INTRODUCTION

Species are involved in complex networks through a variety of interaction types that span the entire spectrum from antagonist to mutualistic relationships and regulate multiple ecosystem functions (Bascompte, 2010; Reiss et al., 2009). Species interaction networks are not random but rather form determined structures, including the ordinalization of interaction links or the presence of modules, which can be driven by traits or extrinsic environmental conditions (Gravel et al., 2013; Laigle et al., 2018; Poelmann & Kessler, 2016). Studying the structure of ecological networks is challenging, since they have multiple entangled causes (Dormann et al., 2017; Laigle et al., 2018). Progress in our understanding of the mechanisms that determine the structure of interaction networks could be achieved by formulating distinct hypotheses that can be tested against empirical data (Pellissier et al., 2018). Furthermore,
the determining factors driving species interaction networks could be context-dependent, where interaction rules may shift along environmental gradients (Pellissier et al., 2018; Welti & Joern, 2015), possibly as a result of modification to the functional or phylogenetic composition of assemblages (Tylianakis & Morris, 2017). Methodological challenges of acquiring network data sets that are spatially distributed along large-scale environmental gradients have limited the study of the spatiotemporal variation in species interactions and their underlying drivers (Pellissier et al., 2018). In this regard, the development of DNA metabarcoding techniques and their application in network ecology has opened new perspectives for addressing the biogeography of species interactions (Roslin & Majaneva, 2016).

The structure of species interaction networks is expected to be driven by species attributes that can modulate the strength and identity of interactions between species (Dormann et al., 2017; González-Varo & Traveset, 2016). Attributes include functional traits (Laigle et al., 2018), phylogeny (Rohr & Bascompte, 2014) and demographic properties (Vázquez et al., 2007), which can favour or hinder species interactions. For example, plant chemical or physical defences can impede insect herbivores’ ability to feed on plants (Monteiro & Faria, 2018), or, in plant–pollinator networks, morphological trait-matching between floral traits and pollinator feeding appendages determines pollination success (Garibaldi et al., 2015). In addition to measuring species functional traits, the phylogenetic position of the interacting species may also explain their degree of interaction strength, as the phylogenetic relationships among taxa can be seen as an axis of integration of the overall species phenotypes (Brousseau et al., 2018; Rohr & Bascompte, 2014). Moreover, some demographic parameters can also explain the degree of species interaction strength; for example, interactions are more probable between abundant species than between rare ones (Canard et al., 2014; Fagundes et al., 2016; Krishna et al., 2008; Vázquez et al., 2007). Therefore, trait values, phylogenetic similarities and species abundances could all shape the structure of ecological networks, but with different influences and thus with variable explanatory power (Tylianakis & Morris, 2017).

The structure of plant–herbivore networks may vary along environmental gradients as a result of turnover in species composition (Pellissier et al., 2018), turnover in species traits (Descombes et al., 2017; Lamanna et al., 2014) and turnover in interaction (Tylianakis & Morris, 2017). Along environmental clines, such as elevation gradients, shifts in abiotic conditions are expected to alter species traits and species compositions (Hodkinson, 2005; Kergunteuil et al., 2018; Rahbek, 1995). Modifications of abiotic conditions are also expected to alter the structuring mechanisms that control the realization, the intensity and the persistence of these interactions (Morris et al., 2015; Ramos-Jiliberto et al., 2010). Moreover, the strength of the mechanisms determining species interactions may vary along climatic gradients, due to a shift in traits that respond to climatic variations (Tylianakis & Morris, 2017), but which also determine the interactions between species (Pellissier et al., 2018). For example, high-elevation harsh climatic conditions may favour the evolution of tough leaves, which in turn may protect plants against herbivory. Further, changes in species abundance and in functional or taxonomic composition along environmental gradients could be associated with shifts in the ecological rules underlying the structure of ecological networks (Hoiss et al., 2012; Mc Cain & Grynnes, 2010). A hypothesis-based approach could help determine whether ecological rules determine species interactions consistently along environmental gradients (Pellissier et al., 2018).

Here, we investigated the role of species traits, phylogenetic history, and species abundance in determining the interactions in plant–orthoptera bipartite networks along elevation gradients. We reconstructed 48 plant–herbivore networks in semi-natural grasslands located along six elevation gradients in the Central European Alps in Switzerland (Figure S1) using a DNA metabarcoding method applied to insect faeces (Pitteloud et al., 2021). We designed hypotheses-based networks driven by: (i) plant abundance, where herbivores feed on the plant species present in a manner proportional to the plant species’ relative cover or biomass (Bernays & Chapman, 1970; Cates, 1980; Descombes et al., 2020); (ii) the phylogenetic position of the plant taxa, where orthoptera are expected to feed on plants based on their taxonomic identity (Baur et al., 2006; Joern, 1979); (iii) mechanical trait matching, where we considered the positive association between mandibular strength and leaf toughness (Ibanez et al., 2013); and (iv) plant traits that reflect nutritional qualities or chemical defences, which should influence herbivore preference (Bernays et al., 1994; Joern & Behmer, 1998). We applied the above-mentioned hypotheses to quantify the relative contribution of these rules in explaining species interactions and to test their variation along the temperature gradient associated with elevation.

2 | MATERIALS AND METHODS

2.1 | Study design, vegetation surveys and temperature data collection

We selected six elevation gradients that are representative of the diversity of macroclimatic conditions and bedrock types in the Central Alps (Figure S1). Each transect consisted of eight study sites ranging from low to high elevation (minimum 578 m; maximum 2417 m), with individual sites separated by an average elevation distance of 240 m. Low- to medium-elevation sites corresponded to pastures and dry meadows (mostly Mesobromion or Stipo-poion grasslands, Delarze et al., 2015) with low impact from agricultural practices, while high-elevation sites were typical alpine meadows with no mowing and occasional grazing. We conducted insect and vegetation inventories during summer 2016 and 2017, gradually surveying from low to high elevation following the phenological peak of each community throughout the season, within a plot of 10 × 10 m that covered the plant–insect interactions found at each site. We identified plant species based on Swiss Floras (Eggenberg & Möhl, 2013; Lauber et al., 2012) and estimated vegetation cover using a nine-level scale.
(<0.25%, 0.25%–0.5%, 0.5%–1%, 1%–5%, 5%–15%, 15%–25%, 25%–50%, 50%–75% and >75%). We first performed the vegetation surveys in a circular subplot of 9 m² located within each 100 m² plot, where the vegetation was the most representative of the entire plant community according to a preliminary survey of the plot. We then extended the surveys to the 100 m² plots, where we searched for less frequent species and refined abundance estimations. We used the median values of the vegetation cover categories in downstream statistical analyses (i.e., 0.125, 0.375, 0.75, 3, 9, 37.5, 62.5, 87.5%). Vegetation surveys resulted in the identification of 492 plant species, representing 264 genera and 62 families. To represent abiotic variation along the elevation gradient, we used data loggers (DS1921G-F5 HomeChip) to record temperature along each transect at the highest, lowest- and two middle-elevation sites. We protected the temperature loggers with silicone capsules, wrapped them in parafilm, and buried them at 4 cm soil depth at the centre of the study plots. Loggers recorded data every 240 min at a resolution of 0.5°C for one year. We inferred temperatures for unsampled sites with separate linear regression models applied to the individual transects, using the relationship between the elevation and the summer mean temperature from 1 May 2018 to 29 September 2018 (Figure S2).

2.2 Reconstruction of trophic networks using DNA metabarcoding

We performed the reconstruction of 48 plant–orthoptera bipartite networks through DNA metabarcoding applied to 403 insect faeces samples. We conducted field surveys within the 100 m² plots during weather conditions maximizing insect activity. We identified orthoptera species (Caelifera and Ensifera) through visual identification (Baur et al., 2006) and sampled faeces from 10 individuals per species on average at each site, resulting in a mean number of 84 individuals collected per sampling location. We placed insects in tubes and then released them again after 2 h of faecal excretion. We kept the collected faeces at 4°C for a maximum of 8 h before storage at ~20°C. After this phase of sampling, we performed DNA extraction using the FastDNA SPIN Kit for Soil (MP Biomedicals). The DNA metabarcoding procedure consisted of two consecutive PCR steps in which we individually tagged samples by double-indexing, that is, the amplicon PCR and the indexing PCR. We chose the ITS2 plant genetic barcode, based on recent findings that this marker performs best for identifying plant taxa to the species level (Moorhouse-Gann et al., 2018). Following library preparation, we sequenced samples on the MiSeq v3 2X300 Illumina platform (Illumina Inc.). The DNA extraction, library preparation procedures and processing of sequenced libraries are described in further detail in the Methods S1. We generated an OTU table of plant sequences by completing the following key steps: read trimming (https://github.com/lh3/seqtk), paired-end merging (Magoc & Salzberg, 2011), primer removal (Martin, 2011), quality filtering (Schmieder & Edwards, 2011), and size selection and ZOTU calling (Edgar, 2016b). We performed the taxonomic assignment of plant sequences using the SINTAX classifier (Edgar, 2016a) against a DNA barcode reference database. We established the reference database by fetching sequences from Genbank (Clark et al., 2016) and producing custom sequences of ITS2. The DNA barcoding reference database that we used spanned the plant species richness observed in the field for 95.2% of the families, 92.2% of the genera and 88.5% of the species, while 50% of the missing species had their genus represented in the database. Based on assignment probabilities provided for each taxonomic level, we retained affiliations above a threshold of 0.95. From the OTU table (1774 plant entries), we removed the taxa identified above the family level (176) and those that were not monophyletic (103). We summed the count of the taxa sharing their affiliation and filtered the taxa count by site-specific species composition, as low count may refer to both sequencing artefacts and low plant consumption. The streaming of the OTU table resulted in 459 unique plant taxa, to which we added 138 taxa that were never consumed by the insects. The list of plant taxa included 495 species, 99 genera and six families that could not be further identified in the field. To account for differences in sequencing depth, we computed the sample relative read abundance (RRA) used in downstream analyses as the quantitative interaction in order to reflect relative dietary preferences (Deagle et al., 2019; Roslin et al., 2019). The methodological procedures used for the reconstruction of the DNA barcode reference database, taxonomic assignment and OTU table filtering are detailed in the Methods S1. In total, we recorded 10,615 realized interactions out of 28,064 possible links between 45 species of orthoptera (29 Caelifera, 16 Ensifera) and 597 plant taxa. We verified the completeness of interaction sampling across the networks using accumulation curves drawn using the vegan package (Oksanen et al., 2020) in R version 3.5.0 (R Core Team, 2019; Figure S3).

2.3 Collection of plant and insect functional traits

For 93% of the orthoptera species identified in the field, we measured the orthopteran incisive strength of six individuals per species collected across their respective elevation and geographical ranges, following the procedure described in Ibanez et al. (2013). For those individuals, we measured incisive strength from images of mouthpieces captured in duplicate with a high-resolution digital microscope (Leica DVM6; Leica Microsystems) averaged across the full range of each species, resulting in one mean value per species. We collected plant leaf functional traits that respond to abiotic variations but are also related to nutritional qualities and resource acquisition, that is, nitrogen content and specific leaf area (SLA; Pérez-Harguindeguy et al., 2013), and to chemical resistance to herbivory, that is, plant metabolomic composition. To represent leaf mechanical resistance, we measured the force required to pierce the lamina of a leaf (i.e., punch strength), which is thought to represent leaf mechanical properties relevant for herbivory (Sanson et al., 2001).
For all traits measured, we collected fully expanded and healthy leaves in triplicate per collecting site for a minimum of six replicate individuals and a maximum of 12 replicates across the species’ full elevation range when the species occurred at more than one site, and a minimum of three replicate individuals when the species occurred only at one site. To measure SLA, we calculated the area of a fresh leaf divided by its dry weight, following standard procedures (Pérez-Harguindeguy et al., 2013). To measure punch strength, we used a digital force gauge (Imada Co., Ltd.) equipped with a measuring tip that pierced the leaf lamina on a circular surface of 2 mm diameter in a position away from major leaf veins. From the measured values, we calculated the punch strength in MN/m². For a few grass species that had a leaf width smaller than the diameter of the measuring tip, we measured the leave width using a digital caliper gauge (0.01 mm precision) to estimate the tip contact area and calculated the punch strength over this area following the method described in Descombes et al. (2020). We used the averaged value of the traits for each species in all analyses. Plant nitrogen content and metabolomic composition were measured on plant material desiccated at 40°C for one week and grounded to powder. We quantified the nitrogen content by dry combustion (CN elemental analyser, NC-2500 from CE Instruments). For untargeted metabolomics analyses, we pooled 20 mg of ground leaf material from each species across replicates, then extracted the samples with 0.5 ml of extraction solvent (MeOH: MilliQ water: formic acid; 80:19.5:0.5). After centrifugation, we placed the supernatant in an HPLC vial and injected 2.5 μl of the pure extract into an Acquity UPLC C18 column (50 × 2.1 mm, 1.7 μm). We then completed analysis via ultra-high-pressure liquid chromatography–quadrupole time-of-flight mass spectrometry (UHPLC-QTOFMS) using an Acquity UPLC coupled to a Synapt G2 MS. We used a binary solvent system consisting of H₂O and acetonitrile, both supplemented with 0.05% formic acid. The chromatographic separation was carried out at a flow rate of 0.6 ml/min at 40°C for 6.0 min using a linear gradient of 2%–100% acetonitrile. We conducted MS detection in positive electrospray ionization over a mass range of 85–1200 Da. We cleaned the MS source before each of the five batches running over 5 days. We acquired data in the data-independent acquisition (DIA) mode, in which all precursor ions from the full mass range are fragmented to yield MS/MS spectra. For peak detection and assignment of the parent mass to each of the fragmented spectra of the DIA data, we used MS-DIAL (Tsugawa et al., 2015). We implemented the output of MS-DIAL in the Global Natural Products Social Molecular Networking (GNPS) system to cluster the MS/MS spectra into compound families of molecules based on their cosine similarity and molecular networking (Wang et al., 2016). We complemented our data set of SLA, N and C traits with published data (Descombes et al., 2017; Descombes et al., 2020; Kattge et al., 2011; Körner et al., 2016). Our plant trait measurements of SLA, nitrogen content, punch strength and plant metabolomic composition covered 79, 83, 76 and 77%, respectively, of the plant species found in the field.

FIGURE 1  (a) Representation of the metaweb for empirical interactions between orthopteran clades and plant families. The width of the connecting lines corresponds to the interaction intensity. (b) A reconstructed network based on plant abundance given as cover category, where observed interactions for one location are plotted in white and the size of the dots is proportional to the relative read abundance (RRA). The RRA accounts for differences in sequencing depth and was analysed as the quantitative interaction to reflect relative dietary preferences. The gradient from blue to red indicates the intensity of the interaction, where red indicates the strongest and blue the weakest interactions. Interactions with a high RRA mainly occur with highly abundant plant species.
2.4 | Definition of hypotheses

We defined five ecological rules that are expected to structure ecological networks: (i) plant relative abundance: we expected orthoptera to preferentially feed on plant species with a higher surface cover (Figure 1); (ii) phylogenetic position of plant taxa: we expected orthoptera to feed preferentially on Poaceae and Cyperaceae, which have lower levels of secondary metabolites; (iii) mechanical trait matching: we expected a stronger interaction intensity for corresponding values of mandibular strength and leaf toughness; (iv) leaf nutritional qualities and (v) chemical defences: we expected orthoptera to preferentially feed on species with a higher leaf nitrogen content and a smaller amount of chemical compounds that decrease palatability. Ecological rules were directly based on the ranking of plant, SLA, leaf nitrogen content and metabolomic composition (phenols and tannins). For each hypothesis, we constructed a metaweb (i.e., all possible feeding links between co-occurring species determined by hypothetical ecological rules) of expected interactions between all species pairs. From these metawebs, we extracted the expected interactions between species present at each sampling site and constructed hypothesis-based local webs. For the phylogenetic rule, we hypothesized that orthoptera interact at lower intensity with plants that are phylogenetically more distant from the grass family. We used a well-resolved phylogeny for European flora (Durka & Michalski, 2012) that we pruned according to the species sampled and made ultrametric (R package ape; Paradis & Schliep, 2019). We collapsed the species tips corresponding to grasses and measured the phylogenetic distance of each plant to the collapsed tip (R package stats; R Core Team, 2019). For the mechanical matching hypothesis, we expected interaction intensity to be highest when mandibular strength corresponded to punch strength, with intensity decreasing when below this correspondence and no interaction possible above this value. We calculated the matching rule by scaling the mandibular strength and plant punch values to be between zero and one, subtracting the mandibular strength from the plant punch value, and adding one to all values. Finally, we set mandibular strengths smaller than the punch force to zero (i.e., the upper half of the matrix). We also added a random rule of interaction that corresponded to a randomization of the empirical interaction values applied to each network individually. Overall, correlation analyses demonstrated that ecological rules were weakly correlated with each other, indicating marginal interdependency of the hypotheses outlined above (Figure S4).

2.5 | Comparison of hypotheses to empirical data

We used both presence–absence and quantitative interaction matrices to assess the explanatory power of each matrix defined by the ecological rules, and to quantify their variation in explanatory power along the elevation gradient and across transects. For quantitative data, we computed the Kendall rank correlation coefficient tau (R package stats) between the observed interaction intensity for each network and each ecological rule. For presence–absence data, we computed the True Skills Statistic coefficient (TSS) from the error matrices obtained by comparing empirical and hypothetical networks (R package caret; Kuhn et al., 2018). To transform quantitative rules into binary ones, we assigned presences of interaction to the highest value of the rule while limiting them to the same number of presences as found in each local empirical network. Since several ecological rules were categorical, with potentially more presences than in the local empirical network, we drew presences randomly 1000 times. We then reconstructed the interaction networks and computed the TSS coefficients. Next, we compared the explanatory power of the ecological rules by testing the difference between tau and TSS coefficients against the randomly generated rule values using linear mixed regression models by including transect identity as a random factor using the lme4 and lmerTest R packages (Bates et al., 2008; Kuznetsova et al., 2017). We quantified the relationship between temperature and the explanatory power of each ecological rule in each network using linear mixed effects regression models with transect identity included as a random factor (Bates et al., 2008; Kuznetsova et al., 2017). Finally, we tested whether the explanatory power of the ecological rules varied across the different transects investigated (R package stats).

3 | RESULTS

3.1 | Overall determinants of interaction networks

We built hypotheses in the form of quantitative metawebs, and evaluated the explanatory power of ecological rules against local empirical interactions using binary and quantitative interaction values. The explanatory power of most ecological rules differed significantly from random expectations and the level of explanatory power varied among the rules (Figures 2a,c; Table 1). We found the highest match between empirical interaction strength and the networks derived from the hypothesis of phylogenetic rule (mean tau = 0.315, p ≤ .001, t-value = 22.67; SD = 0.0139; Figure 2a, Table 1), which provides further evidence that species abundance is a major factor structuring interaction networks. The plant abundance rule showed the second highest match, with dominant plant species being preferably selected by herbivores (mean tau = 0.181, p ≤ .001, t-value = 13.02; SD = 0.0139; Figure 2a, Table 1), which provides further evidence that species abundance is a major factor structuring interaction networks. When considering presence–absence data, the phylogenetic distance and the plant abundance rule had similar explanatory power (phylogenetic rule: mean TSS = 0.157, p ≤ .001, t-value = 10.58; SD = 0.0149; plant abundance rule: mean TSS tau = 0.150, p ≤ .001, t-value = 10.11; SD = 0.0149; Figure 2c, Table 1). Rules based on plant traits further indicated that interaction strength and presence are determined by SLA and leaf nitrogen content (SLA rule tau coefficient: mean tau = 0.074, p ≤ .001, t-value = 5.33; SD = 0.0139, TSS coefficient: mean TSS = 0.115,
We evaluated whether the explanatory power of each hypothesis varied along the elevation gradient and showed a change in the signal for several hypotheses (Figure 2, Table 2). We related the explanatory power of the ecological rules in each network to temperature and tested the significance of the relationship using linear mixed effects regression models. The hypothesis based on SLA and leaf nitrogen content decreased (SLA tau: slope = 0.006, \( p \leq 0.01 \), t-value = 3.10, TSS: slope = 0.009, \( p \leq 0.01 \), t-value = 3.27; leaf nitrogen content tau: slope = 0.007, \( p \leq 0.01 \), t-value = 2.92, TSS: ...
slope = 0.009; \( p \leq .01 \), \( t \)-value = 2.73; Figures 2d,e, Table 2) with decreasing temperature, indicating that plant–orthoptera interactions are more weakly determined by SLA and leaf nitrogen content in cold environments. In contrast, the hypotheses based on phenols and tannins increased (phenols tau: slope = −0.009, \( p \leq .01 \), \( t \)-value = −3.9, TSS: slope = −0.007, \( p \leq .05 \), \( t \)-value = −2.49; tannins tau: slope = −0.005, \( p \leq .05 \), \( t \)-value = −2.00; Figures 2d,e; Table 2) with decreasing temperature, consistent with a greater importance of these traits in determining plant–orthoptera interactions in cold environments (Table 2). The analyses of additional metabolites are provided in (Figure S5, Table S2).

### 3.3 Variation among transects

We compared the explanatory power of the ecological rules across the six transects in the Swiss Central Alps to evaluate the differences among regions associated with macroclimate or bedrock type, which influence community composition of plants (e.g., Michalet et al., 2002) and possibly the structure of interaction networks (Poisot et al., 2020). We found that the ecological rules of plant abundance and tannins had a significantly different explanatory power in the different transects (plant abundance tau coefficient: \( F = 2.71, p \leq .05, MS = 0.02 \), TSS: \( F = 3.65, p \leq .05, MS = 0.02 \); tannins tau coefficient: \( F = 2.97, p \leq .05, MS = 0.02 \), TSS: \( F = 2.72, p \leq .05 \).
In our analyses, the functional and phylogenetic position, abundance and functional traits all partly contribute to explaining species interactions in plant–orthoptera networks (Bersier & Kehrli, 2008; Dormann et al., 2017; Laigle et al., 2018; Vázquez et al., 2007). In particular, we found that the phylogeny and plant abundance rules had the highest explanatory power in the structuring of plant–orthoptera networks, while trait-based rules related to mechanical constraints and nutritive requirements showed weaker explanatory power. Ecological rules determining interaction networks varied along an elevation gradient, suggesting that environmental clines can influence how species interact with each other within food webs. Our results, in line with previous studies (Albrecht et al., 2018; Pellissier et al., 2018), suggest that interactions should be studied in the context of their surrounding environmental conditions.

Functional traits drive trophic interactions between species and influence how they assemble into interaction networks (Gravel et al., 2013; Ibanez et al., 2013). In our analyses, the functional traits we measured, which we expected to be related to plant–herbivore interactions and particularly plant–orthoptera networks (Ibanez et al., 2013), were less important than phylogeny in predicting network structure. The strong phylogenetic signal found in our plant–herbivore interaction system agrees with the findings of Brousseau et al. (2018) and those of Rohr and Bascompte (2014), where phylogenetic information was shown to accurately predict interaction networks in antagonistic systems. In our study plant abundance was the second best predictor for describing plant–herbivore interaction strength across all networks and had a similarly high explanatory power as the phylogenetic rule for the presence–absence of interactions. Interaction intensity should increase with increasing probability of encounter, which should relate to plant species cover or herbivore abundance (Tylianakis & Morris, 2017). Species abundance has similarly been found to be a major determinant of interaction in networks in other systems (Canard et al., 2014; Sam et al., 2017; Vázquez et al., 2007). Further, the signal of other facets of ecological interactions, notably functional traits (Laigle et al., 2018), has been observed to be more detectable after removing the effect of species abundance (Dormann et al., 2017). Accordingly, a dominant effect of phylogeny and species abundance values might mask a direct link between plant and herbivore functional traits during network analyses. Nonetheless, we found that some functional traits partly explained interactions in the studied networks, especially SLA and nitrogen. Because nitrogen is a major limiting resource for insects, plants with higher nitrogen concentration are usually preferred (Joern & Behmer, 1997; Mattson, 1980). Along the same lines, high SLA values are generally associated with leaves that are soft, are more palatable, and have a high nitrogen content (Wright et al., 2004), and thus are preferred by herbivores. Finally, we found that chemical traits, such as the concentration of phenol-based compounds, had explanatory power. While these classes of molecules have classically been associated with lower leaf palatability (Appel, 1993; Rhodeas & Cates, 1976), their role in defence against herbivores has been questioned at several levels (Barbehenn & Peter Constabel, 2011;.

**TABLE 3** Analysis of variance results for the linear models testing the relationships between the transect identity and the tau and TSS coefficients. For each ecological rule tested individually, the F-ratio (F), the p-value (p), the mean squares (MS) and the degrees of freedom (df) are given. The hypothesis-based rule of plant abundance and tannins explained the plant–orthoptera interaction with significant variation among transects.

<table>
<thead>
<tr>
<th>Interaction rule</th>
<th>Test coefficient</th>
<th>F</th>
<th>p-value</th>
<th>MS</th>
<th>Df</th>
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<td>.88</td>
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MS = 0.02; Table 3. In particular, the set of networks in the southern slope of the Alps, located on a siliceous rock type (Figure S1), showed a greater influence of plant abundance but a weaker influence of tannins (Figure S6).

## 4 | DISCUSSION

We used DNA analyses of orthoptera faeces to identify ecological rules underlying interaction networks, and to compare the relative importance of these rules along environmental gradients. Our results indicate that phylogenetic position, abundance and functional traits all partly contribute to explaining species interactions in plant–orthoptera networks (Bersier & Kehrli, 2008; Dormann et al., 2017; Laigle et al., 2018; Vázquez et al., 2007). In particular, we found that the phylogeny and plant abundance rules had the highest explanatory power in the structuring of plant–orthoptera networks, while trait-based rules related to mechanical constraints and nutritive requirements showed weaker explanatory power. Ecological rules determining interaction networks varied along an elevation gradient, suggesting that environmental clines can influence how species interact with each other within food webs. Our results, in line with previous studies (Albrecht et al., 2018; Pellissier et al., 2018), suggest that interactions should be studied in the context of their surrounding environmental conditions.
Salminen & Karonen, 2011), and especially for generalist herbivores such as orthoptera. Nonetheless, regardless of the exact role of each leaf trait in driving plant-herbivore interactions, our results illustrate that it is possible to disentangle the role of functional traits from those of abundance and phylogenetic position in explaining the structure of interaction networks in nature.

Structural properties of species interaction networks can change along environmental gradients (Maunsell et al., 2015; Miller-Struttmann & Galen, 2014; Morris et al., 2015; Ramos-Jiliberto et al., 2010), but previous analyses generally did not consider the potential variation of ecological determinants with changes in abiotic conditions (but see Sonne et al., 2020). Measures of network variation along environmental gradients, for example, show that the specialization of plant–pollinator networks decreases with increasing latitude (Schleuning et al., 2012) or increasing elevation (Miller-Struttmann & Galen, 2014; Pellissier et al., 2012; Rensnider et al., 2019), a pattern that was directly linked to animal feeding traits or floral morphology. In parallel, studies have been conducted to investigate the mechanisms ruling species interactions, including phylogeny, abundance and functional traits (Dormann et al., 2017), but not the influence of abiotic parameters on network structuration. Further, we observed that the rules underlying plant–herbivore interaction networks are not systematically conserved along temperature gradients following elevation clines. We observed a decrease in the importance of SLA and nitrogen in structuring networks with increasing elevation, in parallel to the decrease in the community mean SLA and nitrogen values at higher elevation reported in the same study area (Pitteloud et al., 2020). Plants with a higher nutrient content are expected to be preferentially consumed at high elevations, where the season for completing the life cycle is shorter (Hodkinson, 2005). However, a decline in the community-level average nitrogen content (Pitteloud et al., 2020) might decrease the benefit of diet selectivity, instead favouring diet mixing (Franzke et al., 2010; Joern & Behmer, 1997; Unsicker et al., 2008) and promoting a higher level of generalism at high elevations (Pitteloud et al., 2021). Our findings are also aligned with those from experimental studies in which the consumption of high-nitrogen plants increased in warm environments, indicating a positive relationship between temperature and nitrogen limitation (Lemoine et al., 2013). As a consequence, our results suggest that plant–herbivore interaction networks might be less determined by species differences in leaf quality in cooler environments.

Boardscale spatial variation in species interaction networks (Dalsgaard et al., 2021; McFadden et al., 2022) indicate that rules governing interactions are also organized biogeographically (Gravel et al., 2019). While the scale of our study and the number of transects was limited to some extent (i.e., six transects in the Central European Alps versus large-scale latitudinal gradients), our analyses showed significant differences in the importance of the ecological determinants between transects from distinct biogeographic regions, such as between the calcareous Prealps and the granitic southern slope of the Alps. Bedrock type may influence the magnitude of the effect of interaction rules on ecological networks via changes in leaf properties (Michalet et al., 2002). Moreover, we found that mechanical trait matching had the lowest explanatory power on the transect located in the most arid climate (Figure S1; Salgesch area). Dry conditions are typically associated with plant species with high leaf toughness and dry matter content (low SLA), which might condition the association between plants and herbivores (Bernays & Chapman, 1994). By using empirical data, rather than reconstructed food webs with data sets retrieved from the literature (Albouy et al., 2019; Hattab et al., 2016), together with the assessment of multiple ecological rules, our study highlights the contribution of multiple evolutionary and ecological forces underlying species interaction network changes along environmental gradients. However, we suggest wider spatial replicates should be considered in future work to more comprehensively study the biogeography of interaction networks.

The identification of the mechanisms that determine species interactions serves two important purposes. First, it enables the quantification of structural properties of interaction networks, which in turn can provide information on their changes and resilience when faced with environmental disturbances, such as species extinction (Dunne et al., 2002). Second, knowledge of ecological rules makes it possible to formulate predictions about the properties of current and future interaction networks between species that have not been observed to interact (Albouy et al., 2019; Hattab et al., 2016). Building on previous efforts (e.g., Arstingstall et al., 2021; Kitson et al., 2019), our application of DNA metabarcoding to quantify network structure provided novel insight into the mechanisms determining interactions. While we found that sampling effort tended to reach saturation in the detected interaction, increasing the sampling effort per network, but also the number of networks sampled, will be key to providing more detailed insights into the determinants of species interactions at broader spatial and taxonomic scales (Cuff et al., 2021). Given the existing data set, our results indicate that variability exists in the explanatory power of network ecological rules along spatial and environmental gradients. Our effort contributes to the development of the emerging research field of “spatial network ecology”, which will ultimately help us to understand the nature of the processes shaping the structure of biological communities in space and time.

ACKNOWLEDGEMENTS
The data presented in this study has been generated and analysed in collaboration with the Genetic Diversity Centre (GDC) at ETH Zurich. We are grateful to Christian Roesti, Maude Poirier, Giulia Donati, Fabian Fopp, Joan Casanelles Abella, Marie-Angèle Pitteloud, Nico Baetz, Nadine Sandau, Gabriel Dettling, Daniele Pezzotta, Marco Lanfranchi, Janka Bollenbach and Angéline Bedolla for help with field data collection and Kevin Cazelles and Felix Neff for insightful discussions. We thank James Kitson and Rafael Pinheiro for valuable comments on a previous version of the manuscript. This study was funded by the National Swiss Foundation through research grants 179481 to SR and 162604 to LP. Open access funding provided by Eidgenössische Technische Hochschule Zurich.
AUTHOR CONTRIBUTION
L.P. and C.P. jointly designed this study, C.P., P.D., L.P. and E.D. collected the data. C.P., S.R., C.A. and L.P. analysed and interpreted the data. All the authors C.P., E.D., C.A., P.D., S.R. and L.P. contributed to the writing of the manuscript.

DATA AVAILABILITY STATEMENT
The dataset of plant-herbivore trophic interactions analysed in this article, as well as the plant functional traits are published on the data portal HYPERLINK "https://www.envi.dat.ch" https://www.envi.dat.ch under the following link https://doi.org/10.16904/envi.dat.178. The raw sequencing data of the DNA metabarcoding libraries used to reconstruct the networks have been deposited into NCBI bioprojects database (accession no. PRJNA680586, https://www.ncbi.nlm.nih.gov/bioproject/).

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REFERENCES


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