Phylogenetic relatedness, functional traits, and spatial scale determine herbivore co-occurrence in a subtropical forest

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Abstract. The mechanisms driving species co-occurrence are varied and include biotic interactions, abiotic factors, and scale-dependent processes. Based on a comprehensive dataset of lepidopteran herbivores recorded from a large-scale forest biodiversity experiment, we tested the contribution to herbivore species co-occurrence of herbivore phylogenetic relatedness, plant diversity and functional traits, and spatial scale. We found that Lepidoptera co-occurrence was negatively associated with their phylogenetic relatedness, tree diversity, and defensive traits, but positively associated with nutritional functional traits. Furthermore, species co-occurrence was higher at larger spatial scales (tree species or plot) than at smaller scale (individual trees). Our results provide evidence supporting both environmental filtering and competition exclusion hypotheses, and highlight the significance of functionality in shaping species coexistence of herbivores in plant-arthropod systems.

Key words: BEF-China; checkerboard scores; competition exclusion; environmental filtering; Lepidoptera herbivores; phylogenetic structure; species coexistence.

INTRODUCTION

Species co-occurrence examines whether and why two or more species coexist, which remains a challenging topic in ecology. One approach that has received considerable attention is the use of a phylogenetic framework, which takes into account evolutionary processes and can be used for testing some ecological hypotheses related to species coexistence (Webb et al. 2002, Emerson & Gillespie 2008, Mayfield & Levine 2010, Yan et al. 2016, Ceccarelli et al. 2020). Furthermore, studies have suggested that various biotic and abiotic factors might play important roles in species coexistence, and that these factors are highly correlated with niche partitioning as

well as tradeoffs of species life history (Tokeshi 2009, Trivellone et al. 2017, D'Amen et al. 2018, Nakadai et al. 2018). Three key hypotheses have been used to interpret species coexistence patterns: environmental filtering, competitive exclusion, and neutral assembly processes (Cavender-Bares et al. 2009, Cardillo 2011, Shinen & Navarrete 2014). Assemblages are observed to be phylogenetically clustered if traits are evolutionarily conserved and environmental filtering is significant (Cavender-Bares et al. 2004, Emerson & Gillespie 2008, Yan et al. 2016, Moura et al. 2017). In contrast, the competitive exclusion hypothesis posits that the co-occurrence of relatives should be infrequent due to competition, as relatives usually possess many ecological traits and share resources (Elton 1946, Hardin 1960). Competitive exclusion is expected to lead to "overdispersion," or the inverse of phylogenetic clustering (e.g. Cavender-Bares et al. 2004, Emerson & Gillespie 2008,

Machac et al. 2017; but see Mayfield & Levine 2010). Additionally, Hubbell (2001) proposed a spatial model of neutral communities in which co-occurrences are unaffected by relatedness (Hubbell 2001, Kembel & Hubbell 2006).

Phylogenetic structure of species assemblages needs to be interpreted in the context of spatial scale, because the processes and mechanisms of species assembly have been observed to differ markedly along this axis (Gómez et al. 2010, Jin et al. 2020). At local scales, competitive exclusion is thought to play a key role for community assembly (Swenson et al. 2006, Gómez et al. 2010), while environmental filtering is considered more important at regional spatial scales (Cavender-Bares et al. 2009). However, when being viewed across spatial scales, co-occurrence can be determined by different mechanisms, such as the discrepancy between a species' fundamental and realized niche (Cardillo 2011), conspecific attraction (e.g. habitat selection) and interspecific territoriality for species interactions, and distributional effects of species dispersal and colonization (Ricklefs 1987, 2015, Brown et al. 2000, Gotelli et al. 2010). The role of phylogenetic structure and environmental factors in modulating species co-occurrence has been studied extensively in plants (Cavender-Bares et al. 2009, Elliott & Davies 2017). These studies have shown that phylogenetic distance has significant effects, mediated by environmental filtering based on shared environmental preferences. Some recent studies on vertebrates have shown the importance of spatial factors and phylogeny on species co-occurrence (e.g. Krasnov et al. 2014, Yan et al. 2016, Kay et al. 2018). This is because community assembly processes that link evolution, habitat filtering, and functional trait dispersion, can leave phylogenetic signatures (Emerson & Gillespie 2008, Gerhold et al. 2015, Jarzyna et al. 2021). For insects, studies have suggested high correlations between phylogenetic structure and species co-occurrences across different areas (e.g. Ceccarelli et al. 2020, Seifert et al. 2020, Staab et al. 2020). However, it is still unclear how phylogenetic distance and functional traits affect co-occurrence within two trophic levels (Gotelli & McCabe 2002, Kraft et al. 2015).

In this study, we analyzed co-occurrence of pairs of caterpillar species in a subtropical forest, and examined how these might be influenced by Lepidoptera phylogenetic distance across three spatial scales (tree individual, tree species, and plot levels). We also considered diversity and functional traits of trees, and other environmental predictors, as recent evidence has suggested that herbivores are not only phylogenetically structured for host use, reflecting adaptations to plant functional traits (Volf et al. 2018, Wang et al. 2020), but also are non-random in their association with the host plant's phylogeny (Pellissier et al. 2013, Wang et al. 2020). Specifically, we explored the interrelationships of phylogenetic distance of caterpillars, several measures of tree diversity and tree functional traits on Lepidoptera species co-occurrence. The experiment was conducted on the 'BEF-China' platform, currently the largest tree

diversity experiment worldwide and located in a highly biodiverse region of south-east China (Bruelheide et al. 2014). For tree diversity, we included species richness, functional diversity, and phylogenetic diversity. Furthermore, mean phylogenetic pairwise distance (MPD), and mean nearest taxon distance (MNTD) were considered as metrics of phylogenetic diversity, as these indices differentially reflect phylogenetic structuring (Srivastava et al. 2012).

We expected that lepidopteran phylogenetic distance would show a consistent correlation with species co-occurrence across the three spatial scales and that co-occurrence would also be driven by tree diversity and relevant functional traits of trees. We explicitly hypothesized that (1) Lepidoptera species pair co-occurrence is explained to a large part by their phylogenetic distance, which is the central variable reflecting evolutionary processes; (2) as predicted by environmental filtering theory, these patterns might be in part explained by (phylogenetically structured) adaptations to defensive and nutritional traits of the host plants; (3) the degree of coexistence is lower at smaller spatial scales (e.g. individual tree vs. tree species or plot) as predicted by the competitive exclusion principle. Our study shows that various driving forces play significant roles in determining the herbivore species coexistences, which has important implications for building a comprehensive understanding of herbivore species coexistence mechanisms in species-rich ecosystems.

Methods

Study site

The study was conducted in the BEF-China tree diversity experiment that is located in subtropical south-east China, Jiangxi province, (29°08′–29°11′N, 117°90′–117°93′E). The mean annual temperature is 16.7°C and mean annual precipitation 1,821 mm (Yang et al. 2013). The experiment is replicated in two study sites (Site A and Site B, 4 km apart from each other and comprising a total area of 50 ha), which were established in 2009 (Site A) and 2010 (Site B). In total, 566 plots of 25.8×25.8 m were established on the two sites, and 400 tree seedlings were randomly planted on each plot in a regular arrangement of 20 rows and 20 columns with mean planting distance 1.29 m among trees (Bruelheide et al. 2014). In this study, 64 intensively studied, randomly distributed plots were selected on the two sites (32 plots at each site; Fig. 1; Appendix S1: Fig. S1) and represented a tree species richness gradient from monoculture to 24 species mixtures (16 monocultures, and eight, four, two, one, and one mixtures of two, four, eight, 16, and 24 species, respectively, on each study site). In total, 40 locally common tree species were planted on the two sites. The tree species composition at the two sites differed, with two non-overlapping species pools of 16 broadleaved canopy species at each site. Moreover, an

overlap of eight tree species in the 24-species mixtures was planted on both sites. We excluded nine plots because of high mortality of trees, and therefore, the data from 55 plots were used for further analysis (Fig. 1).

Sampling

Lepidopteran caterpillars were collected once in April, June, and September in both 2017 and 2018 (in total six times for the two years) using a beating method. For each tree individual, the resident caterpillars were knocked down onto a white sheet (1.5 × 1.5 m²) with a padded stick (Schuldt et al. 2014b, Peralta et al. 2015). There is a high probability that the caterpillars actually feed on the trees from which they were collected, because of their poor mobility relative to adults and other flying herbivorous insects (see also Wardhaugh et al. 2012). We sampled the trees from the first rows per plot for a total of 80 living trees in each plot. The sampled trees adequately covered the tree species composition and species richness at the plot level because of the randomized planting design. We collected caterpillars individually in tubes filled with 99.5% ethanol to avoid contamination of samples and stored all samples in a −20°C freezer until further processing.
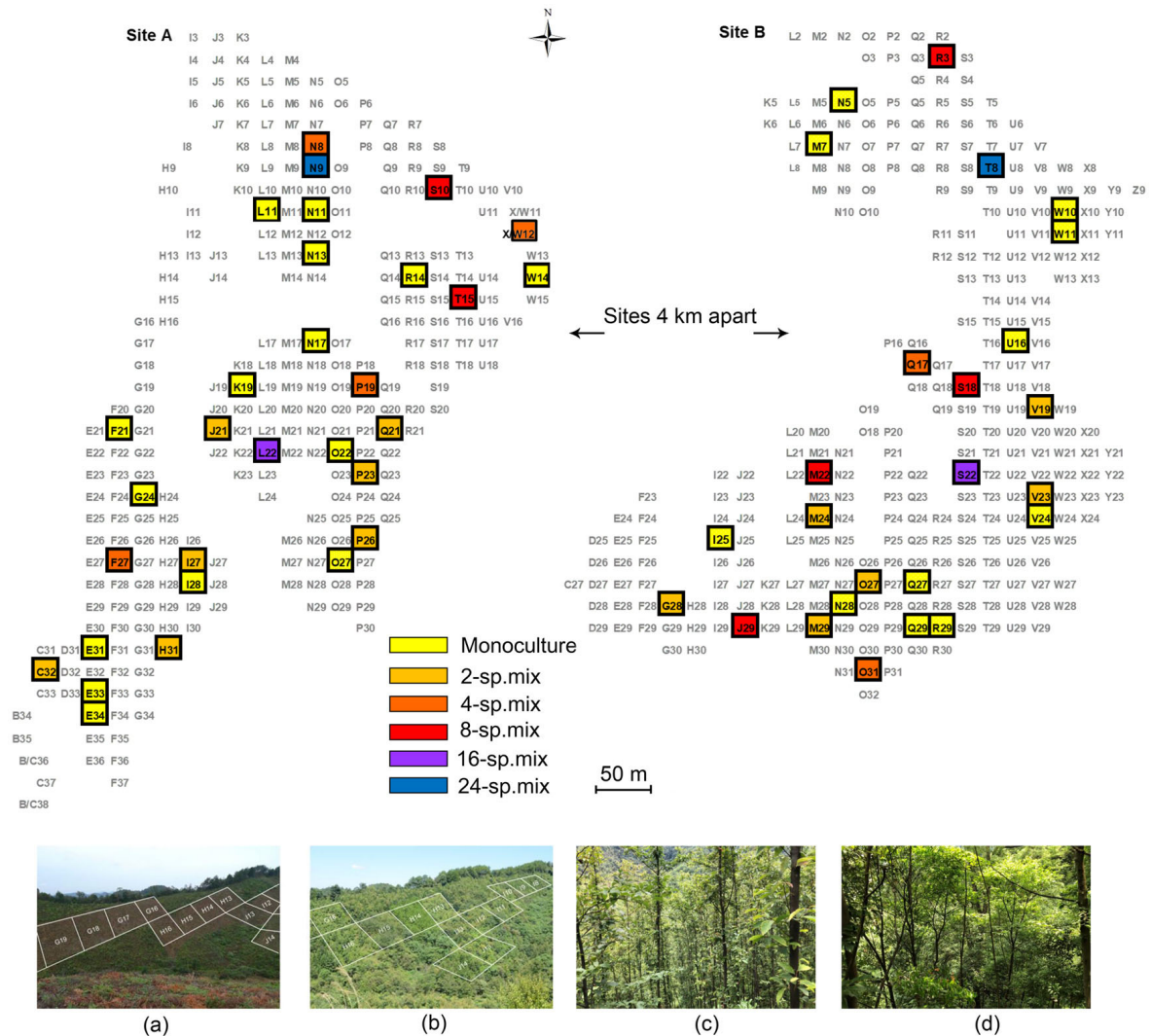

FIG. 1. Overview of the two experimental sites of BEF-China (left: Site A, right: Site B) and the study plots selected for the current study (with bold-black outline). Levels of tree species richness are indicated by color. Each study plot has a size of 25.8 × 25.8 m. (a) Photograph was taken in November 2009 a few months after planting (photograph by Xuefei Yang). (b) Photograph was taken in August 2017, showing fully established trees and closed canopies (photograph by Helge Bruelheide). (c) Monoculture study plot (Q27) of site B (photograph by Ming-Qiang Wang in June 2021). (d) A 24 mixture species study plot (T8) of Site B (photograph by Ming-Qiang Wang in June 2021). For more information on all the very intensively studied plots and the tree species richness gradient of the other plots, see Appendix S1: Fig. S1.

DNA extraction, sequencing, and phylogenetic analysis

Details on DNA extraction, amplification, and sequencing are provided in the appendix (Appendix S1: Section S1). In brief, whole genomic DNA was extracted using DNeasy Blood and Tissue Kits (Qiagen), following the manufacturer's instructions. The mitochondrial cytochrome oxidase subunit I (*COI*) gene (Hebert et al. 2003) was amplified using universal primer pairs and sequenced for all samples. Then, a Perl-based DNA barcode aligner (Chesters 2019) was used to align against a profile alignment. We used three methods for inferred Molecular Operational Taxonomic Units (MOTUs) (Blaxter et al. 2005) from the plot DNA barcodes; threshold-based hierarchical clustering with BLAS-Tclust, Automatic Barcode Gap Discovery (ABGD), and Poisson Tree Processes model (PTP). The BLAS-Tclust module of the NCBI-BLAST package (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used under a clustering threshold of 97.8% identity (Ratnasingham & Hebert 2013). Then we used the online implementation (bioinfo.mnhn.fr/abi/public/abgd/) of ABGD (Puillandre et al. 2012) with the Kimura (K80) model and parameters: $P_{min} = 0.001$, $P_{max} = 0.1$, Steps = 50, $X = 0.5$ and Nb bins = 50. MOTU were also inferred using the PTP model (Zhang et al. 2013), which was run with default parameters on the maximum-likelihood phylogeny of the unique plot-level barcodes. To select the most internally consistent method for further analysis, we used the *clues* package (Wang et al. 2007) in R to calculate the Hubert and Arabie-adjusted Rand index, a measure of clustering congruence (Hubert & Arabie 1985). We constructed a phylogeny of the Lepidoptera barcodes following Wang et al. (2020) and Chesters (2020), in which we used two approaches to improve phylogenetic structure and provide more accurate diversity indicators (Macías-Hernández et al. 2020); the incorporation of a reference framework and a high-quality backbone topology. Reference DNA barcodes were mined and processed as described in Wang et al. (2020). Briefly, all taxonomically annotated Lepidoptera COI barcodes were downloaded from the BOLD API (Ratnasingham & Hebert 2013), for which we removed highly dissimilar or likely to be mislabeled sequences, and aligned them (Chesters 2019). The phylogeny was then constructed in two steps, with constrained phylogenetic inference followed by phylogenetic placement of the MOTU plot. Constraints were inferred through analysis of taxonomic overlap of the reference barcodes with three previously published backbone trees, using the method described in Chesters (2020). The backbone trees selected were of the ditrysian group (Heikkilä et al. 2015), Lepidoptera (Kawahara et al. 2019) and Noctuoidea (Regier et al. 2017). Analysis of taxonomic overlap used both the NCBI and ITIS taxonomic hierarchies. The reference phylogeny was constructed with RAxML version 8 (Stamatakis 2014), then the MOTU plot was added to the fixed reference tree using the same software, with bootstrapped inference to obtain indicators of placement support. MOTU placement support (in the form of bootstrap and distance to the nearest reference branch) was parsed using the Perl script bagpipe_phylo (Chesters et al. 2015). The phylogeny was used for the calculation of phylogenetic distance between Lepidoptera species pairs.

Lepidoptera co-occurrence and phylogenetic distance of paired species

For the co-occurrence metric, we chose one of the most commonly used indices of co-occurrence, a measure of proportional similarity, to characterize paired species co-occurrence probability: Schoener's index (C_{ij}) (Schoener 1970), $C_{ij} = 1 - 0.5\Sigma|p_{ik} - p_{jk}|$, where p_{ik} and p_{jk} are the proportions of occurrences of species *i* and *j*, respectively, in sample *k*, and where values close to 1 indicate high coexistence probability, and lower values indicate low coexistence probability. We used the R package *picante* to calculate the index of co-occurrence across the three spatial scales – tree individual, tree species and study plot – in our study. Therefore, species *i*/*j* represent caterpillar MOTU and *k* represents caterpillars occurring on each tree individual, species or study plot in our case. Our results are expected to be conservative in detecting phylogenetic structure using this index (see also Yan et al. 2016), as previous studies have suggested a high type 1 error (Kembel 2009). We calculated the co-occurrence index and phylogenetic distance of all species pairs at the three spatial scales to test the relationship between the co-occurrence and phylogenetic distance. The phylogenetic distance matrix of paired species was extracted from our constrained phylogeny using the "cophenetic.phylo" function of the *ape* package in R, which was then transposed for further analysis (for more details see Appendix S1: Section S2). To analyze species co-occurrence at the tree species and plot level, the pairwise species co-occurrence of all Lepidoptera species was averaged across the two levels, which enabled us to test how the general co-occurrence patterns were affected by the functional traits and environmental covariates at the two levels. Specifically, for each tree species or plot, we calculated the co-occurrence of the Lepidoptera species occurring on a tree species or in a plot. The species co-occurrence indices for all species pairs were subsequently averaged to represent the mean value of co-occurrence across all Lepidoptera species for the tree species or plot level.

Plant functional traits and environmental covariables

In total, our study considered 11 morphological and chemical traits of the host tree species as biotic predictors that were classified as defensive or nutritional traits, and commonly reported to drive herbivore diversity and community composition (e.g. Pérez-Harguindeguy et al. 2003, Schuldt et al. 2014*a*, Muiruri et al. 2019). This allowed us to characterize plot conditions in accordance

to nutritional quality and potential defense traits of the trees. For morphological traits (Table 1), leaf area (LA), specific leaf area (SLA), leaf dry matter content (LDMC), and leaf toughness (LT) were included in our study. SLA has often been found to be positively related to herbivory rate, because leaves with high SLA are usually more palatable (soft and digestible) to herbivores (Pérez-Harguindeguy et al. 2013). In contrast, leaves with high LDMC and LT are often reduced in palatability (tough and structurally robust leaves) resulting in decreased herbivory (Pérez-Harguindeguy et al. 2003, Poorter et al. 2004). Moreover, we used seven leaf chemical traits in our analysis (Table 1): leaf carbon (C) concentration, the ratio of leaf carbon to nitrogen (C:N), leaf potassium (K), leaf calcium (Ca), leaf sodium (Na) and leaf phosphorus (P) content. Additionally, leaf tannin concentration was considered as a secondary metabolite. Carbon concentration and the C:N ratio of leaves are also related to palatability; low C:N ratios in particular have commonly been found to promote herbivory (Pérez-Harguindeguy et al. 2003, Poorter et al. 2004), while the remaining six traits are likely to be indicators of nutritional quality of the tree species (e.g. Poorter et al. 2004, Borer et al. 2014). We measured all traits on pooled samples of sun-exposed leaves of a minimum of five tree individuals per species (Kröber et al. 2014) following standard protocols (Pérez-Harguindeguy et al. 2013). Because our leaf-sampling design did not allow us to quantify intraspecific variability, single mean values for each species were used. Previous studies in our study region showed that variability in trait–environment relationships is much more significant at the interspecific than the intraspecific level (Kröber et al. 2012, Schuldt et al. 2012).

As abiotic predictors, we also considered environmental covariables to characterize the heterogeneous topography of the study plots. These covariables were plot means of elevation, slope, "eastness" (sine-transformed radian values of aspect) and "northness" (cosine-transformed radian values of aspect). Data were obtained from a 5 m digital elevation model based on differential GPS measurements (Scholten et al. 2017).

Statistical analysis

Samples from the six periods were pooled at each of the three spatial scales before our analysis. In addition, we excluded eight tree species from our analysis at the tree species level, because several functional traits were missing or too few caterpillars were sampled for the tree species.

Lepidoptera co-occurrence and phylogenetic distance.— We tested the relationships between Lepidoptera co-occurrence and phylogenetic distance across the spatial scales using linear regression, with paired species co-occurrence as the response variable and phylogenetic distance as the predictor variable in the model. Moreover, to check whether Lepidoptera species pairs with low phylogenetic distances drove overall effects on co-occurrence, the ranges of phylogenetic distances at the spatial scales were divided into intervals (breaks of intervals = 0.1), then the co-occurrence data on each distance group were averaged across all levels. In this way,

TABLE 1. Overview of the biotic predictors (host tree traits and diversity indices) used in the study.

Predictor	Abbreviation	Role	Function	Trait type
Functional traits				
Specific leaf area	SLA	B/S	O	Morphological
Leaf dry matter content	LDMC	B	NU	Morphological
Leaf toughness	LT	B	D	Morphological
Leaf area	LA	B/S	O	Morphological
Tree height	–	S	O	Morphological
Tree wood volume	TV	N	O	Morphological
Carbon concentration	C	N	NU	Chemical
The ratio of leaf carbon to nitrogen	C:N	N	NU	Chemical
Leaf potassium content	K	N	NU	Chemical
Leaf calcium content	Ca	N	NU	Chemical
Leaf sodium content	Na	N	NU	Chemical
Leaf phosphorus content	P	N	NU	Chemical
Leaf tannin concentrations	–	S	D	Chemical
Tree diversity indices				
Tree species richness	–	ENV	O	Taxonomic diversity
Tree mean pairwise distance	Tree MPD	ENV	O	Phylogenetic diversity
Tree mean nearest taxon distance	Tree MNTD	ENV	O	Phylogenetic diversity
Tree functional diversity	Tree FD	ENV	O	Functional diversity

Notes: The role of predictors can be expected to provide benefit (B), stress (S), nutrition (N) and environment (ENV) for herbivores. All predictors were classified by defensive (D), nutritional (NU) and other (O) functions.

we were able to assess the general variation of Lepidoptera species co-occurrence that occurs with phylogenetic distance. In addition, we used null models to check whether the relationships between co-occurrence and phylogenetic distance are random results by chance (see Appendix S1: Section S3 for information on the null models).

Lepidoptera checkerboard scores.—To check whether the lower co-occurrence at the finest scale (tree individual) is caused by strong competition, we calculated the checkerboard scores (C-scores) (Stone & Roberts 1990) using the package *bipartite* in R for individual trees in each monoculture plot (tree individual level) and across all monoculture plots (plot level). The C-score represents the average number of species pairs across all possible paired combinations; in a matrix with sites (individual trees or monoculture plots in our case) as columns and taxa (Lepidoptera species) as rows, for each unique pair, the C-score is equal to $C_{pq} = (R_p - S)(R_q - S)$ where R_p and R_q are row sums of taxa p, q and S is the number of shared sites in which both p and q are present (Stone & Roberts 1990). The C-score is then calculated as the mean number of checkerboard C_{pq} across all species pairs in the community. For M species, there are $P = M(M - 1)/2$ species pairs, therefore, C-score is calculated: C-score = $\sum_{q=0}^{M} \sum_{p<q} C_{pq}/P$. This allowed us to further test Hypothesis 3 that the degree of coexistence depends on the spatial scale considered, by only comparing the patterns at different scales under consistent conditions (e.g. comparable palatability and phylogenetic conservatism of host plants). Therefore, C-scores of tree individuals from each plot were compared with the C-score across all monoculture plots using the one-sample *t*-test. To test whether the results are affected by potential sampling effects, we used null models across the three spatial scales to compare the C-scores derived from the observed community matrix and randomized matrices from 100 simulated runs, reshuffling the columns (the Lepidoptera species) in the community tables. This allowed us to test whether C-scores observed were significantly different from random expectation, as standardized deviation from random expectation can be considered significant if the absolute values of the effect size is larger than 1.96.

Lepidoptera phylogenetic structure.—To check the general phylogenetic patterns (phylogenetic clustering or overdispersion) of local Lepidoptera assemblages at the three spatial scales, standardized effect size (SES) of MPD or MNTD for each Lepidoptera assemblage was calculated based on 999 null models using a trial-swap algorithm (Miklós & Podani 2004). These are two complementary metrics of phylogenetic structure sensitive to either basal or terminal branching. SES MPD, as a "basal" metric, is more sensitive to deeper (older) branching, while SES MNTD is "terminal" and better

for representing more recent processes at phylogenetic tree terminals (Mazel et al. 2016). Following Kembel (2009), one-sample *t*-tests were performed to determine whether the SES values were significantly lower or higher than zero at different spatial scales. In this way, we will have a clear understanding of the average phylogenetic structure of local Lepidoptera assemblages across the three spatial scales, i.e., phylogenetic clustering if SES values were significantly lower than zero or phylogenetic overdispersion if SES values significantly were higher than zero.

Community-weighted mean trait values, functional and phylogenetic diversity of trees.—At the study plot level, we used the community-weighted mean values (CWMs) of each leaf trait, tree wood volume as well as the functional diversity of these traits as environmental predictors of mean values of Lepidoptera species co-occurrence across plots (see details on statistical methods for CWM values in Appendix S1: Section S3).

The MPD in trait values among tree species (weighted by tree wood volume as a proxy for host plant biomass) was used to calculate tree functional diversity and expressed as Rao's Q (Ricotta & Moretti 2011). We used a maximum-likelihood phylogenetic tree for the tree species of our study region to calculate phylogenetic metrics of trees. The tree phylogeny was inferred through obtaining sequences (matK, rbcL, and ITS region) from Gen-Bank or *de novo* using standard barcoding protocols for all species from a regional species pool, including 438 woody species occurring in the local area (Purschke et al. 2017). Then a maximum-likelihood tree was constructed and dated using non-parametric rate smoothing and using published fossils as age constraints (Purschke et al. 2017). Phylogenetic diversity metrics of the tree communities were quantified by calculating MPD and MNTD of the tree communities, which in the abundance-weighted case (we weighted species by their wood volume to account for differences in resource availability and apparency to herbivores) are equivalent to Rao's Q (Tucker et al. 2017). More details for these two metrics are given in Appendix S1 (Appendix S1: Section: S3).

Linear and phylogenetic regression models.—At the tree species level, we used phylogenetic generalized least squares (PGLS) regression to test the effects of tree species-specific mean trait values on Lepidoptera species co-occurrence (see details on statistical methods for CWM values in Appendix S1: Section S3).

To test for potential relationships between environmental predictors (see Appendix S1: Section S3) and co-occurrence at the level of tree communities (i.e. comparing different levels of tree species richness), the mean values for Lepidoptera species co-occurrence were analyzed also as response variables, with diversity of trees (including species richness and phylogenetic diversity) and study site as predictors. We used the plot-level data

to test for effects of tree species richness, tree functional and phylogenetic diversity, and leaf traits including the CWMs of LA, SLA, LDMC, LT, leaf carbon content, leaf C:N content, potassium content, leaf calcium content, leaf sodium content, leaf phosphorus content, tree wood volume, and abundance of caterpillars. As tree MPD was highly correlated with tree species richness (Pearson's $r = 0.74$, $P < 0.001$), it was excluded in the models where tree species richness was a predictor. However, to test for the effects of tree MPD on co-occurrence, we replaced tree species richness with tree MPD in an alternative model. To check whether there were effects between the two study sites, we included the interactions between site and tree species richness and site and tree functional diversity as predictors. Then, we checked whether our results depended on interactions with the study site. We simplified the linear models based on values of the AICc using a stepwise procedure, and selected the subset models with the lowest AICc. Moreover, we tested the correlations among all predictors (Appendix S1: Figs. S2, S3) and checked variance inflation factors (VIF) of our statistical models to ensure that the analysis were not strongly affected by multicollinearity (see details on linear models in Appendix S1: Section S3).

RESULTS

In total, 8,036 Lepidoptera larvae were collected over the six sampling periods across 2017 and 2018 (from almost 25,440 tree individuals), for which 7,204 COI sequences were successfully sequenced and were inferred into 461, 408 and 524 MOTU by hierarchical clustering, ABGD and PTP, respectively. The results of the hierarchical clustering were selected for further analysis, as they were the most consistent when compared with the other two methods (pairwise clustering congruence measured by the HA-adjusted Rand index; hierarchical clustering vs. ABGD: 0.995; hierarchical clustering vs. PTP: 0.990; ABGD vs. PTP: 0.985). Altogether, 82,621, 58,676 and 49,202 Lepidoptera species pairs were included in our analysis at the individual tree, tree species, and plot level, respectively.

Effects of phylogenetic distance on Lepidoptera co-occurrence

Lepidoptera species co-occurrence probability at the tree individual level was much lower than at the tree species level or plot level (Fig. 2a–c). The regression results indicated that there were significantly negative relationships between phylogenetic distance and co-occurrence (Appendix S1: Table S1, Fig. S4). Moreover, the null model correlation results suggested that phylogenetic distance had significantly negative effects on paired species co-occurrence irrespective of whether Lepidoptera species were observed at the fine (i.e. tree individual level) or at larger spatial scales (tree species

level and plot level) (Table 2). Our null model approach suggested that phylogenetic distance and co-occurrence of observed species were significantly non-random in relation to randomized phylogenetic distance and co-occurrence (Table 2). The negative relationship between Lepidoptera phylogenetic distance and species co-occurrence were obvious when co-occurrence values were averaged across phylogenetic distance classes (Fig. 2d–f). We note that some species pairs showed a high degree of co-occurrence across the three spatial scales, i.e., highest co-occurrence values were found at mid-levels of phylogenetic distance for a small number of Lepidoptera species pairs (Fig. 2a–c). However, these pairs accounted for only a relatively small proportion of the total species pairs across the three scales (for individual trees: 287/82,621 [co-occurrence > 0.1]; tree species scale: 6465/58,676 [co-occurrence > 0.5]; plot scale: 4413/49,202 [co-occurrence > 0.5]). Our regression and null model results indicated negative relationships between Lepidoptera species co-occurrence and phylogenetic distance despite these occurrences, suggesting that they did not affect the overall pattern that co-occurrence decreased with increasing of species phylogenetic distance. The C-scores of co-occurring Lepidoptera species on individual trees in each monoculture plot were significantly higher than the C-score of all monoculture plots (mean = 5.02, $t = 3.6$, df = 24, $P < 0.001$; Fig. 3a). The SES values of MPD and MNTD were significantly lower than zero across individual tree, tree species, and plot level (except for MPD at tree species level, which nevertheless had SES values lower than zero in 19 of the 38 tree species; Table 3; Appendix S1: Tables S2–S5; Fig. 3b–d; Appendix S1: Fig. S5). The three null models of C-scores suggested that the observed results were significantly different from randomized C-scores (absolute values of effect size were 3.37, 2.38 and 3.39 at the scale of individual trees, tree species, and plot, respectively). These significant differences further provide evidence for the general negative relationship between Lepidoptera co-occurrence and phylogenetic distance, which was found to be independent of the number of occurrences and the community structure.

Effects of diversity and functional traits of trees on Lepidoptera co-occurrence

At the tree species level, the phylogenetic regression analysis showed that Lepidoptera species co-occurrence was significantly positively related to tree nutritional traits, such as C:N concentrations and leaf potassium content, while leaf tannin concentration and other leaf chemical traits had no effect (Table 4, Fig. 4a). However, LT (a defensive trait) and tree height had negative effects on Lepidoptera species co-occurrence across tree species level (Table 4, Fig 4b, c).

At the plot level, Lepidoptera species co-occurrence was significantly and negatively related to tree species
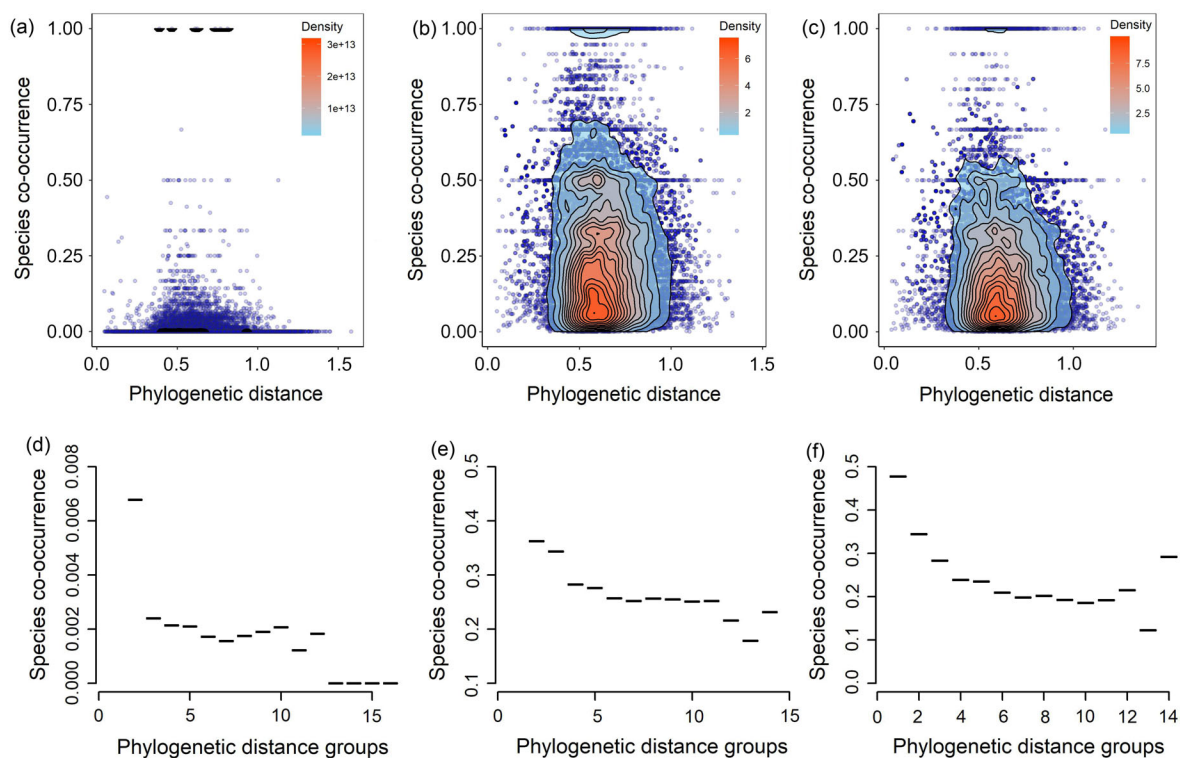
FIG. 2. Relationship between Lepidoptera co-occurrence and phylogenetic distance at (a) tree individual level, (b) tree species level, and (c) study plot level. Relationships between the mean values of co-occurrence and grouped phylogenetic distance at (d) tree individual level, (e) tree species level, and (f) study plot level. Density scatter plots with colors representing density of co-occurring species pairs.

TABLE 2. Correlations between observed co-occurrence and phylogenetic distance, and null model results for comparing randomizations of co-occurrence and phylogenetic distance matrix.

Spatial scale	Observed correlation	P_{obs}	Rank	$P_{obs.\ rand}$
Tree individual level	−0.02	<0.001	1	0.001
Tree species level	−0.03	<0.001	1	0.001
Plot level	−0.04	<0.001	1	0.001

Notes: P_{obs}: *P*-value of observed Pearson correlation of between co-occurrence and phylogenetic distance (standard *P*-value for correlation coefficient, not based on comparison with randomizations); Rank: Rank of observed correlation vs. random; $P_{obs.\ rand}$: *P*-value of observed correlation vs. randomizations.

richness across plots, but unrelated to abundance of caterpillars (Table 4, Fig. 4d). Tree MPD had similar, negative effects on Lepidoptera species co-occurrence as the correlated variable tree species richness when it replaced the latter as a predictor (Appendix S1: Table S6, Fig. 4e). Moreover, CWM carbon concentration, CWM LA, and tree MNTD in site B also had negative effects on species co-occurrence. In contrast, CWM C:N concentrations and CWM phosphorus content were positively associated with Lepidoptera

species co-occurrence. CWM LA also had negative effects on co-occurrence (Table 4, Fig. 4f). The results were not dependent on interactions with the study site, meaning that there was no variability in effects between the two study sites. VIF for linear models were <2.9 in all cases, suggesting that multicollinearity had little influence on the analysis.

DISCUSSION

In this study, we found Lepidoptera species co-occurrence to be negatively associated with phylogenetic distance of both Lepidoptera and trees (although some species with medium phylogenetic distance had higher co-occurrence than the species with lower and higher phylogenetic distance), with phylogenetic clustering patterns across the three spatial scales, supporting an overall strong effect of environmental filtering (Hypothesis 1). We also found that Lepidoptera species co-occurrence was positively associated with nutritional functional traits (e.g. C:N concentrations), but negatively associated with defensive traits (e.g. LT) and the tree diversity of the tree communities, suggesting food abundance enables coexistence, although defensive traits do not, probably due to selection pressure on Lepidoptera leading to niche separation (Hypothesis 2).
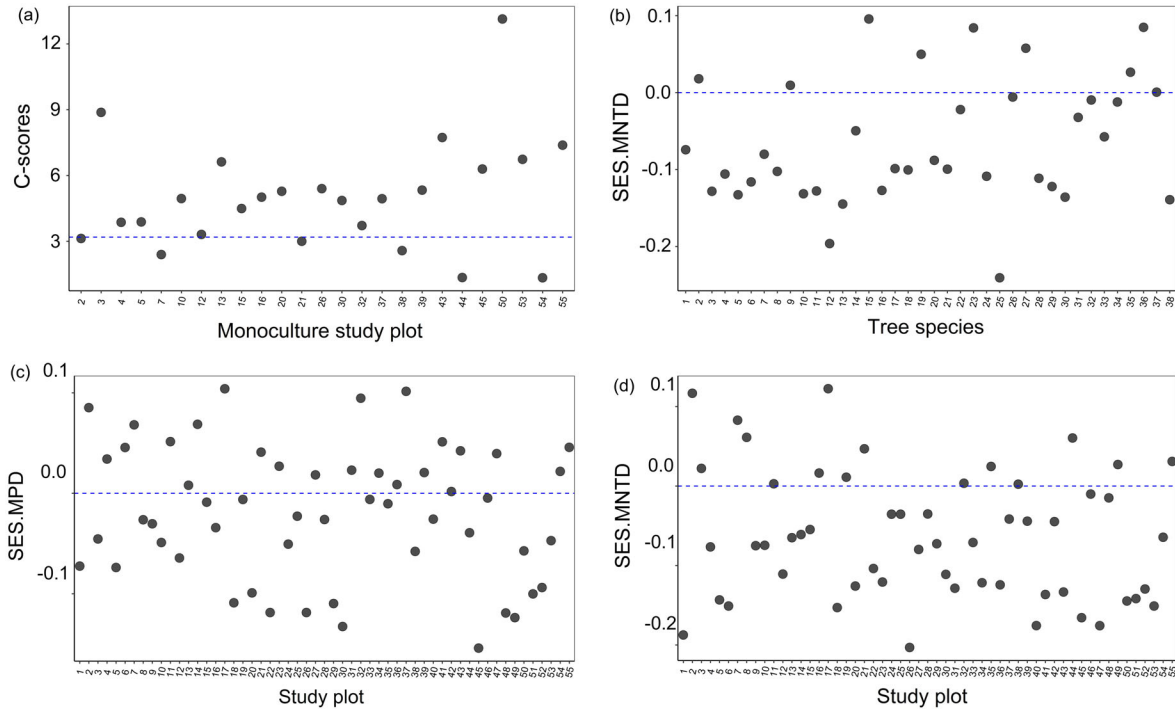
Fig. 3. (a) Pattern of C-scores of individual trees across monoculture plots (black points) compared with all monoculture plots (blue dashed line represent 3.19, which is the C-score of all monocultures; mean = 5.02, $t = 3.6$, $df = 24$, $P < 0.001$); standardized effect size (SES) value of MNTD across all tree species (b); SES value of MPD (c), and MNTD (d) across all plots. The dashed blue line (a) represent C-score of Lepidoptera species across all monoculture plots; the other three dashed blue lines mark zero. For more details and the code of tree species and plot see Appendix S1: Tables S2–S5.

TABLE 3. Summary results of t-tests for standard effective size (SES) value of mean pairwise distance (MPD) and mean nearest taxon distance (MNTD) across tree individual, tree species, and plot level.

	Mean	t	df	P
Tree individual level				
MPD	−0.006	−1.99	214	0.024
MNTD	−0.012	−4.25	214	<0.001
Tree species level				
MPD	−0.012	−0.96	37	0.170
MNTD	−0.065	−4.39	37	<0.001
Plot level				
MPD	−0.021	−2.62	54	0.005
MNTD	−0.068	−6.13	54	<0.001

TABLE 4. Summary results of final linear models for (a) co-occurrence at tree species level, (b) co-occurrence at plot level.

	Est.	SE	t	P
(a) Co-occurrence at tree species level				
(Intercept)	0.249	0.003	95.21	<0.001
C:N	0.012	0.004	3.16	0.006
K	0.010	0.004	2.74	0.015
Ca	0.009	0.004	2.30	0.036
LT	−0.015	0.004	−3.56	0.003
Tree height	−0.010	0.004	−2.73	0.016
ΔAICc	59.03			
(b) Co−occurrence at plot level				
(Intercept)	0.205	0.004	50.39	<0.001
Tree species richness (log)	−0.007	0.003	−2.31	0.026
Tree MNTD	0.004	0.004	1.03	0.310
Site B	0.011	0.007	1.52	0.136
CWM LA	−0.013	0.003	−3.99	<0.001
CWM C:N	0.019	0.004	4.44	<0.001
CWM C	−0.009	0.003	−3.01	0.004
CWM P	0.013	0.004	3.64	<0.001
Tree MNTD: Site B	−0.016	0.005	−2.94	0.005
ΔAICc	51.85			

Notes: Standardized parameter estimates (with standard errors, t and P-values) are shown for the variables retained in the minimal models. ΔAICc values show difference between initial and final model ΔAICc values.

However, Lepidoptera species co-occurrence was much lower at smaller spatial scales (individual tree level), compared with larger (tree species or plot levels), supporting the competition exclusion principles for the smallest spatial scale, i.e. the scale in which direct species interactions are most likely to occur (Hypothesis 3). This result was also confirmed by the checkerboard patterns of species distributions at individual tree level in monoculture plots. Therefore, our results revealed multiple forces driving species coexistences in a plant–insect system.
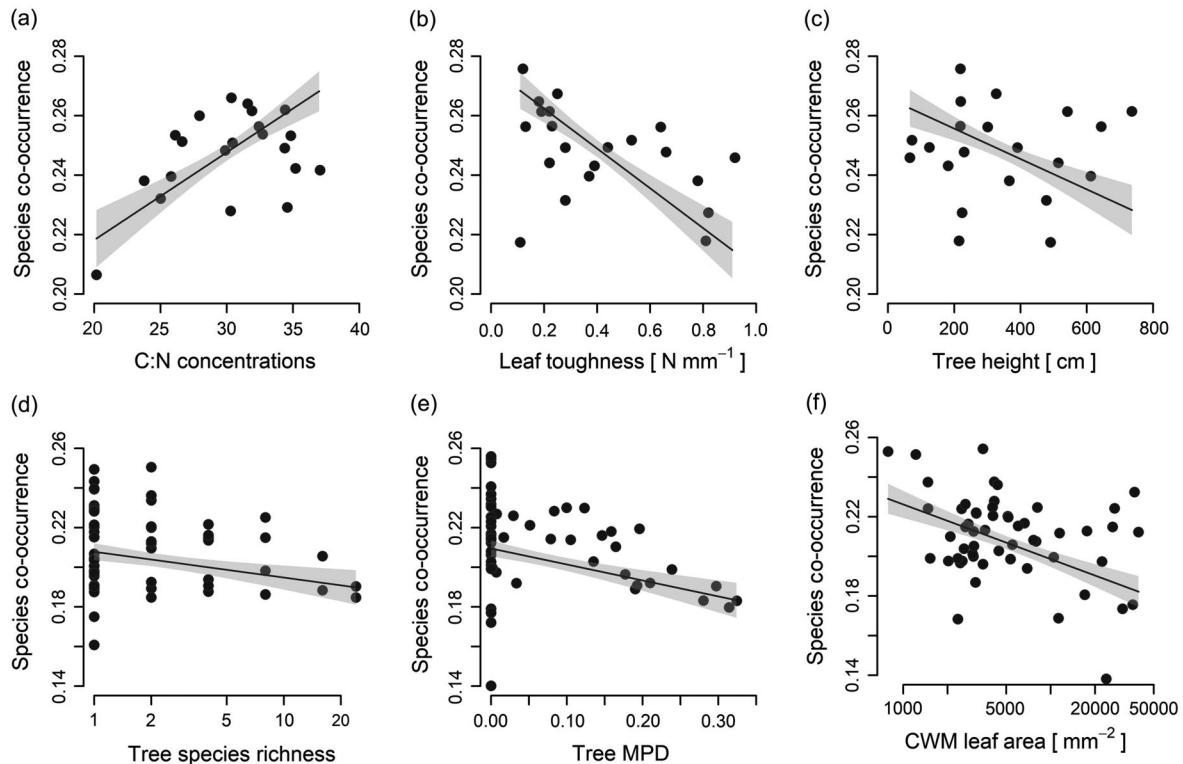
FIG. 4. Relationships between co-occurrence and (a) ratio of leaf carbon to nitrogen concentrations, (b) leaf toughness, and (c) tree height at tree species level; as well as relationships between co-occurrence and (d) tree species richness, (e) tree mean pairwise distance, and (f) community-weighted mean value of specific leaf area at study plot level. Values were adjusted for covariates of the final regression model. Regression lines (with 95% confidence bands) show significant ($P < 0.05$) relationships. Note that axes are on a log scale for tree species richness and community-weighted mean value of specific leaf area.

Environmental filtering

The negative relationship between Lepidoptera species co-occurrence and phylogenetic distance (even the low correlation coefficients between co-occurrence and phylogenetic distance; see also Cavender-Bares et al. 2004) that was observed at each scale (Table 2, Fig. 2; Appendix S1: Fig. S4) suggests that the patterns of co-occurrence are strongly affected by environmental/niche filtering and/or evolutionary processes, as similarly found with vertebrates (Lovette & Hochachka 2006, Cardillo 2011, Yan et al. 2016). Moreover, the key role of environmental filtering for the community assembly of Lepidoptera species is also supported by the significant phylogenetic clustering patterns of SES MPD and SES MNTD across the three spatial scales (except for MPD at tree species level, Table 3, Fig. 3; Appendix S1: Fig. S5). Interestingly, clustering was observed in SES MNTD but not SEM MPD at tree species level, which was unexpected. Previous studies have found that MPD usually shows more overdispersion than MNTD (e.g. Bose et al. 2019), indicating that overdispersion is not only caused by close relatives (lower MNTD from terminal branching), but also by more distinct relatives (basal branching represented by MPD). These results suggest

distinct evolutionary processes and influences of environmental filtering when considering complementary metrics of phylogenetic structure sensitive to either basal or terminal branching (Mazel et al. 2016). A related study at our study sites further showed that Lepidoptera MPD increased with tree species richness (Wang et al. 2019), supporting our finding that Lepidoptera co-occurrence decreased with tree species richness. Moreover, the study also indicated that two tree functional traits (SLA and LT) with a strong phylogenetic signal had significant effects on Lepidoptera MPD and MNTD, respectively, across the study plots (Wang et al. 2019). In general, coexisting relatives are impacted by competition as described by the competitive exclusion principle (Hardin 1960), which gives the prediction that increasing phylogenetic distance should lead to greater degrees of co-occurrence due to decreased resource competition (Weiher & Keddy 1999). Phylogenetic overdispersion and phylogenetic clustering can be caused by competition and environmental filtering, respectively. However, phylogenetic overdispersion (repulsion) can result from either competition or environmental filtering. On the one hand, phylogenetic overdispersion could come from competition between closely related species (low phylogenetic distance) that have similar niches (e.g.

functional traits of trees). On the other hand, phylogenetic overdispersion could also come from environmental filtering of distantly related species (high phylogenetic distance) that have converged on similar niches (Webb et al. 2002). Our study showed that two functional traits (LA and LT) with a strong phylogenetic signal have negative effects on Lepidoptera co-occurrence, while at the same time co-occurrences were negatively related to Lepidoptera phylogenetic distance. This supports the assumption that the main process of Lepidoptera community assembly at the scale of the tree species and of the plot was environmental filtering. This filtering was caused by the two traits LA and LT. However, the more persistent association that a herbivore insect has with its host plant through its life cycle is expected to lead to a conserved pattern in the evolution of interactions between plants and herbivores (Pellissier et al. 2013, Wang et al. 2020). This pattern is more apparent at fine spatial scales in our experimental site, because closely related herbivores tend to share traits of resistance against plant defenses, resulting in similar dispersal patterns and a high probability of co-occurrence. Previous studies in our site have shown non-random associations between herbivores and plants when phylogenetic compositions were taken into account (Wang et al. 2020). It might be that caterpillars are more specialized than other herbivorous insects (Novotny et al. 2010, Forister et al. 2015, Zhang et al. 2017, Wang et al. 2020). Our data showed that environmental filtering is an important determinant for coexistence, which is not consistent with the high speciation–low extinction hypothesis (high co-occurrence with increasing of phylogenetic distance; e.g. Tamma & Ramakrishnan 2015). Alternatively, assembly processes in a highly speciose system could be affected by speciation and colonization rates, although we observed a negative association for some plant species (e.g. Cardillo 2012). A probable explanation is that plants are under selective pressure for rapid growth, prompt maturation, and seed dispersal (Lamont & Markey 1995, Bond & Midgley 2001). Animals enjoy mobility for selection of suitable habitats and attractive niches within a heterogeneous environment (Rosenberg & McKelvey 1999), particularly, herbivores target leaves with high palatability, as commonly measured in leaf functional trait studies (Foley & Moore 2005, Schuldt et al. 2012, López-Carretero et al. 2016).

The competition exclusion effect

We found that co-occurrence was lowest at finer spatial scale, which appears consistent with competition exclusion principles (Hardin 1960). This result also implies that the mechanisms structuring species assemblages may differ according to scale, particularly in their phylogenetic structure (Weiher & Keddy 1999, Cavender-Bares et al. 2006, Emerson & Gillespie 2008, Kooyman et al. 2011). Previous studies have suggested that phylogenetically clustered species assemblages are

frequently observed at larger scales, and that species assemblages with larger phylogenetic distance are more frequently detected at small spatial scales (Cavender-Bares et al. 2004, Emerson & Gillespie 2008, Kooyman et al. 2011). Moreover, for plant communities, phylogenetic clustering/overdispersion had been also shown to be associated with changes in spatial scale in some empirical studies, which found strong competition and high environmental filtering at small and larger spatial scales, respectively (e.g. Kembel & Hubbell 2006, Swenson et al. 2007, Cavender-Bares et al. 2009). Here, our results suggest that competitive exclusion is more apparent at small spatial scales, as the degree of species relatedness in phylogeny will increase along with decreases in spatial scale (see also Roughgarden 1983, Tilman 1994). In line with previous studies, this pattern could be caused by spatial heterogeneity in the environment that can drive coexistence mechanisms and comes into play at larger scales, overriding the competitive exclusion that operates at small scales (Tilman 2004, Davies et al. 2005).

In our case, current coexistence patterns are suggested to be the outcome of competition between two herbivore species over ecological time. As the Lotka–Volterra competition model describes, one species can competitively exclude another in ecological time due to competition for food resources or environmental limitation (Goel et al. 1971, Lotka 2002). We observed that herbivore species co-occur more at larger spatial scales than at small scales, because resource partitioning at larger scales (greater food resources and environmental capacity) results in decreased or even the absence of competition between a species pair. Moreover, the response of species to interspecific competition in evolutionary time often opposes that of ecological time; competitive herbivores generally will specialize in plants with particular palatability (resource types) and coexist (Huntly 1991). It is interesting to note that Lepidoptera species pairs with a phylogenetic distance near the average appear to more easily co-occur at any spatial level (Fig. 2). This means that herbivore species at this level of relatedness show a set of features amenable to co-occurrence, regardless of whether the coexisting pattern was driven primarily by environmental filtering.

Impacts of functional traits

Tree species richness, MPD and leaf traits partly determined Lepidoptera co-occurrence (Fig. 4). The negative relationships between tree species richness/tree MPD and species co-occurrence at plot level were expected, because the pattern of Lepidoptera co-occurrence was mainly driven by environmental filtering based on our results at this spatial scale. Furthermore, the significant effects of tree leaf traits on co-occurrence indicated that traits correlated with leaf palatability can also play a role in the pattern of herbivore coexistence, via interspecific interactions (see also Nakadai et al.

2018, Wang et al. 2020). This pattern was determined mainly by species niche divergence to defensive traits of trees. Taken together, our study showed that phylogenetic distance of herbivores, as well as diversity and functional traits of their host plants, jointly drive herbivore coexistence.

Tree species richness and MPD had negative effects on Lepidoptera co-occurrence, supporting our findings that environmental filtering plays a key role in Lepidoptera co-occurrence (Fig. 2). In general, a tree community with lower tree species richness and MPD reflects Lepidoptera communities with a relatively higher similarity in host plant utilization and homogenous natural resources, which might lead to high co-occurrence probabilities (Nakadai et al. 2018). Additionally, more niche opportunities and resources would be expected to be provided with increasing tree species richness, and species coexistence patterns might be affected by the co-occurrence "dilution effect," which means that some instances (highly associated species; e.g. competitors) could be obscured within the noise of other varied factors (unrelated species) as shown in previous studies (e.g. Gilpin & Diamond 1982, Graves & Gotelli 1993, Both et al. 2011). In our case, the co-occurrence patterns caused by competition might be difficult to predict, because non-competing species (unrelated species) were included in the dataset from higher tree species richness (Gilpin & Diamond 1982).

Our findings provide new insights into species coexistence of insect herbivores, as mediated by host plant diversity. These results might reflect phylogenetic conservatism in interactions between host plants and herbivores, as we found herbivore species with low phylogenetic distance more likely to co-occur on tree species with a similarly low phylogenetic distance (Appendix S1: Fig. S6; see also Wang et al. 2020). Previous findings have suggested that phylogenetically conserved associations between plants and herbivores are responsible for structuring herbivore species co-occurrence patterns to some extent (Pellissier et al. 2013, Wang et al. 2020). More evolution involved phylogenetic conservatism results were found in some plant defensive traits (Winemiller et al. 2015, Zhang et al. 2016, Fontes et al. 2020), and we also found a strong phylogenetic signal in the distribution of trait values among the tree species at our study sites (Wang et al. 2019). Of the 10 leaf traits considered in our study (Table 1), four showed a significant phylogenetic signal (SLA, LDMC, LT, LA; Appendix S1: Table S7). Therefore, such leaf traits might drive the phylogenetic conservatism of herbivore resistance (Petschenka et al. 2017), which can further mediate the pattern of herbivore species co-occurrence through non-random species interactions.

An important finding of our study is that herbivore species co-occurrence can be affected by both nutritional and defensive traits, such as C:N and LT at tree species level. In general, the co-occurrence pattern of herbivores might be negatively affected by lower nutritional

content. For C:N, nitrogen content is often included in N-based compounds that are used as a defense against herbivores (Baraza 2007), and it usually correlated with leaf palatability (Coley & Barone 1996, Eichhorn et al. 2007). In addition, herbivore performance will be higher with increasing nutritional content of their hosts, as predicted by the plant vigor hypothesis (e.g. De Bruyn et al. 2002), which could be another potential interpretation for the herbivore co-occurrence positively relating to nutritional contents. However, as a defensive trait, high levels of LT can restrict host use to rather specialized herbivores (López-Carretero et al. 2016, Wang et al. 2020). Co-occurrence decreased with increasing LT, which could result in a decrease in common caterpillars (and therefore representing lower co-occurrence) because only some specialists can cope with high LT. In contrast, Lepidoptera species that can attack leaves with high concentrations of defense compounds might share similar niches and also probably have close relationships in phylogeny (low phylogenetic distance) (Nakadai et al. 2018). However, LT had no effect on Lepidoptera co-occurrence at the tree community level in our study, possibly because additional tree species with relatively low LT might provide alternative resources for caterpillar competitors at larger spatial scales. In this way, plant defensive traits could lead to niche separation of herbivores and promote coexistence via trait adaptations (e.g. Klauschies et al. 2016). Therefore, the alternative resources for caterpillar competitors could dilute species co-occurrence and lead to complex patterns of co-occurrence. Interestingly, we found that Lepidoptera co-occurrence was negatively affected by tree height, this result is probably caused by larger spatial areas occupied by trees with increasing height. The non-significant relationships between Lepidoptera abundance and co-occurrence at plot level indicate that the coexistence patterns were not affected by Lepidoptera species dispersal, although distributions and co-occurrence patterns of other species were previously interpreted by random dispersal (Ulrich & Zalewski 2006). A previous study showed that the associations between host plants and Lepidoptera herbivores in our study sites are phylogenetically structured, suggesting some degree of phylogenetic conservatism (host specificity) for different Lepidoptera families (Wang et al. 2020). As the effects of phylogenetically conserved plant traits on co-occurrence suggest, Lepidoptera species might be filtered by these plant traits. If competition plays the dominant role at tree species/plot scale, we should expect the opposite pattern (i.e. higher co-occurrence for phylogenetically more distantly related species), because competition would exclude those species with similar resource requirements (which are the closely related ones in our case). We note that our samples are collected from three seasons and might be affected by Lepidoptera phenology. However, because of limited sample size per sampling period, we were unable to analyze phenology effects on co-occurrence patterns at the three spatial

scales for each season (especially for individual trees), which means that our analysis largely represents the co-occurrence patterns for the entire growing season of caterpillars. Of course, it will be an interesting potential perspective for further research to address how such co-occurrence patterns, especially at the level of individual trees, are affected by Lepidoptera phenology and seasonal changes in herbivore communities.

Overall, current knowledge of co-occurrence patterns of herbivore is still inadequate, especially for key herbivore assemblages in highly diverse forests. Our findings provide new insights into how coexistence pattern of herbivores are driven by herbivore phylogenetic relatedness and the functional traits and diversity of their host plants. Our results depict for a highly diverse group of insect herbivores that various driving forces, in particular environmental filtering, play a significant role in determining the species coexistences in herbivore communities via species interactions (see also Nakadai et al. 2018), and that the relative importance of these forces varies from local to larger spatial scales within the host communities. Therefore, trophic interactions should be considered to study problems on species coexistence pattern in the future. This approach has rarely been applied in previous studies of species coexistence, but can provide a better knowledge of comprehensive understanding for species coexistence mechanism.

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Supporting Information

Additional supporting information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecm.1492/full

Open Research

Plot and species data are available on the BEF-China database (plot data: https://data.botanik.uni-halle.de/bef-china/datasets/641; species data: https://data.botanik.uni-halle.de/bef-china/datasets/642). Sequence data can be accessed on GenBank (accession numbers: MN131188–MN132787), and on BOLD (BEFCN001-19–BEFCN1600-19). The FASTA file of the sequences (Wang et al. 2021) is available on the Dryad data repository at https://doi.org/10.5061/dryad.9zw3r22f9. The analysis pipeline and Perl scripts (Chesters 2021) are available on Zenodo at https://doi.org/10.5281/zenodo.5577080