisms and plant traits, and additionally, assessing the potential feedbacks of the observed changes at a community-level for ecosystem functioning.

Acknowledgement

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Table 1. Effect of nitrogen (N) addition over dissolved and microbial carbon and N

Response variable	Effect estimate	F-statistic	P-value	Adjusted R^2
Soil DOC	40 ± 30	1.34	0.27	0.03
Soil DON	60 ± 10	41.4	0.001	0.81
Microbial C	230 ± 230	0.93	0.36	0
Microbial N	170 ± 60	7.74	0.02	0.42
Microbial C:N	-0.66 ± 0.22	8.78	0.01	0.46

Note: Effect estimates (± standard error) were calculated by means of separate general linear models for each environmental variable. The intercepts (not shown) are the ambient conditions. Units of response variables are in parts per million (ppm). All models have 8 degrees of freedom.

Table 2. Relationship between the compositional similarity of the soil hexapod community with dissolved and microbial carbon and nitrogen

Explanatory variables	Effect estimate	F-statistic	P-value	Adjusted R^2
Soil DOC	0.017 ± 0.007	4.63	0.06	0.28
Soil DON	0.02 ± 0.008	11.8	0.008	0.54
Microbial C	0.001 ± 0.001	1.83	0.21	0.08
Microbial N	0.007 ± 0.002	8.53	0.02	0.45

Note: Compositional variability of soil hexapod communities was synthesized as the x-variate from an sPLS-DA. The higher the value of the x-variate (response) the greater was the dissimilarity between control and nitrogen-addition communities (see Figure 2). The effect estimates (± standard error) of each environmental variable were calculated by means of separated general linear models. Units of response variables are in parts per million (ppm). All models have 8 degrees of freedom.

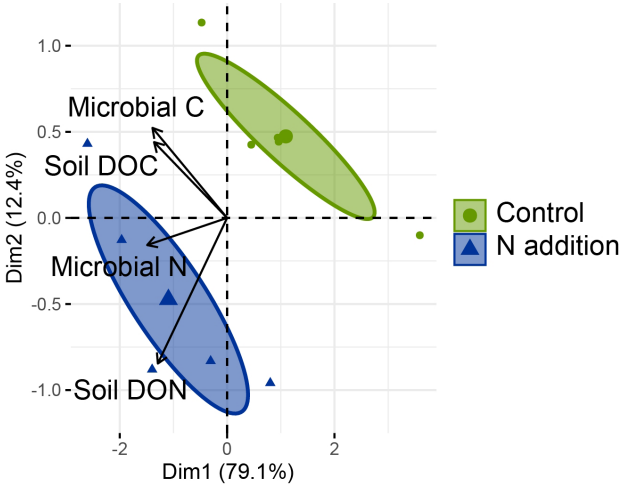

Figure 1. Soil environment. Principal component analysis of the variables describing the soil environment (microbial carbon, microbial nitrogen, soil dissolved organic carbon and nitrogen -DOC and DON, respectively-). Ellipses denote 95% confidence envelopes for the control and the nitrogen addition plots.

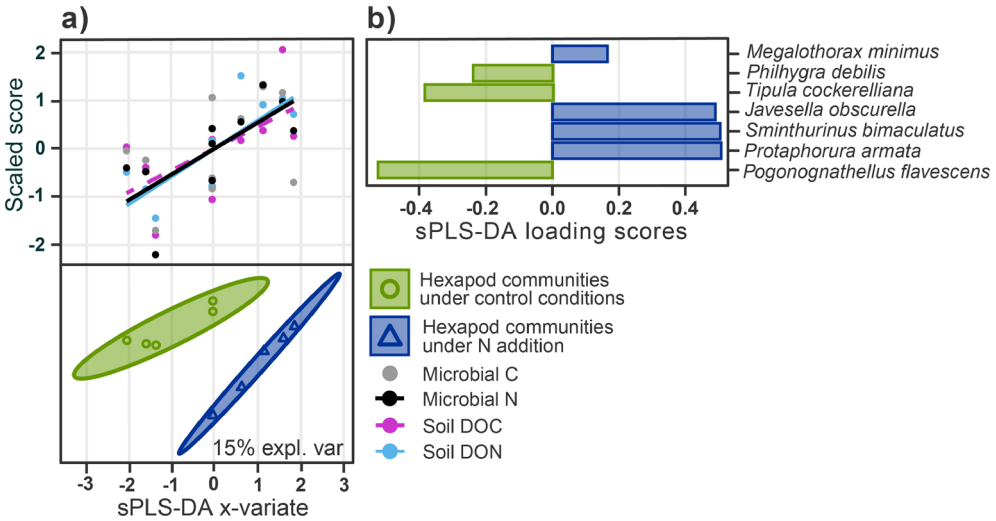

Figure 2. Soil hexapod communities. (a) Compositional variation of the soil hexapod communities based on a single-component sparse partial least squares discriminant analysis (sPLS-DA) between control and N addition plots. The amount of explained variance by the x-variate is shown in the inset. Confidence ellipses are set at 95%. The linear relationships between the x-variate scores with the corresponding soil environmental variables (microbial carbon, microbial nitrogen, soil dissolved organic carbon and nitrogen -DOC and DON, respectively-) are shown on the top of the sPLS-DA ordination. Solid and dashed lines show significant and marginally significant slope parameters ($P < 0.05$ and $P < 0.1$, respectively). See Table 2 for further information on the linear models. (b) Identity of the main hexapod species-level mOTUs driving the compositional differences between the treatments. Only species-level mOTUs with a loading score >0.1 in the corresponding discriminant analysis are shown.

Chapter IV

Responses of soil hexapod communities to warming are mediated by microbial Carbon and Nitrogen in a subarctic grassland*

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Abstract

Warming in subarctic ecosystems will be two-fold higher compared to lower latitudes under current climate change projections. While the effects of warming in northern ecosystems on plants and microorganisms have been extensively studied, the responses of soil fauna have received much less attention, despite their important role in regulating key soil processes. We analyzed the response of soil hexapod communities in a subarctic grassland exposed to a natural geothermal gradient in Iceland with increases of +3 and +6 °C above ambient temperature. We characterized hexapod communities using environmental DNA (eDNA) metabarcoding. We analyzed the amounts of microbial carbon (Cmic), microbial N (Nmic), dissolved organic C (DOC) and dissolved organic N (DON) and then assessed whether these variables could help to account for the compositional dissimilarity of ground hexapod communities across temperatures. The increases in soil temperature did lead to changes in the composition of hexapod communities. The compositional differences caused by +6 °C plots were correlated with a decrease in Cmic and Nmic, soil DOC and DON. Our results highlight the response of soil hexapods to warming, and their interaction with microbial biomass ultimately correlated

1. Introduction

Northern regions are experiencing the fastest warming rate on Earth (Scholze et al., 2006; Williams et al., 2007). Under these new temperature conditions soil microbial activity is accelerated, increasing decomposition rates and reducing the stocks of labile carbon (C; Saad & Conrad, 1993). Despite the initial increase in microbial activity, substrate depletion leads to the loss of microbial C (Cmic), as well as microbial N (Nmic; Marañón-Jiménez et al., 2019; Marañón-Jiménez et al., 2018). The ultimate depletion of labile C and N may have a larger impact on plant productivity than the direct effects of temperature alone, and so the impacts on nutrient dynamics are important for ecosystem functioning in northern latitudes (Rustad et al., 2001; Treseder, 2008; Zhang et al., 2019). However, the impacts of C and N availability and warming on soil biodiversity of subarctic regions are mostly unknown, despite subarctic ecosystems accounting for the largest pool of soil C on the surface of Earth (Scharlemann et al., 2014).

Most information about the response of subarctic species to climate change is limited to the aboveground ecosystems, with soil fauna being mostly unexplored (Eisenhauer et al., 2019). Previous studies have not reached a consensus about how subarctic hexapod diversity responds to warming. While some studies have argued that the direct reaction should be strong, with generalized reductions of

hexapod species abundances and drastic alterations in community composition (Blankinship et al., 2011), other studies have suggested that these changes may only be transient due to their high resilience at a community level (Holmstrup et al., 2018; Peng et al, 2022). Accordingly, some studies have reported that soil warming can even improve the fitness of particular hexapod species, which could even expand their areas of distribution (Deutsch et al., 2008; Sánchez-Bayo & Wyckhuys, 2019). However, global warming can also have multiple indirect effects over soil biodiversity (Bardgett & Wardle, 2010). Thus, downstream shifts in C and N availability as a result of the effect of soil warming over soil microbial communities and vegetation can strongly impact indirectly the communities of soil arthropods (Filser et al., 2016). Nonetheless, the impact of C and N throughout soil food-webs remains poorly studied. Most soil ecosystems are relatively poor in N, and many detritivore hexapods have low-N diets and Nmic is their main pathway for N assimilation (Douglas, 2009). Therefore, the abundances of some hexapod species may decrease with lower C to N ratio (C:N), while other microvorous arthropods may exploit the increases in bacterial and fungal biomass (Peguero et al., 2022). This complexity and the lack of a general pattern linking warming with C and N availability, microbial decomposers and hexapod communities in soil hampers our ability to predict their combined responses to climate change and the consequences for ecosystem functioning, which may be particularly sensitive at higher latitudes.

The role of soil hexapods in C and N cycling is still poorly known despite their prominent contribution to the dynamics of soil nutrients (David, 2014). Hexapod detritivores break down soil organic matter into particles with higher surface to volume ratios, facilitating their further decomposition and mineralization by the local microbial community (Bardgett & van der Putten, 2014; Filser, 2002). Microbial feeders, though, exert a strong top-down control on microbial communities, thus regulating their abundance, diversity and activity (Filser, 2002; Freckman, 1988). The typically cryptic morphological traits used in the identification of soil hexapod

species is likely one of the main reasons hindering our knowledge of these communities. Advanced molecular techniques like metabarcoding have recently facilitated their identification, even using DNA remnants in the soil (Taberlet et al., 2018; Zinger et al., 2016). This environmental DNA (eDNA) can be easily extracted from soil samples and allows a detailed community description and even the discovery of species that otherwise could not be detected (Cristescu, 2014), thereby facilitating the study of soil faunal communities.

Here, we studied the impact of soil warming on soil hexapod community composition in a subarctic grassland. We collected soil samples across a natural geothermal site in Iceland at ambient temperature, +3 and +6 °C above ambient. We characterized soil hexapod communities using eDNA metabarcoding and analyzed the amounts of Cmic, Nmic, soil dissolved organic C (DOC) and soil dissolved organic N (DON). We then assessed whether these variables could help to account for the compositional dissimilarity of hexapod communities across temperatures. We hypothesized that soil warming would have an impact on the structure and composition of soil hexapod communities likely through indirect effects arising from alterations of microbial communities' C and N concentrations.

2. Material and methods

2.1. Site description and experimental conditions

This study was conducted at the ForHot research site in Iceland (Sigurdsson et al., 2016) between August 2017 and June 2018 (64°0'N, 21°11'W). Soil type was a Brown Andosol (Arnalds, 2015). Mean annual temperature at the site was 5.1 °C. The coldest and warmest temperatures in the neighboring village of Eyrarbakki in 2016 were -12.3 °C and 21.6 °C, respectively. Average annual precipitation for the same year was 1153 mm (Icelandic Meteorological Office, 2016). The vegetation was an unmanaged grassland dominated by *Agrostis capillaris* L., *Galium boreale* L. and *Anthoxantum odoratum* L..

Vascular plants cover 46% of the area over a moss mat which covers up to 88% of the ground. Natural N deposition in the area is 1.3 ± 0.1kg N ha^{-1} y^{-1} (Leblans et al., 2014). This grassland has been geothermally warmed since 29 May 2008, when an earthquake transferred geothermal energy from hot groundwater to previously unheated soils (Sigurdsson et al., 2016). Belowground temperatures at 10 cm depth now display a permanent warming gradient reaching +10 °C, with a less severe increase in temperature at the soil surface of +0.2 C. The warming has only been mildly disruptive respective to previous conditions, and the warmed area experiences a similar magnitude of warming and cooling over the seasons. Soil humidity was only marginally affected, with volumetric water content changing from 40% to 38%, and water pH increased from 5.6 in unheated soil to up to 6.3 after warming. Geothermal groundwater has remained in the bedrock and has not reached the root zone, thus avoiding direct eco-toxicological effects (Sigurdsson et al., 2016). The resulting stable conditions and lack of artifacts provide a realistic natural belowground experiment on soil warming under climate change. Even though, we acknowledge that aboveground effects of the on-going warming should lead to an opposite warming gradient across the soil profile (i.e., warmer at the top) and it may also entail complex plant-soil feedbacks that may not be entirely captured by the geothermal warming present at our study site. Five transects were established, each one consisting of three 2 x 2 m plots, and each plot at different temperature: an unheated control, a low warming level of ca. +3 °C and a higher warming level of ca. +6 °C above the ambient reference in the control (henceforth referred as "+3 °C" and "+6 °C").

2.2. Soil core sampling

Soil cores were collected using an auger to a depth of ~10 cm, excluding the O horizon. Soil cores were sampled seasonally four times: August 2017, corresponding to late growing season; November 2017, at start of winter and initial soil freezing; April 2018, with the first

soil thaw in un-warmed soils, and June 2018, in the early part of the growing season. We thus collected a total of 20 core samples for each warming treatment (5 replicates in 4 seasons for 3 temperature levels = 60 samples). All samples were immediately sieved to remove roots and stones larger than 2 mm. Fifteen grams of each sample were then frozen in plastic bags in liquid N in the field to immediately stop all biological processes. All frozen samples were then freeze-dried in the laboratory following the standard protocol of the commercial lyophilizer for this type of sample (FreeZone 2.5 Liter -50C Benchtop Freeze Dryers, LabConco Corp., Kansas City, MO. USA). eDNA was extracted from 15 g soil samples as previously described (Taberlet et al., 2012; Zinger et al., 2016).

2.3. Metabarcoding analysis

The soil hexapod communities were characterized based on Molecular Operational Taxonomic Units (MOTUs) using an eDNA metabarcoding approach. We amplified the 16S mitochondrial rDNA region using the Ins16S_l primer pair (Ins16S_1-F: 5'-TRRGACGAGAAGACCCTATA-3'; Ins16_1-R: 5'-TCTTAATCCAACATCGAGGTC-3'; Clarke et al. 2014). This primer pair, specifically designed for hexapod metabarcoding, introduces a very limited taxonomic bias and performs very well for identifications at the species level throughout the Hexapoda subphylum (e.g. Kocher et al., 2017; Talaga et al., 2017). PCR amplification was performed in triplicate in 20-µL mixtures consisting of 10 µL of AmpliTaq Gold Master Mix (Life Technologies, Carlsbad, USA), 5.84 µL of nuclease-free Ambion water (Thermo Fisher Scientific, Waltham, USA), 0.25 µM each primer, 3.2 µg of bovine serum albumin (Roche Diagnostic, Basel, Switzerland) and 2 µl of DNA template that was diluted 10-fold to reduce PCR inhibition by humic substances. The thermal profile of the PCR amplification was 40 cycles of denaturation at 95 °C (30 s), annealing at 49 °C (30 s) and elongation at 72 °C (60 s), with a final elongation step at 72 °C for 7 min. Tags had at least five differences between them to minimize ambiguities (Coissac

et al., 2012). The sequenced multiplexes comprised extractions/PCR blank controls, unused tag combinations and positive controls (Kocher et al., 2017). The PCR products were then sequenced using the MiSeq platform (Illumina Inc., San Diego, USA), with the expected sequencing depth set at 400 000 reads per sample.

The sequences were processed using ObiTools software (Boyer et al., 2016). Low-quality sequences (containing Ns, alignment scores <50, lengths <140 bp or >320 bp and singletons) were excluded. The remaining sequences were clustered into MOTUs using SUMACLUST (Mercier et al., 2013) at a threshold of sequence similarity of 97%. The final number of MOTUs after curation was 11785. The hexapod MOTUs were taxonomically assigned using Basic Local Alignment Search Tool, (BLAST), with a query coverage criterion of 95%. MOTUs showing <80% similarity with either the local or the EMBL reference databases were removed, leading to 590 MOTUs (Kanz 2004). These retained MOTUs included taxa from classes Insecta and Entognatha, which both belong to the subphylum Hexapoda. All sequences with a frequency of occurrence below 0.05 per MOTU and per run were discarded. This threshold was empirically determined to clear the negative sequencing controls (non-used tag combinations) included in our global data production procedure. We then applied a post-processing pipeline (Zinger et al., 2021) to minimize PCR and sequencing errors, contaminations and false-positive sequences, and a detailed curation of ecologically incongruent assignments also to deal with the ambiguous matches and provide more reliable information about species identifications (e.g., taxa with distributions outside the Palearctic and Nearctic zones, none barcoded MOTUs and redundant assignations). This conservative approach retained a total of 40 identified species. We then used checklists of Icelandic hexapod species and information from previous studies at the same study site (Fjellberg, 2007; Holmstrup et al., 2018) to assess the performance of our eDNA metabarcoding protocol to properly describe the hexapod communities in the soil.

2.4. Environmental variables

Dissolved organic C (DOC), dissolved organic N and total dissolved N (TDN) in all soils were quantified in 1 M KCl extracts. Soil Cmic and Nmic were determined by the chloroform-fumigation extraction method (48 h incubation period), followed by 1 M KCl extraction. All extracts were analyzed for DOC and TDN with a TC/TN-Analyzer (Shimadzu, TOC-VCPH/CPNTNM-1 analyzer). There were no correlations between environmental variables (Table S1-S4).

2.5. Data analysis

All data handling, visualization and statistical analyses were conducted using R v4.0.6 (R Core Team, 2020). We first identified the relationships between all environmental variables (the amounts of DOC and DON, and Cmic and Nmic) across temperatures using principal component analyses (PCAs) and simple general linear models (GLMs), with warming as categorical fixed-effect terms. We then built simple GLMs, with temperature as fixed-effects terms, to identify differences in rarefied mOTU abundance and richness due to warming. Changes in the composition of the hexapod communities for each pair of temperature levels were assessed using sparse partial least squares discriminant analysis (sPLS-DA) as implemented in the mixOmics package (Rohart et al., 2017). sPLS-DA selects variables and classifies the most discriminative taxa in the community matrices. The optimal number of components was chosen based on the error rate of t-tests, which suggested the use of single-component sPLS-DAs in both warming levels. The mixOmics package also delivers P values based on the area under the receiver operating characteristic curve to complement the sPLS-DA performance results. The main sPLS-DA variates (the x-variate, capturing the compositional similarity of soil hexapod communities) were then subjected to simple GLMs against the set of environmental variables: the amounts of soil DOC and DON, Cmic and Nmic.

3. Results

3.1. Environmental variables

The amounts of Cmic, soil DOC and DON decreased with warming, while Nmic did so marginally ($P < 0.05$, $P < 0.05$, $P < 0.05$ & $P = 0.08$, respectively; Table 1). The confidence ellipses of the environmental PCAs overlap between the control and the temperature levels (Figure 1).

3.2. Insect community description

eDNA metabarcoding identified 40 species, corresponding to different lineages within the subphylum Hexapoda: Collembola (14 species), Coleoptera (12 species), Diptera (six species), Hemiptera (five species), Hymenoptera (one species), Dermaptera (one species) and Lepidoptera (one species). Three springtail species were previously unrecorded for Iceland: *Sminthurinus bimaculatus* (244 reads across nine samples), *Pogonognathellus flavescens* (110 reads across three samples) and *Megalothorax perspicillum* (two reads in one sample). The most abundant group was four species of springtails belonging to the order Neelipleona, which together accounted for 42% of all reads. The next most abundant orders were Diptera (18.6%), Entomobryomorpha (9.1%), Hemiptera (9.1%), Symphypleona (8.4%) and Poduromorpha (7.6%).

3.3. Community response to soil warming

The number of eDNA reads did not differ significantly across temperature levels ($P = 0.54$; Figure 2a), nor did the richness of the soil hexapod communities ($P = 0.29$; Figure 2b). The sPLS-DA with the warming levels identified significant compositional dissimilarities between the control, the +3 and +6 °C treatments ($P < 0.01$ for both, Figure 3a and b). The first sPLS-DA variate (x-variate) from the control and the +3 °C treatment accounted for 11% of the variance, the

variate including the control and the +6 °C treatment accounted for 19%, and the variate including both warming levels explained 16% of variance (Figure 3). In all sPLS-DAs, higher scores in the x-variate implied increasing compositional dissimilarity with higher temperatures (Figure 3). Compositional changes with warming were driven mainly by springtails (e.g. *Tomocerus ocreatus*, *Folsomia quadrioculata* and *Megalothorax svalbardensis*), beetles (e.g. *Liogluta microptera* and *Hypnoidus sp. 1*), and the dipteran *Bradysia subvernalis* (Figure 4).

None of the four environmental variables had a significant effect at +3 °C when we assessed the capacity of the environmental variables to account for such differences in the composition of the soil hexapod community. Compositional similarities at +6 °C, however, were negatively correlated with the amounts of Cmic and Nmic and marginally correlated with the amount of DOC and DON found in the control ($P < 0.05$, $P < 0.05$, $P = 0.07$ & $P = 0.06$, respectively; Table 2). The negative correlation between the Control vs +6 °C sPLS-DA x-variate and these environmental variables imply that Cmic, Nmic, soil DOC and DON depletion lead to communities with species compositions similar to those found at the +6 °C (Figure 3).

4. Discussions

Our results indicated that warming causes changes in the compositions of the soil hexapod community in a subarctic grassland. In this case of study, an increase in soil temperature of 6 °C above ambient had an impact on the community composition, which correlated with decreases in the amounts of Cmic, Nmic, DOC and DON. These results highlight how the responses of soil hexapod community assemblages to warming can be associated with changes in microbial biomass and shifts in C and N availability. Moreover, eDNA metabarcoding was an efficient methodological approach to obtain a description of the soil hexapod communities. Additionally, eDNA metabarcoding allowed us to detect three springtail species not previously reported for Iceland but extensively distributed across the northernmost part of continental Europe (GBIF, 2021).

Table 1. Variation of environmental variables across temperatures.

Environmental variable	Estimate	F-statistic	P-value	Adjusted R^2
Microbial C	-0.005 ± 0.002	6.26	0.02 *	0.27
Microbial N	-0.01 ± 0.00	3.58	0.08 •	0.15
Soil DOC	-0.06 ± 0.02	6.18	0.02 *	0.27
Soil DON	-0.20 ± 0.07	7.70	0.01 *	0.32

*Note: Effect estimates followed by their standard errors are the output of general linear models for each environmental variable. The intercept is always the control, and the explanatory variable is always the corresponding environmental variable: microbial carbon (c), microbial nitrogen (n), soil dissolved organic carbon (doc) or soil dissolved organic nitrogen (don). •, $p < 0.1$; *, $p < 0.05$. All models have 13 degrees of freedom.*

Table 2. Relationship between the compositional similarities of the soil hexapod community with the environmental variables across temperatures.

Treatment	Environmental variable	Estimate	F-statistic	P-value	Adjusted R^2
Control vs +3 °C	Microbial C	0.000 ± 0.001	0.10	0.75	0
	Microbial N	0.002 ± 0.004	0.23	0.64	0
	Soil DOC	-0.02 ± 0.01	2.42	0.15	0.13
	Soil DON	-0.02 ± 0.04	0.24	0.63	0
Control vs +6 °C	Microbial C	-0.002 ± 0.000	8.23	0.02 *	0.44
	Microbial N	-0.008 ± 0.003	7.38	0.02 *	0.41
	Soil DOC	-0.03 ± 0.01	4.04	0.07 •	0.25
	Soil DON	-0.07 ± 0.03	4.65	0.06 •	0.28
+3 °C vs +6 °C	Microbial C	-0.001 ± 0.001	0.65	0.44	0
	Microbial N	-0.004 ± 0.005	0.70	0.42	0
	Soil DOC	-0.02 ± 0.04	0.28	0.60	0
	Soil DON	-0.03 ± 0.09	0.12	0.72	0

*Note: Results are general linear models, with the first variate (X-variate 1) from an sPLS-DA of the hexapod communities as the response variable modeled against each environmental variable: microbial carbon (C), microbial nitrogen (N), soil dissolved organic carbon (DOC) or soil dissolved organic nitrogen (DON). Effect estimates are followed by their standard errors. •, $P < 0.1$; *, $P < 0.05$. All models have 8 degrees of freedom. Note that the higher the value of the sPLS-DA x-variate, the higher the similarity with the communities of the higher temperature level of the pair (see Figure 3).*

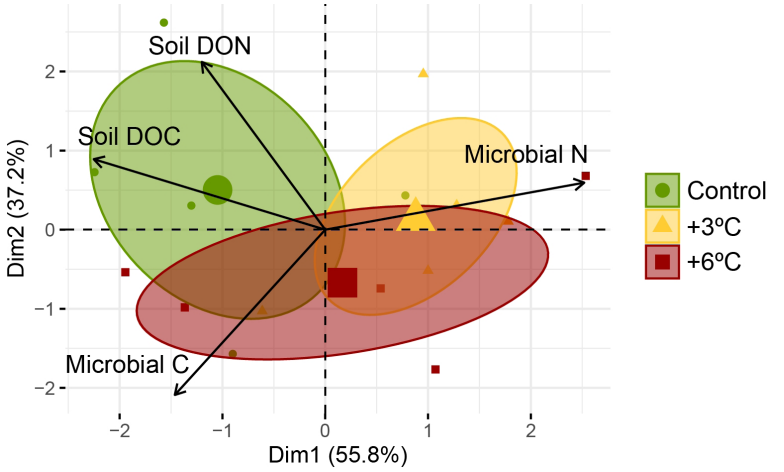

Figure 1. Principal component analyses of the distribution of the environmental variables, with 95% confidence ellipses denoting different treatments.

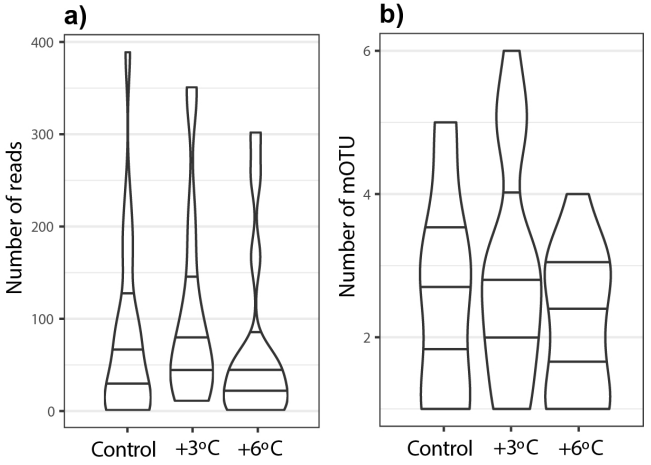

Figure 2. Violin plots of the numbers of reads (a) and molecular operative taxonomic units –mOTU- (b) across sites and treatments. Horizontal lines inside each plot from the lowest to the highest denote the first, second and third quartiles.

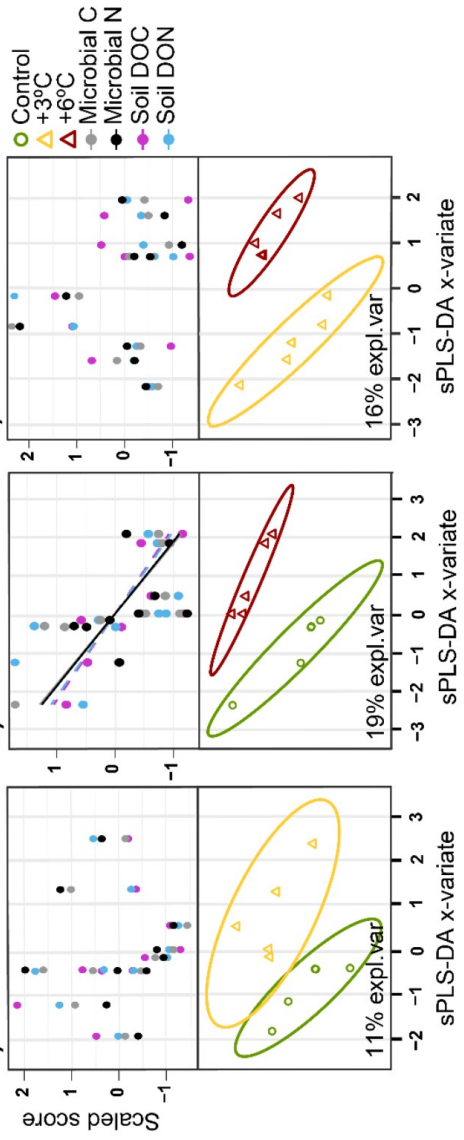

Figure 3. Compositional variation of the soil hexapod communities based on a single-component sparse partial least squares discriminant analysis (sPLS-DA) for control against +3 °C warming (a), control against +6 °C warming (b) and +3 °C against +6 °C (c). The amount of explained variance of the x-variate is shown in each figure. Confidence ellipses are set at 95%. The linear relationships between the x-variate scores with the corresponding soil environmental variables (microbial carbon, microbial nitrogen, soil dissolved organic carbon and nitrogen -DOC and DOC, respectively-) are shown on top of each sPLS-DA. Solid and dashed lines show significant and marginally significant slope parameters ($P < 0.05$, and $P < 0.1$, respectively). See Table 2 for further information on the linear models.

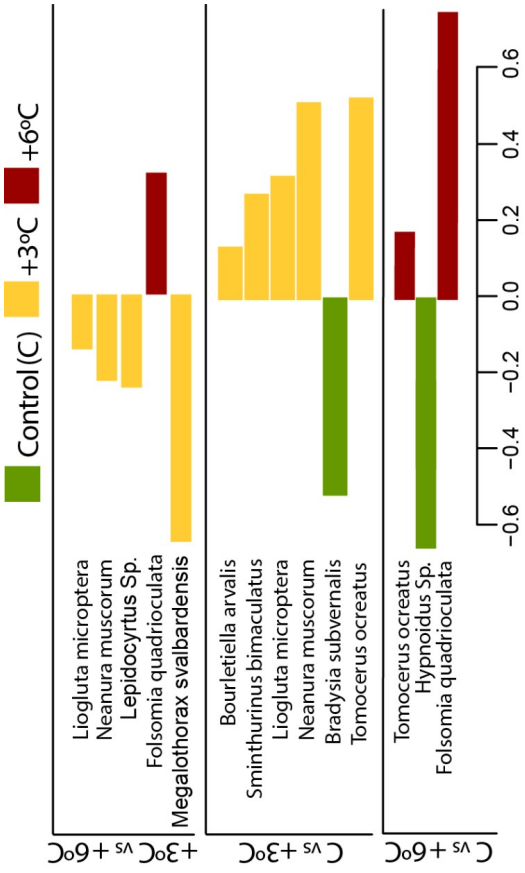

Figure 4. Identity of the main hexapod species driving the compositional differences between the controls and the warming treatments. Only species with a loading score >0.1 in the corresponding discriminant analysis are shown.

Our results support previous findings that exposure to soil warming for over a decade in this subarctic grassland may not result in a shift of either soil hexapod abundance or richness (Holmstrup et al., 2018). We found, however, compositional dissimilarities that may threaten the functionality of soil hexapod communities more than can simple changes in species richness or abundance (Bardgett & van der Putten, 2014; Briones, 2014). Shifts in community composition in our study were mainly driven by springtail, beetle and fly species, which were also the richest lineages with the most DNA reads. The species driving such changes in community composition differed not only between the control and higher temperature levels, but also between the +3 and +6 °C treatments. These differences may have been associated with different temperature optima, sensitivity to temperature maxima or resilience to warming by the populations of hexapod species (Deutsch et al., 2008; Sánchez-Bayo & Wyckhuys, 2019). For instance, the rove beetle *Liogluta microptera* and wireworms of the genus *Hypnoidus* are known to prefer warmer and drier soils (Ottesen 1996; Drahun et al., 2022), while some fungus gnats of the genus *Bradysia* are also known to have a temperature-sensitive phenology (Li et al., 2015) (Figure 4). Changes in food resources caused by warming are also an important factor capable of altering soil hexapod communities (Alatalo et al., 2016; Holmstrup et al., 2018), both by affecting the amount of litter input or the microbial biomass (S. F. Bokhorst et al., 2009).

The impacts on soil hexapod communities under the most extreme conditions of soil warming correlated with the depletion of Cmic and Nmic in the topsoil. Previous studies at the same site have found that warming leads to the loss of soil organic C (SOC) stocks in the topsoil ultimately reducing the amount of Cmic (Marañón-Jiménez et al., 2018; Verbrigghe, Leblans, et al., 2022; Verbrigghe, Meeran, et al., 2022). A proportional loss of Nmic might have followed this loss of C due to the relatively tight C:N stoichiometric balance of microbial metabolism at the site (Marañon-Jimenez et al., 2019, Séneca et al., 2021; Walker et al., 2018). The depletion of labile DOC and DON in the warmest soils in our study, however, only

affected marginally the soil hexapod communities (Table 2, Figure 3). The reduction of Cmic and DOC should have negatively affected important soil hexapod trophic guilds such as microbial feeders and detritivores relying on bacterial and fungal biomass as well as litter input. In contrast, the lack of effect of the moderate +3 °C warming may have been associated with the threshold dynamics of the soil microorganisms found in this subarctic grassland, where only increases in temperature equal or above 6 °C have been shown to lead to abrupt shifts in microbial diversity and composition (Radujković et al., 2017; Weedon et al., 2022).

Global warming is generally expected to increase SOM decomposition leading to higher N availability (Davidson & Janssens 2006; Jenkinson et al., 1991). However, at a decadal scale the impact of warming correlated with lower DOC and DON availability as well as with a loss of Cmic and Nmic. The impact of warming was therefore quite different from the predicted short-term warming response, which should be driven by the initial enhanced availability of N. The requirements of hexapods for C and N, and the sources of C and N, are of great interest for predicting the response of soil hexapods to warming. In our study, we consider it likely that the loss of Cmic and Nmic due to warming depleted the food resources of hexapod bacterivores and fungivores (Gargallo-Garriga et al., 2021), increasing litter concentration and exerting a positive feedback on hexapod detritivores. These differences in C and N availability, together with the species-specific temperature optima and sensitivity, may account for the lack of a common compositional response across warming levels. The stoichiometric balance of microorganisms and hexapods also highlight the importance of the C and N to understand the mechanistic response of soil hexapods to warming. The nutrient requirements and elemental stoichiometry of microorganisms and plants have been well studied (Zechmeister-Boltenstern et al., 2015), but our knowledge of hexapod stoichiometry is a lot less advanced (Moe et al., 2005). For instance, previous work suggests that arthropod stoichiometric response to temperature is not necessarily monotonic in shape (Ruiz et al.

2020), and that resource quality (e.g. C:N and C:P ratios) plays a major role in ground arthropod feeding behavior (Jochum et al., 2017; Ott et al., 2014). Therefore, the complex behavior of arthropods hamper our ability to assess their nutritional requirements (Fagan et al., 2002; Hódar et al., 2002).

Furthermore, it is crucial to consider potential biases in these responses, as the study system differs from global warming in two significant aspects. Firstly, geothermal warming exhibits a contrasting vertical gradient compared to climatic warming, with warmer temperatures increasing from deep soil layers rather than the surface. Previous research indicates that such warming influences the composition of soil hexapod communities across the soil profile. For instance, in regions with a natural gradient of European climatic aridity, specific traits of soil-dwelling springtail species are favored on the soil surface, whereas short-term exposure to drought induces a different response, promoting traits associated with soil conditions (Ferrín et al., 2021). Secondly, global warming can lead to shifts in vegetation communities, which also have implications for hexapod populations. In subarctic regions, one common response to warming is the expansion of shrubs into grasslands and tundra, a process known as shrubification. This vegetation change alters nutrient dynamics, microbial activity, and hexapod communities (Barrio et al., 2016; Jia et al., 2022; Mekkonen et al., 2021). Therefore, it is essential to interpret these results with caution, considering the unique characteristics of the study system and the potential influence of these factors. Nonetheless, experimental and mechanistic approaches allow the identification of the nutritional demands affecting soil hexapods over a multiplicity of biomes, and by extension their community responses to warming, clarifying belowground ecological dynamics. Future studies on this topic are therefore strongly recommended.

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Chapter V

Trait-mediated responses to aridity and experimental drought by springtail communities across Europe*

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Abstract

The capacity to forecast the effects of climate change on biodiversity largely rely on identifying traits capturing mechanistic relationships with the environment through standardized field experiments distributed across relevant spatial scales. The effects of short-term experimental manipulations on local communities, may overlap with regional climate gradients that have been operating during longer time periods. However, to the best of our knowledge, there are no studies simultaneously assessing such long-term macroecological drivers with climate manipulations. We analyzed this issue with springtails (Class Collembola), one of dominant soil fauna groups, in a standardized climate manipulation experiment conducted across six European countries encompassing broad climate gradients. We combined community data (near 20K specimens classified into 102 species) with 22 eco-morphological traits and reconstructed their phylogenetic relationships to track the evolution of adaptations to live at different soil depths, which is key to cope with desiccation. We then applied joint species distribution models to investigate the combined effect of the regional aridity gradient with the local experimental treatment (drought and warming) over the assembly of springtail communities and tested for significant trait-environment relationships mediating their community-level responses. Our results show: (1) a convergent evolution in all three major collembolan lineages of species adapted to

inhabit at different soil strata; (2) a clear signature of aridity selecting traits of more epigeic species at a biogeographic scale, and (3) the association of short-term experimental drought with traits related to more euedaphic life-forms. The hemiedaphic condition is the plesiomorphic state for Collembola while the adaptations for an epigeic life would have been secondarily gained. Epigeic springtails are more resistant to drought but also have a higher dispersal capacity that allows them to seek more favourable micro-habitats after experiencing drier conditions. The observed relative edaphization of the springtail communities after short-term experimental drought may thus be a transient community response. The disparity between macroecological trends and fast community-level responses after climate manipulations highlights the need of simultaneously assessing long-term and short-term drivers at broad spatial scales to adequately interpret trait-environment relationships and better forecast biodiversity.

1. Introduction

Global biodiversity patterns are undergoing rapid shifts driven by on-going climate change (Blowes et al., 2019). Our capacity to forecast such biodiversity responses rely, however, on detailed knowledge of the processes behind species' population rates and the corresponding community reorganizations. Ecological theory predicts that a continued stress will filter out the species with the most vulnerable combinations of traits leading to changes on the functional structure of communities (Mouillot et al., 2013). This selection process may be either the result of the new abiotic conditions impairing the population growth of some species (strict environmental filtering) or the outcome of a reduced competitive performance under the new environmental conditions (Cadotte & Tucker, 2017). Traits, defined as any morphological, physiological, behavioural or phenological feature measurable at an individual level with impacts on species fitness via their effect on growth, reproduction and survival, are thus posited as the common currency for functional biogeography (McGill et al., 2006; Violle et al., 2014). Because trait selection generally precedes diversity loss, it is therefore fundamental to detect those traits capturing mechanistic relationships with stress factors such as climate change if we are to anticipate shifts on species composition and ultimately on ecosystem functioning (Berg et al., 2010; Haddad et al., 2008).

Coordinated and distributed multisite field experiments of climate manipulation are among the best tools available to infer these trait-environment relationships at relevant spatial scales under a scenario of rapid climate change (Halbritter et al., 2020). The effects of short-term experimental manipulations on local communities, however, overlap with regional climate gradients that have been operating during longer time periods which may lead to divergent ecosystem responses across such gradients that hinder our interpretations (Reinsch et al., 2017). This dependency of observed ecological patterns on multiple processes operating across a hierarchy of space-time scales is a classic issue in functional biogeography that strongly affects our predictive ability because general inferences and extrapolations are too often bogged down in local contingencies (Levin, 1992; Mouquet et al., 2015). Thus, for the development of the so-called predictive ecology, a key is to incorporate this complexity when assessing the performance of the analytical methods available in the macroecological toolkit (Mouquet et al., 2015).

Despite the growing concern over the impact of climate change on biodiversity, there has been a disproportionate focus on aboveground communities disregarding the key role of soil fauna on ecosystem functioning (Bardgett & van der Putten, 2014; Filser et al., 2016; Guerra et al., 2021). Among this neglected soil biodiversity there are the springtails (Class Collembola), an abundant and diverse group of small arthropods whose communities show fast reductions in taxonomic, phylogenetic and functional richness due to drought, and additionally, are also a good proxy of ecosystem functions like litter decomposition (Peguero et al., 2019). Springtails present a diverse array of adaptations related to dwelling in specific soil layers (Figure 1). Smaller collembolan species with unpigmented bodies, shorter appendages, reduced sensory organs and mostly parthenogenetic reproduction are associated with more euedaphic habitats (Christiansen, 1964; Chahartaghi et al., 2006; Gisin, 1943; Rusek, 1989; Salmon et al., 2014), while larger species with pigmented bodies, longer appendages, sexual reproduction and conspicuous mechanic and light receptors such as trichobothria and

ocelli respectively, are typical from epigeic environments (Salmon et al., 2014). The species inhabiting the upper soil horizons have a greater drought resistance due to a lower cuticular permeability that allow them to resist desiccation (Kærsgaard et al., 2004). On the other hand, living deeper in the soil profile implies a lower exposure to variability in temperature and soil moisture, and additionally, soil-dwelling species also present biochemical, physiological and behavioural mechanisms to cope with drought (Holmstrup et al., 2001). Accordingly, a multi-site replicated experiment suggested that increasingly drier conditions may eventually favour euedaphic over epigeic springtail species after short-term climate manipulations (Petersen, 2011). Even though, this relative edaphization may show idiosyncratic responses to short-term climate manipulations since in single-site studies at sub-arctic latitudes epigeic species seem to be favoured by experimental warming (Bokhorst et al., 2012; Makkonen et al., 2011). Hence, potentially divergent community responses across large spatial scales may hamper our ability to detect general trends, and in particular to identify those traits behind the current climate-induced community reorganizations. This may be even more difficult if broad climate constraints have had time to differentially shape springtail assemblages across such environmental gradients, although this macroecological dimension, to the best of our knowledge, has never been included in studies assessing soil fauna responses to climate change.

The present study unfolds from a standardized climate manipulation experiment replicated across six natural shrublands encompassing the broad gradients of temperature and precipitation present in Europe (Beier et al., 2004). Here, we investigated the simultaneous effect of short-term experimental climate treatments (i.e. drought and warming) and the regional long-term climate gradient synthesized in the aridity index, an indicator based on mean annual temperature and precipitation, over the composition and trait distribution of springtail communities. First, we extracted data from a comprehensive sampling of nearly 20 thousand springtail specimens consisting of 102 species (Petersen, 2011). We then

combined it with 22 eco-morphological traits (Salmon et al., 2014) to track across the springtail phylogeny the evolution of the suite of adaptations to inhabit specific soil layers (Table 1). Finally, we leveraged this community data to build joint species distribution models (JSDM) to test for significant associations between traits with climate manipulation treatments and aridity that could be mediating the assembly of collembolan communities. We specifically addressed the following hypotheses and predictions: (i) the adaptations that allow springtails to thrive at different layers of the soil matrix will show a pattern of repeated convergent evolution across the main lineages of Collembola; (ii) the long-term environmental impact of the large-scale climatic gradient present across Europe has driven a selection of drought-tolerant springtail species with increasing aridity; (iii) the experimental short-term increase in local dryness and warming will be a major disturbance driving fast responses that eventually favour more euedaphic springtail assemblages.

2. Material and methods

2.1. Study sites and climatic manipulations

The six shrublands studied comprised most of the European climatic regions (Figure S1 and Table S1). Mean annual precipitation (MAP) at the sites ranged from 544 to 1,263 mm, and mean annual temperature (MAT) ranged from 7.4 to 16.1 °C. The major types of shrublands present in the study area were: Atlantic heathland (UK, United Kingdom; NL, The Netherlands; DK, Denmark), continental forest steppe (HU, Hungary), and Mediterranean garrigue (SP, Spain; IT, Italy). In each study site, nine 20 m^2 plots were divided into three blocks and randomly assigned one plot at each block to a warming treatment, a drought treatment, or as a control. Warming plots were covered with reflective covers during the night throughout the year inducing a passive night-time warming, while drought plots were automatically covered by transparent polythene curtains during precipitation events that retracted as soon as rain

stopped thus avoiding any warming effect (Beier et al., 2004). Manipulations of temperature and precipitation were carried out in the same way in all sites, but the timing and duration of the experimental drought were adjusted to the local climate regime (Table S2). The sites were established in 1998 (UK, NL, DK, and SP) and 2001 (HU and IT), and on average the warming treatment produced an increase of 0.5 ºC of MAT and the drought treatment a 35% reduction of soil moisture (Table S2).

2.2. Springtail sampling, phylogeny and trait information

Springtails were sampled sequentially, to equalize mean temperatures across sites, from April to July 2003 as it follows: Italy (Apr. 29–May 4), Spain (May 13–17), Hungary (May 27–June 1), Denmark (June 23–27), Netherlands (July 9–13) and U.K. (July 19–23). Five quadrats of 1.25 m^2 representative of the most dominant plant species were chosen in each plot. Springtails were sampled from all plants in the quadrat by suctioning with an adapted vacuum cleaner connected to a fauna trap. Springtails from the soil surface were sampled using the same suction method, from the same five 1.25 m^2 quadrats in Italy and from smaller 78.6 cm^2 quadrats in the other 5 sites. Specimens dropped were also recovered by placing small polythene boxes with aqueous benzoic acid beneath the plants. Soil springtails were sampled in 10 cm deep soil cores with a surface of 25 cm^2 taken to the lab. After 2 to 6 days stored in refrigerated boxes, samples were put through high-gradient extraction funnels during 10 days (Gjelstrup & Petersen, 1987). These procedures were slightly adapted in UK and IT due to an excess of soil moisture and stones, respectively (Petersen, 2011). Springtails were identified to species level based on Baquero & Jordana (2008), Bretfeld (1999), Carapelli et al. (2001), Fjellberg (1998, 2007), Gisin (1960), Jordana et al. (1997), Mateos (2008), Potapov (2001) and Rusek (2002). And some dubious specimens were revised by Drs. L. Dányí, R. Jordana, and E. Mateos. A total of 19,641 springtail specimens were collected

and classified into 102 species-level entities (Tables S3) and are conserved in glycerol in H. Petersen's personal collection.

We reconstructed the phylogenetic relationships of springtails by means of sequence data from the only two genes available (cox1 and 28s), both gathered from public repositories covering 75% of the species found in our study (accession numbers in Table S3). We applied Maximum Likelihood and Bayesian procedures to obtain a highly-supported consensus tree that agrees with previous systematic works integrating molecular and morphological information (Yu et al., 2016). See supplementary methods and figure S2 for further details.

Additionally, we collated 22 eco-morphological traits from the ColTrait database (Salmon et al., 2014) for 47 up to 64 of the 102 species present in our study sites (Table 1,Table S4 and S5). These traits encompass different dimensions of their ecological niche such as life history (e.g., reproduction strategy), dispersal ability (e.g., leg length) and biotic interactions (e.g., sensory organs and defensive structures). In addition, for each species with available traits data, we calculated its Eco-Morphological Index (EMI, Parisi et al., 2005). This trait-based index provides a simple continuous metric ranging from 1 to 20 and indicates the level of adaptation of a given springtail species to a specific soil layer. Accordingly, they may be classified as atmobiotic (EMI < 2), epigeic (EMI < 6), hemiedaphic (EMI = [6-8]) or euedaphic (EMI > 8, Figure 1).

2.3. Data analysis

All data handling, visualization and statistical analyses were carried out using R v4.0.0 (R Core Team, 2020). First, we investigated the evolution of the adaptations that allow springtail species to live in specific soil layers by means of the reconstruction of the ancestral EMI score across the phylogenetic tree. This was done with the function fastAnc of the R package phylosig (Revell, 2012) and assuming a Brownian-Motion model of evolution of the suite of traits associated to the EMI. We also estimated the correlation between

the similarity in EMI and species' evolutionary distance (i.e. the phylogenetic signal) through Pagel's λ. Then, we assessed how the evolution across Collembola of the suite of traits associated with the EMI may lead to specific patterns in the phylogenetic structure of springtail assemblages in response to the drought and warming treatments separately. Thus, we obtained the mean neighbour taxon distance (MNTD) and the mean pairwise distance (MPD) of each springtail assemblage standardized against 999 community randomizations with the R package picante (Kembel et al., 2010). By accounting for both indexes we explored possible non-random patterns occurring at different phylogenetic depths since MNTD can capture changes at the tips of the phylogeny (e.g. selection of sister species sharing specific adaptations) while MPD can detect shifts in the in-depth phylogenetic structure (e.g. selection of species belonging to specific lineages with highly conserved adaptations) (Cadotte & Davies, 2016).

Second, we assessed the role of aridity and the experimental climate manipulations on the trait-based assembly of springtail communities across Europe. To do so, we carried out a series of generalized linear latent variable models (GLLVM), as implemented in the R package gllvm (Niku et al., 2019). GLLVMs are a type of Joint Species Distribution model (JSDM) that extend the basic generalized linear mixed-effects model to multivariate abundance data incorporating a small number of latent variables accompanied by species-specific factor loadings to model correlations between response variables (i.e. all species in the community matrix). These latent variables have a natural interpretation as ordination axes, but have also the capacity to predict new values controlling for known environmental factors using standard model selection tools (Niku et al., 2019). Additionally, GLLVMs allow us to test for trait-environment associations and the potential effects of biotic interactions. Although the effects of the environment and biotic interactions cannot be teased apart, the inclusion of the latter are a major advance of JSDM compared with previous species distribution tools and 'fourth corner' models that do not include the co-variation of the abundances

between all species within a series of sites or communities (Poggiato et al., 2021; Warton et al., 2015). Hence, we first built an unconstrained model (without environmental predictors) with two latent variables and a negative binomial distribution and a log link function. The selection of the best number of latent variables and distribution family followed the recommendations of Niku et al. (2019), which are based on goodness-of-fit and residual diagnostics. Afterwards, we added sequentially the aridity index and the local climate manipulation treatments as environmental constraints and performed likelihood-ratio tests (LRT) to assess the predictive improvement of these increasingly complex models, and if so, we computed the percentage of deviance explained by the best model. The aridity index of each site was calculated by means of a modified version of the inverse of the Gaussen Index (iGI) as: $iGI = 1/(MAP/(2 \times MAT)) \times 100$ (Reinsch et al., 2017). This index captures the large-scale gradient of aridity currently present across Europe and that can be traced back to the beginning of the Holocene about 10,000 years ago after the last Younger Dryas cold spell came to an end (Hewitt, 1999). Finally, to see whether specific traits were selected by aridity and by the climate manipulation treatments favouring more euedaphic or epigeic springtail species, we built a series of GLLVMs including or not the interaction between a given trait and these two environmental variables and tested their significance via LRT (Niku et al., 2019).

3. Results

Comparative analyses of the EMI revealed multiple independent evolutions of the collembolan traits associated to live in specific soil layers (Figure 2). In all the major lineages of Class Collembola there are groups of species showing either an epigeic habitus (orders Symphypleona and Entomobryomorpha) or, to a lesser extent, a more euedaphic habitus (order Poduromorpha), apparently as a result of a convergent adaptive evolution of a distinctive suite of eco-morphological traits as depicted in Figure 1. The high and significant phylogenetic signal of the EMI (Pagel's $\lambda = 0.757$) pointed out, however,

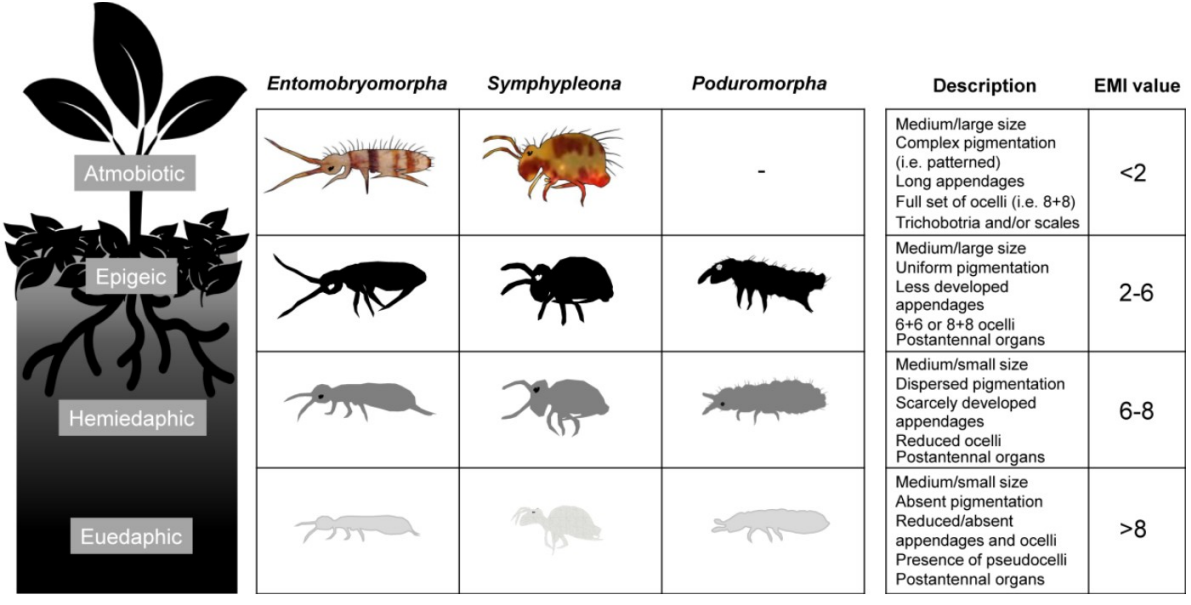

Figure 1. Schematic representation of the morphological and functional adaptations to living in different soil layers by the main phylogenetic lineages of Collembola (Order Entomobryomorpha, Symphypleona and Poduromorpha) along with the corresponding range for the Eco-Morphological Index (EMI). Based on Parisi et al. (2005), Potapov et al. (2016) and Salmon et al. (2014).

Table 1. List of Collembolan traits analysed.

Type	Trait	Type of variable	Values	Species[1]
Body aspect	Length	Continuous	0.18 – 6.5 mm	60
	Shape	Categorical	Spherical Cylindrical Wide-cylindrical	61
Reproductive strategy	Reproduction	Categorical	Parthenogenesis Sexual Mixed	47
Dispersal capacity	Furcula[2]	Discrete Binomial	0 – 1	61
	Furcula length[2]	Semi-quantitative	0 – 4	53
	Leg-body ratio	Continuous	0.11 – 0.69	52
Sensory organs	Antennal length	Semi-quantitative	1 – 3	55
	Antenna-head ratio	Continuous	0.40 – 3.50	51
	Ocelli	Discrete Binomial	0 – 1	62
	Ocelli number	Discrete	0 – 8	62
	Post Antennal Organ (PAO)	Discrete Binomial	0 – 1	58
	PAO number of vesicles	Discrete	0 – 190	56
	Trichobothria	Discrete Binomial	0 – 1	60
Protective features	Scales	Discrete Binomial	0 – 1	61
	Pigmentation	Discrete Binomial	0 – 1	60
	Pseudocelli	Discrete Binomial	0 – 1	64
	Pseudocelli number	Discrete	0 – 56	64

Note: Trait data collated from COLTRAIT database (Salmon et al., 2014). Some continuous or discrete numerical traits (e.g. Length, Ocelli, PAO number, Pseudoccelli) have minimum and maximum values recorded separately. See Tables S4 and S5 for further information on the traits included in this study.

[a] *Number of species with trait data available.*

[b] *Note that furcula (as presence/absence) and its length are also protective features related with defensive evasion.*

that once fixed phenotypic reversions in these set of traits within a clade are rare and only in a few cases sister species show contrasting soil layer preferences (e.g *Tomocerus minor* and *Oncopodura crassicornis*; TOMMIN and ONCCRAS respectively in Figure 2). The analysis of the phylogenetic structure of springtail assemblages showed that under the experimental drought there was a relative increase in phylogenetic clustering according to the MNTD, i.e. the mean distance of the more closely related species in the assemblage was smaller under drought relative to control conditions ($P < 0.01$). However, the MPD showed no differences across climatic treatments

(P = 0.37), suggesting that under all experimental conditions there were rather similar phylogenetic community structure.

The unconstrained ordination of springtail communities displayed a clear grouping of all springtail assemblages according to the country of origin (Figure 3a). This clustered pattern captured the species turnover among our experimental sites, but additionally, the two latent variables implicitly highlighted the steep aridity gradient that pervades the European geography by grouping together the Spanish assemblages close to those from the Italian and the Hungarian sites. Accordingly, this pattern disappeared when the aridity index was included as a constraining predictor (Figure 3b), thus confirming water availability and mean annual temperature as major drivers of springtail communities across Europe. Indeed, the aridity index alone captured 70.6 % of the whole compositional variability (LRT P < 0.05). Regarding the interaction between traits and the environment, the structuring power of aridity was stronger than that of the short-term effect of the experimental climate manipulations (Figure 4). Among the 22 traits tested for trait-environment associations, 8 showed significant (P < 0.05) relationships with aridity, 6 with the experimental drought and only 3 with the warming treatment based on LRTs between equivalent models with and without the corresponding trait-environment interaction (see Table 2 for a complete list of all significant trait-environment relationships).

At a biogeographic scale, all traits showing significant interactions with aridity indicated a clear trend towards more epigeic springtail assemblages as mean annual temperatures increased and precipitation decreased. Indeed, species with predominance for sexual reproduction over parthenogenesis, higher numbers of ocelli and longer furcula and antennae were positively selected by increasing aridity (Figure 4). On the other hand, the lower presence and abundance of pigmented species and with photoreceptors (ocelli) in the plots under the experimental drought, along with the increase of species with pseudocelli as typically euedaphic defensive organ and with a greater minimum and maximum number of vesicles in postantennal organ pointed out to a relative edaphization of the

springtail assemblages after this short-term experimental treatment. Warming only reduced the presence of species with parthenogenesis and slightly increased the number of ocelli in the springtail assemblages.

4. Discussions

The capacity to forecast the effects of climate change on biodiversity largely rely on identifying those traits capturing mechanistic relationships with the environment through standardized field experiments distributed across macro-ecologically relevant spatial scales (Halbritter et al., 2020; Mouquet et al., 2015). Our results demonstrate, however, that the effects of local climate manipulations on communities of springtails overlap with the regional gradients that have been operating during longer time periods. The broad aridity gradient present across Europe promoted assemblages of species with more epigeic traits as water availability decreases and temperature increases. Despite this long-term and strong aridity driver, the effect of the drought treatment led to a relative edaphization of springtail communities. We discuss below how this disparity of community responses against short versus long-term climate restrictions result from the trait differences between epigeic and more euedaphic springtails and why this also cautions that our interpretations of the trait-environment relationships behind the current biodiversity reorganizations of soil fauna may change or even be reversed as climate change continues.

4.1 Evolutionary convergence of euedaphic traits in Collembola

Springtail species adapted to inhabit a specific soil strata typically share a suite of eco-morphological traits and, to some extent, they even share a similar trophic niche irrespective of their phylogenetic affiliation (Ponge, 2000; Potapov et al., 2016; Salmon et al., 2014).

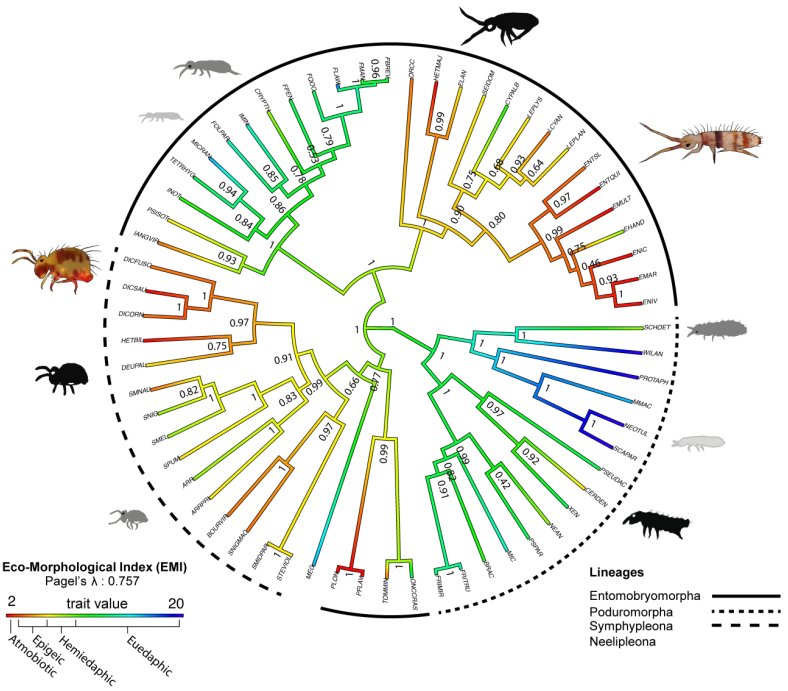

Figure 2. Phylogenetic reconstruction of the Eco-Morphological Index (EMI) across the clade Collembola. Red to yellow indicate lower EMI values associated to atmobiotic and epigeic species, while green to blue denote higher EMI values related to hemiedaphic and euedaphic species. Numbers at the nodes are the posterior probabilities based on ultrametric Bayesian consensus tree. Drawings are placed along with the corresponding species or its closest relative to illustrate their morphological and functional adaptations. See the Supplementary Methods and Table S3 in the Supporting Information for further details about the phylogeny and for the species abbreviations.

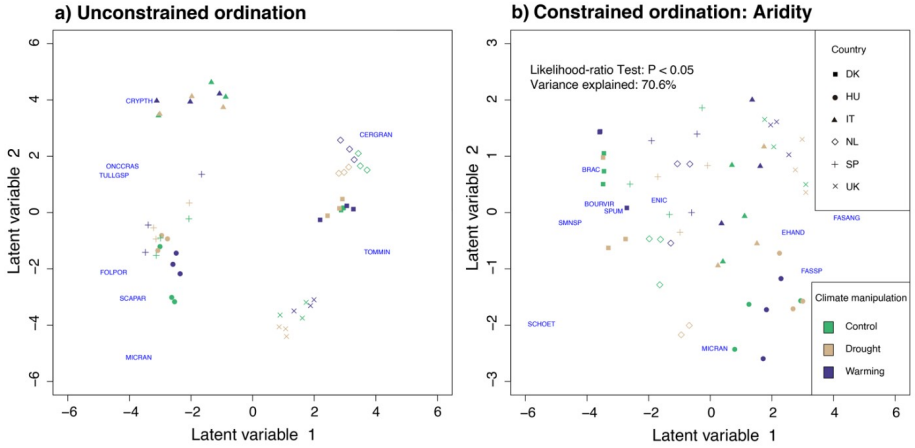

Figure 3. Ordination based on a generalized linear latent variable model fitted to the collembolan community data (102 species) without environmental predictors (a) and after controlling for the effects of the aridity index (b). Symbol shape correspond to the countries included in the study (DK, Denmark; HU, Hungary; IT, Italy; NL, Netherlands; SP, Spain and UK, United Kingdom), and colour refer to the experimental climate manipulation applied to each plot. Taxa with the largest factor loadings are included as indicator species. See Table S3 in the Supporting Information for species abbreviations. Inset in panel b shows the result of a likelihood-ratio test between the unconstrained and the constrained model along with the increase in variance explained by the aridity index.

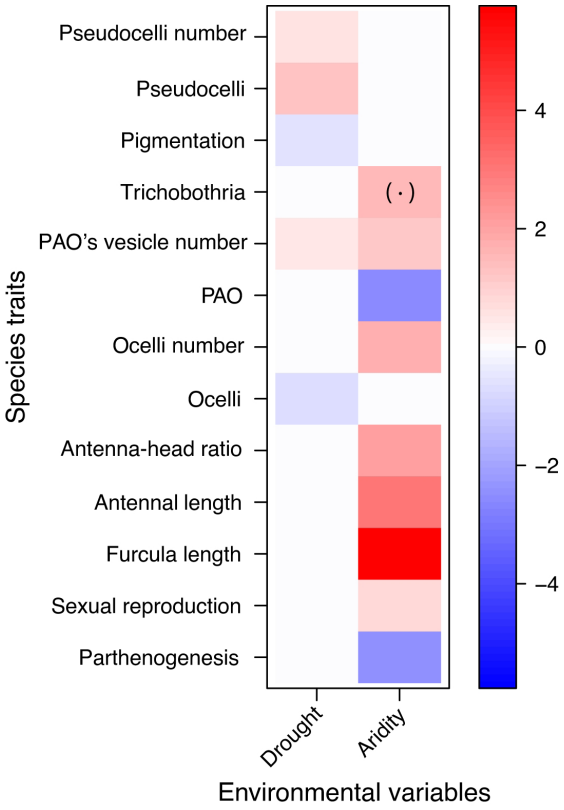

Figure 4. Level plot for the empirical significant interactions between collembolan traits and short-term experimental drought and the long-term large-scale aridity gradient present in Europe according to generalized linear latent variable models (GLLVM). Each trait-environment interaction was modelled with a separate GLLVM maximizing the number of species with trait data available (47 to 64 species). See Table 2 in the Supporting Information for a complete list of interactions and model outputs. Statistical significance was assessed by means of likelihood-ratio tests between equivalent models with or without the trait-environment interaction. Coloured squares show significant interactions ($P < 0.05$) with (·) denoting marginal significance ($P < 0.1$). The colour scale offers an indication of the sign and magnitude of the selection exerted over each trait in the collembolan communities.

Table 2. Statistically significant interactions between springtail traits and environmental conditions.

Trait	Drought	Warming	Aridity	*P*-value	N° of species
Parthenogenesis	-0.63±0.67	-1.19±0.66 *	-2.44±1.20 *	0.008	46
Sexual reproduction	0.05±0.43	0.53±0.43 *	0.87±0.81 *	0.008	46
Furcula length (1)	0.44±1.53	-1.53±1.72	5.76±3.43 *	0.000	53
Furcula length (2)	-0.75±1.47	-0.71±1.44	5.02±2.85 *	0.000	53
Furcula length (4)	-0.05±0.69	0.44±0.66	7.78±1.77 *	0.000	53
Antennal length (2)	-0.47±3.45	-0.26±3.79	7.21±6.10 *	0.000	55
Antennal length (3)	-0.42±0.56	0.26±0.50	3.07±1.34 *	0.000	55
Antenna-head ratio (min.)	0.01±0.38	0.24±0.34	2.05±0.76 *	0.000	51
Antenna-head ratio (max.)	0.07±0.35	0.40±0.32	2.12±0.71 *	0.000	51
Ocelli	-0.65±0.62 *	0.58±0.61	0.61±1.57	0.019	61
Ocelli number (min.)	-0.16±0.23	0.36±0.27 *	3.18±0.86 *	0.001	62
Ocelli number (max.)	-0.09±0.28	0.44±0.27 *	1.73±0.75 *	0.000	62
Post Antennal Organ (PAO)	0.27±0.51	-0.31±0.52	-2.54±1.35 *	0.002	58
PAO number (min.)	0.53±0.54	0.26±0.44	1.30±0.51 *	0.000	56
PAO number (max.)	0.46±0.41 *	0.20±0.36	1.15±0.44 *	0.000	56
Trichobothria	0.07±0.48	0.50±0.45 ·	1.46±1.19 ·	0.082	60
Pigmentation	-0.60±0.59 *	0.53±0.58	-0.09±1.35	0.005	60
Pseudocelli	1.28±0.67 *	-0.31±0.93	-0.82±3.67	0.003	63
Pseudocelli number (min.)	0.53±0.28 *	-0.01±0.24	-0.04±0.58	0.000	63
Pseudocelli number (max.)	0.53±0.28 *	-0.01±0.24	0.01±0.55	0.000	63

Note: Each line corresponds to a fourth corner model based on Generalized Linear Latent Variable Models. Effect estimates are followed by its 95% Confidence Interval. Significant and marginal interactions are represented by "" and "·", respectively. P-values show the result of a Likelihood-ratio test (LRT) against an equivalent model without the corresponding trait-environment interaction. Last column indicates the number of species with data available for each trait and thus included in the models.*

Our results complement these earlier findings demonstrating that this is the result of an adaptive convergence that has repeatedly happened in the lineages of the three major collembolan orders. The ancestral ecology of springtails has been of particular interest in light of the still unknown terrestrialization path followed by the subphylum *Hexapoda* (Ghilarov, 1958; van Straalen, 2021). According to our dataset, the hemiedaphic condition would be the plesiomorphic state for Collembola while the adaptation to a true epigeic life

would have been secondarily gained, particularly by Entomobryomorpha and Symphypleona. This conclusion agrees with previous morphological assessments (D'Haese, 2003), thus bridging together the cladistics approach with our phylogenetic analysis.

The development of an epigeic habitus is associated with larger and pigmented bodies with longer appendages, sexual reproduction and more developed sensory organs, while the specialized euedaphic life-form typically imply a smaller unpigmented body, shorter appendages, reduced or absent sensory organs along with the development of the ability to reproduce through parthenogenesis (Chahartaghi et al., 2006; Salmon et al., 2014). Despite the repeated convergent evolution of these traits at a broad phylogenetic scale, the transitions between epigeic and euedaphic life-forms, however, are rarely reversed within a clade so that sister species usually share a similar habitus and hence soil layer preferences. A previous study at a smaller spatial scale has shown that evolutionary close species tend to co-occur due to their akin ecological preferences and competitive exclusion of sister species may be rare notwithstanding (Ponge & Salmon, 2013). This suggests that the relative phylogenetic clustering observed under the experimental drought according to the MNTD metric may mirror springtail assemblages where sister species are coexisting. The apparent discrepancy with the MPD, which points out to a lack of phylogenetic clustering due to the climate manipulations, may actually arise by the fact that in all major springtail lineages there are species relatively specialized to either an epigeic or euedaphic lifestyle. Thus, the presence of species from all evolutionary lineages under all experimental treatments results in similar in-depth phylogenetic community structure and may preclude any phylogenetic imbalance in the resulting assemblages. Therefore, this adaptive convergence may explain why Collembola phylogenetic diversity measures are less sensitive to species losses or community shifts due to warming and drought than functional richness or other trait-based diversity metrics (Peguero et al., 2019).

4.2 Long-term climate gradients versus short-term climate manipulations

The current climate gradients present in Europe date back to around 10000 years BP and by 6000 year BP the vegetation patterns already resembled that of today (Hewitt, 1999). Atmobiotic and epigeic species have a higher resistance to drought and thermal stress than specialized soil-dwelling springtails due to biochemical and physiological mechanisms that include a lower cuticular permeability, the production of sugars and polyols to regulate internal osmolality and a greater plasticity in the fatty acid composition of their cellular membranes (Dooremalen et al., 2013; Kærsgaard et al., 2004). But additionally, their larger body size, longer appendages and fully functional visual organs provide these epigeic species with a greater dispersal capacity (Ojala & Huhta, 2001), which allows them to migrate to avoid adverse conditions and choose more favourable micro-habitats within their home range (Chauvat et al., 2014; Ponge et al., 2006), ultimately making them more resilient against local disturbances (Lindberg & Bengtsson, 2006; Malmström, 2012).

In contrast to epigeic springtails, the species adapted to live deeper in the soil profile typically migrate downwards to escape from desiccation (Detsis, 2000; Hopkin, 1997). This behavioural difference may be behind the observed relative edaphization of springtail assemblages under the experimental drought. Atmobiotic and epigeic species may have dispersed seeking for better patches nearby (Chauvat et al., 2014; Ojala & Huhta, 2001; Ponge et al., 2006) after experiencing between 2 up to 4 years of drought, while euedaphic species were still there likely retreated below and perhaps migrating across the soil profile tracking the daily variation in moisture (Detsis, 2000; Hopkin, 1997). Indeed, the soil matrix provides a remarkable buffering capacity against environmental variation (Geiger et al., 2009). However, the euedaphic species are generally more vulnerable to desiccation and thermal stress than epigeic collembolans (Dooremalen et al., 2013; Kærsgaard et al., 2004; Liu et al., 2020, 2021) in spite of their biochemical and physiological adaptations

(Holmstrup et al., 2001). The effectiveness of this vertical migration strategy may therefore be limited if the drought episode lasts too long. The response to the warming treatments did not lead to major changes in the functional structure of the communities. Our warming treatment was rather mild, increasing the MAT of the experimental plots from 0.2 to just 0.9 °C over 4 months (Table S2). Additionally, previous research suggests that the diversity and composition of springtail communities may be fairly resistant and resilient to moderate warming (Alatalo et al., 2015; Holmstrup et al., 2013, 2018; Peguero et al., 2019; Petersen, 2011).

5. Conclusions

There is a solid consensus around the fact that the responses of species to withstand climate change can be grouped around two major strategies: to disperse or to adapt (Berg et al., 2010; Jump & Penuelas, 2005). Springtails, and most likely all soil fauna, are no exception and when experiencing increasingly adverse environmental conditions may either 'move or change' (Ponge, 2020). To move implies from local dispersion up to distribution range shifts. The effectiveness of this strategy relies, however, first on the buffering capacity of the local microhabitats, and secondly on the mismatch (or not) between compositional changes of communities tracking environmental suitability and the velocity of climate change itself (Devictor et al., 2012). Dispersal estimates for collembolan species are scarce but lie in the range of few (~5) centimetres per week during the favourable season. Thus springtail assemblages, at a community level, are expected to select habitats by active movement within a diameters of up to 200 meters (Chauvat et al., 2014; Ojala & Huhta, 2001; Ponge & Salmon, 2013; Ponge, 2020; Treasure & Chown, 2013). Taking into account that birds and butterflies, which both have notably high dispersal capacities, are already experiencing climatic debts of 212 and 135 km respectively (Devictor et al., 2012), we may have serious doubts about the ability of Collembola to keep up with climate change through their active dispersal. Like other organisms such as

plants that are unlikely to migrate fast enough to track the rapidly changing climate, adaptation must play an increasingly important role (Jump & Penuelas, 2005; Ponge, 2020). There are hopeful examples of soil fauna showing rapid evolutionary changes in response to climate change (Bataillon et al., 2016). Even though the only example with a springtail species reported some degree of ontogenetic plasticity (i.e. steeper reaction norms of developmental rates of juveniles than those of adults), there was almost no sign of local adaptation to geothermal warming (Kutcherov et al., 2020). If so, this calls for urgent studies assessing the adaptive potential of springtails, and more generally tracking population dynamics and functional trait shifts at a community level (Bardgett & van der Putten, 2014; Berg et al., 2010; Guerra et al., 2021). This is especially relevant considering that for conservation purposes collembolan functional diversity indices may outperform phylogenetic metrics and better correlate with ecosystem functioning (Peguero et al., 2019).

Finally, our study also cautions that our interpretations of the trait-environment relationships behind the current biodiversity reorganizations of soil fauna may change or even be reversed as climate change continues. As pointed out by previous studies (Alatalo et al., 2015; Holmstrup et al., 2013, 2018b), our work also suggests that the observed shifts of springtail communities to climate manipulations may be transient in time, with epigeic species showing faster responses probably due to their higher vagility. Thus, the resultant relative edaphization we have observed in the springtail communities of our experimentally drought plots could change and even be reversed after some time, in light of the clear and strong selection towards more epigeic assemblages that the aridity gradient has exerted at a larger spatiotemporal scale. To the best of our knowledge there are no studies simultaneously assessing long-term macroecological drivers with short-term climate manipulations at relevant spatial scales and this study demonstrates how important this is if we are to adequately forecast soil fauna responses to climate change.

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Chapter VI

Interactive effects of warming, antibiotics and nanoplastics on the gut microbiome of a soil collembolan*

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Abstract

Nanoplastics and antibiotics are among the most abundant chemical pollutants of soils, but their interplay with global warming remains poorly understood. Finding ways to better assess the impact of these xenobiotics in soils is mandatory to protect the biodiversity of soil organisms and ecosystem services. The springtail Folsomia candida (Class Collembola) is a standard model for ecotoxicological assays with potential as a bioindicator of soil xenobiotics. Little is known, however, about their gut microbiome and how it might respond to warming and these pollutants. We exposed populations of F. candida in microcosms to either nanoplastics or an antibiotic under two temperatures. The antibiotic treatment consisted of colistin addition (50 g•kg-1 of dry soil), and the nanoplastic treatment consisted of polystyrene particles 0.044 µm in diameter (0.1 g•kg-1 of dry soil). Both treatments were incubated at 20 and 22 °C for two months, and their bacterial gut microbiomes were then sequenced. Warming strongly interacted with the impact of both xenobiotics on F. candida gut microbiome. Exposure to nanoplastics at 20 °C decreased the abundance of the most dominant bacterial phyla and families, and decreased the evenness of the gut microbiome. Warming of +2 °C, however, increased the abundances and evenness of the dominant families. Surprisingly, Gram-negative bacteria targeted by colistin were not globally affected. And at genus-level, the endosymbiont Wolbachia controlled

the compositional shifts under nanoplastic addition, potentially driving the response of the gut microbiome to the particles. Our results also indicated that warming was a major driver that modulated the impacts of the antibiotic and nanoplastics. We illustrate how the gut microbiomes of springtails are sensitive communities responsive to xenobiotics and provide evidence of the need to combine multiple factors of global change operating simultaneously if we are to understand the responses of communities of soil arthropods and their microbiomes.

1. Introduction

Anthropogenic global change involves multiple drivers that interact simultaneously in complex ways, leading to increased uncertainty in the responses of organisms and ecological communities (Rillig et al., 2019). Advocating for the study of such responses of biodiversity that integrate the effects of climate change and environmental pollution by novel chemical entities is consequently a growing concern (Sigmund et al., 2023). The term "xenobiotics" represents molecules of anthropogenic origin that have a high potential for unwanted geophysical and biological effects (Steffen et al., 2015). Xenobiotics are recognized as a major obstacle to the delivery of ecosystem services and the conservation of biodiversity and interact complexly with other environmental stressors such as global warming (Moe et al., 2013; Rillig, de Souza Machado, et al., 2019; Rockström et al., 2009). These emergent pollutants have long been influencing ecosystems by worldwide littering, and their production has never been so large (European Environment Agency, 2018). We have only recently, however, begun to properly recognize their impact, with the unsettling discovery that we are already beyond the planetary boundary within which human activity and environmental stability are safe (Persson et al., 2022; Zortéa et al., 2017). Our concern for the well-being of life exposed to xenobiotics and their interplay with global warming thus constitutes a crucial gap in our knowledge of the ecology of global change that we must urgently address.

Plastics are one of the most abundant contaminants in the natural environment, but their fate in soil ecosystems is still mostly a mystery (Horton et al., 2017; Rillig, 2012). Of special interest are micro- and nanoplastics. Microplastics have been defined as plastic particles <5 mm (Thompson et al., 2004), and nanoplastics have been described as a subset of microplastics ranging from 1 to 100 nm (Jambeck et al., 2015). The smallness of these particles confer them high mobility and surface-to-volume ratio, which allows them to either directly interact with biosphere but also adsorb and transport other pollutants (Law & Thompson, 2014; Qi et al., 2020; Wiesner et al., 2011). The bioavailability of microplastics on land reduces the growth and survival of earthworms, interferes with nematode reproduction and modifies bee and springtail behavior (Ji et al., 2021; Liebezeit & Liebezeit, 2013). Similarly, antibiotics are widely distributed and highly bioavailable xenobiotics (Jensen et al., 2003). The effects of antibiotic waste and abuse are notably recognizable by antibiotic resistance in target microbial communities (Z. Li et al., 2022; MacFadden et al., 2018; McGough et al., 2020). Animals, however, are also susceptible to antibiotic contamination, causing microbial dysbiosis, a disruption to the microbiome leading to an imbalance in the microbiota and to changes in their functional composition and metabolic activity (Wypych & Marsland, 2018). Such changes in gut microbiota due to antibiotic contamination affect host fitness in bees and springtails (Agamennone et al., 2015; Zortéa et al., 2017).

Experiments assessing single factors may allow a more thorough mechanistic understanding of their individual impact, but current global-change research stresses that the environment is a multifactorial scenario where the impact of xenobiotics is concomitant with other variables such as warming (Rillig, Ryo, et al., 2019). Indeed, interactions are key to the discovery of emergent and non-intuitive properties capable of increasing the loss of diversity and endangering the resilience of ecosystems (Darling & Côté, 2008; Paine et al., 1998; Rillig, Ryo, et al., 2019). Examining high-order interactions among two or more components, however, needs extensive prior knowledge of the factors being examined, which unfortunately is

rarely available (Altenburger et al., 2013). Identifying pairwise interactions is a reasonable approach to study such complexity, because it may lead to a more realistic understanding of the interplay of simultaneous drivers of global change but balancing the high uncertainty. For example, the impact of temperature on the impact of microplastics on aquatic arthropods has been documented (Chang et al., 2022; Lyu et al., 2021). Yet, to the nuanced response of terrestrial species to microplastics in relation to their rearing temperature remain undiscovered. Similarly, the interactions between antibiotics and temperature on terrestrial arthropods remain a field awaiting exploration.

Assessing the impact of xenobiotics in soils is thus mandatory to protect the delivery of the ecosystem services that soils provide around the world, and we need to find new methods that overcome previous limitations. The gut microbiomes of soil fauna link soil environmental conditions with animal physiology and ultimately with fitness (S. Li et al., 2021; Xiang et al., 2019; Q. Zhang et al., 2019). The term "holobiont" refers to the ecological unit comprising a host, its microbiome and their interplay (Margulis & Fester, 1991). Holobionts are useful in soil ecology because hosts and microbes influence each other under warming and soil contamination (Iltis et al., 2022; Zhu, An, et al., 2018; Zhu, Chen, et al., 2018). Assessing the impact of xenobiotics in the gut microbiomes of soil fauna is thus of great interest, because these entities are potential bioindicators of the ecotoxicological response of fauna to environmental pollutants under a scenario of rapid shifts in temperature. Early attempts focused on the microbial diversity of springtail guts, which decreases under antibiotic treatment but increases when exposed to microplastics (Zhu, An, et al., 2018; Zhu, Chen, et al., 2018). Previous studies, however, have also suggested that the responses of gut microbiomes to xenobiotics in soil arthropods is highly variable within and between species (Thimm et al., 1998; C. Zhang et al., 2019), with some bacteria having differential responses between experiments (Agamennone et al., 2015; Pike & Kingcombe, 2009).

We characterized the response of the gut microbiome of a model soil organism experimentally exposed to nanoplastics and an antibiotic under two rearing temperatures (20 and 22 °C). We used *Folsomia candida* Willem (Class Collembola) as a host because of its cosmopolitan distribution and widespread use as a soil ecotoxicological model. We hypothesized that: (1) antibiotic addition would reduce the microbial diversity of the *F. candida* gut and that Gram-negative bacteria would be disproportionately affected because the antibiotic, colistin, specifically targets this polyphyletic group, (2) nanoplastic addition would reduce bacterial diversity by either increasing or decreasing evenness, depending on its preferential impact on dominant or rare taxa, and (3) a moderate increase in temperature would improve abiotic conditions increasing the abundance of all microbial taxa under control conditions, which would not necessarily be associated with compositional differences in the gut microbiome, but the exposure to xenobiotics under a higher temperature could exacerbate the potentially noxious effects of the nanoplastics and antibiotic, leading to larger compositional differences of the treated gut microbiomes compared to the control.

2. Materials and methods

The experiment was conducted between May and July 2019 in the laboratories of the Center for Ecological Research and Forestry Applications (CREAF, Barcelona, Spain). New colonies of the soil collembolan F. candida originated from the permanent culture at CREAF. The antibiotic was colistin (also known as polymyxin E) in the form of colistin sulfate (Katz & Demain, 1977). Its industrial production has been associated with a therapy of last resort against Gram-negative bacteria. It has been widely used in animal production at the global level (Barlaam et al., 2019). And since 2015, stable plasmid-mediated mobile colistin resistance genes have been detected leading to regulations and policies to preserve the efficacy of colistin and side effects (Rhouma et al., 2023). The nanoplastic pollutants we used (Bangs Laboratories, Inc; Indiana, USA) were

polystyrene particles 0.044 μm in diameter. We tested six treatments: the addition of the antibiotic at 20 °C and 22 °C, the addition of nanoplastics at 20 °C and 22 °C and a control at 20 °C and 22 °C. Six replicates of each treatment were incubated at the original 20 °C of the permanent culture and at 22 °C in different climatic chambers, for a total of 36 microcosms (Fountain & Hopkin, 2005).

The soil in this study was collected from a Mediterranean forest dominated by holm-oak (Quercus ilex) at the Autonomous University of Barcelona campus. After air-drying it for two weeks, it was defaunated following a standard procedure of five consecutive cycles of freezing at -30 °C for 24 h followed by thawing and heating to 40 °C for 24 h.

Each microcosm consisted of a glass pot containing 30 g of wet soil, with moisture adjusted to 50% of the water holding capacity. The relative humidity and soil moisture of each microcosm were monitored daily and kept constant along the experiment. At the start of the experiment, 10 *F. candida* individuals, aged 10 to 12 days, were added to each microcosm. Soil samples in the antibiotic treatment received colistin at a concentration of 50 $g \bullet kg^{-1}$, and soil samples in the nanoplastic treatment received nanoplastics at a concentration of 0.1 $g \bullet kg^{-1}$. The control treatment only contained 30 g of untreated soil. The collembolans were not fed in any of the treatments to increase exposure to the xenobiotics. All individuals were extracted after two months using small Berlese funnels and preserved in 80% ethanol.

The gut microbiome of three individual (random selection) from each microcosm was then analyzed. Each collembolan was washed with 0.5% sodium hypochlorite for 10 s to eliminate surface microbial contamination and then rinsed five times in sterile phosphate buffered saline (PBS). The guts were dissected using sterile forceps and individually placed into 1.5 mL centrifuge tubes. DNA was extracted following the protocol of the DNeasy Blood & Tissue Kit (QIAGEN; Hilden, Germany). We defined the gut microbiome by the total DNA extracted from the dissections quantified using high-throughput sequencing. Microbial communities were

characterized using primers 515F and 806R targeting the V4 region of the bacterial 16S rRNA gene (515F: 5'-GTGCCAGCMGCCGCGG-3'; 806R: 5'-GGACTACHVGGGTWTCTAAT-3') (Caporaso et al., 2010). The reverse primer was designed with 24 unique barcodes to distinguish between the samples. Thermal cycling consisted of 95 °C for 5 min and 35 cycles of 95 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s. We used a Qubit 3.0 fluorimeter to determine the concentration of purified amplification products, and 24 products of equal concentration were pooled as a library. The amplification products were purified and then sequenced on an Illumina MiSeq platform (Meiji; Shanghai, China). The high-throughput sequencing data were then analyzed using Quantitative Insights Into Microbial Ecology (QIIME v1.9.1) (Chang et al., 2010). Operational taxonomic units (OTUs) were classified at 97% similarity using the GreenGenes 13.8 bacterial database and UCLUST (Edgar, 2010; Glöckner et al., 2017; Kõljalg et al., 2013). OTUs with only one sequence (i.e. singletons) were discarded to obtain the final OTU table.

The microbiome communities were assessed using general and generalized linear models (GLMs) to detect differences in OTU standardized abundance, richness and evenness (Pielou's index). The GLMs were always built separating the nanoplastic and antibiotic treatments, with the control at 20 °C as a fixed-effect term and the xenobiotics and the +2 °C warming treatment as predictors. Gram-negative species were identified using the FAPROTAX database and OTU phylogeny (Louca et al., 2016) for determining the impact of the antibiotic on the target taxa, conducting a GLM with treatment as a fixed-effect term and standardized abundances as a predictor. Compositional dissimilarities in the gut microbial communities were assessed using a permutational multivariate analysis of variance (PERMANOVA). Percentage analyses, nonmetric multidimensional scaling (NMDS) and a post hoc test were carried out using a GLM focusing only on the variations in the genus *Wolbachia*. All data handling, visualization and statistical analyses were carried out using R v4.0.6 (R Core Team, 2020).

3. Results

3.1 Characterization of the gut microbiota of *F. candida*

The total number of high-quality sequences across all treatments was 14 951. We identified 1022 OTUs after removing unassigned sequences and combining redundant assignments. The most dominant phyla were Firmicutes (46.2%), Proteobacteria (43.7%) and Actinobacteria (8.0%), covering 97.9% of the abundances of all microbial lineages (Figure 1). The main orders (>5% total abundances) were Bacillales (46.3%), Rickettsiales (13.1%), Sphingomonadales (8.3%), Caulobacterales (7.2%), Actinomycetales (7.0%) and Burkholderiales (5.4%). The family Bacillaceae represented nearly half of all bacteria (46.0%) with 50.7% of all identified genus abundances corresponding to *Geobacillus*, followed by family Rickettsiaceae (13.3%) with *Wolbachia* representing 15% of all genus abundances, family Sphingomonadaceae (8.4%) represented mainly by *Sphingomonas* (9.2% of all abundances; dominant species *S. azotifigens*), family Caulobacteraceae (7.3%) with no genus above 5% total abundances, and family Nocardiaceae (6.1%) with genus *Rhodococcus* corresponding to 6.9% of all genus abundances.

3.2 Response of the gut microbial community to addition of colistin and nanoplastics at 20 °C

The exposure to nanoplastics decreased the relative number of sequence reads and hence the abundance of microbes in the gut of *F. candida* (Table 1, and Figure 2a). This smaller gut microbiome did not affect OTU richness compared to the control but was coupled with a lower evenness of the gut microbial communities, indicating that the most dominant taxa became proportionally even more abundant after exposure to the antibiotic and nanoplastics (Table 1, and Figure 2b and c). Moreover, the abundances of Sphingomonadaceae were higher while that of Proteobacteria (including Rickettsiaceae),

Actinobacteria and Firmicutes (excluding Bacillaceae) were lower. *Wolbachia sp.* drove the compositional dissimilarities between the nanoplastic addition treatments and the control conditions (Table 2 and S4), but no other dominant taxon was found. On the other hand, colistin addition did not modify the abundance nor the richness of the overall gut microbial communities, showing a slight decrease in evenness (Table 1, and Figure 2c). Nonetheless, the abundances of the bacteria in the family Sphingomonadaceae were lower with the exposure to the antibiotic, while that of Proteobacteria (including Rickettsiaceae), Firmicutes and Actinobacteria were higher (Figure 1 and Tables S1).

3.3 Interaction between exposure to xenobiotics with +2 °C warming

An increase of 2 °C during the incubation significantly altered how the *F. candida* gut microbiome reacted to the presence of the two chemical pollutants. The increased warming led to a loss of abundances visible in most of the dominant phyla and families with the partial exception of *Sphingomonadaceae* (Table 1 and S1), which was thereby coupled with a decrease in the evenness of the gut microbiome, but without a detectable change in richness.

The effect of the nanoplastics was temperature-dependent (see significant interactions between temperature and treatment for abundance and evenness in Table 1). The exposure to nanoplastics at 22 °C increased the distribution of the abundances across microbial taxa and the evenness of the gut microbiome, suggesting that the most dominant taxa were disproportionately affected by the simultaneous effect of temperature and nanoplastics. However, nanoplastic addition decreased the abundance of all main phyla and Rickettsiaceae but increased the abundances of Sphingomonadaceae (Table S1). On the other hand, antibiotic exposure combined with +2 °C warming did not modify the abundance and richness of microbial taxa in the gut microbiome (Table 1). But main taxa (excluding Baccillaceae) decreased abundances under antibiotic addition at 22 °C

(Table S1). Warming also led to shifts in the composition of the gut microbiome of *F. candida*. The compositional change with warming and antibiotic exposure was minor and consistent with the change in the control and with warming (Figure 3 and Table 2). The proportion of Bacillaceae was higher, and the proportion of Rickettsiaceae was lower (Figure 1). Additional analysis, however, indicated that the abundance of Gram-negative bacteria was not affected by colistin addition ($P = 0.26$; Table S3). In contrast to the control and antibiotic treatments, a significant interaction between warming and nanoplastic exposure (Table 2) indicated that the compositional effect of the nanoplastics on the gut bacterial communities depended on the temperature of the incubation with increases in abundances, evenness and shifts in composition (Table 1 and 2). Under combined warming and nanoplastic addition, we found increases in Actinobacteria, Firmicutes and Proteobacteria (including Rickettsiaceae), but decreases Sphin*gomonadaceae*, which made the gut microbiomes structurally more similar to the microbiomes in the control incubated at 20 °C (Figures 1 and 3).

4. Discussion

The main finding from our experiment was the pivotal role of temperature in the response of the *F. candida* gut microbiome to the antibiotic and nanoplastics. At 20 °C antibiotic addition led to a loss of evenness, while nanoplastic exposure reduced relative abundances and evenness, and caused shifts in composition partially driven by *Wolbachia sp*. However, with a temperature increase of 2 °C, antibiotic exposure increased evenness, and nanoplastics increased abundances and evenness while also producing shifts in composition. Interestingly, at a lower taxonomic level response became taxon-dependent, and target Gram-Negative bacteria were not affected by colistin. This finding raises important concerns about the uncertainty surrounding the responses of soil fauna to the interactive effects of simultaneous drivers of global change. We also provide additional evidence of the sensitivity of the gut microbiomes of soil fauna to

Table 1. Linear models of community species abundance, evenness and richness against each treatment

Measure	Response variable	Antibiotics		Nanoplastics	
		Estimate ± SE	R^2	Estimate ± SE	R^2
Abundance	Intercept	17.0 ± 3.90**	0.24	23.0 ± 3.21**	0.79
	22 °C	-0.51 ± 0.18**		-0.80 ± 0.15**	
	Xenobiotic	-		-39.0 ± 4.56**	
	Interaction	-		1.88 ± 0.21**	
Richness	Intercept	486 ± 304	0.00	169 ± 208	0.00
	22 °C	8.41 ± 14.6		5.08 ± 9.95	
	Xenobiotic	34.4 ± 28.9		9.16 ± 19.9	
	Interaction	-		-	
Pielou evenness	Intercept	0.85 ± 0.00**	0.56	0.85 ± 0.00**	0.88
	22 °C	-0.05 ± 0.01**		-0.04 ± 0.00**	
	Xenobiotic	-0.02 ± 0.01*		-0.05 ± 0.00**	
	Interaction	0.05 ± 0.01**		0.11 ± 0.00 **	

*Notes: GLMs where intercept group are controls incubated at 20 °C. Linear models were done with Gaussian family. Adjusted-R^2 was calculated for all linear models. SE, standard error; *, $P < 0.05$; **, $P < 0.01$. Degrees of freedom = 18 and 17 for the nanoplastic and antibiotic models, respectively.*

Table 2. PERMANOVA of community matrix against each treatment

Response variable	Antibiotic		Nanoplastics	
	Sum of squares	R^2	Sum of squares	R^2
22 °C	0.39	0.06	0.29	0.04
Xenobiotic	0.29	0.05	0.29	0.04
Interaction	-	-	0.70*	0.11

*Notes: Degrees of freedom = 18 and 17 for the nanoplastic and antibiotic treatments and the temperature models, respectively. *, $P < 0.05$. Number of permutations is 999 per model.*

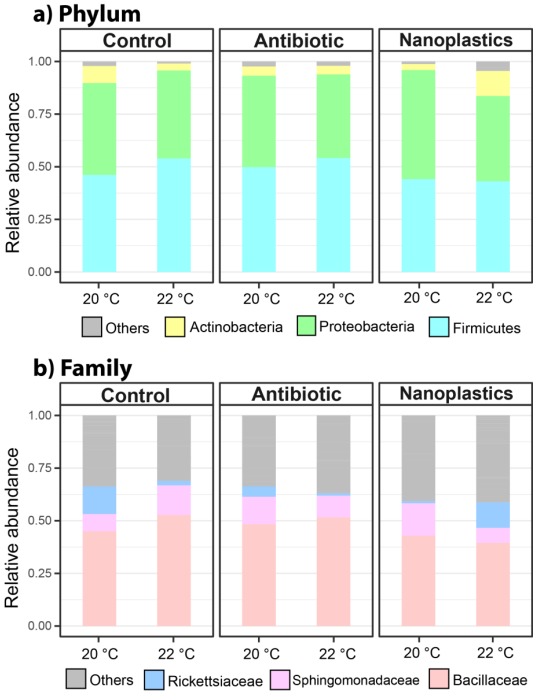

Figure 1. Taxonomic composition of the gut microbiome of *Folsomia candida* at the phylum (a) and family (b) levels for each combination of treatment and rearing temperature. Taxa with relative abundances <5% have been grouped as "Others".

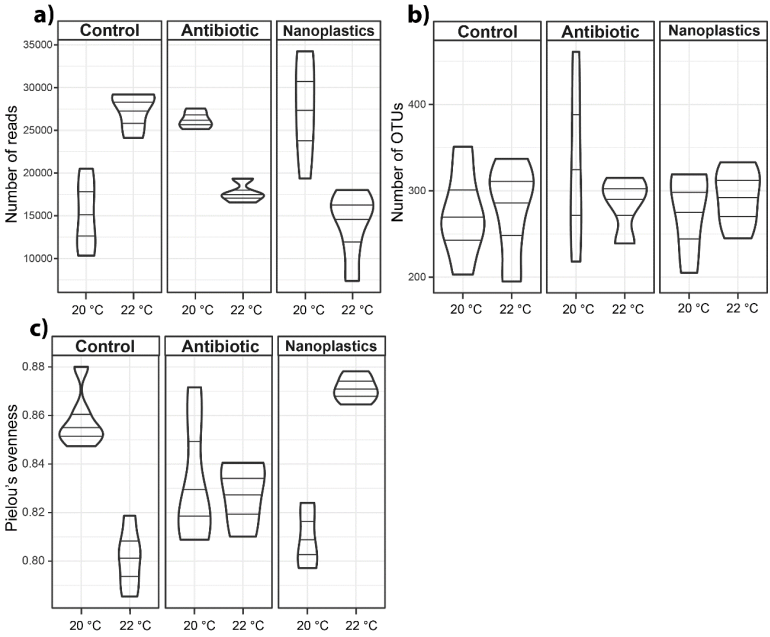

Figure 2. Violin plots of mean species abundance (a), richness (b) and Pielou evenness (c) of the gut microbiome of *Folsomia candida* across experimental treatments. Horizontal lines inside each violin from lowest to highest denote the first, second and third quartiles. Violins were trimmed to adjust to the data.

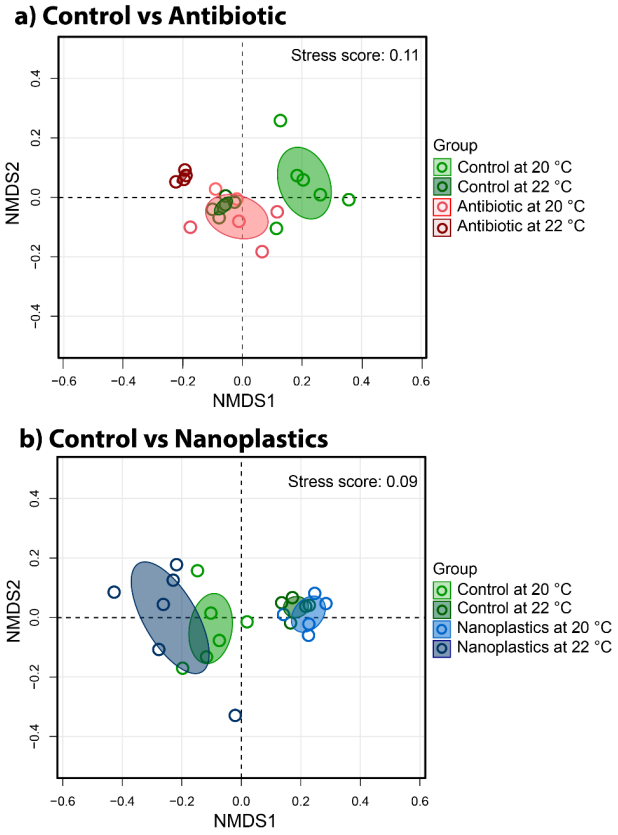

Figure 3. Ordination of the gut microbiome of *Folsomia candida* by non-metric multidimensional scaling showing ellipses and points to separate the two temperature levels of control and antibiotic addition (a) and for the two temperature levels of control and nanoplastic addition (b).

antibiotics and are the first to report the impact of nanoplastic exposure, which was an interesting contrast to previous observations involving larger microplastics (Zhu, Chen, et al., 2018). By highlighting the sensitivity of the gut microbiome in response to changes in temperature and to pollutants, this research emphasizes the need for a comprehensive understanding of how various environmental stressors can interact and potentially amplify their effects in soil ecosystems (Rillig, Ryo, et al., 2019; Sigmund et al., 2023).

4.1 Impact of warming on the gut microbiome

The response to warming suggested a decrease in the fitness of the most dominant taxa in the control treatment. The decrease in abundance, linked with the lack of differences in richness and the decrease in evenness, indicated that the most competitive taxa were disfavored by warming. This finding was somewhat inconsistent with our initial hypothesis that a moderate increase in temperature under control conditions would increase the abundance of all microbial, since the abundance of most of the dominant taxa decreased with warming (Table 1 and S1). Our results did not support previous observations of increases in the relative abundance of *Proteobacteria* in arthropods under experimental warming, although we corroborated the loss of abundance in members of the phylum *Actinobacteria* (Table S1) (Moghadam et al., 2018; Horváthová et al., 2019). Dissimilarities in the composition of the *F. candida* gut microbiome between studies are common (Thimm et al., 1998). Compositional dissimilarities can favor the presence or absence of heat-protective facultative symbionts that alter the thermal performances of microbiomes and hosts (Brumin et al., 2011; Feder & Hofmann, 1999; Iltis et al., 2021; Xi et al., 2008), ultimately generating complex interactions driving the response of the gut microbiome. Our findings generally strengthen the idea that increases in soil temperature drive evenness and compositional shifts in gut microbiome communities by a complex fitness response involving host-microbe interactions (Figure 2 and 3, and Table 1, 2 and S1).

4.2 Interactions between warming and xenobiotics

Warming was a strong driver of shifts in the impact of the novel chemical entities on the *F. candida* gut microbiome. We expected that an increase in temperature of 2 °C during the incubation would amplify the effects of the antibiotic and nanoplastics, but this extra warming radically changed the response of the gut microbiome to these chemical pollutants.

Antibiotic addition decreased evenness, suggesting an unequal increase in taxa abundances that favored some dominant taxa in Firmicutes (albeit not Bacillaceae) but decreased the overall abundances of taxa from the phylum Actinobacteria and Proteobacteria families (e.g. Rickettsiaceae and Sphingomonadaceae) (Table 1 and S1). This finding was surprising, because Actinobacteria are Gram-positive bacteria not targeted by colistin. A comparison of these results with Figure 1, where the relative abundance of Actinobacteria decreased in favor of Firmicutes and Proteobacteria, suggested competitive exclusion under antibiotic addition, but this figure must be interpreted with caution. Interestingly, the response to antibiotic combined with a temperature increase of 2 °C only favored Gram-negative Sphingomonadaceae (Table S1), contrasting with the response under antibiotic addition at 20 °C. These findings were unexpected and suggested a complex interaction between warming and colistin addition. We expected antibiotic exposure under warming to increase the antibacterial activity of colistin, reducing the abundance of vulnerable bacteria (Beveridge & Martin, 1967). Exposure to colistin, however, did not affect the abundance of all Gram-negative bacteria (Table S1), suggesting the up-regulation of genes (e.g. mcr) conferring colistin resistance (Arcilla et al., 2016; Wang et al., 2020). The upregulation of mcr and the subsequent lack of impact on some target Gram-negative bacteria could account for the low impact of the antibiotic (Figure 2 and Table 1). The lack of response from bacteria targeted by colistin nonetheless led to a similar response between Gram-negative and -positive bacteria, suggesting a more transversal impact on the entire microbiome and

therefore accounting for the unexpected lower impact on evenness than the nanoplastics had.

Similarly, the effects of nanoplastic addition varied depending on the temperature of incubation. At 20 °C, the nanoplastics decreased the relative abundances of Firmicutes (but not Bacillaceae), Actinobacteria and Proteobacteria phyla (with the increase in Sphingomonadaceae). But at 22 °C, the response was reversed, decreasing the abundances of all aforementioned taxa except the latter, ultimately increasing evenness (Table 1 and S1). We expected that the effects of the nanoplastics would be more generalized than those of the antibiotic colistin, affecting all microbial taxa in the gut microbiome. The results, however, were similar in magnitude yet the interaction between warming and nanoplastic exposure suggested that different taxa benefited or were impaired by the presence of the nanoplastic particles (Table 1). Interestingly, the nanoplastic treatments differed from the control through *Wolbachia sp.* (Table S2 and S4). A loss of *Wolbachia* under nanoplastic addition at 20 °C is especially important, because they are endosymbionts that control the reproduction of *F. candida* and down-regulate immune responses and responses to heat stress (Table S5) (Xi et al., 2008). Nanoplastic addition may thus have modified the gut microbiome by altering the abundance of *Wolbachia*, thereby altering evenness (Figure 2).

Xenobiotics co-occur and interact under natural conditions (Shen et al., 2019; Arias-Andres et al., 2018; Parthasarathy et al., 2019), so identifying interactions between xenobiotics can be of great interest for better understanding the responses of springtail gut microbiomes.

5. Conclusions and final remarks

To our knowledge, no previous study has focused on colistin, nanoplastics nor the interaction with soil warming in the gut microbiome of *F. candida*. We extended our knowledge of the diverse implications of antibiotic exposure and demonstrated the risks of nanoplastic contamination to soil mesofauna and the central role of temperature. We argue that a greater focus on the size of plastic

particles could produce interesting findings for the response of the gut microbiome to micro- and nanoplastics. The Gram-negative bacteria targeted by the antibiotic were only partially affected, so additional research on mcr genes is necessary to assess their expression in intestinal microbiomes. Finally, we did not address whether changes in the gut microbiome caused detrimental dysbiosis in *F. candida* that would hamper its functions in soil ecosystems. Further study of the impact of the interaction between xenobiotics and soil warming in the functioning of soil ecosystems is therefore recommended.

6. Acknowledgements

This work was supported by the Spanish Government grants PID2020115770RB-I, TED2021-132627 B–I00 and PID2022-140808NB-I00; funded by MCIN; AEI/10.13039/ 501100011033 European Union Next Generation EU/PRTR; the Fundación Ramón Areces grant CIVP20A6621; and the Catalan Government grant SGR 2021–1333.

General Discussion

Our results show a multiplicity of responses to global change, supporting our general objectives and providing surprising insights. In the present dissertation, we aimed to produce new perspectives on soil insect ecology by focusing on the impact of global change across multiple ecosystems. We assessed the relative impact of N and P availability on soil insect community assemblies in tropical hotspots (Chapters I and II), the impact of N deposition on soil insect communities in subarctic regions (Chapter III), the response of subarctic soil insect communities to soil warming (Chapter IV), the response of soil insect communities to warming and drought in continental Europe (Chapter V), and the response of soil insects to xenobiotic particles and whether it is exacerbated by warming (Chapter VI). We corroborate the impact of N and P addition while highlighting the importance of micronutrients. We challenge the assumption that global warming is the primary driver of changes in European soil insects, suggesting instead that drought and aridity are stronger drivers. We provide evidence of the impact of nanoplastics and antibiotics on Folsomia candida, demonstrating the significant role of warming in the gut microbiome's response to xenobiotics. These findings shed light on current research gaps in soil insect ecology and the impacts of global change.

Nutrient availability

Our results achieve our first and second objectives, suggesting that N and P do not drive tropical soil insect communities, albeit N addition influences community composition in subarctic grasslands. These findings reflect the asymmetric nutrient imbalances across hemispheres and suggest that the direct impact of macronutrients on soil insects might be overstated.

One striking finding is that micronutrients can exert a stronger environmental filtering effect than macronutrients, even under N and P addition. Tropical soil insects responded strongly to the availability of Ca, Mg, K, and Na. This dissertation also found that macronutrient addition can indirectly change the availability of micronutrients and microbial N, structuring soil insect assemblages. Furthermore, the responses of insects to nutrient availability do not translate well to lower taxonomic levels. For instance, orders such as Blattodea, Hymenoptera, and Orthoptera share similar responses, while Hemiptera does not. At the species level, dissimilarities arise. These differences are relevant since multiple guilds can be found within a single order, which might affect guilds differently and therefore challenge functional redundancy.

Warming and aridity

We also accomplished our third and fourth objectives. Contrary to expectations, this dissertation did not find a consistent impact of warming on soil insect communities, suggesting no realistic direct impact of human-induced warming over continental Europe and Iceland. Shrubland springtails under a two-decade-long +0.5°C warming did not suffer any major functional shifts, nor did subarctic soil insects living under +3°C warming for a decade. However, we found that a month-long temperature increase of +2°C diminished springtails' gut microbiome abundances and evenness, potentially causing fitness loss in hosts. An extreme +6°C warming in Iceland did drive changes in soil insect community composition, correlating with lower DOC, DON, as well as microbial C and N.

Another significant finding is the high impact of drought. Experimental drought in European shrublands controls springtail functional diversity more than experimental warming. Macro-ecological aridity gradients structure functional traits throughout continental Europe. However, trait selection between local drought and regional aridity favors different life forms (euedaphic and epigeic, respectively). As a result, we argue that drought is an important disturbance to springtails, but local experiments need to be interpreted cautiously if we are to predict soil insects' responses to global warming.

Novel entities

Regarding our fifth objective, we found that xenobiotic particles elicit a response in soil insects' gut microbiome. Exposure to antibiotics reduced evenness, while nanoplastics reduced evenness and relative abundances, leading to shifts in composition. *Wolbachia sp.* was affected by the presence of nanoplastics, which might imply major shifts in host fitness; however, we did not assess whether life history traits were impaired. Our results also support the second half of our hypothesis suggesting an interaction between xenobiotics and warming. A temperature increase of 2°C combined with antibiotic exposure increased evenness, and in combination with nanoplastics, it increased abundances and evenness while also producing changes in community composition. These results are significant because, to our knowledge, no previous experiment has focused on the impact of nanoplastics on soil insects. Additionally, we demonstrate the critical role of warming in the response of *F. candida* gut microbiome when exposed to xenobiotics. These findings are novel, and several questions remain unanswered.

Limitations and future directions

These findings may be somewhat limited by our technical approach. Metabarcoding techniques allow for more reliable characterization of communities, but the quantitative estimates of the original

relative abundances are not entirely trustworthy. We implemented multiple molecular techniques, post-processing approaches, and analyses to explore bulk and eDNA metabarcoding. However, methodological dissimilarities between chapters hamper our ability to draw comparisons. Moreover, technical limitations are still debated among scholars, with constant methodological improvements questioning prior practices. Research needs to continue critically assessing and testing these techniques through systematic comparisons between expertise-based and molecular identifications.

Our results encourage researchers to focus on the impact of micronutrients in tropical ecosystems and drought in temperate regions to predict and protect organisms from planetary boundaries. Research in nutrient imbalance focuses mainly on macronutrients such as N and P due to their human-induced worldwide redistribution. Previous scholarship already suggests macronutrient-driven selection over soil insects. However, a growing body of research accounts for the impact of micronutrients, and our results further highlight their importance. Future studies must go beyond assessing macronutrients and explore shifts in micronutrients and their contribution to soil insect community variance in other hotspots, such as the Mediterranean basin.

Similarly, entomological research on global warming usually focuses on the direct impact of warming, but we argue that the impact of drought and aridity is more important than just temperature increases. Research exploring the fate of soil insects under drought is therefore of great interest. Insect biogeography would benefit from a deeper understanding of natural long-term aridity to predict future.

Moreover, despite the promising results of our third goal, many questions remain. Particularly, our results on xenobiotic particles open a wide array of questions concerning the impact of nanoplastics and the interaction of novel entities with warming. Further experimental studies need to directly assess the fitness of hosts, while observational studies based on novel entities and warming can be carried out in already contaminated areas. Either/or, research on the

impact of novel entities on the soil insect communities are scarce and evidence accretion is needed.

Final outlook

Global change constitutes an emergency acknowledged by researchers and stakeholders, with most evidence focusing on vertebrates and plants. However, worldwide conservation cannot continue to overlook the response of soil organisms. Predicting and protecting soils and their organisms calls for greater academic recognition of soil insects, implying taxa- and region-specific research. This dissertation provides valuable contributions for the future of the discipline, showing the implications of global change on soil insects at multiple scales, regions, and taxa. By assessing such a wide array of contexts, we have pinpointed fruitful paths for future research in soil insect community ecology.

General Conclusions

In this section, we enumerate the main findings of each chapter to highlight the key points of the dissertation in an orderly manner:

1. **Micronutrient influence:** Micronutrients drive tropical soil insect communities primarily through shifts in Ca, K, Mg, and Na availability. Higher K concentration increases insect mOTU richness, higher Na concentration increases insect abundance, and Ca and Mg concentrations control community composition. At a lower taxonomic level, similar drivers are observed among *Blattodea*, *Hymenoptera*, and *Orthoptera*.

2. **Community composition:** P and NP additions drive community composition, but the variances explained by micronutrients are an order of magnitude greater than those observed with P and NP.

3. **Tropical rainforest richness:** In tropical rainforests, insect richness increases with Ca and Mg, and decreases with Na, and community composition changes with K, Mg, and Na.

4. **N addition in subarctic grasslands:** N addition causes shifts in the subarctic grassland soil hexapod community composition, associated with changes in microbial and dissolved N pools.

5. **Geothermal warming:** A natural geothermal temperature increase of 6°C leads to changes in soil insect community composition, associated with changes in microbial and dissolved C and N pools.

6. **European shrubland springtails:** European shrubland springtails exhibit convergent evolution across all three major lineages, with species adapted to different soil strata, and hemiedaphic conditions as their plesiomorphic state.

7. **Aridity and traits:** In European shrublands, aridity selects for traits favoring more epigeic species.

8. **Experimental drought:** Short-term experimental drought favors more euedaphic life-forms, although this may be a transient response due to the high vagility of euedaphic springtails.

9. **Nanoplastics exposure:** Exposure to nanoplastics at 20°C decreases the abundance of the most dominant bacterial phyla and families, reduces the evenness of the gut microbiome, and, at the genus level, the endosymbiont *Wolbachia* controls shifts in composition.

10. **Warming and xenobiotics:** Warming modulates the impact of nanoplastics and antibiotics on F*olsomia candida* gut microbiome.

Supporting information

Chapter I: Micronutrients are drivers of abundance, richness and composition of soil insect communities in tropical rainforests

Supplementary Methods

Section S1: Molecular analyses. The communities of soil insects were characterized to identify molecular operational taxonomic units (OTUs) using DNA metabarcoding. We prepared the bulk insect communities for extracting DNA using one leg of all macrofaunal specimens (wider than approximately 2 mm), and the entire body of all smaller specimens (Braukmann et al., 2019; Ji et al., 2013). DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen, Valencia, USA), and the 16S rRNA region was amplified using the Ins16S_1 primer pair (Ins16S_1-F: 5'-TRRGACGAGAAGACCCTATA-3'; Ins16S_1-R: 5'-TCTTAATCCAACATCGAGGTC-3'; (Clarke et al., 2014)). PCR amplification was performed in 25-µL mixtures containing 2 µL of DNA template, 0.2 µL of AmpliTaq Gold (5 U/µL; Applied Biosystems, Foster City, USA), 2.5 µL of 10' PCR buffer (provided with Amplitaq Gold), 0.5 µL dNTPs (2.5 mM each, Promega, Madison, USA), 1 µL of each primer (10 µM), 0.25 µL of bovine serum albumin (10 mg•mL-1, Promega), 2.5 µL of MgCl2 (25 mM, Applied Biosystems) and nuclease-free water (Promega). PCR conditions consisted of: 95 °C (10 min) followed by 40 cycles at 95 °C (30 s), 50 °C (30 s) and 72 °C (30 s), and a final elongation step at 72 °C (10 min). Different combinations of tags were added to the 5' end of each primer to enable the sequencing of multiple PCR products in a single run. The tags were eight base pairs long at least five differences to minimize ambiguities in the downstream analyses. The sequenced multiplexes comprised extractions, PCR blank controls, unused tag combinations and positive controls to control for potential contaminants and false positives caused by tag-switching (Kocher et al., 2017; Zinger et al., 2018). The PCR products were sequenced using the MiSeq platform (Illumina Inc., San Diego, USA). Paired-end reads were assembled based on barcodes using vsearch, and the primers were removed. Merged sequences were filtered for quality using a threshold of 0.5 for the maximum expected number of errors, retaining reads with a maximum 50% chance to contain an erroneous base. Low-quality sequences (shorter than 50

bp, singletons or containing Ns) were excluded. The sequences were clustered into OTUs using SUMACLUST at a threshold of sequence similarity of 97%. The OTUs were taxonomically assigned using a GenBank blast curated with a local database with >5000 reference sequences from French Guianan insects (Murienne et al. unpublished). We applied a post-processing pipeline to minimize PCR and sequencing errors, contaminants, false-positive sequences and nonfunctional PCRs using conservative criteria to check for quality following Zinger et al. (2018, 2021). The resulting OTU table had a total of 2634 OTUs and 14 thousand reads (Figure S1). Finally, we built matrices of the metabarcoded communities at the plot level, aggregating the data from the sampling points in each plot, leading to 24 insect communities.

Table S1. Abbreviations and brief methodological description of the 18 environmental variables measured.

	Abbreviation	Variable Description	Unit	Method
Belowground (top soil)	Soil C	Carbon content	%	Combustion coupled to an isotope ratio mass spectrometer
	Soil K	Potassium content	%	Acid digestion and ICP-OES
	Soil Mg	Magnesium content	%	Acid digestion and ICP-OES
	Soil N	Nitrogen content	%	Combustion coupled to an isotope ratio mass spectrometer
	Soil Na	Sodium content	mg kg^{-1}	Acid digestion and ICP-OES
	Soil P	Phosphorus content	%	Acid digestion and ICP-OES
	Soil C:N ratio	Carbon to Nitrogen ratio	Mass ratio	---
	Soil C:P ratio	Carbon to Phosphorus ratio	Mass ratio	---
	Soil N:P ratio	Nitrogen to Phosphorus ratio	Mass ratio	---
Aboveground (litter)	Litter C	Carbon content	%	Combustion coupled to an isotope ratio mass spectrometer
	Litter Ca	Calcium content	%	Acid digestion and ICP-OES
	Litter K	Potassium content	%	Acid digestion and ICP-OES
	Litter Mg	Magnesium content	%	Acid digestion and ICP-OES
	Litter N	Nitrogen content	%	Combustion coupled to an isotope ratio mass spectrometer
	Litter Na	Sodium content	mg kg^{-1}	Acid digestion and ICP-OES
	Litter P	Phosphorus content	%	Acid digestion and ICP-OES
	Litter C:N ratio	Carbon to Nitrogen ratio	Mass ratio	---
	Litter C:P ratio	Carbon to Phosphorus ratio	Mass ratio	---
	Litter N:P ratio	Nitrogen to Phosphorus ratio	Mass ratio	---

Table S2. Response of community richness and abundances to site

	Taxa	Estimate	*F*	*P-value*	Adjusted R^2
Abundance	Hexapoda (df = 21)	235 ± 121	3.79	0.06 ·	0.11
	Blattodea (df = 20)	74.7 ± 41.7	3.20	0.08 ·	0.09
	Coleoptera (df = 21)	4.60 ± 6.19	0.55	0.46	0.00
	Hemiptera (df = 20)	10.0 ± 4.33	5.41	0.03 *	0.17
	Hymenoptera (df = 21)	130 ± 74.5	3.04	0.09 ·	0.08
	Orthoptera (df = 13)	-1.77 ± 1.34	1.75	0.20	0.05
Richness	Hexapoda (df = 21)	0.37 ± 15.7	0.00	0.98	0.00
	Blattodea (df = 19)	-10.5 ± 16.8	0.39	0.53	0.00
	Coleoptera (df = 19)	1.56 ± 5.95	0.06	0.79	0.00
	Hemiptera (df = 18)	-0.94 ± 3.84	0.06	0.80	0.00
	Hymenoptera (df = 21)	7.67 ± 7.10	1.16	0.29	0.00
	Orthoptera (df = 18)	-9.81 ± 8.70	1.27	0.27	0.01

*Note: Results are general linear models, with abundance and rarefied richness as the response variable modeled against each site. Intercept corresponds to Paracou. Effect estimates are followed by their standard errors. · and * denote P < 0.1 and P < 0.05, respectively.*

Table S3. Response of community richness and abundances to topography

	Taxon	Topography	Estimate	*t value*	*P*	Adjusted R^2
Abundance	Hexapoda (df = 20)	Intercept	266 ± 105	2.52	0.02 *	0.06
		Slope	255 ± 149	1.71	0.10	
		Top	29.7 ± 154	0.19	0.84	
	Blattodea (df = 19)	Intercept	19.5 ± 37.9	0.51	0.61	0.03
		Slope	82.8 ± 52.0	1,59	0.12	
		Top	19.7 ± 53.7	0.36	0.71	
	Coleoptera (df = 20)	Intercept	20.2 ± 5.05	4.00	0.000 ***	0.05
		Slope	7.12 ± 7.15	0.99	0.33	
		Top	-6.1 ± 7.40	-0.82	0.41	
	Hemiptera (df = 19)	Intercept	9.28 ± 4.31	2.15	0.04 *	0.03
		Slope	6.33 ± 5.90	1.07	0.29	
		Top	4.85 ± 6.09	0.79	0.43	
	Hymenoptera (df = 20)	Intercept	192 ± 64.9	2.96	0.007 **	0.03
		Slope	130 ± 91.8	1.42	0.17	
		Top	-3.01 ± 95.1	-0.03	0.97	
	Orthoptera (df = 12)	Intercept	4.00 ± 1.39	2.86	0.01 *	0.00
		Slope	-0.83 ± 1.80	-0.46	0.65	
		Top	-0.80 ± 1.87	-0.42	0.67	
Richness	Hexapoda (df = 20)	Intercept	34.3 ± 3.00	11.4	0.000 **	0.00
		Slope	-1.85 ± 4.25	-0.43	0.66	
		Top	0.12 ± 4.39	0.02	0.97	
	Blattodea (df = 20)	Intercept	33.2 ± 11.3	2.92	0.008 **	0.00
		Slope	-2.99 ± 16.0	-0.18	0.85	
		Top	-4.61 ± 16.6	-0.27	0.78	
	Coleoptera (df = 20)	Intercept	11.6 ± 3.26	3.56	0.001 **	0.00
		Slope	-0.94 ± 4.62	-0.20	0.84	
		Top	1.99 ± 4.78	0.41	0.68	
	Hemiptera (df = 20)	Intercept	5.97 ± 1.83	3.25	0.003 **	0.00
		Slope	-0.61 ± 2.59	-0.23	0.81	
		Top	-0.96 ± 2.68	-0.35	0.72	
	Hymenoptera (df = 20)	Intercept	20.8 ± 3.59	5.80	0.000 ***	0.02
		Slope	-6.76 ± 5.08	-1.33	0.19	
		Top	0.49 ± 5.25	0.09	0.92	
	Orthoptera (df = 20)	Intercept	9.77 ± 3.06	3.81	0.001 **	0.02
		Slope	-6.27 ± 4.32	-1.45	0.16	
		Top	-5.96 ± 4.47	-1.33	0.19	

*Note: Results are general linear models, with abundance and rarefied richness as the response variable modeled against each topographic level. Intercept corresponds to hill bottoms. Effect estimates are followed by their standard errors. *, ** and *** denote P < 0.05, P < 0.01 and P < 0.001, respectively.*

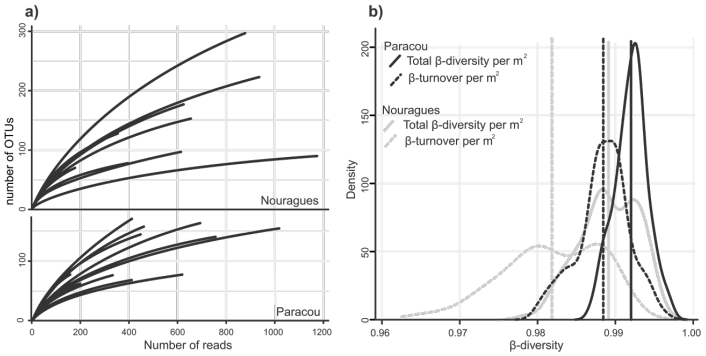

Figure S1. (a) Curves of the accumulation of OTU richness based on DNA sequences for soil Hexapoda in the Nouragues and Paracou. (b) Total β-diversity and turnover of communities of soil Hexapoda at the sampling-point level (i.e. 1 m2) for Paracou and the Nouragues.

Supporting information

Chapter II: Micronutrients are strong drivers of tropical soil insect communities and Phosphorus and Nitrogen fertilization are not

Supplementary Methods

Section S1: Molecular analyses. The communities of soil insects were characterized to identify molecular operational taxonomic units (OTUs) using DNA metabarcoding. We prepared the bulk insect communities for extracting DNA using one leg of all macrofaunal specimens (wider than approximately 2 mm), and the entire body of all smaller specimens (Braukmann et al., 2019; Ji et al., 2013). DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen, Valencia, USA), and the 16S rRNA region was amplified using the Ins16S_1 primer pair (Ins16S_1-F: 5'-TRRGACGAGAAGACCCTATA-3'; Ins16S_1-R: 5'-TCTTAATCCAACATCGAGGTC-3'; (Clarke et al., 2014)). PCR amplification was performed in 25-µL mixtures containing 2 µL of DNA template, 0.2 µL of AmpliTaq Gold (5 U/µL; Applied Biosystems, Foster City, USA), 2.5 µL of 10' PCR buffer (provided with Amplitaq Gold), 0.5 µL dNTPs (2.5 mM each, Promega, Madison, USA), 1 µL of each primer (10 µM), 0.25 µL of bovine serum albumin (10 mg•mL^{-1}, Promega), 2.5 µL of MgCl2 (25 mM, Applied Biosystems) and nuclease-free water (Promega). PCR conditions consisted of: 95 °C (10 min) followed by 40 cycles at 95 °C (30 s), 50 °C (30 s) and 72 °C (30 s), and a final elongation step at 72 °C (10 min). Different combinations of tags were added to the 5' end of each primer to enable the sequencing of multiple PCR products in a single run. The tags were eight base pairs long at least five differences to minimize ambiguities in the downstream analyses. The sequenced multiplexes comprised extractions, PCR blank controls, unused tag combinations and positive controls to control for potential contaminants and false positives caused by tag-switching (Kocher et al., 2017; Zinger et al., 2018). The PCR products were sequenced using the MiSeq platform (Illumina Inc., San Diego, USA). Paired-end reads were assembled based on barcodes using vsearch, and the primers were removed. Merged sequences were filtered for quality using a threshold of 0.5 for the maximum expected number of errors, retaining reads with a maximum 50% chance to contain an erroneous base. Low-quality sequences (shorter than 50 bp, sin-

gletons or containing Ns) were excluded. The sequences were clustered into OTUs using SUMACLUST at a threshold of sequence similarity of 97%. The OTUs were taxonomically assigned using a GenBank blast curated with a local database with >5000 reference sequences from French Guianan insects (Murienne et al. unpublished). We applied a post-processing pipeline to minimize PCR and sequencing errors, contaminants, false-positive sequences and nonfunctional PCRs using conservative criteria to check for quality following Zinger et al. (2018, 2021). The resulting OTU table had a total of 2634 OTUs and 14 thousand reads (Figure S1). Finally, we built matrices of the metabarcoded communities at the plot level, aggregating the data from the sampling points in each plot, leading to 24 insect communities.

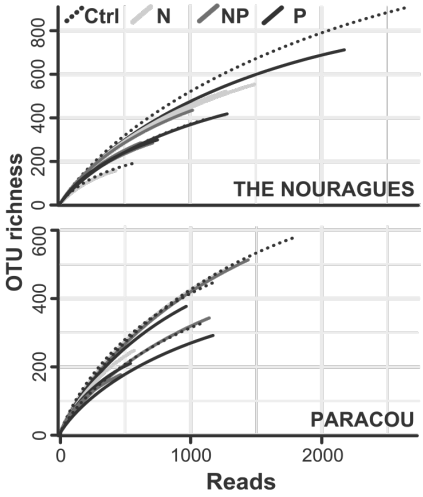

Figure S1. Accumulation of OTU richness per plot with area estimated with Species-Accumulation Curves based on OTU reads (SAC). (Ctrl: control, N: nitrogen, NP: nitrogen-phosphorus, P: phosphorus).

Table S1. Abbreviations and brief methodological description of the seven environmental variables measured.

Compartment	Abreviation	Variable Description	Unit	Method
Aboveground (litter)	Ca	Calcium content	%	Acid digestion and ICP-OES
	K	Potassium content	%	Acid digestion and ICP-OES
	Mg	Magnesium content	%	Acid digestion and ICP-OES
	Na	Sodium content	mg kg^{-1}	Acid digestion and ICP-OES
Belowground (soil)	K	Potassium content	%	Acid digestion and ICP-OES
	Mg	Magnesium content	%	Acid digestion and ICP-OES
	Na	Sodium content	mg kg^{-1}	Acid digestion and ICP-OES

Table S2. Response of Hexapod community abundance and richness to site and topography

Site					
Measure	Estimate		*F*	*P*	Adjusted R^2
Abundance	191 ± 121		2.46	0.13	0.06
Richness	-11.8 ± 12.6		0.88	0.35	0.00
Topography					
Measure	Estimate	*t value*	*F*	*P*	Adjusted R^2
Abundance	412 ± 108	3.79	0.57	0.00**	0.00
	-8.25 ± 153	-0.05		0.95	
	-153 ± 159	-0.96		0.34	
Richness	227 ± 12.7	17.8	1.21	0.000***	0.01
	1.87 ± 18.0	0.10		0.91	
	-23.3 ± 18.0	-1.29		0.21	

*Notes: Results are general linear models, with abundance and richness at a plot level as the response variable modeled against each site and topographic level. Intercept corresponds to Paracou for site and hill-bottom for topography. Estimates of models are followed by their standard errors. Df of all models 21. ** and *** denote P < 0.01 and P < 0.001, respectively.*

Table S3. Response of micronutrients to treatment

Soil layer	Nutrient	Treatment	Estimate	*t*	*P*	R^2
Aboveground (litter)	Ca	Treatment N	-0.43 ± 0.50	-0.85	0.40	
		Treatment NP	0.07 ± 0.50	0.15	0.87	0.05
		Treatment P	0.59 ± 0.50	1.19	0.24	
	K	Treatment N	-0.10 ± 0.14	-0.70	0.48	
		Treatment NP	-0.06 ± 0.14	-0.45	0.65	0.00
		Treatment P	-0.07 ± 0.14	-0.49	0.62	
	Mg	Treatment N	0.00 ± 0.07	-0.11	0.90	
		Treatment NP	-0.02 ± 0.07	-0.29	0.77	0.00
		Treatment P	-0.01 ± 0.07	-0.14	0.88	
	Na	Treatment N	18.7 ± 798	0.02	0.98	
		Treatment NP	-1210 ± 798	-1.51	0.14	0.04
		Treatment P	-1012 ± 798	-1.26	0.22	
Belowground (soil)	K	Treatment N	0.10 ± 0.18	0.57	0.57	
		Treatment NP	0.03 ± 0.18	0.17	0.86	0.00
		Treatment P	0.17 ± 0.18	0.18	0.34	
	Mg	Treatment N	-7.02 ± 124	-0.05	0.95	
		Treatment NP	-43.3 ± 124	-0.34	0.73	0.00
		Treatment P	23.3 ± 124	0.18	0.85	
	Na	Treatment N	33.2 ± 118	0.28	0.78	
		Treatment NP	27.4 ± 118	0.23	0.81	0.00
		Treatment P	119 ± 118	1.01	0.32	

Results are general linear models, with each micronutrient as the response variable modeled against treatment. Intercept corresponds to control. Estimates of models are followed by their standard errors. Abbreviations are: calcium (Ca), magnesium (Mg) and sodium (Na), nitrogen (N), nitrogen-phosphorus (NP) and phosphorus (P). Df for all models is 20.

Supporting information

Chapter IV: Responses of soil hexapod communities to warming are mediated by microbial Carbon and Nitrogen in a subarctic grassland

Table S1. Variation of microbial C and N across temperatures

Environmental variable	Estimate	P-value
Microbial C	-0.016 ± 0.007	0.05 •
Microbial N	-0.017 ± 0.032	0.59
Microbial C : Microbial N	0.000 ± 0.000	0.22

Note: Effect estimates followed by their standard errors are the output of general linear models for each environmental variable. The intercept is always the control, and the explanatory variable is always the corresponding environmental variable: microbial carbon (C) or microbial nitrogen (N). •, P < 0.1. F statistic = 2.97, adjusted R-squared = 0.29, P value = 0.07, Degrees of freedom = 11.

Table S2. Variation of microbial c and soil doc across temperatures

Environmental variable	Estimate	P-value
Microbial C	-0.000 ± 0.000	0.44
Soil DOC	-0.007 ± 0.013	0.59
Microbial C : soil DOC	0.000 ± 0.000	0.79

Note: Effect estimates followed by their standard errors are the output of general linear models for each environmental variable. The intercept is always the control, and the explanatory variable is always the corresponding environmental variable: microbial carbon (c) or soil dissolved organic carbon (doc). F statistic = 2.25, adjusted r-squared = 0.21, p value = 0.13, degrees of freedom = 11.

Table S3. Variation of microbial n and soil don across temperatures

Environmental variable	Estimate	P-value
Microbial N	-0.02 ± 0.02	0.42
Soil DON	-0.44 ± 0.41	0.30
Microbial N : soil DON	0.001 ± 0.001	0.51

Effect estimates followed by their standard errors are the output of general linear models for each environmental variable. The intercept is always the control, and the explanatory variable is always the corresponding environmental variable: microbial nitrogen (n) or soil dissolved organic nitrogen (don). *F* statistic = 2.64, adjusted r-squared = 0.26, *p* value = 0.10, degrees of freedom = 11.

Table S4. Variation of soil doc and don across temperatures

Environmental variable	Estimate	P-value
Soil DOC	-0.03 ± 0.10	0.72
Soil DON	-0.16 ± 0.20	0.44
Soil DOC : soil DON	0.000 ± 0.003	0.91

Effect estimates followed by their standard errors are the output of general linear models for each environmental variable. The intercept is always the control, and the explanatory variable is always the corresponding environmental variable: soil dissolved organic carbon (doc) or soil dissolved organic nitrogen (don). F statistic = 2.33, adjusted r-squared = 0.22, p value = 0.13, degrees of freedom = 11.

Supporting information

Chapter V: Trait-mediated responses to aridity and experimental drought by springtail communities across Europe

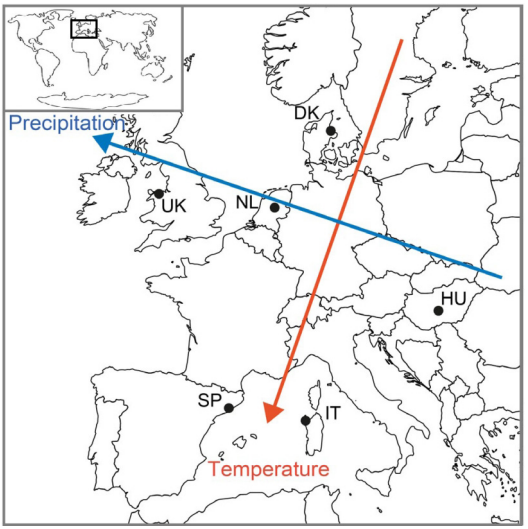

Figure S1. Location of the climatic manipulation experiments in Europe. Arrows depict broad-scale gradients of temperature and precipitation that result in an aridity gradient crossing Europe from North-West to South-East. DK, Denmark; HU, Hungary; IT, Italy; NL, the Netherlands; SP, Spain; UK, United Kingdom.

Table S1. Characteristics of the study sites

Site code	UK	NL	DK	HU	SP	IT
Country	United kingdom	The netherlands	Denmark	Hungary	Spain	Italy
Site name	Clocaenog	Oldebroek	Mols	Kiskunság	Garraf	Capo caccia
Coordinates	53°03′n 3°28′w	52°24′n 5°55′e	56°23′n 10°57′e	46°53′n 19°23′e	41°18′n 1°49′e	40°36′n 8°9′e
Soil type (fao)	Peaty podzol	Haplic arenosol	Sandy podzol	Calcaric arenosol	Petrocalcic calcixerepts	Luvisol and leptosol
Mat (°c)	7.4	8.9	8.7	10.5	15.2	16.1
Map (mm)	1263	1005	669	558	559	544
Growing season	April-september	April-october	April-september	April-september	January-may October-december	January-may October-december
Dominant species	*Calluna vulgaris*	*Calluna vulgaris*	*Calluna vulgaris Deschampsia flexuosa*	*Populus alba Festuca vaginata*	*Erica multiflora Globularia alypum*	*Cistus monspeliensis Helichrysum italicum Dorycnium pentaphyllum*

Mat, mean annual temperature; map, mean annual precipitation.
Mats and maps apply to the study period (see table 2).
Species with relative cover above 10% in the control plots during the study period are listed as dominant species.

Table S2. Experimental manipulations at the study sites

Site code	UK	NL	DK	HU	SP	IT
Start of the experiment (pre-treatment year)	1998	1998	1998	2001	1998	2001
First treatment year	1999	1999	1999	2002	1999	2002
Drought						
Timing	May-september	April-july	May-july	May-june	May-june October-november	April-october
Precipitation excluded (% of yearly total)	25	19	18	22	49	16
Reduction in soil moisture (% of control, 0-20 cm)	45	43	41	23	28	27
Warming						
Timing	Year-round	Year-round	Year-round	Year-round	Year-round	Year-round
Increase in mat (c)	0.2	0.3	0.9	0.4	0.6	0.4

Dk, denmark; hu, hungary; it, italy; mat, mean annual temperature; nl, netherlands; sp, spain; uk, united kingdom.
Drought and warming effects are averages during the study period.
Soil moisture reduction applies to the end of the experimental drought period.

Table S3. List of the 102 springtail species identified. See Petersen (2011) for further information. Accession numbers (from EMBL and BOLT repositories) for the molecular markers (28S rDNA and cox1 mtDNA) used to infer their evolutionary relationships and the availability of trait data are also shown.

Abbreviation	Order	Family	Species	Authority	28S	cox1	Trait data
CYPALB	Entomobryomorpha	Cyphoderidae	Cyphoderus albinus	Nicolet, 1842	KM978338.1	KM617184	Yes
EHAND	Entomobryomorpha	Entomobryidae	Entomobrya handschini	Stach, 1922	KC236317.1	KR119034.1	Yes
ELAN	Entomobryomorpha	Entomobryidae	Entomobrya lanuginosa	Nicolet, 1842	AF483423.1	JN970907.1	Yes
EMAR	Entomobryomorpha	Entomobryidae	Entomobrya marginata	Tullberg, 1871	LK024312.1	GENHP1210-12	Yes
EMULT	Entomobryomorpha	Entomobryidae	Entomobrya multifasciata	Tullberg, 1871	KC236316.1	KM978392.1	Yes
ENIC	Entomobryomorpha	Entomobryidae	Entomobrya nicoleti	Lubbock, 1868	KC236319.1	KM617653	Yes
ENIV	Entomobryomorpha	Entomobryidae	Entomobrya nivalis	Linné, 1758	LK024318.1	KT707112.1	Yes
ENTQUI	Entomobryomorpha	Entomobryidae	Entomobrya quinquelineata cf	Börner, 1901	LK024308.1	KM618447	Yes
ENT	Entomobryomorpha	Entomobryidae	Entomobrya sp				No
ENTSL	Entomobryomorpha	Entomobryidae	Entomobryoides myrmecophilus cf	Reuter, 1886	KC236315.1	KR114833	Yes
HETMAJ	Entomobryomorpha	Entomobryidae	Heteromurus major	Moniez, 1889	JX261654.1	HM397736.1	Yes
LEPAP	Entomobryomorpha	Entomobryidae	Lepidocyrtus apicalis	Mateos & Petersen, 2012			No
LCYAN	Entomobryomorpha	Entomobryidae	Lepidocyrtus cyaneus	Tullberg, 1871	KC236291.1	KM623832.1	Yes
LEPLAN	Entomobryomorpha	Entomobryidae	Lepidocyrtus lanuginosus	Gmelin, 1790	KC236289	JQ801596.1	Yes
LEPLYS	Entomobryomorpha	Entomobryidae	Lepidocyrtus lignorum cf	Fabricius, 1793	LK024362.1	KM623980.1	Yes
LEPSEL	Entomobryomorpha	Entomobryidae	Lepidocyrtus selvaticus cf	Arbea & Ariza, 2007	KC236329	KT707770.1	No
ORCC	Entomobryomorpha	Entomobryidae	Orchesella cincta	Linné, 1758	LK024377.1	KM623648.1	Yes
ORCHSP	Entomobryomorpha	Entomobryidae	Orchesella sp				No
PSALB	Entomobryomorpha	Entomobryidae	Pseudosinella alba	Packard, 1873	KC236295.1	COLNO062-09	No
SEIDOM	Entomobryomorpha	Entomobryidae	Seira domestica	Nicolet, 1841	KC236298.1	KM978395.1	Yes
CRYPALB	Entomobryomorpha	Isotomidae	Cryptopygus albaredai/delamarei	Selga, 1962/ Poinsot, 1970			No
CRYP	Entomobryomorpha	Isotomidae	Cryptopygus sp1				No
CRYPSP	Entomobryomorpha	Isotomidae	Cryptopygus sp2				No
CRYPTH	Entomobryomorpha	Isotomidae	Cryptopygus thermophilus	Axelson, 1900	HQ592746.1	KJ419039.1	Yes
FBREV	Entomobryomorpha	Isotomidae	Folsomia brevicauda	Agrell, 1939	LK024329.1	COLNO068-09	Yes
FLAW	Entomobryomorpha	Isotomidae	Folsomia litsteri	Bagnall, 1939	LK024328.1	COLNO067-09	Yes

FMAN	**Entomobryomorpha**	**Isotomidae**	**Folsomia manolachei**	**Bagnall, 1939**	**LK024327.1**	**COLNO069-09**	**Yes**
FPEN	Entomobryomorpha	Isotomidae	Folsomia penicula	Bagnall, 1939	JN981049.1	GBCO2496-14	Yes
FQOC	Entomobryomorpha	Isotomidae	Folsomia quadrioculata	Tullberg, 1871	JN981050.1	KU373191.1	Yes
FOLPAR	Entomobryomorpha	Isotomidae	Folsomides parvulus	Stach, 1922	JN981041.1	JN981069	Yes
FOLPOR	Entomobryomorpha	Isotomidae	Folsomides portulacensis cf	Gama, 1961			No
ICAER	Entomobryomorpha	Isotomidae	Isotoma caerulea	Bourlet, 1839			No
IANGVIR	Entomobryomorpha	Isotomidae	Isotoma viridis	Bourlet, 1839	JN981061.1	KT808360.1	Yes
IMIN	Entomobryomorpha	Isotomidae	Isotomiella minor	Schäffer, 1896	JN981062.1	KJ186386	Yes
IMOD	Entomobryomorpha	Isotomidae	Isotomodes sp		JN981042.1	JN981070	No
ISMUR	Entomobryomorpha	Isotomidae	Isotomurus graminis cf	Fjellberg, 2007	JN981063.1	JN981085	No
MICRAN	Entomobryomorpha	Isotomidae	Micranurophorus musci	Bernard, 1977	LK024298.1	HM893776	Yes
INOT	Entomobryomorpha	Isotomidae	Parisotoma notabilis	Schäffer, 1896	KJ792158.1	KT808351.1	Yes
PSISOT	Entomobryomorpha	Isotomidae	Pseudisotoma sensibilis	Tullberg, 1876	JN981065.1	JN981086.1	Yes
TETRHYG	Entomobryomorpha	Isotomidae	Tetracanthella hygropetrica cf	Cassagnau, 1954	JN981043.1	HM366594	Yes
TETRAC	Entomobryomorpha	Isotomidae	Tetracanthella sp				No
ONCCRAS	Entomobryomorpha	Oncopoduridae	Oncopodura crassicornis	Shoebotham, 1911	DQ016581.1	KR117530	Yes
PFLAV	Entomobryomorpha	Tomoceridae	Pogonognathellus flavescens	Tullberg, 1871	EU376053.2	KT808376.1	Yes
PLON	Entomobryomorpha	Tomoceridae	Pogonognathellus longicornis	Müller, 1776	KF592009.1	KT808381.1	Yes
TOMMIN	Entomobryomorpha	Tomoceridae	Tomocerus minor	Lubbock, 1862	KF592037.1	KT808378.1	Yes
MEG	Neelipleona	Neelidae	Megalothorax minimus	Willem, 1900	KC900207.1	KC900195.1	Yes
BRAC	Poduromorpha	Brachystomellidae	Brachystomella parvula	Schäffer, 1896	AF483360.1	SMTPP1428-15	Yes
CERDEN	Poduromorpha	Hypogastruridae	Ceratophysella denticulata	Bagnall, 1941	KF684424.1	KU374568.1	Yes
CERGRAN	Poduromorpha	Hypogastruridae	Ceratophysella granulata	Stach, 1949	KF684413.1	HM399009.1	Yes
CER	Poduromorpha	Hypogastruridae	Ceratophysella sp				No
SCHOET	Poduromorpha	Hypogastruridae	Schoettella ununguiculata	Tullberg, 1869	HQ731967.1	HQ732079.1	Yes
WILAN	Poduromorpha	Hypogastruridae	Willemia anophthalma	Börner, 1901	HQ731973.1	KF642103.1	Yes
WIL	Poduromorpha	Hypogastruridae	Willemia sp				No

XEN	Poduromorpha	Hypogastruridae	Xenylla maritima	Tullberg, 1869	LK024444.1	KT808347.1	Yes
XENMED	Poduromorpha	Hypogastruridae	Xenylla mediterranea cf	Gama, 1964	LK024443.1	SMTPF8667-14	No
FRIMIR	Poduromorpha	Neanuridae	Friesea mirabilis	Tullberg, 1871	AF483426.1	GENHP1269-12	Yes
FRITRU	Poduromorpha	Neanuridae	Friesea truncata	Cassagnau, 1958	AF483427.1	GENHP1270-12	Yes
MIC	Poduromorpha	Neanuridae	Micranurida pygmaea	Börner, 1901	AF483435.1	GENHP1168-12	Yes
NEAN	Poduromorpha	Neanuridae	Neanura muscorum	Templeton, 1835	AJ251733.2	KT808329.1	Yes
NEANSP	Poduromorpha	Neanuridae	Neanura sp				No
PSEUDAC	Poduromorpha	Neanuridae	Pseudachorudina meridionalis cf	Bonet, 1929	X90680.1	HG422637.2	Yes
PSAC	Poduromorpha	Neanuridae	Pseudachorudina subcrassus cf	Tullberg, 1871	LK024411.1	LK024514	No
PSPAR	Poduromorpha	Neanuridae	Pseudachorutes parvulus	Börner, 1901	HQ731966.1	KR117034	Yes
XENLOD	Poduromorpha	Odontellidae	Xenyllodes sp		AF483469.1	HQ732089	No
ONYSP	Poduromorpha	Onychiuridae	Onychiurus sp1	(with anal spines)	AF483442.1	HQ732075.1	No
ONYGSP	Poduromorpha	Onychiuridae	Onychiurus sp2	(without anal spines)			No
PROTAPH	Poduromorpha	Onychiuridae	Protaphorura armata	Tullberg, 1869	HQ731965.1	HQ732078	Yes
PROQUER	Poduromorpha	Onychiuridae	Protaphorura quercetana cf	Mateos & Arbea, 1986	LK024409.1	GENHP1278-12	No
MMAC	Poduromorpha	Tullbergiidae	Mesaphorura macrochaeta	Rusek, 1976	AF483375.1	COLNO048-09	Yes
MES	Poduromorpha	Tullbergiidae	Mesaphorura sp				No
NEOTUL	Poduromorpha	Tullbergiidae	Neotullbergia ramicuspis	Gisin, 1953	AF483434.1	COLNO042-09	Yes
SCAPAR	Poduromorpha	Tullbergiidae	Scaphaphorura arenaria	Petersen, 1965	AF483434.1	HQ732072	Yes
TULGSP	Poduromorpha	Tullbergiidae	Tullbergiinae sp1				No
TULLGSP	Poduromorpha	Tullbergiidae	Tullbergiinae sp2				No
ARRPRI	Symphypleona	Arrhopalitidae	Arrhopalites principalis	Stach, 1945	AY239037.1	MHCLB073-09	Yes
ARR	Symphypleona	Arrhopalitidae	Arrhopalites sericus	Gisin, 1947	AF483417.1	MHCLB233-09	Yes
BOURHOR	Symphypleona	Bourletiellidae	Bourletiella hortensis	Fitch, 1863			No
BOURVIR	Symphypleona	Bourletiellidae	Bourletiella viridescens	Stach, 1920	FJ411426.1	KM617234	Yes
BOURPIG	Symphypleona	Bourletiellidae	Bourletiellidae sp		FJ411426.1	BBCCS308-10	No
DEUPAL	Symphypleona	Bourletiellidae	Deuterosminthurus pallipes cf	Bourlet, 1843	FJ411426.1	MHCLB277-09	Yes

FASANG	**Symphypleona**	**Bourletiellidae**	**Fasciosminthurus angulipunctatus**	**Loksa & Bogojevic,1970**			**No**
FASBED	Symphypleona	Bourletiellidae	Fasciosminthurus bedosae cf	Nayrolles, 1994			No
FASSP	Symphypleona	Bourletiellidae	Fasciosminthurus cassagnaui/cugnyi cf	Nayrolles, 1994			No
HETBIL	Symphypleona	Bourletiellidae	Heterosminthurus bilineatus	Bourlet, 1842	FJ411426.1	JF884240	Yes
HETCLA	Symphypleona	Bourletiellidae	Heterosminthurus claviger	Gisin, 1958			No
DICFUSC	Symphypleona	Dicyrtomidae	Dicyrtoma fusca	Lubbock, 1893	LK024301.1	KT808355.1	Yes
DIC	Symphypleona	Dicyrtomidae	Dicyrtoma sp		LK024302.1	KM621738	No
DICORN	Symphypleona	Dicyrtomidae	Dicyrtomina ornata	Nicolet, 1841	LK024305.1	KM623796.1	Yes
DICSAU	Symphypleona	Dicyrtomidae	Dicyrtomina saundersi	Lubbock, 1862	EF199974.2	BBCCS138-10	Yes
DICSP	Symphypleona	Dicyrtomidae	Dicyrtomina sp				No
SMNSP	Symphypleona	Katiannidae	Sminthurinus alpinus cf	Gisin, 1953	LK024422.1	KR117243	No
SMNAU	Symphypleona	Katiannidae	Sminthurinus aureus	Lubbock, 1862	LK024419.1	KU374396.1	Yes
SMEL	Symphypleona	Katiannidae	Sminthurinus elegans	Fitch, 1863	LK024418.1	JQ909220	Yes
SNIG	Symphypleona	Katiannidae	Sminthurinus niger	Lubbock, 1867	LK024423.1	KT808361.1	Yes
SMIGSPS	Symphypleona	Sminthuridae	Sminthuridae sp				No
SMINBOUR	Symphypleona	Sminthuridae	Sminthurus bourgeoisi cf	Nayrolles, 1995			No
SMAC	Symphypleona	Sminthuridae	Sminthurus maculatus	Tömösvary, 1883			No
SNIGMAC	Symphypleona	Sminthuridae	Sminthurus nigromaculatus	Tullberg, 1872	EF199973.2	JN970939	Yes
SPAFLA	Symphypleona	Sminthuridae	Spatulosminthurus flaviceps	Tullberg, 1871			No
SMIDPAR	Symphypleona	Sminthurididae	Sminthurides parvulus	Krausbauer, 1898	DQ016590.1	GENHP1309-12	Yes
SPUM	Symphypleona	Sminthurididae	Sphaeridia pumilis	Krausbauer, 1898	EF192443.1	HM424143	Yes
STEVIOL	Symphypleona	Sminthurididae	Stenacidia violacea cf	Reuter, 1878	AF483455.1	GENHP1309-12	Yes

Table S4. List of the springtail functional traits included in the study. Completeness is the proportion of species with data for a given trait relative to the entire species pool

Trait	Definitions and related functions	No. species with data
Reproduction	sexual vs. parthenogenetic or mixed (reproductive strategy)	47
Body shape	spherical vs. cylindrical or wide_cylindrical (body aspect)	61
Min. length	minimum body length (morphology)	60
Max. length	maximum body length (morphology)	60
Furcula	presence or absence of furcula (evasive organ)	61
Furcula length	furcula length, semi-quantitative scale (escaping ability)	53
Leg-body ratio	leg/body length ratio (locomotive skills)	52
Scales	presence or absence (defensive feature)	62
Pigmentation	presence or absence (protection)	60
Pseudocelli	presence or absence of pseudocelli (defensive structure)	64
Min. number of pseudocelli	minimum number of vesicles in pseudocelli	64
Max. number of pseudocelli	maximum number of vesicles in pseudocelli	64
Ocelli	presence or absence of ocelli (sensory organ, photoreception)	61
Min. number of ocelli	minimum number of ocelli	61
Max. number of ocelli	maximum number of ocelli	61
Antennal length	antennal length, semi-quantitative scale (sensory organ. chemoreception)	54
Min. antenna-head ratio	antenna/head minimum length	51
Max. antenna-head ratio	antenna/head maximum length	51
Post-antennal organ (PAO)	presence or absence of PAO (sensory organ, unknown function)	58
PAO's min. vesicle number	minimum number of PAO vesicles	56
PAO's max. vesicle number	maximum number of PAO vesicles	56
Trichobothria	presence or absence (sensory organ, mechanoreception)	60

Table S5. List of springtail trait values assigned to each species. Reproduction levels are abbreviated as follows: sex = sexual reproduction, par = parthenogenetic reproduction, mix = mixed reproduction with both sexual and parthenogenetic strategies.

Species abbreviation	Reproduction	Body shape	Min. length	Max. length	Furcula	Furcula length	Leg-body ratio	Scales	Pigmentation	Pseudocelli	Min. number of pseudocelli	Max. number of pseudocelli	Ocelli	Min. number of ocelli	Max. number of ocelli	Antennal length	Min. antenna-head ratio	Max. antenna-head ratio	Post-antennal organ (PAO)	PAO's min. vesicle number	PAO's max. vesicle number	Trichobothria
ARR	mix	spherical	0.4	0.8	1	4	0.5	0	1	0	0	0	1	1	1	3	NA	NA	0	0	0	1
ARRPRI	NA	spherical	1	1	1	4	0.5	0	1	1	1	1	1	1	1	3	1.3	1.3	0	0	0	1
BOURVIR	mix	spherical	0.9	1.5	1	NA	NA	0	1	0	0	0	1	8	8	3	1.7	1.7	NA	NA	NA	1
BRAC	sex	wide-cylindrical	0.7	1	1	2	0.25	0	1	0	0	0	1	8	8	1	0.65	0.65	1	4	8	0
CERDEN	sex	cylindrical	0.8	1.8	1	2	0.14	0	1	0	0	0	1	8	8	1	0.45	0.45	1	4	4	0
CERGRAN	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
CRYPTH	sex	cylindrical	1	1	1	4	NA	0	1	0	0	0	1	8	8	NA	NA	NA	1	1	2	0
CYPALB	sex	cylindrical	0.9	1.6	1	4	0.54	1	0	0	0	0	0	0	0	3	2.5	2.5	0	0	0	1
DEUPAL	sex	spherical	0.6	1	1	4	0.48	0	1	0	0	0	1	8	8	3	1.6	2.18	0	0	0	1
DICFUSC	sex	spherical	2	2	1	4	0.62	0	1	0	0	0	1	8	8	3	1.7	1.7	0	0	0	1
DICORN	sex	spherical	2	3	1	4	0.69	0	1	0	0	0	1	8	8	3	1.5	1.5	0	0	0	1
DICSAU	NA	NA	NA	NA	NA	NA	0.69	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EHAND	NA	cylindrical	2	2	1	4	0.44	0	1	0	0	0	1	8	8	3	2.2	3.04	0	0	0	1
ELAN	NA	cylindrical	2	2	1	4	0.47	0	0	0	0	0	1	8	8	3	2.3	2.375	0	0	0	1
EMAR	NA	cylindrical	2	2	1	4	0.43	0	1	0	0	0	1	8	8	3	2.58	2.58	0	0	0	1
EMULT	sex	cylindrical	1.5	2	1	4	0.46	0	1	0	0	0	1	8	8	3	2.375	2.44	0	0	0	1
ENIC	sex	cylindrical	1.5	2	1	4	0.47	0	1	0	0	0	1	8	8	3	2.2	2.875	0	0	0	1

ENV	sex	cylindrical	2	2.5	1	4	0.54	0	1	0	0	0	1	8	8	3	2.9	3.2	0	0	0	1
ENTQUI	NA	cylindrical	NA	NA	1	4	NA	0	NA	0	0	0	1	8	8	NA	NA	NA	0	NA	NA	1
ENTSL	sex	cylindrical	3	3	1	4	0.5	0	1	0	0	0	1	8	8	3	2.1	2.1	0	0	0	1
FBREV	sex	cylindrical	0.8	0.8	1	2	0.27	0	1	0	0	0	1	2	2	1	0.8	0.8	1	1	1	0
FLAW	NA	cylindrical	0.8	0.8	1	NA	0.21	NA	0	0	0	0	0	0	0	1	NA	NA	1	1	1	NA
FMAN	sex	cylindrical	0.6	1	1	3	0.26	0	1	0	0	0	1	2	2	2	1	1	1	1	1	0
FOLPAR	par	cylindrical	0.9	0.9	1	2	0.5	0	0	0	0	0	1	1	2	1	0.9	0.9	1	1	1	0
FPEN	NA	cylindrical	1.6	1.6	1	NA	0.26	0	1	0	0	0	1	2	2	2	NA	NA	1	1	1	0
FQOC	sex	cylindrical	0.8	2.5	1	3	0.26	0	1	0	0	0	1	2	2	1	0.85	0.85	1	1	1	0
FRIMIR	sex	wide-cylindrical	0.7	1.9	0	1	0.22	0	1	0	0	0	1	8	8	1	0.6	0.6	0	0	0	0
FRITRU	NA	cylindrical	0.5	1	0	1	NA	0	1	0	0	0	1	8	8	1	0.68	0.68	0	0	0	0
HETBIL	NA	spherical	NA	NA	NA	NA	0.63	0	NA	0	0	0	NA	8	8	NA	NA	NA	NA	NA	NA	1
HETMAJ	sex	cylindrical	3	3	1	4	NA	1	1	0	0	0	1	8	8	3	2.15	2.15	0	0	0	1
IANGVIR	sex	cylindrical	3	6	1	4	0.3	0	1	0	0	0	1	8	8	3	1.2	1.2	1	1	1	0
IMIN	par	cylindrical	0.7	1.2	1	4	0.31	0	0	0	0	0	0	0	0	3	1.2	1.2	0	0	0	0
INOT	mix	cylindrical	1	1.4	1	4	0.34	0	1	0	0	0	1	2	5	1	0.95	0.95	1	1	1	0
LCYAN	sex	cylindrical	1.5	1.5	1	4	0.38	1	1	0	0	0	1	8	8	3	1.4	1.4	0	0	0	1
LEPLAN	sex	cylindrical	2	2	1	4	0.48	1	1	0	0	0	1	8	8	3	1.6	1.6	0	0	0	1
LEPLYS	sex	cylindrical	1.6	2	1	4	0.48	1	1	0	0	0	1	8	8	3	1.5	1.5	0	0	0	1
MEG	par	spherical	0.4	0.4	1	4	0.51	0	0	0	0	0	0	0	0	1	0.5	0.5	0	0	0	1
MIC	NA	wide-cylindrical	0.4	0.6	0	0	0.2	0	1	0	0	0	1	1	2	1	0.4	0.4	1	5	8	0
MICRAN	par	cylindrical	0.4	0.4	0	NA	NA	0	0	0	0	0	0	0	0	NA	NA	NA	1	1	1	0
MMAC	mix	cylindrical	0.5	0.77	0	0	0.11	0	0	1	14	14	0	0	0	1	0.58	0.85	1	25	46	0
NEAN	mix	wide-cylindrical	2	3.5	0	0	0.11	0	1	0	0	0	1	3	3	1	0.57	0.57	0	0	0	0
NEOTUL	sex	cylindrical	0.9	1.1	0	0	0.14	0	0	1	14	14	0	0	0	1	0.72	0.72	1	50	60	0
ONCCRAS	sex	cylindrical	0.6	0.8	1	4	0.33	1	0	0	0	0	0	0	0	2	1	1	1	1	1	0
ORCC	sex	cylindrical	3	6	1	4	0.54	0	1	0	0	0	1	8	8	3	2	2	0	0	0	1

PFLAV	sex	cylindrical	4	6.5	1	4	0.51	1	1	0	0	0	1	6	6	3	3.5	3.5	0	0	0	1
PLON	NA	cylindrical	4	5	1	NA	0.51	1	1	0	0	0	1	6	6	3	NA	NA	NA	NA	NA	1
PROTAPH	sex	cylindrical	1.1	3	0	1	0.21	0	0	1	56	56	0	0	0	1	0.75	0.75	1	28	40	0
PSEUDAC	NA	wide-cylindrical	0.7	1	1	NA	NA	0	1	0	0	0	1	8	8	NA	NA	NA	1	9	14	0
PSISOT	sex	cylindrical	1.7	1.8	1	4	0.28	0	1	0	0	0	1	8	8	3	1.1	1.1	1	1	1	0
PSPAR	NA	wide-cylindrical	0.8	1.1	1	3	0.16	0	1	0	0	0	1	8	8	1	0.55	0.55	1	6	11	0
SCAPAR	sex	cylindrical	0.35	0.52	0	0	NA	0	0	1	10	16	0	0	0	1	1	1	1	140	190	0
SCHOET	sex	cylindrical	1.2	1.7	1	NA	0.18	0	1	0	0	0	1	8	8	1	0.65	0.65	1	4	4	0
SEIDOM	NA	cylindrical	3	3	1	4	0.55	1	0	0	0	0	1	8	8	3	2.8	2.8	0	0	0	NA
SMEL	sex	spherical	0.7	0.7	1	4	0.6	0	1	0	0	0	1	8	8	3	1.4	1.4	0	0	0	1
SMIDPAR	sex	spherical	0.3	0.55	1	4	0.66	0	1	0	0	0	1	8	8	3	1.3	1.3	0	0	0	1
SMNAU	sex	spherical	1	1	1	4	0.6	0	1	0	0	0	1	8	8	3	1	1	0	0	0	1
SNIG	sex	spherical	1	1	1	4	NA	0	1	0	0	0	1	8	8	3	1.3	1.3	0	0	0	1
SNIGMAC	sex	spherical	1.5	3	1	4	NA	0	1	0	0	0	1	8	8	3	1.9	1.9	0	0	0	1
SPUM	sex	spherical	0.18	0.5	1	4	0.53	0	1	0	0	0	1	8	8	3	1.15	1.15	0	0	0	1
STEVIOL	sex	spherical	0.5	1	1	4	0.68	0	1	0	0	0	1	8	8	3	1	1	0	0	0	1
TETRHYG	NA	NA	0.8	1.2	1	NA	NA	0	1	0	0	0	1	8	8	NA	NA	NA	NA	NA	NA	0
TOMMIN	sex	cylindrical	2	4.5	1	4	0.56	1	1	0	0	0	1	6	6	3	3.15	3.15	0	0	0	1
WILAN	par	cylindrical	0.5	0.7	0	0	0.11	0	0	0	0	0	0	0	0	1	0.9	0.9	1	4	6	0
XEN	NA	cylindrical	1.6	1.6	1	2	0.13	0	1	0	0	0	1	5	5	NA	NA	NA	0	NA	NA	0

Supplementary Methods

Phylogenetic data. The phylogeny of springtails was constructed with sequence data of two genes (cox1 and 28s), obtained from public repositories (GenBank and BOLD). We found information for 77 species (75.5% of all species recorded), although in some cases we had to use sequences for the closest taxa within genus or family level (Table S3). The two genes were aligned separately with CLUSTAL W (Larkin et al., 2007) and then concatenated with GENEIOUS v10.1 (Kearse et al., 2012). The best partition scheme and evolutionary model was selected with PartitionFinder v1.1 (Lanfear et al., 2012) using the BIC criterion. The optimal partition scheme assigned different models to the three codon positions of the cox1 and to the 28s genes (SYM+I+G, GTR+I+G, TM+G+I, GTR+G models, respectively). The best Maximum Likelihood (ML) tree was inferred with the RAxML v.7.2.8 (Stamatakis, 2006) with the help of the RAxML-GUI v1.1 (Silvestro & Michalak, 2012) interface. Each partition was assigned a GTR+CAT model to speed up computation and the best tree and bootstrap support obtained using the ML+ rapid bootstrap option and the 'autoMRE' approach to select the optimal number of bootstrap replicates (Pattengale et al., 2010). Bayesian best trees were inferred with MrBayes v.3.2 (Ronquist et al., 2012), implementing the preferred partition scheme and evolutionary models and running two independent chains for 10 million generations. The correct mixing and the burn-in were evaluated with the help of the program TRACER v1.6 (Rambaut, 2009). Finally, we inferred an ultrametric tree with the program BEAST v1.8.1 (Drummond et al., 2012). The bristletail Dilta littoralis and the dipluran Lepidocampa weberi were used as outgroups, i.e. a group of organisms that allow determining the phylogenetic root of a clade or ingroup with which they are closely related but of which they are not members. Outgroups were removed from the matrix before the analyses and the root of the ingroup was assigned based on the results of the previous phylogenetic analyses. A single relaxed lognormal clock to all partitions was preferred due to the large number of incomplete sequences. The

Birth Death model was selected as tree prior. Because the goal of the analyses was to obtain an ultrametric tree, we assigned an arbitrary rate of 1 to the 'ucld.mean' parameter of the relaxed clock. The accompanying programs LOGCOMBINER and TREEANNOTATOR were used to remove the burn-in generations, to combine the results of three independent chains, and to select the optimal distribution of posterior premasters and tree values. Despite the inherent topological uncertainty of any phylogenetic reconstruction, the tree we obtained was consistent with previous systematic works integrating molecular and morphological information, even in correctly placing the Family Tomoceridae closer to Neelipleona and Poduromorpha than to Entomobryoidae (Yu et al., 2016) (Figure S2).

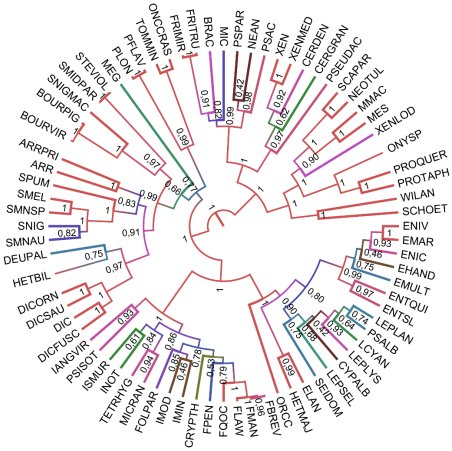

Figure S2. Ultrametric Bayesian consensus tree of the springtails sampled obtained with BEAST under the assumption of lognormal relaxed molecular clock and substitution rates arbitrarily set to 1. Outgroups were removed before the analysis and root was assigned based on previous analyses conducted with maximum likelihood and Bayesian inference including one bristletail and one dipluran as outgroups. Numbers at the nodes are the posterior probabilities, which are correlated with branch color with red denoting the maximum support for a node. See Supplementary Table S3 for interpretation of the species abbreviations.

Supporting information

Chapter VI: Interactive effects of warming, antibiotics and nanoplastics on the gut microbiome of soil collembolan

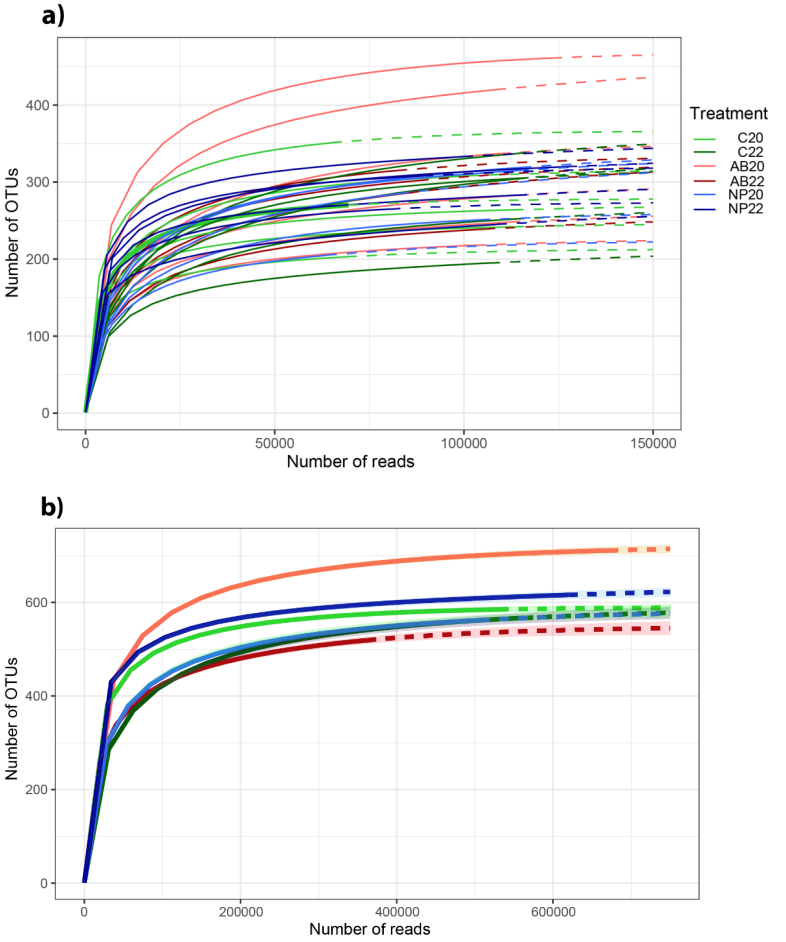

Figure S1. Rarefaction curves for each treatment using replicates (a) and mean reads per replicate (b). Dashed lines correspond to the extrapolated portions of the curves, which were mean-rarefied.

Table S1. Linear models of species abundances for the main phyla and families against each treatment.

		Phyla			
	Response variable	Antibiotic		Nanoplastics	
		Estimate ± SE	Pseudo-R^2	Estimate ± SE	Pseudo-R^2
Actinobacteria	Intercept	2.22 ± 0.88*	0.55	5.03 ± 0.72**	0.86
	22 °C	-0.06 ± 0.04		-0.20 ± 0.03**	
	Xenobiotic	2.80 ± 1.21*		-10.8 ± 1.02**	
	Interaction	-0.13 ± 0.05*		0.52 ± 0.04**	
Firmicutes	Intercept	0.03 ± 0.71	0.37	2.29 ± 1.07*	0.36
	22 °C	0.06 ± 0.03		-0.04 ± 0.05	
	Xenobiotic	2.26 ± 0.97*		-4.67 ± 1.52**	
	Interaction	-0.11 ± 0.04*		0.22 ± 0.07**	
Proteobacteria	Intercept	5.01 ± 2.09*	0.46	11.6 ± 1.25**	0.76
	22 °C	-0.08 ± 0.10		-0.39 ± 0.06**	
	Xenobiotic	6.64 ± 2.87*		-14.4 ± 1.78**	
	Interaction	-0.30 ± 0.13*		0.69 ± 0.08**	
		Families			
	Response variable	Antibiotic		Nanoplastics	
		Estimate ± SE	Pseudo-R^2	Estimate ± SE	Pseudo-R^2
Bacillaceae	Intercept	0.55 ± 0.22*	0.00	0.84 ± 0.29*	0.00
	22 °C	0.01 ± 0.01		0.00 ± 0.01	
	Xenobiotic	0.29 ± 0.31		-0.60 ± 0.41	
	Interaction	-0.01 ± 0.01		0.02 ± 0.01	
Rickettsiaceae	Intercept	1.29 ± 0.35**	0.76	2.47 ± 0.45**	0.72
	22 °C	-0.05 ± 0.01**		-0.10 ± 0.02**	
	Xenobiotic	1.17 ± 0.48*		-4.87 ± 0.64**	
	Interaction	-0.05 ± 0.02*		0.23 ± 0.03**	
Sphingomonadaceae	Intercept	1.14 ± 0.17**	0.58	-0.22 ± 0.22	0.60
	22 °C	-0.03 ± 0.00**		0.03 ± 0.01**	
	Xenobiotic	-1.37 ± 0.24**		1.83 ± 0.31**	
	Interaction	0.06 ± 0.01**		-0.08 ± 0.01**	

*Note: GLMs where intercept group are controls incubated at 20 °C. Linear models for abundances were done with Gaussian family. SE, standard error; **, P < 0.01. Degrees of freedom = 18 and 17 for the nanoplastics and antibiotic models, respectively.*

Table S2. Similarity percentage analyses for each taxon under paired treatments

Paired variables	Taxon	Contribution	Contribution to all taxa contribution
C20-NP20	*Wolbachia* sp.	2.14e-02 ± 5.56e-03 **	5.7e-02

*Note: Discriminating taxon between pairs of treatments using bray-curtis dissimilarities with 999 permutations. Taxa are those with $p < 0.05$ and contributions >5%, if none is shown means that none met the criteria. "contribution" corresponds to taxa contribution to average between-group dissimilarity, and "contribution to all taxa contribution" corresponds to the contribution of a given taxon to the overall taxa contribution totaling 1. Sd, standard deviation; *, $p < 0.05$; **, $p < 0.01$.*

Table S3. Linear model for the abundance of gram-negative species

Response variable	Estimate ± SE	Adjusted-R^2	P-value
Intercept	747 ± 265 **	0.00	0.26
22 °c	506 ± 371		
Colistin	674 ± 397 ·		
Interaction	-1.09e+3 ± 570 ·		

*Note: Linear model with gaussian family for gram-negative taxa at the temperature treatments and colistin. Intercepts are at control 20 ººc. Se, standard error; ·, $p < 0.10$; **, $p < 0.01$. Degrees of freedom = 415.*

Table S4. Linear model for *wolbachia* sp. Abundance with nanoplastic addition and warming

Response variable	Estimate ± SE	Adjusted-R^2	P-value
Intercept	0.35 ± 0.01**	0.89	0.00 **
Nanoplastics	-0.27 ± 0.02 **		

*Note: Linear model with gaussian family for Wolbachia sp. In the temperature and nanoplastic treatments. The intercept is at the control. We found no interaction and no impact from warming, limiting the model to the impact of nanoplastics. **, $p < 0.01$. Degrees of freedom = 9.*

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Soil insects constitute an extraordinarily rich fraction of soil fauna, with impressive taxonomic and functional diversity, offering great potential for research. However, our understanding of insect ecology is still in its infancy, with most scholars neglecting their response to global change. The current characterization of global change emphasizes the worldwide danger of biodiversity loss rates, climate change, P and N cycle alterations, and novel entities' deposition. Soil insect communities are susceptible to all four drivers, yet little research and no consensus exist on their impacts. Moreover, our knowledge gap extends to the possible interactions between drivers and the responses of insect hotspots such as tropical rainforests and the Mediterranean basin. Recent technological advances, particularly in DNA metabarcoding, are aiding researchers in data acquisition, expanding the niche of soil insect ecology to new reaches. It is thus a fruitful moment to develop new perspectives on soil insect community ecology.

In this dissertation, we study the response of soil insect communities to global change (N and P addition, warming, drought, and novel entities) across different ecosystems (tropical rainforests, the Mediterranean basin, and subarctic shrublands). We will utilize various identification tools currently available (expert-based taxonomic identification, bulk DNA metabarcoding, and eDNA). By focusing on such a broad subject, we aim to contribute significantly to soil insect ecology while highlighting the knowledge gaps in this discipline.