Host specificity and interaction networks of insects feeding on seeds and fruits in tropical rainforests.

by Basset, Y., Jorge, L.R., Butterill, P.T., Lamarre, G.P., Dahl, C., Ctvrtecka, R., Gripenberg, S., Lewis, O.T., Barrios, H., Brown, J.W., Bunyavejchewin, S., Buntika, A.B., Cognato, A.I., Davies, S.J., Kaman, O., Klimes, P., Knížek, M., Miller, S.E., Morse, G.E., Novotny, V., Pongpattananurak, N., Pramual, P., Quicke, D.L.J., Sakchoowong, W., Umari, R., Vesterinen, E.J., Weiblen, G., Wright, S.J. and Segar, S.T.

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Abstract

In the tropics, antagonistic seed predation networks may have different properties than mutualistic pollination and seed dispersal networks, but the former have been considerably less studied. We tested whether the structure of antagonistic tripartite networks composed of host plants, insects developing within seeds and fruits, and their insect parasitoids could be predicted from plant phylogenetic distance and plant traits. We considered subsets of the networks ("subnetworks") at three rainforest locations (Panama, Thailand, Papua New Guinea), based on insect families, plant families or plant functional groups. We recorded 3,197 interactions and observed a low percentage of realized interactions, especially in Panama, where insect host specificity was higher than in Thailand or New Guinea. Several factors may explain this, including insect faunal composition, incidence of dry fruits, high fruit production and high occurrence of Fabaceae at the Panamanian site. Host specificity was greater among seed-eaters than pulp-eaters and for insects feeding on dry fruits as opposed to insects feeding on fleshy fruits. Plant species richness within plant families did not influence insect host specificity, but site characteristics may be important in this regard. Most subnetworks were extremely specialized, such as those including Tortricidae and Bruchinae in Panama. Plant phylogenetic distance, plant basal area and plant traits (fruit length, number of seeds per fruit) had important effects on several network statistics in regressions weighted by sampling effort. A path analysis revealed a weak direct influence of plant phylogenetic distance on parasitoid richness, indicating limited support for the "nasty host hypothesis". Our study emphasizes the duality between seed dispersal and seed predation networks in the tropics, as key plant species differ and host specificity tends to be low in the former and higher in the latter. This underlines the need to study both types of networks for sound practices of forest regeneration and conservation.

Key words: Barro Colorado Island; functional group; nasty host hypothesis; plant phylogeny; quantitative food web; seed predation.

Introduction

Community assembly and the relationships among interacting species are frequently studied using ecological interaction networks (Jordano et al. 2003, Blüthgen et al. 2006, Poisot et al. 2015, Dáttilo & Rico-Gray 2018), as the structure of these networks may be critically linked to the dynamics and stability of interacting species within the community (Paniagua et al. 2009). Mutualistic networks involving the processes of pollination and seed dispersal have been relatively well studied in tropical rainforests, often emphasizing vertebrates (e.g., Olesen & Jordano 2002, Schleuning et al. 2012, Escribano-Avila et al. 2018). To date, antagonistic networks, incorporating information on the frequency of each trophic interaction and emphasizing invertebrates in rainforests have been assembled for leaf miners, gallers, leaf-chewers, their hosts and their parasitoids (review in Morris et al., 2014), and, more rarely, for seed predators (Gripenberg et al. 2019).

In tropical rainforests, insects are the main seed predators, especially before seed dispersal (Janzen 1971). Insects that kill seeds either before or after dispersal influence the population dynamics of individual plant species, and ultimately, plant diversity and assemblage composition (Lewis & Gripenberg 2008). In addition to true seed predators, other functional groups of insects, notably in Diptera and Lepidoptera, feed on the fleshy parts of fruits (Ctvrtecka et al. 2016). This guild of "pulp eaters" (as opposed to "seed eaters") can cause fruit abortion and fall, with consequences for plant population dynamics (Stephenson 1981). In the tropics data regarding insect assemblages feeding on seeds/fruits are infrequent (Gripenberg 2018). So far, interaction networks have been built for tephritid flies breeding in tropical flower heads (Prado & Lewinsohn 2004) or in tropical fruits (Novotny et al. 2010), and for the whole assemblage of seed predators in one Panamanian rainforest (Gripenberg et al. 2019).

Concealed insect herbivores, such as seed/fruit predators, are more specialized than insect herbivores that feed externally (Novotny & Basset 2005). Studies in tropical rainforests have often confirmed the high host specificity of seed/fruit predators (Janzen 1980, Hopkins 1983, Nakagawa *et al*. 2003, Copeland et al. 2009, Ctvrtecka et al. 2014, Sam et al. 2017, Gripenberg et al. 2019). Since foliar chemistry and plant phylogeny predict patterns of host use by caterpillars in tropical rainforests with high concordance (Segar et al. 2017, Volf et al. 2017), we expect that plant phylogeny may also influence assemblages of seed/fruit insects in tropical rainforests.

Resource availability, such as the production of young leaves, is key to understanding the local distribution of insect folivores in tropical rainforests (e.g., Basset 2001). Likewise, it may be crucial to explain the structure and species interactions in assemblages of insects feeding on seeds/fruits in tropical rainforests. In mutualistic networks, biotic specialization decreases with increasing

plant diversity, because high plant diversity may reduce relative plant abundance and related plant resources (Schleuning et al. 2012). However, antagonistic and mutualistic networks may be structured differently (Morris et al. 2014).

We expect that plant phylogeny (Segar et al. 2020), local plant diversity and abundance, seed availability and functional plant traits (Basset et al. 2018) may influence interaction networks involving insects breeding in seeds and fruits in tropical rainforests. In this contribution we test whether the structure of antagonistic tripartite networks composed of host plants, insects breeding in seeds and fruits and their insect parasitoids at three representative rainforest locations within different biogeographical regions can be predicted from different plant variables. Because of the high diversity of our study systems (see results), we consider subsets of the overall networks ("subnetworks") at each location, either based on insect families, plant families or plant traits. We answer the following questions.

1. Accounting for host-plant phylogenetic relatedness, does herbivore host specificity vary among (a) insect families or feeding guilds? (b) plant families or functional groups? and (c) three tropical forests?

One important variable accounting for network structure is specialization (Blüthgen et al. 2006), which is positively related to host phylogenetic isolation (Jorge et al., 2017). Host specificity is likely to differ among insect guilds associated with fruit pulp versus seeds, as seeds are better chemically protected than pulp (Janzen 1971). Plant traits may also influence seed predator load and host specificity (Janzen 1971, 1980, Basset et al. 2018, Dahl et al. 2019). Low plant richness may favor high insect host specificity, as suggested by comparisons of insect herbivores in temperate and tropical forests (Novotny et al. 2002).

2. Does plant phylogenetic relatedness or plant functional similarity explain the structure of interaction networks between seeds or fruits and their insect predators across study sites or across different local subnetworks?

Related insect herbivores tend to feed on related host plants (Ehrlich & Raven 1964), because related plants may often (Berenbaum 2001, Rønsted et al. 2012) but not always (Sedio et al. 2018) share similar chemical defences. In turn, specialized herbivores, particularly seed predators, may be adapted to detoxify chemical defences (Kergoat et al. 2005). Hence, the structure of plant-herbivore interaction networks may have a strong phylogenetic signal (Weiblen et al. 2006, Segar et al. 2020). Alternatively, plant apparency theory (Feeny 1976) has been incorporated into a framework of three syndromes of plant defence, including (1) tolerance/escape, (2) low nutritional quality and (3) high nutritional quality and defence (Agrawal & Fishbein 2006). Under this framework, plant functional traits may predict the structure of seed predator networks as well as, or better than, plant phylogenetic relatedness.

3. Does the species richness of parasitoid assemblages feeding on seed/fruit predators reflect the traits of host plants, i.e. do the effects of plants on herbivores cascade upwards to affect the next trophic level?

The nasty host hypothesis proposes that insect herbivores feeding on plant hosts with strong and/or distinctive chemical defenses may support reduced loads of parasitoids because herbivore tissues may be more toxic to parasitoids (Gauld et al. 1992). Thus, this hypothesis predicts that plant phylogenetic relatedness, as a surrogate for plant chemistry (Berenbaum, 2001), (a) should have a significant effect on the species richness of parasitoids, (b) that this effect should be strong, and (c) that this effect should also be positive (i.e., mean phylogenetic distance is predicted to be negatively correlated with parasitoid species richness).

Material and methods

Study sites

Our study sites are three ForestGEO lowland rainforest plots (Anderson-Teixeira et al. 2014) located in different biogeographical regions. Salient characteristics of the plots and seed/fruit samples are detailed in Basset et al. (2018) and in Table 1. Neotropical: Barro Colorado Island (BCI) is a 1,500-ha island created by the opening of the Panama Canal in 1914. The 50-ha plot is in the heart of the island, which is near the center of more than 700 km^2 of protected forests. Oriental: the 24-ha plot at Khao Chong (KHC) is in the protected forest of the Khao Ban Thad Wildlife Sanctuary in southern Thailand. Australasian: the 50-ha plot is located in the 10,000 ha Wanang (WAN) Conservation Area in Papua New Guinea. Marked differences between BCI and the two other sites include lower plant richness, higher percentage of species with dry seeds, higher percentage of Fabaceae species, higher average seed rain per plant species and lower ratio of realized to potential interactions (Table 1).

Plant surveys, phylogeny and functional traits

Field methods were similar for all study sites (details in Basset et al. 2018, Gripenberg et al. 2019). Plant surveys spanned several years at each site (Table 1; Appendix S1A). During the first study year at each site, we indiscriminately surveyed seeds and fruits of locally abundant tree, shrub and liana (more rarely herb) species, to obtain an overview of the local community. During subsequent study years at KHC and WAN, we restricted our sampling effort to the 10 plant families that were most common at each plot. Eight of these focal families were common to all sites: Annonaceae, Arecaceae, Euphorbiaceae, Fabaceae, Lauraceae,

Meliaceae, Rubiaceae and Sapindaceae. Unless specified, results are detailed for all host plant species. Seeds and fruits collected on plants or freshly fallen (without apparent decomposition) were opportunistically surveyed within and/or near permanent plots (from an area < 1,500-ha corresponding to the smallest study area, BCI). Rearing sample units consisted of 1 to 200 seeds and/or fruits collected from a single plant. We targeted as many individual plants as possible for each species, typically > 5. To evaluate the phylogenetic relationships between sampled host plant species at each site, we estimated the relationships between our focal species using the R package S.PhyloMaker (Qian & Yi 2016). We used the updated phylogeny and node ages derived from a sequence-based study by Zanne et al. (2014) as a Megatree. Our focal species were placed within the Megatree where possible and placed to family where not possible. This procedure generates three alternative topologies which differ with respect to the resolution of unplaced taxa. We selected the phylogeny derived from "Scenario 3" as this has been shown to be robust to uncertainty at the higher taxonomic level (Qian & Yi 2016). Note that polytomies in the phylogeny underestimated DSI* slightly for herbivores feeding on a few species within a family. However, we expect this effect to be quite small, especially because it does not apply to monophages that would still have maximum specialization.

To obtain similarity matrices of plant functional traits for each site, we first compiled a matrix of functional traits relevant to seeds and fruits for each plant species, including numerical and categorical variables (Appendix S1B). We then used hierarchical daisy clustering methods to identify functional groups. Finally, we used a mixed Principal Component Analysis (PCA) for numerical and categorical variables to interpret the functional groups. Scores of each plant species on the PCA axes were used to build similarity matrices of functional traits that were used in subsequent analyses. In sum, 1,186 plant species could be assigned to one of five functional groups, coded A, B, C, D or E (A: often fleshy green fruits; B; often dry dehiscent fruits; C: often fleshy orange fruits; D: often red fruits; E: often small fleshly black-green fruits). Appendix S1B provides details about the composition of the matrix of functional traits, computational steps and relevant references, as well as the results obtained.

Insect rearing and processing

Methods for rearing seed/fruits insects are detailed in Basset et al. (2018) and Appendix S1C. Insects were identified with the assistance of taxonomists (see Basset et al. 2018) and/or by molecular techniques (Appendix S1C). Insects reared from seeds/fruits were assigned to the following guild categories (Basset et al. 2018): seed eaters (coded as SE: larva feeding mostly on seed tissue), pulp eaters (PU: larva feeding mostly on mesocarp tissue), and parasitoids (PA: larva feeding on insect hosts). Seed and pulp eaters consisted mainly of seven taxa that represented most of the material reared and are considered in analyses restricted to insect taxa: Bruchinae, Scolytinae, Curculionidae others than Scolytinae (Coleoptera), Tortricidae, Pyralidae (Lepidoptera), Stratiomyidae and Tephritidae (Diptera). Bruchinae were well represented only at BCI. Hereafter for sake of

simplicity, we refer to these seven taxa as “insect families”. For parasitoids, analyses were restricted to Braconidae and Ichneumonidae because they represented most (69%) of the parasitoids reared from samples and their taxonomy was supported by molecular data.

Interactions, topologies and subnetworks

Trophic relationships were inferred from the number of primary consumers reared from samples of seeds/fruits of host plant species at the first trophic level (coded as level 1-2). For the third trophic level, we considered interactions between Braconidae and Ichneumonidae and their insect hosts (coded as level 2-3). Contrary to interactions between the first and second trophic levels, third level data only reflected expected interactions, not documented interactions, because parasitized hosts were not isolated and reared individually, the parasitoids instead being reared from samples including relatively high numbers of seeds and fruits. To assign putative hosts to each parasitoid species, we applied three simple rules, as detailed in Appendix S1D: (1) since many parasitoid lineages are rather conservative in host use, we followed Quicke (2015) to select the most likely host order or family. (2) In case of conflicts, we examined for each parasitoid species the consistency of co-occurrence with the putative host species in all samples from which the parasitoid species was reared. (3) Eventually, we considered the highest abundance of putative host reared in samples in which the parasitoid species was also reared. We considered expected interactions between hosts plants and parasitoids (coded as level 1-3) to answer Question 3.

We constructed tripartite and quantitative interaction networks for the three full networks at BCI, KHC and WAN and for meaningful subsets of the data. This approach was selected because of the complexity of the full networks, which involved an order of magnitude more interacting species than most published networks (see Results; Schleuning et al. 2012, de Aguiar et al. 2019). Breaking a complex network into smaller sub-networks can reveal interesting patterns (Lewinsohn et al. 2006, de Aguiar et al. 2019) and has been performed with networks including insects (Quinto et al. 2012), which are far more diverse than those based on vertebrates (Schleuning et al. 2012). Each of our three full networks can be viewed as a collection of empirical subnetworks built by sampling interactions of a particular taxonomic/functional group within a locality, a general approach which is consistent with most published networks (de Aguiar et al. 2019). In addition, subnetworks were relatively independent from each other, thus motivating analyses at the level of subnetworks (see “subnetwork structure” below). The meaningful subsets (hereafter “topologies” for sake of brevity and in reference to how subnetworks are arranged) were based on (A) insect taxa: the distribution of particular insect families on plant species (n=7 taxa, resulting in 19 subnetworks); (B) plant family: the distribution of insect species within particular plant families (n=8, 24 subnetworks); or (C) plant functional groups: the distribution of insect species on particular plant functional groups (n=5, 15 subnetworks). Topology (A) is more relevant to Questions 1 and 3 of the

Introduction, whereas topologies (B) and (C) are more relevant to Question 2. Topologies (A) and (C) included insects reared from all host plants at each site, whereas for topology (B) we restricted the data to focal plant families. Further, for topologies (B) and (C) we also included in subnetwork illustrations plant species that were surveyed but yielded no reared insects (these "empty hosts" were not considered in the calculation of subnetwork statistics, see below). Variables as surrogates of either resource availability or sampling effort are discussed in Basset et al. (2018). Here, we consider that resource availability is most accurately tracked as the square root of the number of seeds collected for each plant species.

Data analyses

Question 1: herbivore host specificity

Quantitative metrics accounting for network-wide specialization (Blüthgen et al. 2006, Dormann et al. 2009) may be biased by sample size (Morris et al. 2014) and by non-random sampling of the plant phylogeny (Redmond et al. 2018). To overcome these challenges, we calculated herbivore specificity with the rescaled distance-based specialization index (DSI* - Jorge et al. 2014, 2017), which measures trophic specialization by accounting for host phylogenetic relatedness and resource availability. This quantitative metric accounts for differences in abundance and sampling effort of consumers and is largely independent of sample size. Briefly, DSI* measures specialization as a deviation from a random expectation involving the mean pairwise phylogenetic distance between hosts and is rescaled to enable the comparison of consumers that differ in their recorded sample sizes. DSI* varies between −1 (maximum achievable generalization) and 1 (monophages or maximum achievable specialization; Jorge et al. 2017). At each site we calculated DSI* for all seed- and pulp-eating insect species considering all host plant data available. DSI* was not calculated for parasitoid species, as, due to many missing data, we could not build reliably a phylogeny for insect herbivores.

To answer Questions 1a-1c, we first tested for differences in DSI* among herbivore taxa and guilds (pulp and seed eaters), plant families and functional groups, and study sites. We used non-parametric Kruskal-Wallis tests because DSI* values were skewed towards high specialization. Then, we used DSI* as the response variable in a model including taxa, guilds, sites and taxa:site and guild:site interactions as independent variables. To control for variation among insect families on DSI* (excluding Bruchinae, collected only at BCI), we performed a linear mixed model with site and insect guild as fixed effects and insect family as a random effect. To control for variation among plant families, we performed a linear mixed model with mean DSI* of the herbivores feeding on each plant species as the dependent variable, site and plant functional groups as fixed factors, and plant family as a random factor. To evaluate the effect of local plant richness, we calculated the correlation between the average DSI*

of the insect assemblage feeding on each focal plant family at each study site (n=24 site-family combinations) and the species richness of these plant families at each site.

Question 2: subnetwork structure

We addressed Question 2 by modeling indices for network properties at the level of the full network or subnetwork (hereafter "network statistics"). Rather than focusing on any single metric, we calculated the following standard network statistics reflecting network structure (Morris et al. 2014): degree of compartmentalization (number of compartments); weighted quantitative network specialization index H2'; weighted quantitative generality (effective number of host species per consumer species); nestedness (specialization asymmetry); weighted quantitative vulnerability (effective number of consumer species per host plant species); and connectance (degree of redundancy in the study system). Appendix S1E describes these network statistics in greater detail. All network statistics were calculated with the R package Bipartite (Dormann et al. 2018). We also reported the number of species in both trophic levels and the sum of links for each subnetwork, as well as sampling intensity sensu Schleuning et al. (2012). Models were estimated separately for topologies A-C (insect families, plant families and plant functional groups) and trophic levels 1-2. We considered network statistics as independent data points in models, because of (a) no overlap between insect and plant species across study sites; and (b) for a particular site and topology, the average pairwise species overlap between subnetworks was 4.3%, 2.2% and 4.1% for herbivore families, plant families and functional groups, respectively. Network statistics were also calculated for trophic levels 2-3 and 1-3 (see Question 3, below). We did not calculate network statistics when subnetworks were too small (number of species in the lower level < 5).

We used null models to assess how network metrics deviated from those expected from a random distribution of interactions. Null models were implemented for three full networks and 58 subnetworks using Patefield's algorithm ("r2dtable" in Bipartite's nullmodel function¸ Dormann et al. 2018), in which the marginal species totals are constrained as per the respective observed networks. We ran 1,000 randomisations for subnetworks and 200 for full networks (due to the time and CPU demands of running analyses on large sparse networks). We evaluated whether network statistics differed significantly between study sites by performing simple Kruskal-Wallis and Dwass-Steel tests. We refined this analysis by using three types of regression models. To account for the effect of sampling effort, the number of observed interaction events (i.e., the number of links; Schleuning et al., 2012) was used as a weighting factor in each regression. The first type of regression (hereafter "model type I") included mixed models with network statistics as dependent variables, sites and insect guilds as fixed factors and topologies (insect and plant families, plant functional groups) as random factors.

Following Chamberlain et al. (2014), we modeled network statistics with beta regression (H2' and Connectance), generalized linear models with Poisson (Number of compartments) or Gaussian (all other statistics) error distribution, separately for each topology A-C. Models type II parsed the effects of plant phylogeny and of plant ecological variables (resource and functional traits), and were calculated as:

Network statistic = MPD + FDis,

where MPD is the average plant relatedness (mean phylogenetic distance between plant species included in subnetwork, calculated with the function mpd of the R package Picante, Kembel et al. 2010), and FDis is the functional dispersion within the subnetwork, calculated with the function fdisp of the R package FD (Laliberté et al. 2014). FDis quantifies trait diversity as the mean distance in multidimensional trait space of individual plant species to the centroid of all species (Laliberté & Legendre 2010). FDis was calculated with variables accounting for (a) plant resource (no. of stems and basal area in ForestGEO plots, seed rain (g dry weight x m^{-2}), equivalent to total fruit biomass and estimated from litterfall traps, Basset et al. 2018); and (b) plant traits (fruit length and weight (partly related to seed size and biomass), number of seeds per fruit). We used the function betareg of the R package betareg to perform beta regressions (Gruen et al. 2012). For other regressions, we performed model simplification to extract the variables with significant predictive power with the built-in functions glm and step (backward selection of variables) of the R package (R Core Team 2018). We eventually tested the significance of estimators by an ANOVA (type 2 test) with the function anova in the R package 'car' (Fox and Weisburg 2019).

A last series of models considered more specifically the effects of plant variables (hereafter "models type III"):

Network statistic = Plant species richness + MPD + CWM_1 + CWM_2 + … + CWM_n,

where Plant species richness was the number of confamilial species in ForestGEO plots (for topology B) or the number of plant species in functional groups in ForestGEO plots (for topology C), and CWM_n was the community weighted mean of trait *n*, weighted by the number of samples collected and calculated with function dbFD of R package FD (Laliberté et al. 2014; plant species richness could not be included as an independent variable for the topology based on herbivore families). Independent variables accounted for sampling effort, plant species richness, mean phylogenetic distance, plant resource and plant traits (as defined in models type II; only continuous variables). Before analyses, highly correlated variables ($r>0.7$) were removed from models. Regressions were calculated and the significance of estimators tested as described previously.

Question 3: parasitoid species richness

To approach question 3, we computed a path analysis with a bottom-up flow of correlations implying direct and indirect correlations between herbivore species richness, parasitoid species richness (dependent variables) and selected independent variables. This analysis was performed at the level of the plant species, considering all plant species (n= 618) at the three sites from which seed predators were reared. Independent variables included mean phylogenetic distance and variables related to plant resource or plant traits (Appendix S1F). They were selected based on (a) a rationale for each path explained in Appendix S1F; and (b) the best predictors in the regressions performed previously (see previous section and results). The mean phylogenetic distance of a plant species to all other plant species was calculated with the function cophenetic of the R package Picante (Kembel et al. 2010). The model was calculated with the Ωnyx software (von Oertzen et al. 2015).

Data deposition

Interaction data were deposited in figshare, https://doi.org/10.25573/data.11444571.v1. Molecular insect data were deposited in the following Barcode of Life projects (BOLD, www.boldsystems.org): BCI: 2,310 sequences in projects BCISP and PSPLP; KHC: 398 sequences in KHCSP and KHCTE; WAN: 1,646 sequences in WANSP, FRUT and CURCU. Full data for specimens sequenced (including those that failed), including images and host plants, are available on BOLD, accessible by DOI for the datasets dx.doi.org/10.5883/DS-BCISP (BCI), dx.doi.org/10.5883/DS-KHCFRUIT (KHC) and dx.doi.org/10.5883/DS-PNGFRUIT (WAN).

Results

We collected 1,163 kg of seeds and fruits, which produced 80,600 insects across the three sites (Table 1). The composition and species richness of the insect material is discussed elsewhere (Basset et al. 2018). This contribution analyzes the 3,197 interactions across a total of 1,176 plant, 1,015 herbivore and 318 parasitoid species at the three study sites (Table 1). Only 0.58% of the potential 553,160 interactions were realized (Table 1). Since most properties of subnetworks do not represent properties of whole networks (Jordano 2016), we detail network statistics for the full networks (level 1-2) of BCI, KHC and WAN in Table 1, for comparison with other studies. These results emphasize differences between BCI and the other two sites, which we analyze in more depth by considering subnetwork data.

Question 1: insect host specificity

Differences in the median value of DSI* across insect families were significant (W=114.0, $p < 0.001$, d.f.=6; Fig. 1b). Bruchinae were by far the most specialized taxa, followed by Pyralidae, Curculionidae, Scolytinae, Tortricidae, Tephritidae and Stratiomyidae (Fig. 1b). Seed eaters were significantly more specialized than pulp eaters (Mann-Whitney test, U=91.8, $p< 0.001$, d.f.=1; Fig. 1c). There were also significant differences between the median DSI* of insect faunas feeding across plant families (W= 50.9, $p > 0.001$, d.f.=7). For example, on average, insects feeding on Fabaceae were rather specialized whereas those feeding on Meliaceae were less so (Fig. 1d). Differences in median of DSI* for insects feeding across plant functional groups were also significantly different (W= 53.1, $p < 0.001$, d.f.=4). In particular, insects feeding on plants belonging to functional group B (dry dehiscent fruits) were far more specialized than those feeding on group C plants (fleshy orange fruits; Fig. 1e). Overall, insects feeding on dry fruits were significantly more specialized than those feeding on fleshy fruits (Mann-Whitney test, U=39.9, $p< 0.001$, d.f.=1; Fig. 1f). The percentage of true monophagous species (DSI*=1) was higher at BCI (69.5%) than at KHC (25.3%) and WAN (18.9%) and the median of DSI* was significantly different across sites (Kruskal-Wallis test, W=201.8, $p < 0.0001$, d.f.=2; Fig. 1a).

The mixed linear model with DSI* as dependent variable and insect family as random factor indicated that the effect of site was significant, but not that of insect guild (seed eater versus pulp eater; Table S1). When considering the mean DSI* of insect species feeding on plant families, a similar model indicated that the effects of both site and plant functional group (coded A to E, see Appendix S1B) were significant, but not their interaction (Table S1). There was no correlation between the average DSI* of insects feeding on the eight focal plant families at the three study sites and the local species richness of these plant families ($r = -0.16$, $p = 0.45$; Fig. S1).

Question 2: variables affecting the structure of subnetworks

We illustrate nine of the 58 subnetworks studied (Fig. 2) and detail characteristics of all subnetworks in Appendix S2. In null models, most (95.6%) observed network statistics deviated significantly from those expected from a random distribution of interactions (Table S2), with nestednes involved in nearly all cases where the deviation was not significant (Table S2). The distribution of the six main network statistics is summarized in Fig. 3 for the topology based on insect herbivore families and trophic levels 1-2. BCI subnetworks had significantly more compartments, significantly higher degree of specialization (H2'), and significantly lower effective number of host species per consumer species (generality) than KHC and WAN. In particular, for all herbivore families, H2' was also higher at BCI than at other sites (Appendix S2). Subnetworks based on Bruchinae, Tortricidae,

Curculionidae and Pyralidae were in general more specialized than those based on Tephritidae, Scolytinae and Stratiomyidae (Appendix S2). Food webs based on stratiomyid flies were rather unspecialized, rarely parasitized by braconids and their subnetwork at WAN represented the most unspecialized subnetwork of all subnetworks analyzed. Conversely, the most specialized subnetwork was based on Tortricidae at BCI, followed closely by Bruchinae at BCI (Appendix S2). Other network variables were not significantly different between sites (Fig. 3). Mixed models weighting the effect of sampling effort (models type I), for the topology based on insect families, confirmed the strong effect of sites on the number of compartments, H2' and generality (Table S3).

For the topology based on plant families, BCI had significantly more compartments than KHC and WAN, and KHC had significantly larger effective number of consumer species per host plant species (vulnerability) than BCI and WAN (Fig. S1). Nestedness and Connectance were not significantly different between sites for any of the topologies considered (Fig. S1). For the topology based on plant functional groups, the number of compartments and H2' were significantly higher at BCI than at the other sites, whereas generality and vulnerability were significantly lower at BCI (Fig. S2). Mixed models weighting the effect of sampling effort (models type I) for both topologies based on plant families and functional groups confirmed the significant effect of site on all network statistics (Table S3).

When parsing the effects of plant phylogeny and ecological variables (models type II, Table S4), the significance of effects could be ranked overall as mean phylogenetic distance (MPD) > functional dispersion (FDis). MPD was a significant predictor of network statistics calculated for plant functional groups (topology C), while FDis was more important to predict network statistics calculated for plant families (topology B). Note that for models based on plant families, the effect of MPD may be low due to the limited range of MPD within plant families. This also applies for FDIs in models based on plant functional groups.

Not surprisingly, models best explained by CWM (models type III) were related to plant functional groups (65-98% of variance explained, Table 2). Over the different topologies, the variation explained by the type III models was greatest for number of compartments and least for generality. Several variables were reasonably good predictors of subnetwork structure, in order of importance seed rain, mean phylogenetic distance and number of plant species, as well as number of seeds per fruit (Table 2). Plant species richness was a good predictor of network statistics (connectance, nestedness, generality), only for topology B based on plant families. In models describing network statistics for plant functional groups, variables related to plant traits were important to predict H2', while variables related to plant resources were important for number of compartments, vulnerability, connectance and nestedness (Table 2).

Question 3: species richness of herbivores and parasitoids

Our path analysis model explained 19% of the variance in the number of parasitoid species supported by each plant species (Fig. 4). As expected, significant paths existed between plant traits and herbivore species richness, and between plant resources and herbivore species richness. Mean plant phylogenetic distance influenced plant traits but not directly herbivore species richness. The strongest direct paths (as judged from standardized path coefficients) influencing parasitoid species richness originated from herbivore species richness (positive), mean phylogenetic distance (negative) and basal area (positive). Thus, although the effect of mean plant phylogenetic distance was significant and negative on parasitoid species richness (as predicted by the nasty host hypothesis of Gauld et al. 1992), its direct path was about five times smaller than the corresponding direct path originating from herbivore species richness, pointing to other explanations.

Discussion

In this contribution we examined the interaction networks involving seeds and fruits, the insects feeding on them and their parasitoids, at three tropical sites. To analyze the 3,197 interactions reported, we considered three "topologies" (how subnetworks are arranged) resulting in 58 different subnetworks, which were largely independent from each other. This strategy was possible because of the very low overlap of interacting species between subnetworks but may not be applicable to other types of networks, such as mutualistic networks. Topology A, based on families of seed predators, may be useful to entomologists, whereas topologies B and C (based on plant families and functional groups) may be more interesting to botanists. Some interactions may not have been documented in our study system, since attack rates were rather low (8.5% of seeds/fruits attacked, Basset et al. 2018) and substantial sampling effort may be required to rear insects attacking seeds and fruits, For example, Ctvrtecka et al. (2014) consider a minimum sample size of 5kg of fruits/seeds per plant species adequate to rear weevils feeding on fruits/seeds. This condition was achieved for only 3% of our plant species. Low sampling effort may result in inflated insect host specificity and network specialization (Blüthgen et al. 2006). Sampling effort in the field (collecting seeds/fruits, rearing insects) was higher at BCI than at the other sites (Table 1). Hence, we believe that the high host specificity documented at BCI is not an artefact. Another obvious limitation in our study was the indirect documentation of linkages between insect herbivores and parasitoids (see Methods). Some of the linkages reported here will need confirmation but given the limited data on tropical seed predators they are nevertheless valuable.

Insect host specificity

Insect host specificity varied significantly between insect families. While some seed- or fruit-feeding taxa are known to be extremely specialized in rainforests (Bruchinae: Janzen 1980, Curculionidae: Ctvrtecka et al. 2014), others are less so (Tephritidae: Novotny et al. 2010). These trends were confirmed in our study, which also indicated that Stratiomyidae, a taxon rarely considered in studies of frugivorous insects, are less specialized pulp eaters than Tephritidae. Seed-eaters were more host-specific than pulp-eaters, confirming that insect host specificity for tropical herbivore guilds in both temperate and tropical forests decreases in the sequence: seed-eaters > leaf-miners > pulp-feeders > leaf-chewers > sap-suckers > xylophages > root-feeders (Novotny & Basset 2005). This partially reflects the plant's allocation of nitrogen and chemical defences to the tissues consumed by these guilds, as young leaves are sometimes better defended than seeds (Janzen 1971, 1980, Bazzaz et al. 1987, Zangerl & Bazzaz 1992, Kergoat et al. 2005). Insects feeding on dry fruits were also more host specific than those feeding on fleshy fruits. Insect host specificity varied significantly among plant families and functional groups, and the effect of site was important in most of our analyses. Overall, we observed the lowest percentage of realized interactions and highest insect host specificity at BCI. This trend was apparent when considering both entire networks (Table 1) and subnetworks (Figs 1,3). Similar levels of host specificity for entire networks and across subnetworks may be explained by the preponderance of highly specialized fruit/seed consumers, with very few generalist consumers present in more than one subnetwork.

This high insect host specificity at BCI is likely to result from the following factors. (1) Insect faunal composition: highly host specific and diverse Bruchinae are prevalent at BCI and absent from KHC and WAN. (2) Fruit fleshiness: BCI has the highest proportion of dry fruits (supporting highly host specific insects), possibly because of lower rainfall at BCI as compared to our other sites (Kissling et al. 2009). (3) Fruit production: BCI has on average four times higher seed rain per plant species than KHC or WAN (i.e., high fruit production and low plant species richness: Table 1). Our regressions confirmed the importance of variables such as basal area or seed rain to predict insect specialization. (4) Fabaceae: there is a high percentage of Fabaceae species at BCI (Table 1), which support many host-specific insect species.

In mutualistic networks, such as pollination and seed dispersal networks, specialization decreases with increasing plant diversity. An explanation may be that high plant diversity reduces relative plant abundance and related plant resources, resulting in hard evolutionary constraints on specialists (Schleuning et al. 2012, Escribano-Avila et al. 2018). Antagonistic networks may be different (Morris et al. 2014). Lewinsohn and Roslin (2008) discuss the species richness and host specificity of folivorous insects in tropical rainforests, and our BCI data appear to follow their contention that high species richness may be promoted by more insect species per plant species (Table 1) or higher herbivore host specificity (Fig. 1a). In sum, low plant richness and high insect

host specificity at BCI suggests that antagonistic networks based on seed predation may follow the same rules as mutualistic networks, with low plant richness strengthening interactions and favoring high insect host specificity (Novotny et al. 2002).

Subnetwork structure

Our analyses emphasized the strong effects of site on the different network statistics. Plant assemblages at different rainforests may be phylogenetically different or may possess different traits, or both. Tree assemblages are phylogenetically distinct in many rainforests (Webb 2000) and there are important differences in seed functional traits between our rainforest sites (Appendix S1B: Table App2-S1; Dahl et al., 2019). Plant phylogenetic distance had an important effect on subnetwork structure (e.g., specialization H2' and generality in subnetworks based on insect families), but this effect was not overwhelming, as variables related to plant traits or plant resource were also important in this regard. A more explicit inclusion of the hierarchical structure of phylogenies in predicting interaction identities might provide increased explanatory power. Ideally, further analyses would include phylogenies for hosts, herbivores and parasitoids (Ives and Godfray 2006), but herbivore and parasitoid phylogenies are not currently available. Plant traits such as fruit length and number of seeds per fruit, were important predictors of network statistics (Table 2). Other variables related to host phenology, such as the duration of fruiting season and its synchronization within/among years, may well be important in this regard (Janzen 1976), but they could not be tested in this study, for lack of reliable data at all sites. Variables accounting for plant resource (basal area, seed rain) were also important whereas the effect of mean phylogenetic distance was not excessive. This would lend support to the modified plant defence theory (Agrawal & Fishbein 2006). Both plant resource and plant traits were reasonably good predictors of subnetwork structure, particularly for models based on plant functional groups, emphasizing the interest in this topology as a predictive framework for subnetwork structure.

Upward cascades in the subnetworks

The nasty host hypothesis (Gauld et al. 1992) argues that tropical plants often possess highly active chemical defenses, which may lead to greater host specialization and sequestration of secondary compounds in insect herbivores, and reduced loads of parasitoids on particularly well-defended host plants. However, to date, evidence in favor of this hypothesis is mixed (Quicke 2012, Morris et al. 2014). Alternatively, Smilanich et al. (2009) observed that secondary metabolites sequestered by herbivores may compromise their immune response, making them more vulnerable to successful parasitism (the "vulnerable host hypothesis"). In our study system, we used plant phylogenic distance as a surrogate for plant chemistry (Berembaum 2001), as chemical data for tropical fruits and seeds are limited (Gripenberg et al. 2018). Our path model indicated that most of the

455 explained variance in parasitoid species richness on host plants could be attributed to a direct path originating from herbivore
456 species richness, whereas the corresponding path originating from mean plant phylogenetic distance was less important.
457
458 The nasty host hypothesis (Gauld et al. 1992) explains parasitoid loads on plants principally with regard to plant chemistry. Were
459 this hypothesis correct, we would have expected a large direct path from mean plant phylogenetic distance to parasitoid species
460 richness. The larger direct path observed from herbivore species richness to parasitoid species richness seems rather consistent
461 with both the resource concentration and resource base hypotheses (Root 1973, Price 1992), predicting that local assemblages of
462 parasitoids may be more diverse when their herbivore hosts are diverse (Hawkins & Lawton 1987) and vulnerable (Smilanich et
463 al. 2009).
464
465 **Conclusions**
466
467 The stability of mutualistic networks is promoted by a highly connected and nested architecture, whereas stability in antagonistic
468 networks is promoted by a compartmentalized and weakly connected structure (Morris et al. 2014). The subnetworks with the
469 highest number of compartments were those based on Curculionidae, Fabaceae and functional group B (large dry fruits, protected
470 and dehiscent) at BCI. Webs that are strongly compartmentalised (i.e. have high modularity) might be expected to be stable
471 (across modules) because changes in abundance (or extinction) of individual species (within modules) are less likely to cascade to
472 affect nodes in other parts of the network beyond the affected compartment or module (Thébault and Fontaine 2010). In
473 antagonistic insect-plant networks where the host is immobile (a property that distinguishes them from many other food webs),
474 modularity will often result from trait matching and phylogenetic conservatism in plant traits. High levels of trait matching in
475 most cases will make insect herbivores particularly prone to co-extinction following loss of their host plants. Interactions such as
476 those for Stratiomyidae at Wanang which display lower trait matching may be more robust to random plant species loss but the
477 subnetwork overall will be less resilient to the loss of key nodes rich in fleshy fruit (e.g. well-connected plant genera).
478
479 Seed dispersal networks have on average a low specialization (H2') compared to our seed predation subnetworks (Blüthgen et al.
480 2007: average 0.28; average 0.79 for all our subnetworks). Low H2' promotes high redundancy and increased seed dispersal
481 (Blüthgen et al. 2007). Everything else being equal, plant species supporting generalist dispersers but specialized seed predators
482 with low attack rates may be able to produce large number of viable seeds and may be at an advantage over other plant
483 competitors. This is in line with the plant defence syndrome of high nutritional quality and defence (Agrawal & Fishbein 2006).
484 Further, reviewing seed dispersal networks in the tropics, Escribano-Avila et al. (2018) indicated that woody plants bearing small
485 juicy berries containing many tiny seeds often represent keystone species. From the viewpoint of conserving insects feeding on

fruits/seeds, the plants most important in seed predation networks are those which support many insect species (i.e., with high number of consumer species per host plant species, vulnerability). Although we did not study many of the plant families considered by Escribano-Avila et al. (2018), we note that our plant families with high vulnerability (Appendix S2) usually do not bear berries with tiny seeds. This indicates that, from a conservation viewpoint, key plant species in the tropics may differ between networks of seed dispersal and seed predation.

In summary, our study emphasizes the duality between seed dispersal and seed predation networks in the tropics as the former are not very specific whereas the latter are far more specialized and may include different key plant species. From the viewpoint of forest regeneration and conservation, this underlines the need to study both types of network including a variety of potential key plant species.

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635 Supplementary material at xxx.
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638 **Table 1.** Salient characteristics of the study sites and their plant, insect and network interactions. Plot data are from Anderson-

639 Teixeira *et al.* (2014).

640

Variable	BCI	KHC	WAN
Site physiognomy and structure:			
Coordinates	9.15°N, 79.85°W	7.54°N, 99.80°E	5.24°S, 145.08°E
Elevation (m)	120-160	120-330	90-180
Annual average Rainfall (mm)	2551	2665	3366
Annual average daily maximum air temperature (°C)	26.3	27.1	26.5
Number of tree species/genera/families recorded in plot	299/181/59	593/285/82	508/245/77
Percentage of plant spp. with dry seeds/fruits	56.8	26.0	28.0
Percentage of Fabaceae species to total spp. richness in plot	14.0	3.1	4.8
Total fruit production (seed rain; dry g x m-2 x yr-1)	108.0	7.0	10.8
Average fruit production per species (dry g x m-2 x yr-1)*	0.596	0.141	0.157
Plant samples:			
Years of collection	2010-2013	2013-2015	2013-2015
Number of plant species/plant families surveyed	497/82	357/66	332/67
No. plant species surveyed within the 10 focal families	218	171	170
Total number of seeds or fruits collected	208,508	39,252	122,976
Total weight of samples (kg)	380.2	343.2	439.9
Insect samples:			
Total number of insects reared	27,610	17,555	35,434
Number of individuals / species of seed eaters**	11,059/311	2,100/59	3,935/77
Number of individuals / species of pulp eaters**	5,670/214	7,265/161	9,403/193
Number of individuals / species of parasitoids***	775/161	359/61	961/96
Interactions:			
Number of interactions realized / % realized-potential****	892/0.26	917/1.01	1,388/1.20
Plant species with most seeds/fruit reared	*Mikania leiostachya*	*Caryota mitis*	*Mastixiodendron pachyclados*
Most abundant herbivore species	*Pagiocerus frontalis*	*Coccotrypes myristicaceae*	*Coccotrypes* sp.n.3
Most abundant parasitoid species	Dorylinae sp. 156	Alysiinae sp. 13	*Diospilus* sp. 2
Network statistics for full network (level 1-2):			
Average DSI* ± s.e.	0.906±0.013	0.577±0.029	0.503±0.028
Number of compartments	85	20	9
H2'	0.914	0.664	0.657
Generality	1.85	6.84	5.15
Nestedness	0.96	1.61	2.20
Vulnerability	2.23	4.69	6.05
Connectance	0.006	0.021	0.022

641 * Plant species recorded in litterfall traps
642 ** Seven focal taxa only, see methods
643 *** Braconidae and Ichneumonidae only
644 **** No. of interactions between levels 1-2 and 2-3; percentage of realized to potential interactions
645

646 **Table 2**. Summary of the best regression models (type III) with network statistics as dependent variables, for each topology considered (A-C). Independent variables include
647 Mean Phylogenetic Distance (MPD) and the community weighted mean (CWM) of functional traits (see text for details). *** p< 0.001, ** p<0.01, * p<0.05, independent
648 variables listed according to decreasing standardized coefficients, best estimators indicated in bold.

649
650

Topology/ Subnetwork variable	**Significant variables**	**R^2**	**F**	**p**	**AIC**
A. Insect families					
Specialization H2'	**MPD***, Fruit length***, Basal area***, No. seed per fruit***, Seed Rain***	0.400	2457.1	<0.001	-3670.1
Connectance	n.s.	-	-	-	-
No. compartments	**Seed Rain***, Fruit length***, MPD***, Basal area***, No. seed per fruit***	0.581	2637.9	<0.001	26903.0
Generality	**MPD****	0.500	15.9	<0.001	104.1
Nestedness	n.s.	-	-	-	-
Vulnerability	**Fruit length***	0.342	5.2	0.022	94.5
B. Plant families					
Specialization H2'	**No. seed per fruit***, Basal area***, MPD***, Fruit length***, Seed Rain***	0.107	446.1	<0.001	-1999.3
Connectance	**No. plant species***, MPD***, Basal area***, No. seed per fruit***, Fruit length***	0.618	1948.5	<0.001	-7143.4
No. compartments	**Basal area***, Seed Rain***, Fruit length***, No. plant species***, MPD**	0.925	18.5	<0.001	5481.6
Generality	**No. plant species***, Fruit length*	0.351	3.4	0.037	44.6
Nestedness	**No. plant species***	0.467	8.8	0.002	150.4
Vulnerability	n.s.	-	-	-	-
C. Plant functional groups					
Specialization H2'	**No. seeds per fruit***, Basal area***, Seed rain***, MPD***, Fruit length***	0.696	4169.4	<0.001	-10018.1
Connectance	**Seed Rain***, MPD***, Fruit length***, No. seed per fruit***, Basal area***	0.749	5125.6	<0.001	-20987.9
No. compartments	**Seed Rain***, Basal area***, Fruit length***, No. seed per fruit***, No. plant species***	0.987	56.9	<0.001	13211.0
Generality	n.s.	-	-	-	-
Nestedness	**MPD***, Basal area*	0.650	11.2	0.002	60.7
Vulnerability	**Seed rain***, Basal area**, MPD*	0.835	9.1	0.002	52.3

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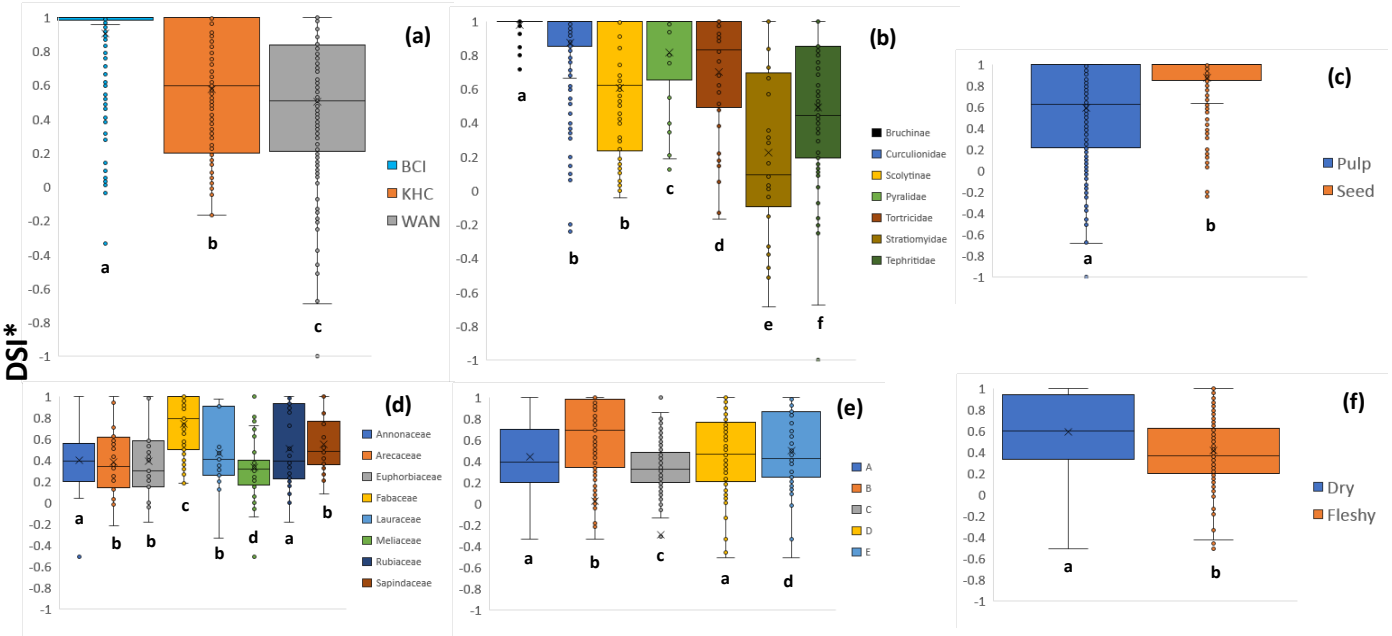
FIGURE CAPTIONS

Fig. 1. Summary distribution of the specialization index DSI*. Box and whisker plots across (a) sites, (b) insect families, (c) insect guilds, (d) plant families, (e) seed functional groups and (f) categories of fruit fleshiness. Groups with different letters are significantly different (Dwass-Steel tests, $p < 0.05$).

Fig. 2. Examples of interaction subnetworks at BCI (left), KHC (middle) and WAN (right). Top: topology based on insect family, here the Curculionidae (without Scolytinae). Middle: topology based on plant family, here the Fabaceae (including plant species lacking insects attacking seeds or fruits). Bottom: topology based on plant functional group, here Group B (dry fruits, protected, dehiscent and relatively large, see Appendix S1B). For each subnetwork, the abundance of parasitoid species (top series of rectangles) and herbivore species (middle series of rectangles) are represented by the number of individual reared, whereas the abundance of plant species (bottom series of rectangles, coloured by plant clades following APG IV: Chase *et al*., 2016) are represented by the square root of the number of seeds collected. Parasitoid families, herbivore orders and plant clades are identified by distinct colours as coded on the right. The scale for each level is also indicated on the right. All subnetwork nodes are ordered as to minimize the number of crossed interactions. From left to right and top to bottom these subnetworks are coded as HB-CURC-BCI, HB-CURC-KHC, HB-CURC-WAN, PL-FABA-BCI, PL-FABA-KHC, PL-FABA-WAN, FG-B-BCI, FG-B-KHC and FG-B-WAN in Appendix S2.

Fig. 3. Summary distribution of the six main network level statistics across study sites (BCI, KHC, WAN) for subnetworks based on insect herbivore families and trophic levels 1-2 (plants-insect herbivores; n=18). The Bruchinae subnetwork for BCI was not included as it has no equivalent at other study sites. Groups with different letters are significantly different (Dwass-Steel tests, $p < 0.05$).

Fig. 4. Results of path analysis testing direct and indirect correlations beween the species richness of seed- and fruit-eating insects (HerbSpp), parasitoid species richness (ParaSpp), mean phylogenetic distance (MPD), plant traits (fruit length, Length and ordination scores delineating plant functional groups, PCA1) and plant resource (basal area, BA and seed rain, SeedRain), for 618 host plant species. Standardized path coefficients are in parentheses. Significant ($p<0.05$) and insignificant paths are indicated by solid and dashed lines, respectively. The rationale of the model is detailed in Appendix S1F.



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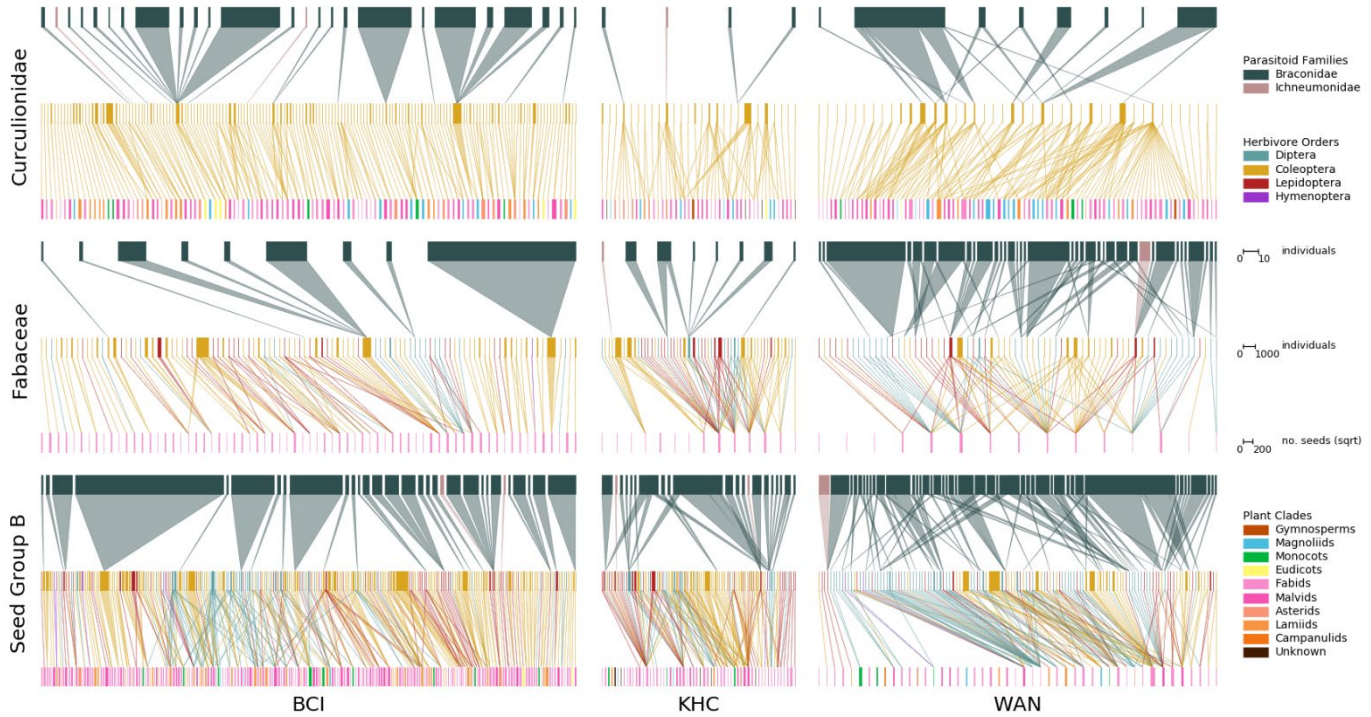

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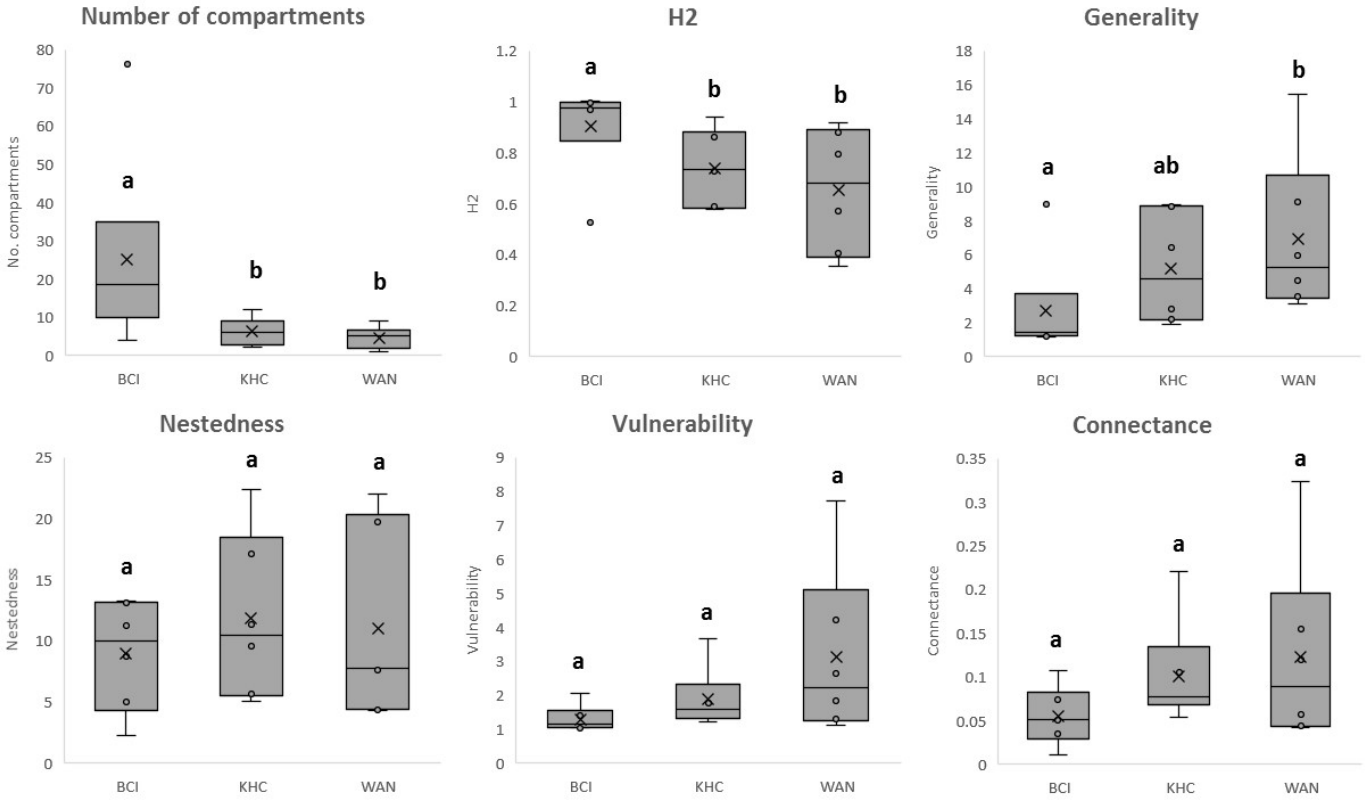

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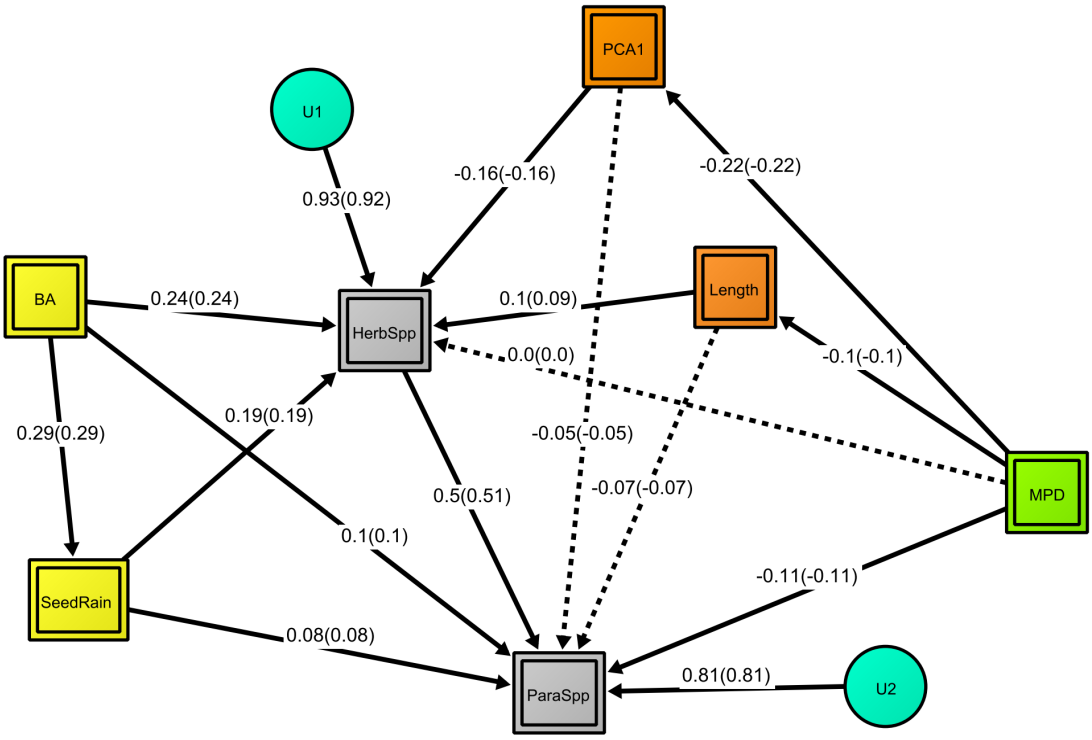

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