Ecological Entomology

Ecological Entomology (2021), DOI: 10.1111/een.13038

Plant-caterpillar food web: Integrating leaf stoichiometry and phylogeny

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Abstract. 1. Trophic interactions and plant-herbivore network structure may be regulated by ecological and evolutionary factors. The allocation of resources to physical and chemical traits may influence plant nutritional values and defence against insect herbivores. However, the elemental consistency of plant leaves is rarely considered a functional trait, despite leaf chemical traits being critical for understanding trophic interactions and network modularity.

2. We constructed a high-resolution network of a plant-caterpillar food web in a subtropical forest in southern China using DNA barcoding. We analysed whether abundance, phylogeny, and functional traits (including leaf chemical elements) significantly differed among modules and conducted linear regressions to explore how these traits could explain the intensity and likelihood of trophic interactions between plants and caterpillars.

3. Caterpillar feeding intensity increased with increasing specific leaf area and leaf nitrogen to phosphorus (N:P) ratio. The intensity and likelihood of feeding decreased with increasing calcium and magnesium levels. Leaf dry matter content and leaf concentrations of potassium, magnesium, copper, and manganese varied among modules. Caterpillar feeding likelihood and intensity were constrained by plant and caterpillar phylogenies, and module phylogenetic clustering was apparent. Species abundance of plants and caterpillars constrained the feeding intensity but not the likelihood of feeding and network module composition.

4. We highlighted the combined roles of functional traits and phylogeny in shaping trophic interactions and modularity in plant-insect herbivore networks and illustrated the importance of leaf stoichiometry in establishing dietary associations.

Key words. Leaf chemical elements, Leaf trait variation, Network modularity, Phylogeny, Plant vulnerability, Trophic interactions.

Introduction

Ecological communities are organised into complex food webs with trophic interactions. Interaction networks often show non-random topological structures and properties, such as modularity, where subsets of species interact more strongly among each other than with the rest of the community (Allesina & Pascual, 2008; Thébault & Fontaine, 2010; Stouffer & Bascompte, 2011). Modular structure is more prominent in antagonistic networks (for example, plant-insect herbivore networks) than in mutualistic networks (for example, seed dispersal networks) due to a higher specialisation that has evolved in antagonistic interactors (Maliet *et al.*, 2020). Modular organisation may be caused by various processes of ecology (i.e.,

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trait-matching) and evolution (i.e., phylogenetic relatedness); however, which process or processes are actually responsible for it remains obscure (Dormann *et al.*, 2017).

Biological processes that structure plant-insect herbivore networks can be grouped into three categories. First, species traits (Ibanez *et al.*, 2013): morphological (related to growth), physical, and chemical plant defence traits will impact the diet preference of insect herbivores (Meskens *et al.*, 2011; Ibanez *et al.*, 2013; Robinson *et al.*, 2015). For instance, the trait-matching of biomechanical traits in plant and Orthoptera species has been hypothesised to control the amount of plant biomass eaten by Orthoptera species (Ibanez *et al.*, 2013). Such diet preferences of insects for a particular plant functional trait will lead to the division of network modules. For example, several plant phenotypes correlated with growth and biomass accumulation (i.e., height and diameter) contribute significantly to module membership (Robinson *et al.*, 2015). Second, phylogenetic history (Cagnolo *et al.*, 2011; Ibanez *et al.*, 2016): phylogeny not only determines host range (Cagnolo *et al.*, 2011; Ibanez *et al.*, 2016) but also regulates the modular structure of plant-insect herbivore networks (Meskens *et al.*, 2011). For instance, two phylogenetic clades of hispine beetles (Cassidinae) specifically prefer to associate with shade-tolerant plant species, which has led to the emergence of two well-defined modules (Meskens *et al.*, 2011). The third category is species abundance, which is thought to control the probability of interactions by mediating encounter rates among individuals of plant and insect herbivores (Canard *et al.*, 2012). The vulnerability of plants to insect herbivores is positively linked with plant abundance, which possibly controls the degree of modularity by impacting resource use (Robinson *et al.*, 2015). The predictability of species abundance to interaction frequency (for example, visitation frequency of pollinators on plants) and some network characteristics (for example, nestedness) has been confirmed in plant-insect pollinator networks (Vázquez *et al.*, 2009; Olito & Fox, 2015; Hu *et al.*, 2019); however, the relative importance of functional traits, phylogeny, and abundance in regulating interaction intensity and modular structures is rarely explored in plant-insect herbivore networks (Rafferty & Ives, 2013; Robinson *et al.*, 2015). In addition, the fundamental importance of leaf chemical elements as a crucial plant functional trait in establishing diet associations among plant-herbivore network structures is often overlooked. To fully understand how ecological networks are organised and predict network structure and community dynamics, it is important to investigate how these traits regulate foraging strategies of insect herbivores.

Chemical elements are the most fundamental building blocks that compose the bodies of living organisms in various proportions (Filipiak & Weiner, 2017a). Animal growth can be impaired if there is an imbalance in stoichiometric ratios in their food (Filipiak & Weiner, 2017a). For instance, phosphorus (P) and nitrogen (N) are the essential building blocks for RNA and protein, respectively. An imbalance in these elements constrains the rate of protein synthesis and, thus, growth rate. A severe scarcity of the basic chemical elements has been reported to hamper or prevent the growth and development of herbivores (Filipiak & Weiner, 2017b; Filipiak, 2018). Moreover, a carbon (C):N:P stoichiometric imbalance in plants can affect plant resistance by mediating the synthesis of secondary chemicals (Abdala-Roberts *et al.*, 2018; Shan *et al.*, 2018) and, thus, insect herbivore foraging behaviour. In order to achieve a balanced diet, insect herbivores could vary which plants they eat or modulate their feeding intensity for food resources of poor nutritional quality, consequently changing plant-herbivore network patterns (Elser *et al.*, 2012; Filipiak & Weiner, 2017a). The limitations imposed by plant chemical elements are rarely incorporated into the research of food web topological structures (Elser *et al.*, 2000; Pokarzhevskii *et al.*, 2003; Moe *et al.*, 2005). Phylogeny, as a proxy for conservative functional traits (i.e., conservative chemical elements), has been used to understand network modular structures (Meskens *et al.*, 2011); however, this method only gives limited information in revealing the rules of network organisation. Therefore, incomplete mechanistic explanations of diet interactions and the structure of plant-insect herbivore networks are formed when using only plant morphology, physical/chemical defence traits, and phylogeny.

Lepidopteran larvae, which exhibit a relatively narrow diet breadth (high host preference), are often used as model organisms to explore the patterns of diet use in phytophagous insects and their corresponding ecological and evolutionary processes (Singer & Stireman III, 2001; Dyer *et al.*, 2007). Nevertheless, existing ecological studies do not use leaf chemical elements to explain feeding likelihood, feeding intensity, and modular composition for plant-caterpillar networks. To fully illustrate what biological processes primarily underpin diet choice in caterpillars and network structure of a plant-caterpillar network, we based this study on a highly resolved whole network of plant-caterpillar interactions and two subnetworks by season (summer and autumn) that were constructed using DNA barcoding, in order to identify lepidopteran and plant species from a subtropical forest plot. We proposed the following three questions:

1. How do leaf morphological and stoichiometric traits affect the likelihood and intensity of caterpillar feeding on plants?
2. Are the likelihood and intensity of caterpillar feeding on plants constrained by phylogenetic history and species abundance?
3. How do the phylogenies and abundances of plants and caterpillars and plant functional traits impact modular structures and the assemblages of plant-herbivore networks?

Materials and methods

Sampling design and network construction

This study was conducted in a 50-ha subtropical forest plot in the Heishiding Natural Reserve Area in southern China. In the reserve, mean annual precipitation is 1750 mm, 79% of which falls from April to September, and mean annual temperature is 19.7 °C. The dry season occurs from October to March. Within the experimental plot, all tree stems with a diameter at breast height >1 cm were mapped. We chose 72 tree species, including common and rare species, out of a total of 213 woody plant species as our focal trees, which accounted for 80% of the total tree abundance in the plot. In total, 13 individuals of each of the

72 tree species were fogged. Fogging took place over 13 months during the wet seasons (April–October) of 2013–2014. Each month, we fogged one individual of each of 72 focal tree species with a swingfog SN50 (Swingtec, Germany) and 1% pyrethrum for 2.5 min. To reduce the possible effect of wind on sampling, the heights of the trees we fogged ranged from 3 to 12 m. We collected fallen insects using a shade cloth spread around the focal tree for 2 h after fogging. Caterpillars were then sorted from the insect samples and preserved in 100% ethanol in plastic bottles and stored in −40/−80 °C freezers.

Mixed plant and caterpillar genomic DNA was extracted from the caterpillar samples (Zhu *et al.*, 2019). Caterpillar DNA was amplified and sequenced using COI primers, and plant residuals in the gut of the caterpillars were amplified and sequenced using three DNA barcodes (*trnL*, *rbcLa*, and ITS2) using the Sanger sequencing method in an ABI 3730 DNA analyser (Thermo Fisher Scientific). We identified caterpillars to operational taxonomic units (OTUs) by 3% COI sequence divergence measured as Kimura 2-parameter (K2P) distance in MEGA 7 software (Kumar *et al.*, 2016). We then assigned caterpillars to taxonomic information according to the best hit taxa in the MegaBlast searches (Morgulis *et al.*, 2008) in Genbank. We identified trophic interactions between plant and caterpillar species by blasting the sequences of three DNA markers (*trnL*, *rbcLa*, and ITS2) to a local DNA database of 213 woody plant species and the NCBI database. We only used those species with best-hit scores to identify plants foraged by the lepidopteran larvae. Out of 2233 caterpillars, we identified 794 individuals to at least the genus level using the three DNA markers (for details, see Zhu *et al.*, 2019). We identified 794 interactions between 102 plant species, including trees, lianas, bryophytes, and bamboos, or species complexes (defined as a group of species identified to the same genus or family, hereafter referred to as species) and 235 caterpillar OTUs (Zhu *et al.*, 2019).

We were unable to measure leaf morphology and the concentrations of leaf chemical elements (used in the following statistical analyses) for all 102 of the plant species. We were able to include 641 interactions between 80 plant species and 194 caterpillar OTUs, after excluding 22 plant species (traits unmeasured) and their associated 41 caterpillar species (no other alternative plant resources). To generate multiple datasets of networks, we divided the whole network into two subnetworks by season. Each subnetwork was conducted repeated analyses to validate the reliability of the conclusion for entire network. The two subnetworks are as follows: summer (April to June), which included 292 interactions (58 plants, 101 caterpillars) and autumn (July to September), which included 321 interactions (64 plants, 122 caterpillars).

Determining plant-caterpillar trophic interactions from traits and phylogeny

We collected data including caterpillar traits (abundance) and plant traits (abundance, leaf morphological, and stoichiometric traits) at the species level. We calculated the abundance of Lepidoptera species as the observed number of individuals present on all fogged trees. Abundances of host-plant species were calculated for individuals with a diameter at breast height larger than 1 cm in the 50-ha stem-mapped plot. Leaf morphological traits included leaf area (LA), specific leaf area (SLA), and leaf dry matter content (LDMC). Chemical elements included concentrations of leaf C, N, P, leaf acidity (pH), concentrations of leaf potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), cooper (Cu), zinc (Zn), iron (Fe), manganese (Mn), boron (B), and aluminium (Al). Stoichiometric traits included carbon to nitrogen ratio (C:N), carbon to phosphorus ratio (C:P), and nitrogen to phosphorus ratio (N:P). All 20 plant functional traits were measured in healthy, fully developed leaves (approximately 20 leaves for each individual), sampled from 3–30 individuals for each woody species previously identified from this forest plot (He et al., 2018; Chen et al. unpublished data). Species-level mean values for all leaf traits, except for the species-level mean value for each of the three morphological leaf traits (LA, LDMC, and SLA) calculated across all leaves (dataset characterised by one trait value per leaf), were calculated across all individuals (dataset characterised by one trait value per tree).

A phylogenetic tree of the plant species (Appendix S1) was reconstructed using four DNA markers (*trnL*, *rbcLa*, *matK*, and ITS2) in RAXML version 8 (Stamatakis, 2014). We used a combination of maximum likelihood and rapid bootstrap methods to search the phylogenetic tree with the highest probability. We performed 1000 runs and the nucleotide substitution model was set as GTR + GAMMA. The phylogenetic tree for the 194 lepidopteran species (Appendix S2) was reconstructed in BEAST version 1.10.4 (Suchard *et al.*, 2018). Two species of Trichoptera (*Lepidostoma pictile* (Banks, 1899) and *Lepidostoma carrolli* (Flint, 1958)) were taken as outgroups, and their COI sequences were downloaded from the NCBI database (http://www.ncbi.nlm.nih.gov/, sequence accession ID = GU714121.1 and HM101894.1). COI sequences of all lepidopterans and the two outgroups were aligned using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/tclustalo/). The caterpillar OTUs in the families of Erebidae, Noctuidae, Geometridae, Xyloryctidae, and Limacodidae, and the superfamily Gelechioidea, were constrained to be monophyletic. We set GTR + Invariant Sites as the nucleotide substitution model. We applied one relaxed molecular clock nucleotide evolutionary model. The lengths of the MCMC chains and the number of burn-ins were 100,000,000 and 3000, respectively. We checked chain convergence using TRACER version 1.7 (Rambaut *et al.*, 2018). Finally, we obtained the maximum creditability phylogenetic tree of the lepidopteran species.

Given that the interaction matrix included many zeros, we assumed that the count number of each caterpillar species on the corresponding plant species followed a zero-inflated Poisson distribution. To detect the underlying mechanisms of plant-caterpillar interactions, we conducted three zero-inflated regression models for the entire observed networks and two subnetworks in the R package *pscl* (Zeileis *et al.*, 2008). To reduce the dimension of leaf traits (strong correlation between several traits, see Appendix S3), the first three principal components of leaf trait variation were used to explain plant-caterpillar interaction patterns. Thus, in the regression models, the number of each caterpillar species observed on the corresponding plant species

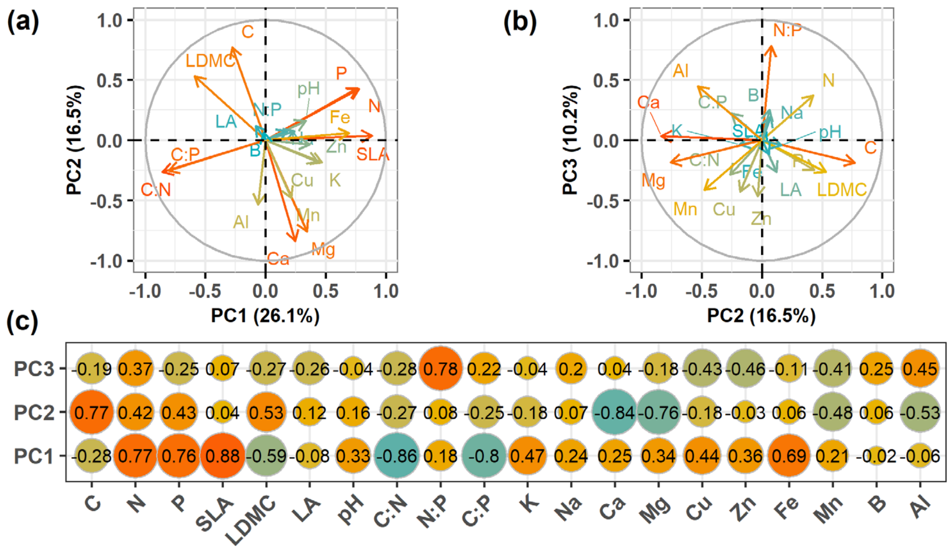

Fig. 1. Biplots and loading values of the first three principal components in the principal component analyses of 20 leaf traits. (a) and (b) The first principal component against the second principal component and the second principal component against the third principal component. (c) A correlation matrix between the values of the first three principal component loadings and plant traits. The length of the arrows on the biplot denotes the contribution of each plant variable to each of the first three principal components. The circle size and values on panel c denote the correlation coefficient of each plant variable with each of the first three principal components. [Colour figure can be viewed at wileyonlinelibrary.com]

was taken as the response variable, and the explanatory variables were abundances of caterpillars and plants, the first three principal components of leaf trait variation, and four interactive terms of the phylogenies of plants and insects along the first two axes.

The first three principal components of leaf trait variation (PC1, PC2, and PC3) were obtained by performing a principal component analysis of 20 leaf traits using the function *prcomp* in the R program (R Core Team, 2015). PC1, PC2, and PC3 together accounted for 52.8% of the leaf trait variation (Fig. 1). PC1 was mostly loaded by SLA, C:N, and C:P; PC2 by concentrations of leaf C, Ca, and Mg; and PC3 by N:P (Fig. 1). Principal coordinates analyses of the phylogenetic relationships between plants and caterpillars were conducted with the phylogenetic distance matrices of plant and caterpillar species using the *cmdscale* function in the R program (R Core Team, 2015). The four interaction terms along the first two axes of plants and insects were then included in our regression model, as they were considered to explain the likelihood of a species interacting with another species based solely on their position in phylogenetic space (Brousseau *et al.*, 2018). We built full regression models for the whole network and the two subnetworks before finally selecting three optimal zero-inflated regression models for the three networks based on the minimum Akaike information criterion (Burnham & Anderson, 2004). Zero-inflated regressions were able to fit the count component (number of caterpillar species on a particular plant species), representing caterpillar feeding intensity, and the binary component (presence/absence of a caterpillar species on a particular plant species) represented the likelihood of caterpillar feeding. By disentangling the likelihood and intensity of caterpillar feeding, this regression model, run in the R package *pscl* (Zeileis *et al.*, 2008), quantified the underlying evolutionary and ecological processes governing trophic interactions.

We measured plant vulnerability (the diversity of insect species attacking plants) using the standardised mean phylogenetic distance of herbivorous insect species on the focal plant species, calculated in the R package *picante* (Kembel *et al.*, 2010). To explore whether the plant variables regulating feeding intensity of insects on plants also affected plant vulnerability, we conducted a multivariate phylogenetic generalised least squares (PGLS) regression, where plant vulnerability was taken as a response variable, and the first three principal components of plant traits and plant species abundance were taken as explanatory variables. The impact of each of the four explanatory variables on plant vulnerability was detected by conducting a univariate PGLS regression. To minimise the influence of insufficient sampling efforts on our results, plants eaten by only one caterpillar species were not included in the regression.

Detecting the impacts of species traits and phylogeny on module composition

To understand how the plant-caterpillar food web was organised, we measured network modularity as the Newman's weighted modularity index (Newman, 2004). The degree of weighted modularity ranged from 0 to 1, with high values representing strong modularity. We searched for the best division of a network into modules in 50 independent runs of the 'Beckett' algorithm (Beckett, 2016) in the R package *bipartite*. The resulting divisions of network modules were classed as optimum if there was no further improvement after 10^6 swaps. We recorded the number of modules and the affiliation of each species to a module for runs with the highest degree of modularity (M).

To examine whether the observed interaction network was more modular than random, we compared the structural metrics

Table 1. The results of a reduced generalised linear regression of the intensity and occurrence of trophic interactions between plants and caterpillars in the entire network (a) and in two subnetworks (b and c).

	(a) Whole network		(b) Summer subnetwork		(c) Autumn subnetwork	
Models	Count	Binary	Count	Binary	Count	Binary
Intercept	(−) 3.907***	(+) 3.140***	(−) 4.413**	(+) 1.121	(−) 2.907***	(+) 3.027
cat.abund	(+) 1.028***	(+) 0.103	(+) 1.258***	(+) 0.472***	(+) 0.635***	(−) 0.009
Plant abundance	(+) 0.264***	(−) 0.089	(+) 0.224*	(−) 0.028	(+) 0.225***	(−) 0.025
PC1	(+) 0.066*	(+) 0.024				
PC2	(−) 0.145***	(−) 0.124**	(−) 0.144***	(−) 0.142*	(−) 0.123***	(−) 0.177**
PC3	(+) 0.360***	(+) 0.109*	(+) 0.544***	(+) 0.450***	(+) 0.236**	(−) 0.014
catpcoa1:plpcoa1			(−) 12.444***	(−) 9.890*		
catpcoa1:plpcoa2	(−) 5.109*	(−) 3.571	(−) 27.947**	(−) 25.007*	(−) 16.917***	(−) 10.094
catpcoa2:plpcoa2	(−) 11.707***	(−) 4.705				

The sign in parentheses indicates whether the relationship is positive or negative. The algebraic value corresponds to estimated regression coefficients 'est', and the stars indicate the 'P-value' of the corresponding test as follows: ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$. PC1, PC2, and PC3 represent the first three principal components of variation of 20 plant leaf traits. 'cat.abund' denotes the abundance of caterpillars. 'catpcoa1:plpcoa2' denotes the interaction of the first principal coordinate analysis (PCoA) axis of caterpillar phylogenetic distance matrix and the second PCoA axis of plant phylogenetic distance matrix. 'catpcoa1:plpcoa1' denotes the interactive term of the phylogenetic distance matrices of plants and caterpillars along with the first PCoA axis, while 'cat.pcoa2:pl.pcoa2' denotes the interaction term of the phylogenetic distance matrices of caterpillars and plants along with the second PCoA axis.

of the observed networks to that of 1000 randomised food webs generated by the *vaznull* function in the R package *bipartite* (Dormann *et al.*, 2009). Having compartmentalised 1000 randomised food webs, we found that the observed plant-caterpillar networks exhibited significantly higher modularity [$M = 0.776$, the most modular result in 50 replicates of the simulations, with a 95% confidence interval of $M = (0.507, 0.651)$].

To test the constraint of phylogeny on species composition within and among modules, we performed a multiple response permutation procedure (MRPP) to compare the dissimilarity of the phylogenetic distance of plants and caterpillars within modules with that between modules using the R *vegan* package (Oksanen *et al.*, 2019). For the MRPP tests, chance corrected within-group agreements (A) estimated whether the phylogenetic dissimilarity within modules was lower/higher than between modules. Due to an imbalance in species distribution between modules, we used a one-way type II ANOVA (Langsrud, 2003) to explore whether modules were associated with the phenotypic attributes of the host plants. Furthermore, we used MRPP to test whether the trait dissimilarity of plants was significantly lower within modules than between modules (Oksanen *et al.*, 2019). All analyses were conducted in the R program (R Core Team, 2015).

Results

The determinants of species traits, phylogeny, and abundance on the likelihood and intensity of caterpillar feeding

PC1 significantly increased the intensity of feeding by caterpillars on plants in the whole observed network but not in the two subnetworks (Table 1 and Fig. 1). The PC2 of leaf trait variation reduced the likelihood and intensity of feeding in the entire observed network and the two subnetworks (Table 1 and Fig. 1). The PC3 of leaf trait variation significantly increased the likelihood and intensity of feeding in the entire observed network and the summer subnetwork, but only significantly increased intensity of feeding in the autumn subnetwork (Table 1 and Fig. 1). The phylogenies of the plants and caterpillars had significant effects on the feeding intensity of caterpillars in all three networks (Table 1).

Concentrations of leaf Ca and Mg were negatively associated, but the concentration of leaf C was positively associated with the axis PC2 of variation in leaf trait (Fig. 1). High concentrations of leaf Ca and Mg could therefore enhance the likelihood and intensity of feeding in the whole network and the two subnetworks (Fig. 1 and Table 1). Similarly, increasing SLA, C:P, and N:P (loaded on PC1, Fig. 1) could enhance caterpillar feeding intensity in the entire observed network only, whereas increasing leaf N:P (loaded on PC3) could enhance feeding intensity in the whole network and the two subnetworks (Fig. 1 and Table 1).

Increasing the abundance of plants and caterpillars enhanced the feeding intensity of the caterpillars but had no significant impact on the likelihood of feeding in either the whole network or the autumn subnetwork (Table 1). Multivariate PGLS regression results showed that only one principal component (PC2) of leaf trait variation significantly reduced plant vulnerability (coefficient = −0.240, $P = 0.049$), whereas plant abundance, or the remaining principal components of leaf trait variation (PC1 and PC3) did not significantly affect plant vulnerability (all P values >0.05, Fig. 2a). The univariate PGLS regression model showed that each of the four explanatory variables (PC1, PC2, PC3, and plant abundance) did not significantly regulate plant vulnerability (all P values >0.05, Fig. 2b).

How species traits, phylogeny, and abundance determine the modular organisation of the plant-herbivore network

The whole modular network of plants and caterpillars was divided into 21 modules (Fig. 3). MRPP tests, along with chance corrected within-group agreements (A), revealed a significantly lower phylogenetic dissimilarity of plants ($A = 0.471$, $P < 0.001$)

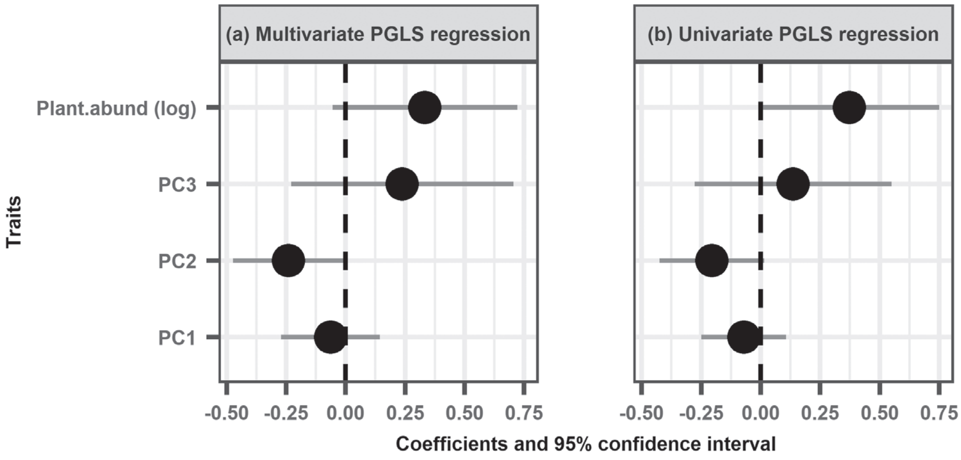

Fig. 2. The impact of plant traits on plant vulnerability in the entire plant-caterpillar food web. (a) and (b) Multivariate and univariate phylogenetic-generalised least square regression results, respectively. Plant vulnerability was measured as the standardised mean phylogenetic distance of insect species on the focal plant species. The regression coefficients, 95% confidence intervals of the three principal components of plant trait variation (PC1, PC2, and PC3), and log-transformed plant abundance (Plant.abund (log)) are shown.

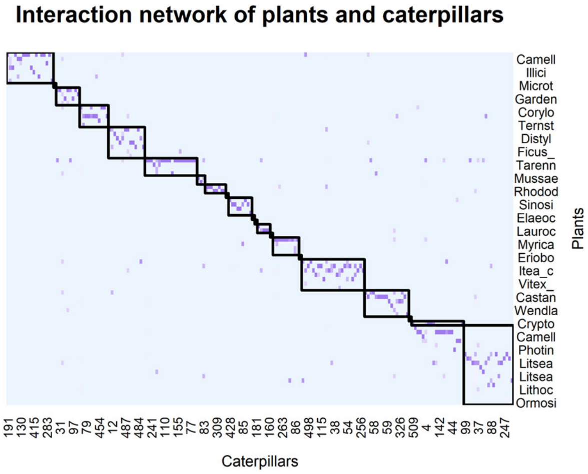

Fig. 3. Interaction patterns and modular structures in the entire plant-caterpillar food web. Some plant species and caterpillar OTUs are labelled on the right and bottom, respectively. An abbreviation for each plant species (first six characters of the Latin name denoting each plant) and each caterpillar OTU (number denoting each OTU) is shown. Each module is framed in a black line. Cells filled in blue denote a plant-caterpillar foraging association, while a blank cell denotes no foraging association. Number of modules = 21, no. of plant species = 80, no. of caterpillar species = 194, and no. of associations = 325. [Colour figure can be viewed at wileyonlinelibrary.com]

and caterpillars ($A = 0.061$, $P = 0.002$) within the same module than that between different modules. The one-way type II ANOVA results indicated that five leaf chemical and morphological traits, including leaf K, Mg, Mn, Cu, and LDMC, along with PC2 of leaf trait variation, were significantly related to the classification of module membership (Table 2a and Fig. 4). Furthermore, the dissimilarity between plants in the five leaf traits (K, Mg, Mn, Cu, and LDMC) and PC2 of leaf trait variation were significantly lower within modules than between modules (Table 2b). Module membership of caterpillars and host plants did not depend on the species abundances of the respective caterpillars (Kruskal-Wallis $\chi^2 = 17.459$, $P = 0.492$, Table 2c) and plants (Kruskal-Wallis $\chi^2 = 19.340$, $P = 0.450$, Table 2c).

Discussion

By disentangling the likelihood and intensity of caterpillar feeding, we assessed the relative importance of species traits and the abundance of species, as well as the importance of phylogeny, on trophic interactions between plants and caterpillars, revealing the evolutionary and ecological processes governing network modular composition in a local subtropical forest community. We found that species abundance tended to determine the feeding intensity of caterpillars rather than feeding likelihood and the modular composition within the network structure. Low concentrations of leaf Ca and Mg (representing mineral nutrients and leaf digestibility) and high leaf C content (representing strong leaf physical structure) reduced the likelihood and intensity of feeding, whereas high SLA (representing leaf palatability) and N:P (representing quality of nutrients) increased both the likelihood and intensity of feeding. Species traits and phylogeny contributed significantly to the modular composition within the plant-herbivore food webs, whereas abundance did not. Our study contributes to understand the assembly rule of plant-caterpillar networks and highlights the importance of leaf morphological and chemical traits and phylogeny in shaping the interaction patterns and modular structure of a plant-insect herbivore food web.

The abundances of plants and caterpillars make no significant contribution to the establishment of trophic interactions and modularity

Consistent with a previous hypothesis (Vázquez & Aizen, 2004), we found that abundant plant/Lepidoptera

Table 2. The impact of plant and caterpillar traits on the module species composition of the entire plant-caterpillar food web.

	(a) ANOVA		(b) MRPP		(c) Kruskal-Wallis	
Attributes	*F*	*P*	*A*	*P*	χ^2	*P*
K	2.228	0.009	0.104	0.048	–	–
Mg	3.447	0.001	0.152	0.013	–	–
Mn	2.177	0.011	0.099	0.049	–	–
Cu	4.181	0.001	0.147	0.045	–	–
LDMC	1.870	0.033	0.116	0.027	–	–
PC2	2.156	0.012	0.129	0.018	–	–
Caterpillar abundance	–	–	–	–	17.459	0.492
Plant abundance	–	–	–	–	19.340	0.450

Three results including (a) one-way type II ANOVA, (b) multiple responses permutation procedure (MRPP), and (c) Kruskal-Wallis chi-square tests. Of the 20 plant traits, only the statistically significant variables are shown.

species exhibited stronger interactions with corresponding partners than that of rare species in the entire network and the two subnetworks (Table 1). This result supports the notion that herbivore feeding intensity increases with the abundance of food plants in a community (Bernays & Chapman, 1970; Singer & Stireman III, 2001). However, the fact that we did not find a significant relationship between the abundance of food plants and the likelihood of feeding in the entire network and the two subnetworks (Table 1) suggests that plant resources may not be the primary limiting factor in establishing plant-herbivore interactions. Therefore, it is not always reliable to predict the occurrence of trophic interactions based on species abundance distributions in a local community. Similar results have been demonstrated in plant-pollinator networks, where species abundance cannot accurately predict species interactions (Olito & Fox, 2015). Despite the fact that some ecological factors that are related to species abundance may contribute to modularity (Matthias *et al.*, 2014), our study demonstrated that neither the species abundance of plants, nor the abundance of caterpillars, contributed to the modular composition of the plant-herbivore food web (Table 2c).

Phylogeny and traits jointly shape plant-caterpillar interactions and network modularity

The stoichiometric ratios of C and other nutritional elements (N, P, K, Na, Ca, Mg, Fe, Zn, Mn, and Cu) are the most important factors for larval development, impacting trophic associations in food webs (Filipiak & Weiner, 2017b). Accordingly, we found that the second principal component that was most positively related to C, and negatively related to Ca and Mg (PC2, Fig. 1), reduced both the likelihood and the intensity of caterpillar feeding across the entire network and in the two subnetworks (Table 1). Moreover, the second principal leaf trait component reduced plant vulnerability (Fig. 2). These results can be attributed to the low concentrations of Ca and Mg reducing plant digestibility (Mládková *et al.*, 2018), and high levels of C from lignin and other indigestible chemical compounds reducing nutritional quality and leaf palatability (Lemoine *et al.*, 2014). Leaf N:P ratios (loaded on PC3, Fig. 1b,c) enhanced the feeding intensity of caterpillars in the entire network and in the two subnetworks (Table 1). High SLA and low C:N and C:P (loaded on PC1, Fig. 1a,c) enhanced the intensity of caterpillar feeding but not the likelihood in the entire observed network (Table 1, PC1 Fig. 1). This indicates that N and P may not be the sole constraining factors that establish dietary interactions between plants and caterpillars, which challenges the notion that the development of insect larvae is limited by N and P deficiencies in food (Lemoine *et al.*, 2014; Filipiak & Weiner, 2017b). The increased intensity of caterpillar feeding on plants with high SLA and N:P (Table 1 and Fig. 1) can be explained by less investment in secondary compounds (i.e., condensed tannins, cellulose, and lignin) by the plants (Abdala-Roberts *et al.*, 2018; Shan *et al.*, 2018). A high intensity of caterpillar feeding on plants with low C:N and C:P (Table 1 and Fig. 1) can be ascribed to the plants potentially allocating less C in producing C-based secondary compounds (i.e., cellulose and lignin) (Shan *et al.*, 2018), and more N and P in producing P-based lipids and N-based proteins.

Furthermore, our study showed that an increase in the content of leaf mineral elements (mostly Fe, Ca, and Mg, see Fig. 1) increased the likelihood of caterpillar feeding in all three networks (Table 1). This suggests that the establishment of dietary associations in the studied lepidopteran larvae and host plants was co-limited by multiple elements (primarily N + P + Mg + Ca + Fe), rather than solely by N and P. This is unsurprising given the importance of essential mineral nutrients in the regulation of insect growth and development (Filipiak & Weiner, 2017b). A scarcity of the mineral elements K, Na, Mg, Zn, and Cu has been reported to limit the development of beetle larvae in dead wood (Filipiak & Weiner, 2017b). Our study suggests that nutrient content and the digestibility of plant resources changed the feeding intensity of the caterpillars, which ultimately shaped the dietary interaction patterns of the plant-insect herbivore food web (Table 1).

Probably constrained by statistical methods or artificial sampling effects, some leaf traits (K, Mn, Cu and LDMC) were not always significantly similar within a module before and after removing modules with single plant/caterpillar species (Table 2, Appendices S4 and S5). Nevertheless, our study identified that two leaf variables (Mg and PC2) were always more similar within modules than between modules (Table 2 and Appendix S4). Principal component analysis showed Mg was mainly

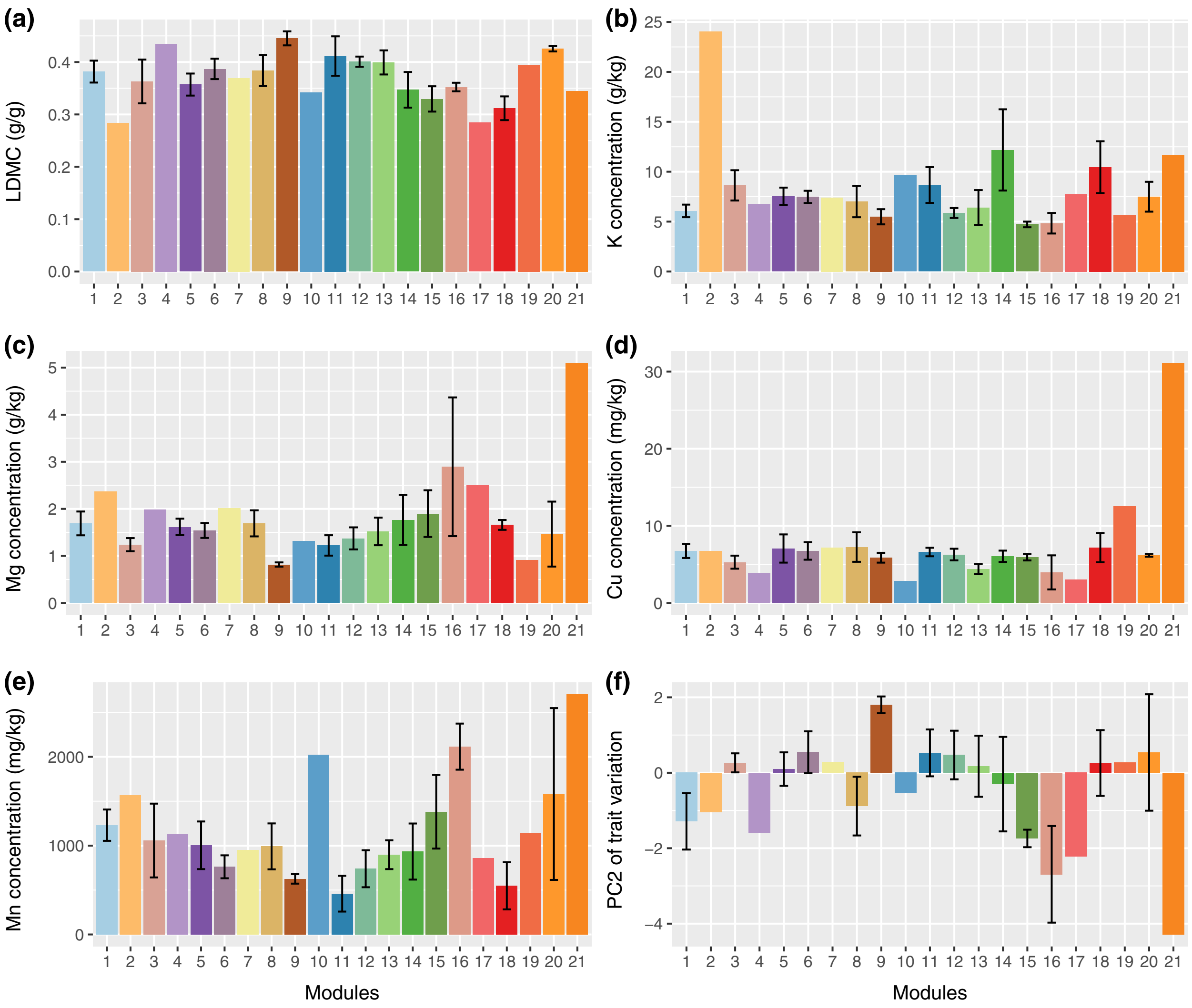

Fig. 4. Differences in leaf functional traits between modules for the entire plant-caterpillar food web. One-way type II ANOVA revealed significant differences in five leaf functional traits (LDMC, K, Mg, Cu, and Mn; a, b, c, d, and e, respectively) and PC2 of leaf trait variation (f) between modules. The height of each bar denotes the mean value of a plant trait in the module. Error bars show the standard error of the mean value of a plant trait in that module. [Colour figure can be viewed at wileyonlinelibrary.com]

loaded on PC2 (Fig. 1). This suggests that the modular plant composition may be the result of the dietary preferences of herbivores that have been driven by the secondary principle axis of leaf trait variation of the host plants. A narrowing diet breadth of generalist insect herbivores regulated by plant resistance can improve network modularity (Robinson & Strauss, 2020). Network modules in plant-insect herbivore food webs often include phylogenetically related species with similar traits (Cagnolo *et al.*, 2011; Donatti *et al.*, 2011; Meskens *et al.*, 2011). Accordingly, we found that phylogenetic histories constrained the modular species composition, with the emergence of phylogenetically clustered plants ($A = 0.471$, $P < 0.001$) and caterpillars ($A = 0.061$, $P = 0.002$) within modules. The phylogenetic clustering of the plants in our plant-caterpillar food web suggests that leaf-trait homogeneity within modules was related to shared ancestry, since most traits showed a phylogenetic signal (Appendix S6). In addition, within-module homogeneity may be mediated by convergent evolution and herbivore choice, not necessarily with a phylogenetic signal.

To conclude, our study has revealed the mechanism that regulate trophic interactions and modularity within a plant-insect herbivore network from both ecological and evolutionary perspectives. We have highlighted the importance of variation in plant trait and phylogenetic relatedness in underpinning terrestrial plant-insect herbivore networks, particularly the role played by plant stoichiometry in controlling the feeding behaviour of caterpillars. However, our phylogeny of Lepidoptera was reconstructed using one DNA barcode with constrained topology for some lepidopteran families (Appendix S2). This phylogeny may have impacted the reliability of our results, and we recommend that more DNA markers be used in further study to investigate the impact of insect phylogeny on module composition. In addition, our results may also be constrained to some extent by insufficient sampling efforts for plants and caterpillars and the low DNA amplification efficiency of the molecular method employed to identify the plant residues in the guts of caterpillars. A combination of the molecular method and the traditional observation method should capture more dietary associations between caterpillars and plants. However, dietary associations identified by direct observation of caterpillars on sample trees without the validation of feeding experiments are not always reliable (Zhu *et al.*, 2019). In the future, incorporating PCR-free high-throughput sequencing technology alongside the observation method should construct a larger network with different structural properties. Nevertheless, increased efforts in sampling and diet identification may have slightly changed our results because phylogeny, abundance, and traits exhibited similar impacts on the trophic interaction patterns in the whole plant-caterpillar network and the two subnetworks (Table 1). We also would expect to find a relationship between the abundances of plants and caterpillars and the establishment of trophic interactions and modular structure. The fact that abundance does not affect modularity, while leaf chemical elements do appear to regulate network structure, confirms the importance of biological nutrient elements in regulating plant-insect herbivore interaction networks.

Acknowledgements

We thank Wen Zhou for providing DNA barcoding sequences for plants. We are particularly indebted to Jianqu Xu, Jie Liang, Dezheng Gao and many others who helped to perform field work and laboratory experiments. We appreciated constructive suggestions from Sébastien Ibanez on this work. This research was funded by the National Natural Science Foundation of China (31925027, 31622014 and 31570426) to CC. All of the authors declare no conflict of interest.

Author contributions

Chunchao Zhu performed molecular experiments and analysed the data. Yongfa Chen provided leaf stoichiometric trait dataset. Deyi Yin collected insect samples. Dong He provided leaf morphological trait dataset. Chunchao Zhu wrote the manuscript, with substantial inputs from Chengjin Chu. Minhua Zhang, M. D. Farnon Ellwood, and Suqin Fang revised the manuscript.

Data availability statement

Representative sequences of lepidopteran species were deposited in the GenBank nucleotide collection under accession numbers: MG986496 - MG986529; MG986531 - MG986544; MG986546 - MG986605; MG986607- MG986631; MG986635- MG986653; MG986655 - MG986696; MG986698 - MG986713; MG986715 - MG986720; MK044350 - MK044392; MK044394 - MK044432; MK044434- MK044446; MK044448 - MK044451; MK044453 - MK044463; MK044465 - MK044486; MK044488 - MK044504; MK044506 - MK044532. They were also deposited in the BOLD system with process ID: from LEPSC001-20 to LEPSC00194-20. Appendices S1-S6 are available in supplement file.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1: A phylogenetic tree of 80 plant species.

Appendix S2: A phylogenetic tree of 194 species of Lepidoptera.

Appendix S3: A Pearson correlation matrix of plant functional traits.

Appendix S4: The impact of plant and caterpillar traits on module composition after removing modules with single plant/caterpillar species.

Appendix S5: The impact of plant leaf traits on module plant composition before and after removing modules with single plant species.

Appendix S6: The details of phylogenetic signals of plant variables.

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Accepted 28 March 2021

Associate Editor: Walter Araújo