

RESEARCH ARTICLE

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

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Abstract

The composition and richness of herbivore and plant assemblages change along climatic gradients, but knowledge about associated shifts in specialization is scarce and lacks controlling for the abundance and phylogeny of interaction partners. Thus, we aimed to test whether the specialization of phytophagous insects in insect-plant interaction networks decreases toward 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 Orthoptera surveys, feeding observations, collection of fecal 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 specialized in warm habitats. Considering phylogenetic relationships of plant resources, however, the specialization 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 specialization of insect herbivores along a climatic gradient, demonstrating that resource phylogeny, availability, and temperature interactively shape the specialization of herbivore assemblages. Instead of low specialization 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 specialization 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.

KEYWORDS

Alps, diet breadth, distance-based specialization index, herbivores, interaction networks, metabarcoding, microclimate, Orthoptera, plant richness, temperature gradient

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1 | INTRODUCTION

Elevational and latitudinal climatic gradients are major drivers of species richness, community composition, and biotic interactions (Gaston, 2000; McCain and 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 in species diversity (Descombes et al., 2017a; Gaston, 2000; McCain and 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., 2016), 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 specialization 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, specialization, and turnover of involved species caused by environmental filtering (Gravel et al., 2019; Pellissier et al., 2018; Pitteloud et al., 2021; Welty & 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 under represented (but see Forister et al., 2015; Pellissier et al., 2012; Pitteloud et al., 2021; Welty 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 generalization 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 generalization (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 defense (Rasmann et al., 2014). Derived from the coevolution of plant defenses and herbivore specialization, generalization is predicted to be favored by lower levels of plant defenses 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 specialization 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 the 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 defenses 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 specialization 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 specialization can be a function of resource diversity. The degree of specialization of phytophagous insects differs, biased by resource abundance. Thus, the availability of diverse plant resources can support populations of different specialized herbivore species, at least in productive climate zones (Novotny et al., 2006). As plant diversity often shows nonlinear 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 specialization 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, while 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, they 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 specialization below the 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 feces 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 specialization of plant-herbivore interactions. We partitioned herbivore specialization in taxonomic identity-based interaction specialization (d') and resource phylogeny-based dietary specialization (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 specialization 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 toward cold and resource species-poor habitats.

2 | MATERIALS AND METHODS

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 2100 m a.s.l. (Figure 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 12–24°C mean summer temperature (seasonal temperatures measured at the sites). Neighboring 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 × 60 m. At these, we 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 ($cor = -0.93$, $t = -16.24$, $df = 39$, $p = <.001$) 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).

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 min 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; Figure S2).

2.3 | Orthoptera turnover

To quantify turnover rates among 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 gradients rate with temperature (Descombes et al., 2017b).

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 m² 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 nonlinear 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 (Figure 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 5000 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 (wMPDs), a common metric of phylogenetic α -diversity, from random communities, maintaining species occurrence frequencies 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.

2.5 | Fecal sampling and feeding observations

To obtain dietary information of the locally occurring Orthoptera populations at the study sites, we collected fecal 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 fecal 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 fecal 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 fecal 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.

2.6 | DNA extraction, amplification, sequencing, and in-silico taxa assignment

Laboratory workflow included DNA isolation from fecal samples, library preparation, indexing, quality control, normalization, pooling, quantification, and sequencing. DNA extractions of fecal 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 recognized 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 μ l of template DNA sample (~ 1 nmol/ μ l) 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 fecal samples of known composition from feeding experiments, processed in parallel to samples, while negative controls contained samples with preservation solution and/or reagents without feces 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, 2016), and definition of amplicon sequence variants (ASVs) (Edgar, 2016), 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 cutoff threshold of 97%, before mapping against a database for Germany. For still unclassified reads, we used SINTAX (Edgar, 2016) 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, Figure S3). ASV counts at the family and genus levels 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; Welty et al., 2019).

2.7 | 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 (Figure S5). For network-level analyses, we calculated the interaction intensity by the multiplication of relative read abundances with the Orthoptera species abundance at the respective study site (Welty et al., 2019).

2.8 | Interaction specialization based on taxonomic identity

Data were analyzed 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 specialization (Shannon entropy, H^2) and herbivore interaction specialization at 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 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 they were outside the 2.5–97.5% quantile interval of the metric obtained from the randomized networks.

2.9 | Interaction specialization based on plant phylogeny

Recent advances in calculating resource specialization 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 specialization index (DSI^*) weighs the degree of specialization by the phylogenetic similarity of resources and accounts for their availability. A reason to include the phylogenetic relatedness of species within measures of host specialization 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 for a given resource spectrum (maximum specialization, $DSI^* = 1$) (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 specialization is a z-value and the rescaled version relies on the calculation of weighted mean pairwise distances (wMPDs, raw measure of phylogenetic diet breadth) between used resources and annealed limits 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 wMPDs at the assemblage level. (DSI^* values for all sampled species in our study are provided

in Table 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 specialization (PSS) as an alternative improved measure for herbivore specialization (see supplementary methods, Pardo-De la Hoz et al., 2022).

2.10 | 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 specialization metrics ($H2'$, d' , DSI^* , $wMPD$, PSS) at the 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 the variance was analyzed with ANOVAs. We used temperature and both plant metrics to explain Orthoptera richness and abundance, while we explained specialization 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 (Figure S6). (The assumed error families are indicated in Table 1 and Table S1). Additionally, we used a subset of abundant Orthoptera species, which occur along a broad fraction of the sampled microclimatic gradient to analyze intraspecific variation in specialization. Averages of responses are presented with corresponding standard deviations.

3 | RESULTS

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 (Table 1; Figure 1a, b). In addition, Orthoptera species richness and abundance increased with plant species richness (Table 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 toward warmer habitats (Table 1; Figure 1c).

Partitioning β -diversity between pairs of Orthoptera 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 (Figure S7). This suggests that the dominance of species within assemblages differed more between assemblages at high temperatures (Figure 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 (Table 1; Figure 1d). Poales were dominant at cold sites and warm meadows, with lowest cover at intermediate and the hottest sites, respectively (Figure 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 (Figure S10). NRI increased with the proportional cover of Poales at the study sites (Table 1).

3.2 | Characteristics of plant-herbivore interaction networks

We sampled feces of 3345 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 fecal samples of grasshoppers, representing a broad range of plant-herbivore interactions (Figure 2). On average, the sampled Orthoptera species in our study fed on 24 (± 9.6) plant species per site (Figure S11).

3.3 | Shifts in taxonomy-based interaction specialization with temperature

We calculated herbivore specialization 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 specialization ($H2'$) with temperature (Table 1; Figure 3a), but $H2'$ was not affected by plant species richness at the study sites. The network specialization was, on average, lower than predicted by null models, and the slope differed from the expected pattern based on network dimensions ($t_{1,39} = -3.93$, $p < .001$, Table S2). At species level, community-weighted herbivore interactions (d') were more specialized in warm habitats and at sites with overdispersed plant assemblages compared to cold habitats with phylogenetically clustered resources (Table 1; Figure 3a; Figure S12a). Intraspecifically, the specialization of common, widespread species increased with temperature (Figure S13a).

3.4 | Shifts in phylogeny-based resource specialization with temperature

In a next step, we analyzed resource specialization by accounting for phylogenetic relatedness of plant species and their availability. Most

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 specialization variables (Network specialization $H2'$, community-weighted mean species-level specialization d' , community-weighted mean distance-based specialization DSI^* , and community-weighted mean pairwise distances $wMPD$) to environmental variables. Predictor variables are mean summer temperature ($Temp$), PSR , proportional cover of Poales (Poa), and NRI

Response	Predictor	Family	N	edf	rdf	χ^2	p	R^2_{adj}	R^2
Orthoptera SR	Temp	Poisson	41	1.84	4	52.88	<.001***	0.68	0.68
	PSR			0.88	4	7.07	.004**		
	NRI			0.00	4	0.00	.935		
Orthoptera ABU	Temp	Quasipoisson	41	2.47	4	27.32	<.001***	0.72	0.77
	PSR			0.95	4	6.71	<.001***		
	NRI			0.34	4	0.18	.208		
Prop. graminivorous	Temp	Quasibinomial	41	0.95	4	4.03	<.001***	0.53	0.49
	PSR			0.00	4	0.00	.868		
	NRI			0.94	4	4.26	<.001***		
Plant SR	Temp	Quasipoisson	41	3.26	4	3.01	.053 ^(*)	0.18	0.24
Plant NRI	Temp	Gaussian	41	0.00	4	0.00	.361	0.28	0.31
	PSR			0.42	4	0.18	.197		
	Poa			0.94	4	3.88	<.001***		
$H2'$	Temp	Beta	41	0.96	4	21.96	<.001***	0.46	0.48
	PSR			0.49	4	0.98	.159		
	NRI			0.85	4	5.01	.011*		
CWM d'	Temp	Beta	41	0.96	4	20.50	<.001***	0.43	0.46
	PSR			0.00	4	0.00	.445		
	NRI			0.85	4	5.61	.010*		
CWM DSI^*	Temp	Beta	41	2.65	4	29.04	<.001***	0.47	0.53
	PSR			1.43	4	3.55	.079 ^(*)		
	NRI			0.74	4	2.78	.052 ^(*)		
CWM $wMPD$	Temp	Quasipoisson	41	2.10	4	3.33	.002**	0.35	0.42
	PSR			1.54	4	1.21	.052 ^(*)		
	NRI			1.21	4	1.16	.030*		

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: ^(*) $p < .1$, * $p < .05$, ** $p < .01$, *** $p < .001$

Orthoptera species had specialized diets ($DSI^* = 0.57$, Figure 3b), ranging from highly specialized ($DSI^* = 0.97$), over nonselective ($DSI^* \sim 0$) to generalist feeders ($DSI^* = -0.71$) (Table S3).

In contrast to taxonomic interaction specialization, the phylogeny-based resource specialization of Orthoptera species had a nonlinear relationship with mean summer temperature. The distance-based specialization index was low at cold sites, peaked at intermediate temperatures, before decreasing again toward the warmest habitats (Table 1; Figure 3b). In other words, herbivore resource specialization 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 Orthoptera's diets at intermediate temperatures (Table S1; Figure S12c). Nevertheless, highly specialized species ($DSI^* = 0.75-1$) occurred along the entire climatic gradient. The phylogeny-based resource specialization and the weighted mean phylogenetic distances

of plants in Orthoptera feces showed a weak peak at intermediate plant species richness, and both decreased with increasing phylogenetic clustering of plant assemblages (Table 1; Table S1). Between populations of widespread species along the microclimatic gradient, the specialization was either constant, showed a mid-temperature peak, or decreased with temperature (Figure S13b). Field observations of feeding interactions also revealed a mid-temperature peak in specialization (Figure S14). Both computed indices characterizing the phylogenetic structure of specialization yielded similar patterns along the gradient (Figure 1; Figure S15).

4 | DISCUSSION

In this study, we empirically tested predictions derived from the 'altitude niche-breadth hypothesis' on herbivore specialization-temperature

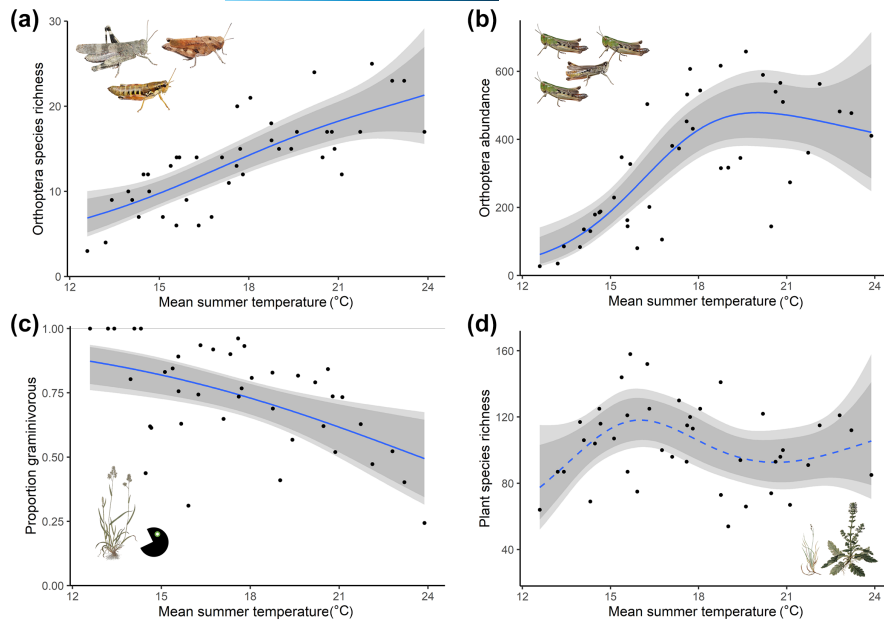


FIGURE 1 Orthoptera were recorded during transect walks at the study sites. Both, species richness (a) and abundance (b) increased with temperature. (c) Orthoptera species with graminivorous-type mouthparts were dominant in this study. Their weighted proportion within assemblages was highest in cold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

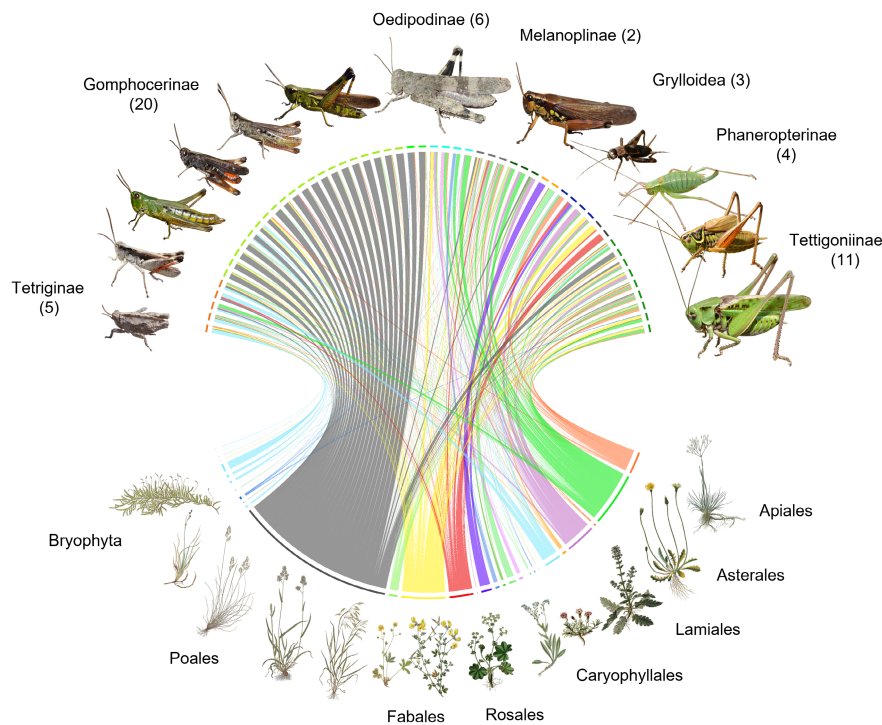


FIGURE 2 Overview of the recorded plant-herbivore interactions, using cumulative interaction strengths (proportions of reads) of plant taxa detected in Orthoptera feces via (DNA-) metabarcoding (Gu et al., 2014). Orthoptera taxonomic subdivisions with representative species (upper half, number of species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

relationships, while controlling for phylogenetic relatedness and availability of plant resources. We observed an increase in network and interaction specialization of Orthoptera and their plant resources with temperature as predicted, but the use of phylogenetic relationships and resource cover unraveled a nonlinear relationship with a peak in specialization at intermediate temperatures.

4.1 | Taxonomy-based interaction specialization

The observed specialization increase of plant-herbivore interactions toward warm habitats is in line with the 'altitude niche-breadth

hypothesis' and observed interaction specialization 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, favoring 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 generalization can also be the result of relaxed plant defenses, but evidence for this is conflicting (Descombes et al., 2017a; Galmán et al., 2019; Leingärtner et al., 2014; Pellissier et al., 2012; Rasmann et al., 2014). Highly

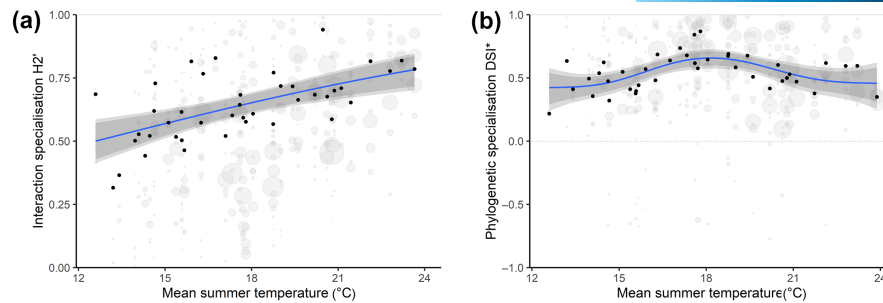


FIGURE 3 Relationship between mean summer temperature and assemblage-level resource specialization metrics. (a) Network specialization (Shannon Entropy $H2'$, black points), and species-level specialization (d' , gray points) increase with temperature. (b) The phylogeny-based dietary specialization (DSI^*)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

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 specialization in our study. Welte et al. (2017) documented increased herbivore generalization 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 specialization in our sampled climatic gradient (Classen et al., 2020). Second, observed 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, unraveled a decrease in specialization 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 specialization.

Alternatively, herbivore species may be forced to escape competition with other species when population densities are high, for example, 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 specialization 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 specialization

with temperature, suggesting that intraspecific changes could contribute to more specialized networks in warm habitats. As evidence of specialization patterns along environmental gradients is conflicting, the inclusion of phylogenetic relationships and resource availability can help to disentangle patterns and underlying processes of biotic specialization of mobile, generalist herbivore taxa such as Orthoptera in ecological networks (Jorge et al., 2017; Pardo-De la Hoz et al., 2022).

4.2 | Phylogeny-based specialization

The phylogeny-based specialization 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 specialization index, we conclude that the phylogenetic structure of plant assemblages also shaped specialization 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 generalization can either be explained by relaxed plant defense 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 specialization and temperature for a part of the covered temperature gradient. However, increasing phylogenetic diet breadths of herbivore assemblages toward the warmest sites were unexpected based on predictions derived from the 'altitude niche-breadth hypothesis'. The observed specialization 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 specialization 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 specialization with temperature, and, therefore, do not sufficiently explain the mid-temperature peak in assemblage-level specialization 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 specialization pattern along the gradient. Therefore, we infer decreases in specialization of common species contributed to low specialization levels in cold habitats, while additional generalist species decreased assemblage-level specialization 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 caerulea* (L.), *Calliptamus italicus* (L.), or *Platycleis albopunctata* (Goeze). Nonetheless, we found highly specialized 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 specialization of Tetrigids or Tettigoniids, however, was not completely captured in this study as they also feed on algae and arthropods, respectively.

The barcoding of fecal samples unraveled 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 over-represented in Orthoptera feces (*Agrostis* (L.), *Bromus* (L.), *Dactylis* (L.), *Festuca* (L.), & *Sesleria* (Scop.), Figure S16). This may be a hint at converging plant traits such as toughness, silica content, or nutrient content, explaining a bias toward 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.

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 levels was found to be less accurate in estimating resource diversity than analyzing 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 specialization 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.

5 | CONCLUSIONS

Incorporating phylogenetic relationships of plant resources and plant cover revealed highest herbivore 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 specialization. We showed that the phylogenetic resource specialization 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' (Rasman et al., 2014). Therefore, we suggest considering the phylogeny of interaction partners, as well as their availability and abundance in studies about biotic specialization along environmental gradients.

Our results suggest that due to nonlinearity, 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 specialized 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, co-existence, and interactions. Thus, novel, and currently unrecognized risks might emerge under climate warming due to mass propagation of herbivores, shifts in plant defense, reduced dietary quality of plant resources for specialized herbivores and reduced stability of plant-herbivore interaction networks.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

S.K., J.K., and I.S.D. designed the research. S.K. and L.B. performed field work, S.K. conducted laboratory work, A.K. performed bioinformatic processing, S.K. analyzed the data. S.K. wrote the first draft of the manuscript with inputs from J.K. and I.S.D. and all authors equally contributed to revisions and gave approval for publication.

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., 2022).

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