Temperature-driven assembly processes of Orthoptera communities: Lessons on diversity, species traits, feeding interactions, and associated faecal microorganisms from elevational gradients in Southern Germany (Berchtesgaden Alps)

Temperaturabhängige Zusammensetzungsprozesse von Heuschreckengemeinschaften: Lektionen über die Diversität, Artmerkmale, Fraßinteraktionen, und Kot-Mikroorganismen von Höhengradienten in Süddeutschland (Berchtesgadener Alpen)

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Table of contents

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I hereby declare that my thesis entitled: “**Temperature-driven assembly processes of Orthoptera communities: Lessons on diversity, species traits, feeding interactions, and associated faecal microorganisms from elevational gradients in Southern Germany (Berchtesgaden Alps)**” is the result of my own work. I did not receive any help or support from commercial consultants. All sources and / or materials applied are listed and specified in the thesis. Furthermore, I verify that the thesis has not been submitted as part of another examination process neither in identical nor in similar form. Besides I declare that if I do not hold the copyright for figures and paragraphs, I obtained it from the rights holder and that paragraphs and figures have been marked according to law or for figures taken from the internet the hyperlink has been added accordingly.

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“If we can walk on the moon or search for life
in outer space, we can fully explore life on Earth.
We know that life occurs here, but we have only an inkling
of its diversity, grandeur, and wonderment”

— *Miller & Rossmann (1996)*

Abstract

Chapter I: Introduction

Temperature is a major driver of biodiversity and abundance patterns on our planet, which becomes particularly relevant facing the entanglement of an imminent biodiversity and climate crisis. Climate shapes the composition of species assemblages either directly via abiotic filtering mechanisms or indirectly through alterations in biotic interactions. Insects - integral elements of Earth's ecosystems - are affected by climatic variation such as warming, yet responses vary among species. While species' traits, antagonistic biotic interactions, and even species' microbial mutualists may determine temperature-dependent assembly processes, the lion's share of these complex relationships remains poorly understood due to methodological constraints. Mountains, recognized as hotspots of diversity and threatened by rapidly changing climatic conditions, can serve as natural experimental settings to study the response of insect assemblages and their trophic interactions to temperature variation, instrumentalizing the high regional heterogeneity of micro- and macroclimate.

With this thesis, we aim to enhance our mechanistic understanding of temperature-driven assembly processes within insect communities, exemplified by Orthoptera, that are significant herbivores in temperate mountain grassland ecosystems. Therefore, we combined field surveys of Orthoptera assemblages on grassland sites with molecular tools for foodweb reconstruction, primarily leveraging the elevational gradients offered by the complex topography within the Berchtesgaden Alpine region (Bavaria, Germany) as surrogate for temperature variation (space-for-time substitution approach). In this framework, we studied the effects of temperature variation on (1) species richness, abundance, community composition, and interspecific as well as intraspecific trait patterns, (2) ecological feeding specialisation, and (3) previously neglected links to microbial associates found in the faeces.

Chapter II: Temperature-driven assembly processes

Climate varies at multiple scales. Since microclimate is often overlooked, we assessed effects of local temperature deviations on species and trait compositions of insect communities along macroclimatic temperature gradients in Chapter II. Therefore, we employed joint species distribution modelling to explore how traits drive variation in the climatic niches of Orthoptera species at grassland sites characterized by contrasting micro- and macroclimatic conditions. Our findings revealed two key insights: (1) additive effects of micro- and macroclimate on the diversity, but (2) interactive effects on the abundance of several species, resulting in turnover and indicating that species possess narrower climatic niches than their elevational distributions might imply. This chapter suggests positive effects of warming on Orthoptera, but also highlights that the interplay of macro- and microclimate plays a pivotal role in structuring insect communities. Thus, it underscores the importance of considering both elements when predicting the responses of species to climate change. Additionally, this chapter revealed inter- and intraspecific effects of traits on the niches and distribution of species.

Chapter III: Dietary specialisation along climatic gradients

A crucial trait linked to the position of climatic niches is dietary specialisation. According to the ‘altitudinal niche-breadth hypothesis’, species of high-elevation habitats should be less specialized compared to their low-elevation counterparts. However, empirical evidence on shifts in specialization is scarce for generalist insect herbivores and existing studies often fail to control for the phylogeny and abundance of interaction partners. In Chapter III, we used a combination of field observations and amplicon sequencing to reconstruct dietary relationships between Orthoptera and plants along an extensive temperature gradient. We did not find close but flexible links between individual grasshopper and plant taxa in space. While interaction network specialisation increased with temperature, the corrected dietary specialisation pattern peaked at intermediate elevations on assemblage level. These nuanced findings demonstrate that (1) resource availability, (2) phylogenetic relationships, and (3) climate can affect empirical foodwebs intra- and interspecifically and, hence, the dietary specialisation of herbivorous insects. In this context, we discuss that the underlying mechanisms involved in shaping the specialisation of herbivore assemblages may switch along temperature clines.

Chapter IV: Links between faecal microbe communities, feeding habits, and climate

Since gut microbes affect the fitness and digestion of insects, studying their diversity could provide novel insights into specialisation patterns. However, their association with insect hosts that differ in feeding habits and specialisation has never been investigated along elevational climatic gradients. In Chapter IV, we utilized the dietary information gathered in Chapter III to characterize links between insects with distinct feeding behaviour and the microbial communities present in their faeces, using amplicon sequencing. Both, feeding and climate affected the bacterial communities. However, the large overlap of microbes at site level suggests that common bacteria are acquired from the shared feeding environment, such as the plants consumed by the insects. These findings emphasize the influence of a broader environmental context on the composition of insect gut microbial communities.

Chapter V: Discussion & Conclusions

Cumulatively, the sections of this dissertation provide support for the hypothesis that climatic conditions play a role in shaping plant–herbivore systems. The detected variation of taxonomic and functional compositions contributes to our understanding of assembly processes and resulting diversity patterns within Orthoptera communities, shedding light on the mechanisms that structure their trophic interactions in diverse climates. The combined results presented suggest that a warmer climate could foster an increase of Orthoptera species richness in Central European semi-natural grasslands, also because the weak links observed between insect herbivores and plants are unlikely to limit decoupled range shifts. However, the restructuring of Orthoptera communities in response to warmer temperatures depends on species' traits such as moisture preferences or phenology. Notably, we were able to demonstrate a crucial role of microclimate for many species, partly unravelling narrower climatic niches than their elevational ranges suggest. We found evidence that not only Orthoptera community composition, specialisation, and traits varied along elevational gradients, but even microbial communities in the faeces of Orthoptera changed, which is a novel finding. This complex restructuring and reassembly of communities, coupled with the nonlinear specialisation of trophic interactions and a high diversity of associated bacteria, emphasize our currently incomplete comprehension of how ecosystems will develop under future climatic conditions, demanding caution in making simplified predictions for biodiversity change under climate warming. Since these predictions may benefit from including biotic interactions and both, micro- and macroclimate based on our findings, conservation authorities and practitioners must not neglect improving microclimatic conditions to ensure local survival of a diverse set of threatened and demanding species. In this context, mountains can play a pivotal role for biodiversity conservation since these offer heterogeneous microclimatic conditions in proximity that can be utilized by species with distinct niches.

Zusammenfassung

Kapitel I: Einleitung

Die Temperatur ist eine wichtige Triebkraft hinter den Artenvielfalts- und Abundanzmustern auf unserem Planeten, was angesichts der Verflechtung der unmittelbar bevorstehenden Biodiversitäts- und Klimakrise besonders relevant ist. Das Klima strukturiert die Artenvielfalt direkt durch abiotische Filtermechanismen oder indirekt durch Veränderungen biotischer Wechselwirkungen. Insekten - wesentliche Bestandteile der Ökosysteme der Erde - sind von klimatischen Veränderungen wie der Erwärmung betroffen, reagieren aber je nach Art unterschiedlich. Während die Merkmale der Arten, antagonistische biotische Interaktionen und sogar die mikrobiellen Partner der Arten temperaturabhängige Zusammensetzungsprozesse bestimmen können, bleibt ein Großteil dieser komplexen Beziehungen aufgrund methodischer Einschränkungen nach wie vor schlecht verstanden. Gebirge, die als Hotspots der Diversität gelten und von sich rasch verändernden klimatischen Bedingungen bedroht sind, können durch Nutzung der großen regionalen Heterogenität der Klein- und Großklimate als natürliche Experimente dienen, um die Reaktion von Insektengemeinschaften und deren trophischen Interaktionen auf Temperaturänderungen zu untersuchen.

Mit dieser Arbeit möchten wir einen Beitrag zum mechanistischen Verständnis der temperaturbedingten Zusammensetzungsprozesse von Insektengemeinschaften leisten, am Beispiel von Heuschrecken, die bedeutende Pflanzenfresser in Grünlandökosystemen der gemäßigten Breiten sind. Hierfür kombinierten wir Felduntersuchungen von Heuschrecken-gemeinschaften in Grünlandstandorten mit molekularen Methoden zur Rekonstruktion von Nahrungsbeziehungen, wobei wir hauptsächlich die Höhengradienten, die die komplexe Topografie der Berchtesgadener Alpenregion (Bayern, Deutschland) bietet, stellvertretend für Temperaturveränderungen verwendeten (Raum-Zeit-Substitutionsansatz). In diesem Rahmen untersuchten wir die Auswirkungen von Temperaturvariation auf (1) den Artenreichtum, die Abundanz, die Zusammensetzung der Gemeinschaft und die inter- und intraspezifischen Merkmalsmuster, (2) die ökologische Nahrungsspezialisierung und (3) die bis dato vernachlässigte Verbindung zu den mikrobiellen Begleitarten im Kot.

Kapitel II: Temperaturabhängige Zusammensetzungsprozesse

Das Klima variiert auf verschiedenen Ebenen. Da Veränderungen im Kleinklima oft vernachlässigt werden, haben wir in Kapitel II die Auswirkungen der lokalen Temperaturunterschiede auf die Arten- und Merkmalszusammensetzung von Insekten-gemeinschaften entlang makroklimatischer Temperaturgradienten untersucht. Hierfür haben wir die Methode der gemeinsamen Artenverteilungsmodellierung verwendet, um zu untersuchen, wie

Artmerkmale die Unterschiede in klimatischen Nischen von Heuschreckenarten auf Grünlandstandorten mit gegensätzlichen mikro- und makroklimatischen Bedingungen beeinflussen. Unsere Ergebnisse brachten zwei wichtige Erkenntnisse zutage: (1) additive Auswirkungen des Mikro- und Makroklimas auf die Vielfalt, aber (2) interaktive Effekte auf die Häufigkeit mehrerer Arten, die sich in Zusammensetzungsunterschieden niederschlagen und auf engere klimatische Nischen hinweisen, als es die Höhenverbreitung vermuten lässt. Dieses Kapitel deutet auf positive Auswirkungen einer Erwärmung auf Orthoptera hin, zeigt aber auch, dass das Zusammenspiel von Makro- und Mikroklima eine Schlüsselrolle bei der Strukturierung von Insektengemeinschaften spielt und beide Elemente bei der Vorhersage der Reaktionen von Arten auf den Klimawandel berücksichtigt werden sollten. Darüber hinaus wurden in diesem Kapitel die inter- und intraspezifischen Auswirkungen von Merkmalen auf die Nischen und die Verbreitung von Arten aufgezeigt.

Kapitel III: Nahrungsspezialisierung entlang von Klimagradienten

Ein entscheidendes Merkmal für die Lage der klimatischen Nische einer Art ist die Nahrungsspezialisierung. Nach der "Hypothese der Höhenlagen-abhängigen Nischenbreite" sollten Arten in hoch gelegenen Lebensräumen weniger spezialisiert sein als ihre Pendants in niedrigen Lagen. Empirische Belege für Verschiebungen in der Spezialisierung von generalistischen, herbivoren Insekten sind jedoch rar und es fehlt eine Berücksichtigung der Häufigkeit und Phylogenie von Interaktionspartnern. In Kapitel III haben wir eine Kombination aus Feldbeobachtungen und Amplikonsequenzierung verwendet, um die Nahrungsbeziehungen von Heuschrecken und Pflanzen entlang eines ausgedehnten Temperaturgradienten zu rekonstruieren. Wir konnten keine engen, sondern flexible Beziehungen zwischen einzelnen Herbivoren- und Pflanzentaxa feststellen. Während die Spezialisierung der Interaktionsnetzwerke mit der Temperatur zunahm, erreichte das korrigierte Muster der Nahrungsspezialisierung auf Gemeinschaftsebene seinen Höhepunkt in mittleren Höhenlagen. Diese differenzierten Ergebnisse zeigen, dass (1) die Verfügbarkeit von Ressourcen, (2) phylogenetische Beziehungen und (3) das Klima intra- und interspezifische empirische Nahrungsbeziehungen und damit die Nahrungsspezialisierung pflanzenfressender Insekten beeinflussen können. In diesem Kontext diskutieren wir, dass die zugrundeliegenden Mechanismen hinter der Nahrungsspezialisierung von herbivoren Insekten entlang von Temperaturgradienten wechseln könnten.

Kapitel IV: Verbindungen zwischen Kotbakteriengemeinschaften, Ernährungsgewohnheiten und Klima

Da Darmbakterien die Fitness und Verdauung von Insekten beeinflussen, könnte die Untersuchung deren Vielfalt neue Erkenntnisse über Spezialisierungsmuster liefern. Ihre Verbindung mit Insekten, die sich in ihren Ernährungsgewohnheiten und ihrer Spezialisierung unterscheiden, wurde jedoch noch nie entlang klimatischer Höhengradienten untersucht. In Kapitel IV verwendeten wir Nahrungsinformationen aus Kapitel III, um mit Hilfe von Amplikonsequenzierung Verbindungen zwischen Insekten mit unterschiedlichem Ernährungsverhalten und mikrobiellen Gemeinschaften in deren Kot zu charakterisieren. Sowohl die Nahrung als auch das Klima hatten Auswirkungen auf die bakteriellen Gemeinschaften. Die große Überschneidung der Mikrobengemeinschaften auf Standortebene deutet jedoch darauf hin, dass gemeinsame Bakterien aus der geteilten Nahrungsumgebung, wie z. B. den von den Insekten verzehrten Pflanzen, stammen. Diese Ergebnisse unterstreichen den Einfluss eines breiteren Umweltkontextes auf die Zusammensetzung der mikrobiellen Gemeinschaften im Insektendarm.

Kapitel V: Diskussion & Schlussfolgerungen

Insgesamt stützen die Kapitel dieser Dissertation die Hypothese, dass klimatische Verhältnisse Pflanzen-Pflanzenfresser-Systeme prägen. Die festgestellten Unterschiede in der taxonomischen und funktionellen Zusammensetzung tragen zu unserem Verständnis der Zusammensetzungsprozesse und daraus resultierenden Diversitätsmustern von Heuschreckengemeinschaften sowie der Mechanismen bei, die deren trophische Interaktionen in verschiedenen Klimazonen strukturieren. Die Kombination der Ergebnisse deutet darauf hin, dass wärmeres Klima eine Zunahme des Heuschreckenartenreichtums in naturnahen Grünlandgebieten Mitteleuropas begünstigen könnte, auch weil die schwachen Verbindungen zwischen den herbivoren Insekten und Pflanzen entkoppelte Arealverschiebungen wahrscheinlich nicht limitieren. Jedoch könnten höhere Temperaturen die Zusammensetzung von Heuschreckengemeinschaften je nach den Merkmalen der Arten wie deren Feuchtigkeitsvorlieben oder der Schlupfphänologie verändern. Darüber hinaus konnten wir nachweisen, dass das Mikroklima für viele Arten eine entscheidende Rolle spielt, da es teilweise engere klimatische Nischen aufdeckt, als ihre Höhenverbreitung vermuten lassen. Wir fanden Hinweise darauf, dass sich nicht nur die Zusammensetzung, Spezialisierung und Merkmale der Heuschreckengemeinschaften entlang der Höhengradienten ändern, sondern dass sogar die mikrobiellen Gemeinschaften im Kot variieren, was eine neue Erkenntnis darstellt. Diese komplexe Umstrukturierung und Neuzusammensetzung von Gemeinschaften in Kombination mit der nichtlinearen Spezialisierung von Interaktionen und einer hohen Vielfalt an assoziierten Bakterien unterstreichen unser noch immer begrenztes Verständnis davon, wie sich Ökosysteme unter zukünftigen Klimabedingungen entwickeln werden, und mahnen zur Vorsicht bei vereinfachten Vorhersagen über die Veränderung der biologischen Vielfalt im Zuge der Klimaerwärmung. Da solche Vorhersagen auf Grundlage unserer Ergebnisse vom Einbezug biotischer Wechselwirkungen und des Mikro- und Makroklimas profitieren können, dürfen Naturschutzverantwortliche eine Verbesserung der mikroklimatischen Bedingungen nicht vernachlässigen, um das lokale Überleben einer Vielzahl bedrohter und anspruchsvoller Arten zu sichern. In diesem Zusammenhang können Berge eine entscheidende

Rolle für den Erhalt der biologischen Vielfalt spielen, da sie in räumlicher Nähe heterogene mikroklimatische Bedingungen bieten, die von Arten mit unterschiedlichen Nischen genutzt werden können.

Chapter I

General introduction

"We should preserve every scrap of biodiversity as priceless while we learn to use it and come to understand what it means to humanity."

Edward O. Wilson (1996)

"[Our] attention must focus on the underlying evolutionary processes that have resulted in such diversity and evaluate these in terms of present human activities."

Terry L. Erwin (1996)

Insects, essential elements of the world’s ecosystems, are declining at alarming rates. While the extent of suitable habitat for insects is decreasing due to conversion and its quality is constantly deteriorating due to intensification or abandonment, insect populations also respond to climate warming – whether it be man-made or natural – emphasizing the role of temperature as a driver of diversity and abundance. Evidence of temperature-driven changes of insect diversity, abundance, and interactions is still limited due to a lack of data and the multitude of confounding factors which correlate with temperature clines. Furthermore, microclimatic variation is often neglected, further complicating inferences drawn from climatic gradients. To assess how changing climatic conditions shape the assembly of insect communities, we employed a space-for-time design with study sites equally distributed across macroclimatic and microclimatic gradients in a region void of intensive management of insect habitats: the Berchtesgaden Alps. Within this framework, we recorded Orthoptera assemblages to assess the effect of traits on the climatic niches formed by the interplay of micro- and macroclimatic conditions (Chapter II). Subsequently, we focussed on empirically assessing trophic dietary specialisation of Orthoptera, which is predicted to be affected by environmental filtering, through reconstructing interactions based on metabarcoding of DNA-contents in faecal pellets, while considering the composition and phylogenetic relationships of local plant communities (Chapter III). There are many overlooked components contributing to the diversity and realisation of ecological interactions. Hence, we finally also conducted a study on the microbial associates found in the faecal pellets of Orthoptera - for the first time along a climatic gradient (Chapter IV). The synthesis of all our findings and their implications is presented in Chapter V.

1.1 *Insect population trends in times of global change*

To the immeasurable diversity of life on earth, insects contribute the lion's share: over a million species described and estimates surpassing five million (Grimaldi & Engel 2005, Larsen et al 2017, Stork 2018). This dominance extends to arthropod biomass, outweighing the carbon mass of humans and other vertebrates by a significant margin (Eggleton 2020). These staggering numbers already hint on the profound significance of insects within ecosystems. Throughout the recent history of modern humanity, insects have played a crucial role in agricultural food production by providing essential services such as pollination or pest control. Conversely, certain species act as pests in forestry or agriculture (Eggleton 2020, Wagner et al 2021, Wilson 1987). An illustrative example is the mention of yield-destroying swarms of locusts, comprising billions of individuals, referred to as the eighth plague in the Old Testament. This historical reference illustrates the dual nature of insects and their significance to human interests. However, with an increasing awareness of the world's biodiversity heritage and growing recognition of the ecological and economical value of insects, there have been first steps of a concerted effort to accumulate knowledge about their population trends.

In this course, alarming rates of about 1% abundance decline of terrestrial arthropods per year have been documented in Europe and North America (van Klink et al 2020). The situation is even more concerning in German protected areas (75% decline in biomass within 27 years) (Hallmann et al 2017) and grasslands in general (78% decline in abundance and 34% decline in richness during 10 years) (Seibold et al 2019), raising concerns about the future stability and functioning of these ecosystems, wherein insects are essential elements (Hochkirch 2016, Kremen 2018). Notably, these trends are not restricted to rare species (Conrad et al 2006, Seibold et al 2019). A recent analysis of European Red Lists estimates almost two million species are threatened worldwide, double the number reported in the latest IPBES report (Hochkirch et al 2023). Thus, identifying the most important drivers of insect population trends is a critical challenge in the context of global change, and understanding the mechanisms is pivotal to refine projections of population trends and develop effective conservation strategies. Summaries of existing literature on insect declines and the threat status of species during the Anthropocene point to land use (change), intensive agriculture, exploitation, nitrification, pollution, and climate change as paramount drivers (Harvey et al 2023, Hochkirch et al 2023, Wagner et al 2021). Among these, climate change was identified as one of the most severe future threats emerging to biodiversity (Hochkirch et al 2023). A recent study re-analysing biomass trends identified by Hallmann et al (2017) concludes that weather anomalies play a crucial role in shaping insect biomass trends (Müller et al 2023), highlighting the already detectable and immediate impact of climate change on Central European insects.

Climate change is apparent in the unprecedented increase in temperatures, driven by elevated global emissions of carbon dioxide, methane, nitrogen oxide, and other greenhouse gases during the industrial era (Masson-Delmotte et al 2021). It encompasses a complex interplay of temperature rise, enhanced frequencies and magnitudes of extreme weather events, and alterations in precipitation patterns. Among the various facets of climate change, rising temperatures pose a proximate and perceptible threat to insects, since these may exceed values above the organisms' critical thermal limits within their habitats, coercing stenothermic species to adjust their phenology, ranges, or physiology (Bellard et al 2012). Simultaneously, warming temperatures foster the abundance and range expansion of a set of other species (Bowler et al 2017, Soroye et al 2020, Thomas 2010). Biodiversity and the composition of species communities have already been affected by climate change (Devictor et al 2012). Shifts in the ranges and distributions of species have been reported as well as invasions of species into novel habitats (Thomas 2010). While some species can track their shifting climatic niche, others exhibit a decoupling of their climatic niches from abundances and distributions (Sunde et al 2023, Viana & Chase 2022). Range shifts have been recorded for butterflies (Habel et al 2016, Hill et al 2021, Kerner et al 2023, Parmesan 2006, Thomas 2010) and bees already (Maihoff et al 2023). Additionally, phenological responses to warming, such as an earlier emergence in spring, are even more prevalent in literature (Roslin et al 2021, Visser et al 2006, Zuna-Kratky & Landmann 2017). These changes are expected to persist and intensify as climate change continues (Devictor et al 2012, Pauli et al 2012).

The impacts of temperature on biodiversity have been a topic of discussion for centuries (von Humboldt & Bonpland 1807), but became a central concern in the context of ongoing global warming (Lenoir et al 2020). Temperature is the predominant environmental driver considered in climate change studies (Ganuza et al 2022, Kerner et al 2023, Redlich et al 2022, Román-Palacios & Wiens 2020). However, the effects of climate change in temperate regions are complex, with local extinctions (Harris et al 2019, Holzmann et al 2023, Martay et al 2017, Wiens 2016) alongside instances of increases in diversity and biomass (Maihoff et al 2023, Marta et al 2021, Müller et al 2023, Thorn et al 2022). Thereby, several authors emphasize effects of mean temperature (Marta et al 2021, Martay et al 2017, Uhl et al 2022), weather anomalies and extreme weather events (Forister et al 2018, Müller et al 2023, Sutton et al 2018), or temperature extremes (Román-Palacios & Wiens 2020). In conclusion, it is evident that climate change is causing a worldwide restructuring, replacement, and reassembly of biotic communities (Antão et al 2022, Habel et al 2016, Halsch et al 2021, Hill et al 2021), while the scale-dependency and multifaceted nature of climate change present challenges in fully understanding it's impacts on species and ecosystems. Since recent distribution patterns result from a mixture of past and current processes

(Moreau 1966), the underlying evolutionary processes that generate and maintain those biodiversity patterns should also be considered as major research questions (Erwin et al 1997).

1.2 *Temperature as driver of biodiversity patterns*

Temperature influences various levels of biodiversity, ranging from cellular processes to organisms to ecosystems to biomes (Bellard et al 2012). It has been acknowledged as a pivotal parameter determining metabolic processes, community assembly, diversity patterns, and ecosystem dynamics for a long time (Mayor et al 2017). With increasing mean temperature, diversity is observed to increase along latitudinal or elevational gradients (Peters et al 2016a). The metabolic theory of ecology provides a framework to explain this pattern, suggesting links between the metabolic rate of an organism, temperature, and mass. Higher temperatures are postulated to accelerate the evolutionary diversification rate, resulting in a larger pool of warm-adapted species compared to cold-adapted ones (Brown 2014). Further, higher temperatures enhance the speed of biotic processes such as the metabolic and growth rates, reproduction, and the activity of organisms (Belmaker & Jetz 2015, Brown 2014, Frazier et al 2006, Savage et al 2004). This acceleration enables the formation of populations with a higher number of individuals, thereby reducing the risks of local extinction risks (O'Grady et al 2004).

Studies on species and systemic responses to warming temperatures are gaining increasing interest and urgency in the light of recent climatic developments. Despite latest efforts on national and international level to implement and harmonize standardized biodiversity monitoring schemes, first reliable results will only become available in the medium term, demanding novel approaches, as the availability of detailed long-term data is often limited. The few existing historic monitoring data are frequently taxonomically biased (Halsch et al 2021), and geographically restricted (Wagner et al 2021). Re-surveys of sites previously probed offer another promising tool to infer population trends, but these assessments are highly sensitive to weather events around the sampling years (Müller et al 2023), given the high interannual fluctuations of insect population sizes, and hence require cautious interpretation. Similarly, comparisons between historical data (e.g., museum collections) (Duchenne et al 2020) and new surveys can provide valuable insights into ecosystem processes under climate change. However, standardised baseline assessments are necessary for such comparisons, but missing for many regions and taxonomical groups. An alternative method, complementary to long-term studies, are space-for-time substitution approaches, which utilize geographical gradients of temperature at specific moments in time as surrogates to derive predictions for colder sites from patterns found at warmer sites (Ganuza et al 2022, Mayor et al 2017, Redlich et al 2022). This method provides a valuable perspective in the absence of existing long-term data. However, implementing such designs poses challenges due to the geographical spread of sites to cover a sufficiently broad temperature gradient, and confounding factors and extinction debts may influence inference (Oliver & Morecroft 2014). To mitigate the organizational challenges of such space-for-time designs, efforts can be streamlined by selecting geographic gradients in areas where temperature variation occurs within short distances. Mountain areas, which are formed by orogenetic processes, are particularly suitable for such space-for-time designs (Classen et al 2015, Hoiss et al 2013, Hoiss et al 2012, Leingärtner et al 2014a, Peters et al 2016a).

1.3 *Mountains as surrogate to study temperature effects*

Along mountain slopes, air temperature is the variable that varies most accurately, steadily, and predictably with a lapse rate of about 0.55 K per 100 m increase in elevation (Körner 2007). While multiple abiotic variables such as wind speed, precipitation, and air pressure change with elevation, temperature is anticipated as the paramount driver behind species turnover (McCain & Grytnes 2010, Rahbek 1995). Given that temperature determines insect performance and distribution, biodiversity and specialisation patterns observed at low elevations would be predicted to shift to higher elevations if global warming continues. Therefore, space-for-time surrogates in mountain systems provide a valuable means to explore community assembly processes and anticipate species' responses to climate warming (Körner 2007, Rahbek et al 2019). Such elevational climatic gradients in mountains are major drivers of species richness, community composition, assemblage structure (Gaston 2000, McCain & Grytnes 2010, Peters et al 2019, Rahbek 1995, Sanders & Rahbek 2012), and are further influenced by biotic interactions that undergo rewiring with changes in elevation (Tylianakis & Morris 2017). Indeed, diversity gradients towards summits paralleling temperature reductions were already recognized through the progressive work of Charles Darwin and Alexander von Humboldt, who found consistent patterns in mountain ranges worldwide, mirroring global biodiversity clines observed from lower to higher latitudes, leading to the development of a conceptual model of elevational zonation (Fig. 1A) (Lomolino 2001, Lomolino et al 2017, von Humboldt & Bonpland 1807). Not only does species richness exhibit a decrease with elevation (Descombes et al 2017a, Gaston 2000, McCain & Grytnes 2010, Sanders & Rahbek 2012, Wettstein & Schmid 1999), but abundance also often follows a similar pattern (Peters et al 2019, Pitteloud et al 2020). The consistent monotonic decline of air temperature with elevation poses a significant constraint on species distributions along elevational gradients (Peters et al 2016a), since climatic harshness and its temporal variation are considered major stressors that cause the impoverishment of fauna and flora towards mountain summits (Buckley & Huey 2016, Hoiss et al 2012, Körner 2003, Laiolo et al 2023, Laiolo et al 2018, McCain & Grytnes 2010, Sierra-Almeida et al 2009). Alternatively, the reduction of land area (species–area relationship) and increased isolation (island biogeography theory) may also contribute to this pattern observed by fuelling stochastic processes of species extinction with low rates of immigration (Bertuzzo et al 2016, Jiménez-Alfaro et al 2021, Laiolo et al 2023, Peters et al 2016a). However, empirical elevational diversity patterns are variable, and thus alternative hypothesis explain mid-elevation-peaks by considering the random distribution of species ranges within a geographically restricted area (mid-domain effect) (Colwell et al 2004).

Another factor that makes mountain systems attractive to study species responses to climate change is their predisposition to climate warming, as high-elevation regions have been shown to heat up faster than low elevation regions (Nigrelli & Chiarle 2021, Pepin et al 2015). For instance, average temperatures in the European Alps increased by 1.5 K compared to the global average increase of 0.7 K over the last century (Beniston et al 1997, Nogués-Bravo et al 2007). Further, a warming of 1.5 K is expected within the first half of the 21st century (Gobiet et al 2014). At the same time, mountains are of exeptional importance for biodiversity as the serve as climatic refugia for cold-adapted species (Berger et al 2010, Laiolo et al 2018, Trew & Maclean 2021). Due to the high diversity of climatic conditions, natural dynamics, and often low land-use intensity, these regions harbour an extraordinarily high biodiversity, serving as hotspots for various groups such as amphibians, birds, or mammals (Rahbek et al 2019). Furthermore, ice-free mountain regions acted as refugia during glacial extremes and as nuclei for the recolonization of surrounding regions within the cycles of warming and cooling periods in temperate regions (Laiolo et al 2018, Nogués-Bravo et al 2016). Consequently, these regions also harbour a high proportion of small-range endemics due to climatic fluctuations and recurring separation of populations, contributing to local diversity patterns (speciation hypothesis) (Jiménez-Alfaro et al 2014, Laiolo et al 2023, Laiolo et al 2018, Lomolino et al 2017). The elevational climatic variation in mountains allows species to shift according to their specific climatic requirements, and small streams provide humid areas even in drought periods, facilitating the survival of moisture-dependent species (Fjeldsaå & Lovett 1997, Scherrer et al 2011). Despite the expected sensitivity of mountain biodiversity to climate warming (Albrich et al 2020, Nogués-Bravo et al 2007), we lack a detailed understanding of the vulnerability of these unique ecosystems to recent climatic change (Trew & Maclean 2021).

1.4 *The role of microclimatic variation in mountains*

Climatologists make predictions about the local manifestations of climate warming at a coarse spatial resolution using a network of exposed weather stations installed at a height of 2 m above a flat surface (Lembrechts 2023, World Meteorological Organization 2008). Thus, ecological studies often rely on interpolated, gridded temperature maps such as provided by ERA5-Land (Muñoz-Sabater et al 2021) or WorldClim (Fick & Hijmans 2017). However, macroclimatic variations explain only part of the temperature conditions insects experience, since these are mainly determined by local conditions – the meso- and microclimate (Kankaanpää et al 2021, Ohler et al 2020, Scherrer et al 2011), and it is not well characterized how these conditions will change with ongoing climate warming (Pincebourde & Woods 2020). Indeed, many species experience temperature regimes which differ strongly from the macroclimate predicted (Potter et al 2013). While temperature is buffered in dense vegetation due to shading and transpiration (Geiger et al 2003), animals dwelling on the soil surface or exposed leaves need to cope with temperature maxima up to 20 K higher than the surrounding air temperature (Miller et al 2021, Pincebourde & Suppo 2016, Vandewiele et al 2023). Variation at this scale is therefore crucial for many organisms, as the suitable range of temperatures experienced could be exceeded. Including microclimate data was already demonstrated to improve abundance predictions of insect pests (Rebaudo et al 2016). Due to the buffering capacity of microclimate (Bennie et al 2013, Senf & Seidl 2018, Stark & Fridley 2022), species' responses to climate warming may be overestimated in case predictions fail to account for microclimatic conditions (Scherrer et al 2011).

In the context of microclimate, mountains are extraordinary valuable. Despite facing elevation-dependent above-global-average temperature increases (Pepin et al 2015), mountains provide numerous meso- and microclimatic site conditions in geographic proximity (Fig. 1B). These differences result from variations in aspect/exposition/orientation and slope (summarized as topographic heterogeneity), radiation, wind speed, substrate, and vegetation structure at the meso- and microscale (Fig. 1C) (Albrich et al 2020, Gril et al 2023, Körner & Hiltbrunner 2021, Ohler et al 2020, Rita et al 2021, Scherrer & Körner 2011). Average soil temperatures at north- and south-facing slopes at similar elevations can differ by amplitudes comparable to 500 m elevational difference (Fig. 1D) (Ohler et al 2020, Scherrer et al 2011), reflecting effects of the duration of direct insolation, the incidence-angle-dependent incoming radiation dose, and self-shading (Scherrer & Körner 2011, Scherrer et al 2011).

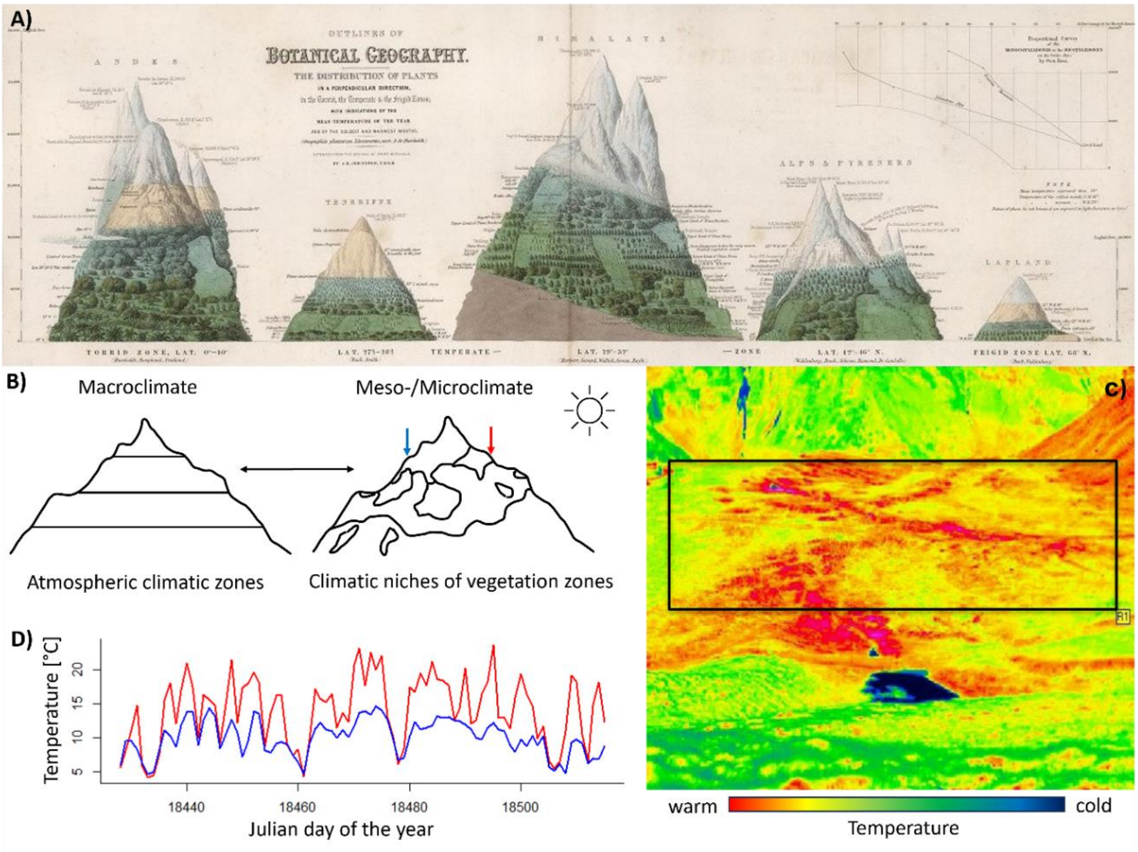


Figure 1. Variation in microclimatic conditions. A) Clinal zonation of plants along elevation (Johnston, 'The physical atlas of natural phenomena', 1980, image downloaded from Wikimedia commons public domain collection). B) Macroclimate-dependent layering of biomes based on atmospheric temperature clines along elevation (left) compared to climatic zonation based on topographic and exposure differences along elevation (right). Arrows indicate positions of study sites at the same elevation. C) Thermal image showing microclimatic heterogeneity of surface temperatures, red represents warm temperatures and blue represents colder temperatures. The image was modified from Ohler et al (2020), Figure 1. D) Daily average soil-level temperatures of two sites at the same elevation, measured with three temperature loggers each during summer 2020. The blue line corresponds to a shaded, north-exposed site, while the red line represents mean temperatures measured at a south-exposed study site.

This microclimatic heterogeneity enables the survival of a high number of species with distinct climatic niches, translating into high β-diversity (Fontana et al 2020, Sponsler et al 2022, Tello et al 2015, Zografou et al 2017). Within such a mosaic of tessellated climatic conditions, species can match their climatic requirements by macroclimate-dependent use of specific microclimates (relative niche-consistency, Dobrowski 2011, Feldmeier et al 2020). While microclimate temperature offsets (i.e., difference to nearby open habitats) have already been characterized for forests in the study region (Fig. 2) (Vandewiele et al 2023), data on microclimate temperature deviations close to the soil from the atmospheric macroclimatic zonation along the elevational gradient are lacking in open habitats.

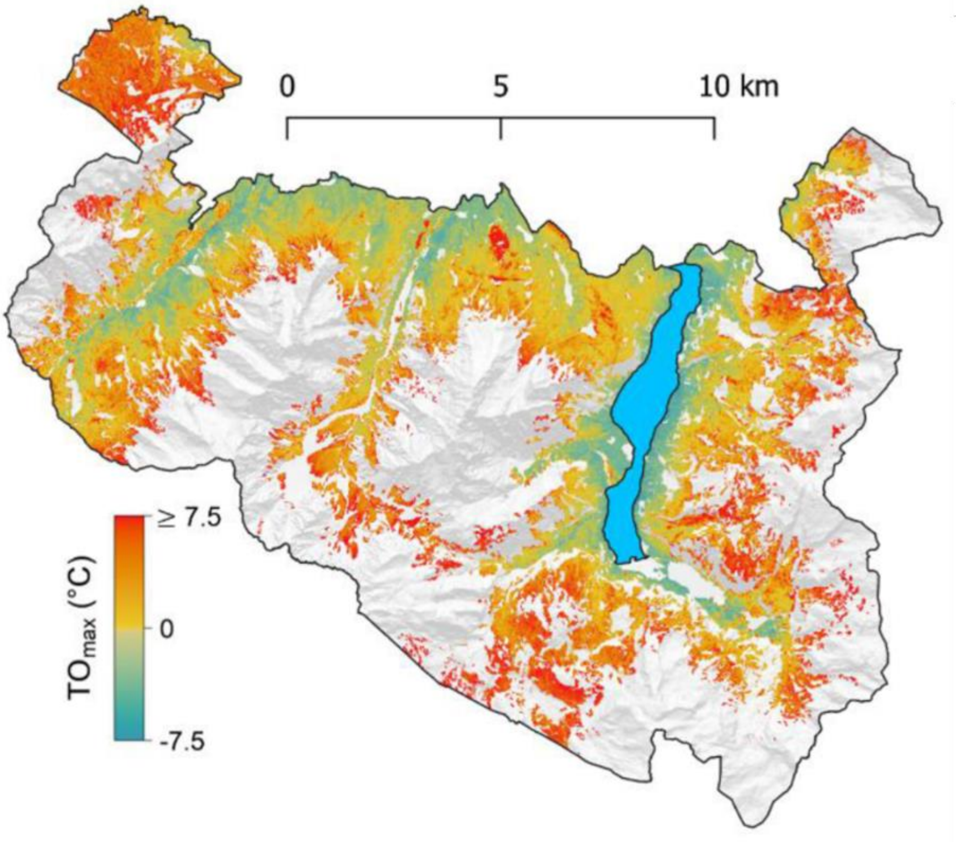


Figure 2. Geographic variation in microclimate temperature offsets in forests of the study region Berchtesgaden National Park. Modelled offset of the temperature maximum in summer based on 150 microclimate loggers (TMS-4, TOMST). The figure was modified from Vandewiele et al (2023), Figure 3.

Locations where certain species are found thus differ markedly in the local climatic site conditions along elevational gradients. Generalist species that tolerate a broad range of environmental conditions are found under a vast range of macroclimate, regardless of the specific microclimate at the sites (Fig. 2A+B). These are therefore considered temperature-indifferent species. However, for most species, climatic niches are more clearly defined. They can be narrow and primarily shaped by microclimatic conditions at the site (stenothermic species). For example, a thermophilic species will only occur, when the microclimate is favourable (Fig. 2D+F), provided the appropriate scale to measure microclimate was identified for this species (Fig. 2C). A thermophobic species, on the other hand, will only occur at sites with cold microclimatic conditions when macroclimate is warm, e.g., at shaded sites of low elevations. However, under unfavourably cold macroclimatic conditions, such species could make use of warm microclimatic conditions, enabling their persistence along a broader range than macroclimatic conditions would suggest. In this case, the preference for cold microclimate would vanish or even change to a positive impact of warm microclimate, depending on the macroclimate (Fig. 2F). Therefore, macro- and microclimate might interact to form species niches. Such phenomena are known from edges of distributional ranges of species, where we often observe a higher specificity for certain climatic conditions than at the centres (Antão et al 2022, Deutsch et al 2008, Sunday et al 2012). Examples for such macro-microclimate interactions include species common in Northern Europe being restricted to mountains and cold habitats in the south, species common in the Mediterranean being restricted to dry, warm habitats in northern regions, or species common in meadows in regions with high rainfall being restricted to wetlands elsewhere.

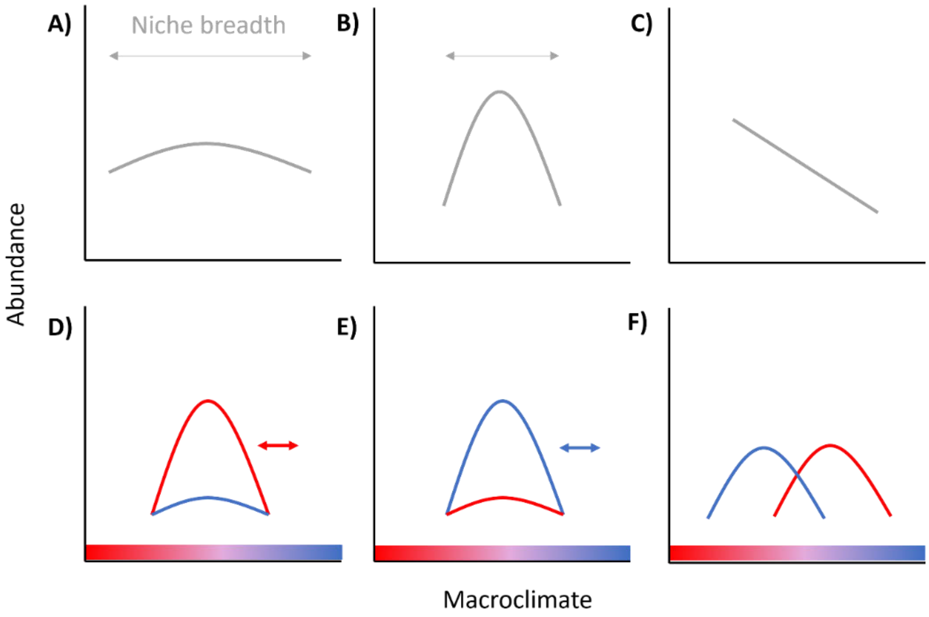


Figure 2. Possible scenarios how species' microclimatic niches are shaped along macroclimatic temperature gradients. A) A species occupies a broad range of macroclimatic conditions independent of microclimate. B) A species occupies a small range of macroclimatic conditions independent of microclimate. C) A monotonic relationship of abundance and macroclimate is detected since the species is not close to its thermal limits across the studied gradient. D) A species prefers warm microclimatic conditions along its entire macroclimatic range, E) a species prefers cold microclimatic conditions along its entire macroclimatic range, or F) a species preference for warm or cold microclimate depends on the macroclimate.

In mountainous terrain, species can track their climate preferences by making use of the heterogeneous topography, e.g., moving upslope or from hotter sun-facing slopes to cooler sites (Scherrer & Körner 2011). Previous research has documented such range shifts along mountain slopes for plants (Bertrand et al 2011, Elmendorf et al 2015), but see (Bässler et al 2013, Kerner et al 2023), birds (Freeman et al 2018, Princé & Zuckerberg 2015), and selected invertebrate taxa (Cerrato et al 2019, Kerner et al 2023, Maihoff et al 2023, Zografou et al 2014). While the role of microclimate in shaping responses to climate change has been included for plants in several studies, evidence for microclimate effects on insect distributions along macroclimatic gradients is still scarce. Understanding synergistic and interactive effects of macro- and microclimate is crucial for evaluating risks and opportunities for biodiversity amidst global environmental changes and can help to improve predictions of climate-induced range shifts. However, studies on how the interplay of both facets of climate (macro vs. micro) shapes biological communities in mountain regions are lacking.

1.5 *Trait analysis for a mechanistic understanding of community responses*

Charismatic orders such as Lepidoptera, bees, and syrphids have been in the focus of numerous studies on climate change effects on insects (Duchenne et al 2020, Halsch et al 2021, Sunde et al 2023). Typical examples include climate-induced temporal mismatches which can lead to the disruption of trophic relationships at the community level (Hill et al 2021). An illustrative case involves the disruption of synchrony between oak bud burst and the emergence of an associated herbivore caterpillar during warmer springs (Visser & Holleman 2001). This temporal mismatch causes fitness losses for the moth but also decreasing predation due to misaligned timing of bird feeding and caterpillar occurrence (Asch & Visser 2007, Visser et al 2006). Studies have revealed instances of earlier adult appearance in response to climate warming for Orthoptera in various locations, including Austria, Greece, and the US (Nufio & Buckley 2019, Zografou et al 2015, Zuna-Kratky & Landmann 2017). In mountain regions, rapid range shifts to higher elevations as a response to temperature increase have been shown for insect communities (Bässler et al 2013, Kerner et al 2023, Lenoir et al 2020, Maihoff et al 2023, Ogan et al 2022). However, it is crucial to recognize that species responses to rising temperatures are often dependent on the taxon studied (Bässler et al 2013, Ganuza et al 2022, Lenoir et al 2020, Peters et al 2016a) and species-specific (Diamond et al 2011, Engelhardt et al 2022, Hickling et al 2006, Neff et al 2022, Poniatowski et al 2020). While a preference for high temperature was found to be a reliable predictor for abundance increases and range expansions (Bowler et al 2017, Engelhardt et al 2022, Neff et al 2022, Poniatowski et al 2020, Thorn et al 2022), several studies highlight that species occurrence losses have been most severe for narrow-ranged, specialised, and cold-adapted species over the past three to four decades (Engelhardt et al 2022, Neff et al 2022).

If climate determines the composition of communities, filtering leads to assemblages of species with similar traits. Using ecological and morphological traits of species when studying temperature-driven assembly processes may therefore help to understand which species are especially threatened or which may profit from increasing temperatures, as they determine extinction risks (Chichorro et al 2022) and distribution patterns of species along climatic gradients (Chichorro et al 2022, Classen et al 2017, Hoiss et al 2012, Leingärtner et al 2014b, Peters et al 2016a). Identifying traits that confer an adaptive advantage under specific climatic conditions is therefore crucial to assess the sensitivity of populations and assemblages to the impacts of climate change (Diamond et al 2011). Hence, changes in trait composition can be interpreted as an early warning system (Mouillot et al 2013).

In cold habitats, the brevity of growing seasons imposes constraints on completing life cycles. This circumstance necessitates thermoregulatory and developmental adaptations (e.g., faster-heating colour phenotypes, Dieker et al 2018, Fernandez et al 2023, Harris et al 2013, Köhler & Schielzeth 2020). Responsive traits to temperature variation include body size (Berner et al 2004, Levy & Nufio 2015, Tiede et al 2018), phenology (Ingrisch & Köhler 1998, Kankaanpää et al 2021), wing length (Hodkinson 2005, Laiolo et al 2023, Leihy & Chown 2020, Tiede et al 2018), and specialisation (Engelhardt et al 2022, König et al 2022a, Neff et al 2022, Pitteloud et al 2021, Rasmann et al 2014). These traits not only influence the thermal performance (Peters et al 2016b), but also the dispersal potential and therefore the capability for shifting ranges (Stevens et al 2014). Temperature preferences are probably the most apparent trait to change with temperature variation and are highly informative to reveal patterns in temporal studies (Engelhardt et al 2022, Löffler et al 2019, Poniatowski et al 2020, Thorn et al 2022). However, many temperature-preference measures available were derived from species distributions, reflecting elevational distributions, and offering limited additional information when studying trait clines along climatic gradients without a temporal component.

Evidence for temperature filtering processes has been traditionally demonstrated by clines of mean trait values without considering intraspecific variability, assuming that the difference in trait values is larger between species than within species (Jung et al 2010). However, increasing numbers of studies emphasize that intraspecific variability, stemming from heritable adaptation or plasticity (Violle et al 2012), can hint on underlying mechanisms (Classen et al 2017, Jung et al 2010, Laiolo et al 2023, Tiede et al 2018).

1.6 *Trophic interactions and temperature: The 'altitudinal niche-breadth hypothesis'*

Moreover, species do not exist as isolated entities within a community, but interact with many others, forming ecological networks that ultimately shape the ecosystem dynamics (Proulx et al 2005). Thus, not only biodiversity, but also species interactions are fundamental for the stability of ecosystems (Bascompte et al 2006, Tylianakis et al 2008). As interaction strengths and the functional traits of species that mediate interactions tend to vary along elevational gradients, the resulting turnover in the communities also alters co-occurrence patterns of interaction partners and therefore the realization and manifestation of interactions between species across space (Dormann et al 2017, Pellissier et al 2018, Tylianakis & Morris 2017, Welti & Joern 2015). When interacting species shift their elevational ranges in response to climatic changes with different velocities, spatial (Kerner et al 2023, Schweiger et al 2008) and temporal mismatches can occur

(Kharouba et al 2018), which is critical in case of close dependencies between interaction partners (Geppert et al 2021). For instance, studies reported that range shifts of mobile insects outpace expectations (Bässler et al 2013) and surpass those of plants, which often lag behind anticipated shifts based on temporal changes of average temperatures (Alexander et al 2018, Bässler et al 2013, Lenoir et al 2010). Such a turnover of interaction partners may result in interaction turnover, rewiring, loss of interactions, or novel interactions (Descombes et al 2020, Pellissier et al 2018). Additionally, the strength of interspecific interactions may be influenced by temperature clines (Pellissier et al 2018). A comprehensive understanding of how species interactions are influenced by climate warming is crucial for deciphering the impacts on community and ecosystem-level processes (Hill et al 2021, van der Putten et al 2004). Climatic gradients provide a valuable framework for assessing changes in interaction networks between herbivores and plants (Pellissier et al 2018). Linking specialisation patterns to temperature variation may thus facilitate the prediction of foodweb stability under climate change. Incorporating these intricate interactions is necessary to predict reactions of communities to climate change (Abrego et al 2021).

Along climatic gradients, interspecific interactions are often observed to be less specialized in regions with cold temperature regimes (Classen et al 2020, Forister et al 2015, Hoiss et al 2015, Pellissier et al 2018, Pellissier et al 2012, Pitteloud et al 2021). Explanations for a higher generality of insect herbivore consumers in cold environments are complex. One hypothesis for increasing dietary niche breadths of consumers with decreasing temperature transfers potential mechanisms from the ‘latitude niche-breadth hypothesis’ to elevational gradients. Integrating biotic and abiotic processes, the ‘altitude niche-breadth hypothesis’ predicts trends towards polyphagy of species which inhabit high elevations compared the ones from low elevations based on differences in the variability of temperature, precipitation, and wind speed (Rasmann et al 2014). These conditions are expected to trigger generalism based on different mechanisms: Colder temperatures could reduce search and digestive efficiency in ectotherms (Hodkinson 2005), necessitating the evolution of broader diets in habitats characterized by high environmental unpredictability (Macarthur & Levins 1967, Pitteloud et al 2021) and lower resource availability (Tylianakis & Morris 2017). There, variation in population sizes is expected to reinforce generalisation (Rasmann et al 2014). Alternatively, potential competitive pressure for resources is released in cold environments, as herbivore densities decline with temperature. Therefore, the need to maintain specialized interactions to escape competition might be relaxed at high elevations (Hodkinson 2005, Macarthur & Levins 1967, Rasmann et al 2014). Furthermore, there is (ambiguous) evidence for reduced plant defence mechanisms in high elevations attributable to abiotic processes and decreases in herbivory (Descombes et al 2017a, Pellissier et al 2012, Rasmann et al 2014). Such increases in plant palatability may favour broader resource use of herbivores in high elevations (Descombes et al 2017a, Pellissier et al 2012, Rasmann et al 2014). In contrast, traits of plants associated with physical resistance were observed to intensify in harsh high-elevation zones, which can also shape interactions (Descombes et al 2017a, Körner 1989).

However, empirical network studies on plant - insect-herbivore relationships are scare (but see Forister et al 2015, Pellissier et al 2012, Pitteloud et al 2021, Welti et al 2019), because the assessment of interactions is challenging and ecological mechanisms of multiple origins are entangled (Dormann et al 2017). At the same time, most studies published use taxonomic identity to calculate specialisation metrics along environmental gradients (Classen et al 2020, Pitteloud et al 2021). However, the dietary niche breadth is not only determined by the arithmetic number of resources, but also by characteristics of the resources. Traits such as toughness, specific leave area, nutritional composition, and defence mechanisms are often shared by phylogenetically related taxa (Goberna & Verdú 2016, Swenson 2014, Swenson & Enquist 2007). Thus, phylogeny-informed analyses could help to estimate the dietary niche breadths of herbivores more realistically by integrating costs and nutritional rewards of resource use from a consumer perspective (Jorge et al 2017, Pardo-De la Hoz et al 2022, Rasmann & Agrawal 2011). Despite its importance for shaping species interactions, the role of abundance-based processes in driving interaction networks is furthermore often overlooked (Canard et al 2014, Carnicer et al 2009, Vázquez et al 2007). The paucity of studies which control for the availability and phylogenetic relationships of resources (but see Novotny et al 2006) and the difficulties to record interactions at larger spatial scales are reasons why underlying processes are little understood (Pringle & Hutchinson 2020). While dietary specialisation patterns were already recorded for wild bees, and some herbivorous insects with a high number of specialized taxa, such as Jewel Beetles Buprestidae, (Rasmann et al 2014) and caterpillars (Forister et al 2015, Novotny & Basset 2005, Pellissier et al 2012), patterns might deviate if a herbivore taxon with broad environmental niches and a higher proportion of generalist species is assessed. Combining these traits, Orthoptera can serve as a study system to assess the effect of temperature on feeding interactions.

1.7 *Orthoptera assemblages as a study system*

Insect herbivores are extraordinarily diverse, with around half a million described species (Hardy et al 2020), and play essential systemic roles: Their impact extends beyond direct plant consumption; they induce plant defence mechanisms (Fuchs et al 2017), and thereby alter dominance patterns in plant assemblages, fostering plant coexistence (Bagchi et al 2014, Descombes et al 2020). Moreover, herbivores constitute vital links in ecosystems by transforming nutrients stored in plant tissues into forms that are digestible for higher trophic levels (Leingärtner et al 2014a), thereby affecting both, above- and belowground decomposition and soil enzyme activity (Prather et al 2017). In temperate mountain grassland habitats, Orthoptera (Caelifera &

Ensifera) are among the most numerous insect herbivores. 29,000 species have been described globally so far (Cigliano et al 2023), from which around 160 occur in Central Europe, from which a share of 50 are inhabiting the study area. Since they first appeared during the Permian age (300 - 250 Mio. years ago) (Ingrisch & Köhler 1998, Song et al 2015, Song et al 2020, Zuna-Kratky & Landmann 2017), these insects have significantly been affecting the ecosystem. They chew on phanerogam plant parts (leaves, flowers, or even dead parts), removing up to 30% of the biomass and, therefore, influencing nutrient cycling by concentrating nitrogen in faecal pellets and bodies (Blumer & Diemer 1996). Often, they exhibit tissue-specific feeding behaviour, consuming relatively nutrient-rich parts of the leaf blade and leaving low-nutrient biomass behind (Zhang et al 2011). At times, this biomass drops to the ground during the feeding processes without being consumed by the grasshopper (Ingrisch & Köhler 1998, Zhang et al 2011). These feeding habits are, in part, dependent on the evolutionary history of the insects. While many species adopt a strategy of consuming plant species from more than one family to enhance fitness, categorizing them as generalists (Bernays & Bright 2005, Bernays et al 1994, Berner et al 2005, Franzke et al 2010, Joern 1979, Unsicker et al 2008), some lineages exhibit certain shared feeding behaviours: Gomphocerinae (Grasshoppers) often select species from the family Poaceae (grasses), while some Oedipodinae (Band-winged Grasshoppers) preferably feed on either forbs (dicotyledonous plants) or grasses and other members of the Oedipodinae and Melanoplinae display high levels of generalism, feeding on many different forb and grass species simultaneously (Ingrisch & Köhler 1998, Joern 1979). Groundhoppers (Tetrigidae), an evolutionary old lineage originating from the Triassic period (approximately 250 Mio. years ago) (Song et al 2015), consume the ancient groups algae, lichen, and mosses in high quantities (Hochkirch et al 2000). These behaviours may be based on co-evolutionary constraints, where mandible morphology is adapted to structural properties of the resource plant (Franzke et al 2010, Ibanez et al 2013a, McClenaghan et al 2015, Stockey et al 2022). In the suborder Ensifera, omnivorous lifestyles have developed. With a few exceptions, most of the species which occur in Central Europe feed on grass seeds, flowers of grasses and forbs, as well as soft leaves and other soft plant tissues (Ingrisch & Köhler 1998). Additionally, some Ensifera species prey on smaller insects and, in some cases, even Caelifera. Especially the larger ones regularly consume arthropods in high quantities. The only solely carnivorous taxa in Central Europe are oak crickets and the Saginae (Ingrisch & Köhler 1998). Furthermore, Orthoptera are critical elements in food webs, as they serve as important food source for various animals at higher trophic levels, including birds, spiders, wasps, flies, horsehair worms, reptiles, and mammals (Ingrisch & Köhler 1998).

Orthoptera inhabit the majority of terrestrial ecosystems from the arctic and alpine tundra to tropical forests and subtropical deserts. However, most of the species are concentrated in tropical and subtropical regions, indicating a prevalent thermal demand within this insect order. This pattern is also evident in Europe, where out of the 1,082 Orthoptera species recorded, 160 are found in Central Europe. Southern European countries are inhabited by up to 400 species, while Great Britain and Scandinavia only host 30 and 50 species, respectively (Hochkirch & Nieto 2016). In temperate regions, Orthoptera are therefore strongly constrained by cold climatic conditions and often thermophilic, requiring warm temperatures for the completion of their development and reproductive life cycles (Geppert et al 2021, Ingrisch & Köhler 1998, Schmitz et al 2016, Willott 1997, Willott & Hassall 1998). Thus, climatic conditions determine the distribution and abundances of Orthoptera species in Central Europe like in many other insects, leading to a decline in diversity towards higher elevations and northern latitudes, or in general regions with shorter summer seasons (Alexander & Hilliard 1969, Geppert et al 2021, Hochkirch & Nieto 2016, Zuna-Kratky & Landmann 2017). Orthoptera are anticipated to be particularly sensitive to temperature changes and hence represent a suitable model group to study the impact of climate warming. Although studies reported correlations between climate and grasshopper abundance (Edwards 1960), macroclimate alone cannot fully explain patterns at the local scale. Larger-scale effects of climate are modified within the boundary layer near to the ground (Geiger et al 2003), where plants intercept radiation, transpire, and affect air movements (Willott 1997). And Orthoptera occupy this boundary layer. They are thus also demanding in terms of specific microclimatic requirements on their habitat (many species are thermophilic in Central Europe) (Gardiner & Dover 2008), highlighting their suitability as indicators for environmental changes (Bazelet & Samways 2011, Fartmann et al 2012). Habitat quality metrics such as vegetation structure, which also influence the microclimate, have been shown to be important determinants of abundance and species richness (Fonderflick et al 2014, Fumy et al 2023, Löffler & Fartmann 2017, Marini et al 2009, Schirmel et al 2019, Schirmel et al 2011). Given that this taxon is regularly used in ecological and conservation assessments on national and international level, monitoring data are - in parts and albeit with limitations - available. This combination leads to the fact that already several studies based on monitoring or observational data (Engelhardt et al 2022, Neff et al 2022, Poniatowski et al 2020), and re-surveys (Fumy et al 2020, Löffler et al 2019, Ogan et al 2022, Thorn et al 2022) could highlight recent changes in the composition and distribution of species, which could be linked to climatic changes. Since comparing abundances between different assessments can be challenging and is prone to observer or methodological bias, most studies work with presence-absence data, weakening the detectability of assembly processes.

Central European species are well characterized, with numerous ecological and morphological traits available, summarized by Ingrisch & Köhler (1998). Body sizes vary considerably from the tiny Ant Crickets (4.5 mm) to the huge Predatory Bush-crickets of the genus *Saga* (>80.0 mm) (Harz 1969, Harz 1975). Furthermore, the green-brown colour polymorphism is widespread among Orthoptera taxa and represents a suitable study system to examine the effects of climatic gradients on colour patterns (Dieker et al 2018, Köhler et al 2017, Varma et al 2023). The impact of these numerous herbivorous insects on plant communities has prompted several studies to investigate dietary preferences (Williams 1954). Some of them employed laboratory settings or cafeteria experiments to determine the plants consumed (Ibanez et al 2013b, Kaufmann 1965, Unsicker et al 2008). However, under natural conditions, these preferences might change based on climatic impacts or differences in the availability of resources. As observing trophic interactions can be challenging since grasshoppers are not feeding most of the time (Illich & Winding 1989), alternative methods were used to assess natural dietary habits. One method involves dissecting the animals and visually analyse the crop and gut contents under a microscope (Capello et al 2011, Gandar 1982, Green 1987, Hyslop 1980), a time-consuming approach. Researchers made use of different microscopic structures that can be used to determine plants in some cases reaching the species-level, or employed stable-isotope ratios and fatty acid profiles to determine prey items at coarse resolution (Traugott et al 2013). Nevertheless, comprehensive datasets on dietary spectra of many species under natural conditions are lacking due to methodological constraints which have only recently been solved by the application of molecular techniques (Roslin et al 2019, Vacher et al 2016).

Since the advent of amplicon sequencing (DNA-metabarcoding) paved the ways to efficiently identify DNA contents from environmental samples in a cost-and resource-saving manner (Deagle et al 2007), a few research facilities have started to adapt protocols to extract DNA from gut or faecal samples of Orthoptera and identify the plants consumed. A positive side effect of using faecal samples to assess dietary spectra is that the animals can be released after collecting the tissue, thereby omitting negative impacts on population sizes – a crucial consideration particularly for threatened species with small populations (Fig. 3). As a result, this approach has already revealed differentiation of feeding niches within meadows (Ibanez et al 2013b, McClenaghan et al 2015) or morphological trait matching (Deraison et al 2015, Ibanez et al 2013a). However, comprehensive data on resource use along broad ecological gradients is still scarce (but see Pitteloud et al 2021, Senzaki et al 2023, Welti & Joern 2015), but will help to identify specialisation patterns that hint on environmental filtering or assembly processes. Additionally, such data provide valuable information on the resource spectrum of certain species, hinting on important vegetation elements within grasshoppers' habitats, which could be relevant from either an economical or a conservation perspective.

Figure 3. Female of Eisentraut's Bow-winged Grasshopper producing faeces. *Chorthippus eisentrauti* is a rare, regionally restricted grasshopper species only recently documented in Germany. This female was feeding on the Mountain Smallreed (*Calamagrostis varia*). Faecal pellets are often kicked away from the tip of the abdomen by the grasshopper after excretion. Photo credit: Sebastian König.

1.8 *Microbes and their role for Orthoptera*

Microbial symbionts have enabled organisms across the tree of life to access otherwise unobtainable nutrients and outsource crucial functions such as immune defence (Cornwallis et al 2023). The insects' microbiome is hence acknowledged as a 'hidden' player shaping insect traits, including feeding niches (Cornwallis et al 2023, Paniagua Voirol et al 2018). Thus, associations between the highly diverse groups of insect herbivores and bacteria are common (Engel & Moran 2013). On the one hand, beneficial microbes support the insect with digestion, nutrient absorption, detoxification, or immunity (Buchon et al 2013, Dillon & Charnley 2002, Engel & Moran 2013, Idowu et al 2009, Kaufman & Klug 1991, Zheng et al 2021). On the other hand, the intestine represents a nutrient-rich niche a high number of microbes utilizes (Hirata et al 2023). Despite their ecological and economical significance, the microbiome of orthopterans is poorly understood (Muratore et al 2020). Although insects eventually profit from the symbionts, these relationships are often not fixed. For instance, caterpillar guts are frequently dominated by leaf-associated bacteria, suggesting that in some groups, resident, host-specific symbionts are sparse or absent (Hammer et al 2017, Hird et al 2014, Zhou et al 2022). This would correspond to a neutral assembly of gut bacteria (Deb et al 2019), where bacterial frequencies from the feeding environment are reflected in the samples obtained from insects.

First studies on the microbiome of orthopterans, which were often restricted to some species of migratory locusts (Dillon & Charnley 2002, Dillon & Charnley 1995, Dillon et al 2005, Dillon et al 2008) and crickets (Engel & Moran 2013, Smith et al 2017), highlight potential contribution to the synthesis of an aggregation pheromone, compartmentalisation, and repeatedly report some shared groups of microorganisms, which often comprise Enterobacteriaceae cells. These were shown to produce extracellular digestive enzymes (Idowu et al 2009). Studies in natural environments reveal similar patterns, while the relative abundances of certain bacteria such as members of the genus *Pantoea* or *Pseudomonas* were correlated with differences in cellulose digestibility (Ling et al 2022). Furthermore, insects feeding behaviour was observed to influence the bacteria composition and diversity (Muratore et al 2020).

Gut symbionts differ in their thermal niches, including temperature optima, temperature sensitivity, and resistance to thermal stress (Hammer et al 2021, Moghadam et al 2018, Palmer-Young et al 2018). If insects are dependent on mutualist bacteria, this relationship may represent a vulnerability in their responses to climate change (Wernegreen 2012). At least, insect herbivores likely rely on their associated microbes to extract nutrients from plant tissues challenging to digest (Hansen & Moran 2014). However, the interplay between the gut microbiome composition and diversity and temperature is not yet known for Orthoptera. The ability to react to changing or extreme climatic conditions could thus at least partly be mediated by associated microbes. Most of the research on insects' microbiome composition has focused on charismatic bees (Engel et al 2012, Leonhardt et al 2022, Voulgari-Kokota et al 2019a) or caterpillars (Hammer et al 2017). Here, a recent study provides initial evidence for microbial community variation along an elevational gradient in Eastern Africa (Mayr et al 2021).

1.9 *Study design*

Effects of temperature variation on assembly processes, diversity, abundances, traits, and biotic interactions can be studied along elevational gradients, where the climatic context gradually changes. To address the knowledge gaps highlighted in the sections above, we assessed Orthoptera assemblages, plants, and their antagonistic interactions, as well as their microbial associates at different climatic zones in the Berchtesgaden Alps (Fig. 4).

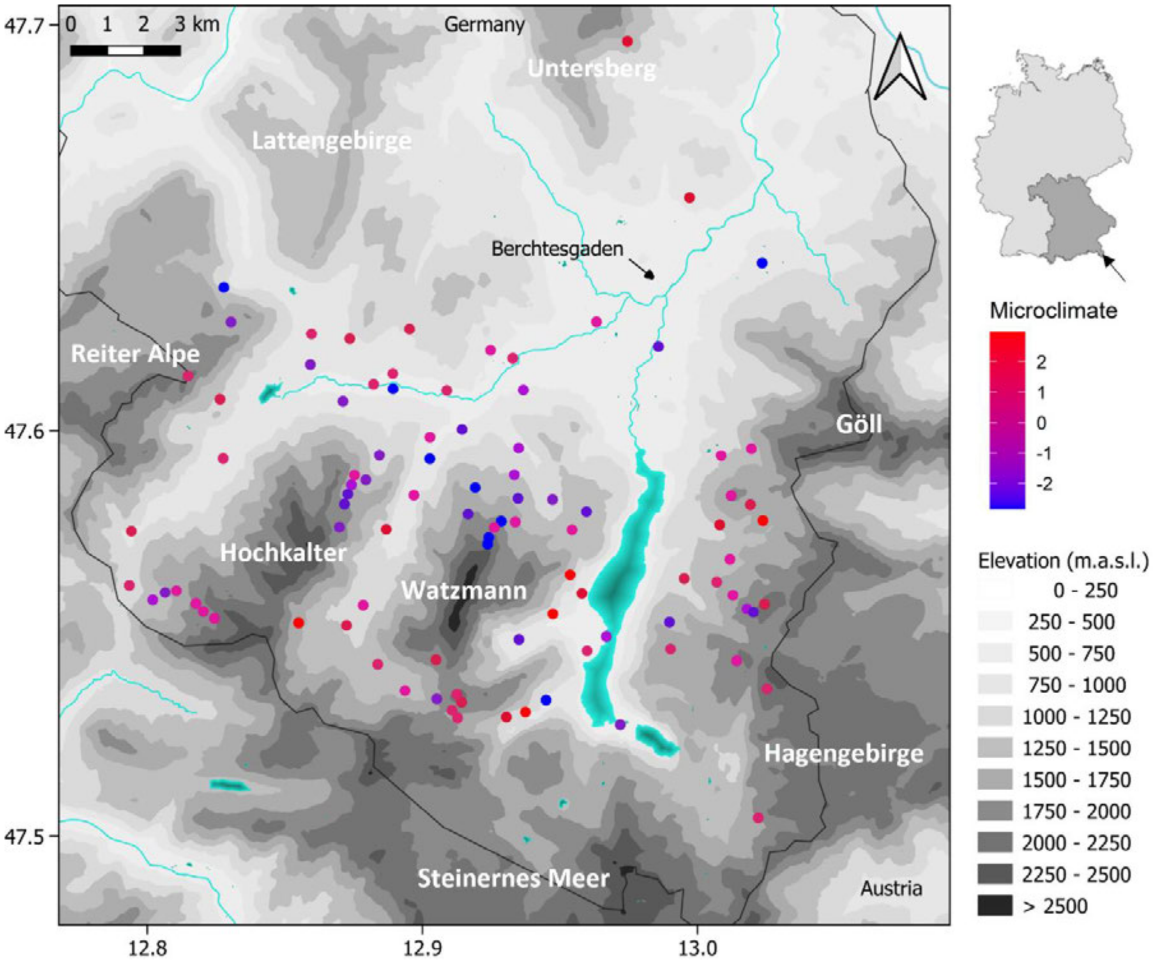


Figure 4. Overview of the study region Berchtesgaden Alps located in the South-East of Bavaria (Germany). We selected 93 study sites located in grassland along elevational gradients. Within similar elevational zones, we chose sites differing in orientation and therefore in microclimate. Sites were located along the slopes of the mountain massifs 'Untersberg', 'Göll', 'Hagengebirge', 'Watzmann', 'Hochkalter', 'Lattengebirge', and the 'Reiter Alpe'. Darker shades of grey indicate higher elevations, and red points represent sites with warm microclimate while blue points indicate cold conditions. Figure adapted from König et al. (*under review*).

1.10 *Study region*

The study region is located in the southeast of Germany (47°6′ N, 12°9′ E). This region encompasses the typical layering of vegetation zones along temperate elevational gradients (Thom et al. 2022), resulting in tessellated landscapes of formerly afforested production fir forests, mixed submontane forests, extensive hay meadows and pastures at lower elevations (600 – 850 m.a.s.l.). Moving upwards, there are mixed montane forests and extensive pastures in the montane zone (850 – 1,250 m.a.s.l.), coniferous forests in the high-montane zone (1,250 – 1,500 m.a.s.l.), krummholz in the subalpine zone (1,500-1,750 m.a.s.l.), and alpine grasslands as well as bare rock in the alpine and nival zones (1,750 - 2,713 m.a.s.l.). Throughout all elevational zones, natural open successional elements are interspersed due to terrain dynamics (Fig. 5). The annual mean temperature range covers +7 °C to -2 °C, and the annual mean precipitation ranges between 1,500 mm and 2,600 mm, depending on the elevation (Hoiss et al 2013). The landscapes in and around the Berchtesgaden National Park (20,808 ha, established in 1978) have a long history of traditional pastoral systems, dating back at least a thousand years. However, over the last century, many of the former pastures in the region have been abandoned. A total of 93 grasslands within the study region were selected. At each of the grasslands, a study site (60 m x 60 m quadrats, Fig. 6) was established. 34 of the sites were already part of an earlier research program in this area (FORKAST, Hoiss et al 2012, Hoiss et al 2015, Leingärtner et al 2014a) and repeatedly sampled in parallel to assessments of this work (ADAPT, Kerner et al 2023, Maihoff et al 2023, Sponsler et al 2022). As such temperate natural and semi-natural grasslands are threatened by habitat fragmentation, nitrogen pollutants, global warming or lost to intensive agricultural practices, they rank among the most modified biomes on Earth (Dengler et al 2020, Fuhlendorf et al 2018), while they harbour and maintain a high diversity of plants and arthropods, also in the Alps (Blumer & Diemer 1996, Hodgson et al 2005, Kampmann et al 2008, Poschlod & WallisDeVries 2002, Tattoni et al 2017, Zong et al 2018).

Figure 5. Different facets representing the landscape diversity in the study region Berchtesgaden Alps. A) ‘Watzmann’ (2,713 m.a.s.l.) from the south, B) rocky alpine grassland at the ‘Hirschwieskopf’ (2,000 m.a.s.l.), C) ‘Wimbach’ valley (1,500 m.a.s.l.), D) mouth of the stream ‘Eisbach’ into the lake ‘Königssee’ (600 m.a.s.l.), E) open habitat of early successional stage at the alluvial grassland ‘Mühlsturzgraben’ (1,000 m.a.s.l.). F) Submontane pasture close to Berchtesgaden, and G) montane pastures in the eastern part of the National Park. Photo credits: Sebastian König.

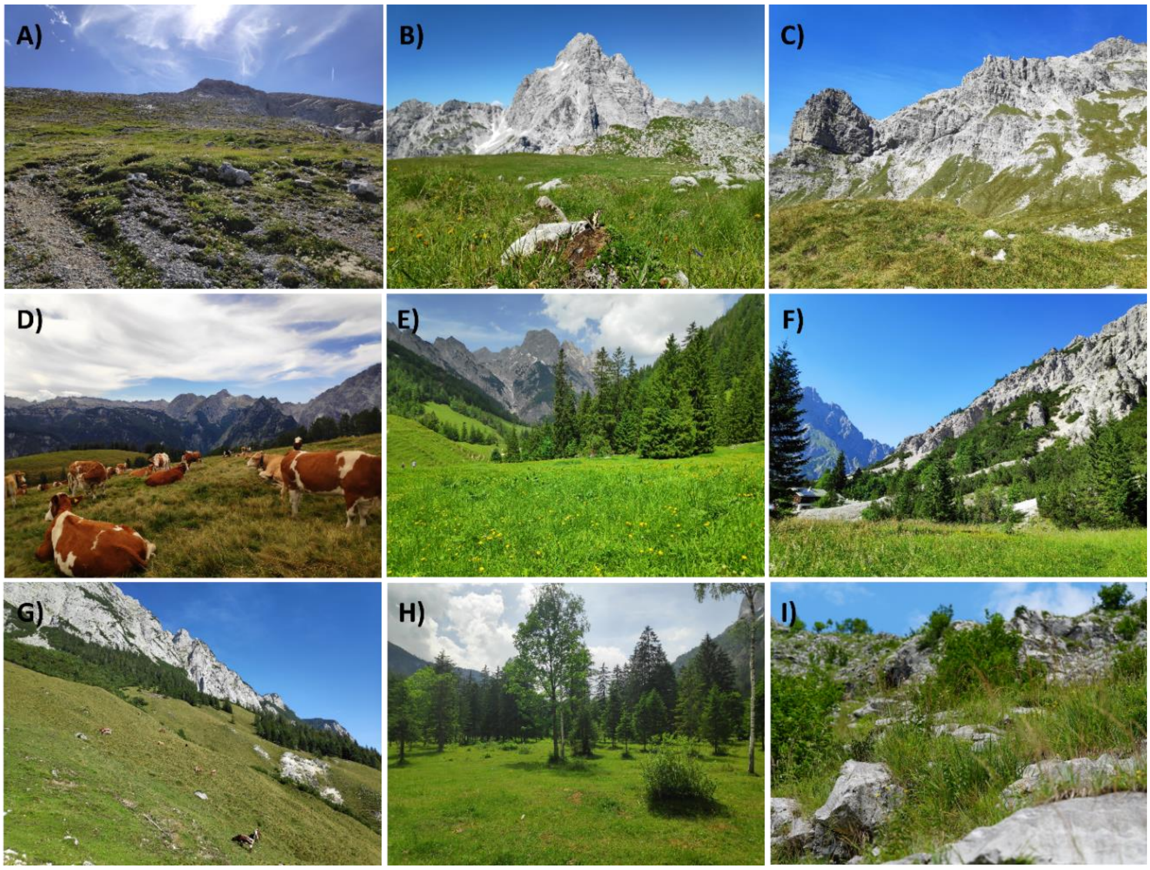


Figure 6. Impressions from representative grassland study sites established along elevational gradients in the Berchtesgaden Alps. A) ‘Hocheck’ (alpine grassland), B) ‘Hirschwieskopf’ (alpine grassland), C) and D) ‘Gotzenalm’ (subalpine pasture), E) ‘Bindalm’ (high-montane pasture), F) ‘Wimbachgrieshütte’ (high-montane natural grassland), G) ‘Halsalm’ (montane pasture), H) ‘Lahnwaldalm’ (submontane pasture), and I) ‘Rinnkendlsteig’ (submontane natural grassland). Photo credits: Sebastian König.

1.11 *Methods in a nutshell*

To record Orthoptera assemblages, which were the core of my thesis, we employed different methods. Transect counts were the central part of the surveys. Therefore, we visited all study sites twice between July and October in 2019 and 2020, following the phenology from the valleys towards the summits. At each visit, all stridulating and seen Orthoptera individuals were counted along variable 10 min transects, which were repeated five times. Transects were selected in field to cover all microhabitats within the defined margins of the study site. The transect walks were conducted, when weather conditions were suitable (sunny, temperature above 20 °C, wind below Beaufort scale 3). All species were identified directly in the field, following the latest nomenclature (Cigliano et al 2023). We supplemented these transect assessments with quadrat samplings in 2020 to validate that no bias occurred due to differences in slope or vegetation structure during transect counts (Fig. 7A). Therefore, we set an isolation quadrat covering an area of 1 m^2 20 times randomly distributed at each site, within which we counted and identified all Orthoptera individuals before releasing them. Both methods are regularly used in Orthoptera assessments and regional and national monitoring schemes. While transect counts are suitable for rapid detection of many species by experienced observers, quadrat counts produce reliable abundance data (Gardiner et al 2005). To record crickets, we additionally visited the sites in May and June 2020, when singing activity peaks. Groundhoppers (Tetrigidae) were also separately assessed by specific search in suitable microhabitats. For recording species, which are difficult to hear due to high-pitched stridulation, we deployed a bat detector. Lastly, we used a beating tray to assess tree-dwelling species. For analyses, community data were pooled at site-level.

Faecal pellets of all populations which consisted of more than 10 individuals within the margins of the study sites were collected. Therefore, we caught 5 male and 5 female grasshoppers, transferred them into sterile 50 ml Falcon tubes and waited until each hopper produced 2 – 3 faecal pellets, before releasing the animals directly at the collection site again. The pellets were then pooled per species and site, covered with preservation/stabilizing solution and later, the DNA content in the sample, which we extracted in a laboratory dedicated to DNA analysis, was used to reconstruct feeding interactions and microbial associates via amplicon sequencing.

Vegetation assessments consisted of two visits to the study sites, at which all plants were recorded in ten 2 m x 2 m quadrats randomly distributed across each study site. Their cover was estimated using the DOMIN-scale.

Local microclimate was measured with three temperature sensors (ibuttons, Maxim Integrated) per site measuring temperature at soil-level every two hours over the whole sampling period between May and October 2020. As we could install the loggers only after snowmelt, the last high-elevation sites were installed at the beginning of June. Therefore, we excluded all datapoints from the other loggers before June to cover the same period with all sensors.

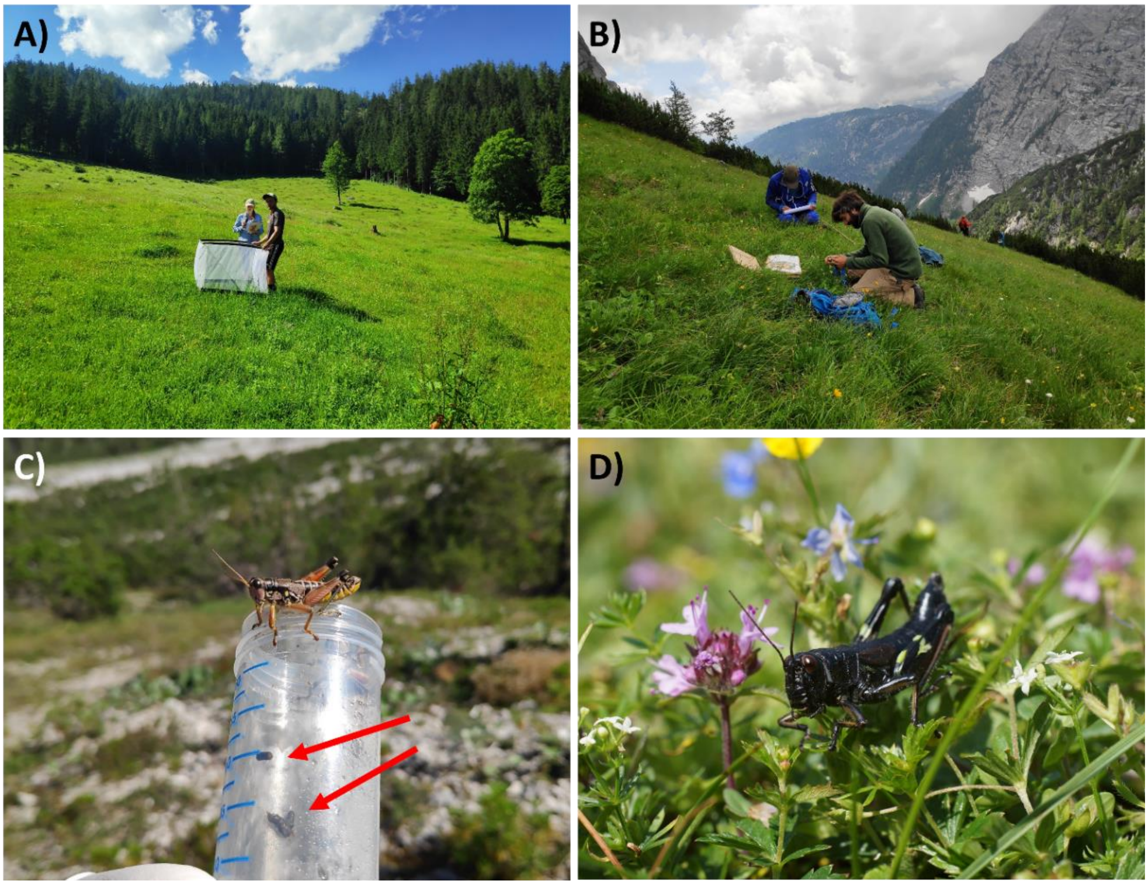


Figure 7. Methods overview. A) Isolation quadrat sampling. Students Carolin and Maximilian doing repeated isolation quadrat assessments at the montane pasture ‘Schapbach’. All Orthoptera individuals caught within the square were counted and identified. B) Vegetation assessments at the subalpine mountain pass ‘Trischübel’. Three botanists recorded plant assemblages at 20 randomly selected plots of 2 x 2 m each during two visits to the study sites (10 plots in June/July and 10 in August/September). All plants were identified, and cover classes estimated following the DOMIN-scale. C) Faecal sampling. Release of a male Common Mountain Grasshopper (*Podisma pedestris*, Melanoplinae) after defecation into a sterile 50 ml Falcon tube at a study site in the ‘Wimbach’ valley. The grasshopper was feeding on the Briskly Hawkbit (*Leontodon hispidus*, Asteraceae), Bladder Campion (*Silene vulgaris*, Caryophyllaceae), and Einsele's Columbine (*Aquilegia einseleana*, Ranunculaceae). Faecal pellets (dark marks in the tube, indicated by red arrows) were collected, pooled per site and species from 10 individuals each, and covered with DNA/RNA-shield solution to prevent degradation of the DNA before storage, DNA-extraction, and amplicon sequencing. D) Feeding observation of a female Green Mountain Grasshopper (*Miramella alpina*, Melanoplinae) chewing on the Bedstraw (*Galium anisophyllon*, Rubiaceae) at a species-rich subalpine grassland ‘Hocheisalm’. Photo credits: Sebastian König.

1.12 *Objectives: Research questions*

Considering the gaps in knowledge identified in the preceding sections combined with the opportunities presented by the Berchtesgaden alpine region, we aimed to answer three complementary questions (Fig. 8):

1) How does the interaction between microclimate and macroclimate shape the structure and diversity of Orthoptera communities based on links between the climatic niches of species and morphological or ecological traits? (Chapter II)
2) How does climatic variation shape the trophic specialisation of Orthoptera communities? (Chapter III)
3) How are microbial communities in the faeces of Orthoptera populations affected by the feeding habits and specialisation of their hosts, and by climatic variation? (Chapter IV)

To answer these questions, we employed a space-for-time substitution approach utilizing elevational gradients, which offer extensive macroclimatic variation. Study sites were selected to cover an evenly distributed gradient of macroclimatic mean seasonal temperature. By selecting sites within a relatively small spatial extend, we aimed to minimize effects of differences in local species pools arising from geographical dispersal barriers and historical circumstances, which could affect inference of patterns along macroclimatic gradients, such as latitudinal gradients. Differences in land-use intensity often overlay the climate responses measured in studies. This circumstance makes it especially difficult to separate effects of climate change *per se* from variation in land-use intensity, which itself affects the microclimate. All study questions take advantage of the nature protection status of the study region, primarily situated in the core and management zone of the Berchtesgaden National Park. In this protected area, intensive management of grassland habitats is not permitted (i.e., no intensive mowing on meadows, low densities of livestock (cattle, sheep) on pastures or no management). Therefore, we guarantee comparability of the study sites and exclude effects of intensive management of grassland sites when assessing the climate response of species. All additional sites outside of the protected area were selected fulfilling the same criteria mentioned above. Central to all questions is the detailed assessment of Orthoptera assemblages at the study sites.

Chapter II leverages the topographic and structural heterogeneity of the Berchtesgaden Alpine region. There is a gap in our comprehension of species climatic niches, which makes predictions to climate change difficult or sometimes even wrong. Failing to consider microclimate will lead to an overestimation of responses of species to climate change (Scherrer et al 2011). Our study area, the Berchtesgaden Alps (Bavaria), sets the stage to address the impact of contrasting microclimatic conditions on the community composition of insect herbivores along a broad gradient of macroclimatic conditions. Accounting for phylogenetic relationships as well as morphological and ecological traits of species can provide valuable information to derive assembly processes of Orthoptera communities, that can be used to infer predictions about future changes in the communities with ongoing climate change. Additionally, assessing intraspecific trait variation could reveal to what extend plasticity or adaptation is possible in response to climatic variation. In this chapter, we explore how micro-, and macroclimate interact to shape the niches of Orthoptera species using joint species distribution modelling. We link the niche parameters to their respective traits and derive species richness, abundance, trait, and β-diversity patterns along the climatic gradients from the joint niches modelled. Inference along the climatic gradients was based on I) individual, II) population, and III), community responses. The information recorded on biodiversity, abundance, and community composition serves as reference baseline for monitoring changes over time in future studies.

Chapter III focusses on studying the effects of climate on the trophic specialisation of Orthoptera communities - an ecological trait believed to be subject to environmental filtering processes. Therefore, we observed feeding interactions and collected faecal pellets to reveal feeding specialisation by amplicon sequencing. Specialisation metrics were calculated, accounting for resource availability and phylogenetic relationships.

In Chapter IV, we dive deeper into feeding specialisation and introduce another level to the biotic trophic interactions - the diversity and composition of microbial associates. Potential effects of climate change on feeding interactions have not been assessed at this level before, as there is a knowledge gap regarding the functions of microbial communities in Orthoptera guts and the factors that influence them. In this chapter, we aim to make a first contribution to identify drivers of diversity and composition of microbial communities of Orthoptera by studying the combined effects of feeding habits, feeding specialisation, and the climate of origin.

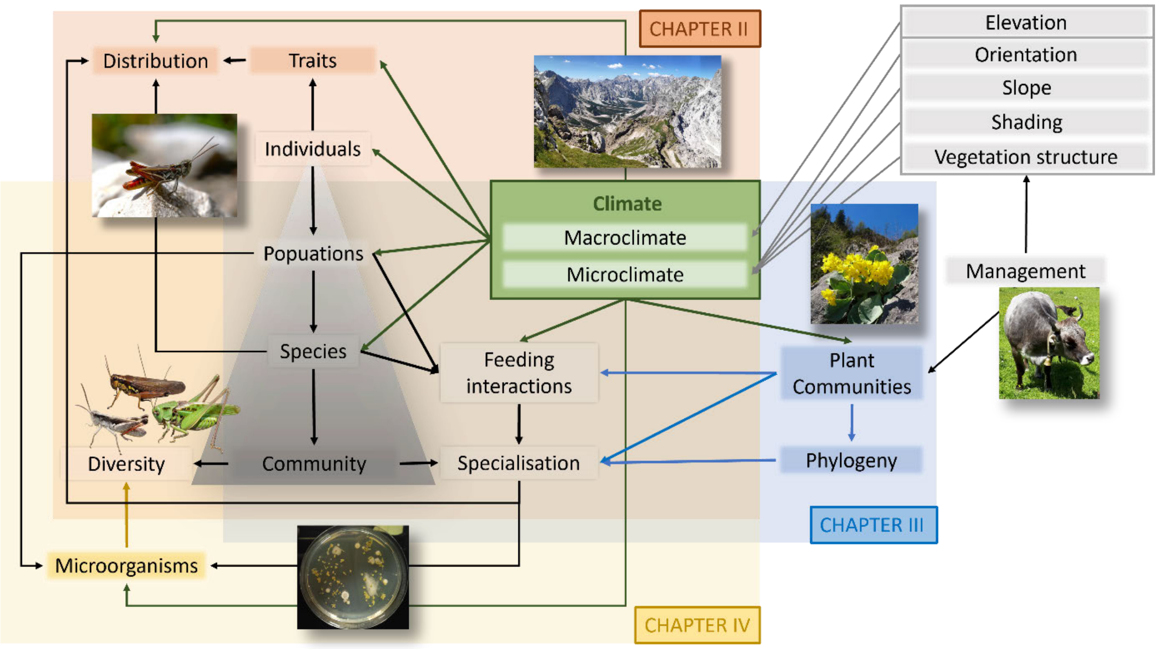


Figure 8. Diagram linking research topics. Chapter II (red) focusses on effects of micro- and macroclimate on the distribution and abundance of Orthoptera species in the study region, linking diversity, community composition, and species' traits to their climatic niches. Chapter III (blue) empirically assesses dietary specialisation of Orthoptera populations along the climatic gradient through metabarcoding of DNA from faecal samples while accounting for the availability and phylogenetic relatedness of host plants. Chapter IV (yellow) describes links between feeding habits and microbial communities in the faeces along the same climatic gradient. Photo credits: Sebastian König.

Chapter II

Micro- and macroclimate interactively shape diversity, niches, and traits of Orthoptera communities along elevational gradients

> “To insects, the little things that run the world”
>
> — *Edward O. Wilson (1987)*

> “To paraphrase a famous sentence, nothing makes sense in biology if the organisms studied are not identified and named, as their taxonomic placement in special units, the taxa, provides irreplaceable information on their characters, relationships, and evolution.”
>
> — *Alain Dubois et al. (2013)*

Temperature is one of the main drivers shaping species diversity and assembly processes. Yet, site-specific effects of the local microclimate on species and trait compositions of insect communities have rarely been assessed along macroclimatic temperature clines.

Bayesian joint species distribution models were applied to investigate how ecological and morphological traits drive variation in the climatic niches of 32 Orthoptera species on 93 grassland sites with contrasting microclimatic conditions along a steep elevational macroclimatic gradient in an Alpine region in Central Europe.

Species richness and abundance decreased along the elevational macroclimatic gradient, and both benefitted from warm microclimate. Interactive effects of elevation and microclimate on the abundance were, however, species-specific, and partly mediated by traits: Warm microclimatic conditions facilitated the occurrence of demanding xerophilic and late-hatching species, resulting in marked community dissimilarities at mid-elevations where colder sites harboured only a subset of the species. The latter mainly occurred at low elevations together with long-winged species. Abundance peaks of non-xerophilic species were further upslope when microclimate was warm. Intraspecifically, the body sizes and wing lengths of the larger females, but not the males, decreased with elevation akin the community mean, and brown colour morphs were more frequent at sites with warm microclimate.

Our results reveal that trait-dependent responses of species to microclimate play a key role in the assembly and structuring of insect communities along macroclimatic gradients. Since microclimate preferences changed with elevation, we conclude that species temperature niches are narrower than the elevational range suggests and both macro- and microclimatic conditions must be considered when predicting species responses to climate change. Microclimatic contrasts among sites at similar elevations enhanced species turnover mediated by moisture preferences and phenology, highlighting the invaluable conservation importance of mountains as climatic refugia where species with diverging niches can persist in proximity.

2.1 *INTRODUCTION*

Climate change is causing a restructuring and reassembly of biotic communities worldwide (Habel et al 2016, Halsch et al 2021, Hill et al 2021). Mountain regions are particularly at risk as temperatures rise rapidly there (Nigrelli & Chiarle 2021, Pepin et al 2015) while they serve as exceptionally important refugia for cold-adapted and often endemic species (Berger et al 2010, Laiolo et al 2018, Trew & Maclean 2021). Since air temperature declines steadily with elevation (lapse rate of ~ -5.5 K / 1,000 m), gradients along mountain slopes can be used as space-for-time surrogates to study community assembly processes and anticipate species' responses to climate warming (Körner 2007, Rahbek et al 2019). However, the temperature conditions species are exposed to within their habitats are not only determined by the elevational macroclimatic gradient *per se* but also significantly modified by local conditions – the microclimate (Kankaanpää et al 2021, Ohler et al 2020, Scherrer et al 2011). As the microclimate can buffer changes in the macroclimate (Bennie et al 2013, Senf & Seidl 2018, Stark & Fridley 2022), neglecting it may lead to an overestimation of species' responses to climate warming (Scherrer et al 2011). Yet, the scale at which species respond to climatic variation (micro vs. macro) depends on the size, area requirements, and dispersal distances of species (Poggiato et al 2023), and studies on how the interplay of both facets of climate shapes biological communities in mountain regions are lacking.

Despite facing above-average temperature increase (Pepin et al 2015), mountains provide a variety of microclimatic conditions in proximity based on differences in aspect/orientation/exposition and slope (topographic heterogeneity), radiation, wind speed, substrate, and vegetation structure at the meso- and microscale (Albrich et al 2020, Körner & Hiltbrunner 2021, Ohler et al 2020, Rita et al 2021). Especially on sunny days, soil temperatures of north and south facing slopes can differ starkly at similar elevations, comparable to differences expected from about 500 m elevational difference for seasonal average soil temperatures (Ohler et al 2020, Scherrer et al 2011), with hottest conditions reached at steep sun-facing slopes with low vegetation cover (Maclean et al 2019). This climatic heterogeneity facilitates the persistence of species with diverging niches in proximity, resulting in high rates of β-diversity in mountains (Fontana et al 2020, Sponsler et al 2022, Tello et al 2015, Zografou et al 2017). Within such a mosaic of climatic conditions, species are known to match their climatic requirements by macroclimate-dependent preferences for specific suitable microclimates (relative niche-consistency, Dobrowski 2011, Feldmeier et al 2020), but this has rarely been linked to species traits.

Rapid range shifts of insect communities to higher elevations in mountain regions due to temperature increase have been shown (Kerner et al 2023, Maihoff et al 2023, Ogan et al 2022). However, responses to increasing temperatures are species-specific (Engelhardt et al 2022, Hickling et al 2006, Neff et al 2022, Poniatowski et al 2020). Since certain ecological and morphological traits can be beneficial under either warm or cold climatic conditions, they determine distribution patterns of species along climatic gradients (Chichorro et al 2022, Classen et al 2017, Hoiss et al 2012, Leingärtner et al 2014b, Peters et al 2016a). At high elevations, short growing seasons and cold temperatures shorten the time available to complete a life cycle, demanding thermoregulatory adaptations (e.g., faster-heating colour phenotypes, Dieker et al 2018, Fernandez et al 2023, Harris et al 2013, Köhler & Schielzeth 2020) and rapid development, which is associated with small adult body size (Berner et al 2004, Levy & Nufio 2015, Tiede et al 2018), or early hatching phenology to prolong the season (Ingrisch & Köhler 1998, Kankaanpää et al 2021). Additionally, the reduction of wing length independent of body size is a common adaptation in cold environments (Laiolo et al 2023, Leihy & Chown 2020, Tiede et al 2018), as it may be advantageous to allocate resources to reproduction rather than wing development (energy trade-off, Hodkinson 2005, Laiolo et al 2023, Tiede et al 2018). Cold habitats may also require utilizing a broad range of food items, thus favouring less specialised species (König et al 2022a, Pitteloud et al 2021, Rasmann et al 2014). Despite the urge to understand the position of species' climatic niches to estimate potential threats for the systems in the context of climate change, it is largely neglected how microclimate interacts with macroclimate to form the climatic niches of species and how trait combinations promote or constrain the use of microclimatic refugia under a warmer macroclimate according to predictions.

Evidence for climatic filtering processes has been demonstrated by approaches based on mean trait values without considering intraspecific variability, assuming that the difference in functional trait values between species is larger than within species (Jung et al 2010). However, a growing number of studies have suggested that intraspecific variability can hint on underlying filtering mechanisms (Classen et al 2017, Jung et al 2010, Laiolo et al 2023, Tiede et al 2018).

In mountain grasslands, Orthoptera (Caelifera & Ensifera) are an abundant and diverse taxon with significant functional importance as primary consumers (Blumer & Diemer 1996, Samways 2005). Like most insects in temperate regions, they are restricted by (micro-) climate and often require warm temperatures to complete their development and life cycle (Geppert et al 2021, Ingrisch & Köhler 1998, Willott 1997). Hence, the distribution of Orthoptera species in Europe is predominantly determined by climatic conditions, leading to diversity decreases towards northern latitudes and high elevations, which are characterised by less favourable climatic conditions and, therefore, shorter seasons (Geppert et al 2021, Hochkirch & Nieto 2016). The specific demands of Orthoptera to the microclimate of their habitat make them suitable indicators for environmental changes (Bazelet & Samways 2011, Fartmann et al 2012).

Here, we ask: How does the interplay of macro- and microclimate drive diversity patterns and the assembly of insect herbivore communities? Therefore, we studied orthopteran assemblages along an elevational gradient in a topographically heterogeneous mountain region in southern Germany to test the following expectations: 1) The diversity and abundance of Orthoptera increase with micro- and macroclimatic temperature since species' climatic niches are constrained by harsh temperature conditions at cold sites or high elevations. Due to the overall cold and humid macroclimate and the complex topography in the northern Alps, microclimatic effects were expected to be particularly evident. 2) Differences in community compositions between sites peak at mid-elevations, where lowland species are still fostered by warm microclimate and overlap with mountain species. 3) Climatic niche parameters are related to species traits. Cold micro- & macroclimatic conditions filter the species pool towards smaller (body size), short-winged (wing length), less specialised (dietary breadth), early-hatching (phenology), darker (coloration), and less xerophilic (moisture preference) species (predictions Tab. S1). 4) Intraspecifically, body sizes, wing lengths, and coloration should follow the same clines as the community-level trait patterns.

2.2 MATERIALS AND METHODS

2.2.1 Study region & study sites

We studied Orthoptera communities at grassland sites on calcareous bedrock along elevational gradients in southern Germany (Bavaria). Within a region of heterogeneous landscapes in the Northern Limestone Alps (Berchtesgaden Alps), characterized by tessellated mountain pastures in a matrix of (mainly) coniferous forest and bare rock, we selected 93 study sites along the slopes of several mountains, covering a gradient of 7°C – 0°C mean annual temperature, 1,500 – 2,600 mm annual precipitation and ranging from 600 – 2,150 meters above sea level (m.a.s.l.) elevation (Fig. 1) (Hoiss et al 2013). The treeline is on average just above 1,500 m in this seasonal temperate and humid region (Sponsler et al 2022). When selecting the sites, care was taken to ensure a balanced and even distribution of orientation and elevation spanning five elevational zones (submontane: 600 – 825 m.a.s.l., montane: 825 – 1,200 m.a.s.l., high-montane: 1,200 – 1,500 m.a.s.l., subalpine: 1,500 – 1,825 m.a.s.l., and alpine zone: 1,825 – 2,150 m.a.s.l.). By focusing on a single mountain region, we attenuate large-scale spatial variation in the species pool resulting from historical or biogeographical circumstances, which allows direct inference on assembly processes caused by local climatic variation. All grassland sites were either extensively managed (*n* = 48; one cut per year on meadows, extensive cattle or sheep grazing on pastures with 0.5 – 1.5 livestock units per ha) or unmanaged (*n* = 45). The established grassland study sites covered 60 x 60 m each.

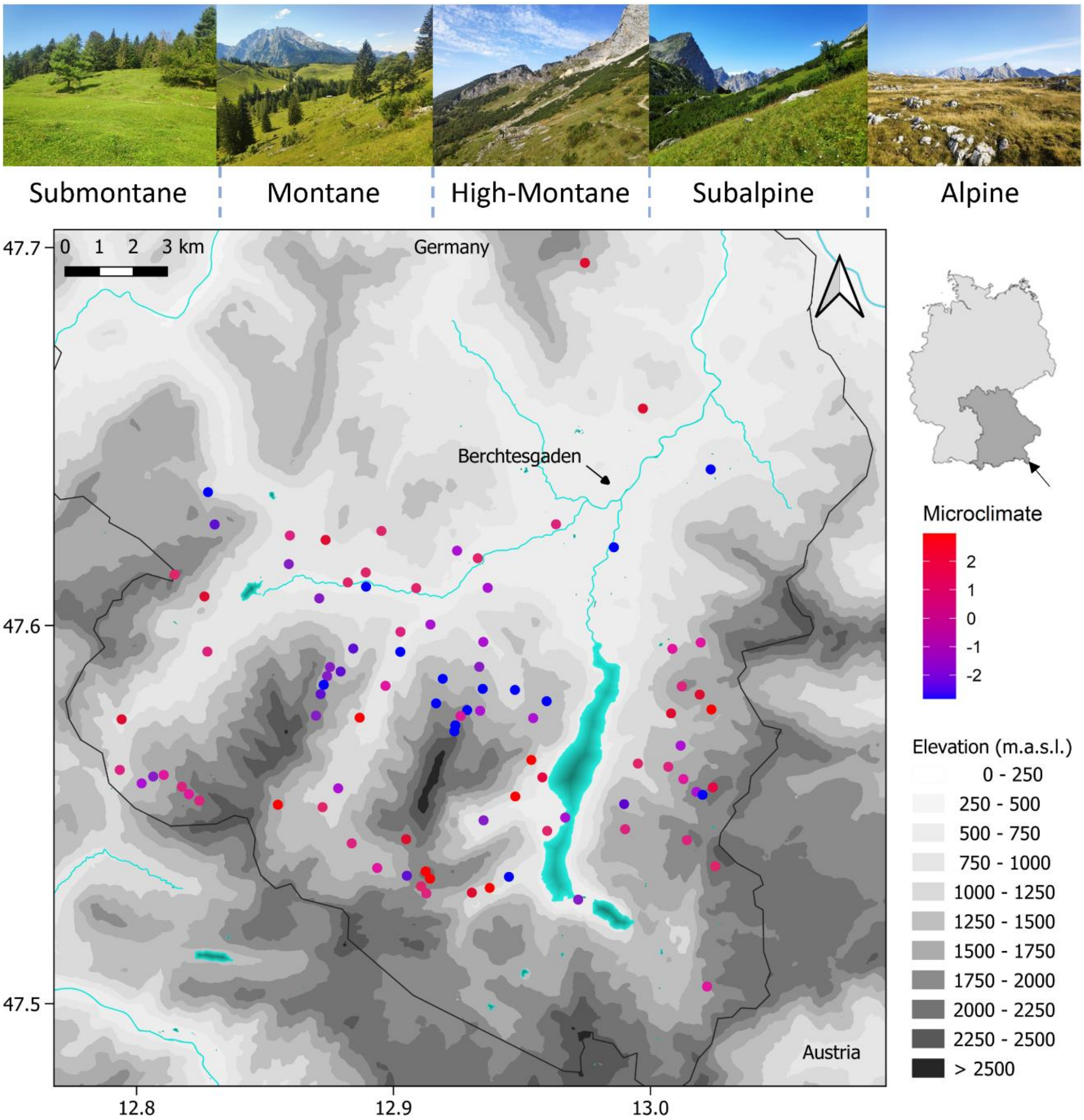


Figure 1. Location of the 93 study sites along elevational gradients ranging from 600 to 2,150 m.a.s.l. (greyscale) in the Northern Limestone Alps (Berchtesgaden, Bavaria, Germany). All study sites were either extensively managed (grazed/mown) or unmanaged open grassland sites. Point colour scale corresponds to measured local microclimatic conditions (red = warm, purple = intermediate, blue = cold). Example images of study sites from the five sampled elevational zones were added from low to high elevation (left to right: submontane, montane, high-montane, subalpine, and alpine zone).

2.2.2 Climatic variables

We used the mean elevation of the study sites as a proxy for macroclimatic temperature variation along the gradient (hereafter referred to as macroclimate/elevation, correlation with summer-seasonal mean temperature derived from a climate model based on neighbouring local climate station temperature data: $r = -0.98$, $df = 1{,}92$, $p < 0.001$; Kerner et al 2023). At each of the study sites, we additionally recorded temperature in 2-h intervals from June until October 2020 with three covered temperature loggers (ibuttons, Maxim Integrated) installed 2 cm above the soil-level in the vegetation to account for average near ground temperature deviations from macroclimate resulting from vegetation structure, aspect/orientation, inclination/slope, exposition, topography, wind speed, solar radiation, atmospheric moisture, and cloud cover (Hodkinson 2005, Hoiss et al 2013, König et al 2022a). From the logged temperatures, we calculated the mean of daytime and nighttime temperatures during the sampling period from June to October. The local microclimate was defined as the residuals of a regression of the in-field measured average temperatures with the modelled macroclimatic temperatures at the site and therefore represents local temperature deviations near ground. Positive values for microclimate indicate – on average – warmer microclimatic temperature conditions at the study site than expected based on elevation (Fig. S1).

2.2.3 Orthoptera field surveys

To record insect herbivore communities, we conducted two surveys at all study sites between following phenology from the valleys to the summits. To avoid double counts of grasshoppers at the sites, we carried out one early survey (beginning of July 2020 – end of August 2020) and one later in the season (end of August 2020 – end of October 2020). Each of the two variable transect counts per site consisted of five sub-transects of 10 minutes within the plots margins to cover most of the microhabitat variation (König et al 2022a). Surveys were performed on warm, sunny days, representing maximum activity conditions for most Orthoptera in the region (König & Krauss 2019). Orthoptera species were identified by their stridulation and visually in field. Additional late afternoon surveys with bat detectors and vegetation beatings were performed to also record species with late and high-pitched song activity as well as tree-dwelling species. Grylloids and Tetrigids were additionally recorded in May and June by listening to their songs (evening/night) or specific search in suitable microhabitats, respectively. Due to low detectability, we excluded the soil-dwelling species *Myrmecophilus acervorum* from sampling results, leading to near complete assemblage assessments. Recorded abundances of the species from the two surveys and the additional assessments were aggregated at site-level to focus on community patterns along the climatic gradients. We restricted our analyses to data gathered on adult specimens as identification of nymphs in field is difficult for several species.

2.2.4 Orthoptera traits

We collected traits of the occurring Orthoptera species both empirically and from literature (Tab. S2). Ecological traits were the species' moisture preferences ranging between hygrophilic and xerophilic species based on classifications by Harz (1969), Harz (1975), Oschmann (1991), Ingrisch and Köhler (1998), Fauna Info CSCF (2022), Thorn et al (2022), and Dvořák et al (2022), a diet breadth index, which specifies the range of used resource items with respect to evolutionary relationships (range between oligophagous species, mainly feeding on a narrow range of phylogenetically related resources, to polyphagous species which regularly use a broad range of resources) based on field-recorded feeding interactions, barcoding of faecal samples, feeding experiments, and published literature (Descombes et al 2017a, Descombes et al 2020, Ibanez et al 2013b, Ingrisch & Köhler 1998, Fauna Info CSCF 2022, König et al 2022a, Pitteloud et al 2021), and the monthly triad marking the beginning of larval hatching as a phenological trait (own observations, Ingrisch & Köhler 1998, Schlumprecht & Waeber 2003, Zuna-Kratky & Landmann 2017) (Tab. S2).

Species-level morphological traits included were mean female body size, relative wing length of females, and the predominant body coloration of the species (brown/green). We decided to include morphometrical measurements (Detzel 1998, Harz 1969, Harz 1975) of females rather than males due to the sexual dimorphisms of many Orthoptera species (Laiolo et al 2013, Laiolo et al 2023). Larger species are often capable of producing more offspring than smaller species (Ingrisch & Köhler 1998). Relative wing length is considered as a measure of resource allocation, where short winged species may be worse dispersers due to reduced flight ability but invest more into reproduction (Laiolo et al 2023, Tiede et al 2018).

Intraspecifically, we measured pronotum lengths as a proxy for body size and wing lengths of the Common Green Grasshopper *Omocestus viridulus* (L., 1758), a graminivorous, long-winged species with broad elevational distribution and no clear preference for warmer sites, and the graminivorous, long-winged Rufous Grasshopper *Gomphocerippus rufus* (L., 1758), which prefers warm microclimates all along the elevational gradient, with digital callipers to the closest 0.1 mm in field. Therefore, we caught – wherever possible – 10 individuals (males and females) at every site the species was present, measuring each parameter twice to reduce measurement error. Additionally, we scored body coloration and colour morphs of the Common Green Grasshopper (green, dorsal green - lateral brown, brown) in the field to calculate colour morph frequencies. This green/non-green polymorphism is common in Orthoptera and similar ratios between the sexes in local populations suggest a shared genetic or environmental control (Dieker et al 2018).

2.2.5 Statistical analysis

Data were analysed in R 3.6.4 (R Core Team 2019) with the packages ‘vegan’ (Oksanen et al 2020), ‘betapart’ (Baselga & Orme 2012), ‘mgcv’ (Wood 2011), ‘brms’ (Brückner 2018), and ‘Hmsc’ (Tikhonov et al 2020).

We started assessing the impact of climatic variation on Orthoptera assemblages at the community level. First, we employed permutational multivariate analysis of variance (PERMANOVA, adonis2 function in the ‘vegan’ package) based on Bray-Curtis distances between Orthoptera communities, including elevation, microclimate, and their interactive term as fixed effects to study the community compositional dissimilarity (β-diversity). To plot the ordination based on nonmetric multidimensional scaling (NMDS) of the Bray-Curtis-dissimilarity matrix, the metaMDS function in the ‘vegan’ package was used. We then computed β-diversity rates as the abundance-based Bray-Curtis dissimilarities between all pairs of communities within a moving elevational distance window of 200 m to examine at which part of the elevational gradient community composition differences peaked, (Descombes et al 2017b, König et al 2022a). Therefore, we partitioned the total differences into balanced variation in abundances (turnover equivalent of incidence-based β-diversity) and abundance gradients, in which one community is a subset of another (nestedness-resultant equivalent of incidence-based β-diversity) by using the package ‘betapart’ (Baselga 2017, König et al 2022a). Low values in balanced variation indicate a greater proportion of shared species abundances between site pairs, while high values of abundance gradients indicate that communities with low abundances are subsets of communities with high abundances of similar composition (Baselga 2017, König et al 2022a). We related all β-diversity indices to the mean elevation of each pair of sites as well as to corresponding microclimatic differences and their interaction with beta-regressions (logit-link) using generalized additive models (Wood 2023), setting $k = 3$. Deviations from an intercept-only model indicate non-constant balanced variation or abundance gradients rates with temperature and microclimate (Descombes et al 2017a, König et al 2022a). Additionally, we tested the effect of elevational difference and microclimatic differences on compositional dissimilarities with permutational mantel tests based on Pearson product-moment correlation.

Second, we used a multivariate hierarchical generalized linear mixed modelling approach (latent variable model) fitted with Bayesian inference to jointly model species elevational and microclimatic niches to assess the impact of climatic variation on species richness, abundance, and species-specific responses (Hmsc, Drag et al 2023, Ovaskainen et al 2017, Tikhonov et al 2020). When assessing the impact of the environment on traits, it is necessary to control for the tendency of related species to resemble each other more than species drawn at random from the same tree (phylogenetic independence, Abrego et al 2017, Münkemüller et al 2012, Ovaskainen et al 2017). Therefore, a phylogeny of the occurring Orthoptera species was reconstructed (supplementary text).

We excluded eight species with low prevalence (occurrence ≤ 10 sites) from the recorded communities, as statistical inference may not be trustworthy, resulting in a dataset of occurrences and abundances of 32 Orthoptera species at 93 study sites.

As sampling units, we aggregated the abundances observed at the individual visits to the study sites to yield one abundance estimation per species and study site. Due to zero inflation of our count data, we applied a hurdle approach, i.e., one model for presence-absence (probit regression) and another one for abundance conditional on presence (abundance COP model, linear regression of abundances with log-normal error distribution, declaring zeros as missing data, Whalen et al 2023).

We included the mean elevation of the study sites (linear and quadratic effect) and the sites' microclimatic temperature deviations (microclimate) as focal fixed effects. We allowed microclimate to interact with elevation to capture elevation-dependent differences in microclimatic niches. The site-level random effect controls for additional unexplained variation at the site level on top of the explicitly modelled, uncorrelated climatic covariates (Fig. S2A).

Hierarchical modelling of species communities includes a hierarchical structure assessing how species' responses to environmental covariates depend on species traits and phylogenetic relationships (Abrego et al 2017). Thus, we examined, if species with a similar set of traits had more similar climatic niches than species with converging trait expressions. As uncorrelated traits, we included body size, relative wing length, coloration, moisture preference, dietary specialisation, and hatching phenology (Fig. S2B). After determining the phylogenetic signal in the traits (Fig. S3), we examined if the variation in species niches after accounting for the species' traits was phylogenetically structured, i.e., if closely related species had more similar climatic niches than distantly related species.

We fitted the HMSC hurdle model with the R package Hmsc (Tikhonov et al 2020) assuming default prior distributions and generating a total of 1,000 posterior samples after thinning (model fitting and validation details in the supplementary text, Ovaskainen & Abrego 2020). Combining both statistically independent parts of the hurdle model, we predicted Orthoptera species abundances from the models' β-parameters, species richness, cumulative abundance, and community-weighted mean trait patterns along the elevational gradient for cold (-1 standard deviation (SD) of microclimate), intermediate, and warm (+1 SD) microclimate by multiplying the predictions for occurrence probabilities of each species from the presence-absence model with the conditional abundance predictions from the abundance COP model using the full 1,000 posterior samples.

To address our main study question, how and which traits modulate species responses to elevation and microclimate, we first examined ***1)*** the **peak elevations** of all species' abundances (elevational/macroclimatic optima). As we included the first and second order polynomial term of elevation, we did not directly infer elevational patterns from the β- and γ-parameters of the single models but derived the predicted elevational peak within the range of sampled elevations for each species from the combined models' full posterior predictive distribution (total effect). ***2)*** We further assessed which species showed a positive or negative response to microclimate (**microclimate slope**) with at least 0.95 posterior probability (linear effect across the range of elevations, weighted by the sample frequency within the five elevation bins, resulting in 1,000 slopes summarized as median and 0.95 credible intervals (CIs)). ***3)*** The third climatic niche parameter we calculated from the models' posterior distribution was the elevational abundance **peak shift** due to microclimate, addressing the interaction between macro- and microclimate. Therefore, we derived the differences between predicted peak elevations for warm microclimate (+ 1 SD) and for cold microclimate (– 1 SD).

Then, we asked if the elevational distribution of median values of species' abundance peaks, their peak shifts, and their microclimate slopes (posterior median) could be explained by their traits using phylogenetic generalized least squares regression with maximum likelihood estimation of the phylogenetic signal λ (Orme et al 2018), since the residual errors are not independent.

To examine the effect of elevation and microclimate on intraspecific trait distributions we used Bayesian generalized mixed effects models fitted with 'brms' (Brückner 2018). Thus, we used the empirically measured body sizes, relative wing lengths (tegmen length divided by pronotum length, gaussian regression with identity-link), and body colouration score frequencies (logistic regression with logit-link) as responses and the mean elevation, microclimatic temperatures, and the corresponding three-way-interaction with sex as explanatory variables. Further, we included the sampling site as random factor, as well as the species identity as random effect in the models where necessary.

2.3 *RESULTS*

2.3.1 Orthoptera diversity along the elevational gradient

We counted a total of 35,306 individuals of 40 Orthoptera species during transect surveys; 27 of which were Caelifera species and 13 belonged to the order Ensifera with an average of 11.7 (+/- 6.2) species and 194.0 (+/- 173.0) individuals per site (Fig. 2). The most abundant species, comprising more than 55% of the counted individuals, were members of the subfamily Gomphocerinae, the Bow-winged Grasshopper *Chorthippus biguttulus* (6,420), Rufous Grasshopper *Gomphocerippus rufus* (5,297), the Meadow Grasshopper *Pseudochorthippus parallelus* (4,419), and the Common Green Grasshopper *Omocestus viridulus* (3,252).

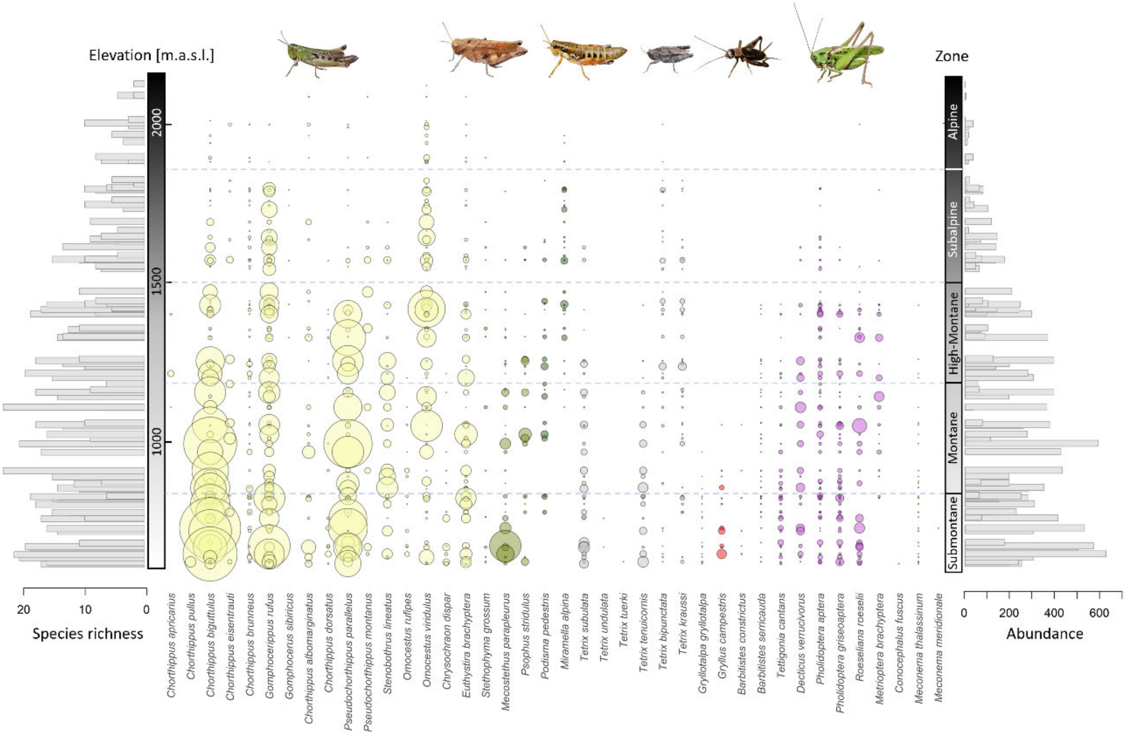


Figure 2. Orthoptera communities recorded at the study sites. Circle size is proportional to the abundance of the species recorded during surveys. Sites are ordered vertically according to their mean elevation from valleys (bottom) to summits (top), where dashed horizontal lines delimit the submontane, montane, high-montane, subalpine, and alpine elevational zones. Species richness is shown as bars on the left and summed abundances on the right. Orthoptera species are ordered and coloured according to their phylogeny with representative species images (from the left to the right: Gomphocerinae, Oedipodinae, Melanoplinae, Tetriginae, Gryllotalpinae, Gryllinae, Phaneropterinae, Tettigoniinae, Conocephalinae, and Meconematinae).

Orthopteran species richness decreased with decreasing macroclimatic temperature along the elevational gradient from 16 to three species (Fig. 3A) and abundances decreased from more than 500 to five individuals (Fig. 3B). At similar elevations, species richness and abundance were higher at sites with warmer microclimates than at sites with colder microclimates, implying additive effects of micro- and macroclimate on *α*-diversity (Fig. 3). Thus, species richness and abundances approached zero faster at cold sites of high elevations than at sun/south-exposed sites. Community composition changed along the elevational gradient, with sites characterized by a warm microclimate harbouring different Orthoptera communities compared to those from cold sites, especially in the submontane and montane zone (Fig. 3C). Therefore, Orthoptera *β*-diversity rates were pronounced at mid-elevations (where balanced variation of abundances between sites peaked) and decreased towards high elevations (with increasing abundance gradients) (Fig. 3D, Fig. S4, Tab. S3). The balanced variation of abundances component of *β*-diversity increased with elevational distance and with microclimatic differences between the study sites (Fig. S5).

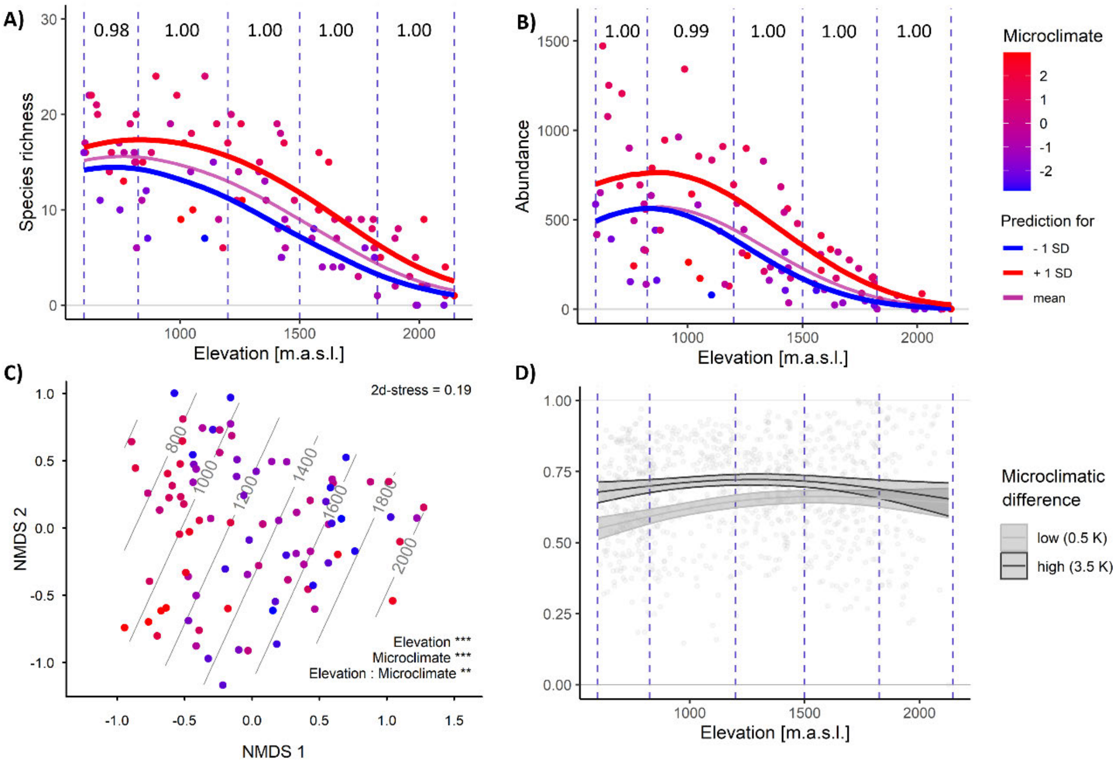


Figure 3. Effect of microclimatic variation on community-level patterns of Orthoptera communities along an elevational gradient. Predicted species richness (A) and abundance (B) decreased with elevation and were both consistently higher under warm (red lines) than under intermediate (purple lines) and cold microclimatic conditions (blue lines) at similar elevations (numbers indicate the posterior probability of a positive impact of microclimate within each elevational zone). Both, elevation ($F = 22.97$, $p < 0.001$, $R^2 = 0.19$), and microclimate ($F = 6.42$, $p < 0.001$, $R^2 = 0.06$), as well as their interaction ($F = 2.86$, $p = 0.010$, $R^2 = 0.02$) influenced the composition of Orthoptera assemblages (C). Compositional dissimilarity between Orthoptera communities was high at mid-elevations, reflected in the abundance-based *β*-diversity rate (D), which peaked at intermediate elevations and was higher when microclimatic conditions between sites differed, except at high elevations (Tab. S3). Point colours represent microclimatic conditions at the sites (red = warm, purple = intermediate, blue = cold). Vertical dashed lines separate the submontane, montane, high-montane, subalpine, and alpine elevational zone from left to right in A), B), and D).

2.3.2 Species climatic niches

The diversity patterns emerged from underlying species-level climatic niches. Accounting only for positive and negative effects with 0.95 posterior probability, 94% of the species were more abundant at the low than at the high range limit of the elevational gradient. However, 12 of the 32 species analysed had broad elevational ranges and occurred in all elevational zones along the entire 1.5 km gradient. On average, species abundances showed a hump-shaped pattern and peaked between the submontane and montane elevational zone at around 824 m.a.s.l. (range: < 600 – 1,410 m.a.s.l., Fig. S6, Fig. S7, Tab. S4).

Warmer microclimates were favourable for a high proportion (41%) of the species across the entire elevational gradient (e.g., *Chorthippus eisentrauti, Decticus verrucivorus, Gryllus campestris, Psophus stridulus, Stenobothrus lineatus, Tetrix tenuicornis*), while the remaining 59% did not differ in abundance between warm and cold microclimate with high statistical support, which could either result from a preference for intermediate microclimate, indifferent behaviour, or a change in preference with elevation (Fig. S6, Fig. S8, Fig. S9, Tab. S5).

Despite relatively broad elevational ranges, our assessment revealed narrow temperature niches for some species, which they either find at sites with cooler conditions in low elevations or at higher, more sun-exposed sites (e.g., *Miramella alpina, Omocestus viridulus, Pholidoptera aptera, Pseudochorthippus montanus, Tettigonia cantans*). Hence, microclimate effects depended on elevation for those species. Elevational distributions along gradients with warm microclimatic conditions were higher up than those along gradients with cold conditionsfor most species (Fig. S6, Fig. S10). None of the species had a higher occurrence probability with 0.95 posterior probability nor a higher abundance under cold microclimatic conditions at high elevations in the subalpine and alpine zone (Fig. S6, Fig. S10, Tab. S6).

2.3.3 Trait-environment interactions

Species ecological traits influenced species' climatic niche parameters. Especially, moisture preferences and hatching phenology of species were important predictors for species responses, since brown-coloured, xerophilic, and late-hatching species were likely to increase in occurrence probability and abundance at sites with warm microclimates (Tab. 1). Less xerophilic and mesophilic species peaked in abundance further up the elevational gradient at sites with warm microclimates than at cold microclimates (Tab. 1). Similar to the effect of microclimate, the warm macroclimate at low elevations supported late hatching species. Likewise, long-winged species mainly occurred in the valleys (Tab. 1). Though average body sizes at community level got smaller with increasing elevation, the morphological trait body size did not systematically affect the responses to any of the environmental covariates with high statistical support on species level (Tab. 1).

Furthermore, the predicted community-weighted mean traits changed along the elevational gradient, revealing a consistently higher share of xerophilic, large, and late-hatching individuals within the communities at warm microclimate sites (Fig. 4). Average community hatching phenology and diet breadth decreased along the elevational gradient, while moisture preference and wing length increased (Fig. 4). Body sizes and wing lengths of individuals within communities were larger only at warm sites of low elevations, but not in high elevations. A distinct change in community-level trait patterns became evident in the subalpine belt around 1,500 m.a.s.l.; above this elevation, predicted communities mainly consisted of the alpine specialist *Miramella alpina* and two species with broad environmental niches, *Gomphocerippus rufus* and prominently *Omocestus viridulus*, shaping the communities' traits (Fig. 4).

Table 1. Effects of traits on median values of climatic niche parameters derived from joint species distribution modelling. *Peak elevation* describes the median elevation, where predicted abundances of the species peaked, *microclimate slope* represents the median estimate of a specie's response to warming microclimate and *peak shift* is the median of the predicted difference between abundance peak elevations, if microclimate is either warm or cold. We highlight effects of the phylogenetic generalized least squares regression which are significantly positive in red or negative in blue and marginally significant slopes in pale.

Response	**Trait**	**Df**	**Est**	**SE**	***t***	***F***	***p*-value**	R^2_m	R^2_a	λ
Peak elevation	Body size	1,30	-1.86	7.12	-0.26	0.07	0.795	0.00	-0.03	0.00
	Rel. wing length	1,30	-315.56	158.85	-1.99	3.95	0.056(*)	0.12	0.09	0.00
	Coloration	1,30	16.15	128.58	0.13	0.02	0.901	0.00	-0.03	0.00
	Hatching phenology	1,30	-48.95	16.56	-2.96	8.74	0.006**	0.23	0.20	0.00
	Moisture preference	1,30	-19.01	41.32	-0.46	0.21	0.649	0.01	-0.03	0.00
	Diet breadth	1,30	99.57	73.92	1.35	1.81	0.188	0.06	0.03	0.22
Micro-climate slope	Body size	1,30	0.01	0.02	0.56	0.31	0.581	0.01	-0.02	0.00
	Relative wing length	1,30	0.49	0.35	1.43	2.04	0.164	0.06	0.03	0.00
	Coloration	1,30	0.53	0.26	2.06	4.25	0.048(*)	0.12	0.09	0.00
	Hatching phenology	1,30	0.13	0.03	4.10	16.77	<.001***	0.36	0.34	0.00
	Moisture preference	1,30	-0.33	0.06	-5.17	26.72	<.001***	0.47	0.45	0.50
	Diet breadth	1,30	0.15	0.13	1.14	1.30	0.264	0.04	0.01	0.00
Peak shift	Body size	1,21	0.60	1.37	0.44	0.19	0.664	0.01	-0.04	0.00
	Rel. wing length	1,21	31.63	26.45	1.20	1.43	0.245	0.06	0.02	0.00
	Coloration	1,21	-23.34	21.68	-1.08	1.16	0.294	0.05	0.01	0.00
	Hatching phenology	1,21	3.11	4.53	0.69	0.47	0.501	0.02	-0.02	0.00
	Moisture preference	1,21	25.14	4.07	6.17	38.11	<.001***	0.65	0.63	0.00
	Diet breadth	1,21	-10.44	10.23	-1.02	1.04	0.320	0.05	-0.00	0.00

Abbr.: *Df* = degrees of freedom, *Est* = Parameter estimate, *SE* = standard error, R^2_m = marginal R^2, R^2_a = adjusted R^2, λ = phylogenetic signal.

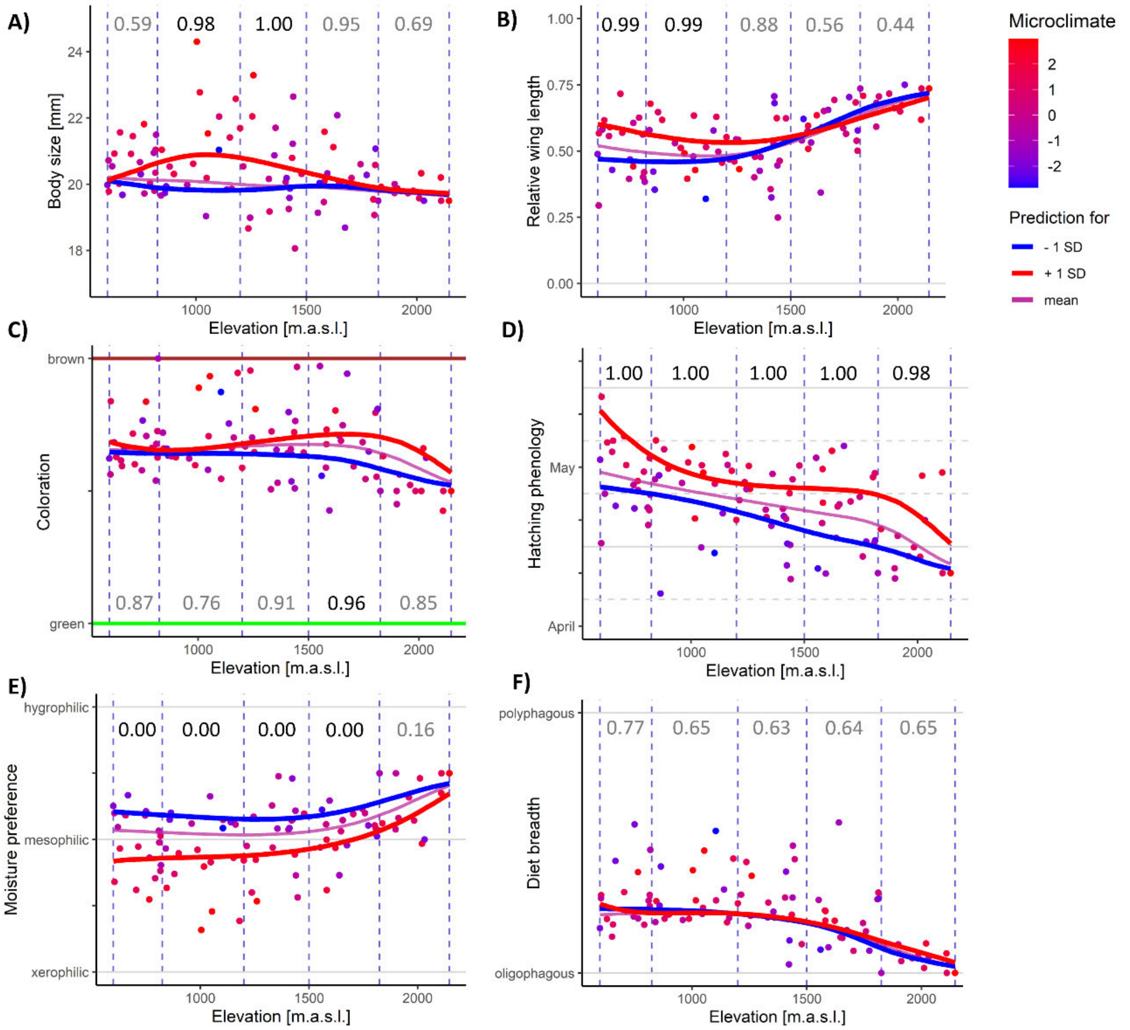


Figure 4. Effect of microclimatic variation on community-level abundance-weighted Orthoptera traits along an elevational gradient. Shown are predictions for warm (red line), intermediate (purple line), and cold microclimatic conditions (blue line) along the elevational gradient (numbers indicate the posterior probability of a positive impact of microclimate within each elevational zone). Xerophilic, large, and late-hatching individuals had a higher share of the communities at sites with warm microclimate. Point colours represent microclimatic conditions at the sites (red = warm, purple = intermediate, blue = cold). Vertical dashed lines separate the submontane, montane, high-montane, subalpine, and alpine elevational zone from left to right in each panel.

Empirical morphometric measurements of pronotum lengths and relative wing lengths of two Orthoptera species revealed changes for females, but not males, along the elevational and microclimatic gradient with high statistical support (Fig. 5, Tab. S7). Females were generally larger than males and tended to be smaller and with shorter wings (only *Gomphocerippus rufus*) at high elevations than at low elevations, particularly when microclimatic conditions were cold, whereas male size did neither vary systematically along the macro- nor microclimatic gradient. The proportion of brown colour morphs in *Omocestus viridulus* populations did not systematically vary with elevation but was higher when the microclimate was warmer (Fig. S11, Tab. S8).

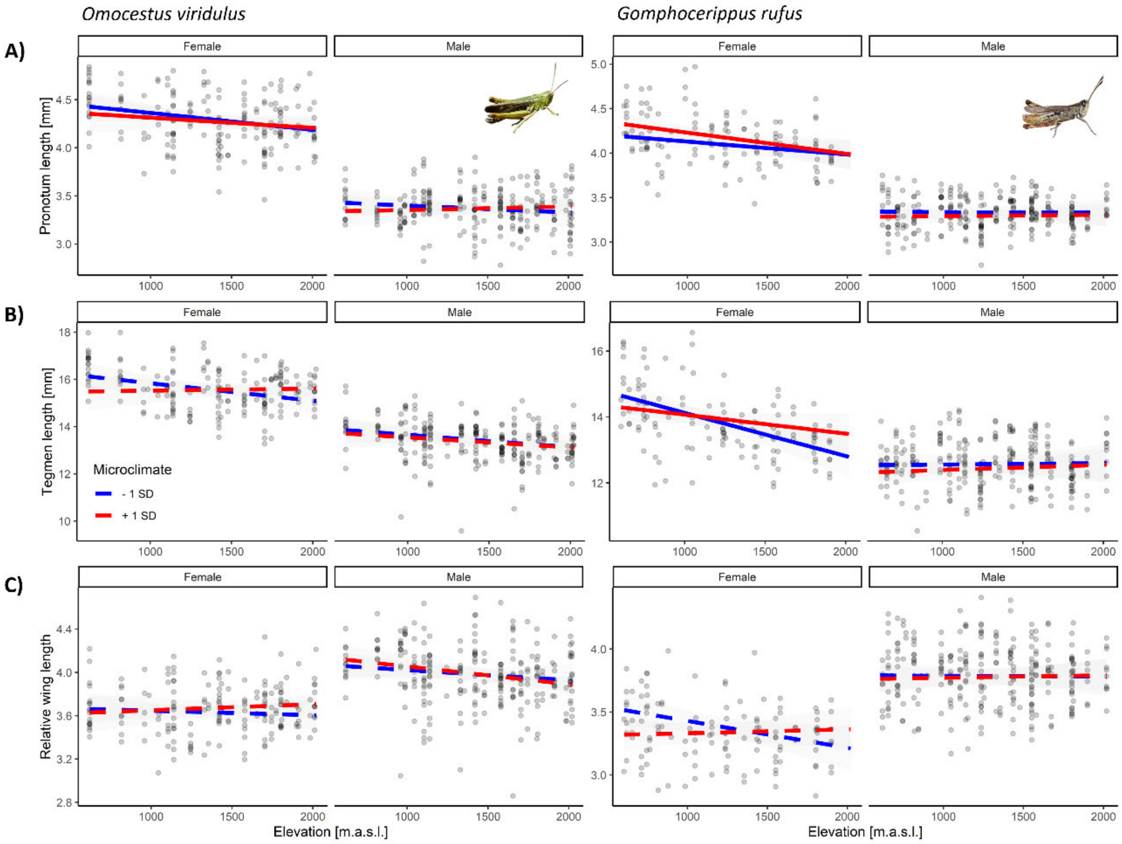


Figure 5. Empirical morphometric measurements of pronotum lengths (A), tegmen lengths (B) and their index, relative wing length (C) of two grasshopper species along the elevational gradient. The left panels show *Omocestus viridulus* and the right panels show *Gomphocerippus rufus*. Females were on average smaller at higher elevations than at low elevations (Tab. S7). In *Gomphocerippus rufus* females, wing length decreased with elevation, stronger when microclimate was cold.

2.3.4 Effects of phylogenetic relationships

The selected ecological trait moisture preference ($\lambda = 0.00$, $p = 1$; Blomberg's $K = 0.11$, $p = 0.140$) showed no raw phylogenetic signal for the 32 species involved in the HMSC analysis, whereas we detected moderate phylogenetic correlations for the species body coloration ($\lambda = 0.91$, $p = 0.177$; Blomberg's $K = 0.20$, $p = 0.022$) (Fig. S3). Very strong indications for phylogenetic signals in traits were found for species hatching phenology ($\lambda = 0.91$, $p < 0.001$; Blomberg's $K = 0.22$, $p = 0.022$), their diet breadth ($\lambda = 0.98$, $p < 0.001$; Blomberg's $K = 0.93$, $p = 0.001$), body size ($\lambda = 0.99$, $p < 0.001$; Blomberg's $K = 0.67$, $p = 0.001$) and relative wing length ($\lambda = 0.92$, $p = 0.005$; Blomberg's $K = 0.26$, $p = 0.013$).

Concerning trait-environment interactions, we detected a moderate phylogenetic signal only for the effect of species moisture preferences on the microclimate slopes and for the effect of diet breadth on the elevational distribution (Tab. 1).

2.4 DISCUSSION

There is growing evidence that microclimatic conditions modulate the response of species to macroclimatic variation and, therefore, to climate change. Our study revealed strong patterns of elevational structure in the richness, abundance, and β-diversity of mountain Orthoptera communities. Though richness and abundance of Orthoptera peaked in the valleys, community dissimilarity was highest in the montane and high-montane zone. By extending our macroclimatic analysis with microclimatic contrasts between sites, we were able to empirically disentangle effects of local microclimatic and macroclimatic variation not only on the distribution but also on the abundance of a functionally important insect group. Especially sites with warm microclimate supported almost the full spectrum of species, while some were regularly absent at sites with colder microclimate. Thus, we found additive effects of macro- and microclimate for diversity, but many species experienced interactive effects, highlighting an elevation-dependent effect of microclimate, which suggests narrower temperature niches than the elevational distribution indicates. Particularly, moisture preferences and hatching phenology were linked to the differentiation of climatic niches. While both traits explained the response to microclimate, the phenology and wing length also determined the position of the species' macroclimatic niches.

2.4.1 Orthoptera diversity thrives under warm climatic conditions: Additive and interactive effects of the local microclimate and macroclimate

Mountains are ecological theatres where the complex interplay of orientation and slope affect the local temperature and water balance and lead to heterogeneous microclimates at small spatial scales (Scherrer et al 2011). Such topography-based combinations of micro- and macroclimates in mountain areas enable species to track thermally optimal habitats within short distances (Rebaudo et al 2016). Temperature had the expected strong impact on Orthoptera communities in our study system. Like in many other taxa (Kerner et al 2023, Maihoff et al 2023), Orthoptera richness and abundance exhibited an almost monotonic decline with elevation (Descombes et al 2017a, Geppert et al 2021, Pitteloud et al 2020), decelerating in the valleys. As mostly thermophilic insects, they are favoured by the rising mean annual temperature towards the valleys since low ambient temperatures limit available biomass and crucial physiological processes, leading to reduced performance and fitness (Berner et al 2004, Ingrisch & Köhler 1998, Willott & Hassall 1998). Meeting our expectations, our results suggest that higher temperatures due to climate warming result in a diversification of mountain communities that are currently limited by temperature. Since most species peaked in abundance between the submontane and montane zone, richness and abundance did not continue to increase in the valley, which could hint on a lack of more thermophilic species in the regional species pool or a lack of suitable microhabitats at the lowest elevations. Although most species were more abundant at lower elevations, many had broad elevational distribution ranges, spanning the entire 1.5 km gradient which highlights their ability to survive in colder macroclimates by utilizing sun-exposed sites with warm microclimate.

Local microclimatic conditions close to the ground varied considerably (up to 5 K) at similar elevations across the entire elevational gradient, equalling several hundred meters of elevational difference in atmospheric temperature. Such variation can buffer against the effects of regional warming, as species that evade unsuitable warm macroclimatic conditions can survive at colder sites within short distances (e.g., north-facing slopes in the northern hemisphere), making them potential stepping stones or recolonization nuclei (Albrich et al 2020, Bennie et al 2013, Körner & Hiltbrunner 2021, Scherrer et al 2011, Senf & Seidl 2018, Stark & Fridley 2022, Suggitt et al 2018). Like for macroclimate, we expected consistent effects of microclimate on the Orthoptera species. As predicted, we found more individuals and species when microclimatic conditions were warmer throughout the entire gradient (Weiss et al 2013).

However, not all taxa reacted equally to the microclimate along the gradient. Especially species that are vertically oriented and usually dwell in longer swards were more abundant at sites with a colder microclimate or did not profit from warm microclimate, particularly at low elevations. If the climatic niche of species is narrow and stable, this would imply that their microclimate preference changes with elevation. While for some species microclimate had no impact or colder sites were favoured under warm macroclimatic conditions, this effect vanished at the high-elevation tail of the species' distributions or even changed to a positive impact of warm microclimate, a phenomenon referred to as elevation-dependent microclimate preference (Dobrowski 2011, Feldmeier et al 2020).

2.4.2 High community dissimilarity in the montane and high-montane zones

The peak of β-diversity of Orthoptera communities at intermediate elevations probably reflects the fading dominance of typical low-elevation species like most grasshoppers and crickets, and simultaneously a highly diverse mountain community at mid-elevations, which could result from the interplay of macro- and microclimate in this transition zone. Decreasing richness and abundance with elevation suggest that harsh abiotic conditions close to the summits formed specific communities out of a small species pool, leading to more similar communities at high elevations (Fontana et al 2020, Laiolo et al 2023, Tello et al 2015). However, we also found an impact of the microclimate on the dissimilarity at site pairs in low and mid elevation zones, probably reflecting reciprocal abundance patterns of thermophilic and thermophobic species in a zone where ambient temperatures are suitable to facilitate the development of many different species. As thermophilic species require a sufficient amount of external heat, they are more strongly bound/restricted to warm microclimate sites (Geppert et al 2021). Warm microclimatic conditions facilitate their presence at higher elevations such as the montane and high-montane zone, where cold sites harbour only a subset of the species in the pool, which increases the dissimilarity between sites. Interestingly, the tree line did not constitute a discrete transition to a new equilibrium of species composition, but rather the beginning of an accelerating decline in abundance as found for bumblebees (Sponsler et al 2022). This implies that communities decrease in the total number of individuals but are of similar composition, probably because elevational distributions of many species were rather broad in contrast to other taxonomic groups (Fontana et al 2020).

2.4.3 Eco-morphological trait-interactions with climatic niches

As season length declines along elevational or latitudinal gradients, univoltine insects with a long generation time are expected to follow the converse of Bergmann's rule (Classen et al 2017). Thus, predominantly positive effects of larger body sizes on fecundity, thermoregulatory ability, and desiccation resistance prevail under warm conditions (Schellenberger Costa et al 2018, Tiede et al 2018). While there was no clear evidence of smaller body sizes of species that inhabit high elevations or cold microclimates than their low elevation relatives, we found a higher share of large individuals within communities at warm, low elevation sites, and similar intraspecific body size clines for female grasshoppers. This matches findings of Levy and Nufio (2015) that larger females react stronger than males to climatic variation because their fitness may be more sensitive to changes in season length and climatic conditions (Laiolo et al 2013), or to decreases in nutritional quality of food plants resulting from elevational turnover. The mechanism behind the body size reduction at high elevations was shown to be a local adaptation in form of lower size thresholds to adult moulting (Berner et al 2004).

Since high temperatures facilitate insect flight (Prinster et al 2020), we expected a wing length reduction with elevation and microclimate. Indeed, long-winged species occurred more often in low elevations, corroborating findings of Tiede et al (2018) and Laiolo et al (2023), but not at sites with warmer microclimate. As reported in other studies, however, relative wing lengths of long-winged species did not vary with elevation intraspecifically. There it was argued that only species with low dispersal ability are locally adapted and show reductions in wing lengths with increasing elevation, highlighting the impact of dispersal potential on size clines (Levy & Nufio 2015). While there may be frequent genetic exchange of long-winged dispersing species along elevational gradients (Levy & Nufio 2015), especially species of low mobility contribute to *β*-diversity patterns (Marini et al 2012).

We expected a higher share of darker animals in cold environments based on thermoregulatory benefits (Köhler & Schielzeth 2020). However, no consistent effect of macro- and microclimate on body coloration and colour morph frequencies was detected, neither for species distributions nor community traits. Intra- and interspecifically, warm microclimates were associated with a higher proportion of brown individuals, contrary to our expectation. That doesn’t necessarily exclude the proposed impact of local microclimate and macroclimate on the coloration as found in other studies (Köhler et al 2017), but suggests that other effects such as the advantage of matching background/vegetation colour features to avoid predators, UV protection, or precipitation differences interfere with temperature effects (Dieker et al 2018). This phenomenon is referred to as crypsis-thermoregulation trade-off (camouflage, background-matching, predator avoidance) (Dieker et al 2018, Köhler & Schielzeth 2020), underlining the multifaceted nature of colour patterns and morph frequencies.

We found a strong impact of hatching phenology on the climatic niche parameters and community patterns. A later hatching may be risky for univoltine species, as the summer season length may be too short to complete their development and reproduce successfully in cold and high elevation habitats. Much of the differences in hatching phenology could be explained by differences in post-diapause-development, development speed, and oviposition sites (Ingrisch & Köhler 1998, Kankaanpää et al 2021).

Since the study area is humid with high levels of summer and winter precipitation, we found that xerophilic species had higher abundances at sites with a warm microclimate. This is in line with our prediction and could be caused by drier conditions at microclimatically warm sites due to run-off dynamics, increased evaporation or lower vegetation cover (e.g., at sun-exposed, steep sites) (Häring et al 2013), or because xerophilic species are often also thermophilic (Ingrisch & Köhler 1998). Following the same line of argumentation, hygrophilic species were more restricted to north-exposed, cool sites at low elevations, but the effect of microclimate changed with increasing elevation, leading to upslope shifts in the distributions from cold to warm microclimate sites. This elevation-dependent preference for microclimate or aspect was especially evident for less xerophilic species.

The elevational-niche breadth hypothesis suggests broader dietary spectra of species which occur further up the mountain (Rasmann et al 2014). However, a recent empirical study on dietary specialisation, which includes a broader climatic range and phylogenetic relationships of food plants, suggests a unimodal relationship with most pronounced dietary specialisation at intermediate elevations (König et al 2022a), offering explanations for the lack of such a pattern. For several traits, species niches and community mean patterns differed. Such differences may result from intraspecific trait variation (Classen et al 2017) or variation in elevational niche breadths of different species, as some species such as *Omocestus viridulus* displayed broader climatic niches than others such as the specialist *Miramella alpina*, but dominate the communities in terms of numbers of individuals, potentially blurring the understanding of environmental filters.

Trait expressions are often correlated with evolutionary relationships between species, as also demonstrated in our study, since closely related species often share similar characteristics. However, we also found evidence for phylogenetic signals in trait-environment interactions, highlighting that not only the traits we focused on contribute to species' climatic niches (e.g., thermal tolerances, thermoregulatory capacities).

2.4.4 Caveats: The scale of microclimate and associated covariates

We found the highest number of species at sun-exposed extensive pastures in line with other studies (Chisté et al 2016, Gardiner & Dover 2008, Klein et al 2020, Marini et al 2009, Weiss et al 2013), conditions which offer a mosaic of warm microclimate but also facilitate structurally rich vegetation, that could be used as shelter. Within-site microclimatic variation at even smaller scales than measured in our study (0.01 – 1 m) could also be crucial for the persistence of certain species, as shown for plants in alpine habitats (Ohler et al 2020, Scherrer et al 2011), possibly dampening the microclimate response we measured with this study. Likewise, species responses derived may be interfered by factors interacting with climate, such as moisture (Dvořák et al 2022, Powell et al 2007), management (Humbert et al 2021, Marini et al 2009), vegetation structure (Gardiner 2022, Löffler & Fartmann 2017, Schirmel et al 2019), composition (Tobisch et al 2023), and diversity (Fournier et al 2017, Ramos et al 2021).

2.4.5 Conclusions

The limited potential of montane assemblages to respond to climate change is of major concern to conservationists. Our findings imply that macroclimatic as well as microclimatic changes in temperature have the potential to restructure, reassemble, and replace Orthoptera communities in temperate mountain grasslands. Here, we demonstrate additive effects on diversity, but also community composition and functional traits are affected, as the interaction of elevation and microclimate shapes species niches. Since species can shift their elevational distribution not only upward but also northward to sites with cooler microclimates, climate change impacts might be mitigated by the complex topography in mountain areas (Feldmeier et al 2020, Suggitt et al 2018). Our results suggest that this turnover is the result of differences in abiotic conditions at similar elevations, highlighting the importance of mountains as climatic refugia, which support species with diverging preferences or requirements in proximity. Under future climate warming, we expect a less pronounced dissimilarity pattern in low elevations, as thermophobic species retreat and thermophilic species equally spread. This is referred to as biotic homogenization (Thorn et al 2022). At higher elevations, the arrival of thermophilic species at warm-microclimate-sites and the retreat of thermophobic species to cold-microclimate-sites is expected, increasing dissimilarity in the high-montane zone. Further, our results underline the extraordinary value of traditional extensive pastoral systems including different slope exposures and therefore contrasting microclimatic conditions to conserve biodiversity in mountains.

Our results suggest that microclimate preferences of a species in its core distribution are not always reflected at the edges, where they may be more specialized. For example, less demanding species concerning temperature conditions in their core distribution may be more restricted at the edges. Therefore, possible shifts of microclimate preferences should be acknowledged not to overestimate range reductions or expansions. Since microclimate data and small-scale modelling approaches become available (Maclean et al 2019), and local deviations to downscaled macroclimate are often high (+/- 2 K), there is an urgent need to incorporate high-resolution microclimate data into species distribution models for an accurate estimation of the availability of suitable conditions for future species distributions (Stark & Fridley 2022).

Combinations of traits help explain species' complex ecological niches and thus should prove useful in predicting their responses to future climatic changes in their habitats. Increasing temperatures in combination with drought events will likely increase diversity and the fraction of xerophilic Orthoptera species, but possibly force moisture dependent and high-temperature sensitive species to retreat to higher elevations and/or north-facing slopes. As macroclimatic average temperatures are increasing with climate change, so does the frequency of extreme weather events, which can differentially affect future distributions of species (Feldmeier et al 2018). In the course of climate change, upslope shifts and population growth of thermophilic species at higher elevations is likely but can also be hampered or reversed by late snowfall or unsuitable extreme events, which regularly occur in mountain systems.

2.5 *SUPPLEMENTARY MATERIAL TO CHAPTER II*

Additional habitat covariates

As site-specific environmental parameters we estimated the cover of 1) bare soil/rocks, 2) short vegetation (< 15 cm), 3) tall swards (> 15 cm), 4) dwarf shrubs/shrubs, and trees visually in 20 1 m^2 plots per site. Hereafter, we classified vegetation structural diversity by counting structural categories representing more than 5% of the study site area. Average vegetation height was recorded by measuring the height of plants at 20 random sampling points per study site in August 2020. We did not use these additional site-level microhabitat variables in the hierarchical joint species distribution models but report their correlations with microclimate and elevation in Fig. S2.

Orthoptera phylogeny

When assessing the impact of the environment on traits, it is necessary to control for the tendency of related species to resemble each other more than species drawn at random from the same tree (phylogenetic independence, Abrego et al 2017, Münkemüller et al 2012, Ovaskainen et al 2017). Therefore, a phylogeny of the occurring Orthoptera species was reconstructed with Bayesian inference based on 'MUSCLE' alignments (Edgar 2004) of Cytochrome-c-Oxidase subunit I (COI) sequences (trimmed to 567 bp), which were retrieved from BOLD (boldsystems.org), including *Forficula auricularia* (L.) as an outgroup. We first used 'jModelTest 2' (Darriba et al 2012) to determine the best-fitting substitution model via AIC and BIC, which was GTR+I+G. Then, a Markov Chain Monte Carlo (MCMC) chain was run for 10,000,000 generations, sampling every 1,000 generations, and discarding a burn-in of 10% in Mr. Bayes (Ronquist et al 2012). Convergence of the model was checked by calculating the potential scale reduction factors (Gelman & Rubin 1992) and viewing trace plots. Posterior probabilities are given as statistical branch support.

Statistical analysis: HMSC modelling details

We fitted the HMSC model with the R package Hmsc (Tikhonov et al 2020) assuming default prior distributions (Ovaskainen & Abrego 2020). We sampled the posterior distribution with four Markov Chain Monte Carlo (MCMC) chains, each of which was run for 3,750,000 iterations, of which 125,000 were removed as burn-in. The chains were thinned by 1,000 to yield 250 posterior samples per chain and therefore 1,000 posterior samples in total. We examined MCMC convergence by checking the potential scale reduction factors (Gelman & Rubin 1992) and trace plots of the model parameters.

We examined the explanatory and predictive powers of the probit model through species-specific AUC (Pearce & Ferrier 2000) and Tjur's R^2 (Tjur 2009) values. The explanatory and predictive powers of the abundance COP model were measured by conditional pseudo R^2. To compute explanatory power, we made model predictions based on models fitted to all data. To compute predictive power, we performed 5-fold cross validation, in which the sampling units were assigned randomly to five folds, and predictions for each fold were based on a model fitted to the data of the remaining four folds.

Convergence and model fit

The Markov Chain Monte Carlo (MCMC) convergence of the hierarchical multivariate generalized linear mixed models was satisfactory. The potential scale reduction factors of the β-parameters that measure the response of the species to environmental covariates were on average 1.00 for the presence-absence model and 1.09 for the abundance COP model.

The HMSC hurdle model sampled with four Markov Chain Monte Carlo (MCMC) chains showed a good fit to the data (presence-absence model: explanatory power Tjur's $R^2 = 0.42$, AUC = 0.91, predictive power Tjur's $R^2 = 0.21$, AUC = 0.77, abundance COP model: explanatory power $R^2 = 0.57$, predictive power $R^2 = 0.29$).

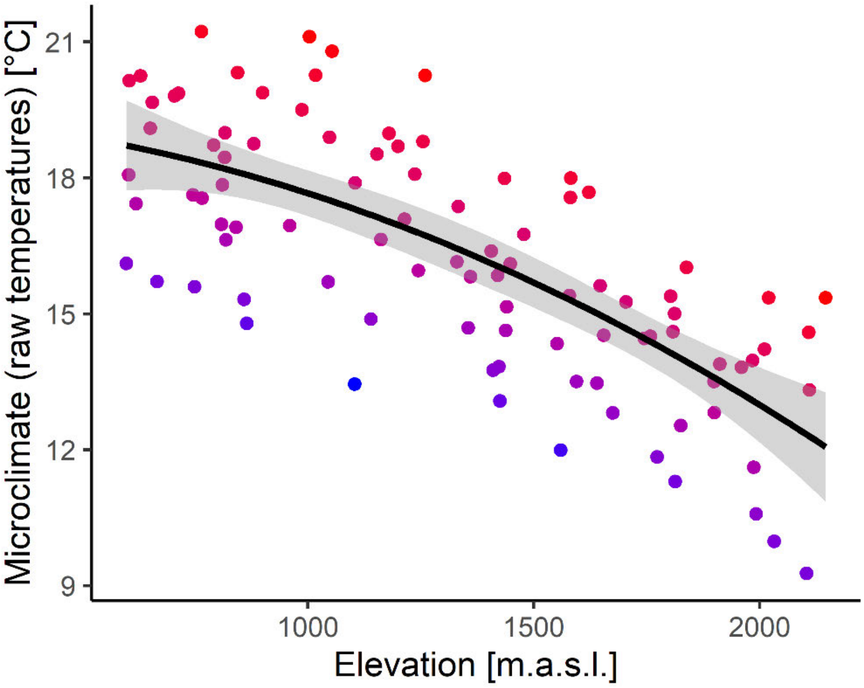


Figure S1. Average microclimatic temperatures measured during summer with three temperature loggers per study site, plotted against the mean elevation of the study sites. Point colours refer to the deviation of the local temperature close to the ground from the predicted temperatures based on an elevation-informed climate model calibrated with near-by climate stations (blue = colder than expected, red = warmer than expected, black line: prediction).

Table S1 Predictions for ecological and morphological traits to study responses of Orthoptera communities to climatic variation.

Trait	Units	Description	Prediction
Body size	1.1 -50.0 mm	Morphometric. Average length of females measured from apex of the head to the apex of the abdomen (Detzel 1998, Harz 1969, Harz 1975)	Resource intensive traits like body size are smaller at high elevations and cold microclimates due to conditions favouring short developmental times (Berner et al 2004, Tiede et al 2018)
Relative wing length	0 - 1.4	Morphometric. Average length of female alae divided by average body length (Detzel 1998, Harz 1969, Harz 1975)	Shorter wing length at high elevations due to investment in reproduction over dispersal (Köhler et al 2017, Laiolo et al 2023, Tiede et al 2018). Flight / Dispersal propensity facilitated under warm macro- / microclimatic conditions (Prinster et al 2020)
Colour	0 - 1	Morphologic. Average body coloration score ranging from mainly green body colouration/ morph frequencies (0) over mixed (0.5) to mainly brown/grey body colouration or morph frequencies (1) (Detzel 1998, Schlumprecht & Waeber 2003)	Higher frequency of brown morphs in cold habitats due to thermoregulatory advantages (faster heating) (Dieker et al 2018, Köhler et al 2017, Köhler & Schielzeth 2020)
Diet breadth	0 - 1	Ecology. Index based on the proportion of used food items from different evolutionary lineages (specialisation) (König et al. unpublished)	Broader diets in climatically unstable habitats (cold / high-elevation / exposed habitats) (König et al 2022a, Pitteloud et al 2021, Rasmann et al 2014)
Moisture preference	0 - 1	Ecology. Classification of species as hygrophilic (1), mesophilic/indifferent (0.5), or xerophilic (0) (Info Fauna CSCF, Dvořák et al 2022, Harz 1969, Harz 1975, Ingrisch & Köhler 1998)	As xerophilic species are restricted to dry habitats, they should be supported by conditions favouring fast evapotranspiration/drought, such as warm macro- and microclimates (Schlumprecht & Waeber 2003)
Hatching phenology	0 - 36	Ecology. Average monthly triad marking the beginning of larval hatching of the species (phenology) (Detzel 1998, König et al. unpublished, Schlumprecht & Waeber 2003)	While late emerging species may only be able to complete their life cycle in warm habitats, early emerging species can also inhabit colder micro- and macroclimates (Ingrisch & Köhler 1998, Kankaanpää et al 2021)

Table S2. Traits to study the response of Orthoptera species to environmental variation.

subfamily	**species**	**Hatching phenology [monthly triad]**	**Moisture preference**	**Color**	**Diet breadth index**	**Body size [mm]**	**Relative wing length**
Gomphocerinae	*Chorthippus dorsatus*	15	0.5	0.50	1.25	22.00	0.68
	Chorthippus albomarginatus	15	0.5	0.50	1.25	20.25	0.69
	Chorthippus biguttulus	14	-1.0	0.75	1.25	19.50	0.76
	Chorthippus brunneus	13	-1.0	1.00	1.25	21.50	0.84
	Chorthippus eisentrauti	16	-2.0	1.00	1.50	22.00	0.75
	Gomphocerippus rufus	15	0.0	1.00	1.25	20.00	0.63
	Stenobothrus lineatus	14	-1.0	0.25	1.00	23.50	0.67
	Omocestus viridulus	12	1.0	0.50	1.00	19.50	0.74
	Omocestus rufipes	13	-0.5	0.75	1.00	16.50	0.89
	Pseudochorthippus parallelus	14	0.5	0.50	1.25	20.00	0.17
	Pseudochorthippus montanus	15	2.0	0.25	1.00	21.00	0.39
	Euthystira brachyptera	10	0.0	0.00	1.00	20.50	0.18
	Chrysochraon dispar	11	1.0	0.50	1.00	25.00	0.26
Oedipodinae	*Mecostethus parapleurus*	16	1.5	0.00	1.25	28.00	0.80
	Stethophyma grossum	16	2.0	0.50	1.00	32.00	0.77
	Psophus stridulus	16	-1.5	1.00	2.00	33.00	0.59
Podisminae	*Podisma pedestris*	12	-2.0	1.00	2.50	27.00	0.14
	Miramella alpina	11	1.0	0.00	2.50	24.50	0.19
Tetriginae	*Tetrix tenuicornis*	21	-1.0	1.00	1.75	09.45	0.77
	Tetrix subulata	15	2.0	1.00	1.50	12.75	0.80
	Tetrix kraussi	14	-1.0	1.00	2.00	10.60	0.36
	Tetrix bipunctata	14	-1.0	1.00	2.00	10.60	0.66
Tettigoniinae	*Pholidoptera griseoaptera*	12	0.0	1.00	3.00	18.00	0.00
	Pholidoptera aptera	11	0.0	1.00	3.00	23.00	0.00
	Decticus verrucivorus	11	0.0	0.50	3.00	35.50	0.79
	Roeseliana roeselii	13	1.0	0.50	1.50	17.75	0.32
	Metrioptera brachyptera	13	0.5	0.75	3.00	19.00	0.42
	Tettigonia cantans	12	1.0	0.00	3.00	31.00	0.82
Phaneropterinae	*Barbitistes serricauda*	14	-1.0	0.50	2.00	20.50	0.00
Meconematinae	*Meconema thalassinum*	14	-0.5	0.00	1.00	13.00	0.90
Gryllinae	*Gryllus campestris*	20	-1.0	1.00	3.00	23.00	0.59
Gryllotalpinae	*Gryllotalpa gryllotalpa*	18	1.5	1.00	2.00	43.50	0.69

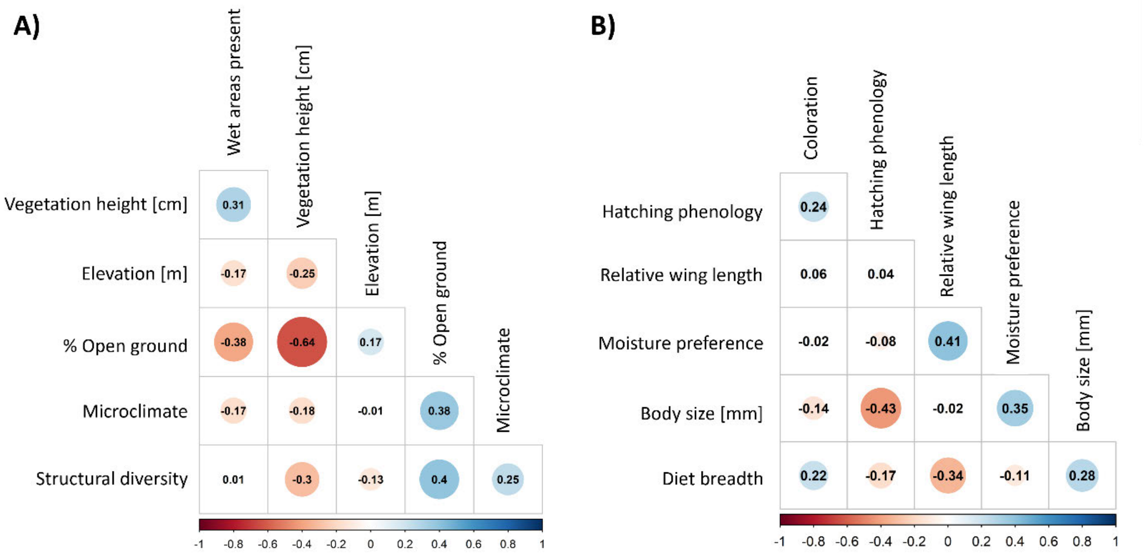


Figure S2. A) Pearson-correlations between environmental covariates included in the analyses. *Elevation* represents the mean elevation of the study sites, *Microclimate* represents the microclimatic temperature deviations, derived from a regression of temperatures measured by temperature loggers and the modelled temperatures. *Structural diversity* is a measure of structural diversity of the vegetation at the study site, *Vegetation height* reports the average of 20 random plant height measurements at all sites. The factor *Wetness* evaluates whether study sites included wet areas or not. B) Pearson-correlation of Orthoptera species-level traits included in the analyses. *Hatching phenology* is a phenological measure depicting the start of the larval hatching; *Moisture preference* measures the species preference for wet habitats; *Diet breadth* is an index calculated based on data on relative contributions of food items of different evolutionary linages included in the diet, which were derived from literature and observational data; *Body size* was included as mean length of females in mm; *Relative wing length* was calculated as the length of female alae divided by the mean body length of females. *Coloration* describes a score for main colour morphs of the species in the region.

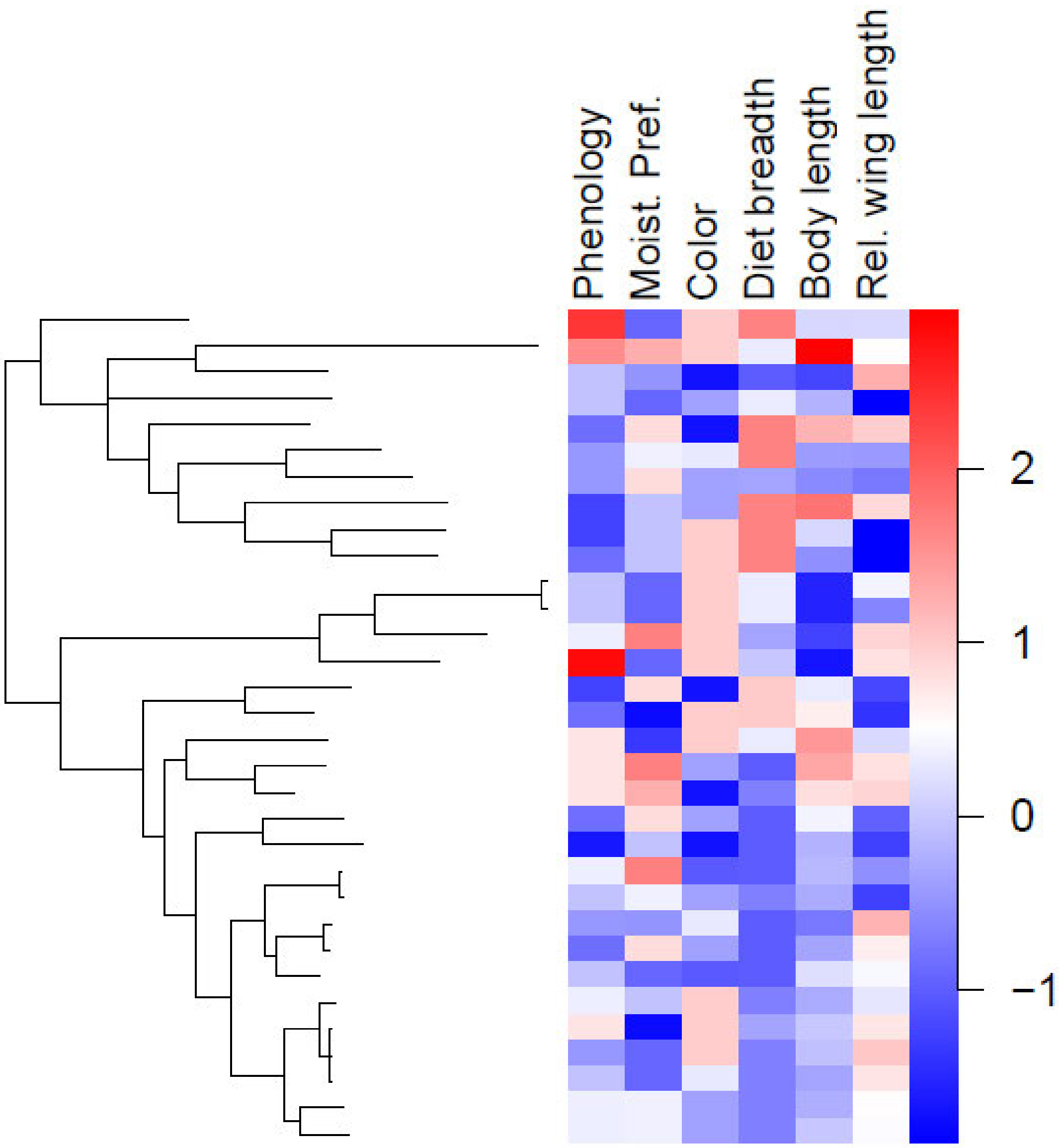


Figure S3. Scaled Orthoptera species-level traits included in the analyses. Species are ordered according to their phylogenetic relationships (Ensifera at the top and Caelifera at the bottom). A phylogenetic signal becomes evident for the traits *Diet breadth*, *Body size*, *Relative wing length*, and *Hatching phenology*. Colours of fields refer to the standardized trait expression (blue = low value, red = high value).

Table S3. Effects of elevation and microclimatic contrasts on Orthoptera β-diversity estimated with beta-regression (logit-link). Total β-diversity of Orthoptera communities (Bray-Curtis dissimilarity, partitioned into its components: abundance gradients (abundance-based nestedness equivalent, and balanced variation of abundances (abundance-based turnover equivalent, derived from pairwise site comparisons within elevational intervals of 200 m. *edf* = effective degrees of freedom, *Est* = Estimate, *SE* = standard error, R^2_a = adjusted R^2, *DE* = Deviance explained.

Response	**Covariate**	***n***	**edf**	**Est**	**SE**	z / χ^2	***p*-value**	R^2_a	**DE**
β-diversity (Bray-Curtis)	s(Elevation)	956	1.80	-	-	21.21	<.001***	0.10	0.07
	Micro. contrast		-	0.11	0.05	2.24	0.025*		
	s(Elevation) : Micro. contrast		0.86	-	-	6.14	<.006**		
β-diversity (Balanced variation in abundances)	s(Elevation)	956	1.98	-	-	195.49	<.001***	0.00	0.02
	Micro. contrast		-	-0.01	0.12	-0.13	0.901		
	s(Elevation) : Micro. contrast		0.96	-	-	21.57	<.001***		
β-diversity (Abundance gradients)	s(Elevation)	956	0.82	-	-	4.53	0.014*	0.10	0.06
	Micro. contrast		-	-0.05	0.07	0.87	0.842		
	s(Elevation) : Micro. contrast		1.69	-	-	10.10	0.002**		

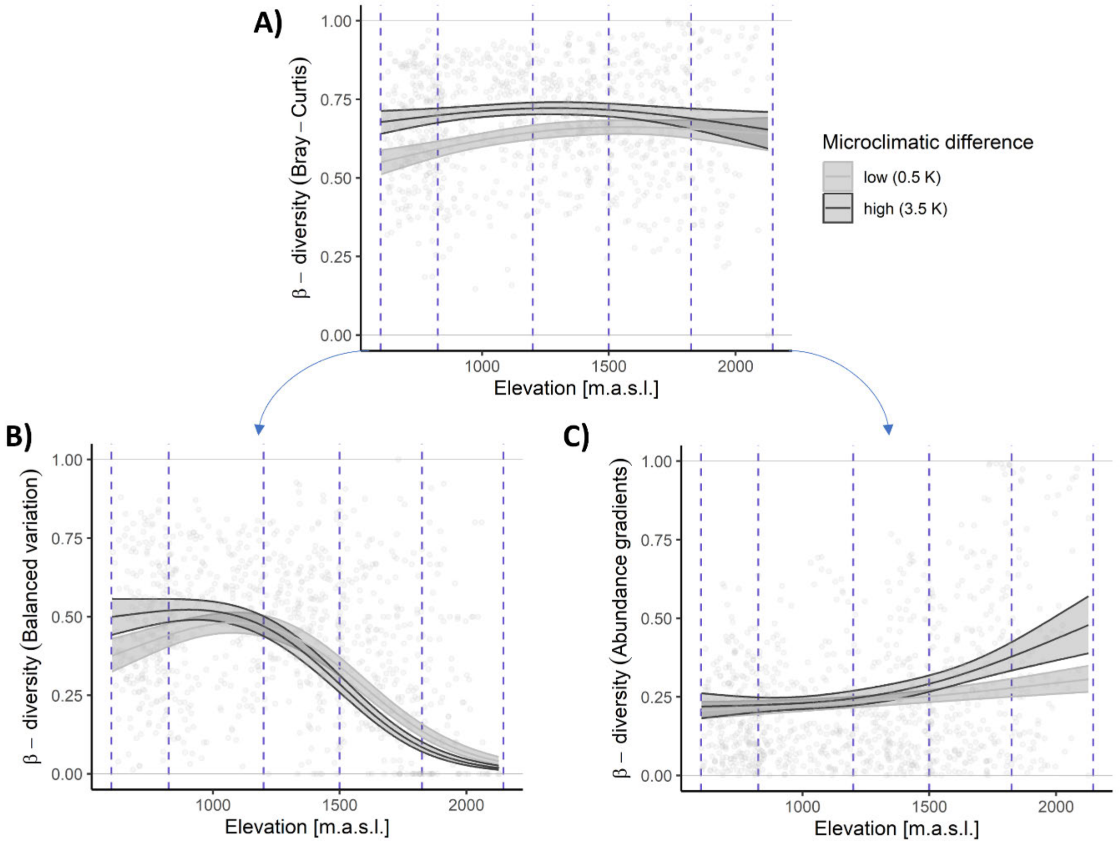


Figure S4. Total *β*-diversity of Orthoptera communities (Bray-Curtis dissimilarity, A), partitioned into its components: abundance gradients (abundance-based nestedness equivalent, B) and balanced variation of abundances (abundance-based turnover equivalent, C) derived from pairwise site comparisons within elevational intervals of 200 m. We repeated this analysis with intervals of 50, 100, 200, and 300 m to test, whether chosen intervals affect *β*-diversity rates along the gradient. Nestedness-equivalent rates increased with elevation for site pairs with high differences in microclimatic temperatures. Turnover-equivalent rates peaked at intermediate elevations and turnover contributed more to *β*-diversity then nestedness. Vertical dashed lines separate the submontane, montane, high-montane, subalpine, and alpine elevational zone from left to right in each panel.

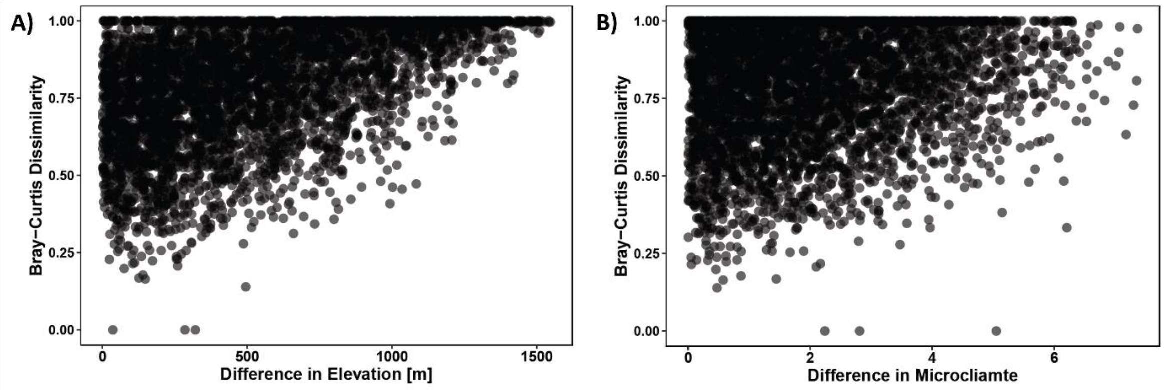
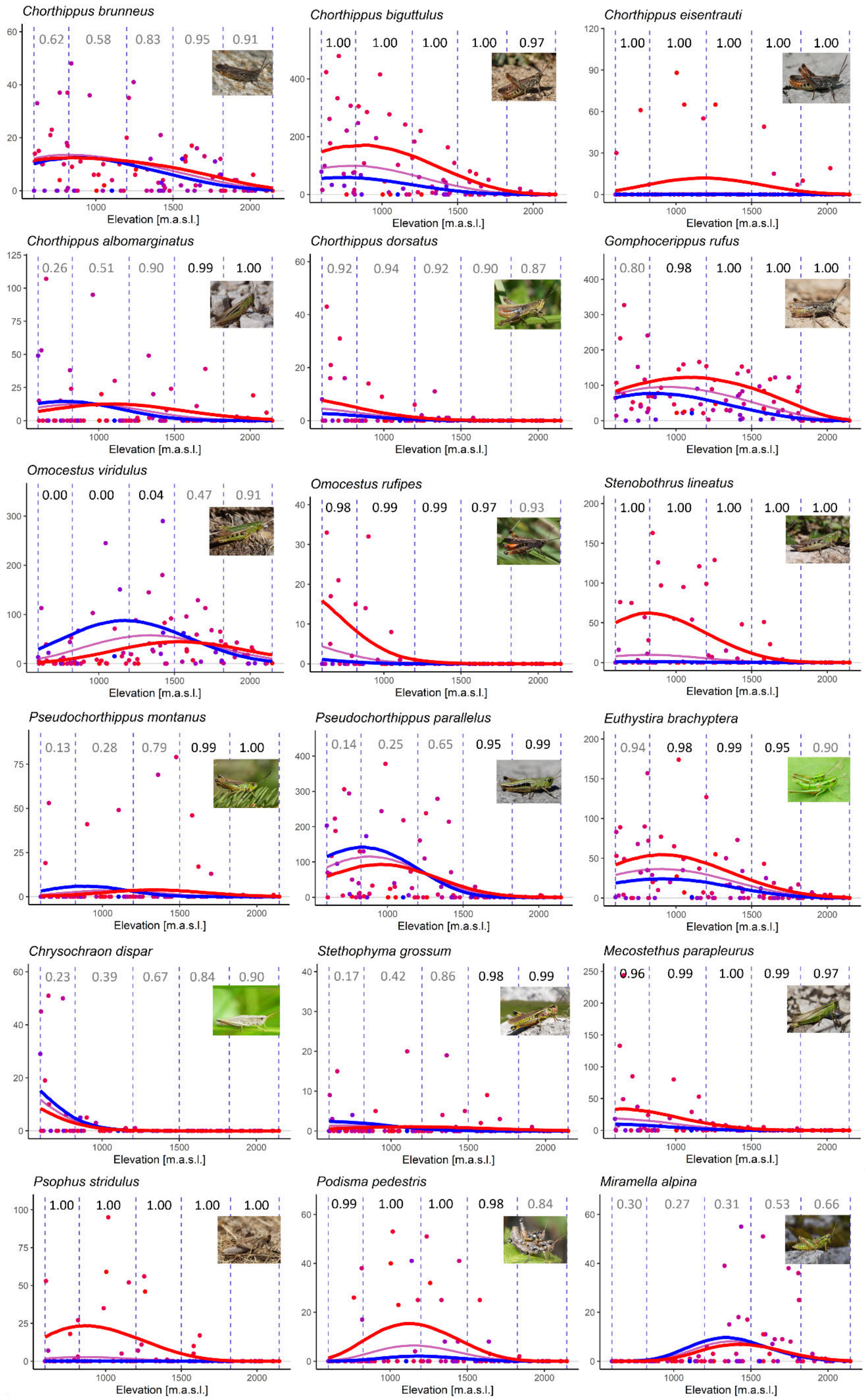


Figure S5. Mantel-tests based on Pearson's product-moment correlations demonstrating the matrix correlation of the Bray-Curtis dissimilarity of Orthoptera communities between site pairs and the A) elevational distance between the site pairs ($r = 0.46$, $p = 0.001$) and B) microclimatic contrasts between site pairs ($r = 0.19$, $p = 0.001$).


Chorthippus brunneus
0.62 0.58 0.83 0.95 0.91
60 40 20 0
1000 1500 2000
Elevation [m.a.s.l.]
Chorthippus biguttulus
1.00 1.00 1.00 1.00 0.97
400 200 0
1000 1500 2000
Elevation [m.a.s.l.]
Chorthippus eisentrauti
1.00 1.00 1.00 1.00 1.00
120 90 60 30 0
1000 1500 2000
Elevation [m.a.s.l.]
Chorthippus albomarginatus
0.26 0.51 0.90 0.99 1.00
125 100 75 50 25 0
1000 1500 2000
Elevation [m.a.s.l.]
Chorthippus dorsatus
0.92 0.94 0.92 0.90 0.87
60 40 20 0
1000 1500 2000
Elevation [m.a.s.l.]
Gomphocerippus rufus
0.80 0.98 1.00 1.00 1.00
400 300 200 100 0
1000 1500 2000
Elevation [m.a.s.l.]
Omocestus viridulus
0.00 0.00 0.04 0.47 0.91
300 200 100 0
1000 1500 2000
Elevation [m.a.s.l.]
Omocestus rufipes
0.98 0.99 0.99 0.97 0.93
40 30 20 10 0
1000 1500 2000
Elevation [m.a.s.l.]
Stenobothrus lineatus
1.00 1.00 1.00 1.00 1.00
200 150 100 50 0
1000 1500 2000
Elevation [m.a.s.l.]
Pseudochorthippus montanus
0.13 0.28 0.79 0.99 1.00
75 50 25 0
1000 1500 2000
Elevation [m.a.s.l.]
Pseudochorthippus parallelus
0.14 0.25 0.65 0.95 0.99
400 300 200 100 0
1000 1500 2000
Elevation [m.a.s.l.]
Euthystira brachyptera
0.94 0.98 0.99 0.95 0.90
200 150 100 50 0
1000 1500 2000
Elevation [m.a.s.l.]
Chrysochraon dispar
0.23 0.39 0.67 0.84 0.90
60 40 20 0
1000 1500 2000
Elevation [m.a.s.l.]
Stethophyma grossum
0.17 0.42 0.86 0.98 0.99
40 30 20 10 0
1000 1500 2000
Elevation [m.a.s.l.]
Mecostethus parapleurus
0.96 0.99 1.00 0.99 0.97
250 200 150 100 50 0
1000 1500 2000
Elevation [m.a.s.l.]
Psophus stridulus
1.00 1.00 1.00 1.00 1.00
100 75 50 25 0
1000 1500 2000
Elevation [m.a.s.l.]
Podisma pedestris
0.99 1.00 1.00 0.98 0.84
60 40 20 0
1000 1500 2000
Elevation [m.a.s.l.]
Miramella alpina
0.30 0.27 0.31 0.53 0.66
60 40 20 0
1000 1500 2000
Elevation [m.a.s.l.]

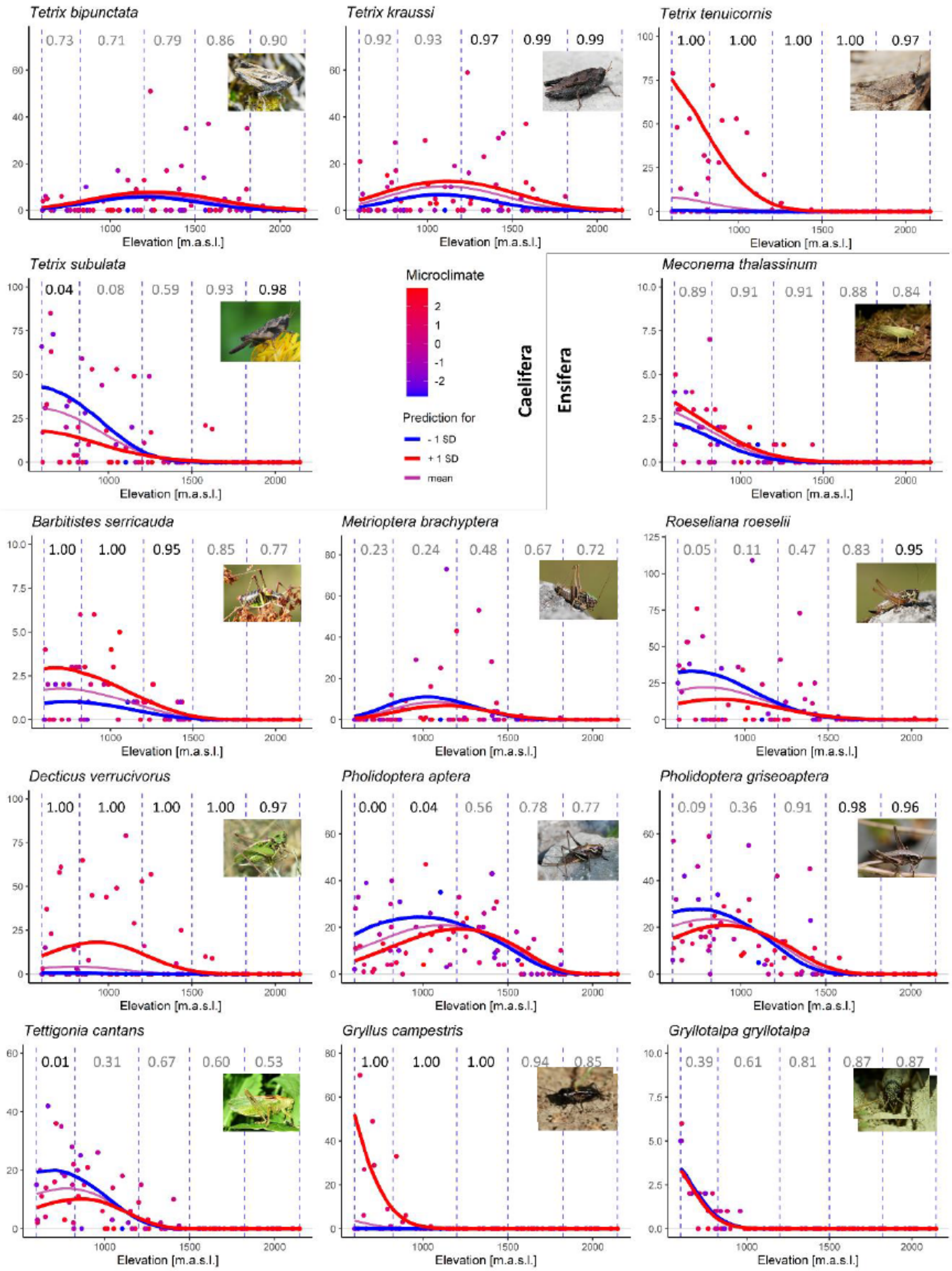


Figure S6. Orthoptera species responses along the elevational gradient. Shown are predicted median species abundances based on the combination of the presence-absence model and the abundance-conditional-on-presence model along the elevational gradient under warm (red line, + 1 SD microclimate), intermediate (purple line, mean microclimate), and cold microclimatic conditions (blue line, - 1 SD microclimate). Point colours represent microclimatic conditions at the sites (red = warm, purple = intermediate, blue = cold). Vertical dashed lines separate the submontane, montane, high-montane, subalpine, and alpine elevational zone from left to right. Numbers indicate the posterior probability of a positive impact of microclimate within each elevational zone. The black horizontal line divides species of the Caelifera and Ensifera suborder. Photo credits: Sebastian König, Louis Puille, Benjamin Dignal.

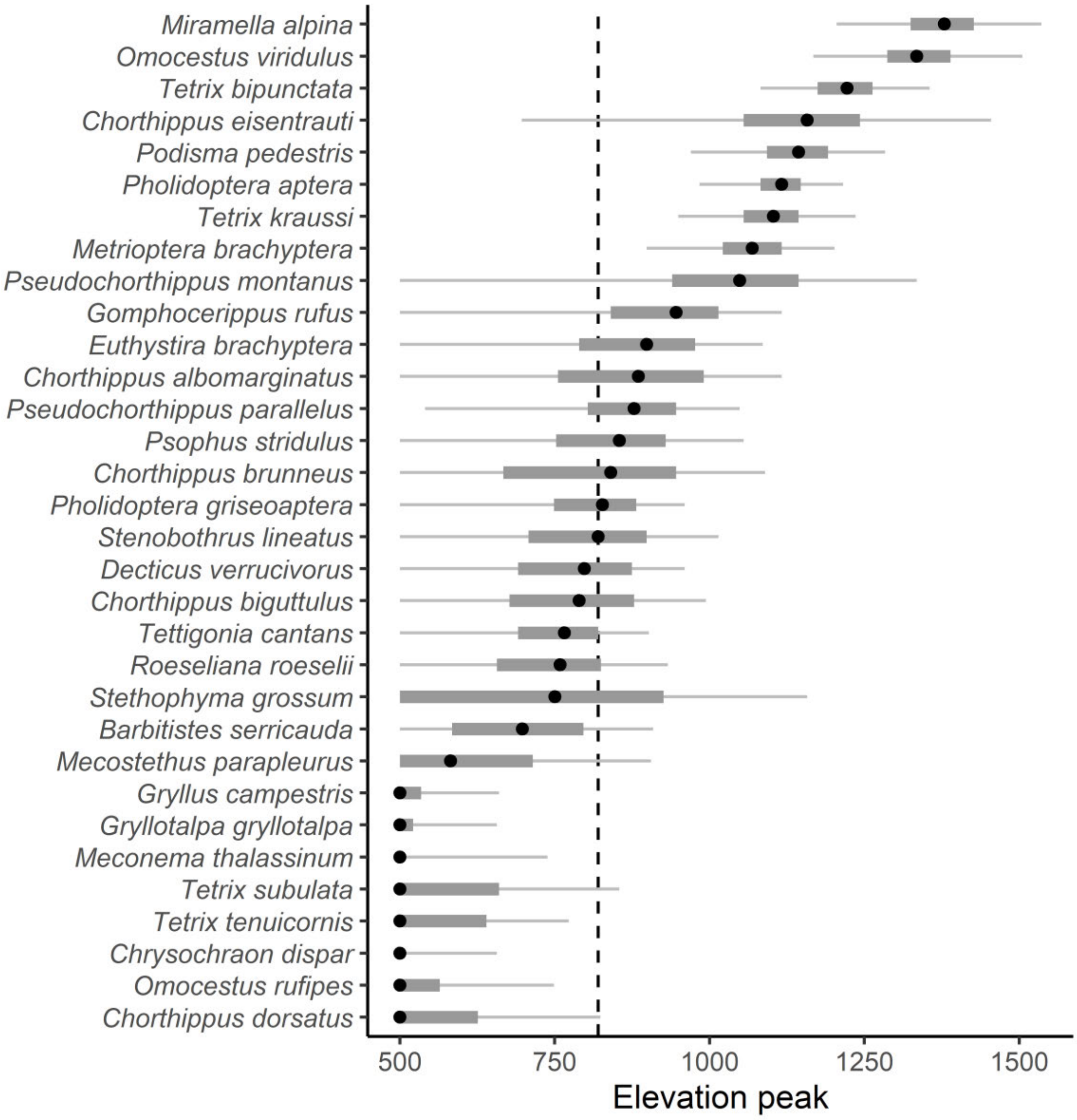


Figure S7. Elevational abundance peak estimates (estimated niche optima, total effect of elevation, m.a.s.l.) of Orthoptera communities (32 species) derived from HMSC analysis. Depicted are the median peak estimates (points), as well as the 75% (thick line) and the 95% credible intervals (thin line) for each species. Note that we did not predict abundance peaks below 500 m.a.s.l. due to the limited extent of our elevational gradient. The dashed line indicates the average species median elevational abundance peak at 824 m.a.s.l.

Table S4. Summarized elevational abundance peak estimates (m.a.s.l.) of Orthoptera communities (32 species) derived from HMSC analysis (total effect of elevation). Note that we did not predict abundance peaks below 500 m.a.s.l. due to the limited gradient extent.

species	**2.5%**	**25%**	**median**	**75%**	**97.5%**
Chorthippus dorsatus	<500	<500	<500	626	824
Chorthippus albomarginatus	<500	756	885	991	1,117
Chorthippus biguttulus	<500	677	790	878	994
Chorthippus brunneus	<500	667	841	946	1,090
Chorthippus eisentrauti	697	1,055	1,158	1,243	1,454
Gomphocerippus rufus	<500	841	946	1,014	1,117
Stenobothrus lineatus	<500	708	820	899	1015
Omocestus viridulus	1,168	1,287	1,335	1,389	1,505
Omocestus rufipes	<500	<500	500	565	749
Pseudochorthippus parallelus	541	803	878	946	1,049
Pseudochorthippus montanus	<500	939	1,048	1,144	1,335
Euthystira brachyptera	<500	790	899	977	1,086
Chrysochraon dispar	<500	<500	<500	<500	657
Mecostethus parapleurus	<500	<500	582	715	905
Stethophyma grossum	<500	<500	750	926	1,158
Psophus stridulus	<500	752	854	929	1,055
Podisma pedestris	970	1,093	1,144	1,192	1,284
Miramella alpina	1,205	1,324	1,379	1,427	1,536
Tetrix tenuicornis	<500	<500	<500	640	773
Tetrix subulata	<500	<500	<500	660	854
Tetrix kraussi	950	1,055	1,103	1,144	1,236
Tetrix bipunctata	1,082	1,175	1,222	1,263	1,355
Pholidoptera griseoaptera	<500	749	827	882	960
Pholidoptera aptera	984	1,083	1,117	1,147	1,215
Decticus verrucivorus	<500	691	798	875	960
Roeseliana roeselii	<500	657	759	824	933
Metrioptera brachyptera	899	1,021	1,069	1,117	1,202
Tettigonia cantans	<500	691	766	820	902
Barbitistes serricauda	<500	584	698	796	909
Meconema thalassinum	<500	<500	<500	<500	738
Gryllotalpa gryllotalpa	<500	<500	<500	521	657
Gryllus campestris	<500	<500	<500	534	660

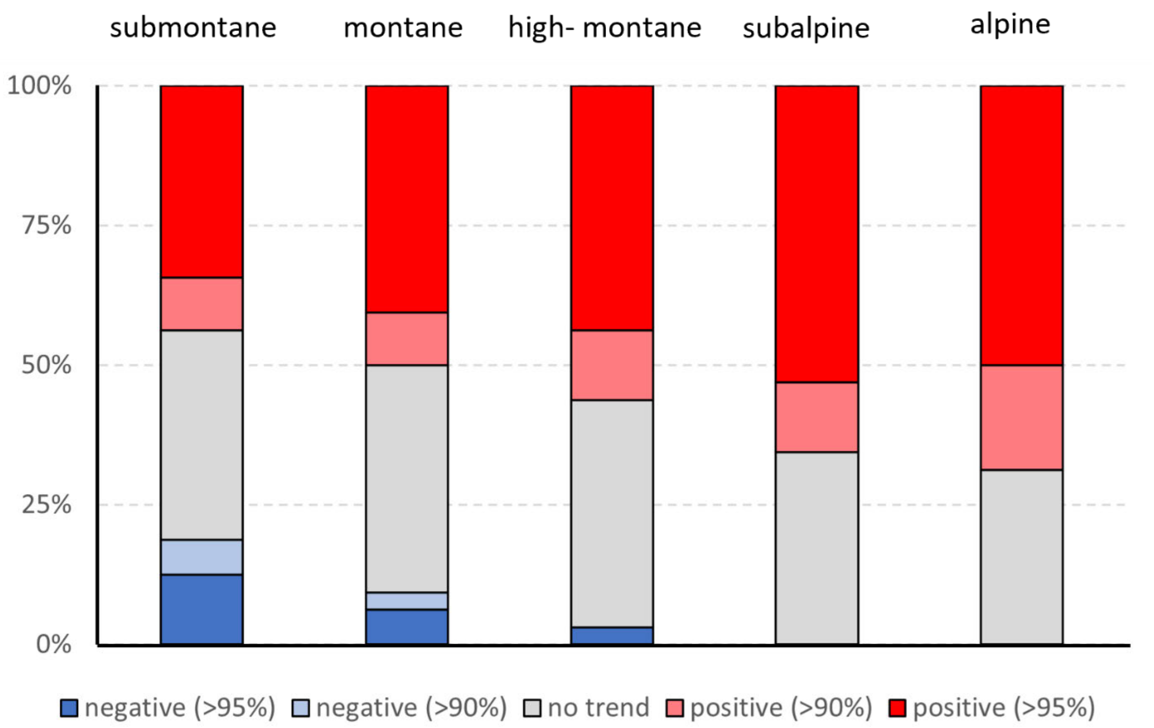


Figure S8. Orthoptera species responses to microclimate along the elevational gradient. Shown are the frequencies of higher, indifferent, or lower abundances of species at warm microclimate sites compared to cold microclimate sites averaged within the submontane, montane, high-montane, subalpine, and alpine elevational zone (red = positive response to microclimate with 0.95 posterior probability, pale red = positive response to microclimate with 0.90 posterior probability, blue = negative response to microclimate with 0.95 posterior probability, pale blue = negative response to microclimate with 0.90 posterior probability, grey = indifferent response to microclimate based on our data).

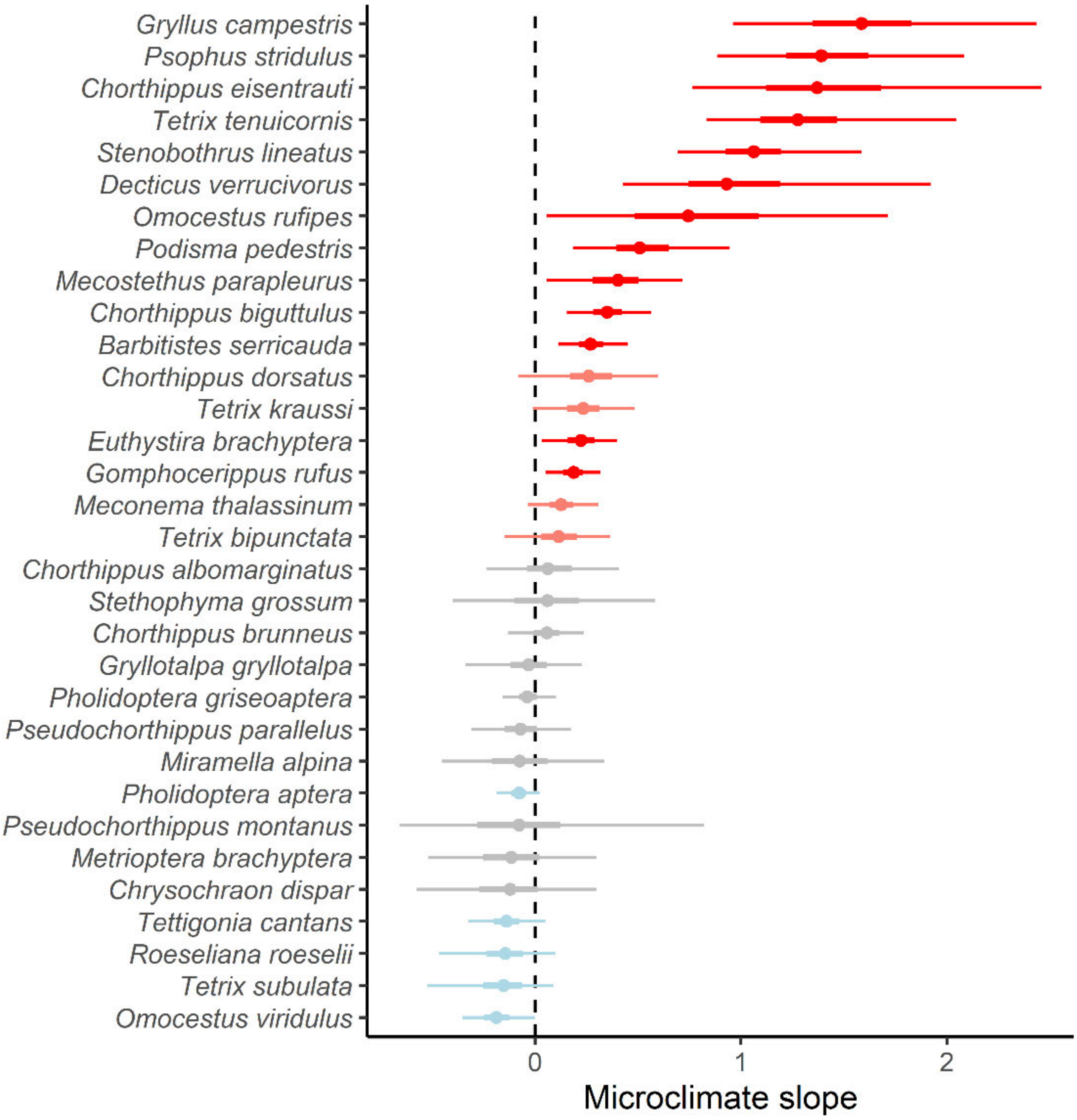


Figure S9. Summarized microclimate slope estimates derived from HMSC analysis of Orthoptera communities (32 species), averaged across all elevational zones and weighted by sampling intensity within the zones. Depicted are the median peak estimates (points), as well as the 75% (thick line) and the 95% credible intervals (thin line) for each species. If 95% credible intervals do not overlap with zero, we consider the effect of microclimate on the abundance of the species to be positive (red) or negative (blue) with high posterior probability, if the 75% CIs do not overlap with zero, there was only weak statistical support for positive (light red) or negative (light blue) effects and else no support for an overall effect of microclimate (grey).

Table S5. Summarized predicted microclimate slope estimates derived from HMSC analysis of Orthoptera communities (32 species), averaged across elevational zones and weighted by sampling intensity.

species	2.5%	25%	median	75%	97.5%	trend
Chorthippus dorsatus	-0.08	0.17	0.26	0.37	0.60	no trend
Chorthippus albomarginatus	-0.24	-0.04	0.06	0.18	0.41	no trend
Chorthippus biguttulus	0.15	0.28	0.35	0.42	0.57	increasing
Chorthippus brunneus	-0.13	-0.01	0.06	0.12	0.24	no trend
Chorthippus eisentrauti	0.76	1.12	1.37	1.68	2.46	increasing
Gomphocerippus rufus	0.05	0.14	0.19	0.23	0.32	increasing
Stenobothrus lineatus	0.69	0.93	1.06	1.20	1.59	increasing
Omocestus viridulus	-0.35	-0.25	-0.19	-0.12	0.00	no trend
Omocestus rufipes	0.06	0.48	0.74	1.09	1.71	increasing
Pseudochorthippus parallelus	-0.31	-0.15	-0.07	0.01	0.18	no trend
Pseudochorthippus montanus	-0.66	-0.28	-0.08	0.12	0.82	no trend
Euthystira brachyptera	0.03	0.16	0.22	0.29	0.40	increasing
Chrysochraon dispar	-0.58	-0.27	-0.12	0.01	0.30	no trend
Mecostethus parapleurus	0.05	0.28	0.40	0.50	0.72	increasing
Stethophyma grossum	-0.40	-0.10	0.06	0.21	0.58	no trend
Psophus stridulus	0.89	1.22	1.39	1.62	2.08	increasing
Podisma pedestris	0.18	0.40	0.51	0.65	0.95	increasing
Miramella alpina	-0.45	-0.21	-0.08	0.06	0.34	no trend
Tetrix tenuicornis	0.83	1.09	1.28	1.47	2.05	increasing
Tetrix subulata	-0.52	-0.25	-0.15	-0.06	0.09	no trend
Tetrix kraussi	-0.01	0.16	0.23	0.31	0.49	no trend
Tetrix bipunctata	-0.15	0.03	0.11	0.20	0.37	no trend
Pholidoptera griseoaptera	-0.16	-0.08	-0.04	0.01	0.10	no trend
Pholidoptera aptera	-0.19	-0.12	-0.08	-0.04	0.02	no trend
Decticus verrucivorus	0.43	0.75	0.93	1.19	1.92	increasing
Roeseliana roeselii	-0.47	-0.24	-0.14	-0.06	0.10	no trend
Metrioptera brachyptera	-0.52	-0.25	-0.11	0.02	0.30	no trend
Tettigonia cantans	-0.33	-0.20	-0.14	-0.08	0.05	no trend
Barbitistes serricauda	0.11	0.21	0.27	0.33	0.45	increasing
Meconema thalassinum	-0.04	0.07	0.13	0.19	0.31	no trend
Gryllotalpa gryllotalpa	-0.34	-0.12	-0.03	0.06	0.23	no trend
Gryllus campestris	0.96	1.35	1.59	1.83	2.44	increasing

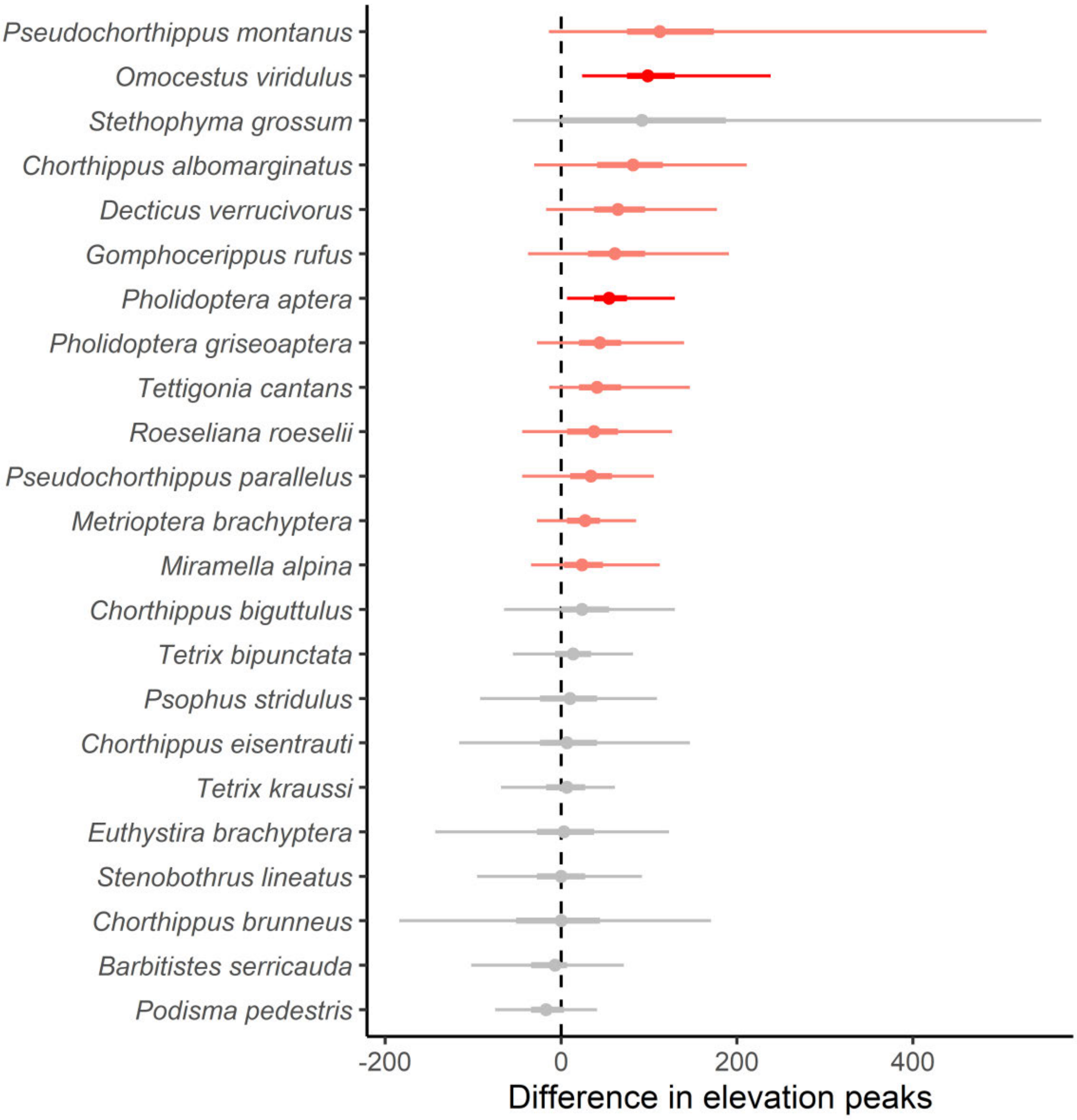


Figure S10. Predicted shifts in elevational abundance peaks (elevational optima [m]) between cold sites (- 1 SD microclimate) and warm sites (+ 1 SD microclimate) derived from HMSC analysis of Orthoptera communities (23 species). Note that we did not predict abundance peak shifts for species which did not peak above 600 m.a.s.l. elevation due to the limited extent of our elevational gradient. Depicted are the median peak shift estimates (points), as well as the 75% (thick line) and the 95% credible intervals (thin line) for each species. If 95% credible intervals do not overlap with zero, we consider the effect of microclimate on the abundance of the species to be positive (red) or negative (blue) with high posterior probability, if the 75% CIs do not overlap with zero, there was weak statistical support for positive (light red) or negative (light blue) effects and else no support for an effect of microclimate on the elevational abundance peaks (grey).

Table S6. Summarized predicted shifts in elevational abundance peaks [m] between cold sites (- 1 SD microclimate) and warm sites (+ 1 SD microclimate) derived from HMSC analysis of Orthoptera communities (24 species). Note that we did not predict abundance peak shifts for species which did not peak above 600 m.a.s.l. due to the limited extent of our elevational gradient.

species	**2.5%**	**25%**	**median**	**75%**	**97.5%**
Chorthippus albomarginatus	-30.66	40.88	81.76	115.83	211.22
Chorthippus biguttulus	-64.73	0.00	23.85	54.51	129.46
Chorthippus brunneus	-183.97	-51.1	0.00	44.29	170.51
Chorthippus eisentrauti	-115.83	-23.85	6.81	40.88	146.58
Gomphocerippus rufus	-37.47	30.66	61.32	95.39	190.87
Stenobothrus lineatus	-95.39	-27.25	0.00	27.25	91.98
Omocestus viridulus	23.85	74.95	98.8	129.46	238.48
Pseudochorthippus parallelus	-44.29	10.22	34.07	57.92	105.61
Pseudochorthippus montanus	-13.71	74.95	112.42	173.75	483.94
Euthystira brachyptera	-143.09	-27.25	3.41	37.47	122.73
Stethophyma grossum	-54.68	0.00	91.98	187.37	546.03
Psophus stridulus	-91.98	-23.85	10.22	40.88	109.1
Podisma pedestris	-74.95	-34.07	-17.03	3.41	40.88
Miramella alpina	-34.15	3.41	23.85	47.7	112.42
Tetrix kraussi	-68.14	-17.03	6.81	27.25	61.41
Tetrix bipunctata	-54.51	-6.81	13.63	34.07	81.76
Pholidoptera griseoaptera	-27.25	20.44	44.29	68.14	139.76
Pholidoptera aptera	6.81	37.47	54.51	74.95	129.46
Decticus verrucivorus	-17.03	37.47	64.73	95.39	177.24
Roeseliana roeselii	-44.29	6.81	37.47	64.73	126.14
Metrioptera brachyptera	-27.25	6.81	27.25	44.29	85.26
Tettigonia cantans	-13.63	20.44	40.88	68.14	146.49
Barbitistes serricauda	-102.2	-34.07	-6.81	6.81	71.54

Table S7. Summarized effects of elevation and microclimate on measured morphological characters of *Omocestus viridulus* and *Gomphocerippus rufus* individuals. *Est* = Posterior Mean Estimate, *Est.Er* = Estimate error, *2.5%* = lower 0.95 credible interval, *97.5%* = upper 0.95 credible interval, *B.ESS* = bulk effective sample size, *T.ESS* = tail effective sample size, *Rhat* = potential scale reduction factor on split chains.

Response	Species	Covariate	Est	Est.Er	2.5%	97.5%	Rhat	B.ESS	T.ESS
Tegmen length [mm]	*Omocestus viridulus* (*n* = 428)	Intercept	**15.55**	0.10	15.34	15.75	1.00	2,006	2,621
		Elevation	-0.11	0.11	-0.31	0.11	1.00	1,717	2,101
		Microclimate	0.01	0.08	-0.14	0.16	1.00	1,937	2,311
		Sex	**-2.15**	0.09	-2.34	-1.96	1.00	2,938	2,835
		Elevation : Microclimate	0.13	0.08	-0.03	0.29	1.00	2,020	2,390
		Elevation : Sex	-0.08	0.10	-0.27	0.11	1.00	2,602	2,974
		Microclimate : Sex	-0.04	0.08	-0.20	0.12	1.00	2,560	2,733
		Elevation : Microclimate : Sex	-0.12	0.07	-0.26	0.04	1.00	2,705	2,938
	Gomphocerippus rufus (*n* = 393)	Intercept	**13.84**	0.10	13.66	14.03	1.00	1,478	2,511
		Elevation	**-0.40**	0.10	-0.59	-0.20	1.00	1,370	1,900
		Microclimate	0.05	0.06	-0.08	0.17	1.00	1,711	2,366
		Sex	**-1.33**	0.08	-1.49	-1.16	1.00	3,420	3,279
		Elevation : Microclimate	0.10	0.07	-0.02	0.24	1.00	1,506	2,043
		Elevation : Sex	**0.44**	0.08	0.28	0.60	1.00	3,490	2,675
		Microclimate : Sex	-0.10	0.06	-0.22	0.02	1.00	2,886	2,772
		Elevation : Microclimate : Sex	-0.09	0.06	-0.21	0.03	1.00	2,806	3,001
Pronotum length [mm]	*Omocestus viridulus* (*n* = 438)	Intercept	**4.28**	0.03	4.23	4.33	1.00	2,207	2,847
		Elevation	**-0.06**	0.03	-0.11	0.00	1.00	1,883	2,754
		Microclimate	-0.01	0.02	-0.05	0.03	1.00	2,150	2,507
		Sex	**-0.91**	0.03	-0.96	-0.86	1.00	3,596	2,908
		Elevation : Microclimate	0.01	0.02	-0.03	0.05	1.00	1,924	2,417
		Elevation : Sex	**0.05**	0.02	0.00	0.10	1.00	3,754	3,126
		Microclimate : Sex	0.01	0.02	-0.03	0.05	1.00	3,612	3,042
		Elevation : Microclimate : Sex	0.01	0.02	-0.03	0.04	1.00	2,758	2,934
	Gomphocerippus rufus (*n* = 394)	Intercept	**4.12**	0.03	4.07	4.18	1.01	1,503	1,785
		Elevation	**-0.08**	0.03	-0.13	-0.02	1.00	1,347	2,011
		Microclimate	0.03	0.02	-0.01	0.06	1.00	1,559	2,339
		Sex	**-0.81**	0.02	-0.85	-0.76	1.00	4,614	2,757
		Elevation : Microclimate	-0.01	0.02	-0.05	0.02	1.00	1,535	1,989
		Elevation : Sex	**0.08**	0.02	0.03	0.12	1.00	3,821	3,070
		Microclimate : Sex	**-0.04**	0.02	-0.07	-0.01	1.00	2,927	2,725
		Elevation : Microclimate : Sex	0.02	0.02	-0.02	0.05	1.00	3,070	2,984
Relative wing length	*Omocestus viridulus* (*n* = 436)	Intercept	**3.65**	0.02	3.60	3.70	1.00	3,759	3,186
		Elevation	-0.00	0.02	-0.04	0.05	1.00	2,767	2,565
		Microclimate	0.02	0.02	-0.02	0.05	1.00	2,940	2,997
		Sex	**0.33**	0.03	0.28	0.39	1.00	4,842	3,106
		Elevation : Microclimate	0.02	0.02	-0.02	0.05	1.00	3,193	2,725
		Elevation : Sex	**-0.06**	0.03	-0.11	-0.00	1.00	3,576	2,754
		Microclimate : Sex	-0.02	0.02	-0.06	0.03	1.00	3,558	3,287
		Elevation : Microclimate : Sex	-0.03	0.02	-0.07	0.02	1.00	3,489	2,935

	Gomphocerippus rufus (*n* = 393)	Intercept	**3.36**	0.03	3.31	3.41	1.00	2,665	3,014
		Elevation	-0.04	0.03	-0.09	0.01	1.00	1,908	2,395
		Microclimate	-0.01	0.02	-0.05	0.02	1.00	2,160	2,772
		Sex	**0.42**	0.03	0.37	0.48	1.00	3,289	3,255
		Elevation : Microclimate	**0.03**	0.02	-0.00	0.07	1.00	2,207	2,486
		Elevation : Sex	0.04	0.03	-0.01	0.10	1.00	3,676	3,358
		Microclimate : Sex	0.01	0.02	-0.03	0.05	1.00	3,119	3,025
		Elevation : Microclimate : Sex	-0.03	0.02	-0.07	0.01	1.00	3,383	2,748

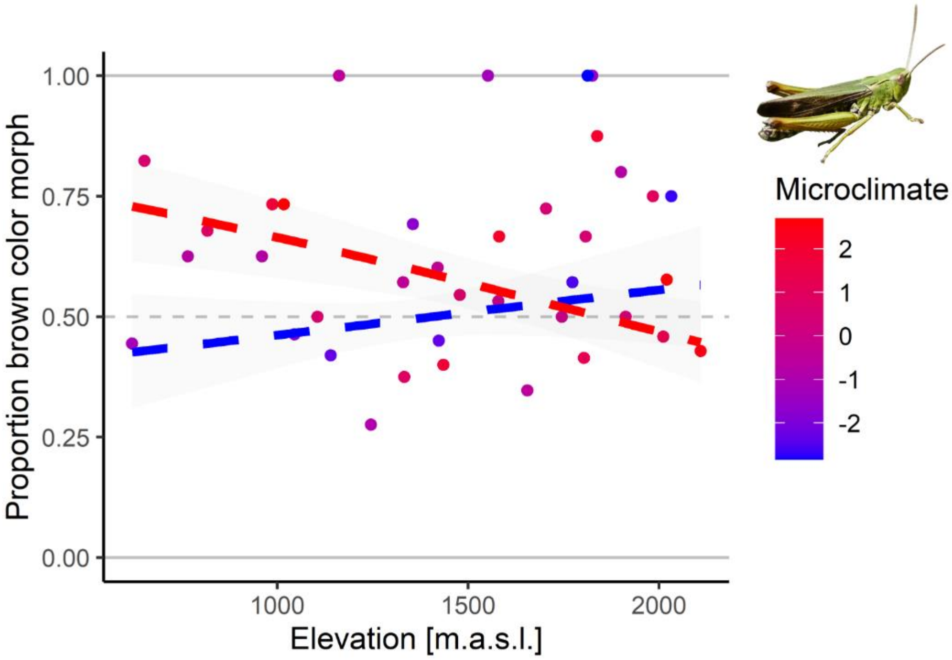


Figure S11. Color morph frequencies (brown morph vs. green morph) within populations of *Omocestus viridulus* did not significantly vary along the elevational gradient, but the frequency of brown morphs was higher when microclimate at the study site was warm (Tab. S8).

Table S8. Summarized effects of elevation and microclimate on the proportion of brown color morphs within *Omocestus viridulus* populations (n = 38). *Estimate* = Posterior Mean Estimate, *2.5%* = lower 0.95 credible interval, *97.5%* = upper 0.95 credible interval, *B.ESS* = bulk effective sample size, *T.ESS* = tail effective sample size, *Rhat* = potential scale reduction factor on split chains.

Covariate	**Estimate**	**Est.Error**	**2.5%**	**97.5%**	**Rhat**	**Bulk.ESS**	**Tail.ESS**
Intercept	0.44	0.30	-0.13	1.03	1.00	5,157	4,085
Elevation	-0.00	0.00	-0.00	0.00	1.00	5,277	3,536
Microclimate	0.65	0.20	0.26	1.03	1.00	1,692	1,718
Elevation : Microclimate	-0.00	0.00	-0.00	-0.00	1.00	1,818	2,072

Chapter III

Phylogenetic relatedness of food plants reveals highest insect herbivore specialisation at intermediate temperatures along a broad climatic gradient

“What escapes the eye, however, is a much more insidious kind of extinction: the extinction of ecological interactions”

— *Daniel Janzen (1974)*

“How sad to think that nature speaks and mankind doesn't listen”

— *Victor Hugo*

“Three hundred trout are needed to support one man for a year. The trout, in turn, must consume 90,000 frogs, that must consume 27 million grasshoppers that live off 1,000 tons of grass.”

— *G. Tyler Miller Jr*

The composition and richness of herbivore and plant assemblages change along climatic gradients, but knowledge about associated shifts in specialisation is scarce and lacks controlling for the abundance and phylogeny of interaction partners. Thus, we aimed to test whether the specialisation of phytophagous insects in insect - plant – interaction networks decreases towards cold habitats as predicted by the ‘altitude niche-breadth hypothesis’ to forecast possible consequences of interaction rewiring under climate change.

We used a non-invasive, standardized metabarcoding approach to reconstruct dietary relationships of Orthoptera species as a major insect herbivore taxon along a broad temperature gradient (~12°C) in southern Germany. Based on orthopteran surveys, feeding observations, collection of faecal pellets from > 3,000 individuals of 54 species, and parallel vegetation surveys on 41 grassland sites, we quantified plant resource availability and its use by herbivores.

Herbivore assemblages were richer in species and individuals at sites with high summer temperatures, while plant richness peaked at intermediate temperatures. Corresponding interaction networks were most specialised in warm habitats. Considering phylogenetic relationships of plant resources, however, the specialisation pattern was not linear but peaked at intermediate temperatures, mediated by herbivores feeding on a narrow range of phylogenetically related resources.

Our study provides empirical evidence of resource specialisation of insect herbivores along a climatic gradient, demonstrating that resource phylogeny, availability, and temperature interactively shape the specialisation of herbivore assemblages. Instead of low specialisation levels only in cold, harsh habitats, our results suggest increased generalist feeding due to intraspecific changes and compositional differences at both ends of the microclimatic gradient. We conclude that this non-linear change of phylogeny-based resource specialisation questions predictions derived from the ‘altitude-niche breadth hypothesis’ and highlights the currently limited understanding of how plant-herbivore interactions will change under future climatic conditions.

3.1 *INTRODUCTION*

Elevational and latitudinal climatic gradients are major drivers of species richness, community composition, and biotic interactions (Gaston 2000, McCain & Grytnes 2010, Peters et al 2019, Rahbek 1995, Sanders & Rahbek 2012). However, understanding the evolutionary and ecological processes underlying biodiversity patterns and predicting responses to future climate change are crucial challenges due to the complex interplay among environmental drivers and biotic responses (Descombes et al 2020, Devictor et al 2012, Masson-Delmotte et al 2021, Tylianakis et al 2008). Along environmental gradients, a decrease in temperature is often paralleled by declines of species diversity (Descombes et al 2017a, Gaston 2000, McCain & Grytnes 2010, Sanders & Rahbek 2012) and species abundances (Peters et al 2019, Pitteloud et al 2020). Diversity declines may be caused by lower rates of biotic processes or lower evolutionary diversification rates (Peters et al 2016a), likely influencing the strength of interspecific interactions (Pellissier et al 2018).

Interaction network analysis is a powerful tool to study responses of biotic interactions to environmental variation. Quantitative metrics allow a characterization of specialisation and robustness of communities and unravel niche breadths of species (Blüthgen et al 2006). Along environmental gradients, the structure of networks changes due to shifts in resource partitioning, specialisation, and turnover of involved species caused by environmental filtering (Gravel et al 2019, Pellissier et al 2018, Pitteloud et al 2021, Welti & Joern 2015). Thereby, interspecific interactions are often observed to be less specialized under cold, variable temperature regimes (Classen et al 2020, Forister et al 2015, Hoiss et al 2015, Pellissier et al 2018). Such patterns can be the result of trait-driven or stochastic processes, while evidence for the significance of partner availability in driving ecological interactions is growing (Simmons et al 2019). However, empirical network studies on plant - insect-herbivore relationships remain underrepresented (but see Forister et al 2015, Pellissier et al 2012, Pitteloud et al 2021, Welti et al 2019).

Due to their ubiquity and significance in most terrestrial ecosystems, insect herbivores play a key role in shaping plant communities and fostering the coexistence of species through density dependent resource exploitation (Bagchi et al 2014, Connell et al 1971, Janzen 1970). With approximately half a million described species (Hardy et al 2020), phytophagous insects are exceptionally diverse, and most of them are highly specialized. However, reasons for their specificity remain debated (Hardy et al 2020) and characteristics of diet breadth are poorly documented (Maunsell et al 2015). Besides genetic constraints, environmental clines such as temperature gradients may influence the diet breadth of insect herbivores (Hardy et al 2020, Pellissier et al 2012).

Higher levels of generality of herbivore consumers in cold habitats can be explained by different mechanisms. Summarizing biotic and abiotic processes, the ‘altitude niche-breadth hypothesis’ predicts higher levels of generalisation of phytophagous species which inhabit high-elevation habitats compared to lowland species (Rasmann et al 2014). Variability of temperature, precipitation, and wind speed increase with elevation. As assumed in the ‘latitude niche-breadth hypothesis’, such conditions would be characterized by considerably varying population sizes, triggering generalisation (Rasmann et al 2014). Cooler and more variable temperatures might reduce search and digestive efficiency in ectothermic organisms (Hodkinson 2005), which could, together with environmental unpredictability, necessitate the evolution of broader diets in cold habitats (Macarthur & Levins 1967, Pitteloud et al 2021). Furthermore, high herbivore densities in warm environments might reinforce specialized interactions to escape competition (Hodkinson 2005, Macarthur & Levins 1967) and plant defence (Rasmann et al 2014). Derived from the coevolution of plant defences and herbivore specialisation, generalisation is predicted to be favoured by lower levels of plant defences in cold habitats caused by abiotic mechanisms and release from herbivore pressure (Descombes et al 2017a, Pellissier et al 2012, Rasmann et al 2014). Nevertheless, climate change could rewire trophic interactions between plants and herbivores (van der Putten et al 2004), as range shifts of insects have been shown to be faster than those of plants (Alexander et al 2018, Bässler et al 2013, Lenoir et al 2010).

Evidence of expected shifts in consumer specialisation with temperature is scarce, regularly lacks controlling for abundance and phylogenetic relationships of resources (but see Novotny et al 2006) and the underlying processes are little understood (Pringle & Hutchinson 2020). Most studies currently use taxonomic identity to calculate interaction network metrics when explaining variation of network characteristics along environmental gradients (Classen et al 2020, Pitteloud et al 2021). Regardless of the number of plant species in the diet of a phytophagous insect, its dietary niche breadth is also determined by characteristics of the ingested resources, as plants differ in their costs and gains of use. Since phylogenetically related taxa often share similar traits (Goberna & Verdú 2016, Swenson 2014, Swenson & Enquist 2007), phylogenetic distances can be interpreted as an integration of plant traits such as chemical defences and mechanical properties and, thereby, estimate the trait-based dietary niche breadth of herbivore species (Jorge et al 2017, Pardo-De la Hoz et al 2022, Rasmann & Agrawal 2011). Thus, measuring specialisation levels of herbivores requires the inclusion of functional traits or phylogenetic relationships for a more realistic assessment of its niche breadth (Jorge et al 2017, Pardo-De la Hoz et al 2022).

On the other hand, resource specialisation can be a function of resource diversity. The degree of specialisation of phytophagous insects differs, biased by resource abundance. Thus, the availability of diverse plant resources can support populations of different specialised herbivore species, at least in productive climate zones (Novotny et al 2006). As plant diversity often shows non-linear relationships with temperature (Descombes et al 2017a, Hoiss et al 2013), the analysis of steep climatic gradients could help to disentangle the drivers of herbivore specialisation and foster the predictive power of interaction rewiring under climate change (Descombes et al 2020).

Orthoptera are among the dominant insect herbivores in temperate open grassland habitats with crucial impact on the functioning of these ecosystems. Their broad spectrum of resource use in grassland ecosystems is unique, as they remove up to 30% of the phanerogam plant biomass and accelerate nutrient cycling by concentrating nitrogen (Blumer & Diemer 1996). As most Orthoptera species are thermophilic in Central Europe (Poniatowski et al 2020), they are expected to track changes in climate especially fast while plants lag, leading to novel plant-herbivore interactions (Descombes et al 2020). Most species are categorized as generalist feeders, thus consume plant species from numerous families (Franzke et al 2010, Joern 1979), and mix diets to increase their fitness (Bernays & Bright 2005, Berner et al 2005, Unsicker et al 2008). However, some subfamilies show distinct preferences for certain plant families; for example, grasshoppers (Gomphocerinae) often prefer grasses (Poaceae), whereas species of the subfamily Melanoplinae prefer forbs (dicotyledonous plants), and groundhoppers (Tetrigidae) regularly select algae, lichen, and mosses as food resources based on phylogenetic, co-evolutionary constraints, as well as morphological and physiological trait matching, or still unknown mechanisms (Franzke et al 2010, Ibanez et al 2013a, McClenaghan et al 2015, Stockey et al 2022). Nevertheless, context dependent resource specialisation below family level is largely unknown due to methodological constraints which have only recently been solved using molecular techniques (Roslin et al 2019).

In this study, we applied an adapted metabarcoding protocol to in-field collected Orthoptera faeces to reconstruct dietary information at the assemblage level. Orthoptera and plants were recorded on 41 study sites along a broad temperature gradient in southern Germany. We aimed to understand how temperature and differences in resource availability explain the range of used plant resources and shifts in the specialisation of plant-herbivore interactions. We partitioned herbivore specialisation in taxonomic identity-based interaction specialisation (*d'*) and resource phylogeny-based dietary specialisation (*DSI**, *PSS*). Derived from the ‘altitude niche-breadth hypothesis’, we expected herbivore species to be less selective in cold habitats, which would lead to (1) a decrease in interaction specialisation at species level (and subsequent effects at ecological network level) along with declining temperatures and resource diversity, and (2) increasing dietary niche breadths (less phylogenetically clustered diets) of herbivores towards cold and resource species-poor habitats.

3.2 *MATERIALS AND METHODS*

3.2.1 Study region and sites

We studied open grassland sites on calcareous bedrock along a temperature gradient in southern Germany (Bavaria). Within this heterogeneous region, we selected 41 study sites between 250 and 2,100 m a.s.l. (Fig. S1) along the slopes of several mountains in the northern limestone Alps (National Park Berchtesgaden) and in the lowlands (Lower Franconia), covering an evenly distributed microclimatic gradient of 12°C – 24°C mean summer temperature (seasonal temperatures measured at the sites). Neighbouring sites in the Alps had at least 200 m difference in elevation. All grasslands were either extensively managed (low impact) or unmanaged. Our recordings at the established study sites were concentrated to a homogeneous area, covering 60 x 60 m. At these, we also recorded aboveground temperature at intervals of 2 h from May until October 2019 with 4 - 5 temperature-loggers (ibutton, Maxim Integrated) per site, installed 2 cm above soil level. We used the mean temperature measured during the observation period (summer) instead of elevation (cor = -0.93, t = -16.24, df = 39, p = <0.001) as a proxy for microclimatic variation along the gradient, as it also accounts for microclimatic differences resulting from vegetation structure, inclination, exposition, wind speed, atmospheric moisture, and cloud cover (Hodkinson 2005, Hoiss et al 2013).

3.2.2 Orthoptera field surveys

Orthoptera assemblages were recorded with four surveys at all study sites between June and October 2019. During each survey round, the sites were visited within two weeks from lowlands to highlands, following Orthoptera phenology. Each variable transect walk consisted of 5 sub-transects of 10 minutes within the study sites' margins to cover the microhabitat variation. Surveys were performed on warm, sunny days, representing maximum activity conditions for Orthoptera in the region (König & Krauss 2019). Species were identified visually and by their stridulation in field. Due to low detectability, we excluded the soil dwelling species *Gryllotalpa gryllotalpa* (L.) and *Myrmecophilus acervorum* (Panzer) from sampling results, leading to near 100 % sampling completeness (Abundance-based Coverage Estimator based on sub-transects; ACE = 0.99; Fig. S2).

3.2.3 Orthoptera turnover

To quantify turnover rates amongst Orthopteran assemblages along the temperature gradient, we computed β-diversity as the abundance-based dissimilarities between all pairs of assemblages with a temperature difference below 1.5°C. We partitioned the total differences into balanced variation in abundance (turnover equivalent of incidence-based β-diversity) and abundance gradients, in which one assemblage is a subset of another (nestedness-resultant equivalent of incidence-based β-diversity) by using the package ‘betapart’ (Baselga 2017). Low values in balanced variation indicate a greater proportion of shared species abundances between site pairs, while high values of abundance gradients indicate that assemblages with low abundances are subsets of assemblages with high abundances of similar composition (Baselga 2017). We related the β-diversity indices to the mean temperature of each pair of sites using generalized additive models, allowing penalization to zero. Deviations from an intercept-only model would indicate a non-constant balanced variation or abundance gradient rate with temperature (Descombes et al 2017b).

3.2.4 Plant field surveys

We recorded vegetation composition and cover of vascular plants and mosses at all study sites in ten randomly selected 4 m^2 plots in June - July as well as in August - September 2019, respectively. Species identification followed Oberdorfer (2001) and cover was estimated using the DOMIN-scale (Currall 1987). The DOMIN-scale consists of a non-linear ten-step classification of mean percent cover values for plants (1 = 0.05%, 2 = 0.5%, 3 = 2.5%, 4 = 7.0%, 5 = 17.0%, 6 = 28.5%, 7 = 41.0%, 8 = 62.0%, 9 = 84.5%, 10 = 97.5%). Species cover estimates were transformed back and averaged across the 20 plots per site before normalization to estimate percent cover of the occurring plant species (Hoiss et al 2013). For subsequent phylogenetic analyses, we employed a dated phylogeny of all vascular plant species in our study (Durka & Michalski 2012), which was expanded by a phylogeny of the occurring bryophyte species (Fig. S3). The bryophyte phylogeny was estimated based on two molecular markers (ITS2: 258 bp, TrnL-TrnP: 396 bp). We aligned sequences downloaded from GenBank with the MUSCLE algorithm (Edgar 2004), trimmed, and concatenated both alignments. A phylogenetic tree was calculated based on Bayesian inference with BEAST v.2.6.2 (Bouckaert et al 2014), with linked trees, GTR + G (ITS2) and HKY + I (TrnL-TrnP) substitution models for the partitions, and a point-calibrated molecular clock. The clock was calibrated using the split of mosses and liverworts estimated at around 465 Ma (Morris et al 2018), while choosing a relaxed clock and a Yule speciation process as tree priors. Monophyly was enforced for Bryidae and Dicranidae using age estimates obtained from Laenen et al (2014). We ran the analyses for 50,000,000 generations, sampling every 5,000 steps before generating a consensus tree, discarding a burn-in of 10%. The resulting phylogeny was linked to the vascular plant phylogeny at an estimated divergence time of crown embryophytes at around 500 Ma (Morris et al 2018). Based on this phylogeny, we calculated the phylogenetic community structure of plant assemblages as the deviation of weighted mean pairwise distances (w*MPD*s), a common metric of phylogenetic α-diversity, from random communities, maintaining species occurrence frequency and sample species richness

(‘Independent swap algorithm’). We multiplied the standardized effect size by -1 to obtain the net relatedness index (*NRI*), where negative values indicate phylogenetic overdispersion and positive values indicate clustering.

3.2.5 Faecal sampling and feeding observations

To obtain dietary information of the locally occurring Orthoptera populations at the study sites, we collected faecal samples in August 2019. We caught 10 (± 1) adult individuals (5 males and 5 females) per species on each site (hereafter referred to as population, omitting small populations < 10 individuals), transferred them separately into sterile falcon tubes, waited until each animal produced 2 - 3 faecal pellets and released them straight at the site. With this non-invasive method, we aimed to minimize the impact on local Orthoptera population densities. After release, we carefully collected and pooled faecal samples into one common sample per distinct population (one sample per species and study site). To avoid degradation of DNA, we immediately added DNA/RNA shield (ZymoBIOMICS) to the samples. The faecal samples were then used to reconstruct the trophic network using DNA metabarcoding techniques. To form a basis for reviewing our network reconstructions and compare methodologies, we additionally visually recorded feeding interactions (evidence for insects chewing on vegetation) between Orthoptera and their resource plants on all study sites.

3.2.6 DNA extraction, amplification, sequencing, and in-silico taxa assignment

Laboratory workflow included DNA isolation from faecal samples, library preparation, indexing, quality control, normalization, pooling, quantification, and sequencing. DNA extractions of faecal samples were performed using the ZymoBIOMICS DNA Miniprep Kit, following the manufacturer’s instructions. After DNA extraction, we amplified the ITS2 rDNA region, which is recognised as a universal plant DNA barcode to identify species at a high taxonomic resolution (Moorhouse-Gann et al 2018, Sickel et al 2015). We followed a dual-indexing strategy to generate a pooled amplicon library with a combination of plant barcoding primers expanded for Illumina conformity (Sickel et al 2015).

PCRs of samples and controls were conducted in triplicates to reduce stochastic effects of random amplification bias with 1 µl of template DNA sample (~1 nmol/µl) in each reaction. This procedure improves the robustness of PCR reactions. We used PCR Phusion Master Mix (New England Biolabs, UK), along with the two indexed primers in a unique combination for each sample. PCR conditions were adjusted according to the primer’s guidelines. Samples were initially denatured at 95°C for 4 min and then amplified with 37 cycles of 95°C for 40 s, 49°C for

40 s, and 72°C for 5 min. For final extension, the program ended with a step of 72°C for 10 min. After amplification, multiplexed triplicates were combined, and PCR success was checked through gel electrophoresis in a 1% agarose gel. As positive controls, we used faecal samples of known composition from feeding experiments, processed in parallel to samples, while negative controls contained samples with preservation solution and/or reagents without faeces taken at multiple steps of the workflow to control for possible contaminations in the field and laboratory.

The ITS2 rDNA library was pooled after DNA normalization to equimolar ratio (Invitrogen SequalPrep Plate Normalization Kit, Thermo Fisher Scientific, Life Technologies). Fragment length distributions were verified with the Bioanalyzer 2200 (Agilent) with High Sensitivity DNA Chips and dsDNA concentration was quantified using a Qubit II Fluorometer and the dsDNA High-Sensitivity Assay Kit (Thermo Fisher Scientific, Life Technologies). The pooled amplicon libraries were sequenced in-house on a MiSeq platform (Department of Human Genetics, University of Wuerzburg, Germany), using a v2 2x250 cycle reagent cartridge spiked with custom index, Read1 and Read2 sequencing primers to bind to the unique ITS2 primers (Sickel et al 2015), and 5% PhiXv3, a control library for low diversity Illumina sequencing runs (Illumina 2019).

We used VSEARCH v2.14.2 (Rognes et al 2016) to join paired ends of forward and reverse reads. We also used VSEARCH to remove reads shorter than 150 bp, quality filtering (EE < 1) (Edgar & Flyvbjerg 2015), de-novo chimera filtering (following UCHIME3) (Edgar 2016b), and definition of amplicon sequence variants (ASVs) (Edgar 2016b), as previously done for plants (Elliott et al 2021). The ITS2 rDNA reads were first directly mapped with global alignments against a floral ITS2 reference database for Berchtesgaden generated with the BCdatabaser (Keller et al 2020) tool also with VSEARCH and an identity cut-off threshold of 97%, before mapping against a database for Germany. For still unclassified reads, we used SINTAX (Edgar 2016b) to assign taxonomic levels as deep as possible with a global reference database (Ankenbrand et al 2015).

The ASV community matrix was filtered by retaining only counts of embryophyte plant taxa identified at the focal sites and the surroundings using our field surveys and additional plant inventories, as well as regional species occurrence lists (~9% of the reads per sample, filtering was independent of the temperature gradient, Fig. S3). ASV counts at the family and genus level were conservatively allocated among the affiliated species occurring at the sites using our feeding observations. To control for unbalanced sequencing depths, we computed relative read abundances (RRAs) that were further used as a semi-quantitative estimations of interaction intensities (Deagle et al 2019, Descombes et al 2020, Welti et al 2019).

3.2.6 Network reconstruction

We complemented site-level interaction networks by adding occurring, but not eaten plant species to the trophic networks with an interaction intensity of zero (Descombes et al 2020). Finally, we compared the filtered amplicon sequence table with observed feeding interactions from the study sites to check congruence of molecular and traditional methods (Fig. S5). For network-level analyses, we calculated the interaction intensity by multiplication of relative read abundances with the Orthoptera species abundance at the respective study site (Welti et al 2019).

3.2.7 Interaction specialisation based on taxonomic identity

Data were analysed in R 3.6.4 (R Core Team 2019) with the packages phyloseq v1.22.3 (McMurdie & Holmes 2013), vegan v2.5-7 (Oksanen et al 2020), and mgcv (Wood 2011). We computed network metrics using the R package econullnetr (Vaughan et al 2018), which is a wrapper for bipartite functionalities (Dormann et al 2008). As we expect covariation of network size along the sampled temperature gradient, we used measures such as network specialisation (Shannon entropy, *H2'*) and herbivore interaction specialisation on species level (Kullback-Leibler distance, *d'*), which are independent of variation in network-size and sampling intensity (Blüthgen et al 2006). Hence, to disentangle the effect of the non-random interactions (e.g., resource preference and avoidance) on the metric from the influence of inherent bias of network metric calculation (e.g., network size, relative abundance of different taxa, sampling completeness), we used a null model approach. We selected null models, which predict interaction strengths for each pair of consumer and resource species depending on resource availability. In this regard, proportions were drawn from a Dirichlet distribution with shape parameters determined by the relative abundance of the different resources (Vaughan et al 2018). Thus, 500 random network matrices were rearranged for each study site according to their herbivore species composition and plant resource cover. Deviances of the observed metric and the one resulting from random networks were considered significant, if it was outside the 2.5 – 97.5% quantile interval of the metric obtained from the randomized networks.

3.2.8 Interaction specialisation based on plant phylogeny

Recent advances in calculating resource specialisation of herbivores were proposed by Jorge et al (2017) and Pardo-De la Hoz et al (2022). Rather than using counts of resource species or higher taxonomic categories to measure diet breadths of herbivores, the rescaled standardized distance-based specialisation index (*DSI**) weighs the degree of specialisation by the phylogenetic similarity of resources and accounts for their availability. A reason to include the phylogenetic relatedness of species within measures of host specialisation is that similar species will likely share comparable defensive adaptations and mechanical properties, so that herbivores would encounter familiar costs of use (Jorge et al 2017). In this framework, a specialist is defined as a species that selects a subset of resource species more related (phylogenetically clustered) than expected by chance from a given resource spectrum (maximum specialisation, *DSI** = 1) (Jorge et al 2017, Redmond et al 2019). On the other hand, non-selective feeders use resource species proportional to their availability (*DSI** = 0) and generalists consume resources less related than expected by chance (maximum overdispersion, *DSI** = -1). The distance-based resource specialisation is a *z*-value and relies on the calculation of weighted mean pairwise distances (w*MPD*s, raw measure of phylogenetic diet breadth) between used resources and annealed maxima based on cover-weighted resource availability. It was calculated separately for every species and network using the observed plant cover as reference for null models. Thereafter, we calculated the community-weighted mean values of the *DSI** and w*MPD*s at assemblage level. (*DSI** values for all sampled species in our study are provided in Tab. S3). Combining *d’* and phylogenetic α-diversity of resources by decoupling the null models for interaction frequency and phylogenetic distance, we also computed the phylogenetic structure of specialisation (*PSS*) as an alternative, improved measure for herbivore specialisation (see supplementary methods, Pardo-De la Hoz et al 2022).

3.2.9 Variation of metrics along the climatic gradient

Relationships between mean summer temperature, plant species richness, phylogenetic community structure of plant assemblages (*NRI*), Orthoptera species richness, Orthoptera abundance, as well as the observed weighted specialisation metrics (*H2’, d’, DSI**, w*MPD*, *PSS*) at assemblage level were tested using generalized additive models penalized to k = 5 with extra penalty for every term to allow penalizing to zero, smoothing parameter estimation by restricted maximum likelihood, and variance was analysed with ANOVAs. We used temperature and both plant metrics to explain Orthoptera richness and abundance, while we explained specialisation metrics (from barcoding and field observations) with plant richness, the phylogenetic structure of plant assemblages, and temperature. All predictors used in one model were not significantly correlated (Fig. S6). (The assumed error families are indicated in Tab. 1 and Tab. S1.) Additionally, we used a subset of abundant Orthoptera species, which occur along a broad fraction of the sampled microclimatic gradient to analyse intraspecific variation in specialisation. Averages of responses are presented with corresponding standard deviations.

3.3 *RESULTS*

3.3.1 Variation of Orthoptera and plant assemblages along the climatic gradient

Within our studied grassland sites, we detected 54 Orthoptera species via transect walks; 34 of which were Caelifera species and 20 belonged to Ensifera with an average of 13 (± 5.4) species per site. Orthopteran species richness increased more than threefold along the temperature gradient from 6 to 20 species, while abundances increased tenfold from 50 to almost 500 individuals, with an average number of 333 individuals per site (Tab. 1, Fig. 1a, b). In addition, Orthopteran species richness and abundance increased with plant species richness (Tab. 1). Within orthopteran assemblages, graminivorous species were dominant in cold habitats and at sites with phylogenetically clustered plant assemblages, while the proportion of omnivores and forb-feeders increased towards warmer habitats (Tab. 1, Fig. 1c).

Partitioning β-diversity between pairs of assemblages along the temperature gradient revealed a high contribution of balanced variation to β-diversity (0.49 ± 0.22), increasing with temperature, and a low contribution of abundance gradients (0.14 ± 0.14), peaking at the coldest sites (Fig. S7). This suggests that the dominance of species within assemblages differed more between assemblages at high temperatures (Fig. S8).

Via field surveys, we recorded 595 plant species in total. Plant species richness ranged between 54 and 158 species per site with an average of 107 (± 24.4), peaking at intermediate temperatures (Tab. 1, Fig. 1d). Poales were dominant at cold sites and warm meadows, with lowest cover at intermediate and the hottest sites, respectively (Fig. S9). Plant assemblages differed in their phylogenetic structure ($NRI = 0.18 \pm 0.46$), ranging from overdispersed ($NRI = -0.74$) to clustered ($NRI = 1.14$) assemblages, but neither a clear trend with temperature nor plant species richness was detected (Fig. S10). *NRI* increased with the proportional cover of Poales at the study sites (Tab. 1).

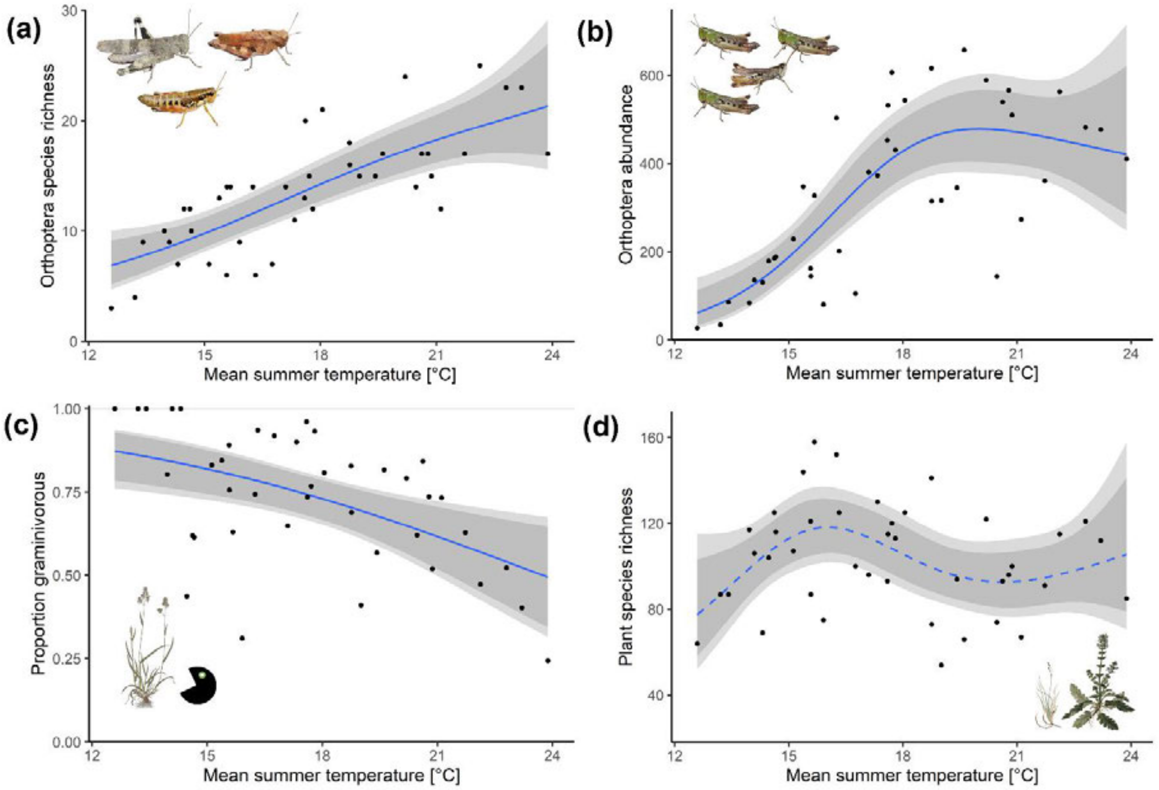


Figure 1. Orthoptera were recorded during transect walks at the study sites. Both, species richness (a) and abundance (b) increased with temperature. (c) Orthoptera species with graminivorous-type mouthparts were dominant in this study. Their weighted proportion within assemblages was highest in cold habitats. (d) Plant species richness peaked at 16°C summer temperature along the sampled microclimatic gradient. Smooths were fitted using gams (Tab. 1). Grey areas depict 95% point-wise (narrow) and simultaneous (wide) confidence intervals of smooths.

Table 1. Results obtained from generalized additive models (gams) with smoothing parameter estimation by restricted maximum likelihood, relating assemblage-level diversity (Orthoptera species richness (*SR*), Orthoptera abundance (*ABU*), proportion graminivorous species, plant species richness (*SR*), and the net relatedness index (*NRI*) of plant assemblages) and specialisation variables (Network specialisation *H2'*, community-weighted mean species-level specialisation *d',* community-weighted mean distance-based specialisation *DSI**, and community-weighted mean pairwise distances w*MPD*) to environmental variables. Predictor variables are mean summer temperature (*Temp*), *PSR*, proportional cover of Poales (*Poa*), and *NRI*. *edf* = effective degrees of freedom, *rdf* = reference degrees of freedom; *N* = number of observations; *family* = error distribution family; R^2_{adj} = adjusted R^2; R^2 = Deviance explained; Significance levels: . $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

response	*predictor*	*family*	*N*	*edf*	*rdf*	χ^2	*p*	R^2_{adj}	R^2
Orthoptera _SR_	Temp	poisson	41	1.84	4	52.88	<0.001 ***	0.68	0.68
	PSR			0.88	4	7.07	0.004 **		
	NRI			0.00	4	0.00	0.935		
Orthoptera _ABU_	Temp	quasipoisson	41	2.47	4	27.32	<0.001 ***	0.72	0.77
	PSR			0.95	4	6.71	<0.001 ***		
	NRI			0.34	4	0.18	0.208		
Prop. graminivorous	Temp	quasibinomial	41	0.95	4	4.03	<0.001 ***	0.53	0.49
	PSR			0.00	4	0.00	0.868		
	NRI			0.94	4	4.26	<0.001 ***		
Plant _SR_	Temp	quasipoisson	41	3.26	4	3.01	0.053.	0.18	0.24
Plant _NRI_	Temp	gaussian	41	0.00	4	0.00	0.361	0.28	0.31
	PSR			0.42	4	0.18	0.197		
	Poa			0.94	4	3.88	<0.001 ***		
H2'	Temp	beta	41	0.96	4	21.96	<0.001 ***	0.46	0.48
	PSR			0.49	4	0.98	0.159		
	NRI			0.85	4	5.01	0.011 *		
CWM _d'_	Temp	beta	41	0.96	4	20.50	<0.001 ***	0.43	0.46
	PSR			0.00	4	0.00	0.445		
	NRI			0.85	4	5.61	0.010 *		
CWM _DSI*_	Temp	beta	41	2.65	4	29.04	<0.001 ***	0.47	0.53
	PSR			1.43	4	3.55	0.079.		
	NRI			0.74	4	2.78	0.052.		
CWM w_MPD_	Temp	quasipoisson	41	2.10	4	3.33	0.002 **	0.35	0.42
	PSR			1.54	4	1.21	0.052.		
	NRI			1.21	4	1.16	0.030 *		

3.3.2 Characteristics of plant-herbivore interaction networks

We sampled faeces of 3,345 individuals of 347 Orthoptera populations at the 41 study sites (8.5 ± 3.4 populations per site, range 3 - 17). The metabarcoding provided an average sequencing depth of 14,498 high-quality reads after bioinformatic processing including filtering. We identified 407 plant species from 38 orders in faecal samples of grasshoppers, representing a broad range of plant-herbivore-interactions (Fig. 2). On average, the sampled Orthoptera species in our study fed on 24 (± 9.6) plant species per site (Fig. S11).

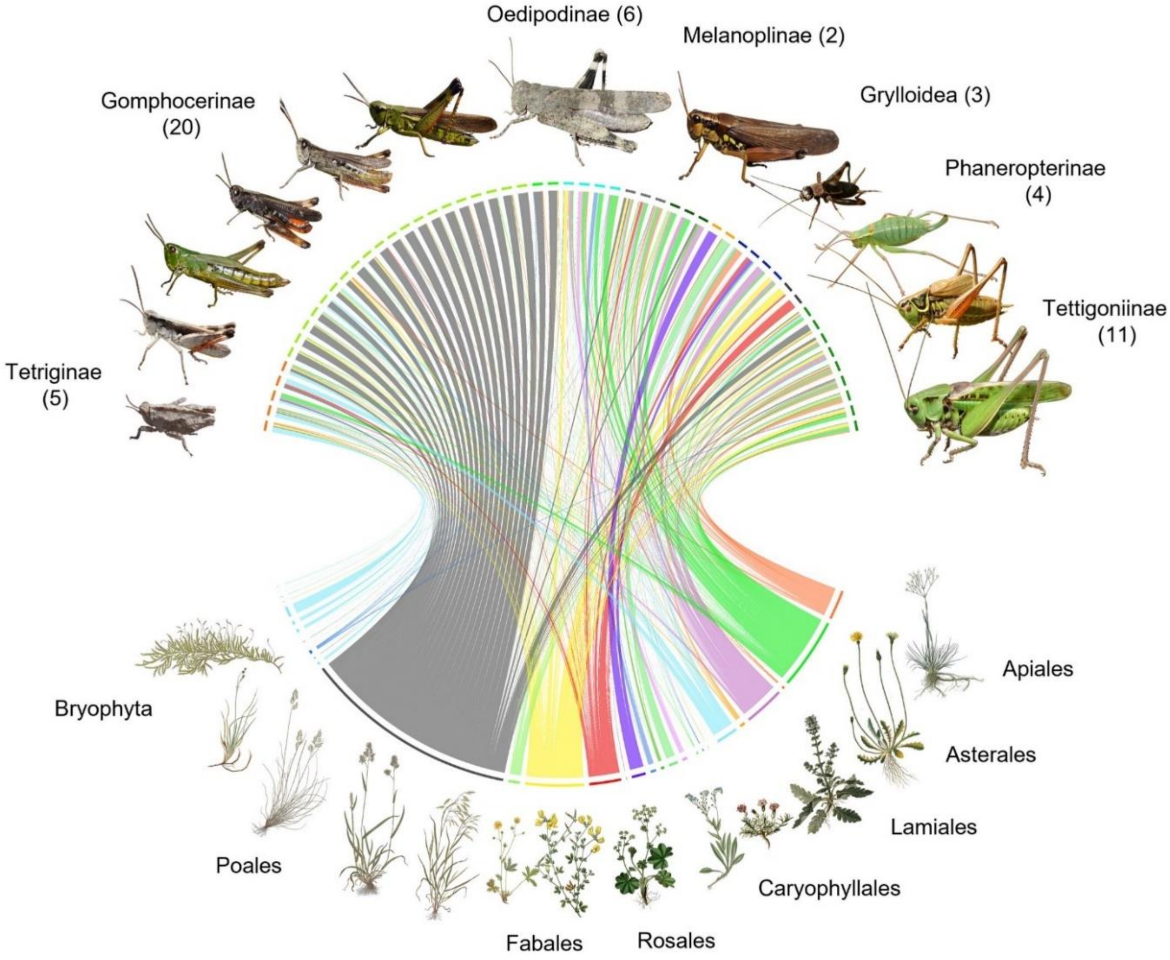


Figure 2. Overview of the recorded plant-herbivore interactions, using cumulative interaction strengths (proportions of reads) of plant taxa detected in Orthoptera faeces via (DNA-) metabarcoding (Gu et al 2014). Orthoptera taxonomic subdivisions with representative species (upper half, number of species in brackets) and prominent embryophyte plant orders (lower half) are depicted.

3.3.3 Shifts in taxonomy-based interaction specialisation with temperature

We calculated herbivore specialisation metrics at assemblage and species level for each site to reveal responses to environmental gradients in our study region. Networks were more generalized (and less modular) in cold habitats, indicated by an increase of network specialisation (*H2'*) with temperature (Tab. 1, Fig. 3a), but *H2'* was not affected by plant species richness at the study sites. The network specialisation was, on average, lower than predicted by null models, and the slope differed from the expected pattern based on network dimensions ($t_{1,39} = -3.93$, $p < 0.001$, Tab. S2). At species level, community-weighted herbivore interactions (*d'*) were more specialised in warm habitats and at sites with overdispersed plant assemblages compared to cold habitats with phylogenetically clustered resources (Tab. 1, Fig. 3a, Fig. S12a). Intraspecifically, the specialisation of common, widespread species increased with temperature (Fig. S13a).

3.3.4 Shifts in phylogeny-based resource specialisation with temperature

In a next step, we analysed resource specialisation by accounting for phylogenetic relatedness of plant species and their availability. Most Orthoptera species had specialized diets (*DSI** = 0.57, Fig. 3b), ranging from highly specialized (*DSI** = 0.97), over non-selective (*DSI** ~ 0) to generalist feeders (*DSI** = -0.71) (Tab. S3).

In contrast to taxonomic interaction specialisation, the phylogeny-based resource specialisation of Orthoptera species had a non-linear relationship with mean summer temperature. The distance-based specialisation index was low at cold sites, peaked at intermediate temperatures, before decreasing again towards the warmest habitats (Tab. 1, Fig. 3b). In other words, herbivore resource specialisation was reduced at low and at high temperatures, respectively. This finding was in line with the detection of, on average, smallest mean phylogenetic distances of plant species in Orthopteras' diets at intermediate temperatures (Tab. S1, Fig. S12c). Nevertheless, highly specialised species (*DSI** = 0.75 - 1) occurred along the entire climatic gradient. The phylogeny-based resource specialisation and the weighted mean phylogenetic distances of plants in Orthoptera faeces showed a weak peak at intermediate plant species richness, and both decreased with increasing phylogenetic clustering of plant assemblages (Tab. 1, Tab. S1). Between populations of widespread species along the microclimatic gradient, the specialisation was either constant, showed a mid-temperature peak, or decreased with temperature (Fig. S13b). Field observations of feeding interactions also revealed a mid-temperature peak in specialisation (Fig. S14). Both computed indices characterizing the phylogenetic structure of specialisation yielded similar patterns along the gradient (Fig. 1, Fig. S15).

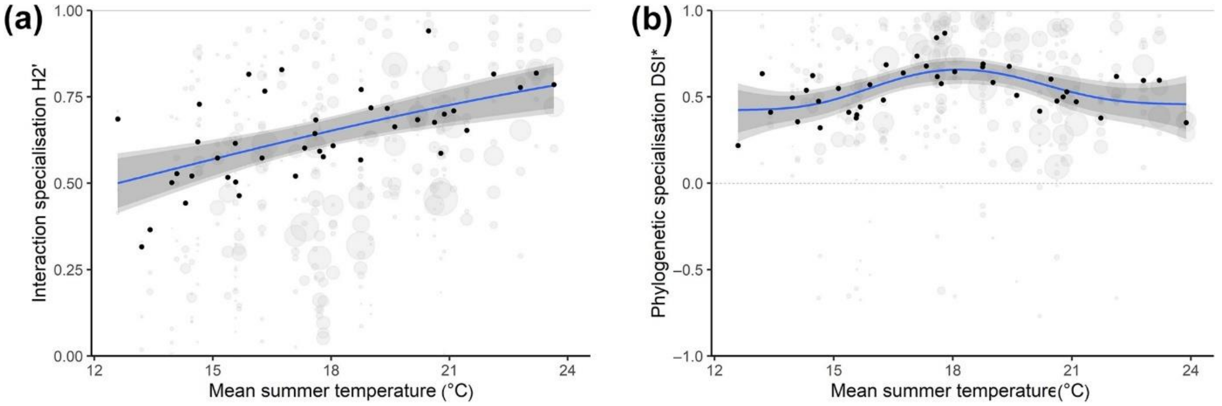


Figure 3. Relationship between mean summer temperature and assemblage-level resource specialisation metrics. (a) Network specialisation (Shannon Entropy *H2'*, black points), and species-level specialisation (*d'*, grey points) increase with temperature. (b) The phylogeny-based dietary specialisation (*DSI**) peaks at intermediate temperatures. Black points represent community-weighted means and grey points depict species-level diets with point sizes proportional to the abundance of the species at the site. Smooths were fitted using gams (Tab. 1). Grey areas correspond to 95% point-wise (narrow) and simultaneous (wide) confidence intervals of smooths.

3.4 *DISCUSSION*

In this study, we empirically tested predictions derived from the ‘altitude niche-breadth hypothesis’ on herbivore specialisation – temperature relationships, while controlling for phylogenetic relatedness and availability of plant resources. We observed an increase in network and interaction specialisation of Orthoptera and their plant resources with temperature as predicted, but the use of phylogenetic relationships and resource cover unravelled a non-linear relationship with a peak in specialisation at intermediate temperatures.

3.4.1 Taxonomy-based interaction specialisation

The observed specialisation increase of plant-herbivore interactions towards warm habitats is in line with the ‘altitude niche-breadth hypothesis’ and observed interaction specialisation patterns, e.g., plant-pollinator relationships from tropical and temperate mountains (Classen et al 2020, Hoiss et al 2013, Pitteloud et al 2021). A high overlap in resource use in cold environments and, therefore, broad dietary niches can be explained by energetic constraints, favouring the survival of generalist species, which can cope with resource stochasticity (Pitteloud et al 2021). In line with this prediction, generalist herbivore species such as *Miramella alpina* (Kollar) occurred in cold habitats. Increasing herbivore generalisation can also be the result of relaxed plant defences, but evidence for this is conflicting (Descombes et al 2017a, Galmán et al 2019, Leingärtner et al 2014a, Pellissier et al 2012, Rasmann et al 2014). Highly specialized interactions between insect herbivores and their plant resources in warm habitats can be explained by parallel diversity gradients of possible resources to feed on. This diversity-increase with temperature increases the probability to encounter resources that match with nutritional demands and mechanical traits, thereby decreasing niche overlap between species (Pitteloud et al 2021). Contrary to this prediction, plant resource species richness was only a poor predictor of herbivore specialisation in our study. Welti et al (2017) documented increased herbivore generalisation with increasing plant diversity. A lack of this pattern can be explained by two mechanisms. First, it may indicate that energetic constraints rather than interspecific interactions (e.g., competition for resources) shape insect specialisation in our sampled climatic gradient (Classen et al 2020). Second, plant richness may not resemble a proper measure of ‘true’ resource diversity of a given herbivore taxon, as only a fraction of possible plant resources is (actually) consumed by the herbivores. Additional to occurring non-resource plants, phenology and abiotic impacts may also modulate the attractiveness of resources under different climatic conditions. However, including the phylogenetic structure of plant assemblages, which can serve as a measure of trait relatedness of resource communities, unravelled a decrease in specialisation with increasingly clustered plant assemblages. The clustering was correlated with the proportional cover of Poales. Poales-dominated assemblages supported the abundance of typical grass-feeding Orthoptera, sharing the same set of host plants and, therefore, decreasing interaction specialisation.

Alternatively, herbivore species may be forced to escape competition with other species when population densities are high, e.g., in warm habitats as shown in this study. Nevertheless, we expect low levels of competition for resources in mobile generalist herbivores such as Orthoptera in open grasslands, where plant biomass is not limiting (Joern 1979, Pitteloud et al 2021). Therefore, increases in interaction specialisation could either be the result of enhanced resource partitioning among herbivores or turnover of species, as warm ambient temperatures allow species with distinct microhabitat requirements to persist. The analysis of *d’* of widespread species along the gradient revealed increasing specialisation with temperature, suggesting that intraspecific changes could contribute to more specialized networks in warm habitats. As evidence of specialisation patterns along environmental gradients is conflicting, the inclusion of phylogenetic relationships and resource availability can help to disentangle patterns and underlying processes of biotic specialisation of mobile, generalist herbivore taxa such as Orthoptera in ecological networks (Jorge et al 2017, Pardo-De la Hoz et al 2022).

3.4.2 Phylogeny-based specialisation

The phylogeny-based specialisation depends on the phylogenetic structure of resources and resource availability. Based on the relationship between the phylogenetic α-diversity of plant assemblages and the phylogeny-based specialisation index, we conclude that the phylogenetic structure of plant assemblages also shaped specialisation patterns along our sampled climatic gradient (Pardo-De la Hoz et al 2022). We detected wide diet breadths of herbivores at cold and warm sites, respectively. Such broad diets in cold, harsh habitats have been reported for different herbivore taxa such as caterpillars and jewel beetles before (Pellissier et al 2012, Rasmann et al 2014). This generalisation can either be explained by relaxed plant defence mechanisms, reduced competition for resources, or environmental unpredictability in cold habitats (Pellissier et al 2012, Pitteloud et al 2021). Therefore, our results are in line with general hypotheses on relationships of resource specialisation and temperature for a part of the covered temperature gradient. However, increasing phylogenetic diet breadths of herbivore assemblages towards the warmest sites were unexpected based on predictions derived from the ‘altitude niche-breadth hypothesis’. The observed specialisation pattern was mainly shaped by the dominant subfamily Gomphocerinae, which often select grasses as preferred food source (Descombes et al 2020, Ibanez et al 2013b). Wherever grasses were dominant, they contributed to phylogenetic clustering of resources, which in turn decreased the specialisation of the dominant grass-feeding species. At sites with a low proportion of grasses, typical grass-feeders also decreased in abundance and, consequently, the amount of dicotyledonous plants in the diets of Orthoptera increased. Analyses of intraspecific patterns of widespread species along the climatic gradient revealed either increases, no relationships, or hump-shaped trends of specialisation with temperature, and, therefore, do not sufficiently explain the mid-temperature peak in assemblage-level specialisation alone. Yet, high values of the balanced variation partition of β-diversity suggest varying dominances of different Orthoptera species at sites with high temperatures, implying that not only few very common Gomphocerinae species shaped the observed specialisation pattern along the gradient. Therefore, we infer decreases in specialisation of common species contributed to low specialisation levels in cold habitats, while additional generalist species decreased assemblage-level specialisation at high temperatures. Presumably, either the proportion of water-rich plants in diets increases when temperatures and evaporation rates are very high, nitrogen-rich plants are added because of increasing nutrient demands of fertile females when temperatures are high, or availability of grasses is low, supporting generalist herbivores such as *Oedipoda caerulescens* (L.), *Calliptamus italicus* (L.), or *Platycleis albopunctata* (Goeze). Nonetheless, we found highly specialised Orthoptera species along the entire sampled gradient, highlighting the possibility to remain specialized under different climatic conditions. Especially grasshoppers (Gomphocerinae), which evolved distinct mandibular morphology to cut through tough plant material (Ibanez et al 2013a), consistently fed on a narrow phylogenetic range of food plants along the covered temperature gradient. Resource specialisation of Tetrigids or Tettigoniids, however, was not completely captured in this study as they also feed on algae and arthropods, respectively.

The barcoding of faecal samples unravelled preferences of Orthoptera for certain food resources below family level, e.g., within Poaceae. For instance, *Anthoxanthum* spp. (L.) and

Brachypodium spp. (P. Beauv.) were generally consumed less than expected by their cover, while some genera were consumed proportional to their cover or even overrepresented in Orthoptera faeces (*Agrostis* (L.), *Bromus* (L.), *Dactylis* (L.), *Festuca* (L.), & *Sesleria* (Scop.), Fig. S16). This may be a hint at converging plant traits such as toughness, silica content, or nutrient content, explaining a bias towards some species of the plant assemblage during the sampling period. Despite its limitations, our study revealed such resource preferences within a generalist herbivore taxon, underpinning the need for detailed analyses of plant resource characteristics and how they change along temperature and precipitation gradients.

3.4.3 Caveats of metabarcoding and comparison to field-observations

Even if advances in molecular methods such as high-throughput sequencing revolutionized the field of molecular analysis of interactions, semi-quantitative results of metabarcoding analyses need to be treated with caution. Using relative read abundances (RRAs) often provides a more accurate representation of population-level diets compared to occurrence counts, which overestimate rare food items (Deagle et al 2019). Nevertheless, using universal primers could lead to a bias by enhanced amplification of certain taxa, as polymerases perform dependent on GC-content of sequences, and DNA extraction could cause unequal recovery rates depending on plant-material-specific features. Additionally, pooling of dietary samples on population level was found to be less accurate in estimating resource diversity than analysing several samples per individual (Mata et al 2019). However, where gathering field observations of interactions is challenging, this method can advance disentangling biotic relationships. We detected a higher number of insect – plant interactions via metabarcoding compared to traditional field surveys, but this did not lead to inferring different patterns of specialisation along the gradient. While we missed many interactions by means of field observations, the detected ones corresponded to the most frequent interactions from barcoding, generating similar patterns and highlighting the robustness of our analysis.

3.4.4 Conclusions

Incorporating phylogenetic relationships of plant resources and plant cover revealed highest herbivore-plant specialization at intermediate temperatures along a broad temperature gradient ranging from lowlands to alpine summits. Orthoptera are generalist herbivores, but our study revealed that many species have preferences for certain plant taxa, which partly shift with temperature. Mean summer temperature was closely linked to herbivore specialization, but plant resource composition also influenced herbivore specialisation. We showed that the phylogenetic resource specialisation of herbivores was highest at intermediate temperatures; a novel finding, which challenges the expected linear relationship of diet breadth and temperature derived from the ‘altitude niche-breadth hypothesis’ (Rasmann et al 2014). Therefore, we suggest considering the phylogeny of interaction partners, as well as their availability and abundance in studies about biotic specialisation along environmental gradients.

Our results suggest that due to non-linearity, dietary interactions of generalist herbivores with ongoing climate change are less predictable than assumed. Orthopterans are expected to increase in abundance and species richness in alpine grasslands due to climate warming, which would lead to more specialised assemblages at high elevations according to our findings. On the other side, dense assemblages in warm habitats could further increase in generality, boosting herbivory pressure on several plant taxa with unknown consequences for plant and herbivore community composition, coexistence, and interactions. Thus, novel, and currently unrecognised risks might emerge under climate warming due to mass propagation of herbivores, shifts in plant defence, reduced dietary quality of plant resources for specialised herbivores and reduced stability of plant-herbivore interaction networks.

3.5 *SUPPLEMENTRARY MATERIAL TO CHAPTER III*

Supplementary methods

1) Indices of phylogenetic resource specialization

The assembly, evolution, and resilience of biological entities relies on specialization patterns (Pardo-De la Hoz et al 2022). Thus, capturing species interactions is of great interest and got boosted by means of molecular methods to assess increasing numbers of interactions (Roslin et al 2019). Based on recent advances in the field of inferring phylogenetic structure from biotic interactions, novel approaches are available to characterize specialization levels of interaction partners. We used the rescaled distance-based specialization index, which measures the phylogenetic α-diversity based on weighted mean pairwise distances, compared to a null model which draws interactions from a pool of resources based on their availability (method presented in the main text). Since our (DNA-) metabarcoding-derived data are frequencies (relative read abundances to account for unequal sequencing depth), which we converted to integers, all samples have the same sum of interactions. Hence, all species within one assemblage are compared based on equal interaction frequencies, what decreases sample-level bias in w*MPD* computation. Nevertheless, we also calculated the recently published phylogenetic structure of specialization index (*PSS*) following Pardo-De la Hoz et al (2022), since for this novel metric calculation, the null models also control for the number of interaction partners instead of only interaction frequencies. Furthermore, this metric is a standardized effect size (SES), thus it provides straight-forward interpretability for comparisons among datasets. Here, the mean pairwise distances are not only weighted by interaction frequencies but the KL-factor (kl*MPD*, Kullback-Leibler weighted *MPD*), similar to computations of the *d'* specialization index. Blüthgen's *d'* (Blüthgen et al 2006) and the net relatedness index (*NRI*) measure specialization using availability and phylogenetic structure, respectively. *PSS* integrates both complementary sources of information to assess specialization, and by that avoids bias from uneven partner availabilities by uncoupling the null models for interaction frequencies and phylogenetic distances (Pardo-De la Hoz et al 2022). Negative values indicate clustering, while positive values indicate overdispersion. In both directions, species could be categorized as specialists, interacting more intensely with partners than expected by chance, if the observed values are outside the 95% confidence interval limits of null distributions.

We analysed our empirical (DNA-) metabarcoding-based and field observation-based interaction networks between Orthoptera and plant species in parallel with both phylogenetic specialization indices, which yielded similar patterns of assemblage-level specialization along the microclimatic gradient (Tab. 1, Tab. S1).

2) *Orthoptera phylogeny*

For plotting, a phylogeny of the occurring Orthoptera species was reconstructed with a maximum likelihood approach (RAxML-ng, Kozlov et al 2019), based on MUSLE alignments (Edgar 2004) of Cytochrome-c-Oxidase subunit I (COI) sequences (567 bp), which were retrieved from BOLD (boldsystems.org). The RAxML-ng options were HKY +I, 10,000 bootstraps with a cut-off percentage of 0.25, and 100 random starting trees. We constrained the phylogeny by enforcing monophyly for Grylloidea, Tettigoniidea & Phaneropteridae, and used *Forficula auricularia* (L.) as outgroup.

Supplementary results

We identified 270 plant species in Orthoptera diets by directly observing feeding interaction of grasshoppers and plants at the study sites. On average, the sampled Orthoptera species in our study fed on 8.75 (± 4.70) plant species per site.

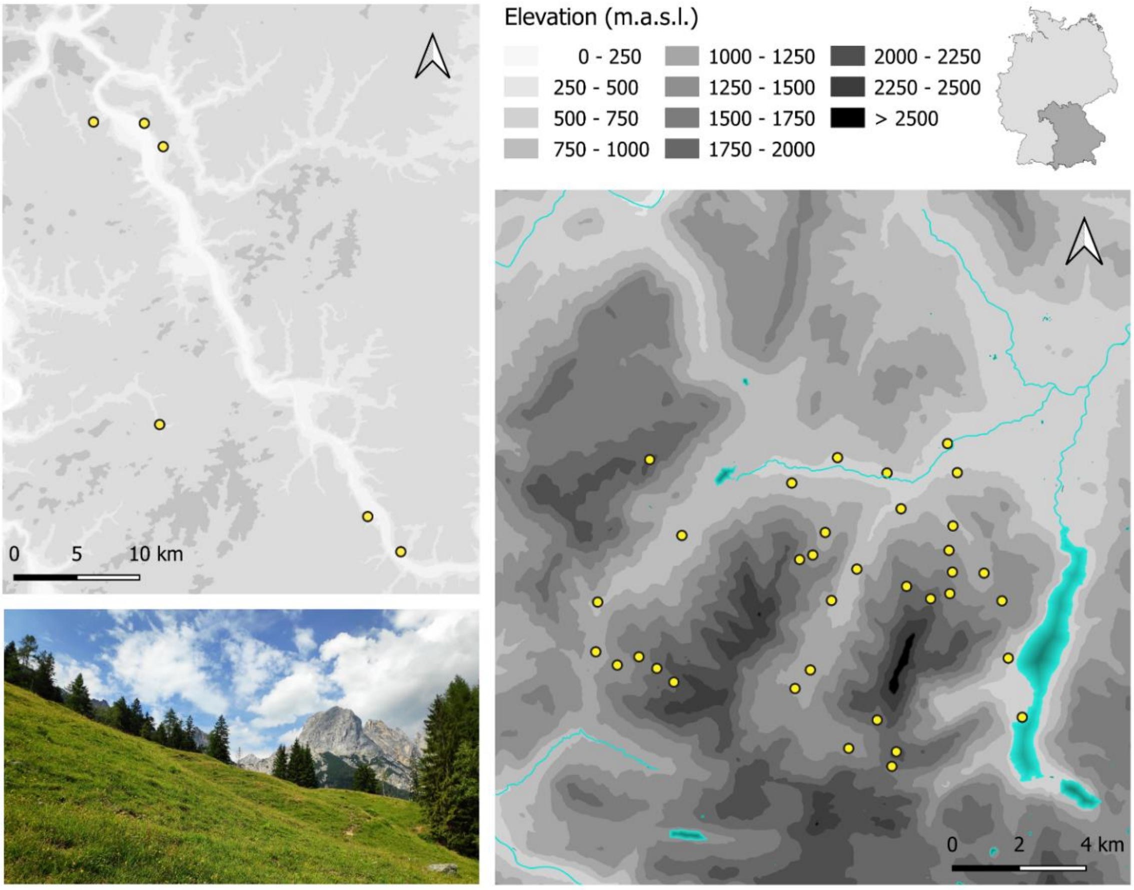


Figure S1. Location of the study sites within Bavaria (Germany). Study sites were extensively managed by low-impact grazing/mowing or unmanaged open grassland sites (60 x 60 m). Grasslands were selected along a gradient of microclimate, ranging from 12°C – 24°C seasonal temperature (measured with temperature loggers at the sites), to capture a broad range of microclimatic conditions, which makes it possible to infer patterns of specialization.

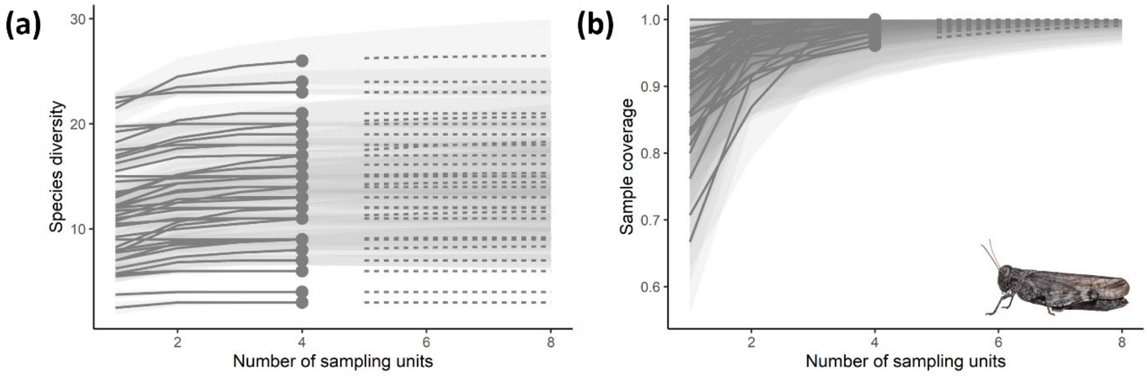


Figure S2. Species accumulation (a) and sample coverage curves (b), calculated based on four survey rounds to assess Orthoptera assemblages at the study sites, computed with the iNEXT package (Hsieh et al. 2020). Assemblage assessments were highly complete (ACE = 0.99).

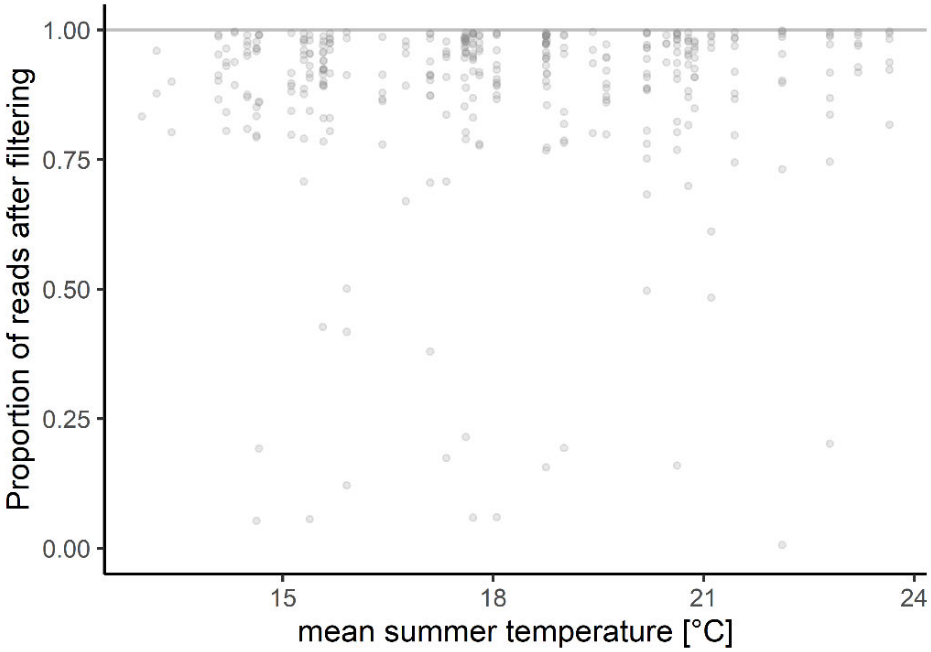


Figure S3 Filtering of reads from (DNA-) metabarcoding, sequenced at a MiSeq platform. Filtering did not systematically vary along the microclimatic gradient. After bioinformatic processing (e.g., filtering of low-quality reads), we retained only counts of embryophyte plant taxa identified at the focal sites and their surroundings using our field surveys and additional plant inventories. Reads of lower taxonomic levels, as well as reads from taxa other than embryophytes were discarded. Samples with low proportions of retained reads mainly correspond to Tetrigid samples (Orthoptera: Tetrigidae), which contained high proportions of chlorophytes (algae, Fig. S4). As we neither assessed algae nor lichen composition at the sites, we discarded corresponding reads for further downstream analyses of dietary spectra.

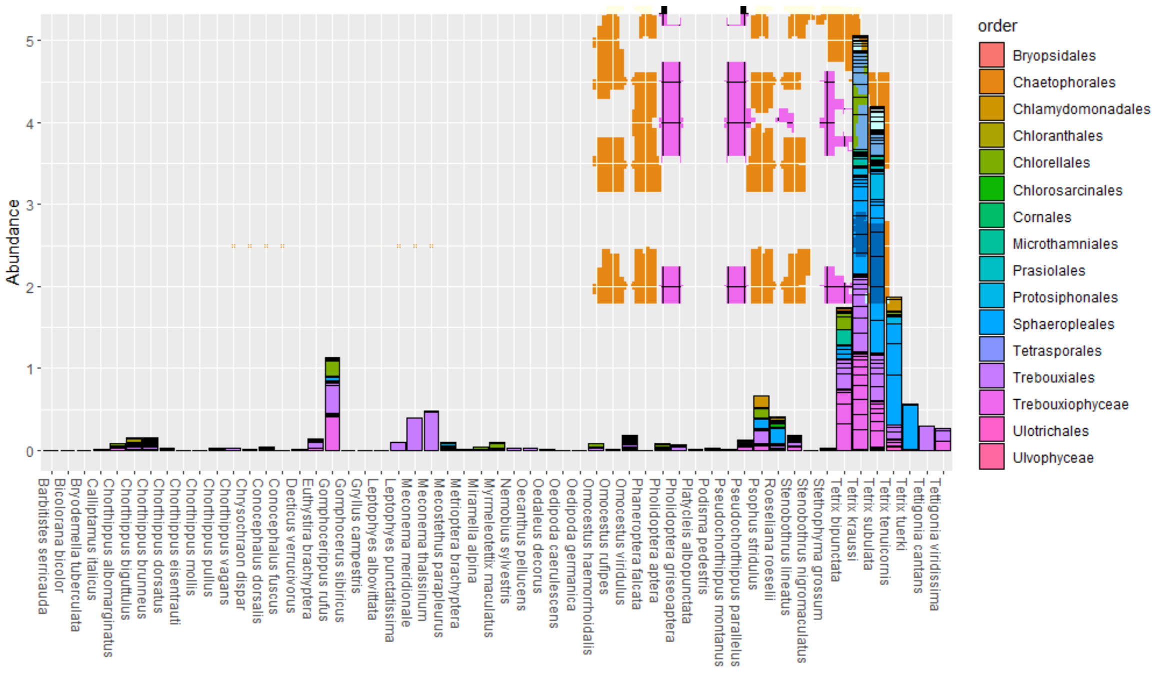


Figure S4. Summed relative read abundances (proportions of reads) of Chlorophyta orders in fecal samples of Orthoptera species. Significant proportions of these orders were detected in all samples of the Tetrigidae subfamily. Tetrigidae are known to feed on detritus, lichen, fungi, and algae besides mosses and other vascular plants (Ingrisch & Köhler 1998).

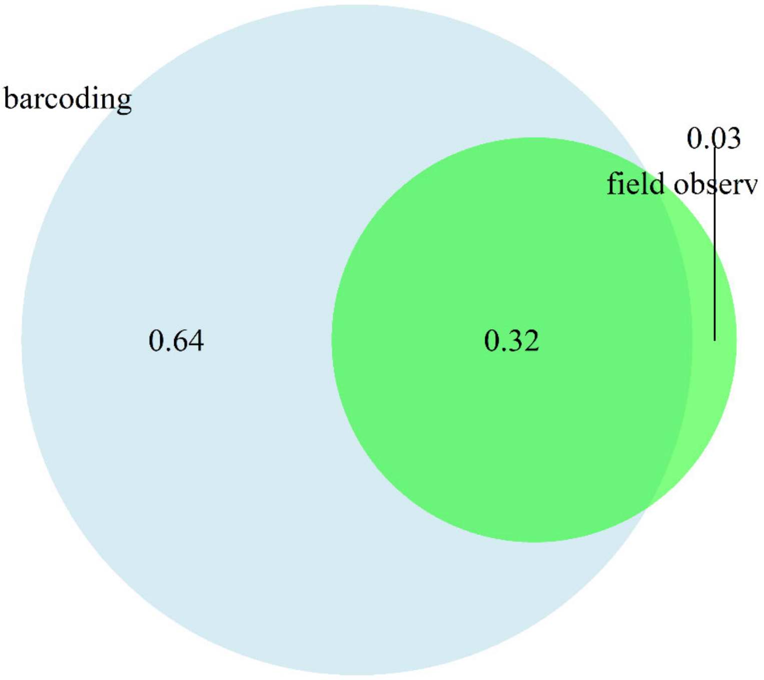


Figure S5. Comparison of (DNA-) metabarcoding-derived (blue) and field observation-based (green) insect-plant interactions (incidence-based). Gathering large numbers of observational data of plant-herbivore interactions is time-consuming and challenging, especially for rare and cryptic species, which are difficult to observe in the field. Hence, it is not surprising that interaction data from field observations are incomplete. For this comparison, we used incidence data on detected herbivore-plant interactions per single sample (Orthoptera species population/site). 32% of all interactions were jointly detected by barcoding and field observations. However, additionally detected plant-herbivore interactions by metabarcoding of faecal samples were mainly interactions of low frequencies (low proportions of reads), so that 83 % of the summed relative read abundances were distributed among interactions also detected by observations. This led to robust and similar patterns of specialization between the two alternative approaches.

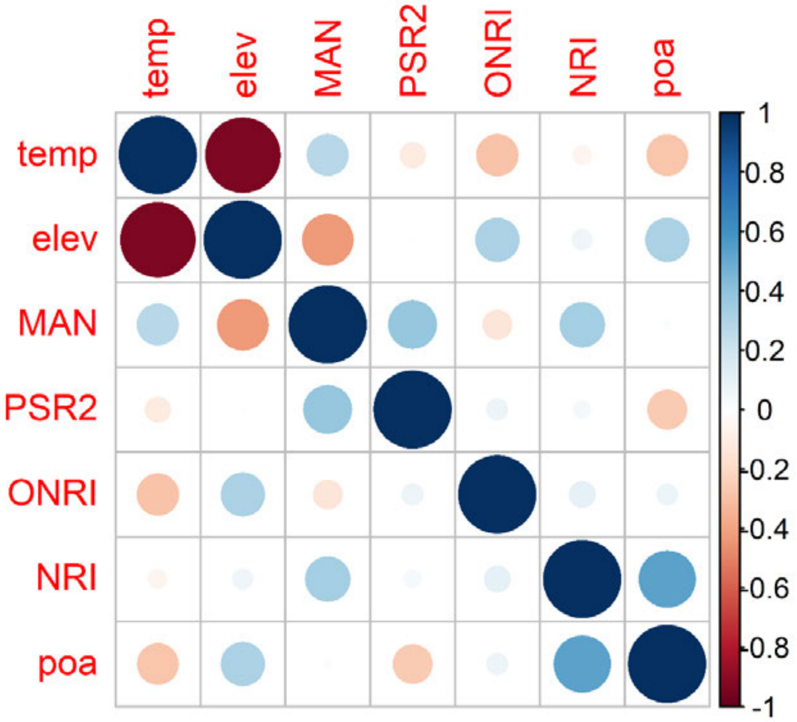


Figure S6. Correlation plot (Pearson correlations) of scaled predictor variables. Temperature (mean seasonal microclimatic temperature, *temp*) was negatively correlated with elevation (*elev*). Sites at high elevations were more often unmanaged than sites at low elevations. The proportional cover of Poales (*poa*) was positively correlated with the net relatedness index (*NRI*) and moderately negatively correlated with temperature/elevation and plant species richness (*PSR2*).

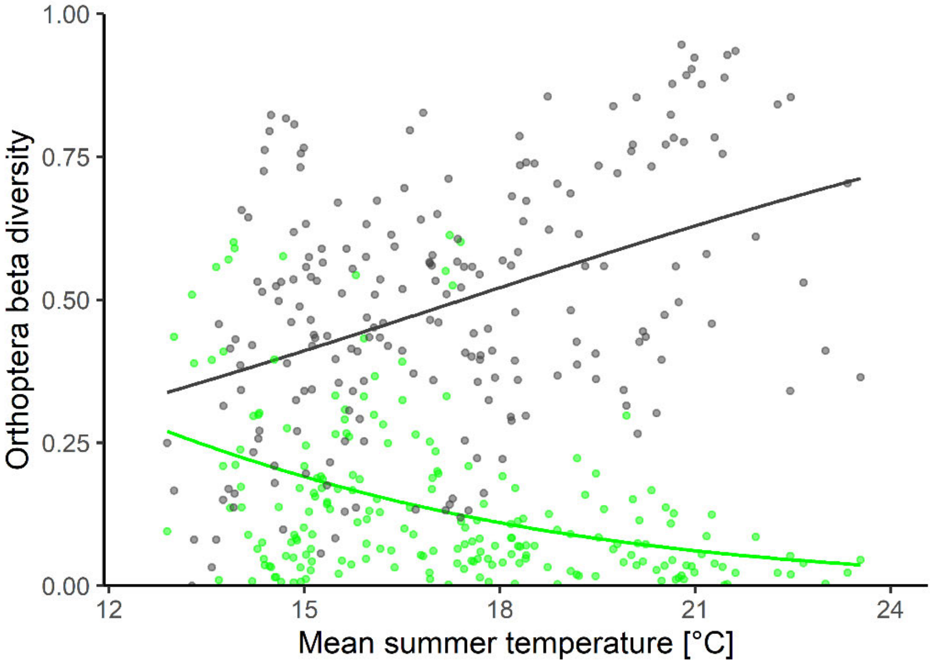


Figure S7. *β*-diversity of Orthoptera assemblages partitioned into balanced variation (abundance-based turnover component, gray) and abundance gradients (abundance-based nestedness component, green) of pairwise site comparisons within temperature intervals of 1.5°C. We repeated this analysis with intervals of 0.5, 1.0, 1.5, and 2.0 °C to test, whether chosen intervals affect *β*-diversity rates along the gradient. Turnover rates increased with temperature. Nestedness was highest in cold habitats.

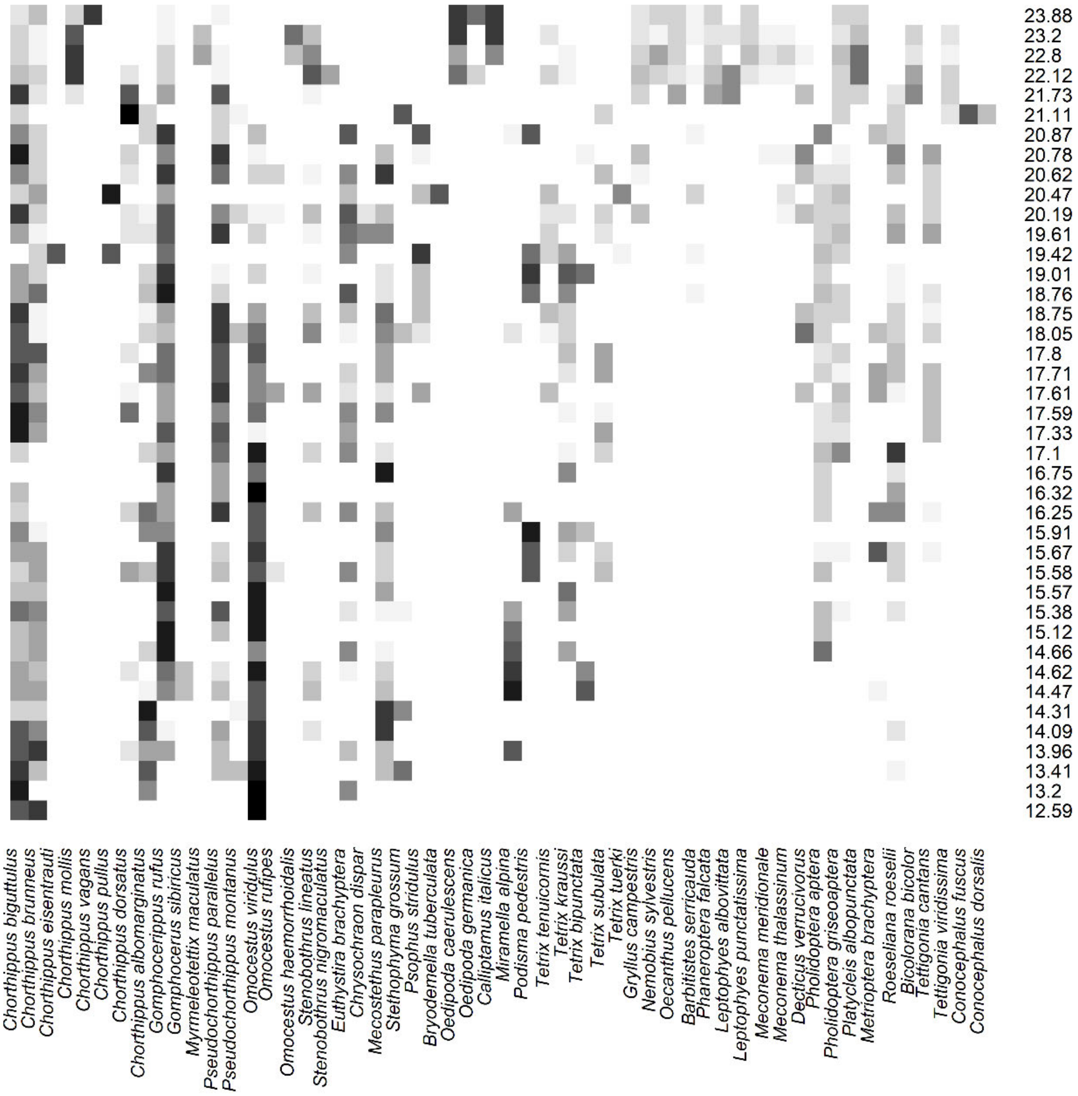


Figure S8. Relative abundances of Orthoptera species within assemblages. Sites are ordered corresponding to mean summer temperatures (seasonal microclimate) measured at the sites. Orthoptera species are ordered on subfamily-level beginning (left) with Gomphocerinae (20), Oedipodinae (6), Calliptaminae (1), Melanoplinae (2), Tetriginae (5), Gryllinae (1), Nemobiinae (1), Oecanthinae (1), Phaneropterinae (4), Meconematinae (2), Tettigoniinae (9) & Conocephalinae (2).

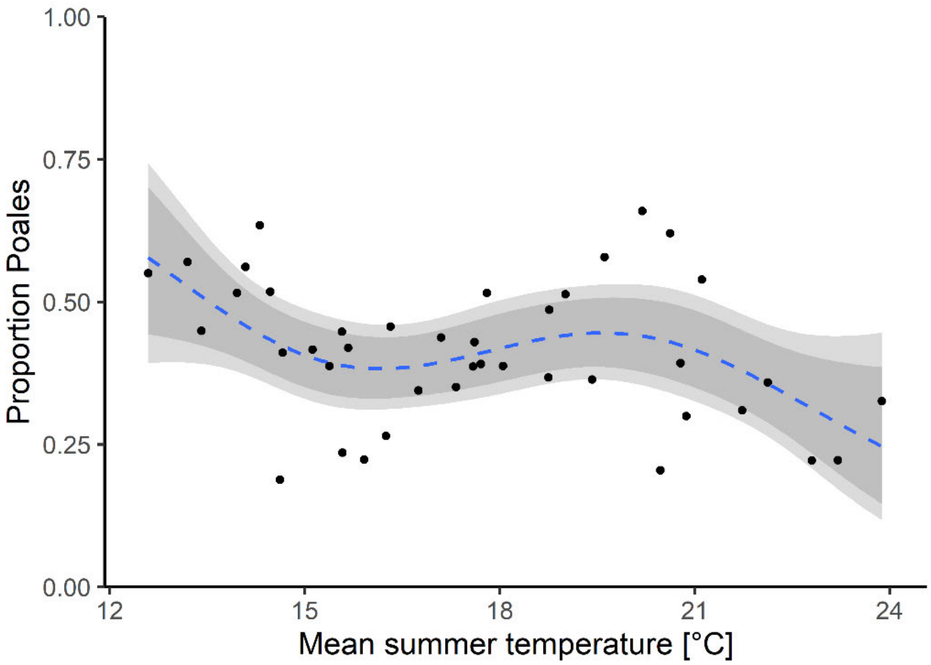


Figure S9. Proportional cover of summed species scores belonging the plant order Poales along the temperature gradient. Gray areas correspond to 95% point-wise (narrow) and simultaneous (broad) confidence intervals of gam-smooths (Tab. S1).

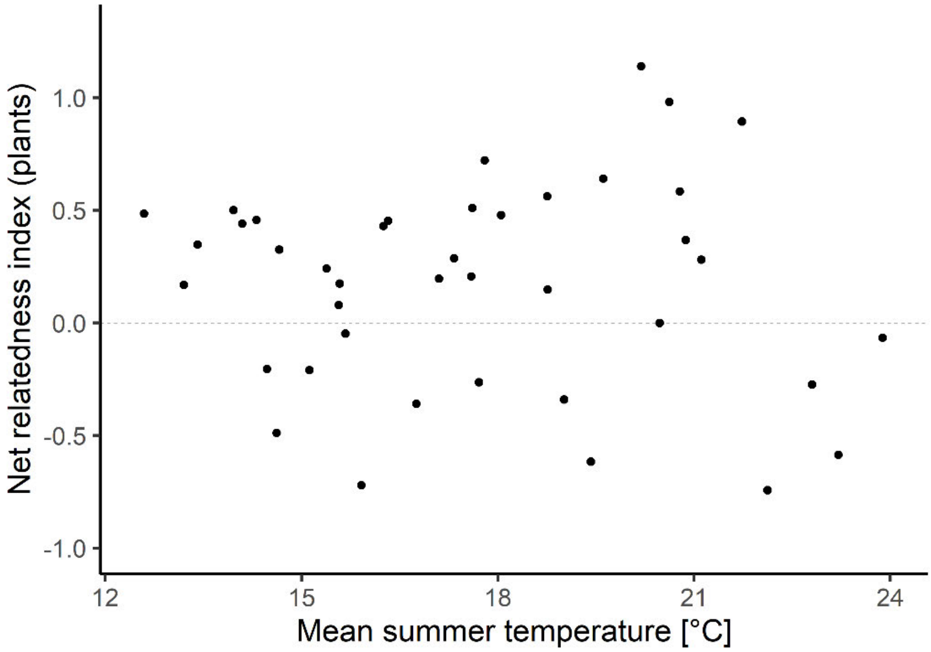


Figure S10. Cover-weighted net relatedness index (*NRI*) of plant assemblages did not systematically vary with temperature. High values (> 0) indicate phylogenetic clustering and low values (< 0) indicate phylogenetic overdispersion compared to random assemblages.

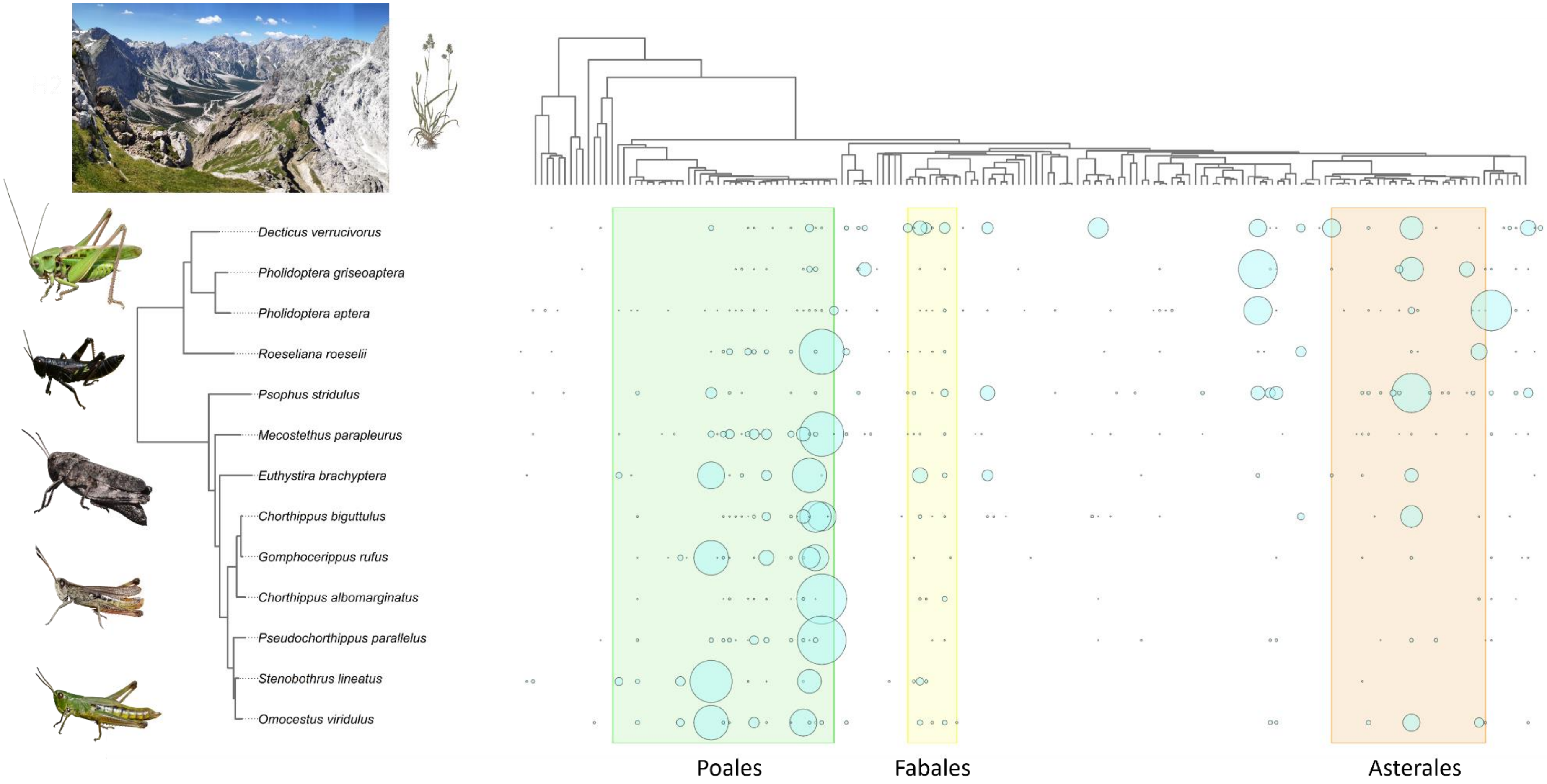


Figure S11. Reconstruction of an example site-level trophic network between Orthoptera species and plant resources at study site H2. Orthoptera species are rows and plant species are columns. Blue points represent documented interactions, while point size is proportional to interaction strength measured as relative read abundances from (DNA-) metabarcoding. Rectangles depict plant taxa of the orders Poales (green), Fabales (yellow), and Asterales (orange).

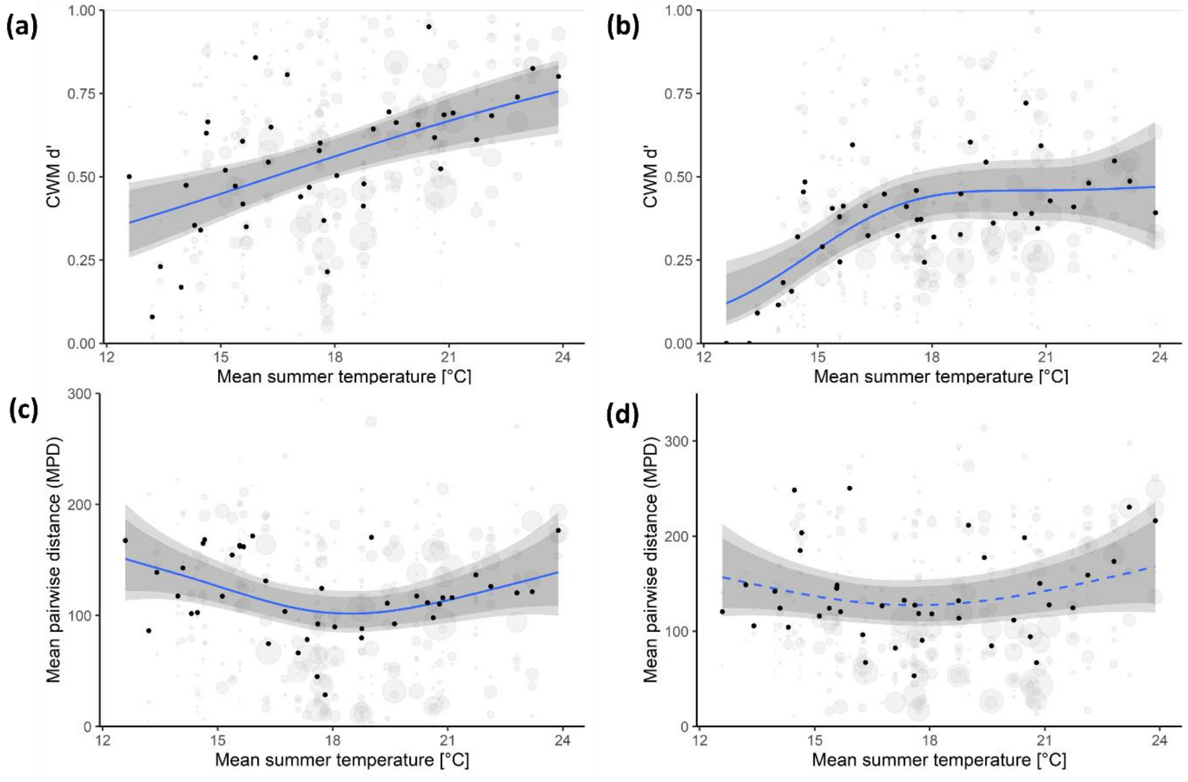


Figure S12. Relationship between mean summer temperature and community-weighted mean (assemblage-level) specialization metrics. (a) species-level identity-based interaction specialization (*d'*) based on (DNA-) metabarcoding of fecal samples of Orthoptera increased with temperature. (b) species-level *d'* based on direct field feeding observations increased with temperature, but the rate slowed down at high temperatures. (c) The weighted mean pairwise distances (w*MPD*, phylogenetic *α*-diversity) of plants in diets of Orthoptera based on (DNA-) metabarcoding of feces were lowest at intermediate temperatures. (d) The w*MPD* of plants in diets of Orthoptera based on direct field feeding observations was lowest at intermediate temperatures. Black points represent community-weighted means, while gray points depict species-level diets with sizes proportional to the abundance of the species at the sites. Smooths were fitted using gams (Tab. 1, Tab. S1). Gray areas correspond to 95% point-wise (narrow) and simultaneous (broad) confidence intervals of smooths.

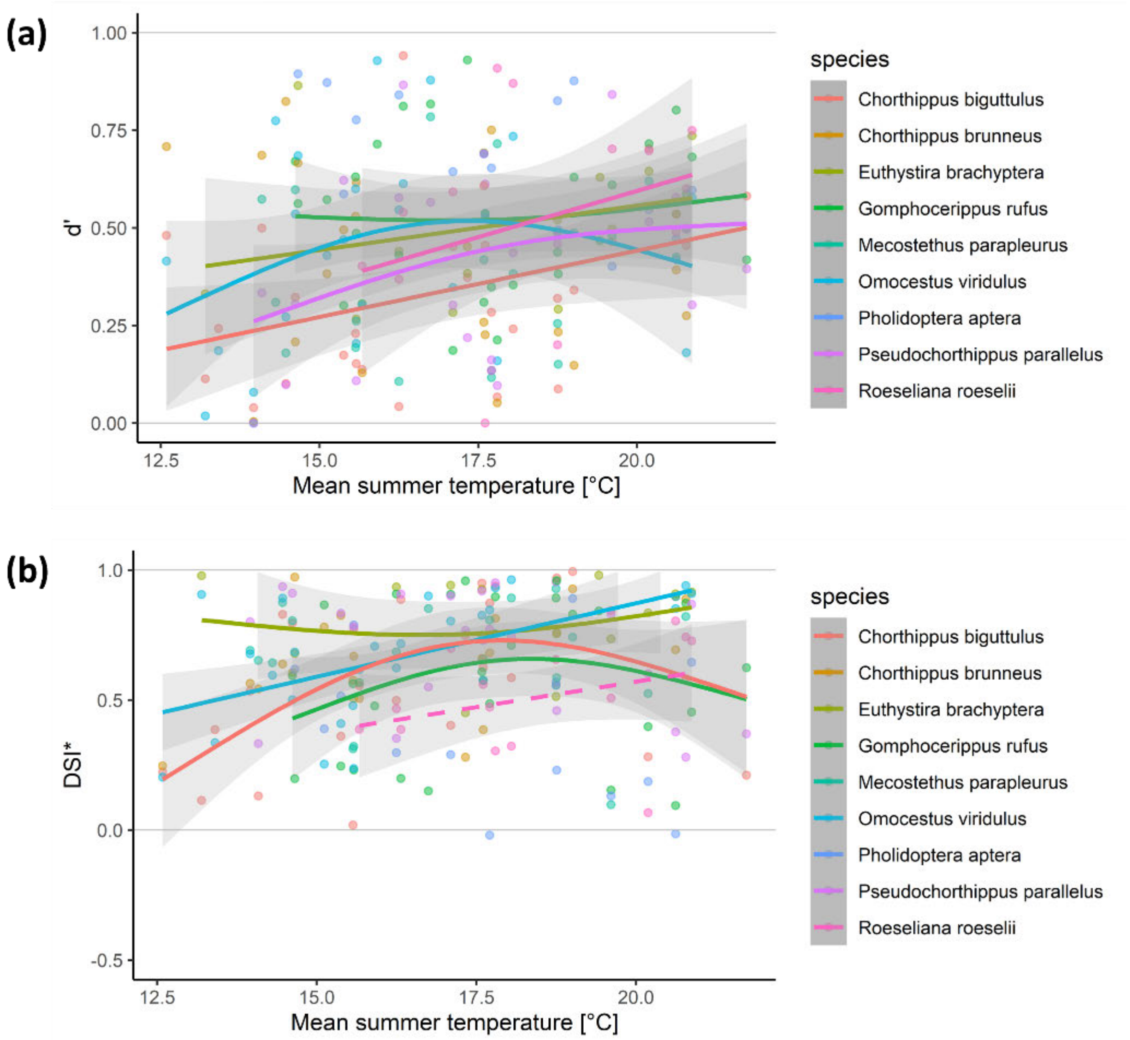


Figure S13. (a) Intraspecific changes in interaction specialisation (*d'*) of nine dominant, widespread Orthoptera species along the temperature gradient based on (DNA-) metabarcoding of Orthoptera feces. Three species did not change in specialization along the gradient, while five increased in specialization with temperature and one peaked at intermediate temperatures. (b) Intraspecific changes in distance-based specialization (*DSI**) of nine dominant, widespread Orthoptera species along the temperature gradient based on (DNA-) metabarcoding of Orthoptera feces. Four species did not change in specialization along the gradient, while three increased in specialization with temperature and two peaked at intermediate temperatures.

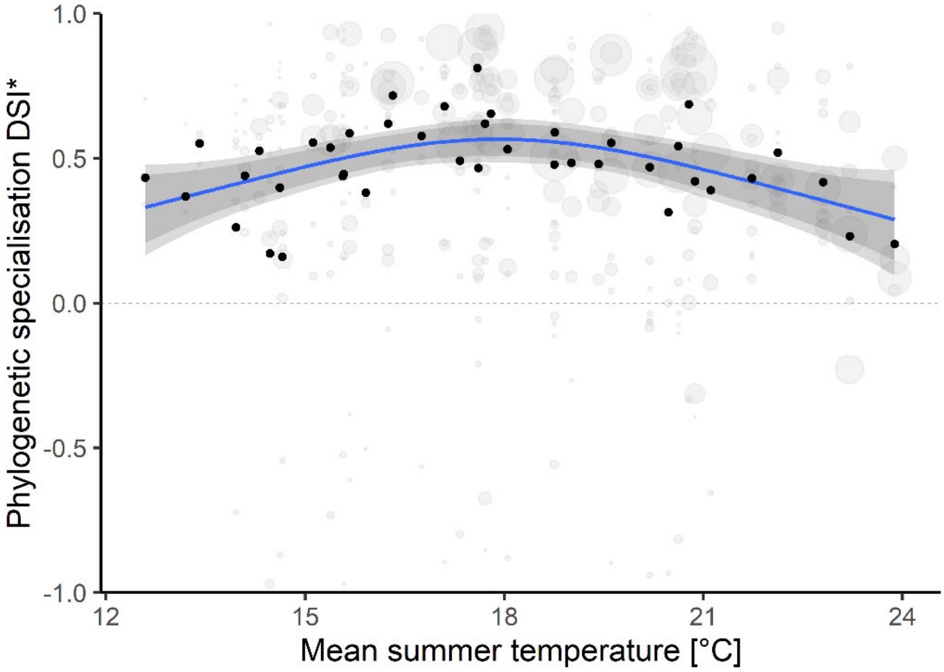


Figure S14. Relationship between mean summer temperature and community-weighted mean (assemblage-level) distance-based resource specialization (*DSI**) based on field observations of Orthoptera chewing on vegetation. The specialization at assemblage level peaked at intermediate temperatures. Gray areas correspond to 95% point-wise (narrow) and simultaneous (broad) confidence intervals of gam-smooths (Tab. S1).

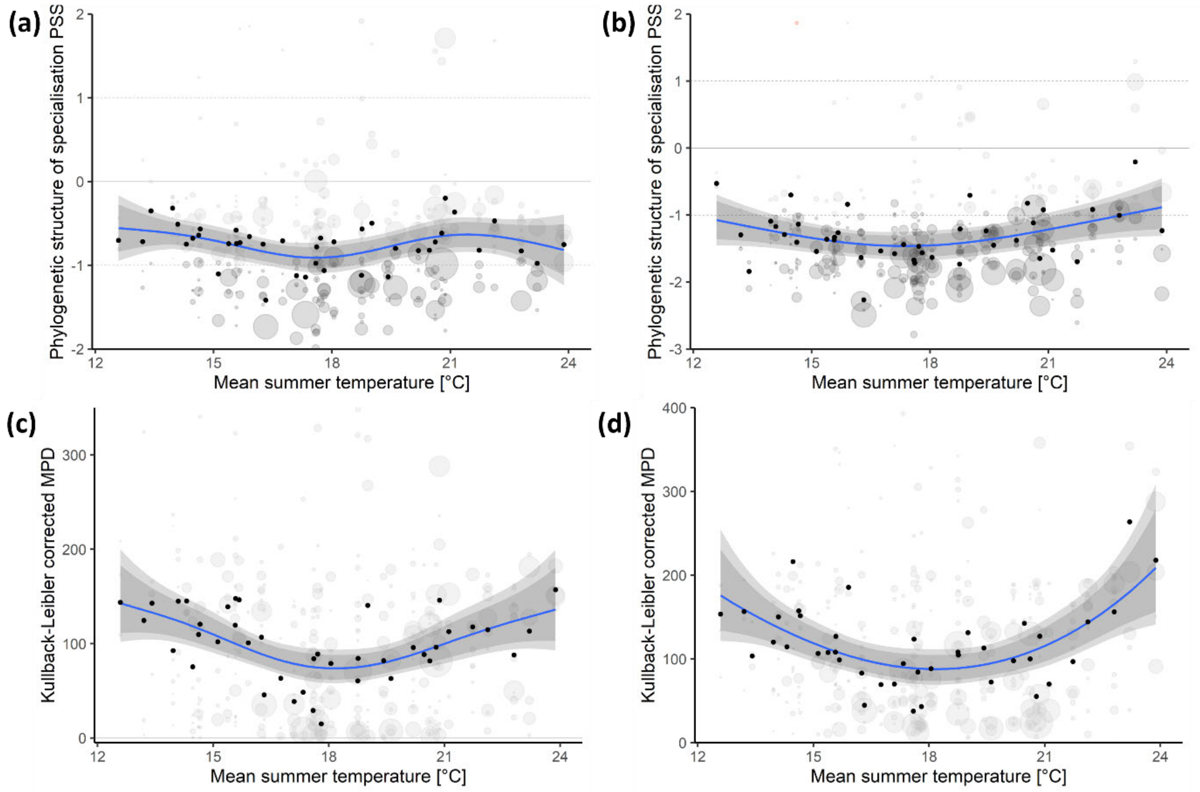


Figure S15. Relationship between mean summer temperature and community-weighted mean (assemblage-level) specialization metrics. (a) The phylogenetic structure of specialization (*PSS*) based on (DNA-) metabarcoding of Orthoptera feces was lowest at intermediate temperatures, indicating clustering (specialization). (b) *PSS* based on direct field observations of feeding interactions. Clustering was pronounced at intermediate temperatures. (c) The Kullback-Leibler corrected mean pairwise distances (kl*MPD*) of plants in diets of Orthoptera based on (DNA-) metabarcoding of feces were lowest at intermediate temperatures. (d) The kl*MPD* of eaten plants based on direct field observations of feeding interactions were lowest at intermediate temperatures. Black points represent community-weighted means, while gray points depict species-level diets with sizes proportional to the abundance of the species at the sites. Smooths were fitted using gams (Tab. 1, Tab. S1). Gray areas correspond to 95% point-wise (narrow) and simultaneous (broad) confidence intervals of smooths. Lines at y = -1 and 1 in (a) and (b) indicate thresholds obtained from 95% confidence intervals of null distributions.

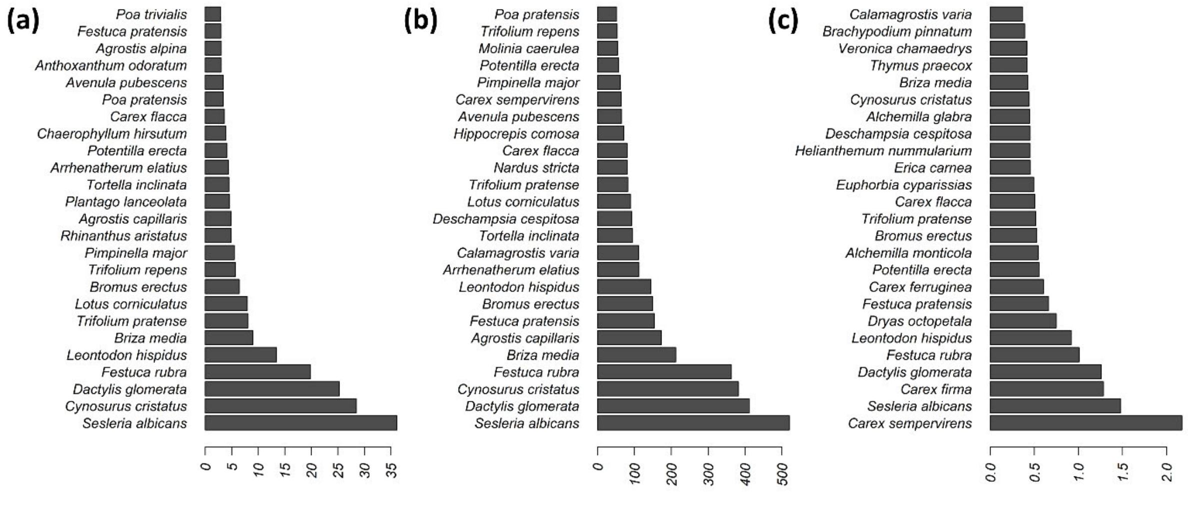


Figure S16. Overview of the 25 most abundant plant species recorded by (a) (DNA-) metabarcoding of Orthoptera feces (summed relative read abundances), (b) direct feeding observations in field (summed observations), and (c) plant assessments at the study sites (summed relative cover values).

Table S1. Results obtained from generalized additive models (*gams*) relating diet breadth variables (community-weighted, Kullback-Leibler corrected mean pairwise distances kl*MPD*, weighted mean pairwise distances (w*MPD*), the phylogenetic structure of specialisation (*PSS*), *d'*, *H2*', the distance-based specialization index (*DSI**), cover of the plant order Poales and community-weighted phylogenetic structure of specialisation *PSS*) to environmental variables. (*obs*) indicates field-observation-based interaction networks, while no indication corresponds to (DNA-) metabarcoding-derived results. Predictor variables are mean summer temperature (*Temp*), net relatedness index of plant assemblages (*NRI*), and plant species richness (*PSR*). *family* = error distribution family; *edf* = effective degrees of freedom, *rdf* = reference df; R^2_{adj} = adjusted R^2; R^2 = Deviance explained; Significance levels: . p < 0.1, * p < 0.5, ** p < 0.1, *** p < 0.05.

response	***predictor***	***family***	***N***	***edf***	***rdf***	χ^2	***p***	R^2_{adj}	R^2
CWM *DSI (obs)**	Temp	Beta	41	2.11	4	17.12	<0.001 ***	0.31	0.35
	PSR			0.00	4	0.00	0.956		
	NRI			0.28	4	0.34	0.273		
CWM w*MPD* (obs)	Temp	Quasipoisson	41	1.42	4	0.87	0.097.	0.44	0.47
	PSR			0.00	4	0.00	0.764		
	NRI			0.96	4	6.37	<0.001 ***		
CWM *d'* (obs)	Temp	Quasipoisson	41	3.68	4	15.38	<0.001 ***	0.72	0.75
	PSR			0.00	4	0.00	0.768		
	NRI			0.95	4	4.72	<0.001 ***		
***H2'* (obs)**	Temp	Beta	41	2.22	4	43.86	<0.001 ***	0.60	0.67
	PSR			0.39	4	0.19	0.187		
	NRI			0.95	4	17.54	<0.001 ***		
Prop. Poales	Temp	Quasibinomial	41	2.82	4	2.56	0.059.	0.20	0.28
	PSR			0.70	4	0.58	0.262		
Prop. Poaceae	Temp	Beta	41	1.37	4	5.53	0.017 *	0.12	0.16
	PSR			0.00	4	0.0	0.828		
CWM *PSS*	Temp	Gaussian	41	3.42	4	2.67	0.027 *	0.18	0.25
	PSR			0.00	4	0.00	0.336		
	NRI			0.00	4	0.00	0.621		
CWM kl*MPD*	Temp	Quasipoisson	41	2.56	4	7.09	<0.001 ***	0.49	0.50
	PSR			1.64	4	1.76	0.020 *		
	NRI			0.00	4	0.00	0.621		
CWM *PSS* (obs)	Temp	Gaussian	41	1.76	4	1.79	0.019 *	0.35	0.41
	PSR			0.61	4	0.39	0.116		
	NRI			1.30	4	2.17	0.004 **		
CWM kl*MPD* (obs)	Temp	Quasipoisson	41	2.23	4	7.61	<0.001 ***	0.59	0.58
	PSR			0.00	4	0.00	0.985		
	NRI			0.93	4	3.49	<0.001 ***		

Table S2. Results obtained from comparing observed network specialization (*H2’*) to null model distributions (econullnetr, 500 simulations). We show observed metrics (*Observed*), means of null models (*Null*), lower (*Lower.CL*) and upper confidence limits (95 %, *Upper.CL*) and the standardized effect sizes (*SES*).

site	index	Observed	Null	Lower.CL	Upper.CL	SES
H1	*H2‘*	0.68	0.71	0.60	0.83	-0.49
H2	*H2‘*	0.57	0.75	0.62	0.86	-3.11
H3	*H2‘*	0.61	0.73	0.61	0.85	-2.02
H4	*H2‘*	0.57	0.79	0.67	0.89	-3.71
H5	*H2‘*	0.62	0.77	0.61	0.90	-2.01
H6	*H2‘*	0.52	0.64	0.44	0.83	-1.11
H7	*H2‘*	0.32	0.67	0.28	0.99	-1.70
HO1	*H2‘*	0.60	0.78	0.57	0.92	-2.01
HO2	*H2‘*	0.52	0.73	0.57	0.86	-2.94
HO3	*H2‘*	0.52	0.71	0.58	0.82	-3.14
HO4	*H2‘*	0.73	0.70	0.50	0.86	0.27
K2	*H2‘*	0.72	0.70	0.56	0.85	0.16
KA	*H2‘*	0.80	0.82	0.62	0.97	-0.24
KB	*H2‘*	0.65	0.78	0.66	0.89	-2.17
KC	*H2‘*	0.78	0.76	0.63	0.86	0.29
MA	*H2‘*	0.82	0.80	0.62	0.95	0.16
SH	*H2‘*	0.81	0.71	0.58	0.83	1.44
SW	*H2‘*	0.71	0.77	0.48	0.96	-0.48
T1	*H2‘*	0.60	0.75	0.60	0.88	-2.08
T3	*H2‘*	0.67	0.62	0.47	0.77	0.68
T4	*H2‘*	0.67	0.64	0.51	0.77	0.43
T5	*H2‘*	0.66	0.66	0.53	0.81	0.07
V	*H2‘*	0.94	0.78	0.40	0.99	1.00
W1	*H2‘*	0.77	0.63	0.44	0.82	1.46
W2	*H2‘*	0.70	0.66	0.48	0.83	0.49
W3	*H2‘*	0.83	0.82	0.60	0.97	0.08
W4	*H2‘*	0.72	0.64	0.46	0.82	0.79
W5	*H2‘*	0.83	0.68	0.42	0.90	1.11
W6	*H2‘*	0.62	0.71	0.47	0.92	-0.76
W7	*H2‘*	0.53	0.69	0.43	0.90	-1.32
W8	*H2‘*	0.44	0.74	0.46	0.96	-2.22
WMA1	*H2‘*	0.64	0.71	0.53	0.86	-0.74
WMA2	*H2‘*	0.58	0.77	0.63	0.90	-2.83
WMA3	*H2‘*	0.77	0.86	0.65	0.98	-1.02
WMA4	*H2‘*	0.50	0.76	0.59	0.93	-2.95
WMA5	*H2‘*	0.69	0.63	0.21	0.96	0.24

WMB1	*H2‘*	0.59	0.73	0.62	0.85	-2.35
WMB2	*H2‘*	0.46	0.79	0.63	0.92	-4.41
WMB3	*H2‘*	0.52	0.77	0.61	0.90	-3.16
WMB4	*H2‘*	0.57	0.80	0.59	0.97	-2.32
WMB6	*H2‘*	0.37	0.73	0.35	0.97	-2.12

Table S3. Mean distance-based resource specialization values (*DSI**, *PSS*) and the corresponding mean pairwise distances (w*MPD*, kl*MPD*) of food plants in the feces of the sampled Orthoptera populations in this study. The specialization values were calculated based on the phylogenetic composition in the sample with respect to resource cover on the study site and were weighted respective to the abundances of the species on the sites. *DSI** values range between -1 and 1, indicating perfect generalisation or specialisation, respectively. Negative *PSS* values indicate clustered diets and positive values indicate overdispersed diets. The Kullback-Leibler corrected *MPD*s (kl*MPD*) are *MPD*s corrected for interaction partner frequency. (*RRA*) indicates (DNA-) metabarcoding-derived indices and (*obs*) indicates indices based on direct field-observations of feeding interactions.

Family	**Subfamily**	**Species**	**DSI* (RRA)**	**DSI* (obs)**	**wMPD (RRA)**	**wMPD (obs)**	**klMPD (RRA)**	**PSS (RRA)**	**d‘ (RRA)**	**d‘ (obs)**
Acrididae	Gomphocerinae	*Chorthippus brunneus* (Thunberg, 1815)	0.67	0.63	82.52	93.69	67.26	-0.88	0.33	0.18
		Chorthippus biguttulus (Linnaeus, 1758)	0.64	0.67	82.68	76.04	64.10	-0.80	0.40	0.28
		Chorthippus eisentrauti (Ramme, 1931)	0.54	0.65	157.91	119.63	129.37	-1.12	0.93	0.42
		Chorthippus mollis (Charpentier, 1825)	0.67	0.46	102.86	168.04	71.05	-0.88	0.68	0.51
		Chorthippus pullus (Philippi, 1830)	0.74	0.52	82.54	152.61	54.21	-1.43	0.73	0.53
		Chorthippus vagans (Eversmann, 1848)	0.37	0.50	171.39	136.03	182.16	-0.59	0.93	0.47
		Chorthippus albomarginatus (De Geer, 1773)	0.40	0.58	151.05	105.75	158.86	-0.47	0.37	0.30
		Chorthippus dorsatus (Zetterstedt, 1821)	0.35	0.52	143.24	106.56	140.02	-0.51	0.65	0.33
		Gomphocerippus rufus (Linnaeus, 1758)	0.54	0.62	106.67	91.10	93.99	-0.54	0.56	0.38
		Gomphocerus sibiricus (Linnaeus, 1767)	0.62	0.37	112.64	175.86	27.93	-1.36	0.34	0.05
		Myrmeleotettix maculatus (Thunberg, 1815)	0.60	0.39	118.93	183.07	93.04	-0.71	0.82	0.47
		Pseudochorthippus montanus (Charpentier, 1825)	0.63	0.71	82.49	68.69	74.20	-0.91	0.66	0.55

		Pseudochorthippus parallelus (Zetterstedt, 1821)	0.68	0.75	72.34	59.25	54.65	-0.83	0.49	0.27
		Omocestus haemorrhoidalis (Charpentier, 1825)	0.79	0.53	63.50	139.22	72.55	-1.09	0.63	0.43
		Omocestus rufipes (Zetterstedt, 1821)	0.92	0.70	19.38	67.88	21.02	-1.17	0.55	0.14
		Omocestus viridulus (Linnaeus, 1758)	0.64	0.70	91.35	74.78	73.35	-1.04	0.46	0.30
		Stenobothrus lineatus (Panzer, 1796)	0.81	0.74	50.88	71.01	37.17	-0.93	0.44	0.33
		Stenobothrus nigromaculatus (Herrich-Schäffer, 1840)	0.97	0.95	10.50	16.85	7.25	-1.27	0.36	0.34
		Chrysochraon dispar (Germar, 1834)	0.71	0.56	53.14	80.56	8.59	-0.85	0.83	0.37
		Euthystira brachyptera (Ocskay, 1826)	0.82	0.79	41.23	49.33	22.72	-1.29	0.55	0.33
	Oedipodinae	*Mecostethus parapleurus* (Hagenbach, 1822)	0.73	0.62	59.72	85.80	51.23	-1.32	0.43	0.28
		Stethophyma grossum (Linnaeus, 1758)	0.54	0.60	105.11	91.11	109.04	-0.72	0.48	0.28
		Oedipoda caerulescens (Linnaeus, 1758)	0.46	0.07	159.18	272.16	140.65	-0.58	0.86	0.63
		Oedipoda germanica (Latreille, 1804)	0.44	0.04	153.42	261.00	138.84	-0.67	0.60	0.47
		Bryodemella tuberculata (Fabricius, 1775)	0.46	0.00	151.71	283.79	114.21	-0.42	0.99	0.81
		Psophus stridulus (Linnaeus, 1758)	0.58	0.31	119.96	195.47	98.04	-0.97	0.72	0.70
	Calliptaminae	*Calliptamus italicus* (Linnaeus, 1758)	0.40	0.19	172.71	234.34	160.53	-1.02	0.79	0.45
	Melanoplinae	*Miramella alpina* (Kollar, 1883)	0.40	0.12	156.50	221.46	109.64	-0.85	0.58	0.53
		Podisma pedestris (Linnaeus, 1758)	0.43	0.11	169.71	282.59	155.31	-0.78	0.83	0.72
Tetrigidae	Tetriginae	*Tetrix bipunctata* (Linnaeus, 1758)	0.06	-0.44	342.11	516.16	305.31	0.67	0.64	0.59
		Tetrix kraussi (Saulcy, 1888)	0.05	-0.11	319.64	383.91	280.64	0.83	0.79	0.81

		Tetrix tenuicornis (Sahlberg, 1891)	-0.29	-0.20	370.95	381.18	407.47	2.01	0.65	0.89
		Tetrix tuerki (Krauss, 1876)	0.22	-0.93	218.91	583.64	183.43	-0.33	0.84	0.76
		Tetrix subulata (Linnaeus, 1758)	-0.39	-0.78	414.10	603.51	363.22	1.30	0.82	0.80
Trigonidiidae	Nemobiinae	*Nemobius sylvestris* (Bosc, 1792)	0.41	0.22	175.58	231.37	84.08	-0.28	0.91	0.67
Gryllidae	Gryllinae	*Gryllus campestris* (Linnaeus, 1758)	0.33	0.01	163.75	219.51	155.20	-0.53	0.73	0.44
	Oecanthinae	*Oecanthus pellucens* (Scopoli, 1763)	0.48	0.57	114.52	94.72	76.53	-0.81	0.77	0.77
Phaneropteridae	Phaneropterinae	*Barbitistes serricauda* (Fabricius, 1794)	0.63	-0.39	89.97	413.29	76.88	-0.60	0.01	0.51
		Leptophyes albovittata (Kollar, 1833)	0.60	0.35	110.94	183.03	104.91	-0.77	0.91	0.50
		Leptophyes punctatissima (Bosc, 1792)	0.09	0.40	270.24	182.24	158.66	-0.42	0.96	0.77
		Phaneroptera falcata (Poda, 1761)	0.63	0.41	87.24	150.82	76.38	-0.61	0.75	0.73
Tettigoniidae	Meconematinae	*Meconema meridionale* (Costa, 1860)	0.78		65.45		43.29	-0.43	0.96	
		Meconema thalassinum (De Geer, 1773)	0.80		60.46		47.83	2.83	1.00	1.00
	Conocephalinae	*Conocephalus dorsalis* (Latreille, 1804)	0.74	-0.66	56.13	259.17	43.69	-0.03	0.74	0.66
		Conocephalus fuscus (Fabricius, 1793)	0.71	0.07	63.02	201.20	14.24	-0.56	0.90	0.56
	Tettigoniinae	*Decticus verrucivorus* (Linnaeus, 1758)	0.20	0.06	176.59	210.37	147.27	-0.74	0.71	0.68
		Pholidoptera aptera (Fabricius, 1793)	0.43	0.17	136.66	204.85	108.30	-0.66	0.66	0.64
		Pholidoptera griseoaptera (De Geer, 1773)	0.40	0.12	138.49	206.59	98.08	-0.53	0.80	0.79
		Bicolorana bicolor (Philippi, 1830)	0.42	0.27	157.85	194.72	136.23	-0.31	0.64	0.32
		Metrioptera brachyptera (Linnaeus, 1761)	0.35	0.19	171.18	211.60	129.26	-0.84	0.63	0.61

Roeseliana roeselii (Hagenbach, 1822)	0.55	0.54	104.28	107.01	87.99	-0.59	0.55	0.30
Platycleis albopunctata (Goeze, 1778)	0.48	0.30	162.30	219.93	148.46	-0.76	0.82	0.44
Tettigonia cantans (Fuessly, 1775)	0.35	0.14	143.02	191.34	110.93	-0.67	0.77	0.86
Tettigonia viridissima (Linnaeus, 1758)	0.69	0.32	92.75	187.00	67.49	-0.55	0.81	0.53

Chapter IV

Food plants, feeding niches, grazing, and climate affect microbial communities in Orthoptera faeces

“Look deep into nature, and then you will
understand everything better”

— Albert Einstein

“Just because you cannot see something,
does not mean it is not there”

— Willard Wigan

“We cannot avoid the fact, that everything we are doing
has an influence on the whole thing.”

— Albert Einstein

Gut microbes affect the ecological fitness of insect hosts by contributing to digestion, nutrient uptake, and immunity. Since grasshoppers thrive under a vast range of climatic conditions and utilize a broad range of resources, from specialists for specific plants to generalists that even scavenge or prey on other arthropods, their associated microorganisms likely depend on the diet and the local climatic conditions. However, the factors shaping such microbial communities are largely unknown.

Here, we aimed to characterize links between specialist or generalist insects and faecal microbes along a climatic gradient. We expected (i) contrasting microbiome compositions of grasshoppers using different types of resources, and lower microbial diversity (ii) in cooler climates and in (iii) highly specialised species. We used a non-invasive amplicon sequencing approach of rDNA (combination of 16S and ITS2) from faecal pellets from 52 Orthoptera species (316 populations) to simultaneously assess diets and microbiota at 32 study sites along elevational gradients in southern Germany (Central Europe).

Although abundant microbial taxa (mostly Enterobacteriaceae) were shared and sampling site explained most of the compositional variation, differences between Orthoptera with distinct feeding niches were found. Particularly, microbial compositions varied within the group of generalist Orthoptera, as well as between specialized algae feeders and all other groups. Additionally, the phylogenetic composition of ingested plants correlated with the dissimilarity between bacterial communities. In contrast to insects, microbial communities were more diverse at higher elevations, while relative abundances of taxa varied along the elevational gradient and with grazing.

Our study provides empirical evidence of dynamic links between insects and microbes along a climatic gradient, demonstrating that feeding niches, consumed plants, and climate shape the microbial composition and diversity detected in insect faeces. However, the large overlap of taxa – particularly at site level – suggests that common, generalist bacteria are mainly acquired from the feeding environment such as the phyllosphere/anthosphere of eaten plants. Yet, this does not deprive them of important functions and highlights our limited understanding of functional links of insects and their potential symbionts.

4.1 *INTRODUCTION*

A lion’s share of the complexity of interactions between herbivorous insects and their environment remains unexplored, especially when zooming from the macro scale, where insects can be observed feeding on certain plant species, to the micro scale, where a rich set of microorganisms inhabiting the phyllosphere get consumed by herbivores, settle in their digestive tracts, or are transmitted as hitchhikers through the food web (Douglas 2015, Engel & Moran 2013, Keller et al 2021, McFall-Ngai et al 2013). High-throughput sequencing paved the ways for the detection of such microbial associations with insects (Roslin et al 2019), triggering recent interest to explore their functional feedbacks and relationships. Because such interactions might change the ecological fitness among populations of insects, co-evolving gut bacteria may have played an important role in the diversification of insects and are possibly involved in shaping feeding interactions (Cornwallis et al 2023, Kaufman & Klug 1991). Climate change leads to fast range shifts in insects and exposes them (and their microbiome) to novel feeding environments (Kerner et al 2023, Maihoff et al 2023). This also reshuffles the cards for insect-microbe interactions and consequences for the ecosystem are still unknown.

Since the insect hindgut represents a nutrient-rich ecological niche, a plethora of microbes are associated with them (Hirata et al 2023). Most of them belong to the domain bacteria (Engel & Moran 2013). Bacteria found in frass of insects can represent the full spectrum from closely linked to the biology of their hosts (obligate symbionts) towards transiently passing through the digestive system (facultative symbionts, commensals, or pathogens). Gut-colonizing bacteria regulate a variety of host processes linked to the insect’s ecological fitness (Buchon et al 2013), such as the nutrient absorption and digestion of food by secreting digestive enzymes, compensating for nutrient-deficient diets, and detoxifying plant defence compounds (Dillon & Charnley 2002, Engel & Moran 2013, Idowu et al 2009, Kaufman & Klug 1991, Zheng et al 2021). Thereby, they affect various components and processes of the ecosystem such as nutrient cycling (Noman et al 2020).

The community structure of microorganisms in insect guts depends on many factors, such as feeding behaviour, phylogenetic position, life stage, sex, and the external environment (Douglas 2015, Zheng et al 2021). While for eusocial insects, some beetles, flies, cicadas, butterflies, and bugs, vertical transfer of endosymbiotic microbiota was demonstrated (Kaltenpoth et al 2009, Paniagua Voirol et al 2018, Zhou et al 2022), the opportunity to transfer gut symbionts between conspecifics of solitary insects with non-overlapping generations is limited (Voulgari-Kokota et al 2019b, Zhou et al 2022). Additionally, shedding the fore- and hindgut during development interferes with colonizing bacteria populations (Zhou et al 2022). Thus, and due to a simple gut structure, caterpillar gut microbiomes are frequently characterized by a prevalence of leaf-associated bacteria, implying that in some groups, resident, host-specific symbionts are sparse or absent (Hammer et al 2017, Hird et al 2014, Zhou et al 2022). In such cases, the microorganisms are often obtained from and reflect the insects' feeding environment (Voulgari-Kokota et al 2020). Insects with comparable diets therefore often share similar microbial communities, e.g., termites, dragonflies, bees, and beetles (Deb et al 2019, Kudo et al 2019, Mikaelyan et al 2015, Voulgari-Kokota et al 2019a). Based on the assumption that different taxonomic units of plants harbour distinct epi- and endophytic/zooic bacterial communities (Gaube et al 2021, Trivedi et al 2020) that also differ from those of detritus or living arthropods, it is reasonable to assume that microbes in faeces vary depending on the insect's last meals, even within general feeding groups. Additionally, various plant parts such as flowers, roots, and leaves are inhabited by distinct epiphytic bacteria based on microhabitat filtering due to osmotic niches and a limited time frame for flower colonization (Gaube et al 2021, Junker & Keller 2015, Trivedi et al 2020). Variation in the diet can dynamically affect the structure of the insect's microbiome with the potential to alter the growth and development rate of the insect. Further, hosts can also select and filter to limit microbial communities, e.g., by specific redox conditions in the insects' hindgut, which select for the proliferation of certain bacterial groups, including members of the Enterobacteriaceae family (Dillon & Charnley 2002, Dillon et al 2000, Dillon et al 2008). Such bacteria could be beneficial in coping with plant defences or assisting with digestion of certain food items which are difficult to disintegrate, a prerequisite for conserved microbiota of specialized insects (Cornwallis et al 2023).

Bacterial diversity is also influenced by feeding behaviour, since omnivorous, polyphagous insects often harbour a more diverse microbial community than stenophagous insects (Deb et al 2019, Yun et al 2014). Generalist insects feeding on a diverse set of host plants or select arthropods, carrion, and detritus may encounter a larger number of bacteria which could settle in their digestive tract, because the food source is more diverse.

Most of the research on insect microbiomes has focused on charismatic hymenopterans and lepidopterans (Engel et al 2012, Leonhardt et al 2022, Voulgari-Kokota et al 2019a). Orthoptera (Ensifera, Caelifera) are significant insect herbivores with a broad resource spectrum in temperate grassland habitats with integral impact on these ecosystems, ecologically and economically (Blumer & Diemer 1996). However, their microbiome is still poorly understood. Most species are classified as generalist feeders, hence consuming and blending plant species from various families (Franzke et al 2010, Joern 1979), but feeding preferences can differ markedly (König et al 2022a). Even closely related co-occurring species may partition food resources due to microclimatic demands or plant traits (Ibanez et al 2013b). This broad range of feeding habits in related insects and their vast environmental niches make them suitable study organisms to characterize differences in microbial associates. At least, insect herbivores, like orthopterans, likely rely on their microbiomes to extract nutrients from plant tissues which are difficult to digest (Hansen & Moran 2014) and disperse microbes via defecation or contact over longer distances within their feeding environment due to their mobility.

Species richness and community composition is shaped by clinal variation of climatic conditions (Gaston 2000, McCain & Grytnes 2010, Peters et al 2019, Rahbek 1995). Along elevational gradients e.g., a reduction in temperature often causes declines of species diversity (Descombes et al 2017a, Gaston 2000, McCain & Grytnes 2010), as previously shown for Orthoptera the same study system as the study presented (König et al 2022a), attributed to lower rates of biotic processes or reduced rates of evolutionary diversification (Peters et al 2016a). As bacteria are, on the one hand, thought to have low dispersal ability and distinct temperature optima, temperature sensitivity, and resistance to thermal stress (Hammer et al 2021, Palmer-Young et al 2018), abiotic climatic clines could lead to rapid turnover rates along the gradient. On the other hand, close associations with their hosts could cause conserved links between insects and microbes across climatic gradients. The ability of Orthoptera to cope with changing or extreme climatic conditions could at least partly be mediated by associated microbes. However, little is known about the temperature-dependence of such interactions in a natural context and how environmental factors shape the microbiome.

In this study, we applied DNA metabarcoding to Orthoptera faeces collected in the field to reconstruct dietary information and links to the bacterial microbiome. We investigated the microbial composition and diversity in four co-occurring superfamilies of Orthoptera (Acridoidea, Grylloidea, Tetrigoidea, and Tettigonioidea) with different feeding niches (specialists for grasses or algae & mosses, generalist herbivores & omnivores) along a climatic gradient. Based on the expectation that the environment mainly determines the associated microorganisms in grasshoppers, we considered the role of general feeding niches, direct links to eaten plants, and site-specific abiotic climatic conditions and hypothesized that *I)* microbial diversity is higher in generalists than in specialists, and community composition differs between Orthoptera with different feeding niches, that *II)* plants eaten affect the microbial composition detected in the faecal samples and that *III)* bacteria diversity decreases along the climatic gradient, paralleling insect diversity declines, and alongside bacterial community composition changes.

4.2 MATERIALS AND METHODS

4.2.1 Study region and sites

We studied Orthoptera and their associated microbial communities at 32 grassland sites on calcareous bedrock in southern Germany (Bavaria). Sites were selected along elevational gradients spanning six elevational zones (colline: < 600 m.a.s.l., submontane: 600 – 825 m.a.s.l., montane: 825 – 1,200 m.a.s.l., high-montane: 1,200 – 1,500 m.a.s.l., subalpine: 1,500 – 1,825 m.a.s.l., and alpine: > 1,825 m.a.s.l.) and ranged between 250 and 2,100 m.a.s.l. mean elevation, covering an evenly distributed cline of mean annual temperature. The grassland sites covered 60 x 60 m of rather homogeneous vegetation and were either extensively managed (low impact grazing, $n = 15$) or unmanaged ($n = 17$).

4.2.2 Orthoptera field surveys, classification, and faecal sampling

Orthoptera were sampled at all study sites between July and September 2019. The sites were visited from low elevations to high elevations, following Orthoptera phenology (König et al 2022a). Surveys were performed on warm, sunny days, which are considered favourable activity conditions for the majority of Orthoptera species in the region (König & Krauss 2019). We identified species by their stridulation and visually directly in the field (Tab. 1). Observations of *Gryllotalpa gryllotalpa* (L.) and *Myrmecophilus acervorum* (Panzer) were excluded from our assessments since we only sporadically caught individuals of those soil dwelling species. All occurring Orthoptera species were classified as either generalist herbivores (primarily feeding on leaves and flowers of different forbs), generalist omnivores (regularly feeding on leaves and flowers of plants, arthropods and carrion, including species which primarily feed on other arthropods in adult stage), specialized grass feeders, or specialized algae & moss feeders based on literature (Ingrisch & Köhler 1998, König et al 2022a). The only specialist carnivorous species, *Meconema meridionale*, was included in the omnivorous group for the analyses.

To characterize microbial associates of the locally occurring Orthoptera populations, we collected frass samples. Therefore, we captured 10 (± 1) imagines (5 males / 5 females) per species at each of the study sites (hereafter referred to as population, excluding small populations with fewer than 10 individuals). We then transferred them individually into sterile falcon tubes, waited until each animal produced 2 – 3 faecal pellets, and released them directly at the collection site (König et al 2022a). By employing this non-invasive method, we aimed to minimize the impact on local Orthoptera densities within the protected areas. Following release, we meticulously gathered and combined the faecal samples into a single shared sample for each distinct population (per species and study site). To prevent degradation of the DNA-samples, we suspended the samples in DNA/RNA shield (ZymoBIOMICS) (König et al 2022a).

Table 1. Taxonomical (suborder, subfamily, species) and ecological (feeding niche, literature survey) classifications of Orthoptera species from which faecal samples were collected. The number of populations sampled (10 ± 1 individuals each) is shown in the column *n*.

Suborder	**Subfamily**	**Species**	**Feeding niche**	***n***
Caelifera	Calliptaminae	*Calliptamus italicus*	Generalist: Herbivore	3
	Gomphocerinae	*Chorthippus albomarginatus*	Specialist: Grasses	12
		Chorthippus biguttulus	Specialist: Grasses	30
		Chorthippus brunneus	Specialist: Grasses	28
		Chorthippus dorsatus	Specialist: Grasses	6
		Chorthippus eisentrauti	Specialist: Grasses	3
		Chorthippus mollis	Specialist: Grasses	2
		Chorthippus pullus	Specialist: Grasses	2
		Chorthippus vagans	Specialist: Grasses	1
		Chrysochraon dispar	Specialist: Grasses	1
		Euthystira brachyptera	Specialist: Grasses	15
		Gomphocerippus rufus	Specialist: Grasses	26
		Gomphocerus sibiricus	Specialist: Grasses	1
		Myrmeleotettix maculatus	Specialist: Grasses	1
		Omocestus haemorrhoidalis	Specialist: Grasses	1
		Omocestus rufipes	Specialist: Grasses	4
		Omocestus viridulus	Specialist: Grasses	31
		Pseudochorthippus montanus	Specialist: Grasses	2
		Pseudochorthippus parallelus	Specialist: Grasses	26
		Stenobothrus lineatus	Specialist: Grasses	12
	Melanoplinae	*Miramella alpina*	Generalist: Herbivore	4
		Podisma pedestris	Generalist: Herbivore	10
	Oedipodinae	*Bryodemella tuberculata*	Generalist: Herbivore	1
		Mecostethus parapleurus	Specialist: Grasses	26
		Oedipoda caerulescens	Generalist: Herbivore	3
		Oedipoda germanica	Generalist: Herbivore	2
		Psophus stridulus	Generalist: Herbivore	8
		Stethophyma grossum	Specialist: Grasses	5
	Tetriginae	*Tetrix bipunctata*	Specialist: Algae & Mosses	5
		Tetrix kraussi	Specialist: Algae & Mosses	12
		Tetrix subulata	Specialist: Algae & Mosses	10
		Tetrix tenuicornis	Specialist: Algae & Mosses	3
		Tetrix tuerki	Specialist: Algae & Mosses	1
Ensifera	Conocephalinae	*Conocephalus dorsalis*	Generalist: Omnivore	1
		Conocephalus fuscus	Generalist: Omnivore	1
	Gryllinae	*Gryllus campestris*	Generalist: Omnivore	3
	Meconematinae	*Meconema meridionale*	Specialist: Carnivore	1
	Nemobiinae	*Nemobius sylvestris*	Generalist: Omnivore	1
	Oecanthinae	*Oecanthus pellucens*	Generalist: Omnivore	2
	Phaneropterinae	*Barbitistes serricauda*	Generalist: Herbivore	1
		Leptophyes albovittata	Generalist: Herbivore	2
		Leptophyes punctatissima	Generalist: Herbivore	2

		Phaneroptera falcata	Generalist: Herbivore	1
	Tettigoniinae	*Bicolorana bicolor*	Generalist: Omnivore	2
		Decticus verrucivorus	Generalist: Omnivore	10
		Metrioptera brachyptera	Generalist: Omnivore	7
		Pholidoptera aptera	Generalist: Omnivore	16
		Pholidoptera griseoaptera	Generalist: Omnivore	8
		Platycleis albopunctata	Generalist: Omnivore	2
		Roeseliana roeselii	Generalist: Omnivore	14
		Tettigonia cantans	Generalist: Omnivore	8
		Tettigonia viridissima	Generalist: Omnivore	2

4.2.3 Amplicon sequencing workflow and taxa assignment

Metabarcoding (amplicon sequencing) analyses were performed for plant feeding with the ITS2 rDNA marker as described in Sickel et al (2015), while workflows for the processing of the 16S bacterial microbiome followed (Kozich et al 2013), Voulgari-Kokota et al (2019a). In a laboratory dedicated to DNA analysis, we performed DNA isolation from faecal samples, library preparation, indexing, quality control, normalization, pooling, quantification, and sequencing (Campos et al 2021, König et al 2022a, Kozich et al 2013). We extracted DNA of frass samples with the ZymoBIOMICS DNA Miniprep Kit, following the manufacturer's instructions. Following DNA extraction, the ITS2 rDNA region, and the 16S rRNA gene V4 region were amplified with a dual-indexing strategy (Kozich et al 2013). To mitigate stochastic effects, PCRs of both, samples and controls were carried out in triplicates using the PCR Phusion Master Mix (New England Biolabs, UK). To monitor potential contaminations arising from the field and laboratory procedures, negative controls (samples with preservation solution and/or reagents without faeces taken at multiple steps) were incorporated. Following DNA normalization to equimolar ratio (Invitrogen SequalPrep Plate Normalization Kit, Thermo Fisher Scientific, Life Technologies) libraries were pooled, fragment length distributions examined (Bioanalyzer 2200, Agilent), and concentrations quantified (Qubit II Fluorometer, Thermo Fisher Scientific, Life Technologies). The final libraries were then sequenced in-house on a MiSeq platform (Biocentre, University of Wuerzburg, Germany) using v2 2x250 cycle reagent cartridges spiked with custom index, Read1, and Read2 sequencing primers (Sickel et al 2015), and 5% PhiXv3 (Kozich et al 2013).

Bioinformatic cleanup followed the pipeline available at https://github.com/chiras/metabarcoding_pipeline (Leonhardt et al 2022) making use of VSEARCH (Rognes et al 2016). Paired ends of forward and reverse reads were joined, and all reads shorter than 150 bp were removed. Furthermore, quality filtering (EE < 1) as described by Edgar and Flyvbjerg (2015) and *de-novo* chimera filtering following UCHIME3 (Edgar 2016b) was performed. VSEARCH was also used to define amplicon sequence variants (ASVs) (Edgar 2016c). For bacteria, ASVs were directly mapped with global alignments with and cut-off threshold of 80 against the RDP database v6 using the VSEARCH implementation of SINTAX (Edgar 2016a). For plant classification, we followed a two-step classification strategy: By using VSEARCH against an ITS2 reference database for plant species of the sampled region, reads were directly mapped with global alignments with an identity cut-off threshold of 97%. The reference database was compiled with the BCdatabaser (Keller et al 2020) based on a list of plant species of Berchtesgaden and curated (Quaresma et al 2023). To classify remaining reads without taxonomic allocation at this point, SINTAX was used with a reference database including global plant species (Quaresma et al 2023, Sickel et al 2015).

To account for differences in reads per sample, we transformed the sequence reads of each sample to relative read abundances (equals proportional abundances of ASVs) that were further used as a semi-quantitative estimations of interaction intensities (Deagle et al 2019, König et al 2022a, Welti et al 2019). We aggregated all ITS2 ASVs at the genus-level and removed ASVs only classified at the family or higher levels. To omit sequencing artefacts, we omitted all ASVs with read numbers below 50 in at least 1% of the samples in both datasets for subsequent analyses.

To estimate phylogenetic relationships of detected plants, we used a pseudo-phylogeny based on taxonomic relationships provided by ‘phyloT’ (Fig. S1). For bacteria, we computed a fastTree on the web server ‘CIPRES’ based on ‘MAFFT’-alignments of the individual sequences recovered from metabarcoding (Fig. S2).

Data were analysed in R 3.6.4 (R Core Team 2019) with the packages ‘phyloseq’ v1.22.3 (McMurdie & Holmes 2013), ‘microVIZ’ (Barnett et al 2021), and ‘vegan’ v2.5-7 (Oksanen et al 2020).

4.2.4 Statistical analyses

I) Differences in α-diversity of microbial communities from faecal samples between populations of different feeding groups were assessed with wilxcon-rank-sum tests (Holm-correction for pairwise comparisons, unfiltered dataset), and community compositions between and within groups were compared using permutational analysis of variance (PERMANOVA, adonis2 - function of ‘vegan’) after testing for differences in multivariate homogeneity of dispersions between the feeding groups (β-diversity, intra-group variability, measured as distance to centroid, betadisper – function of ‘vegan’). As dissimilarity (β-diversity) measure we used generalized UniFrac distances, which include phylogenetic information, and the Bray–Curtis dissimilarity metric, which accounts for both ASV presence and absence, as well as relative abundances. For the comparison of bacterial communities of populations with different feeding niches, we omitted high elevation samples of grass feeders, since high elevation communities were strongly biased towards Gomphocerinae, and included sampling site as cofactor. This reduced sample size to 234. Additionally, we used beta-binomial regression with logit-link (corncob, Martin et al 2020), which was developed to model challenging sequencing data, to detect differentially abundant taxa (relative abundances, hierarchical modelling across taxonomic ranks down to genus-level) between the feeding groups, while including elevation and management as possibly confounding covariates and correcting *p*-values for multiple testing following the Benjamini-Hochberg method.

II) Additional to the coarse ecological classification of feeding habits, we aimed to identify links between the plant community composition found in frass samples and the associated microbial community in more detail. Therefore, the relationship between the phylogenetic dissimilarity matrix of the plants recovered from the 316 faecal samples and the phylogenetic dissimilarity matrix of bacteria communities (generalized UniFrac-distances, GUniFrac) from the same samples was assessed with a permutational Mantel test statistic based on Pearson's product-moment correlation (999 permutations).

III) To analyse the effect of the external environment (elevation/climate) on bacterial communities in Orthoptera faecal samples, we used permutational analysis of variance, followed by a logistic regression model for testing differential abundance in compositional microbiome data with Sandev’s adjustment of *p*-values ('LOCOM', Hu et al 2022) to identify gradients in relative read abundances of bacterial ASVs. This modelling approach was developed for high-dimensional compositional sequencing data with high proportions of zeros, preserving false discovery rate (Hu et al 2022). As we expected potential strain-level variation along the climatic gradient resulting from variation in climatic optima, we conducted this analysis on ASV level instead of higher taxonomic aggregates. For this analysis, we only used members of the grass feeding Orthoptera guild, which were common across the whole elevational gradient, to exclude effects of feeding habits, and thus reducing sample size to 186. Additionally, we excluded all taxa with a prevalence below 10%. We also included subfamily as cofactor in this analysis to account for possible differences resulting from phylogeny.

4.3 RESULTS

We collected a total of 316 pooled faecal samples (per species and site) of more than 3,000 individuals from Orthoptera populations, which belong to 52 Orthoptera species (33 Caelifera, 19 Ensifera). Sequencing for the 16S rDNA yielded on average 17,492 (± 9,490 SD) high-quality reads (5,706,113 high-quality reads), while we recovered 13,372 (± 7,103 SD) ITS2 rDNA reads per sample after quality and control filtering (4,728,304 high-quality reads). After mapping to the reference databases with an identity threshold of 97%, 213 unique plant genera from 87 families and 49 orders, and 13,264 unique bacterial ASV clusters were assigned. Taxonomic classifications of these microbial ASV clusters revealed 24 phyla, including three low abundant archaeal taxa, 123 orders, and 262 families, respectively. On average, the sampled Orthoptera populations fed on 7.2 (± 3.5) plant genera, and we identified 633.6 (± 471.2) microbial ASVs in each sample of pooled frass from 10 individuals per Orthoptera species and site.

Microbes of Orthoptera frass samples were dominated by members of the bacteria phylum Proteobacteria. Three of the six most represented ASVs were assigned to the genus *Pantoea* (Gammaproteobacteria, Enterobacteriaceae), two to *Pseudomonas* (Gamma-proteobacteria, Pseudomonadaceae), and one to *Sphingomonas* (Alphaproteobacteria, Sphingomonadaceae). Cumulatively, Proteobacteria had the highest relative abundance on phylum level, before Bacteroidetes, Firmicutes, and Actinobacteria.

4.3.1 Feeding niches

First, we compared the community compositions of bacteria (community structure) from 234 Orthoptera populations regarding their distinct feeding niches based on literature (30 generalist herbivore populations, 72 generalist omnivore populations, 29 specialist algae & moss feeder populations, 104 specialist grass feeder populations), which revealed a total of 9,781 unique microbial ASVs. On population level, all feeding groups hosted rich microbial communities (Fig. 1A). Bacterial α- and β-diversity differed among and between insects with different feeding niches (Fig. 1A & B, Fig. S3). Since the community dispersions varied between feeding groups ($F_{3,\ 214}$ = 5.77, p = 0.002), results from the analysis of the community composition need to be treated with caution and we focus on discussing differences in dispersions. However, differentially abundant bacterial phyla, orders, families, and genera detected by beta-binomial regression (Fig. 2, Tab. 2) indicate also valid compositional/structural differences between Orthoptera populations of different feeding niches (Fig. 1C). Still, there was also a large overlap of frequent and abundant bacterial taxa in the faeces of Orthoptera of different feeding habits (Fig. 1D). The variance in the community structure of microbes was mainly explained by study site (R^2 = 0.24, $F_{30,\ 200}$ = 2.48, p = 0.001), indicating a large overlap of shared microorganisms at each study site, and a stronger effect than feeding niche (R^2 = 0.10, $F_{3,\ 200}$ = 10.33, p = 0.001) or management (R^2 = 0.01, $F_{1,\ 200}$ = 3.52, p = 0.004) had on the composition (Fig. 1C).

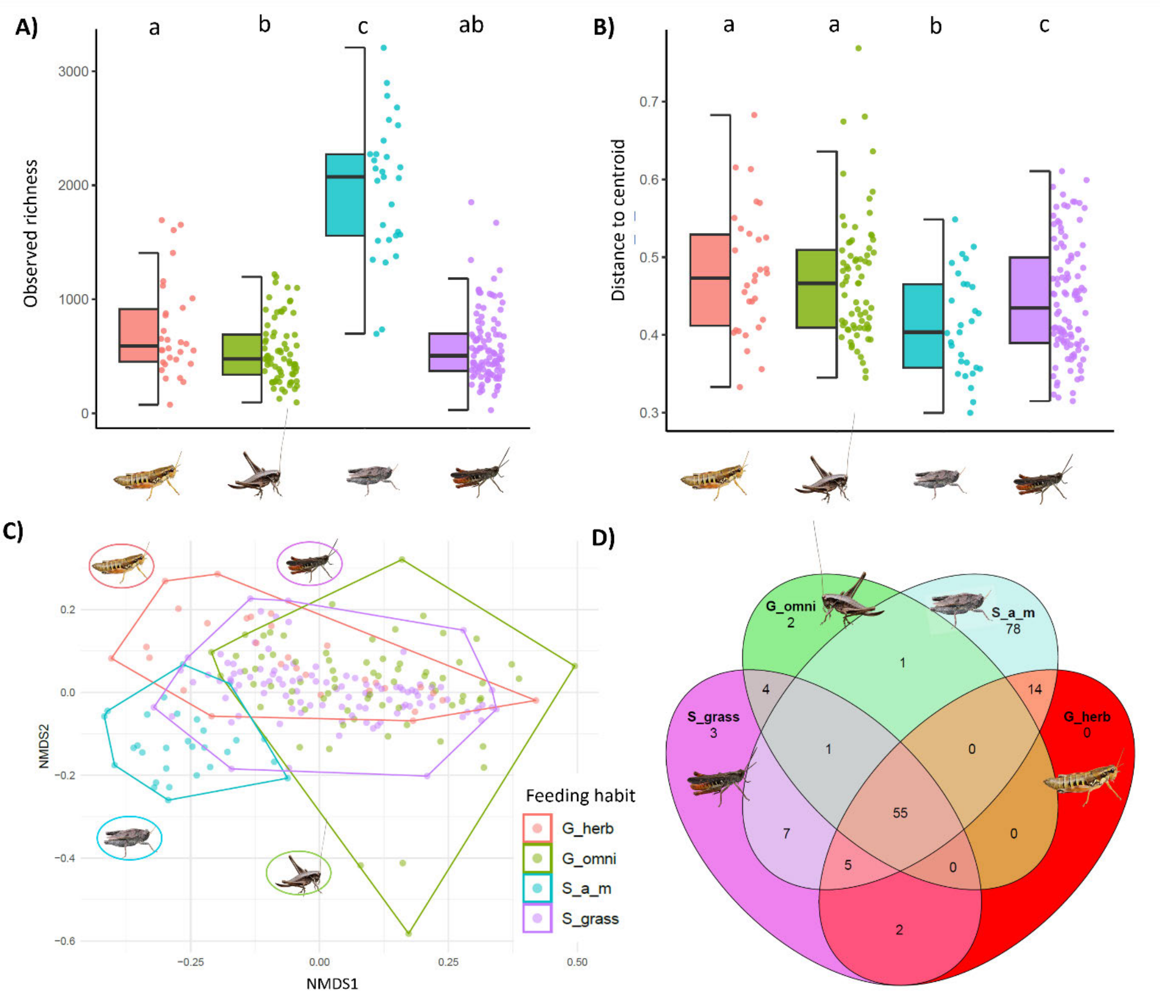


Figure 1. A) Observed ASV richness (α-diversity) of frass-microorganisms from herbivorous generalists, omnivorous generalists, specialists for algae & mosses, and grass-feeding specialists. B) Specialists differed less in their phylogenetic community composition (β-diversity) than generalists, which showed the highest heterogeneity. C) NMDS (stress = 0.14) depicting the generalized UniFrac dissimilarities between bacteria compositions from samples of different feeding groups. Grazing explained 1% (p = 0.004), site 24% (p < 0.001), and feeding niche 10% (p < 0.001) of the variance of the community structure. D) Venn-diagram of overlapping core taxa (25% prevalence and 1 % relative abundance) of the four different feeding groups. Red: *G_herb* = generalist herbivores, green: *G_omni* = generalist omnivores, blue: *S_a_m* = specialists for algae and mosses, violet: *S_grass* = specialists for grasses. Letters indicate statistical significance at a level of 0.05.

Generalist herbivores

In faeces of generalist herbivorous Orthoptera, the most abundant microbial taxa were ASVs belonging to the genera *Pantoea*, *Pseudomonas*, *Sphingomonas*, *Erwinia* (Gammaproteobacteria, Enterobacteriaceae), and *Roultella* (Gammaproteobacteria, Enterobacteriaceae). Differentially more abundant taxa compared to all other feeding groups were e.g., *Methylobacterium* (Alphaproteobacteria, Methylobacteriaceae), *Raoultella*, *Rhizorhabdus* (Alphaproteobacteria, Sphingomonadaceae), and other Proteobacteria (Fig. 2D, Tab. 2). Proteobacteria were more abundant in generalist herbivores and grass-feeding specialists, both groups feeding mainly on higher plants (monocots and dicots). The ASV richness detected was lower than that of algae & moss feeding specialists ($p < 0.001$), not different to that of grass-feeding specialists ($p = 0.150$), but higher than that of omnivorous generalists ($p = 0.070$, Fig. 1A). Communities recovered from generalist herbivore populations were more dissimilar than those of specialist grass ($p = 0.029$) and algae & moss feeders ($p < 0.001$), respectively (Fig. 1B).

Generalist omnivores

The most abundant microbial taxa in the faeces of omnivorous Orthoptera were ASVs belonging to the genera *Pantoea*, *Pseudomonas*, *Obesumbacterium* (Gammaproteobacteria, Enterobacteriaceae), *Hafnia* (Gammaproteobacteria, Enterobacteriaceae), and *Sphingomonas*. Differentially more abundant taxa compared to all other groups were e.g., *Obesumbacterium*, *Lactococcus* (Firmicutes, Steptococcaceae), *Pluralibacter* (Gammaproteobacteria, Enterobacteriaceae), *Stenotrophomonas* (Gammaproteobacteria, Enterobacteriaceae), Pseudomoadaceae, and other Alphaproteobacteria (Fig. 2C, Tab. 2). The ASV richness detected was lower than that of algae & moss feeding specialists ($p < 0.001$) and herbivorous generalists ($p = 0.070$, Fig. 1A), but communities recovered from generalist omnivore populations were as heterogeneous as those of herbivorous generalists ($p = 0.639$) and more heterogeneous than those of specialist grass ($p = 0.025$) and algae & moss feeders ($p < 0.001$), respectively (Fig. 1B).

Specialized algae and moss feeders

For specialized, mostly algae and moss feeding Orthoptera, the most abundant genera in the faeces samples were ASVs belonging to the genera *Pantoea*, *Pseudomonas*, *Sphingomonas*, and members of the phylum Cyanobacteria. Cyanobacteria, Chitinophagaceae, Armatimonadetes, and Acidobacteria were more abundant than in samples from Orthoptera with other feeding habits (Fig. 2A, Tab. 2). The ASV richness detected was on average more than twice that of all other groups ($p < 0.001$, Fig. 1A), but the communities recovered from specialized algae & moss feeding populations were phylogenetically more similar (less heterogeneous) to each other than samples from all other groups, indicating a conserved phylogenetic clustering (Fig. 1B).

Specialised grass feeders

The most abundant microbial genera in the faeces of mainly grass feeding Orthoptera were ASVs belonging to the genera *Pantoea*, *Pseudomonas*, and *Sphingomonas*. *Williamsia* (Actinomycetota, Nocardiaceae), *Hymenobacter* (Cytophagia, Hymenobacteraceae), *Serratia* (Gammaproteobacteria, Enterobacteriaceae), *Pantoea*, and Sphingomonadales were more abundant in grass feeders than in other groups (Fig. 2B, Tab. 2). The ASV richness observed was not different to that of generalist omnivores ($p = 0.410$) or herbivores ($p = 0.150$) but was lower than that of algae & moss feeders ($p < 0.001$, Fig. 1A). Samples from grass feeders differed among themselves less than communities of generalists ($p = 0.03$), but more than samples from algae & mosses feeding specialists ($p = 0.04$, Fig. 1B).

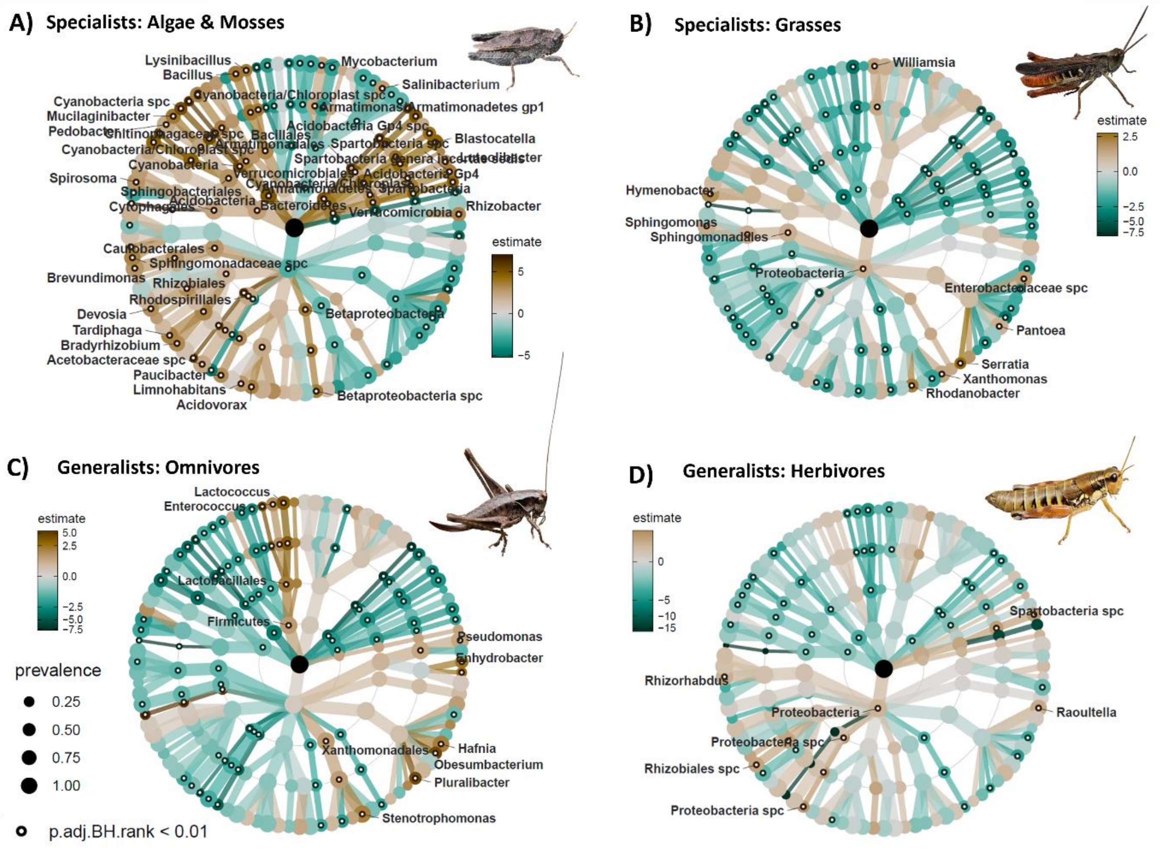


Figure 2. Differentially abundant bacteria genera derived from beta-binomial regression of samples of A) specialists on algae and mosses, B) grass-feeding specialists, C) omnivorous generalists, and D) herbivorous generalists compared to samples from all other groups, respectively. Circle size is proportional to the prevalence of the individual taxa (taxa key in Fig. S5), white circles indicate significant taxa after Benjamini-Hochberg correction of *p*-values, brown colours represent differentially more abundant taxa (relative abundance), and blue indicates less abundant taxa (Tab. 2). Names were added only for core taxa (prevalence above 25%), which were significantly more abundant in the respective feeding group of Orthoptera.

Table 2. Differentially more abundant bacteria taxa found in samples of specialists on algae & mosses, grass-feeding specialists, herbivorous generalists, and omnivorous generalists compared to samples of all other groups derived from beta-binomial regression (hierarchical modelling across ranks). *P*-values were corrected following Benjamini-Hochberg. We show the top 10 most significant taxa for each feeding niche (full modelling results in electronic supplement). *Est.* = estimate, *S.E.* = standard error.

Feeding niche	**Taxon**	**Rank**	**Est.**	**S.E.**	***t*-value**	***p*-value**
Specialists: Algae & Mosses	f: Acidobacteria Gp4	family	3.64	0.24	14.97	<0.001
	g: *Blastocatella*	genus	3.64	0.24	14.97	<0.001
	p: Cyanobacteria	phylum	4.57	0.33	13.70	<0.001
	p: Armatimonadetes	phylum	4.75	0.35	13.52	<0.001
	o: Acidobacteria Gp4	order	3.48	0.26	13.57	<0.001
	p: Acidobacteria	phylum	3.11	0.24	12.91	<0.001
	o: Armatimonadales	order	4.45	0.38	11.81	<0.001
	o: Cyanobacteria	order	5.21	0.44	11.82	<0.001
	f: Chitinophagaceae	family	3.89	0.33	11.85	<0.001
	f: Armatimonadaceae	family	4.45	0.38	11.81	<0.001
	f: Spartobacteria	family	3.69	0.35	10.64	<0.001
Specialists: Grasses	g: *Hymenobacter*	genus	1.59	0.18	8.91	<0.001
	g: *Rhodanobacter*	genus	2.43	0.45	5.35	<0.001
	g: *Serratia*	genus	2.97	0.64	4.67	<0.001
	g: *Williamsia*	genus	1.05	0.25	4.26	<0.001
	g: Enterobacteriaceae spc.	genus	0.90	0.22	4.02	<0.001
	g: *Sphingomonas*	genus	0.56	0.14	3.96	<0.001
	f: Nocardiaceae	family	0.77	0.21	3.63	0.001
	g: *Pantoea*	genus	0.52	0.15	3.40	0.002
	p: Proteobacteria	phylum	0.52	0.15	3.45	0.003
	f: Sphingomonadaceae	family	0.47	0.14	3.37	0.003
	o: Sphingomonadales	order	0.47	0.14	3.37	0.003
Generalists: Omnivores	g: *Obesumbacterium*	genus	4.22	0.44	9.52	<0.001
	g: *Pluralibacter*	genus	4.17	0.52	7.99	<0.001
	o: Alphaproteobacteria	order	5.46	0.73	7.43	<0.001
	f: Alphaproteobacteria spc.	family	5.46	0.73	7.43	<0.001
	g: Alphaproteobacteria spc.	genus	5.46	0.73	7.43	<0.001
	g: *Stenotrophomonas*	genus	1.89	0.26	7.33	<0.001
	f: Streptococcaceae	family	3.04	0.47	6.44	<0.001
	g: *Lactococcus*	genus	3.04	0.47	6.44	<0.001
	o: Lactobacillales	order	1.89	0.43	4.45	<0.001
	f: Leuconostocaceae	family	3.84	0.88	4.36	<0.001
	g: *Weissella*	genus	3.84	0.88	4.36	<0.001
	p: Firmicutes	phylum	1.22	0.31	3.89	<0.001
Generalists: Herbivores	o: Proteobacteria spc.	order	3.33	0.68	4.87	<0.001
	g: Proteobacteria spc.	genus	3.33	0.68	4.87	<0.001
	f: Proteobacteria spc.	family	3.33	0.68	4.87	<0.001

	g: *Rhizorhabdus*	genus	1.35	0.29	4.68	<0.001
	p: Proteobacteria	phylum	0.94	0.22	4.21	<0.001
	f: Rhizobiales spc.	family	2.01	0.49	4.11	<0.001
	g: *Raoultella*	genus	1.38	0.45	3.04	0.010
	f: Spartobacteria spc.	family	2.03	0.67	3.02	0.011
	g: *Methylobacterium*	genus	0.64	0.21	2.98	0.012
	f: Methylobacteriaceae	family	0.64	0.21	2.98	0.012

Management: Grazing on pastures

Since we sampled Orthoptera at either grazed pastures or unmanaged grassland sites, we included management as covariate in the analysis of variance and the regression model. Community structure ($F_{1,\,200}$ = 3.52, p = 0.001) and heterogeneity ($F_{1,\,233}$ = 11.73, p = 0.001) were affected by grazing with less heterogeneous communities at grazed sites, while some bacterial taxa, such as *Serratia*, *Acinetobacter*, *Bacillus*, some Clostridiales, and Actinobacteria such as *Williamsia* were more abundant on grazed sites compared to unmanaged sites (Fig. S6, Tab. S1).

4.3.2 Plant-microbe phylogenetic conservatism

The grass genera *Cynosurus*, *Dactylis*, *Sesleria*, and *Festuca* (all members of the family Poaceae) were the plants most frequently detected in Orthoptera faecal samples. Especially, we found a high proportion of grass genera in frass of Orthoptera classified as grass feeders, while plants from different families such as Apiaceae, Asteraceae, Caryophyllaceae, and Fabaceae were regularly detected in samples from herbivorous and omnivorous generalists (Fig. 3A). Algae made up the majority of ITS2 reads of algae & moss specialists (Fig. 3A). We detected a statistically significant Mantel-correlation of the phylogenetic dissimilarities of bacterial communities from all 316 Orthoptera populations with the respective phylogenetic dissimilarities of plant communities (r = 0.24, p < 0.001, Fig. 3E, Table 2).

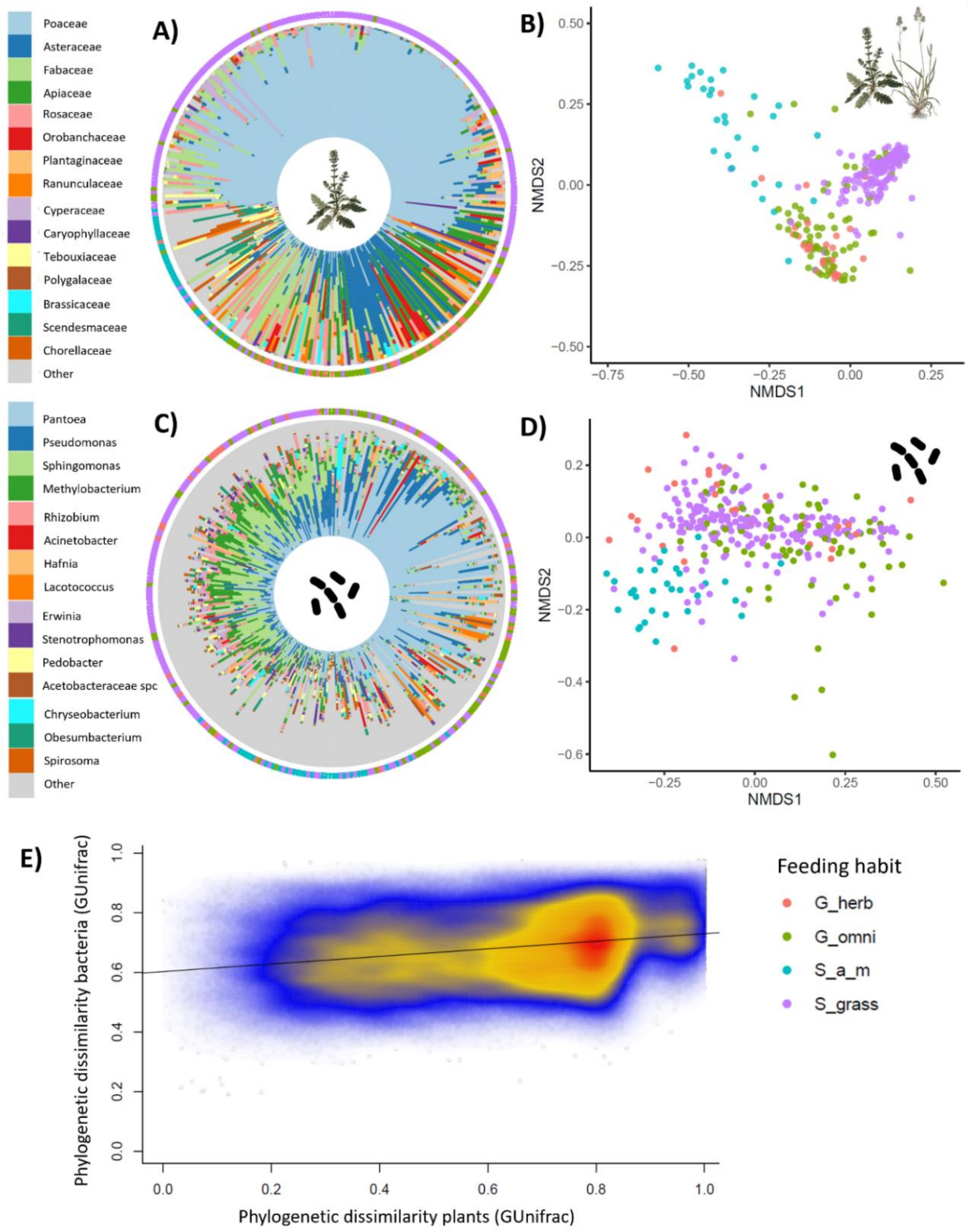


Figure 3. Compositional barplots of A) plants found in 316 Orthoptera faecal samples pooled from more than 3,000 individuals. Genera of the family Poaceae (blue) had the highest relative abundance in many samples. B) Corresponding NMDS plot (stress = 0.13) of the generalized UniFrac (weighted, GUniFrac) dissimilarities of the plant communities found in the faecal samples. C) Compositional barplots of the bacterial communities in faecal samples of Orthoptera. Proteobacteria such as *Pantoea spec.* (blue) were most represented in faecal samples. D) Corresponding NMDS plot (stress = 0.15) of the generalized UniFrac dissimilarities of the bacterial communities found in the faecal samples. E) Mantel-correlation between the phylogenetic dissimilarity matrices of bacteria and plant communities (r = 0.24, p < 0.001). Red: *G_herb* = generalist herbivores, green: *G_omni* = generalist omnivores, blue: *S_a_m* = specialists for algae and mosses, violet: *S_grass* = specialists for grasses, grey: Cumulative representation of all other less abundant taxa in A) and C).

4.3.3 Effect of elevation on bacterial communities

We compared 186 samples from the group of grass-feeding Orthoptera along the elevational, climatic gradient and found that the ASV richness, as well as Shannon diversity of microbes was highest in the high-montane elevational zone and lowest in the submontane zone towards the valleys (Fig. 4B, Fig. S7, Tab. S2). Communities did not differ in their dissimilarity when comparing samples within the four elevational zones ($F_{3,\ 182}$ = 0.69, p = 0.560, Fig. 4C), but compositions differed along the elevational gradient (R^2 = 0.07, $F_{1,\ 149}$ = 16.48, p = 0.001, Fig. S8), since many of the 11,213 ASVs of bacteria detected in the subset of grass feeding Orthoptera were more abundant under warm conditions in the valleys, and some taxa such as *Polaromonas* (Pseudomonadota, Comamonadaceae) were more abundant at high elevations (Tab. 3, Fig. S9, Fig. S10). Within ASVs of the same genera of bacteria, there were cases of increasing and decreasing abundance trends with elevation, highlighting that there can be strain- or species-level differences in the environmental response (Tab. 3).

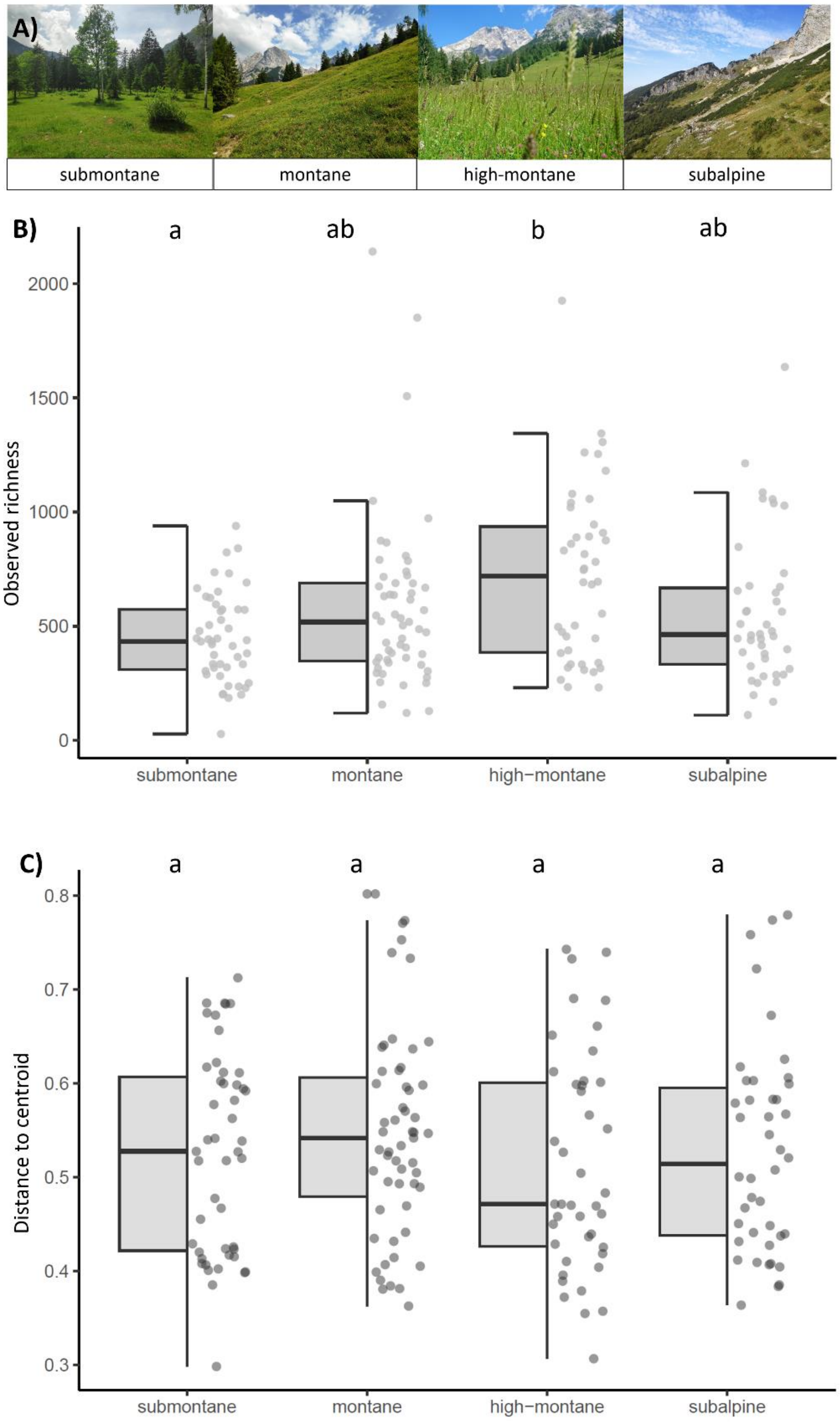


Figure 4. A) Images of typical study sites in the four sampled elevational zones (submontane: 600 – 825 m.a.s.l., montane: 825 – 1,200 m.a.s.l., high-montane: 1,200 – 1,500 m.a.s.l., subalpine: 1,500 – 1,825 m.a.s.l.). B) Observed ASV richness (α-diversity) of bacteria in samples of specialists on grasses from the four elevational zones. C) UniFrac compositional heterogeneity (β-diversity) of microbial communities of grass-specialist samples did not differ between elevational zones along the elevational gradient. Letters indicate statistical significance at a level of 0.05.

Table 3. Bacteria ASVs, which are increasing or decreasing most with elevation, from grass feeders, ordered according to their effect sizes derived from a logistic regression model ('LOCOM') with Sandev's adjustment of *p*-values. We included subfamily and management in the model to account for possible confounding effects. *Rel. Abu.* = relative read abundance.

ASV	**Genus**	**Rel. Abu.**	**Prevalence**	***p*-value**	**Effect size**
Decreasing					
ASV.12041	*Methylobacterium*	0.00004	0.13	0.005	-0.009
ASV.5043	*Pseudomonas*	0.00032	0.13	0.005	-0.009
ASV.8393	*Mucilaginibacter*	0.00012	0.19	0.005	-0.008
ASV.6861	*Spirosoma*	0.00021	0.31	0.005	-0.007
ASV.7056	*Spirosoma*	0.00032	0.30	0.005	-0.007
ASV.12058	*Methylobacterium*	0.00004	0.13	0.005	-0.006
ASV.6949	*Spirosoma*	0.00023	0.27	0.005	-0.006
ASV.5806	*Pantoea*	0.03532	0.76	0.005	-0.006
ASV.7292	*Chryseobacterium*	0.00078	0.32	0.005	-0.005
ASV.13469	Sphingomonadaceae	0.00004	0.25	0.005	-0.005
ASV.5078	*Pseudomonas*	0.00012	0.24	0.005	-0.005
ASV.12589	*Rhizobium*	0.00016	0.32	0.047	-0.004
ASV.13570	*Sphingomonas*	0.00001	0.17	0.005	-0.004
ASV.5008	*Pantoea*	0.00005	0.18	0.005	-0.004
ASV.12582	*Rhizobium*	0.00003	0.16	0.026	-0.004
ASV.6705	*Hymenobacter*	0.00002	0.13	0.005	-0.004
Increasing					
ASV.6674	*Hymenobacter*	0.00018	0.20	0.048	0.006
ASV.12482	*Devosia*	0.00018	0.25	0.005	0.006
ASV.12511	*Devosia*	0.00010	0.14	0.005	0.005
ASV.6334	*Polaromonas*	0.00011	0.20	0.005	0.005
ASV.6317	*Limnohabitans*	0.00027	0.18	0.026	0.005
ASV.831	*Homoserinibacter*	0.00005	0.17	0.012	0.004
ASV.8316	*Sphingobacterium*	0.00018	0.18	0.018	0.004
ASV.6278	*Pseudorhodoferax*	0.00024	0.29	0.029	0.004
ASV.6672	*Hymenobacter*	0.00030	0.27	0.015	0.004
ASV.8482	*Pedobacter*	0.00022	0.22	0.042	0.003
ASV.5864	*Acinetobacter*	0.00028	0.20	0.033	0.003
ASV.7582	Chitinophagaceae	0.00017	0.20	0.012	0.003
ASV.13119	*Rhizorhabdus*	0.00005	0.18	0.009	0.003
ASV.8546	*Mucilaginibacter*	0.00012	0.22	0.005	0.003
ASV.8480	*Pedobacter*	0.00017	0.26	0.009	0.003
ASV.5111	*Pseudomonas*	0.01255	0.90	0.035	0.003
ASV.8632	*Mucilaginibacter*	0.00008	0.25	0.005	0.003
ASV.10171	*Paenibacillus*	0.00005	0.16	0.033	0.003
ASV.10136	*Finegoldia*	0.00028	0.24	0.021	0.003

4.4 *DISCUSSION*

While studying insects' microbial associations has gained increasing interest in the past years, there is growing evidence of functional interactions, but still little knowledge about the factors shaping the assemblages of microorganisms. In this study, bacterial communities from frass were characterized across 52 Orthoptera species with different feeding habits along a climatic gradient. We show that the bacterial composition differs with respect to the feeding niches of Orthoptera and, for the first time, along a natural climatic gradient. However, the large overlap and high shared prevalence of many microbial ASVs among grass feeders, herbivores, and omnivores suggest little specificity of the bacterial communities but rather that the feeding environment and, in particular, the diets consumed are drivers of bacterial microbiomes found in frass of Orthoptera.

Similar to the gut microbial communities reported in other studies, Orthoptera faeces mainly harbored bacterial taxa of Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and Cyanobacteria (Dillon et al 2008, Liu et al 2023, Muratore et al 2020, Wang et al 2020, Zhao et al 2022, Zheng et al 2021, Zhou et al 2022), including taxa with well-known associations with plants or soils (Muratore et al 2020, Walterson & Stavrinides 2015). Many of these bacterial ASVs in our study were ubiquitous and shared among different feeding groups and along elevation, suggesting that either, *I)* there are specific conditions in the insect's gut which select for the proliferation of the same set of taxa acquired from the environment, as shown for the pronounced representation of Enterobacteriaceae cells such as *Pantoea* in locusts hindguts (Dillon & Charnley 2002), or *II)* different components of the environment share generalist bacteria which are equally ingested by Orthoptera with different diets. Thus, epiphytic bacteria communities associated with different plant species or plant parts (phyllosphere / anthosphere / rhizosphere) should be similar in that case. Recent evidence indicates that plant parts and plant species have common phyllosphere bacteria (Massoni et al 2020), while other studies highlight differences in the bacteria communities from flowers and leaves (Gaube et al 2021, Junker & Keller 2015), as well as between species (Gaube et al 2021, Kembel et al 2014). All studies agree that a majority of bacterial taxa are generalists with broad niches and can occupy a variety of different microhabitats (Gaube et al 2023). Most of the bacteria inhabiting the phyllosphere are Proteobacteria, followed by Actinobacteria and Bacteroidetes (Gaube et al 2021, Massoni et al 2020, Trivedi et al 2020). Similar ratios between microbial phyla from our frass samples and typical bacteria communities of plant assemblages could be one explanation why Orthoptera feeding on different plant species had the same bacteria in their faeces and thus a hint on the environmental origin of the bacteria in grasshopper guts, reflecting the feeding environment (Hammer et al 2017). On a lower taxonomic resolution, however, studies reported a higher abundance of Alphaproteobacteria compared to Gammaproteobacteria on plant leaves (Dillon & Charnley 2002, Gaube et al 2021, Massoni et al

2020). The high abundance of Enterobacteriaceae (Gammaproteobacteria) detected in our study could imply an overrepresentation of these bacteria in the guts of Orthoptera compared to the environment (Dillon 2002). However, this still needs to be confirmed since we did not sample plant microbiota directly. Sampling site explained most of the variation in the microbial community composition in insect frass samples, which can be interpreted as a further hint on the impact of the close feeding environment on the bacteria within insects' guts (Hird et al 2014). Site-specific communities, which could be based on differences in the microenvironment, are also expected based on differences in the phyllosphere bacteria between different sites (Gaube et al 2021). However, we still detected differences of the microbiome of Orthoptera with distinct general diets.

4.4.1 Impact of feeding niche on the composition and diversity of bacteria

Feeding niches contribute to shifts in prevalence of bacterial groups in the gut. Some Orthoptera subfamilies have phylogenetically conserved preferences for specific plant taxa; e.g., grasshoppers (Gomphocerinae) are often specialized on grasses (Poaceae), whereas groundhoppers (Tetrigidae) choose algae, lichen, and mosses as main source of food (Ibanez et al 2013a, Ingrisch & Köhler 1998, König et al 2022a, McClenaghan et al 2015). By contrast, omnivorous, polyphagous Tettigonids and Gryllids mix leaves and flowers of plants, and even other arthropods to differing partitions in their diets (Ingrisch & Köhler 1998). We found bacteria such as members of the Lactobacillaceae and Pseudomonadaceae, which are often particularly associated with flowers (Vannette 2020) or pollen-foraging insects (Mayr et al 2021, Voulgari-Kokota et al 2019a), predominantly in samples of omnivorous Orthoptera. Even though our plant barcoding does not include information if the sampled specimens were feeding on flowers, observational and literature evidence supports this link. Many of the species from the study area were regularly observed feeding on flowers of Fabaceae, Caryophyllaceae, Asteraceae, Apiaceae, and Rosaceae flowers for example (Ingrisch & Köhler 1998), possibly ingesting flower associated bacteria which can be found in the frass. Since the generalist Orthoptera differ in their individual or species-specific preferences, or are unselective, it is not surprising that the differences in community compositions of associated bacteria were advanced compared to specialist feeders with conserved and predictable diets.

In specialist grass feeding Orthoptera, microbes such as *Pantoea* were found to correlate with the digestive ability of certain species (Ling et al 2022). This could be due to the secretion of cellulases (Idowu et al 2009), which may assist the insects own cellulases with digesting cellulose of eaten plant fibres, since the *Pantoea* has been shown to be responsible for plant cell-wall polymers break down (Bozorov et al 2019). This is particularly relevant for Orthoptera feeding mainly on tough plant material such as grasses. In line with this, we also found a higher prevalence of such members of the family Enterobacteriaceae (Gammaproteobacteria) in the group of grass feeding species.

The clearest difference between bacterial communities was found between specialist algae & moss feeders compared to all other diet groups. The Tetrigids are an evolutionary ancient group of grasshoppers, and their feeding niche is most distinct from that of other orthopterans, which might explain the high number of unique bacterial taxa in their frass. Many of these were associated with soil, humid conditions, or were Cyanobacteria, common in their main food (algae, lichen, mosses, detritus), which is not regularly consumed by other Orthoptera in the study region (König et al 2022a). The higher diversity of associated microbes than that of generalist Orthoptera was unexpected (Yun et al 2014) but may result from many ASVs belonging to Cyanobacteria, which grow in their microhabitat and are eaten, or from their ground-dwelling lifestyle, exposing them to a high diversity of other soil- and root-associated bacteria not often encountered by phyllosphere insects. Hence, the classification of specialist algae & moss feeders does not account for the many other food sources which are included in addition to those main food items, decreasing their feeding specialisation (König et al 2022a).

4.4.2 Impact of ingested plants on the composition of bacteria

We found a weak effect of the phylogenetic composition of food plants on the phylogenetic composition of bacteria in Orthoptera faeces. The more dissimilar feeding niches are in terms of plants ingested, the more dissimilar the associated microbial communities are. This suggests that plant lineages can act as a phylogenetic filter on the assembly of associated phyllosphere bacteria as found for microbiomes of tropical trees (Kembel et al 2014), for example through phylogenetically conserved trait matching.

4.4.3 Impact of elevation on the composition and diversity of bacteria

Along elevational gradients, the steep decline in temperature causes a turnover in both plant and Orthoptera communities (Hoiss et al 2012, König et al 2022a), which impacts plant-herbivore interactions (König et al 2022a, Pitteloud et al 2021). Additionally, pathogen pressure also decreases towards high elevations (Harvell et al 2002, Hodkinson 2005). By comparing frass of grass-feeding Orthoptera populations along an elevational gradient, we found indications for an elevational effect on gut microbes. On the one hand, our results suggest potential strain-level variation of associated bacteria. ASVs, which could represent either species of a genus or strains, vary in relative abundance from low to high elevations. Thermal properties that support optimal growth in cooler environments, or the changing plant and thus food assemblages could be the reason for the abundance clines we found for some bacteria (Hammer et al 2021). On the other hand, we also found variation of bacterial associates at a higher taxonomic level than the ASVs, such as a higher relative abundance of *Polaromonas*, a genus adapted to harsh abiotic conditions at high elevations and a high dominance of Enterobacteriaceae such as *Pantoea* in the warm valleys, corroborating findings of other studies on temperature-dependent microbiome responses (Berg et al 2016, Horváthová et al 2019, Jaramillo & Castañeda 2021, Moghadam et al 2018). Since *Pantoea* was shown to produce precursors of antimicrobial substances, it is speculated to play an important role in the defense against pathogens (Dillon & Charnley 1995, Dillon et al 2005). If there is a close link of the function of some microbial inhabitants of the digestive system, such as Enterobacteriaceae like *Pantoea* spec., with grasshoppers, either for immunity or nutrient breakdown or uptake, the relaxed dominance of these in samples from high elevations could indicate a higher risk of infection or decreased digestive efficiency. Warmer conditions due to climate change may stabilize these associations also at higher elevations with benefits for the insect's fitness.

Microbial diversity of grass-feeding Orthoptera peaked at higher elevations, a finding that was not expected. This could result from an increase in the availability of niches, as the dominance of Proteobacteria decreased with elevation. If these dominant phyla have their growth optima at higher temperatures higher diversity could result at higher elevations where they cannot dominate the community anymore. Along elevational gradients, similar trends were shown for bumblebees in Berchtesgaden and bees in Tanzania, as the core microbiome was increasingly difficult to maintain and unstable with decreasing temperature towards high elevations (Mayr et al 2021, Maihoff et al. unpublished). However, this could also be an indication of less intensive management of grassland at high elevations, since less diverse and more homogeneous microbial communities on plants were found at grassland sites of higher land use intensity (Gaube et al 2021, Gossner et al 2016).

4.4.4 Impact of grazing

Common bacterial indicators of livestock grazing such as *Serratia*, *Acinetobacter*, *Bacillus*, Clostridiales, and some Actinobacteria were even found in grasshopper faeces on grazed sites (Tab. S4). Bacteria disseminated in cow dung are therefore either directly consumed by orthopterans feeding on the dung itself (Ingrisch & Köhler 1998) or they colonize the phyllosphere by transmission from the soil or by trampling (Bhatt & Maheshwari 2019, Gaube et al 2021). As with arthropods (Gossner et al 2016), these communities from pastures were more similar to each other than communities from unmanaged grassland, suggesting that management could lead to a homogenization of bacterial communities in the environment (Gaube et al 2021), since disturbance events such as grazing and mowing alter the microhabitat and induce stress on the host plant (Ilmarinen & Mikola 2009, Medina-Roldán et al 2012). We showed that cow grazing has an weak impact on the environmental pool of bacteria and then successively impacts the arthropod microbial communities, an alarming finding. Therefore, we should keep in mind to consider how antimicrobial treatments of cattle could pass through the entire grazed ecosystem with unknown consequences.

4.4.5 Caveats

For most of the Orthoptera investigated, feeding niches correlate strongly with their phylogenetic positions. Therefore, inference regarding the impact of feeding niches on the community composition of microorganisms could also reflect an effect of phylogenetically conserved interactions *per se*. However, some species such as the Meadow Bush-cricket (*Roeseliana roeselii*) show tendencies of a transition from an omnivorous lifestyle typical for other katydids towards a specialisation on grasses (Ingrisch & Köhler 1998). Our study showed its community composition clusters with that of typical grass-feeder communities, suggesting a superordinate role of the diet over phylogeny, even though other studies found an impact of the taxonomy on the microbiome (Ling et al 2022).

Apart from metabarcoding-workflow-associated biases in the representation of different microbial taxa, the detection of bacteria by means of amplicon sequencing is neither a direct indication of functions nor of permanent residency nor of the viability of the identified microbes. Nevertheless, it provides a first overview of the associated bacteria, which may have a functional role in the gut, while others may be plant or insect pathogens for which the grasshopper may serve as vector (Humphrey & Whiteman 2020). Additionally, we use relative abundances which are no direct indication for abundances/colony-forming units. It would thus be interesting to compare actual bacterial loads along climatic gradients.

Although there was a strong indication for an environmental origin of microbes and uptake through feeding, we could not directly trace the origin of the bacteria found in the faeces with our study, and thus it remains to be determined whether abundances differ from those in the phyllosphere due to a selective environment in the grasshopper gut.

4.4.6 Conclusions: The feeding environment matters

In contrast to bacterial assemblages of honeybees, those colonizing the guts of generalist insect herbivores are not yet sufficiently characterized. To our knowledge, we provide a first study that examined bacterial community compositions of insects with different feeding niches along an elevational gradient. Our results indicate a dynamic link between grasshoppers and microbes, where the local environment and the diet affect the community composition of bacteria with a large overlap of mainly Alpha- and Gammaproteobacteria. This is consistent with the hypothesized close but not compelling association between grasshoppers and bacteria, whose high generalism provokes interesting questions about the resilience of these associations to land-use change and climatic challenges. Given the magnitude of possible interactions between herbivores, plants, and microbes, a detailed characterization of the bacterial diversity and community composition of different feeding groups of insect herbivores represents a first step towards a better understanding of the interplay and functional role of these different trophic compartments of an ecosystem.

4.5 *SUPPLEMENTARY MATERIAL TO CHAPTER IV*

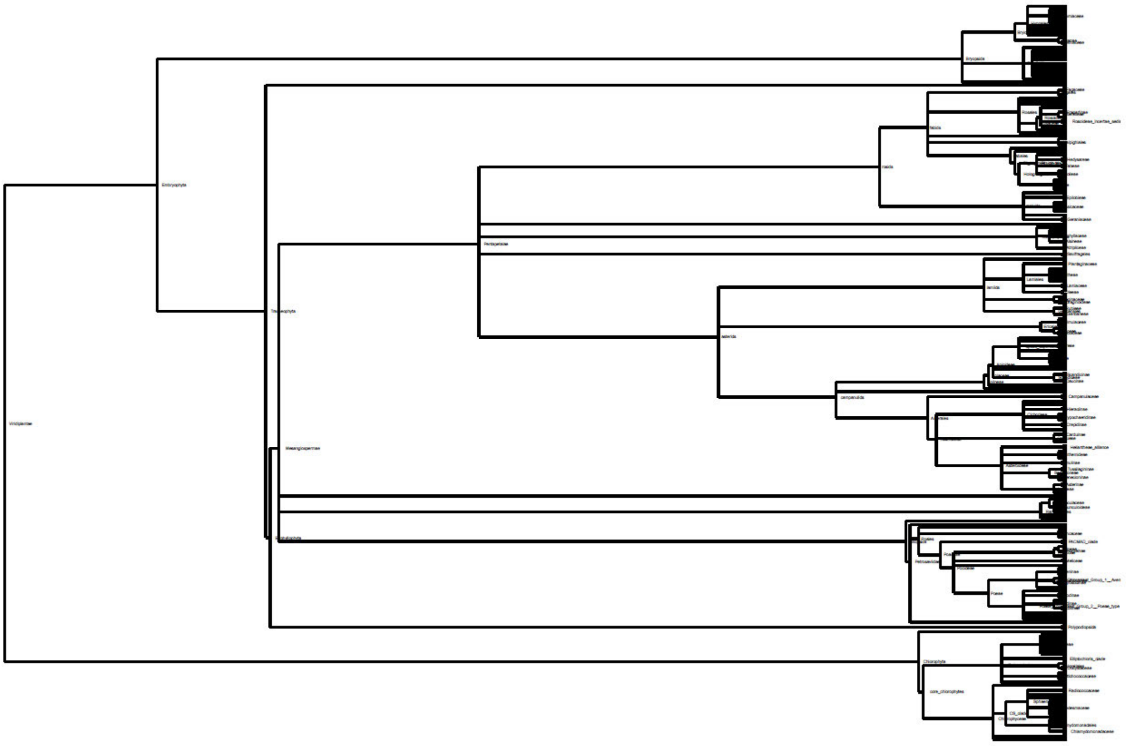


Figure S1. Pseudo-phylogeny of plants detected in frass samples of Orthoptera. The phylogenetic tree was estimated based on the taxonomic relationships between taxa (NCBI taxonomy) with the web program ‘phyloT’.

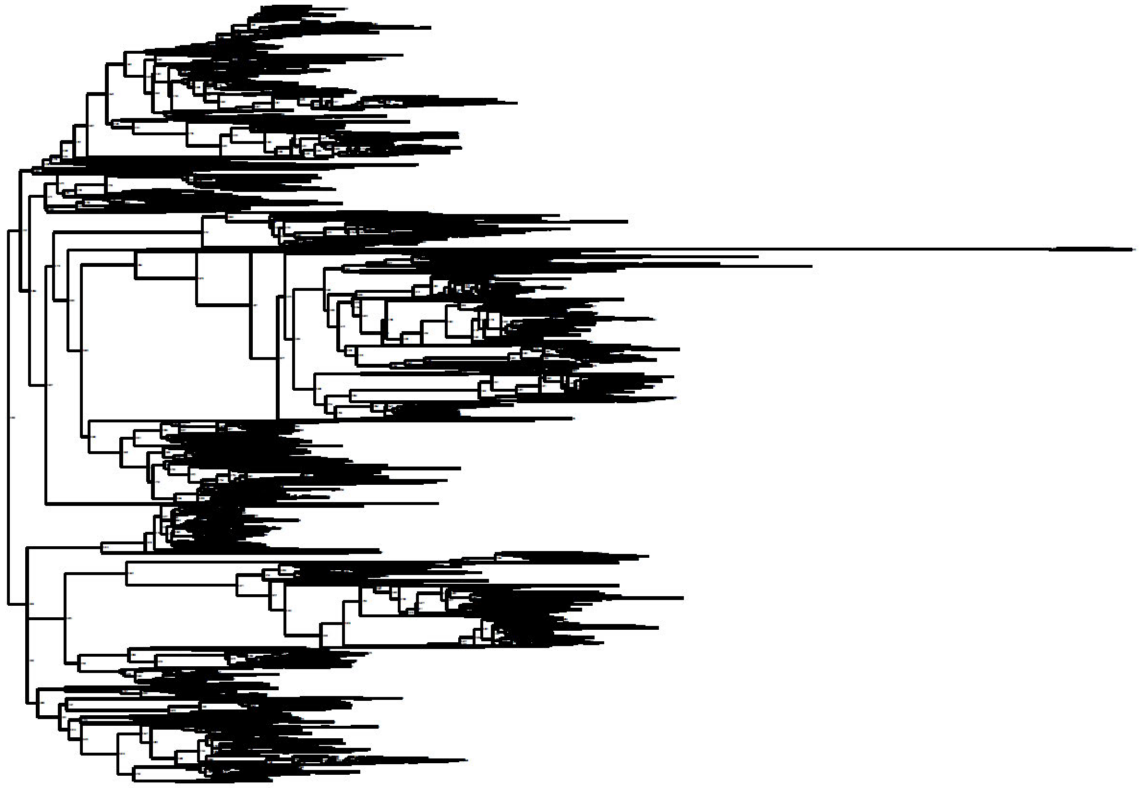


Figure S2. Pseudo-phylogeny of microbial taxa found in Orthoptera frass samples. The phylogenetic tree was estimated based on a 'MAFFT'-alignment of sequenced 16S rDNA fragments with 'fastTree' run on the 'CIPRES' web server.

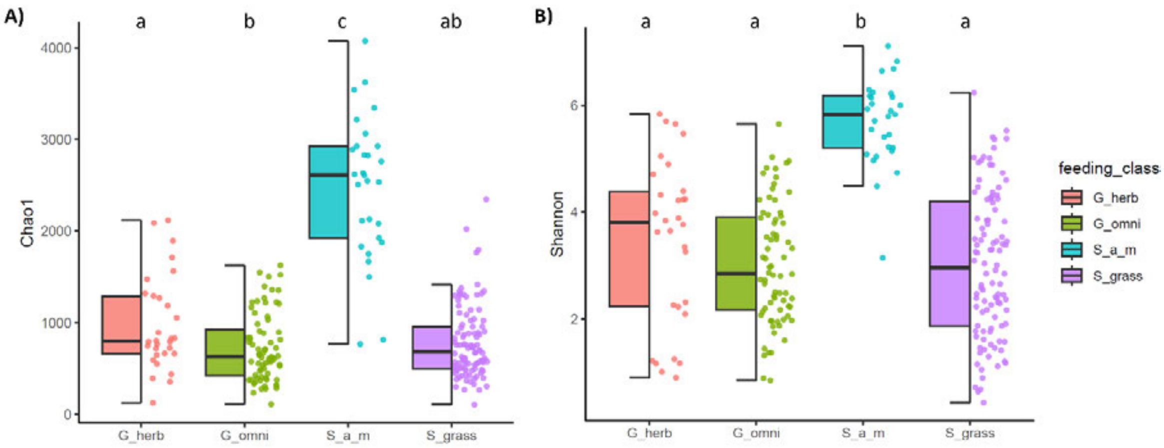


Figure S3. α-diversity indices of microbial communities recovered from Orthoptera populations with different feeding niches. Shown are the corrected richness of microbial ASVs (Chao1, A) as well as the Shannon diversity (B). Red: *G_herb* = generalist herbivores, green: *G_omni* = generalist omnivores, blue: *S_a_m* = specialists for algae and mosses, violet: *S_grass* = specialists for grasses.

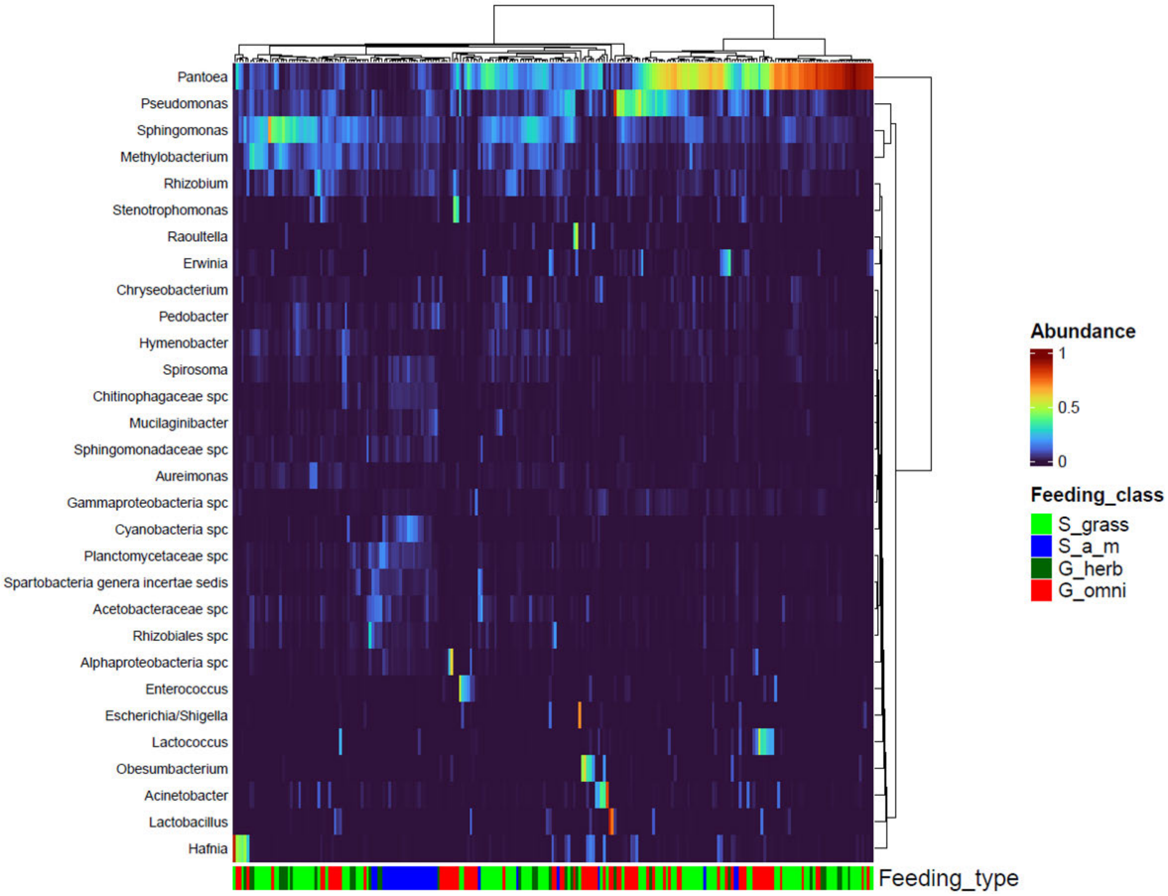
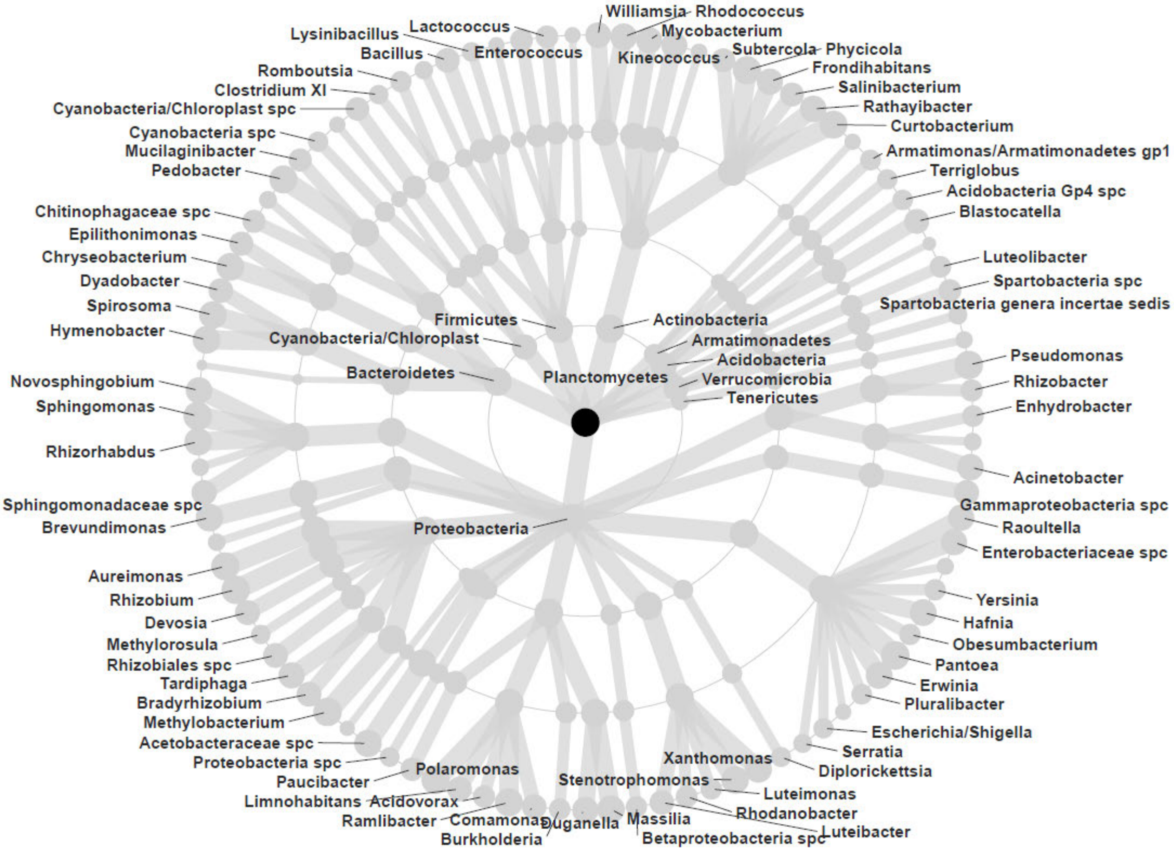


Figure S4. Heatmap of the relative abundances of the 30 most frequent genera of bacteria in 235 Orthoptera faecal samples clustered based on compositional similarity. Red: *G_herb* = generalist herbivores, green: *G_omni* = generalist omnivores, blue: *S_a_m* = specialists for algae and mosses, violet: *S_grass* = specialists for grasses. Red indicates high relative abundance and dark blue indicates no presence in the sample.



Key

Figure S5. Key to bacterial taxa included in the beta-binomial regression to detect differentially abundant taxa between feeding groups. We added all names of genera with at least 25% prevalence in all samples as well as of phyla.

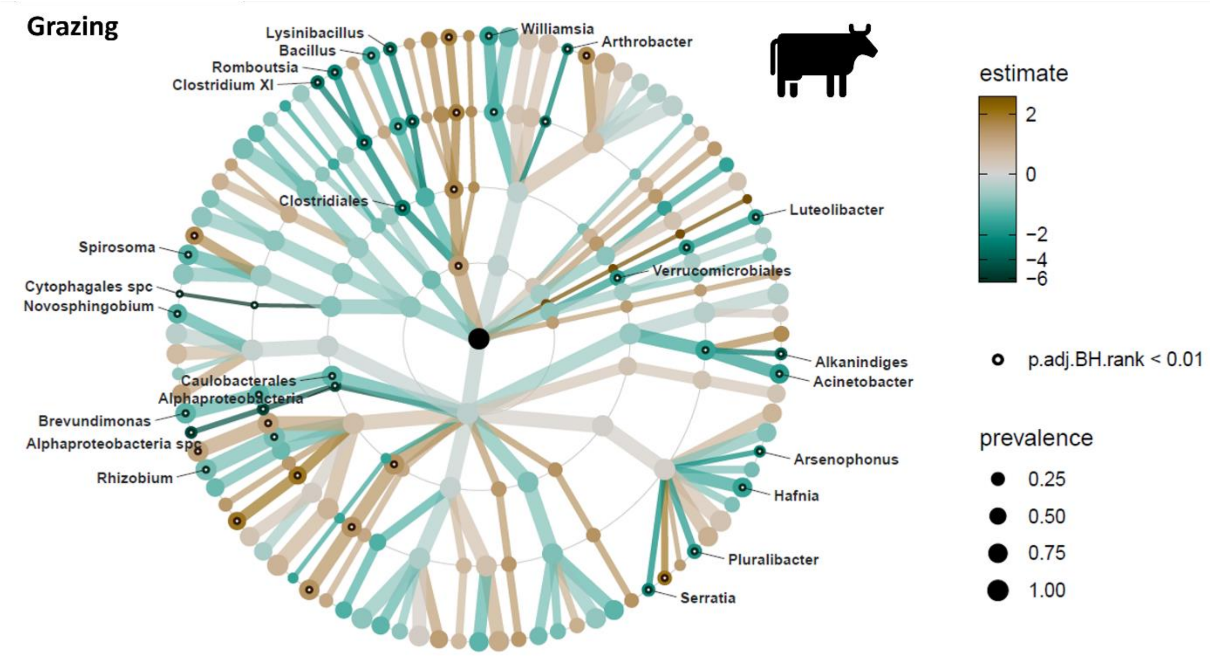


Figure S6. Differentially abundant bacteria genera derived from beta-binomial regression of samples from either unmanaged grassland sites or cattle/sheep grazed sites. Circle size is proportional to the prevalence of the individual taxa (key in Fig. S5), white circles indicate significant taxa after Benjamini-Hochberg correction of *p*-values, brown colours represent differentially more abundant taxa at unmanaged sites and blue indicates less abundant taxa (Tab. S1). We added names of significantly less abundant core taxa with a prevalence above 25%. Many of the differentially abundant bacteria found in faeces of grasshoppers at grazed sites were common bacteria in the digestive system of cows such as *Clostidium* or *Serratia*.

Table S1. Differentially more abundant bacteria taxa of Orthoptera inhabiting cattle/sheep grazed-pastures or unmanaged grassland sites derived from beta-binomial regression (hierarchical modelling across ranks). *P*-values were corrected following Benjamini-Hochberg. We show the most significant more abundant taxa for pastures (full modelling results are available in electronic supplementary material).

Taxon	**Rank**	**Estimate**	**SE**	***t*-statistic**	***p*-value**
o: Alphaproteobacteria	order	-4.62	0.62	-7.45	<0.001
f: Alphaproteobacteria spc.	family	-4.62	0.62	-7.45	<0.001
g: Alphaproteobacteria spc.	genus	-4.62	0.62	-7.45	<0.001
g: *Williamsia*	genus	-1.39	0.26	-5.42	<0.001
o: Clostridiales	order	-2.52	0.48	-5.24	<0.001
f: Peptostreptococcaceae	family	-2.52	0.48	-5.24	<0.001
f: Cytophagales spc	family	-6.36	1.34	-4.76	<0.001
g: Cytophagales spc	genus	-6.36	1.34	-4.76	<0.001
g: *Acinetobacter*	genus	-1.54	0.32	-4.75	<0.001
o: Verrucomicrobiales	order	-1.59	0.36	-4.46	<0.001
f: Verrucomicrobiaceae	family	-1.59	0.36	-4.46	<0.001
f: Planococcaceae	family	-2.90	0.66	-4.36	<0.001
f: Moraxellaceae	family	-1.26	0.29	-4.35	<0.001
g: *Luteolibacter*	genus	-1.59	0.36	-4.46	<0.001
g: *Romboutsia*	genus	-2.35	0.53	-4.41	<0.001
g: *Lysinibacillus*	genus	-2.90	0.66	-4.36	<0.001
f: Nocardiaceae	family	-0.93	0.22	-4.18	<0.001
g: *Hafnia*	genus	-1.27	0.31	-4.11	<0.001
g: *Clostridium* XI	genus	-2.94	0.73	-4.03	<0.001
g: *Alkanindiges*	genus	-3.26	0.84	-3.86	0.001
g: *Serratia*	genus	-2.28	0.59	-3.85	0.001
f: Caulobacteraceae	family	-0.76	0.21	-3.62	0.002
g: *Pluralibacter*	genus	-1.84	0.51	-3.61	0.002
g: *Brevundimonas*	genus	-0.76	0.21	-3.62	0.002
o: Caulobacterales	order	-0.76	0.21	-3.62	0.002
g: *Spirosoma*	genus	-0.78	0.22	-3.56	0.002
g: *Novosphingobium*	genus	-0.78	0.22	-3.51	0.002
f: Micrococcaceae	family	-2.67	0.83	-3.23	0.006
f: Rhizobiaceae	family	-0.52	0.16	-3.22	0.006
g: *Arthrobacter*	genus	-2.67	0.83	-3.23	0.006
g: *Rhizobium*	genus	-0.52	0.16	-3.22	0.006
f: Bacillaceae	family	-1.08	0.34	-3.19	0.006
g: *Bacillus*	genus	-1.08	0.34	-3.19	0.006
g: *Arsenophonus*	genus	-2.27	0.73	-3.09	0.008

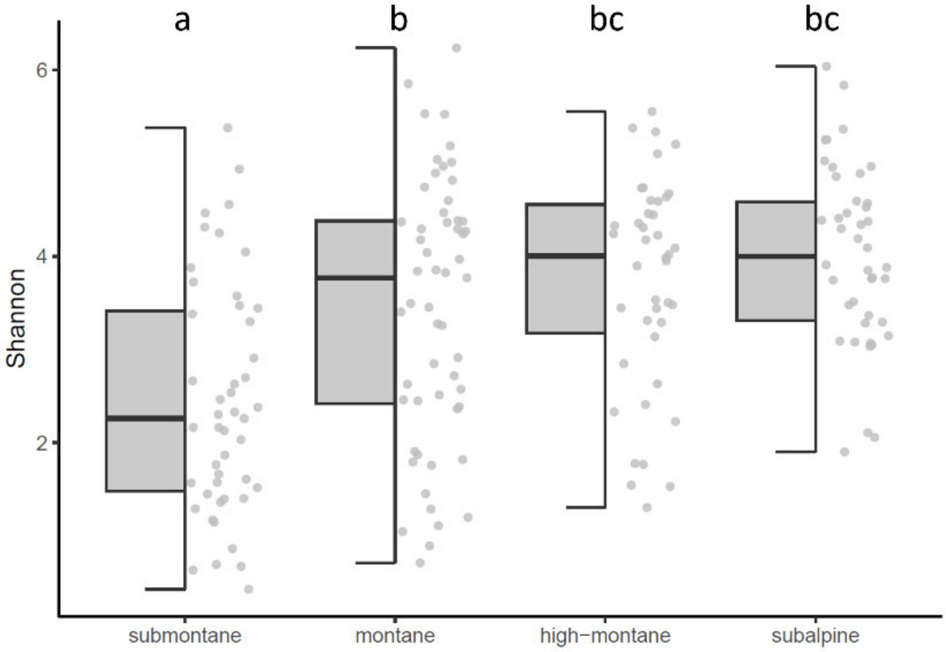


Figure S7. Shannon diversity of bacteria in samples of specialists on grasses from the four elevational zones (Tab. S2). Letters indicate statistical significance at a level of 0.05.

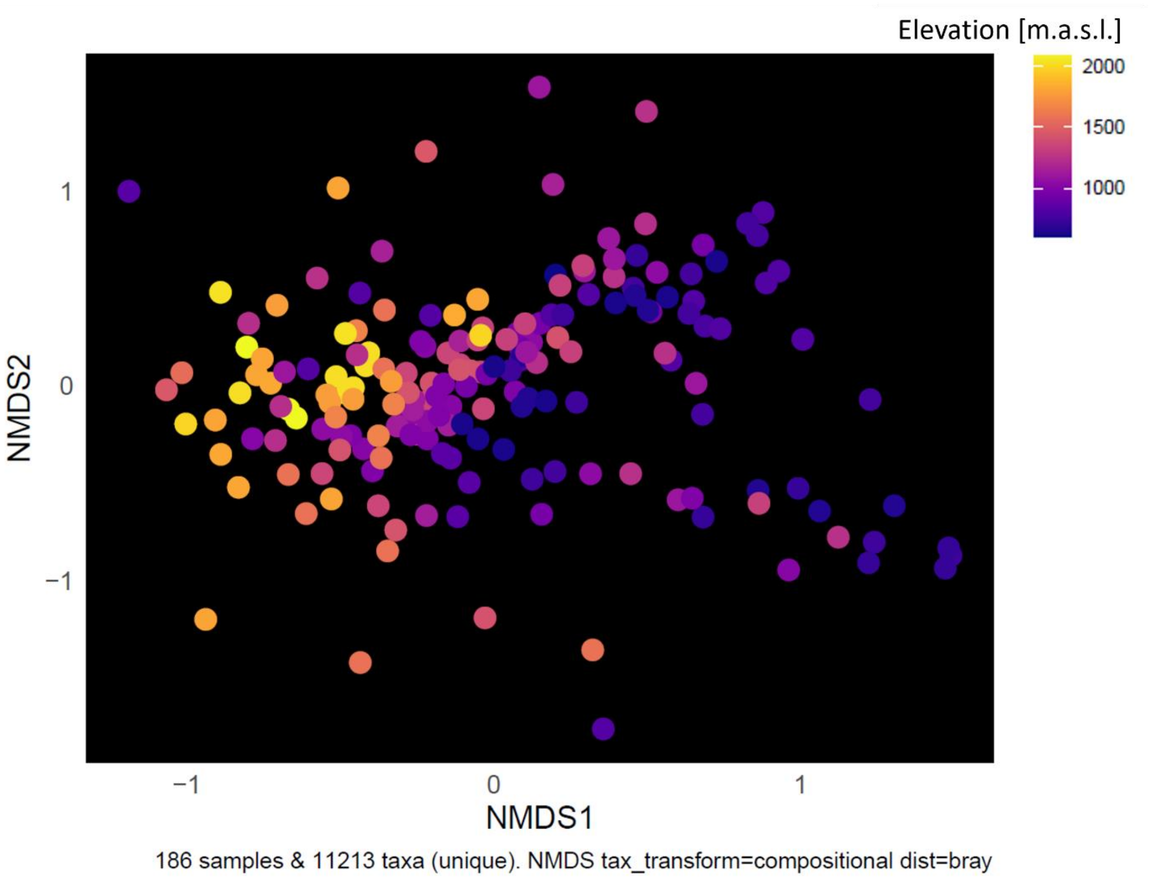


Figure S8. NMDS plot (stress = 0.21) of the community composition of bacteria found in faecal samples of grass-feeding Orthoptera populations from different elevations. Samples are coloured based on the mean sampling elevation from dark blue (low elevation) to yellow (high elevation). Elevation explained 7% of the variation.

Figure S9. Volcano-plot of differentially abundant ASVs of bacteria found in faecal samples of grass-feeding Orthoptera derived from a logistic regression model ('LOCOM') with Sandev's adjustment of *p*-values. We included subfamily and management in the model to account for possible confounding effects. Points are red if the *p*-value is below 0.05.

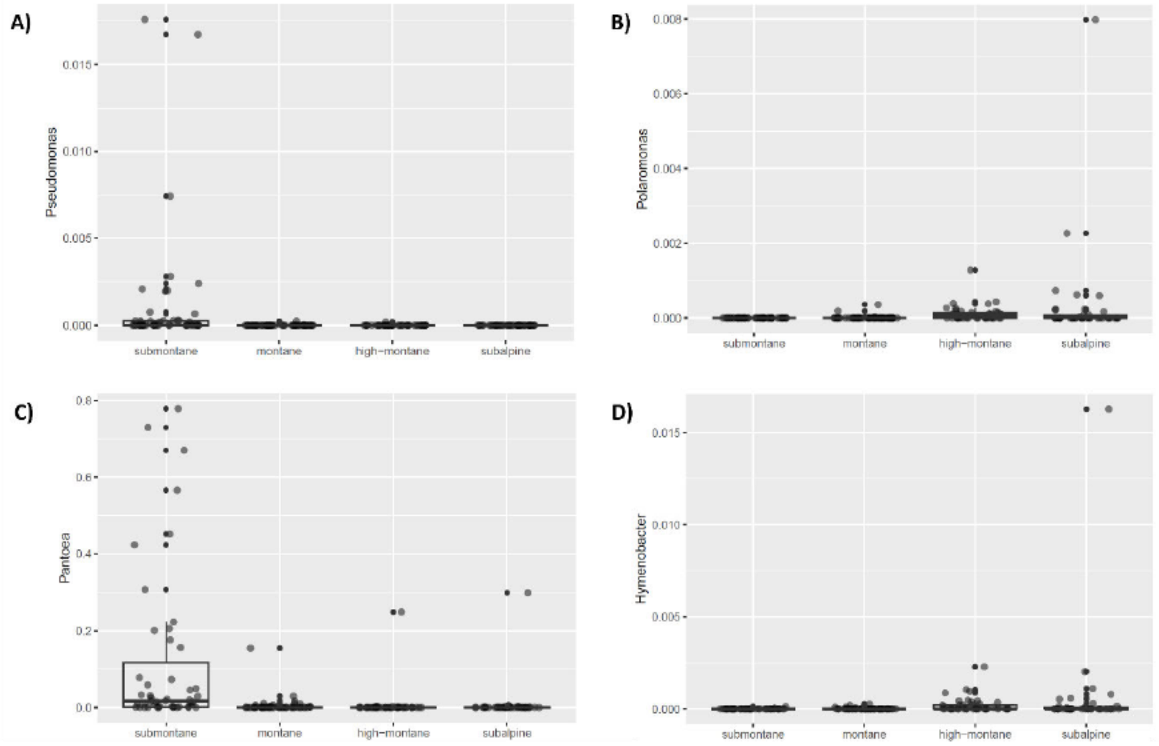


Figure S10. Examples of bacterial ASVs, which are either decreasing (Pseudomonas: A, Pantoea: C) or increasing (Polaromonas: B, Hymenobacter: D) in their relative abundance with elevation identified by a logistic regression model ('LOCOM') with Sandevs adjustment of *p*-values.

Table S2. Results from pairwise Wilcoxon-rank-sum-tests comparing ASV richness and Shannon diversity of frass bacteria of the four different elevational zones. *P*-values were corrected for multiple testing following Holm.

Response	**Comparison**	***p*-value**
ASV richness	Montane vs. High-montane	0.145
ASV richness	Montane vs. Subalpine	0.785
ASV richness	Montane vs. Submontane	0.246
ASV richness	High-montane vs. Subalpine	0.152
ASV richness	High-montane vs. Submontane	0.002
ASV richness	Submontane vs. Subalpine	0.383
Shannon diversity	Montane vs. High-montane	0.641
Shannon diversity	Montane vs. Subalpine	0.147
Shannon diversity	Montane vs. Submontane	0.002
Shannon diversity	High-montane vs. Subalpine	0.641
Shannon diversity	High-montane vs. Submontane	<0.001
Shannon diversity	Submontane vs. Subalpine	<0.001

Chapter V

General discussion

> "A man may have lived all of his life in the gray, and the land and trees of him dark and somber. The events, even the important ones, may have trooped by faceless and pale. And then -the glory- so that a **cricket song** sweetens his ears, the smell of the earth rises chanting to his nose, and dappling light under a tree blesses his eyes. Then a man pours outward, a torrent of him, and yet he is not diminished. And I guess a man's importance in the world can be measured by the quality and number of his glories. It is a lonely thing but it relates us to the world. It is the mother of all creativeness, and it sets each man separate from all other men."
>
> — *John Steinbeck*

Climate change affects all levels of biodiversity, from genes, to individuals, to populations, to species, to interactions, to assemblages, and ultimately entire systems. The primary objective of my dissertation was to study assembly processes of Orthoptera communities along temperature gradients, with a focus on feeding specialisation and associated microorganisms. The results of this thesis provide evidence of temperature-dependent effects on Orthoptera communities across different facets of biotic organization. Our findings demonstrate additive and interactive effects of macroclimate and microclimate on diversity, abundance, and species distributions, respectively. Climatic conditions had contrasting effects among Orthoptera taxa, and these differences were partly explained by ecological and morphological traits of the species. Intraspecifically, body size and wing length responses were consistent with community patterns, suggesting general temperature filtering effects. However, body size had no clear effect on the distribution of climatic niche positions of species. One consistent trait-based response revealed was the positive influence of warm macro- and microclimate on the abundance of later hatching species, suggesting that Orthoptera species which require longer developmental times could benefit from climate change. Temperature effects also became evident at the level of biotic interactions, as community-specialisation patterns varied along the climatic gradient. In Chapter II and III, we found abundance clines and turnover in plant and Orthoptera communities with temperature, which might in turn shape specialisation. Consequently, we predicted that abiotic clines and biotic turnover would result in changes in plant-herbivore interaction specialisation with elevation. On the one hand, interactions were more specialized under warm conditions, but on the other hand, the phylogenetically corrected specialisation of the community peaked at intermediate temperatures. This non-linear relationship revealed questions earlier predictions of a simple specialisation-increase-rule with increasing temperature derived from the ‘altitude niche-breadth hypothesis’ and demonstrates the complexity of ecological interactions under natural conditions. Yet temperature impacts were found on another level, neglected in interaction research in Orthoptera: their associate microbial communities. Changes in temperature can thus have significant consequences on insect populations at different levels of organization. A detailed understanding of how species respond to temperature rise is needed to develop conservation strategies that ensure the protection of biodiversity. In warmer climates, there is the potential of increasing diversity of thermophilic insects, such as Orthoptera, in Central Europe. Especially, because observed feeding specialisation is low in these insects, shifts are therefore decoupled from plant responses. However, since the patterns found in the framework of this thesis were derived in landscapes of well-connected habitats without severe land-use effects, such theoretical increases are to be interpreted with caution, as they may not apply to fragmented, predominantly agricultural landscapes, or only to species which are good dispersers. Additionally, such α-diversity increases may lead to biotic homogenization of communities under global change (Ganuza et al 2022, Gossner et al 2023, Ogan et al 2022, Thorn et al 2022).

5.1 *Additive effects of macro- and microclimate on orthopteran diversity and abundance*

Temperature is among the main parameters affecting the diversity of herbivorous insects (Bale et al 2002, Geppert et al 2021, McCain & Grytnes 2010, Peters et al 2016a). As expected, we found an almost linear decrease of species richness of Orthoptera with increasing elevation in Chapter II. With increasing mean seasonal temperature, which is equivalent to decreasing elevation, both species richness and abundance increased in our study design. Such positive relationships between diversity and temperature were reported from different regions in the Alps (Descombes et al 2017a, Geppert et al 2021, Pitteloud et al 2020) and other mountain ranges all around the world (Kati et al 2012, Nufio et al 2010, Peters et al 2016a, Ramos et al 2021, Tiede et al 2018). This pattern is generally in line with latitudinal diversity clines found in Orthoptera (Hochkirch & Nieto 2016). Aligned with the metabolic theory of ecology (Brown 2014), lower temperatures can impose direct constraints on physiological processes or indirectly limit population sizes by reducing resource productivity (Bale et al 2002, Geppert et al 2021). The increase in richness could therefore be attributed to evolutionary processes that shape the distribution of species along elevational gradients, with fewer species adapted to cooler conditions compared to warmer ones. Nevertheless, the impact of temperature on richness can also be mediated by abundance since warmer temperatures facilitate larger population sizes, increasing the likelihood of detecting a species (Maihoff et al 2023). Taking a closer look at the patters derived from joint species distribution modelling (Chapter II), we actually found a peak of Orthoptera diversity and abundance between the submontane and montane zone, but we only discuss linear increases of both variables with temperature. The divergence in interpretation arises from the decision to exclude certain low-elevation species from the joint species distribution model (jsdm). These species had an insufficient number of occurrences along the temperature gradient, making it unreliable to infer their distribution accurately. Thus, our modelling results at the lower edge should be interpreted with caution.

The majority of Orthoptera species in Central Europe are thermophilic, meaning they thrive under warm temperatures. Thus, lower temperatures impose restrictions on their essential physiological processes, resulting in a reduced fitness and performance (Geppert et al 2021, Schmitz et al 2016, Willott & Hassall 1998). One explanation for their high temperature requirements is their long developmental time. Hatching usually happens in late spring when soil temperatures exceed 15°C for a longer time (Ingrisch & Köhler 1998). Following several weeks of nymphal development, the reproductive phase commences later in the year compared to many other arthropods. Hence, successful reproduction during summer is limited to regions with a sufficiently lengthy summer season. This dependency on high temperature is reflected in the positive effects of both, microclimate at the sites and macroclimate on Orthoptera species richness and abundance. We thus found further evidence for the importance of high temperatures for this insect taxon. However, in contrast to a large body of literature documenting a positive relationship of temperature and Orthoptera richness, several other studies also reported increases of diversity with elevation or mid-elevation peaks of Orthoptera diversity (Fartmann et al 2022, Laiolo et al 2023, Laiolo et al 2018, Zografou et al 2017). In such special cases, other factors such as moisture gradients, seasonal sampling, or land-use-intensity may be confounded with elevation and influence patterns detected. Thus, we emphasize that not a single factor such as increasing average temperature forms realized biodiversity patterns.

5.2 *The importance of warm microclimate for Orthoptera in an Alpine region*

Microclimate varied significantly between sites at similar elevations. We showed that average summer temperatures can differ by up to 5 K at similar elevations, but most of the variation between sites was below 2 K. While temperatures were rather similar at rainy or heavily clouded days, there was high variation of temperatures close to the soil layer at sunny days. This effect was especially pronounced when comparing shaded, north-exposed sites with steep south-exposed sites. These contrasting climatic conditions translated into differences in species composition, particularly with thermophilic species found exclusively at sites with warm microclimates, regardless of elevation. As demonstrated for numerous species in the Austrian Alps, some can inhabit higher elevations in the southern ridge or Central Alps compared to the northern part, owing to favourable climatic conditions (Zuna-Kratky & Landmann 2017).

Microclimate was a strong predictor for abundance patterns of Orthoptera in a rather cold region of Central Europe, akin to findings for ground beetles and flies in an arctic environment (Kankaanpää et al 2021, Peña-Aguilera et al 2023). With a few exceptions such as the Green Mountain Grasshopper (*Miramella alpina*), the Common Green Grasshopper (*Omocestus viridulus*), or the Dark Bush-crickets (*Pholidoptera sp.*), most of the studied species profited from warm microclimatic conditions (Chapter II). In particular, *Chorthippus biguttulus*, *Chorthippus eisentrauti*, *Decticus verrucivorus*, *Gryllus campestris*, *Mecostethus parapleurus*, *Omocestus rufipes*, *Psophus stridulus*, *Stenobothrus lineatus*, and *Tetrix tenuicornis* were closely associated with warmer local temperature deviations from macroclimate. This matches expert opinions on the ecology of these species (Harz 1969, Harz 1975, Ingrisch & Köhler 1998, Oschmann 1991), but does not correlate closely with the species temperature index (STI) and other indices which describe the macroclimatic temperature niche of those species. Thus, microclimatic preferences are not necessarily reflected by distribution-derived indices and could, for instance, be modified mechanistically by differences in thermoregulatory capacities, transpiration, or thermal limits between grasshopper species (Ingrisch & Köhler 1998, Willott 1997). At low ambient temperatures, the Field Grasshopper (*Chorthippus brunneus*) and the Common Green Grasshopper (*O. viridulus*) were shown to be able to rise their body temperature more efficiently than thermophilic species such as the Stripe-winged Grasshopper (*S. lineatus*) or the Common Club Grasshopper (*Myrmeleotettix maculatus*) (Willott 1997), which were only able to occupy sites with warm microclimatic conditions. Our assessments have also revealed that microclimate preferences can change with macroclimate. We classified the Leek Grasshopper (*M. parapleurus*) and Rufous Grasshopper (*Gomphocerippus rufus*) as thermophilic species in the northern Alpine region, but they are associated with cooler conditions south of the Alps (Geppert et al 2021). This highlights the importance of reassessing habitat preferences of species under different macroclimates. Given that some species alter their preferences while others remain thermophilic across the entire gradient, caution should be taken when interpreting reduced climatic niche parameters such as temperatures measured in field. A thermophilic species may not be present at a cold site within a region of warm macroclimate, even if the average temperature falls within the range of the species' climatic niche. We therefore emphasize the essential necessity to include microclimate data in modelling species distributions and responses to climate change (Lembrechts 2023, Lembrechts et al 2019, Meyer et al 2023). Since microclimate is influenced not only by topography, but also by the structure of the vegetation (Vandewiele et al 2023), management practices that modify structural elements of the vegetation can serve as a tool to alter microclimatic conditions. According to literature, the highest number of Orthoptera species can be supported by a mosaic of tall vegetation as well as open patches of bare ground, a combination achieved by extensive grazing, for instance (Weiss et al 2013), or low-intensity mowing (Fumy et al 2023).

5.3 *Assembly processes along an alpine temperature gradient*

How does a typical mountain grasshopper look like and why?

Our analyses revealed species which have their distributional abundance peaks further up and can therefore be considered mountain species. These were characterized by broader dietary spectra, shorter wings, and earlier hatching phenology than average grasshoppers of lower elevations in the study system. Laiolo et al (2023) and Tiede et al (2018) also found a reduction in relative wing length to be a common feature of high-elevation insects and explain this pattern by a trade-off between reproduction and dispersal, which is costly in cold environments. Hatching early increases the likelihood of completing development in time to successfully reproduce in habitats with short seasons, where early snowfall frequently curtails the time available for reproduction. A flexible diet, as observed in the Green Mountain Grasshopper (*M. alpina*), which broadly feeds on various grasses, forbs, and mosses could be of advantage in high elevation habitats. This dietary flexibility reduces the time spent searching for food (Hodkinson 2005, Rasmann et al 2014, Wong et al 2019). High up in the mountains, climate exhibits higher variability compared to low elevations. This variability includes variation in the length of the growing season from one year to another and the occurrence of unpredictable freezing events even during summer (Körner 2003). The resemblance of these conditions at high elevations to those found in high latitudes forms the basis for postulating the ‘altitude-niche-breadth hypothesis’ (Rasmann et al 2014). According to this hypothesis, relaxed predation pressure, plant defence, and environmental unpredictability facilitate dietary generalism. Furthermore, temperature is suggested to affect diet breadth of herbivorous insect directly by affecting the insect’s capability to handle chemical contents of plants, as shown for herbivorous beetles (Lemoine et al 2013). Moreover, high-elevation grasshoppers seem to have an association with a diverse array of microbes, and their partner communities are less dominated by highly abundant bacteria found at lower elevations. If there is a close link between gut microbes and nutrient acquisition or immune defence in Orthoptera, the relaxed association with specific bacteria could potentially pose challenges for the herbivores in alpine habitats. However, further research is necessary to investigate and confirm such potential relationships between herbivorous insects and bacteria along temperature gradients. Our analysis of body size distributions and colour patterns along the climatic gradient was inconclusive, emphasizing the complexity of processes which determine phenotype-environment links. However, we observed a higher proportion of brown grasshopper individuals at sites exhibiting warm microclimatic conditions compared to sites with cold conditions. These locations were also characterized by a significant amount of bare ground and rocks, suggesting that the frequencies of green colour morph are sustained at sites with dense vegetation cover, hinting at potential crypsis effects (Dieker et al 2018, Varma et al 2023). Apart from colour morph clines, the effects of temperature on colour may also be mediated by differences in pigmentation (melanisation), leading to darker individuals independent of the colour morph, which we did not consider in our studies. Although temperature had a positive effect on intraspecific body sizes and community mean sizes, as reported in Tiede et al (2018), we did not find a prevalence of larger species over smaller ones distributed in warmer climatic conditions. While a greater number of larger individuals was supported by higher temperatures, some smaller species also had their distribution peak in warmer climates. This may hint on the presence of opposing mechanisms that simultaneously drive trait patterns of organisms along climatic gradients at different levels of biotic organization (Classen et al 2017, Vellend et al 2014). Cherrill (2002) found that grasshoppers are smaller when hatching later in the season and weather is warmer, offering an alternative mechanism to temperature-size clines.

5.4 *How will future grasshopper assemblages in the study region look like?*

Based on our combined results, high-elevation assemblages may increase in richness and abundance if annual temperatures continue to increase. In mid-elevations, long-winged, grass-feeding grasshoppers dominate the Orthoptera communities. These species, including *P. parallelus, C. biguttulus*, and *G. rufus*, are expected to take advantage of increasing temperatures at higher elevations, especially at high elevation sites with warm microclimatic conditions, e.g. south-exposed sites. As a consequence of rising temperatures, new areas, that were previously unsuitable primarily at higher latitudes or elevations, will become available for colonisation by some more thermophilic Orthoptera species, as already shown for butterflies in the study region and northern Europe (Kerner et al 2023, Sunde et al 2023). Shifting temperature regimes may thus lead to more specialized herbivore communities in the subalpine and alpine elevational zones, akin to those observed in the montane zone. Such temperature increases could also lead to an upslope shift of dominant bacteria from low elevations if these are restricted by their temperature niches. We also observed regular colonization attempts of mobile species such as the Leek Grasshopper (*M. parapleurus*), Roesel’s Meadow Bush-cricket (*Roeseliana roeselii*), and the Large Marsh-Grasshopper (*Stethophyma grossum*) to high elevations. Especially at the upper range margins, high proportions of long-winged individuals of wing-dimorphic species such as *Euthystira brachyptera, Metrioptera brachyptera, R. roeselii, Podisma pedestris, Pseudochorthippus montanus,* and *Pseudochorthippus parallelus* were detected in 2019 and 2020 following warm and dry weather conditions. These long-winged individuals were shown to be important for dispersal (Poniatowski et al 2012). Such dispersal events are likely dependent on weather variation (Prinster et al 2020). However, rather long developmental times of the following generations of these newcomers may still limit their ability to form stable high-elevation populations in the study region.

Descombes et al (2020) demonstrated through experimental manipulation that Alpine plant communities are affected if densities of these grasshoppers increased in Alpine grasslands. Due to conserved preferences of grasshoppers for tough leaves of grasses, the grass biomass will be reduced, creating space for low-stature plants. The density manipulation experiment was based on the assumption that densities of Orthoptera will increase at high elevations in the course of climate change. However, the climatic variation and harshness of high-elevation grasslands leads to high annual fluctuations of population sizes of high-elevation Orthoptera communities (Illich & Zuna-Kratky 2022). This unpredictability of weather conditions could slow the successful immigration rate. Furthermore, a more detailed interpretation of our findings concerning the interaction of micro- and macroclimate may hint at an alternative scenario. The two species with the highest elevational distribution and dominance in some of the subalpine and alpine grasslands, both do not benefit from warm microclimate. Consequently, increasing temperatures at high-elevation sites may therefore fail to result in increased abundance; instead, it could have opposite effects. This implies that while more thermophilic species from low elevations move uphill, thermophobic and moisture-dependent species could decrease instead, resulting in a turnover with no net effect on grasshopper abundance. Evidence for such developments was presented by Neumayer (2020), who found a marked decrease of *O. viridulus* and *M. alpina* abundance in subalpine grasslands in the study area within 30 years. Thermophobic species can be directly affected by high temperatures, since these surpass their thermal limits, or because they may not be able to lower their body temperature via behavioural thermoregulation, as shown for *O. viridulus* (Willott 1997). If *O. viridulus* individuals get substituted by upslope-shifting grasshoppers such as *C. biguttulus*, *G. rufus*, and *P. parallelus,* feeding pressure on the plant communities may not change drastically, since these insects share similar resources (conserved preferences within the Gomphocerinae family, Descombes et al 2020, Pitteloud et al 2021). This aligns with the notion of high stability of such high-elevation systems regarding climate change, as shown for plant communities (Körner & Hiltbrunner 2021), and to long-term data analysed in Zuna-Kratky and Landmann (2017). The latter did not observe general trends indicating upslope shifts. However, detailed re-surveys based on baseline assessments like the ones presented as part of this thesis are required to evaluate which of the scenarios will happen.

At intermediate elevations, we expect abundance increases of typical low-elevation Orthoptera species during climate warming. At warm microclimate sites, thermophilic species such as *C. biguttulus* and *G. rufus*, and *M. parapleurus* might profit, while *P. parallelus* is expected to increase independent of the microclimate. These assemblages at intermediate elevations are already species-rich and diverse and therefore of great value for the conservation of mountain biodiversity. If we assume that the microclimate and macroclimate will become warmer as climate change progresses, could we identify characteristics beneficial for a grasshopper? Species profiting from climate warming may exhibit a variety of traits, including being either brown or green, large, or small (with a tendency of larger species being favoured), dietary specialists or generalists, hygrophilic or xerophilic, and more likely to have longer wings. The absence of clear linearly responsive traits underscores the challenges of predicting species responses to climate change based on simple morphological or ecological traits. However, species which hatch later may be facilitated by warming in general.

5.5 *Are our predictions valid for other regions in Central Europe under climate change?*

The predictions we derived from the elevational gradient in an Alpine region may be a special case because of the geographic and climatic circumstances that differ from lowland regions. However, our predictions match reports of recent range expansions of some Central European species published by Poniatowski et al (2020). Species which recently profited were on average hatching later than species which did not expand their ranges. Also, most of them were long-winged and rather large, corroborating our predictions derived from the space-for-time substitution approach.

We excluded effects of intensive management by selecting study sites only at extensively managed or unmanaged grassland, and we expect that the distribution of species along the entire gradient is not limited by a lack of moisture as the macroclimatic annual precipitation in the whole region is high (DWD 2020). Therefore, temperature-richness-relationships identified in this thesis suggest that further warming could lead to local increases in richness and abundance of Orthoptera. Indeed, several studies re-surveying sites in Lower Franconia, North Rhine-Westphalia, Rhineland-Palatinate, or Baden-Württemberg, have already reported increases in α-diversity of Orthoptera over the last 20 to 30 years, likely associated with parallel increases in mean annual temperatures (Fumy et al 2020, Löffler et al 2019, Ogan et al 2022, Thorn et al 2022). It might thus be tempting to assume Orthoptera as 'winners' of climate change. However, the term 'winners of climate change' can be misleading, as this impression is often only the result of local observations. Even species that are increasing in abundance and expand their ranges may lose habitat at the opposite climatic end of their distribution. For example, strong occupancy decreases of *O. viridulus* and *P. montanus* were already highlighted by re-surveys from sites at low elevations (Löffler et al 2019, Ogan et al 2022, Thorn et al 2022). The shift of species distributions toward the poles and higher elevations, and the transformation of species assemblages toward thermophilic taxa, is more complex than macroclimatic temperature changes alone predict (Ash et al 2017, Devictor et al 2012). Species can only track their shifting climatic niche or zone if there are no dispersal barriers or enough suitable, connected, or reachable habitat on their way. In modern agricultural landscapes, this becomes a limiting factor for many species, preventing them from following their climatic niche shifts and leading to a decoupling of climatic niches and species distributions, as shown in studies on butterflies and birds (Sunde et al 2023, Viana & Chase 2022). Specialized species, often poor dispersers, may be particularly affected, as they are more demanding in terms of habitat quality and may struggle to use habitats of intermediate suitability as stepping stones during dispersal. This emphasizes the importance of being a good disperser in times of global change, as already outlined by several studies (Engelhardt et al 2022, Neff et al 2022, Poniatowski et al 2020, Thorn et al 2022). Another potential reason for the lack of climate niche matching could be unsuitable local microclimatic conditions. The denser vegetation resulting from increased nutrient availability in agricultural landscapes of Central Europe creates a colder microclimate close to the ground (Gardiner & Hassall 2009, Humbert et al 2021), counteracting the warmer macroclimatic conditions. Hence, to enhance our understanding of biotic responses to rising temperatures and to improve projections of climate change effects, a better understanding of the spatial variability of climate at different scales, and a comprehension of the interaction between macro- and microclimate is necessary (Bennie et al 2013, Scherrer et al 2011, Vandewiele et al 2023). On the other hand, a number of generalist and specialist species are indeed spreading in Central Europe with ongoing climate warming (Engelhardt et al 2022, Poniatowski et al 2020), which decreases β-diversity on landscape-level, a phenomenon referred to as biological homogenization (Ganuza et al 2022, Gossner et al 2016, Gossner et al 2023, Ogan et al 2022, Thorn et al 2022). One indication towards increasing biotic homogenization at warmer climates was detected in Chapter II, where we showed decreasing rates of β-diversity towards low elevations. In other words, a higher proportion of species and individuals in distinct communities was shared at warmer temperatures in our assessment. If temperatures rise at intermediate elevations, this could lead to a spread of low elevation species and a retraction of cold-adapted species, which may cause a loss of β-diversity in this elevational zone. In this context, we could speculate that biotic homogenization with climate change observed by several studies might be the result of homogenization of microclimatic conditions in different habitats within a landscape (Thorn et al 2022).

5.6 *Alternative factors that influence diversity and specialisation*

Temperature may also control resource availability (Peters et al 2016a), influencing herbivores. However, we did neither find direct effects of the plant community composition nor plant richness on the specialisation and richness of Orthoptera communities in mountain grasslands in Chapter III. We showed that links between Orthoptera species and specific plant species were weak. Although we observed the conservation of feeding niches at higher taxonomic levels, such as Gomphocerinae and Poaceae (Descombes et al 2020, Ibanez et al 2013a, Ibanez et al 2013b, Ingrisch & Köhler 1998, Pitteloud et al 2021), the identity of grass species consumed was flexible and dependent on the local abundance and dominance of the grasses growing at the sites. This lack of close dependencies of Orthoptera on the presence of certain plant species implies that the quantity of resources present at a site is more critical than the composition (Perner et al 2005). This corroborates findings of several other studies which highlight that vegetation structural properties are more important for Orthoptera than plant species richness or composition *per se* (Geppert et al 2021, Miao et al 2018, Unsicker et al 2008). Different structural features of the vegetation offer microsites for oviposition, shelter from heat or natural enemies (Zhu et al 2017), and various microclimatic habitats (Geppert et al 2021, Kruess & Tscharntke 2002, Löffler & Fartmann 2017, Marini et al 2009, Miao et al 2018, Schirmel et al 2019, Schirmel et al 2011, Torrusio et al 2002, Walcher et al 2017). A reason for the superordinate role of temperature over vegetation composition for Orthoptera might be attributed to their low feeding specialisation, rendering them less dependent on the presence of specific plant species. As a result, rapid range shifts can be realized without the risk of losing essential food resources, given that plants usually shift ranges more slowly than mobile insects. This decoupled relationship could be a contributing factor why many Orthoptera species appear to thrive under climate change in Central Europe.

Plants within the Poaceae family exhibited higher cover than forbs at most sites and were of key importance for Orthoptera in our study. Especially, genera such as *Agrostis*, *Arrhenatherum*, *Briza*, *Bromus*, *Calamagrostis*, *Cynosurus*, *Dactylis*, *Deschampsia*, *Festuca*, *Poa*, and *Sesleria* were frequently consumed. Since Caelifera dominated our communities along the entire gradient, our interpretation of community patterns primarily focuses on this suborder. The observed preference of many Caelifera for grasses could be based on differences in the palatability and digestibility of food plant with – on average – a lower count of secondary metabolites in comparison to dicotyledonous plants (Bernays et al 1994, Joern 1979, Pitteloud et al 2021). Also, the Ensifera did not cover such a broad temperature gradient as Caelifera did, indicating a stronger temperature limitation at higher elevations for this group which could be linked to thinner exoskeletons, making them susceptible to cold temperatures and freezing, probably defining their distribution (Detzel 1998, Illich & Zuna-Kratky 2022, Ingrisch & Köhler 1998, Nadig 1986, Zuna-Kratky & Landmann 2017). And the Ensifera studied are to a large proportion omnivorous, feeding on plants and arthropods, leaving little variation for specialisation analysis. The distribution of Ensifera might therefore not primarily be shaped by feeding specialisation, but temperature effects *per se*. However, quantifications of feeding behaviour considering proportions of animal and plant components may shed light on specialisation patterns of Ensifera.

Pitteloud et al (2022) showed that rules shaping interactions vary geographically. Thus, other factors than temperature *per se* may influence the feeding specialisation. One hypothesis includes moisture as an additional important factor. According to our observations, being a generalist feeder might be of advantage under warm conditions, particularly if plants are potentially under drought stress. At sites in a summer-dry habitat, species restricted to feeding on grasses may face a disadvantage due to the low water content of grasses during drought conditions. This assumption is supported by other studies highlighting decreases in number of individuals of grasshoppers in severe drought conditions (Fartmann et al 2022), as also shown for moths in an Italian protected area (Uhl et al 2022). For instance, the abundance of grass-feeding Orthoptera has been documented to decrease in water-stressed plots compared to watered plots (Lenhart et al 2015). In line with this, we also observed declines in the numbers of typical grass-feeding grasshoppers, such as the Bow-winged Grasshopper (*C. biguttulus*) or the Stripe-winged Grasshopper (*S. lineatus*) in dry calcareous grasslands around Würzburg during dry years (König et al. unpublished). Being flexible and including forbs in their diet instead may be a strategy to cope with such conditions. The Lesser Grasshopper (*Chorthippus mollis*), the Heath Grasshopper (*Chorthippus vagans*), or Eisentraut's Bow-winged Grasshopper (*C. eisentrauti*) are examples for grasshoppers that regularly supplement their grass-based diet with forbs, as observed in Chapter III and reported in literature (Ingrisch & Köhler 1998). The increase in metabolic rates with rising temperatures could lead to higher nutritional demands, potentially explaining the shifted proportion of high-nutrient forbs and low-nutrient grasses in diets of these grasshoppers, as demonstrated for beetles (Lemoine et al 2013). In extreme cases, the diet shifts completely from grass-based to a Fabaceae-dwarf-shrub-based diet, as shown for the Red-legged Grasshopper (*Chorthippus binotatus*) in Mediterranean alluvial grasslands (Löffler et al 2016, Picaud et al 2003). Another indication for the link between warm temperatures and broad diets is the high relative abundance of the Blue Band-winged Grasshopper (*Oedipoda caerulescens*) and the Italian Locust (*Calliptamus italicus*) at the warmest and driest sites of our study in Chapter III, while more specialized Oedipodinae only occurred at sites with higher moisture. There, grass-water-content might not be a limiting factor. In fact, drought-driven effects on food quality and quantity were revealed to be important for grasshopper dynamics (Joern et al 2012). Under such conditions, drought-tolerant forbs are still vital due to deeper root systems than many grasses have, which increases their attractivity for herbivores (Löffler et al 2016). As we did not consider moisture variation in our analyses, the interplay of temperature and moisture however demands further research. Following nutritional theory, forb feeders are expected to be more specialized on specific groups, as phylogenetic lineages of forbs differ in chemical defence compounds (Swenson 2014). Broad resource spectra of forb feeders may therefore represent an adaptation to dilute defensive substances (Joern 1979, Pitteloud et al 2021). Analysing the secondary metabolites of plant species could be a promising approach to unravel the relationship between chemical defence and herbivory by revealing mechanisms behind feeding preferences for certain plant species (Pitteloud et al 2022).

Foodweb structure of has been linked to various parameters in the past, including the abundance patterns of species, their specific traits, and evolutionary relationships between species (Vázquez et al. 2007; Eklöf & Stouffer 2016; Laigle et al. 2018). Although the nutritional quality of plants and biomechanical constraints were identified as drivers of feeding interactions within experimental settings (Joern & Behmer 1997; Ibanez et al. 2013), first studies under natural conditions found weaker signals of trait matching (Pitteloud et al 2021), underscoring the complexity of influences. For further studies, we suggest the phylogenetic aspect of feeding specialisation between herbivore species should be considered, since temperature niches of Orthoptera groups can differ. While Melanoplinae were distributed in colder regions, Gomphocerinae diversity and abundance peaked at intermediate temperatures and Oedipodinae were common in warmer regions. These groups were shown to display phylogenetic conservation of feeding patterns (Descombes et al. 2021).

We demonstrated empirically that species occurring at higher elevations exhibited more generalized feeding habits than those at intermediate elevations. Such relaxed links between herbivores and plants in high elevations were both, theoretically proposed and empirically found for Orthoptera in this thesis. However, the assemblages at the warmest sites also displayed a decrease in specialisation. In Chapter II, we did not find a linear relationship of species elevational distribution and diet breadth. Taking a closer look into this relationship in Chapter III, we identified a peak of specialisation at intermediate elevations rather than in the lowlands. Further evidence of the relationship between feeding specialisation and temperature may emerge when considering more species and a larger gradient. Temperature niches of 102 species occurring in Switzerland were published by Neff et al (2022), considering their entire European distributional ranges. When examining the distribution of their median temperature niches, which can be interpreted as macroclimatic temperature preferences (Engelhardt et al 2022, Neff et al 2022, Poniatowski et al 2020), along with subfamily-level feeding type classifications (Ingrisch & Köhler 1998), two observations emerge: 1) There are indications of feeding specialisation peaking at intermediate temperatures (Fig. 1), supporting our findings. 2) Nevertheless, the distribution of temperature preferences is strongly influenced by phylogeny, which could contribute to the hump-shaped specialisation pattern we detected. For example, there is a trend that Ensifera cannot occur at as cold conditions as Caelifera can, which might be linked to their thinner cuticle (Nadig 1986). We therefore suggest a combination of mechanisms can lead to the specialisation pattern we unravelled in our studies. While environmental filtering at high elevations may select for generalist herbivores following predictions derived from the 'altitudinal-niche-breadth hypothesis', different drivers which include insect physiological requirements or constraints could act under warm climatic conditions. Furthermore, we want to highlight that species can cope with harsh conditions through a range of adaptations, e.g., through shortened developmental time, which enables the persistence of food-specialist herbivores such as the Club-legged Grasshopper (*Gomphocerus sibiricus*) or the Alpine Thick-necked Grasshopper (*Aeropedellus variegatus*) at the highest elevations (Zuna-Kratky & Landmann 2017).

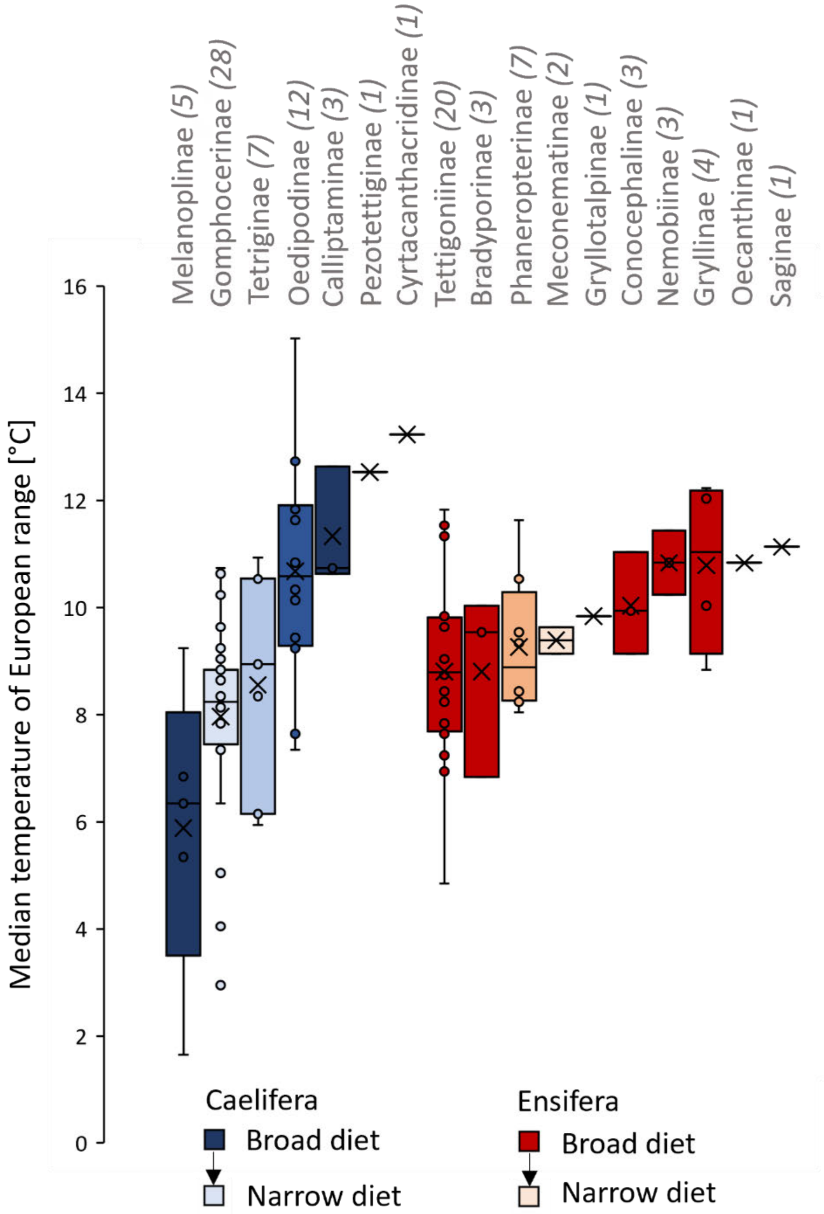


Figure 1. Temperature preferences of 102 Orthoptera species found in Central Europe (Switzerland). Subfamilies were separated, with Caelifera in shades of blue and Ensifera in shades of red. Temperatures were derived from their European distribution records published in Neff et al. (2022). Colour lightness corresponds to subfamiliy-level feeding classifications.

5.7 *Why don't trait patterns reflect community responses?*

Our analysis revealed inconsistent patterns of trait variation depending on the method: community-weighted means of traits, community means of traits, intraspecific variation, or the distribution of niche positions. These differences can be explained by significant variation in environmental niche breadths and species abundances. Generalist species that thrive under a broad range of climatic conditions and in high numbers tend to dominate communities, potentially overshadowing the responses of specialist species with narrow environmental niches. Even though high elevations may not be their preferred conditions, these generalist species could still occur there, sometimes in higher numbers than high-elevation specialists. In such cases, the mean traits of communities sampled at high elevations may not accurately reflect traits of high-elevation species. In our case, species with broad elevational ranges such as the Common Green Grasshopper (*O. viridulus*) and Rufous Grasshopper (*G. rufus*), and not Alpine specialists such as *M. alpina* dominated high elevation communities. However, the interpretation of such trait clines can differ. While relating traits to niche positions can reveal evolutionary adaptations, community patterns and intraspecific clines still have the potential to reveal environmental filtering.

In this context, it is important to highlight the strength of our approach: We did not solely rely on raw presence-absence distribution data to infer species niche parameters. Several studies adopted this approach, overlaying distribution records with coarse climatic variables, e.g., at a scale of 1 km^2, which is the standard resolution of commonly available climate data (Engelhardt et al 2022, Neff et al 2022, Poniatowski et al 2020). However, the number of occurrences detected in different climatic zones can be highly influenced by biases in sampling intensity and the number of potential habitats. Given the decreasing land area with elevation, accurately estimating climatic niches can be challenging. Our approach, which involves deriving species climatic niches based on abundances along carefully selected climatic gradients, offers a more detailed understanding of their climatic requirements. Additionally, we were able to differentiate between the effects of microclimate and macroclimate, which is crucial for predicting range shifts of species under climate change (Bennie et al 2013, Scherrer et al 2011, Willott 1997).

5.8 *Management effects*

Livestock grazing can have diverse effects on insect communities. Extensive grazing can increase structural heterogeneity, halt succession, and prevent transition to woodland, thereby providing habitat conditions for a high number of species. However, numerous studies have reported negative effects of more intensive grazing. In our system, we focused on extensively managed pastures. The peak grasshopper abundances at some grazed sites may result from the high productivity of these sites. However, we did not observe differences in the species richness and community feeding specialisation of Orthoptera assemblages between grazed sites and natural open habitats without management practices applied. However, community compositions differed for Orthoptera, since a high number of farmland-Orthoptera such as *C. biguttulus, Chorthippus dorsatus, P. parallelus, P. montanus, S. lineatus, M. parapleurus, Tetrix subulata, G. campestris, Gryllotalpa gryllotalpa, Tettigonia cantans, R. roeselii, and D. verrucivorus* were significantly more abundant at grazed sites. Similarly, plant community compositions differed between natural and managed sites (Chapter III) (Hoiss et al 2013, Kerner et al 2023, Maihoff et al 2023). Both of these compositional changes did not trace to the structure of feeding interactions, hinting on the stability of food webs. Despite the conservation of feeding specialisation, we surprisingly showed that cow grazing influences the environmental pool of bacteria and successively affects the microbial communities of herbivorous arthropods. This is an alarming finding, as it illustrates how microbes are transmitted through the entire ecosystem and, therefore, antimicrobial treatments of cattle could have cascading downstream effects on arthropods with unknown consequences for the system. It remains to be tested if bacterial associates at pastures have effects on the survival, growth, immunity, or reproduction of herbivorous insects. Such far-reaching effects of antibacterials on the microbiota of non-target organisms have already been shown for beetles (Hammer et al 2016). In case the affected microbes fulfil critical roles in the biology of the host insect, treatments of sheep and cattle could indirectly negatively influence the insect populations at the pastures.

A simple management implication derived from our results can be that practitioners should not neglect improving microclimatic conditions for the conservation of Orthoptera species. Depending on the focal species, this could involve keeping areas of longer sward-height, or of shading by single shrubs or trees in otherwise open habitats. These elements decrease microclimatic temperatures close to the ground, which is relevant for species sensitive to high temperatures. On the other hand, some species profit from the creation of patches of bare ground which heat up quickly and generate warm microclimatic conditions. However, such actions need to be targeted and depend on the focal species. A mosaic of different microclimatic site conditions may be beneficial for the conservation of a high number of species with different climatic niches and simultaneously offer microsites for oviposition.

5.9 *Example species summarizing our findings: Eisentraut's Bow-winged Grasshopper*

An exemplar species that encapsulates our findings regarding the significance of microclimate in mountains and the factors defining feeding specialisation patterns is *Chorthippus eisentrauti* (Ramme, 1931), a novelty for the fauna of Germany.

Eisentraut's Bow-winged Grasshopper is a thermophilic and (partly) geophilic species, which was long considered to be endemic and restricted to the southern parts of the Alpine Arc (Austria, Italy, France, Switzerland) (Ingrisch 1995). However, historically, and more recently, there were several references of ambiguous individuals found in more northern regions of the Alps (Koschuh 2012, personal comm. Illich 2023, image databases for naturalists, Zuna-Kratky & Landmann 2017), which are subject to taxonomical discussions as this species is young (from an evolutionary point of view), and difficult to differentiate from sister species such as the Bow-winged Grasshopper (*C. biguttulus*) or the Lesser Grasshopper (*C. mollis*). Many of these northern observations could not be confirmed or were revised and retracted. In the context of the assessments presented in thesis, we detected populations of Eisentraut's Bow-winged Grasshopper in substantial numbers in the Berchtesgaden Alps in 2017, 2019, and 2020. Therefore, we can confirm the presence of this species for the fauna of Germany, in which it was never officially included before, since historical collection material was ambiguous (Schlumprecht & Waeber 2003).

Identification characteristics were a combination of the male stridulation (short verses: ~1.2 s, repeated 2 - 6 times), the steadily diverging subcostal field of the tegmina of males, bright red hind tibiae, and orange-red abdominal tips in both sexes (Fig. 2) (Ingrisch 1995, Ingrisch & Bassangova 1995, Koschuh 2012, Ramme 1941). Additionally, we used ddRADSeq (i.e., double digest restriction-site associated DNA-sequencing) for SNP recovery (i.e., single nucleotide polymorphism) to screen parts of the genome of samples collected in Berchtesgaden for differences to the co-occurring Bow-winged Grasshopper. Indeed, the northern samples clustered with *C. eisentrauti*, samples collected in the southern Alps, confirming their status (Schmidt, Hawlitschek et al. unpublished). However, to date we cannot exclude possible hybridization with the common sister-species in the study area.

Another difference to the related *C. biguttulus* is its ecological niche. The focal species benefits from natural disturbance dynamics in the study region, since it requires rock-dominated open habitats, which often result from avalanches, mudslides, or can be found at floodplains along river systems with natural flooding and gravel dynamics, or alluvial grasslands (Fig. 3). There, the succession and transition from open to wood-dominated habitats are attenuated in early stages by natural dynamics, crucial for the survival of other open-habitat-specialist species such as the Gravel Grasshopper (*Chorthippus pullus*) or the Speckled Buzzing Grasshopper (*Bryodemella tuberculata*) in the Alps (Reich 1991). Even though the study region is characterized by high amounts of precipitation, these microhabitats dry quickly at the surface due to exposure, runoff dynamics, and high proportions of bare rock. All the sites where we found this species were characterized by a high diurnal and annual fluctuation of temperatures. From all our measurements in the context of this thesis, peak soil-level temperatures during sunny summer days were highest in the habitats of this species. Such extreme locations are usually not inhabited by the sister species *C. biguttulus,* which is more common at pastures and meadows with a higher vegetation cover. Nevertheless, single trees, shrubs, and dwarf-shrubs (e.g., *Amelanchier, Coryllus, Erica, Juniperus, Larix, Pinus, Salix*) and larger rocks also provide shaded refuges even in such extreme habitats. At these sites, also a range of endangered butterflies such as the Mountain Apollo (*Parnassius apollo*), the Old-World Swallowtail (*Papilio machaon*), and *Arctia matronula* were found in high numbers, implying a significant value of such habitats for conserving demanding thermophilic species. The grasshopper was never found at managed sites such as montane or subalpine pastures or submontane meadows in the study region, but warm microclimates allowed its occurrence within a surprisingly broad range of elevation ranging between 600 and 2,100 m.a.s.l along the slopes of the ‘Hochkalter’, ‘Reiter Alpe’, and ‘Watzmann’ massifs (Fig. 4). This species therefore serves as a compelling example of the crucial role that microclimate plays defining the distribution of a species (Willott 1997). The spectrum of regularly co-occurring grasshopper species includes *C. brunneus*, *C. pullus*, *G. rufus*, *E. brachyptera*, *O. viridulus*, *P. pedestris*, *P. stridulus*, *Tetrix kraussi*, *Tetrix bipunctata*, *Barbitistes serricauda*, *Pholidoptera aptera*, and *Pholidoptera griseoaptera*.

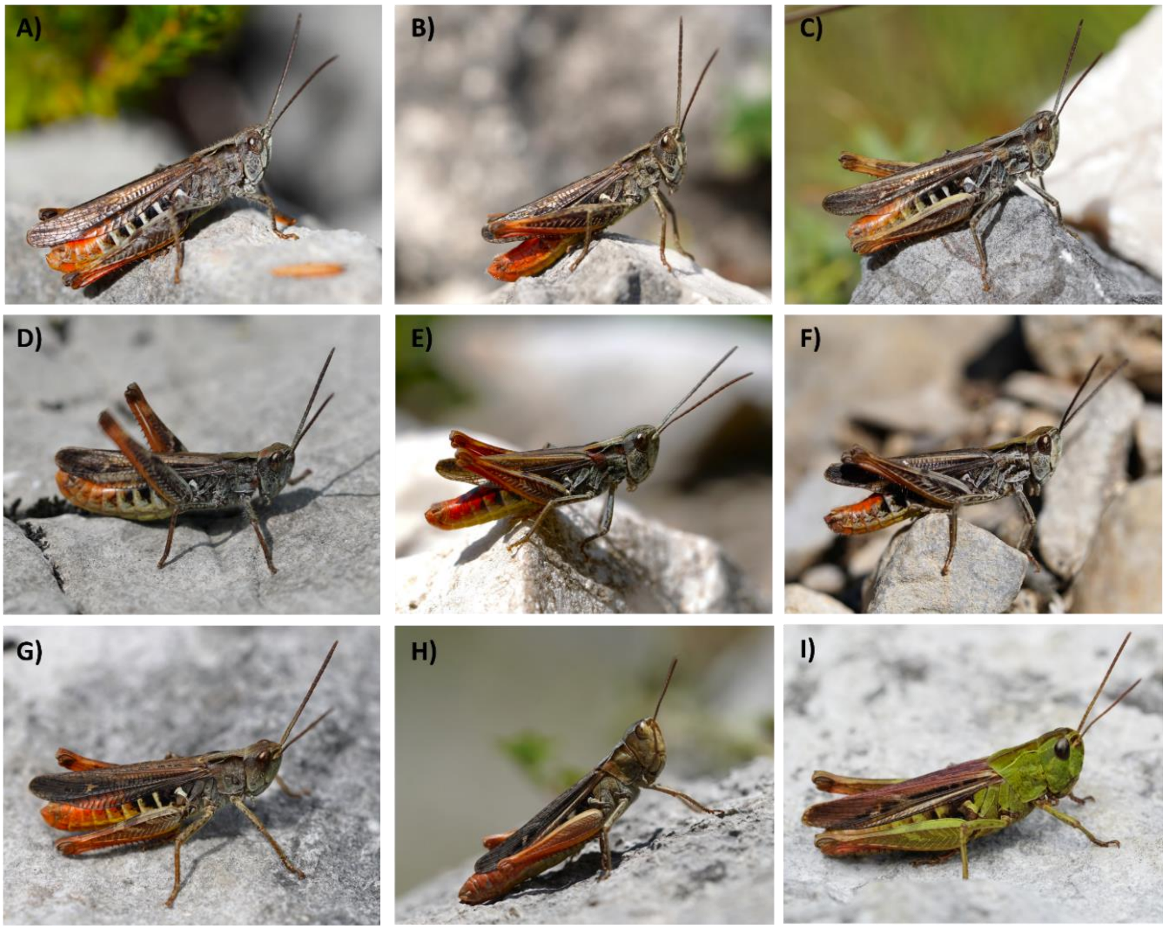


Figure 2. ***Chorthippus eisentrauti*** **individuals from the Berchtesgaden Alps.** A)-G) Images of male grasshoppers. Striking characters are their large heads, long antennae, subcostal fields of the tegmina that are steadily diverging towards the hind margins, reddish tips of the abdomen and reddish hind tibiae. H) and I) Images of female grasshoppers. Animals were slightly larger than the co-occurring, closely related sister-species *C. biguttulus*. I) The green colour morph was common at some more grassy study sites. Photo credits: Sebastian König.

Figure 3. Habitats of *Chorthippus eisentrauti*. This species occupies a broad elevational macroclimatic gradient in the study region, ranging from 600 – 2,100 m.a.s.l. However, all its habitats were characterized by a warm microclimate, high sun exposure, and included a high proportion of bare rock. Dominant Poaceae species within the habitats were the Mountain Smallreed (*Calamagrostis varia*) and the Purple Moor-grass (*Molinia caerulea*) at low elevations, and the Blue Moor-grass (*Sesleria caerulea*) at high elevations, respectively. A) Rocky alpine grassland, B) and C) steep, rocky high-montane slopes, D) rocky subalpine grassland, E) rocky montane alluvial grassland, F), H), I) steep, sun-exposed, rocky montane slope, G) steep rocky submontane slope. Except at the margins, these habitats were not inhabited by the related sister species Bow-winged Grasshopper (*C. biguttulus*). Photo credits: Sebastian König.

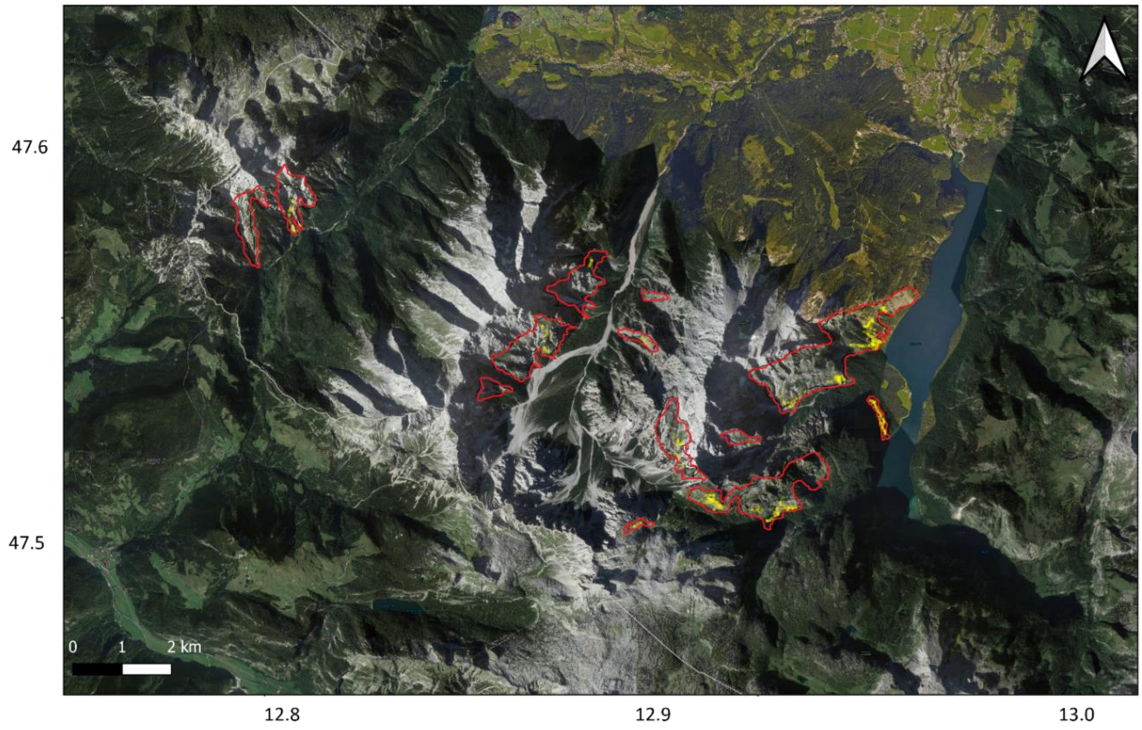


Figure 4. Potential distribution of *Chorthippus eisentrauti* in the Berchtesgaden Alps based on the assessments of this thesis. Yellow marks indicate sites, where *Chorthippus eisentrauti* individuals were found, red lines demark possible extents of occurrence. More occurrences, especially at high elevations, are likely based on the difficult accessibility of the species' habitats. Further occurrences were confirmed by Inge Illich at Austrian parts of the 'Hagengebirge', the 'Loferer Steinberge' and the bordering 'Tennengebirge'. The satellite image was downloaded from: maps.google.com.

We proposed several possible explanations for increases in diet breadth of herbivorous insects. As expected, based on the elevational distribution (peak in cold macroclimate) but the high temperatures in its habitat, the dietary resource use of this grasshopper was broader than that of many other related grasshoppers. We regularly observed it feeding on a range of grasses, woodrushes, and sedges (*Agrostis*, *Calamagrostis*, *Carex*, *Deschampsia*, *Festuca*, *Luzula*, *Molinia*, *Poa*, *Phleum*, *Sesleria*), with highest frequencies of the Mountain Smallreed and the Blue Moor-grass in faecal samples, but also on many plant genera from other lineages such as *Allium*, *Campanula*, *Erica*, *Leontodon*, *Ligusticum*, *Lotus*, *Myosotis*, *Silene*, *Thesium*, *Thymus*, *Trifolium*, *Vincetoxicum*, and even regularly on lichen and mosses. Building upon the mechanisms we suggested, the flexibility in resource use displayed by this species could be of advantage in extreme habitats where resources are scattered and scare, and water availability in grasses is low, but the grasshopper's enhanced rates of transpiration may demand compensation. Additionally, the warm temperatures likely elevate their metabolism rate, necessitating a high nutrient intake. Compensatory feeding and food plant selection based on nutritional value has been demonstrated in grasshoppers before (Berner et al 2005). Furthermore, vegetation cover was low in its habitats, which would increase search time and efficiency for a highly selective herbivore species. This could additionally facilitate the utilisation of broader dietary spectrum and highlights the importance of food plant abundance affecting the specialisation of herbivore assemblages.

5.10 *Limitations*

This work was subject to limitations that demand caution when interpreting the findings. Since the abundance of insect species can fluctuate significantly across years (Wagner et al 2021), influenced by weather anomalies (Müller et al 2023), field data collected in one season are always just snapshots and patterns found may deviate annually. However, fieldwork was conducted in 2017, 2019, and 2020, and diversity patterns were not significantly different for study sites sampled in all three seasons, emphasizing the robustness of the interpreted samples. The warm and dry summer of 2019 (DWD 2020) reflects conditions expected under ongoing climate change (Williams & Newbold 2020). As average temperatures are rising with climate change, so does the frequency of extreme weather events increase, which we did not consider but were shown to be an important driver of insect distributions and biomass variation (Feldmeier et al 2018, Müller et al 2023). Apart from macroclimate, microclimate was unravelled as driver for Orthoptera species' distributions. However, not only the average microclimatic temperature but also temporal and spatial variation of microclimate may be important determinants for Orthoptera diversity. However, local microclimatic heterogeneity was not assessed in this thesis. While we measured the microclimate response of species, other factors defining or interacting with climate, such as moisture (Dvořák et al 2022, Powell et al 2007), management (Humbert et al 2021), vegetation structure (Gardiner 2022, Löffler & Fartmann 2017, Schirmel et al 2019), vegetation composition (Tobisch et al 2023), and vegetation diversity (Fournier et al 2017, Ramos et al 2021) were not assessed in our studies, but could interfere with the climate response we derived.

We studied dietary interactions of Orthoptera via metabarcoding of DNA from faecal samples. There are many potential sources of bias associated with this method (see Chapters III and IV) and the use of relative read abundances. However, we showed that there was a good match between observed feeding behaviour and reconstructed diets based on amplicon sequencing in our study. Additionally, we omitted non-plant dietary components, which can make up significant proportions of the diet of some of the Tettigoniidae studied. Nevertheless, those species were still found to have broad diets in our study, since they fed on a range of different resource plants, corroborating their expected classification as polyphagous generalists even without considering non-plant resources. Another limitation of the diet assessment we conducted was that we ignored ontogenetic differences in feeding behaviour. Feeding preferences for specific plants were found to shift between nymphs or adults (Ingrisch & Köhler 1998). How these developmental shifts in preferences translate into the structure of interaction networks still requires investigation. Dietary specialisation was recently found to be influenced even by noise, possibly through decreases in predation pressure, highlighting the multitude of environmental influences on food webs (Senzaki et al 2023). In this regard, interactions between temperature and moisture on feeding patterns are especially interesting in terms of climate change research, as both variables are expected to vary locally with ongoing climate warming. The feeding niches we identified correlated with the phylogenetic history of the Orthoptera species. Whether the specialisation patterns we detected are causally related to climatic conditions or dependent on other phylogenetically conserved traits of the species remains to be tested. To this end, experimental settings in which a variety of plants are offered to a selection of Orthoptera under defined climatic circumstances (cafeteria experiment) could provide an opportunity to test the predictions we proposed and generate novel mechanistic insights. The bacterial associates we identified via metabarcoding are not necessarily viable or closely linked to the biology of the grasshoppers. While we found differences in the composition and diversity between feeding groups, their role in affecting the growth, digestion, detoxification, immunity, or reproduction of the hosts still requires further investigation. Thus, the work presented in this thesis raised multiple further questions that suggest future research directions.

5.11 *Perspectives*

Following the limitations identified, we want to propose next steps that should be considered to form a proper basis for detailed predictions of species responses to climate change. As we could not include several climatic variables in this work but relied on average seasonal temperatures, it would be important to include more environmental variables such as precipitation, fine-scale soil moisture (Dvořák et al 2022), seasonal, daily, and spatial variation of microclimate (Elsen et al 2020), temperature extremes, and larger macroclimatic ranges of insect species. In fact, our study design including detailed assessments along climatic gradients provides a perfect opportunity to acquire further environmental parameters and conduct re-surveys to determine whether our predictions are realized over the course of a few years.

As niches were shown to differ between species, care should be taken not to generalize findings from single taxa. We found trait clines such as body size variation or hatching phenology variation along the elevational gradients, but other traits not considered in this study could have even stronger effects on species distributions. One candidate for an adaptive trait along elevational gradient apart from behavioural or physiological thermoregulatory capacities are thermal tolerances. These limits could influence the range of ambient climatic conditions a species can cope with (Birkett et al 2018, Sunday et al 2012). Systematic assessments of thermal tolerances may therefore help to understand the macro- and microclimatic habitat use of insects and predict future challenges.

Furthermore, we showed that plant-herbivore interactions can also vary with temperature. However, these results could change if more individuals, individual preferences and behaviours, and ontogenetic differences were analysed. A simple follow-up study could focus on a single species and determine individual-level feeding niches in more detail and their associated changes in time. While recent developments in sequencing technology and bioinformatics allow cost-effective microbial diversity screenings, experiments are needed that identify the functions of bacteria that have non-obligatory interactions but are associated with herbivorous insects for the hosts. Only then we can understand the complex nature of feeding specialisation and how the environment affects it. Until now, we are still far from comprehending the spatio-temporal dynamics of interactions in natural insect communities.

5.12 *Concluding remarks*

The evaluation of insects' responses to temperature variation has increasingly garnered attention due to the imminent entanglement of a biodiversity and climate crises. This is particularly crucial for gaining insights into how assemblages respond to global change. Thus, we need to make progress in understanding effects of global change drivers on biodiversity, which is essential to halting biodiversity loss. With this thesis, we provide a detailed overview of the structuring of the Orthoptera fauna in the Berchtesgaden Alpine region along elevational macro- and microclimatic gradients. The space-for-time approach enables the recognition of species' adaptations to climatic circumstances. Our findings imply that macroclimatic as well as microclimatic changes have the potential to restructure, reassemble, and replace Orthoptera communities in temperate mountain grasslands. Here, we demonstrate additive effects on diversity, but also community composition and functional traits are affected, as the interaction of elevation and microclimate shapes species niches. We conclude that those microclimatic differences are crucial to generate and maintain the high diversity of insects in mountain regions. This topographic heterogeneity may have the potential to mitigate climate change effects on regional scale. We found a maximum species richness of Orthoptera at low elevations. With warming climate, I) diversity increases, and II) species turnover modified by microclimatic preferences and species traits are expected. At higher elevations, thermophilic species may arrive at warm-microclimate-sites and thermophobic species may retreat to sites with cold microclimate, increasing assemblage dissimilarity. Species which hatch later in the season are expected to profit by near-future temperature changes experienced within habitats in Central Europe. Together with high-resolution microclimate data, possible shifts of microclimate preferences of species with macroclimate should be incorporated in species distribution modelling to estimate the extent and distribution of suitable habitat in a warming world. Understanding microclimatic preferences of species could thus be an important step towards anticipating climate change effects.

The findings presented in this dissertation suggest that warmer climates could lead to changes in plant-herbivore interaction networks. In conclusion, phylogenetic turnover of plants and herbivores along the temperature gradient, abundance clines of certain plant species, and the phylogenetic structure of the plant communities were associated with herbivore specialisation, corroborating studies that found structural variation of networks of herbivorous insects and plants across environmental gradients (Pitteloud et al 2021, Tylianakis & Morris 2017, Welti & Joern 2015). We were thus able to demonstrate empirically that temperature affects specialisation of a rather generalist herbivore taxon with a peak at intermediate climatic conditions. Several mechanisms such as trait matching, nutrient or water requirements of the herbivores, nutritional and defence clines in plants, environmental unpredictability, and microbial associates may be linked to the specialisation patterns detected. However, further research is needed for a more mechanistic understanding. Novel, currently unrecognised risks for both, plants and herbivores, might emerge under climate warming that are linked to climate-induced gradation or range shifts of herbivores or altered nutritional quality or quantity of plant resources. However, we anticipate orthopterans to respond directly to temperature warming due to weak associations with specific plant species (Geppert et al 2021).

Yet temperature effects were found on another level, neglected in interaction research in Orthoptera: their associate microbial communities. To our knowledge, we provide first insights into bacterial community compositions found in faeces of insect with different feeding niches along a climatic gradient, as we demonstrated that temperature change was linked to compositional variation of bacteria. The high diversity but large overlap of associated bacteria is consistent with the hypothesized close but not compelling association between grasshoppers and bacteria which are acquired from the feeding environment and provokes interesting further questions about the functions and resilience of these associations to upcoming climatic challenges.

Additionally, this thesis contributes to understanding feeding niches of Orthoptera in Bavaria. We showed that the use of genetic tools enables the effective and fast collection of an impressive number of feeding interactions and can thus contribute to filling the knowledge gap of unknown food plants, interactions, and dependencies of insects and plants. In this context, we accumulated thousands of empirical observations of plant-herbivore interactions to begin a weighted database for feeding interactions of Orthoptera in Central Europe, which can provide valuable insights in important food plants (also from a conservation perspective) and differences in the diet breadths of these herbivores. We therefore suggest applying this methodological framework to organisms where information on interactions is still limited, as such knowledge may be crucial to predict threats under climate change.

Through explorations of species diversity, abundance, assemblage composition, species traits, and both, trophic and mutualistic species interactions, our results can help to comprehend assembly processes across climatic gradients. We highlight the necessity to incorporate interaction ecology to attain a multifaceted understanding of systems under both, current and future climatic conditions. To validate predictions of ecosystem responses to climate change derived from space-for-time substitution approaches, we underscore the need for long-term monitoring initiatives that regard abundances of the focal organisms as a measure of habitat and climate suitability. Our data can serve as a valuable foundation of such long-term projects. Finally, our results underline the extraordinary value of traditional extensive pastoral systems covering areas of different orientation and hence offering a range of microclimatic conditions to conserve open habitats invaluable for biodiversity in mountains. These serve as climate refugia and need to be protected.

“The outcome of any
serious research can only be to make
two questions grow where only one grew before”

— *Thorstein B. Veblen*

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Author contributions

Chapter II

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Data accessibility statement

All data used for the analyses are uploaded to the public Dryad repository DOI: https://doi.org/10.5061/dryad.dfn2z3580.

Chapter III

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Data availability statement

The data that support the findings of this study are openly available in Dryad at the repository: https://doi.org/10.5061/dryad.612jm645j (König et al 2022b)

Chapter IV

Chapter IV is prepared for submission to the journal Molecular Ecology as: König, S., Krauss, J., Keller, A., and Steffan-Dewenter, I. Food plants, feeding niches, grazing, and climate affect microbial communities in Orthoptera faeces.

Author contributions

Conceptualization: S.K., J.K., A.K, and I.S.-D. Data curation: S.K. Methodology: S.K., J.K., A.K., and I.S.-D. Data collection: S.K., Formal analysis: S.K. and A.K., Visualization: S.K. Writing—original draft: S.K. Writing—review & editing: S.K., J.K., A.K., and I.S.-D. Funding acquisition: I.S.-D.

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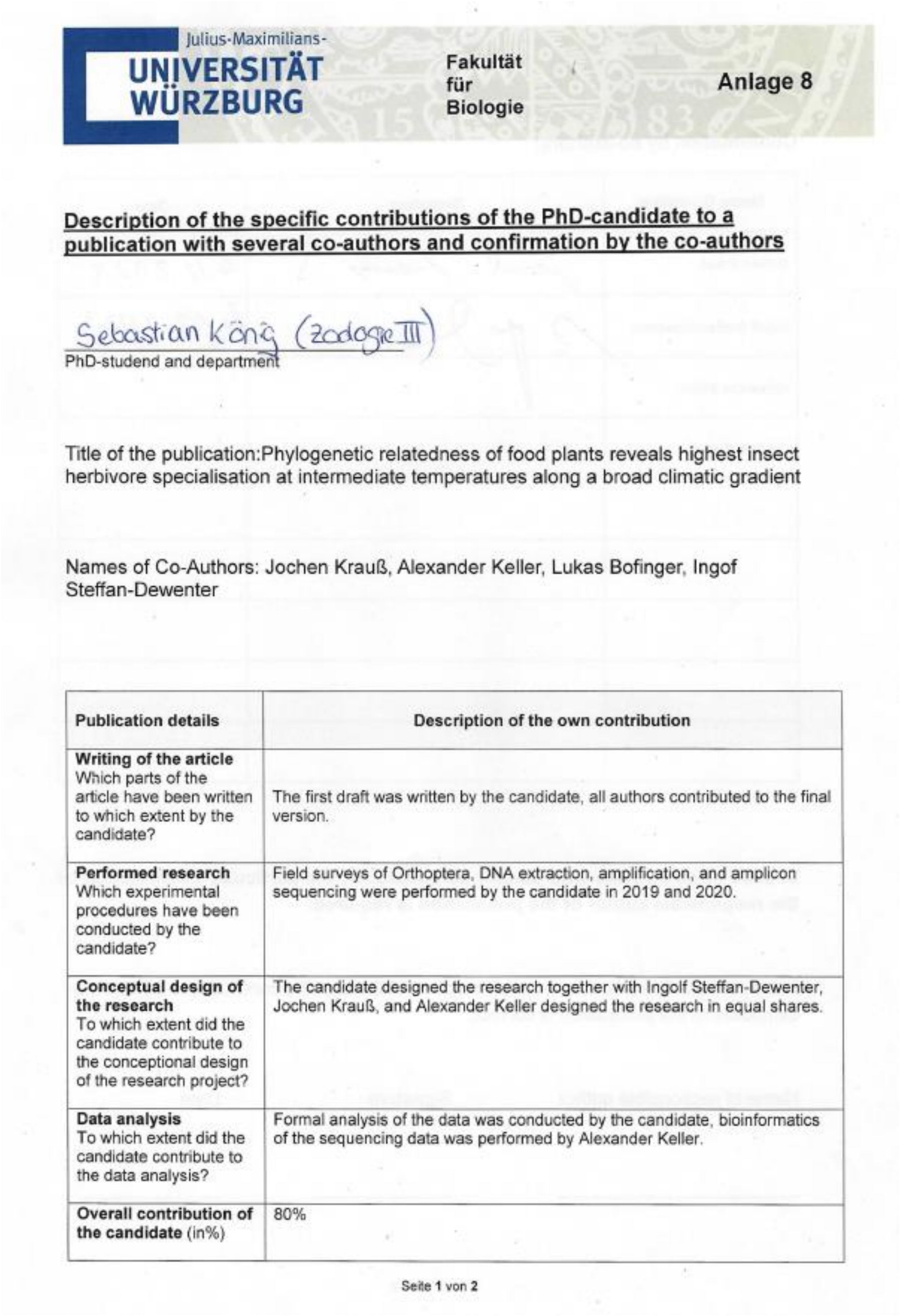

Fakultät
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Biologie

Anlage 8

Description of the specific contributions of the PhD-candidate to a publication with several co-authors and confirmation by the co-authors

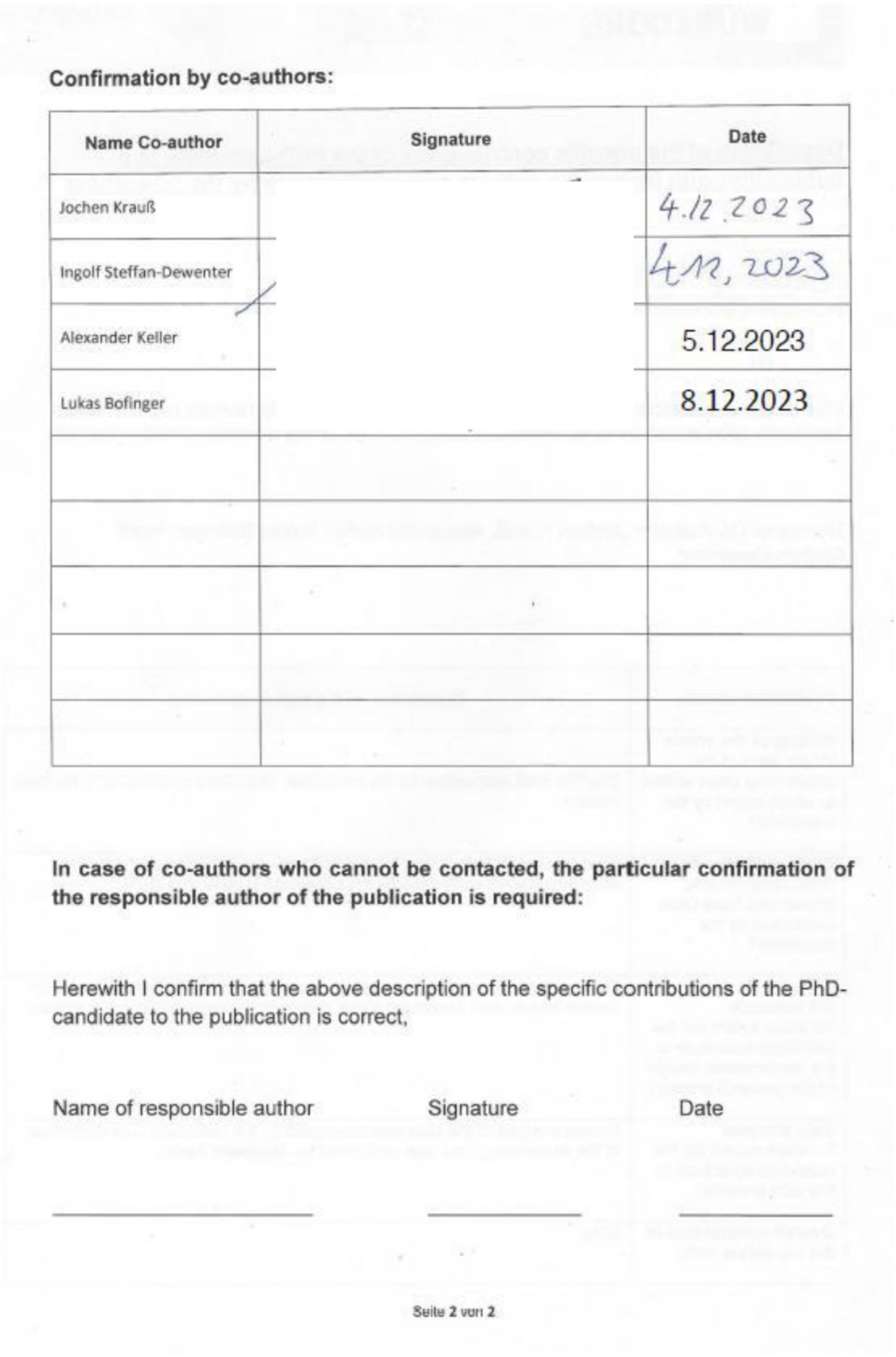
Sebastian König (Zoologie III)
PhD-studend and department

Title of the publication:Phylogenetic relatedness of food plants reveals highest insect herbivore specialisation at intermediate temperatures along a broad climatic gradient

Names of Co-Authors: Jochen Krauß, Alexander Keller, Lukas Bofinger, Ingof Steffan-Dewenter

Publication details	Description of the own contribution
Writing of the article Which parts of the article have been written to which extent by the candidate?	The first draft was written by the candidate, all authors contributed to the final version.
Performed research Which experimental procedures have been conducted by the candidate?	Field surveys of Orthoptera, DNA extraction, amplification, and amplicon sequencing were performed by the candidate in 2019 and 2020.
Conceptual design of the research To which extent did the candidate contribute to the conceptional design of the research project?	The candidate designed the research together with Ingolf Steffan-Dewenter, Jochen Krauß, and Alexander Keller designed the research in equal shares.
Data analysis To which extent did the candidate contribute to the data analysis?	Formal analysis of the data was conducted by the candidate, bioinformatics of the sequencing data was performed by Alexander Keller.
Overall contribution of the candidate (in%)	80%

Seite 1 von 2



Confirmation by co-authors:

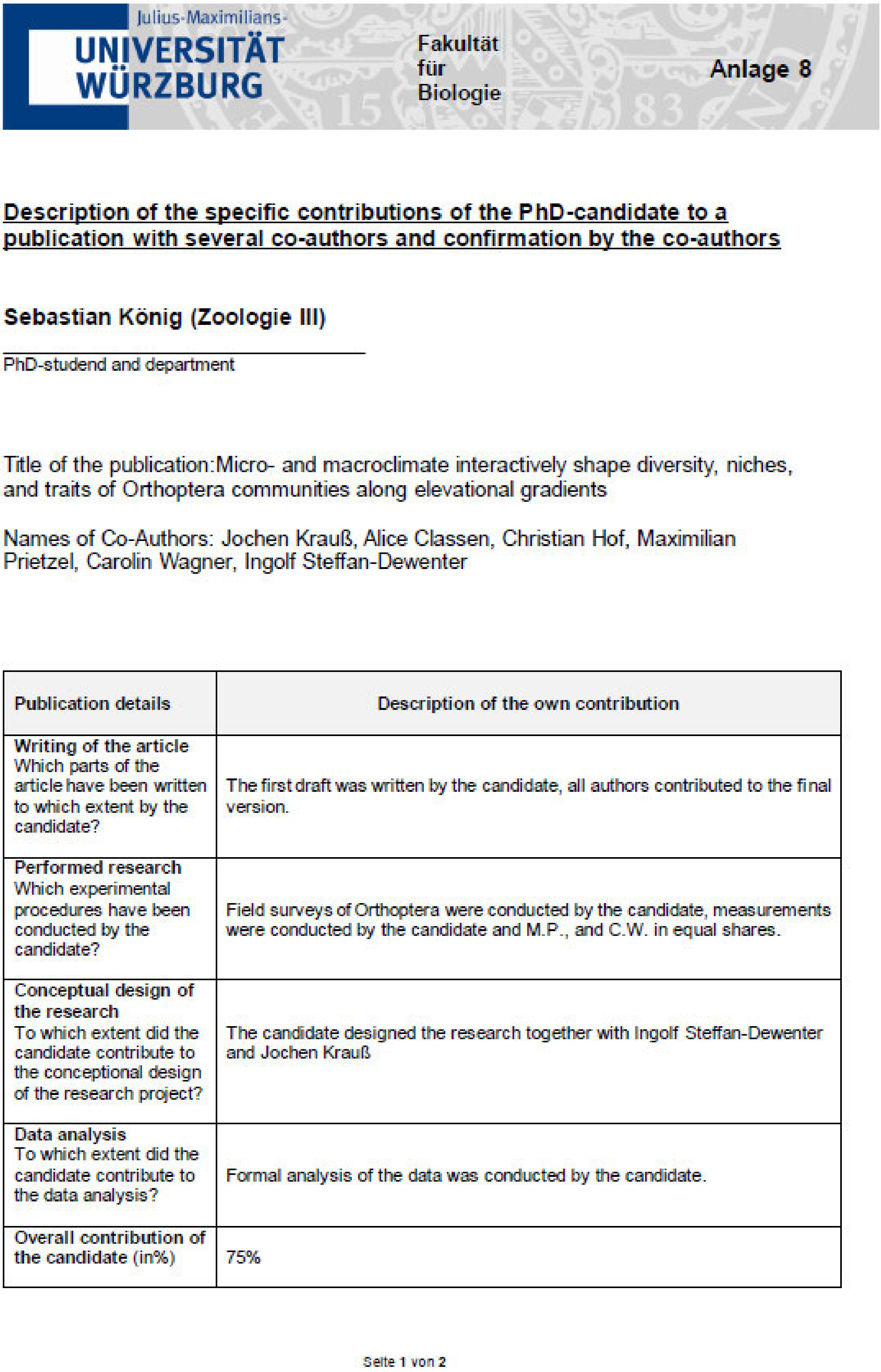
Name Co-author	Signature	Date
Jochen Krauß	[redacted]	4.12.2023
Ingolf Steffan-Dewenter	[redacted]	4.12.2023
Alexander Keller	[redacted]	5.12.2023
Lukas Bofinger	[redacted]	8.12.2023

In case of co-authors who cannot be contacted, the particular confirmation of the responsible author of the publication is required:

Herewith I confirm that the above description of the specific contributions of the PhD-candidate to the publication is correct,

Name of responsible author　　Signature　　Date

Seite 2 von 2



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Biologie

Anlage 8

Description of the specific contributions of the PhD-candidate to a publication with several co-authors and confirmation by the co-authors

Sebastian König (Zoologie III)

PhD-studend and department

Title of the publication:Micro- and macroclimate interactively shape diversity, niches, and traits of Orthoptera communities along elevational gradients

Names of Co-Authors: Jochen Krauß, Alice Classen, Christian Hof, Maximilian Prietzel, Carolin Wagner, Ingolf Steffan-Dewenter

Publication details	Description of the own contribution
Writing of the article Which parts of the article have been written to which extent by the candidate?	The first draft was written by the candidate, all authors contributed to the final version.
Performed research Which experimental procedures have been conducted by the candidate?	Field surveys of Orthoptera were conducted by the candidate, measurements were conducted by the candidate and M.P., and C.W. in equal shares.
Conceptual design of the research To which extent did the candidate contribute to the conceptional design of the research project?	The candidate designed the research together with Ingolf Steffan-Dewenter and Jochen Krauß
Data analysis To which extent did the candidate contribute to the data analysis?	Formal analysis of the data was conducted by the candidate.
Overall contribution of the candidate (in%)	75%

Seite 1 von 2

Confirmation by co-authors:

Name Co-author	Signature	Date
Jochen Krauß		14.12.2023
Ingolf Steffan-Dewenter		
Christian Hof		
Alice Classen		14.12.2023
Carolin Wagner		
Maximilian Prietzel		

In case of co-authors who cannot be contacted, the particular confirmation of the responsible author of the publication is required:

Herewith I confirm that the above description of the specific contributions of the PhD-candidate to the publication is correct,

Name of responsible author　　Signature　　Date

14. 12. 2023

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Publication list

Publications that integrate this doctoral dissertation

König, S., Krauss, J., Keller, A., Bofinger, L., Steffan-Dewenter, I. (2022). Phylogenetic relatedness of food plants reveals highest insect herbivore specialization at intermediate temperatures along a broad climatic gradient. *Global Change Biology* 28: 4027 - 4040. DOI:10.1111/gcb.16199

König, S., Krauss, J., Classen, A., Hof, C., Prietzel, M., Wagner, C., and Steffan-Dewenter, I. (*under review - minor revision*). Micro- and macroclimate interactively shape diversity, niches, and traits of Orthoptera communities along elevational gradients. *Diversity and Distributions*

König, S., Krauss, J., Keller, A., and Steffan-Dewenter, I. (*in preparation*). Food plants, feeding niches, grazing, and climate affect microbial communities in Orthoptera faeces.

Other publications

Thorn, S.*, **König, S.***, Gombert, J., Griese, J. and Fischer-Leipold, O. (2022). Temperature preferences drive additive biotic homogenization of Orthoptera assemblages. *Biology Letters* 18: 20220055. doi:10.1098/rsbl.2022.0055

Ditzel, P., **König, S.**, Musembi, P., and Peters, M.K. (2022). Correlation between Coral Reef Condition and the Diversity and Abundance of Fishes and Sea Urchins on an East African Coral Reef. *Oceans* 3: 1-14. doi:10.3390/oceans3010001

Boetzl, F.A., Krauss, J., Heinze, J., Hoffmann, H., Juffa, J., **König, S.**, Krimmer, E., Prante, M., Poppenburg-Martin, E.A., Holzschuh, A., and Steffan-Dewenter, I. (2021). A multitaxa assessment of the effectiveness of agri-environmental schemes for biodiversity management. *Proceedings of the National Academy of Sciences* 118: e2016038118. doi:10.1073/pnas.2016038118

König, S. and Krauss, J. (2019). Get larger or grow longer wings? Impacts of habitat area and habitat amount on Orthoptera assemblages and populations in semi-natural grasslands. *Landscape Ecology* 34: 175-186. doi:10.1007/s10980-018-0762-5

under review

Klimm, F.S., Bräu, M., **König, S.**, Mandery, K., Sommer, C., Zhang, J., and Krauss, J. (*under review – minor revision*). Importance of habitat area, quality and landscape context for Heteropteran diversity in shrub ecotones. *Landscape Ecology*

Kerner, J.M., **König, S.**, Maihoff, F., Bofinger, L., Sauer, N., Ssymank, A., Väth, P., and Classen, A. (*under review – minor revision*). Transect walks and malaise traps differ in temperature sensitivity but reveal consistent drivers of pollinator richness. *Insect conservation and diversity*

*shared first-authorship

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I need to start my acknowledgements at the origins of my fascination for arthropods in general, and for Orthoptera specifically. As most of you know, this obsession was probably the fault of my grandpa Günther, who took me to the fields as a young boy, where I explored the meadows and got addicted to the little hoppers, that occurred there in large numbers and great diversity, and were unable to escape my quick hands after no time. He made nature tangible for me and supported me taking some critters home, as we could not stay out in the field all day, so that I could observe them and their behaviour. I would like to dedicate this work to his memory.

Jumping about 13 years ahead, I owe thanks to Markus Engstler, dad of my school friend Henrik, who was instrumental in helping me chose my career the day we met. Even though I was still a naturalist at heart and as a hobby, I had no concrete plans for after high school. Your enthusiasm for biology and all the facets I could explore by studying Biology in Würzburg was the advice I needed to decide my further education.

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Importantly, the working environment needs to be a place you like to be at, and a refugium for developing ideas. And what makes a working environment so amazing is the people that fill it. That means a long list will follow:

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