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Research

High specialization and limited structural change in plant-herbivore networks along a successional chronosequence in tropical montane forest

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Secondary succession is well-understood, to the point of being predictable for plant communities, but the successional changes in plant-herbivore interactions remains poorly explored. This is particularly true for tropical forests despite the increasing importance of early successional stages in tropical landscapes. Deriving expectations from successional theory, we examine properties of plant-herbivore interaction networks while accounting for host phylogenetic structure along a succession chronosequence in montane rainforest in Papua New Guinea. We present one of the most comprehensive successional investigations of interaction networks, equating to > 40 person years of field sampling, and one of the few focused on montane tropical forests. We use a series of nine 0.2 ha forest plots across young secondary, mature secondary and primary montane forest, sampled almost completely for woody plants and larval leaf chewers (Lepidoptera) using forest felling. These networks comprised of 12 357 plant-herbivore interactions and were analysed using quantitative network metrics, a phylogenetically controlled host-use index and a qualitative network beta diversity measure. Network structural changes were low and specialisation metrics surprisingly similar throughout succession, despite high network beta diversity. Herbivore abundance was greatest in the earliest stages, and hosts here had more species-rich herbivore assemblages, presumably reflecting higher palatability due to lower defensive investment. All herbivore communities were highly specialised, using a phylogenetically narrow set of hosts, while host phylogenetic diversity itself decreased throughout the chronosequence. Relatively high phylogenetic diversity, and thus high diversity of plant defenses, in early succession forest may result in herbivores feeding on fewer hosts than expected. Successional theory, derived primarily from temperate systems, is limited in predicting tropical host-herbivore interactions. All succession stages harbour diverse and unique interaction networks, which together with largely similar network structures and



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consistent host use patterns, suggests general rules of assembly may apply to these systems.

Keywords: ecological gradients, food webs, herbivory.

Introduction

Examining interaction network properties along ecological gradients is an increasingly popular avenue of research. Such studies provide insights into factors underpinning community assembly and stability (see recent reviews by Tylianakis and Morris 2017, Pellissier et al. 2017). For antagonistic networks, studies commonly focus on spatial change, typically along latitudinal (Novotný et al. 2006, Morris et al. 2014), altitudinal (Morris et al. 2015) or land use gradients (Tylianakis et al. 2007), while temporal change through succession has been comparatively overlooked (but see Villa-Galaviz et al. 2012). This is surprising as ecological succession is not only one of the few community-level processes that we understand and can predict, but secondary regeneration is also increasingly prominent in tropical landscapes, arising through major land use changes and forest disturbance (Chazdon 2014).

Rainforests regenerate by secondary succession in response to natural disturbance events such as treefalls or landslides, and anthropogenic disturbance including selective logging or swidden agriculture. Early regeneration in these gaps is typically dominated by pioneer woody species, possessing distinct life history traits (Turner 2001). Pioneers have short-lived leaves with high nitrogen and water content, photosynthetic capacity and dark respiration rate, while having low mass per area. This contrasts with most mature-forest species which lay on the opposite end of the leaf economics spectrum (Wright et al. 2004). Pioneer plants generally maximize growth rate and, according to the resource availability hypothesis, do so at the expense of protection against herbivores and pathogens, for example by energy-intensive, C-based metabolites (Coley et al. 1985). Fast growing, poorly defended pioneers often suffer higher herbivory and compensate for damage by rapid growth (Fine et al. 2006, Whitfeld et al. 2012b). This well-established ecological theory leads to the assumption that secondary succession is driven by an interplay of plant dispersal and inter-specific competition, with the outcome determined by plant functional traits such as growth rate and dispersal abilities.

While we have a good understanding of successional change, many of the underlying principles have been derived from studies of temperate systems. In temperate zones, early succession communities are often dominated by short-lived herbaceous plant species. This can lead to lower specialisation of herbivores in early succession, where mono- or bivoltine herbivores respond to temporally unpredictable and small-sized pioneer plants, mostly annuals (Novotný 1995). However, Lepš et al. (2001) showed that herbivore specialisation on a subset of host species remained constant during succession in lowland rainforests. In tropical rainforests, even short-lived pioneer trees with a life span < 20 yr represent

a relatively permanent and large resource for their often polyvoltine insect herbivores, obviating a supposed advantage of polyphagy on pioneer vegetation. Thus, how herbivores respond to various succession trajectories, and to what extent general succession theory can be used to predict these responses on the community level, remains unanswered.

We investigate these changes in the context of a montane forest. Such forests generally receive less attention than lowland systems, despite one third of global terrestrial plant diversity being found on mountains (Barthlott et al. 1996). Montane-forest communities are subject to distinct environmental conditions compared with lowland forest, such as lower temperature and land area (Körner 2007). This generates changes in diversity, community composition, functional traits and biotic interactions of plants and herbivores (Sundqvist et al. 2013). Further, studies of forest plant-herbivore interactions generally focus on arbitrary subsets of hosts in the community, often phylogenetically controlled (Lepš et al. 2001), limited to common species, and sampled with equal sampling effort (Novotný et al. 2004). These methods arguably generate subjective and somewhat unrealistic representations of real-life networks (Godfray et al. 1999). Thus, we explore successional change using what we refer to as ‘whole-forest’ networks. A whole-forest approach, i.e. where all woody species in a given area are completely sampled, produces networks which are truly quantitative. Similar proportional biomass sampling approaches are relatively uncommon in host-herbivore interaction network studies, and have focused on quite distinct systems, including temperate forest (Volf et al. 2017), or tropical dry forest communities (Villa-Galaviz et al. 2012).

A whole-forest sampling approach in the tropics will almost invariably encounter a species-rich plant community containing a range of congeners and distantly related species. As such, understanding host relatedness is important given consumer-resource interactions are largely influenced by evolutionary dynamics of species traits (Futuyma and Agrawal 2009). Herbivores can circumvent only a limited set of plant defensive traits due to genetic, physiological and behavioral constraints (Becerra 2015). Thus, community-wide levels of specialisation may be driven by host community phylogenetic diversity, where diverse defenses drive herbivores towards greater specialisation. Using the recently developed distance based specialisation index (DSI), we may account for the relatedness of hosts in a standardized manner (Jorge et al. 2014, 2017; see methods). More traditional approaches do not account for host phylogeny, but are informative in their own right, and can be seen as complementary. Here, species specificity index (SSI) serves this purpose (Julliard et al. 2006). DSI, however, enables more robust cross-community comparisons between communities with varying levels of community phylogenetic diversity, as is often the case along a successional chronosequence. For example, studies of lowland forest in Papua New Guinea (PNG) have shown that community phylogenetic diversity increases as succession progresses, where early succession communities are typically dominated by a few large genera such as *Macaranga* and

Trichospermum (Whitfeld et al. 2012a). The same trend has also been shown in Neotropical forests (Letcher et al. 2012). On the other hand, temperate forests in China followed the opposite trajectory, becoming more phylogenetically uniform with time (Chai et al. 2016).

Here we compile a successional chronosequence of plant-herbivore interaction networks through the sampling of distinct phases of succession within a humid tropical montane forest in PNG. By combining established successional theory (Coley et al. 1985) with advances in host range and network analyses, we derive several key expectations. 1) Limiting resources in later succession stages promotes investment in host defenses, driving herbivore specialization, increasing DSI and SSI. 2) The greater palatability and nutritional quality of early succession stages will lead to high vulnerability (number of herbivores per host) relative to later stages, and by the same reasoning, to high generality (number of hosts per herbivore). 3) With this finer partitioning of resources, we may also expect late succession networks to have reduced connectance (realised proportion of total potential interactions), while becoming more modular (species organising into strongly interacting subsets delineated by host phylogeny) with time. 4) Finally, higher plant species richness and specialisation in primary forest will result in higher network beta diversity (turnover of network components) than is observed in earlier succession stages due to a more limited pool of pioneer hosts, and a greater prevalence of generalist herbivores.

Materials and methods

Field site and succession series

Nine 0.2 ha plots near Yawan village (−6.16388°N, 146.83833°W), Morobe Province, Papua New Guinea were sampled using destructive felling at locations earmarked for clearance for swidden subsistence agriculture by the local land-owning community between July 2010 and November 2012. These plots were spatially separated by an average distance of approximately 200 m. Plots were intermingled to avoid pseudoreplication where possible, however potentially hazardous felling conditions and local restrictions limited plot location selection (Supplementary material Appendix 1 Fig. A1). Plots fell within a range of 1720–1860 m a.s.l. Three distinct phases of succession were identified, namely primary, mature secondary and young secondary forest, based on local accounts regarding previous land use, and plant community structure and composition, where young secondary was ~12–15 yr, mature secondary ~25–30 yr and primary forest >100 yr old. The nine plots comprised of four primary, three mature secondary and two young secondary plots. This approach enabled us to develop a temporal series ‘substituting space for time’ (Pickett 1989). Before sampling, woody plants with a diameter > 5 cm dbh were identified to species or morphospecies. Plots were located in a mosaic of primary and secondary forest, where the latter largely results from slash and burn agricultural practices. This subsistence agriculture is small-scale (~1 ha plots) and low-intensity. Lands

are then often abandoned after 2–3 yr, allowing natural succession to take place.

Host and herbivore sampling

Each plot was divided into four 22 × 22m subplots to facilitate sampling in a systematic manner. After clearing the understorey, trees > 5 cm dbh were felled and sampled, beginning with midstorey trees. Sampling started from the lowest subplot and proceeded in steps. Trees tangled with lianas had the potential for damaging other trees when felled, thus lianas were cut with machetes where possible. Tree felling was directed into gaps created by previous plot clearance, allowing for easier collection. Collection was carried out immediately upon felling by a team of ~15 locally recruited collectors supervised by on-site researchers. Collection involved searching for live caterpillars (Lepidoptera), both free feeding and semi-concealed, and placing them in plastic collections pots. In the field lab, trophic links were confirmed with 24-h no-choice feeding trials using host leaves. Specimens were reared to adults where possible and mounted for later taxonomic identification. Identifications were made using existing literature, COI-5P DNA barcoding and dissection of genitalia where necessary. Data are deposited on Genbank (accession numbers KP849894–KP851000), see Miller et al (2015) for further details. Where rearing failed, larvae were preserved in ethanol, morphotyped and a subset (1–11 individuals per morphotype) identified using molecular methods (data are deposited on Genbank accession numbers MK019196–MK020093). In total, we attempted to sequence 1187 adults and 1045 larvae. Of these 1132 adults and 897 larvae were successfully barcoded. Limitations on rearing, barcoding, and difficulties in discerning tropical larval Lepidoptera prevented reliable species level identification of the entire community (see Supplementary material Appendix 2 for more details).

Foliage fresh weight of each tree represented a measure of plant resource abundance for herbivores. This was attained by manually stripping trees of their foliage, placing it in sacks and weighing in the field with a hanging scale or electronic balance. Ten leaf discs (diameter 2.3 cm) were cut from fresh mature leaves and dried in silica gel for phylogenetic analysis (see below). These discs are stored in −80°C frozen tissue collections at the Univ. of Minnesota (St Paul, Minnesota, USA).

Host phylogenetic diversity

The host phylogeny was reconstructed using two loci: *rbcl*, and *psbA-trnH*, by Bayesian inference (phylogeny is presented and its construction detailed in Supplementary material Appendix 1 Fig. A2). Data deposited on Genbank (accession numbers MH826413–MH826635 and MH826636–MH827001). This was used to create a phylogenetic distance matrix from which phylogenetic diversity of host communities, measured as mean pairwise distance (MPD) and mean nearest taxon distance (MNTD), could be determined. MPD is more strongly affected by deep tree topology and

relationships between distantly related hosts, while MNTD more closely reflects relationships between the tips of the phylogeny (Webb et al. 2002) and thus the presence of alternative, closely related host plant species for herbivores. Both MPD and MNTD were weighted by plant abundance. Standardised effect sizes (SES) were calculated by comparing observed plot means to the plot mean distance under a null distribution. The null model was derived by randomly shuffling species occurrences within the community distance matrix, maintaining total abundance of each species i.e. row sums. This null model is suitable for detecting patterns resulting from species interactions and has a low Type I error rate (Gotelli 2000). Species occurrence differences among sites are assumed to be stochastic. Negative values reflect clustering, while positive values indicate overdispersion. Differences were evaluated using a one-way ANOVA with pairwise contrasts carried out using Tukey post hoc comparison.

Herbivore specialisation

DSI values were calculated for species within each of the three succession stages following the approach detailed in Jorge et al. (2014, 2017). DSI weights the degree of specialisation by the phylogenetic similarity of hosts and their availability, rather than using counts of host species, or higher taxonomic categories. In this sense, DSI measures phylogenetic specialization accounting for differences in the pool of available hosts. The rationale to include the phylogenetic similarity of species within measures of host specialisation is derived from the premise that the more similar a set of species are, the more likely that they will share comparable defensive adaptations. As such, their herbivore communities will encounter familiar costs of use for these resources. In this DSI framework, a specialist is defined as a species that selects a subset of host species more related than is expected by chance. On the other hand, a generalist uses host species that are less related than expected by chance.

The relatedness of host species was measured using MPD, and the deviation from expectations determined using null models that sample the pool of available resources. Here we used a rescaled version of DSI, referred to as DSI*, where differences in abundances and sampling intensities are accounted for, making this measure more amenable to cross-community comparisons. The rescaled upper bounds of DSI* were 1 (monophagy) and the lower bounds -1 (maximum generalisation). DSI* was calculated separately for species in young secondary, mature secondary and primary forest. As undersampling can strongly bias estimates of DSI* by inflating the number of monophages, we chose to use only species that were represented by a minimum of ten individuals in each stage. This threshold was chosen as it provides a more accurate reflection of host use, while retaining almost half of the species in the community, and 90% of individuals. Species specificity index (SSI) was calculated for the same set of herbivores as above (see Julliard et al. 2006, Poisot et al. 2012a for details). This more traditional measure of host use does not account for phylogenetic relatedness. Instead, it

quantifies specialization as the coefficient of variation of average herbivore densities among hosts, thus taking into account host diversity and variation in herbivore density among hosts. SSI is bound between 0 and 1, representing low and high specificity respectively. Differences in DSI* and SSI between succession stages were evaluated by linear mixed effect models where species and succession stage were taken as random factors. Significant effects were determined by likelihood ratio test.

Network analysis

Network structural analyses included all tree species > 5 cm dbh, and reliably documented herbivore interactions defined as having $n > 1$ observations. Each of the nine plots were characterized by simple species richness measures of lower (host) and higher (herbivore) trophic levels, and quantitative network metrics: 1) Weighted generality, average number of hosts used by each herbivore species, weighted by their marginal totals, 2) Weighted vulnerability, average number of herbivores using each host species, weighted by their marginal totals, 3) Weighted connectance, linkage density (i.e. diversity of interactions per species weighted by marginal totals) divided by the number of species in the network, 4) Modularity, the tendency of interacting species to assemble into strongly interacting subgroups, which interact weakly with species outside of their module (see Supplementary material Appendix 3 for details of these metrics). These metrics cover a range of network properties of interest including structure, stability and specialisation, and were calculated following Bersier et al (2002), Tylianakis et al (2007), Dormann (2009) and Dormann and Strauss (2014). As numerous network metrics are affected by network size (Morris et al. 2014), we accounted for the size of each network within our models by including it as a model covariate. Modularity (Q) is not only affected by network size, but also the number of links and the number of interactions. Thus, to make measures of modularity more amenable to comparison, we compared z-scores standardized by null models obtained by the r2d randomization method, which rearranges the interaction matrices keeping marginal sums fixed. These z-scores were then compared across networks (Dormann and Strauss 2014). Differences in network metrics were evaluated using independent models, with habitat type as the explanatory variable (model 1), plus network size as a covariate (model 2). Analyses were performed using the R statistical environment ver. 3.1.3 (R Development Core Team), with 'bipartite' (Dormann et al 2008) and 'multcomp' (Hothorn et al 2008) packages.

Interaction network beta diversity

Network beta diversity was partitioned into four components using a modification of the 'betalink' R package (Poisot et al. 2012b) by Simanonok and Burkle (2014). The complementary beta diversity measure (β_{cc}) was decomposed into

turnover of plants (β_p), herbivores (β_h), both plants and herbivores (β_{ph}) and their interactions (β_o), following Novotný (2009), so that: $\beta_{cc} = \beta_p + \beta_h + \beta_{ph} + \beta_o$. For more details see Supplementary material Appendix 3b.

As this is a presence/absence measure of interaction turnover, matrices were converted to binary format for computation. Pairwise contrasts were performed for both within and between succession stages. As we have only two replicate plots in young secondary forest, and thus only a single measure of within stage beta diversity, we omitted this pairwise comparison from the within and between habitat categorical comparisons.

Data deposition

Host-herbivore interaction data are available Dryad Digital Repository <<http://dx.doi.org/10.5061/dryad.bh2rc50>> (Redmond et al. 2018). Herbivore barcode sequences can be accessed through BOLD (dx.doi.org/10.5883/DS-YAWAN2) Plant barcode sequences can be accessed through BOLD (dx.doi.org/10.5883/DS-YAWANPL).

Results

Host and herbivore communities

830 individual trees from 89 species across 37 families hosted herbivores (Supplementary material Appendix 1 Table A1). Analysis of plant community composition revealed distinct clustering of plots by successional stage (Supplementary material Appendix 1 Fig. A3), with contrasting dominant species (Supplementary material Appendix 1 Fig. A4). Host phylogenetic diversity, measured as MPD, differed between succession stages (ANOVA, $F_{2,6} = 16.15$, $p = 0.004$), where young secondary forest is phylogenetically overdispersed, mature secondary is close to random, becoming significantly less diverse in the phylogenetically clustered primary forest (Fig. 1). When community phylogenetic diversity was measured by MNTD, the same overall trend emerged, however

there were no significant effects due to large within stage variance of secondary forest types (ANOVA, $F_{2,6} = 1.53$, $p = 0.29$).

Mean herbivore abundance was greatest in young secondary forest plots (3046 ± 489 SE), followed by primary forest plots (2461 ± 735 SE) and then mature secondary forest (mean 1087 ± 293 SE). Herbivore abundance per kilogram foliage followed this same order; young secondary (1.29 ± 0.02 SE) – primary (0.97 ± 0.25 SE) – mature secondary (mean 0.49 ± 0.08 SE). We found no evidence of community-wide seasonality effects, as the numbers of herbivores collected per day per leaf area sampled did not fluctuate notably throughout the collection period (Supplementary material Appendix 1 Fig. A5). Abundance, both total and per unit foliage weight, were not statistically different between succession stages, principally due to an outbreak of two cryptic *Leucoma* spp. (Erebidae, Lymantriinae) (treated as a single species complex due to difficulties discerning them taxonomically, see Supplementary material Appendix 2 for details). Removal of this *Leucoma* spp. complex, which represented 4412 individuals found only in primary forest, caused large reductions in mean abundance of primary plots (1359 ± 142 SE). This leads to differences in total abundance (ANOVA, $F_{2,6} = 17.38$, $p = 0.003$), and abundance kg^{-1} foliage (ANOVA, $F_{2,6} = 32.7$, $p < 0.001$), where both are significantly higher in young secondary forest than in primary and mature secondary forest.

Network properties and herbivore specialisation

12 357 herbivore individuals, from 292 species across 29 families (Supplementary material Appendix 1 Table A2), were identified to species and used in subsequent network analyses. Representative network plots for each successional stage are presented in Fig. 2. There were significant differences in host richness between succession stages (ANOVA, $F_{2,6} = 11.185$, $p = 0.009$) (Fig. 3a). Host richness was greatest in primary forest plots, however richness did not increase linearly as expected, as young secondary forest was also relatively host rich (Fig. 3a). Herbivore richness also varied along the

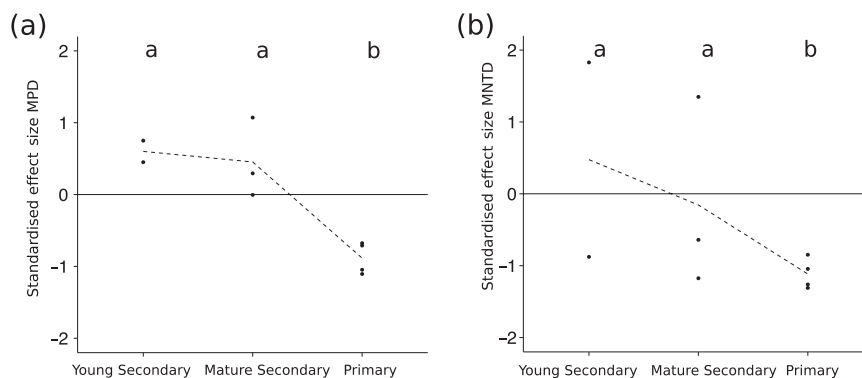


Figure 1. Standardised effect size for weighted mean pairwise distance (MPD) (a) and weighted mean nearest taxon distance (MNTD) (b) of young secondary, mature secondary and primary forest in montane forest in Papua New Guinea. The zero line represents a random structure, while values above zero tend towards overdispersion, and the values below, phylogenetic clustering. Different letters highlight significant differences between succession stages following Tukey post hoc comparisons. Dotted line passes through average values.

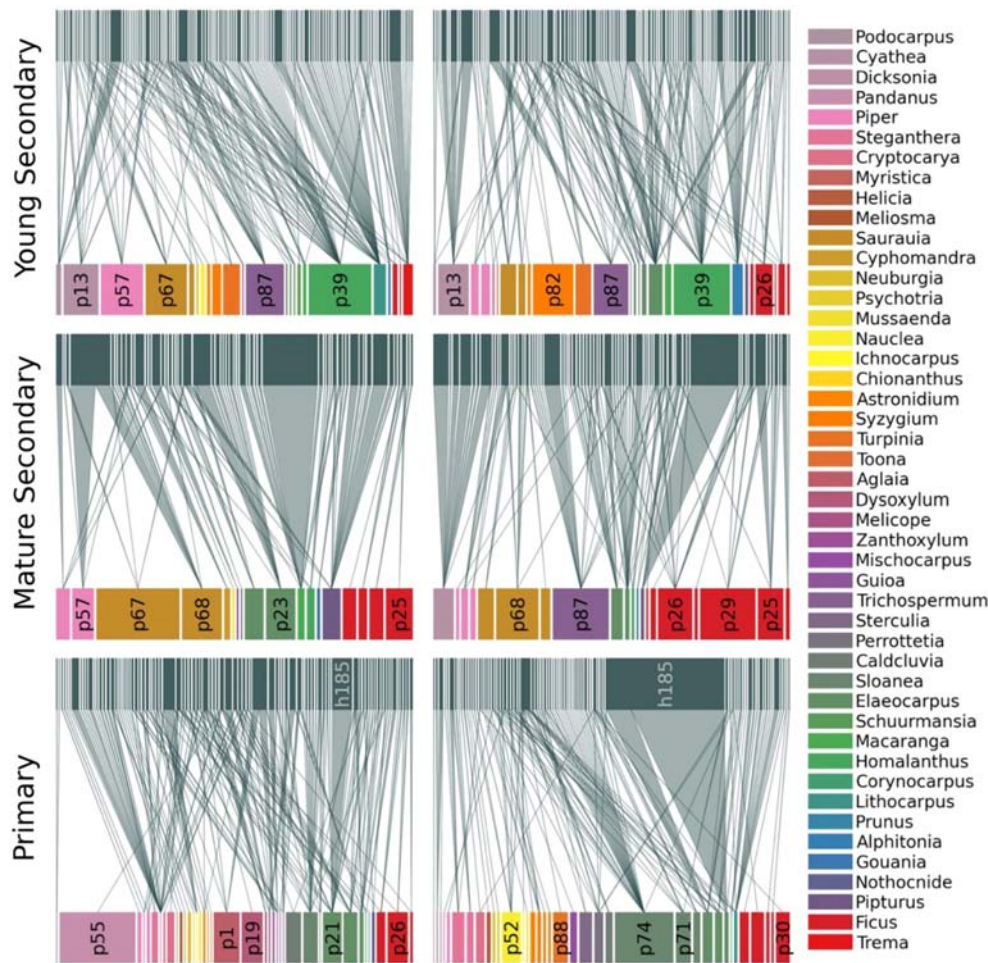


Figure 2. Representative bipartite networks from each of the three stages of succession – young secondary, mature secondary and primary forest. Only two plots per stage are shown for brevity, selected on the basis of network parameters being closest to the mean for that stage. Lower trophic level is coloured according to host genus, and the width of the bars is proportional to host biomass. Hosts are ordered by most basal to most recent (left to right). Only hosts with herbivore interactions are included. The labels of the five most abundant host species in each network is shown, as is the outbreak *Leucoma* spp. complex (h185+h186). Details of hosts and herbivores are given in Supplementary material Appendix 1 Table A1–A2.

chronosequence (ANOVA, $F_{2,6} = 6.44$, $p = 0.032$), with peak richness in young secondary forest, but again comparable to that of primary forest (Fig. 3b).

Habitat stage had a significant effect on weighted vulnerability and weighted connectance under model 1 (Table 1). These effects diminish after controlling for network size, revealing mature secondary as an intermediate phase, where only pairwise contrasts between young secondary and primary forest remain significant for both vulnerability (Tukey-Kramer, $z = 2.721$, $p = 0.016$) and connectance (Tukey-Kramer, $z = 2.786$, $p = 0.013$). There were no differences in generality when considering successional stage only and when controlling for network size (Table 1, Fig. 3d). Degree distributions of herbivore species can be found in Supplementary material Appendix 1 Fig. A6. All networks were highly modular compared with null models, and succession stage had no effect on modularity (Table 1, Fig. 3f). Herbivore communities were consistently highly phylogenetically specialized (DSI*) (linear mixed effect model, $\chi^2 = 1.46$, $p = 0.481$) (Fig. 4a). Three

families (Choreutidae, Gelechiidae and Nolidae) were consistently monophagous or near monophagous throughout all stages of succession (Supplementary material Appendix 1 Table A3). Eupterotidae were the least specialized family and were found only in young secondary and mature secondary forest (Table A3). More species-rich families had a broader range of host use, with high specialization being pervasive throughout (Supplementary material Appendix 1 Table A3, Fig. A7). SSI largely reflected DSI*, here however, succession stage had an effect on species specificity (linear mixed effect model, $\chi^2 = 7.27$, $p = 0.026$) where specificity was lower in primary forest (Fig. 4b).

Beta diversity of networks

Overall network beta diversity across all pairwise contrasts was high throughout our study system (mean $\beta_{cc} = 0.93 \pm 0.01$ SE), approaching its upper limits. Overall beta diversity differed significantly between the five pairwise categories,

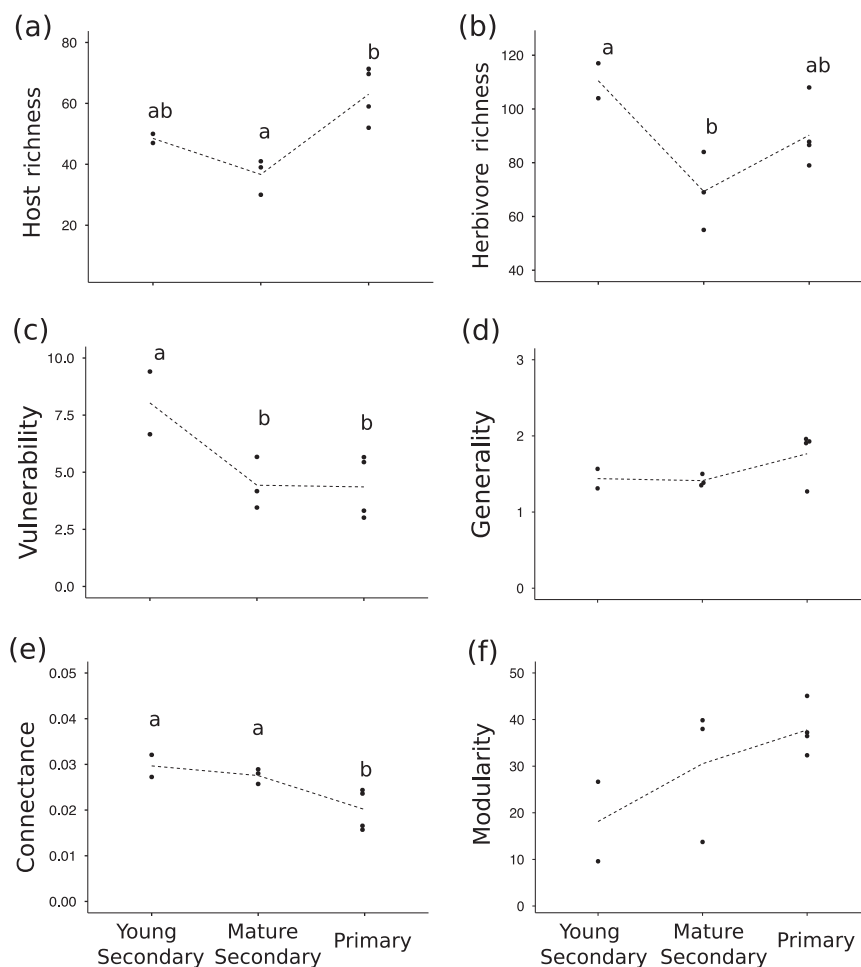


Figure 3. Network metrics for each plot along the successional chronosequence – young secondary, mature secondary and primary forest: (a) host richness, (b) herbivore richness, (c) weighted vulnerability, (d) weighted generality, (e) weighted connectance, and (f) modularity z-scores. Different letters indicate significant differences between succession stages under model 1. Dotted lines pass through the stage mean.

with pairwise contrasts of different habitat types being significantly higher than within habitat type contrasts (Fig. 5, ANOVA, $F_{30,4} = 12.29$, $p < 0.001$). Contributions to overall network beta diversity, calculated across all pairwise contrasts, were partitioned into plant species turnover (mean $\beta p = 0.20 \pm 0.01$ SE), herbivore turnover (mean $\beta h = 0.23 \pm 0.01$ SE), plant and herbivore turnover (mean $\beta ph = 0.33 \pm 0.02$ SE), and interaction turnover (mean $\beta o = 0.16 \pm 0.01$ SE).

Discussion

Tropical forest succession is a dynamic process where plant species compete for newly available space and resources, resulting in changes to community composition and functional traits (Guariguata and Ostertag 2001, Whitfeld et al. 2014). Despite this, we found that patterns of herbivore host use were more similar than expected and that underlying network properties changed little. Generality did not decrease in primary forest, rather it remained at comparable

levels throughout all three stages. This expands the findings of Lepš et al. (2001) to whole communities, where previously only a subset of hosts was examined. Similarly, herbivores in all three succession stages were quite highly specialized when phylogenetic diversity and availability of hosts was taken into account (DSI*). SSI largely reflected this also. However, using SSI, primary forest herbivore specificity was unexpectedly lower than secondary stages, thus overestimating host

Table 1. Effects of succession stage on network metrics when considering succession stage alone (model 1), and controlling for network size (model 2). Asterisks indicate significant differences at $p \leq 0.05$.

Network Metric	~Succession stage		+ Network size	
	F	p	F	p
Weighted vulnerability	5.128	0.050*	4.879	0.067
Weighted generality	2.106	0.202	2.192	0.207
Weighted connectance	5.833	0.039*	4.974	0.065
Modularity	2.365	0.175	3.327	0.121

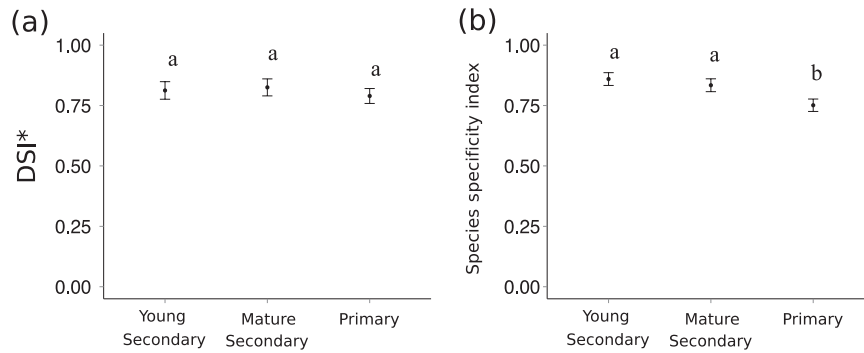


Figure 4. Mean (a) distance based specialisation (DSI*) and (b) species specificity index (SSI) of herbivores along the successional chronosequence – young secondary, mature secondary and primary forest. Error bars represent +/- standard error.

use relative to DSI*. High herbivore specialisation is often reported using both traditional and phylogenetically based measures of specialisation. For instance, lepidopteran communities in Mexican dry forest (Villa-Galaviz et al. 2012) were highly specialized measured using traditional measures, while herbivore communities from four distinct functional groups in PNG were highly phylogenetically specialised (Jorge et al. 2017).

Host plant community composition and the phylogenetic structure of the three succession stages may explain some of these unexpected findings. Phylogenetic diversity decreased with succession, contrasting investigations of lowland systems where succession trajectories typically lead to overdispersion arising from niche differentiation (Whitfeld et al. 2012a). This is likely due to the lack of dominant, diverse genera such as *Ficus* and *Macaranga* in the studied secondary montane systems. Given that herbivore communities along the successional chronosequence are similarly phylogenetically limited in their host use, the rather unexpectedly low generality in secondary forest may result from relatively high phylogenetic diversity. Diversity of host defenses closely correlate with their phylogenetic diversity (Agrawal 2011). Despite lower defensive investment in young secondary forest species (Poorter et al 2004, Endara and Coley 2011), a greater diversity of host defenses may prevent herbivores from utilizing multiple hosts in more phylogenetically diverse secondary forest communities. Indeed, host plant chemical diversity was reported to affect herbivory in several cases (Salazar et al. 2016, Massad et al. 2017). Further, phylogenetic limitations to host use have been well documented, typically occurring on the level of host genus, for example; herbivorous communities of lowland forest in PNG (Weiblen et al. 2006), and neotropical dry seasonal forest (Janzen 2003). Thus, in host communities of low phylogenetic diversity, herbivores should have a greater utilizable resource pool, accounting for the decrease in SSI in primary forest herbivores.

Successional theory did however predict some network interactions more accurately. Vulnerability, or the number of herbivore species using a given host, was greatest in young secondary forest. According to the resource availability hypothesis, this finding may reflect greater palatability and nutritional quality of early succession hosts where

investment in growth is preferred over defensive investment (Coley et al. 1985, Poorter et al 2004, Endara and Coley 2011). Such hosts have been shown to increase herbivore growth rate and development, shortening maturation to a reproductive state and thus greatly increasing herbivore fitness (Coley et al. 2006). Indeed, we found highest herbivore abundance in young secondary forest, both total, and per unit foliage weight, further indicating a preference for these early succession hosts, and reflecting findings of lowland forest in PNG (Whitfeld et al. 2012b). Network connectance was low throughout all stages of succession. Low connectance is typical of antagonistic networks contrasting that of mutualistic networks, where distinct evolutionary processes generate networks of higher connectance (Thébault and Fontaine 2010). Connectance decreased in primary forest, despite the increased phylogenetic similarity

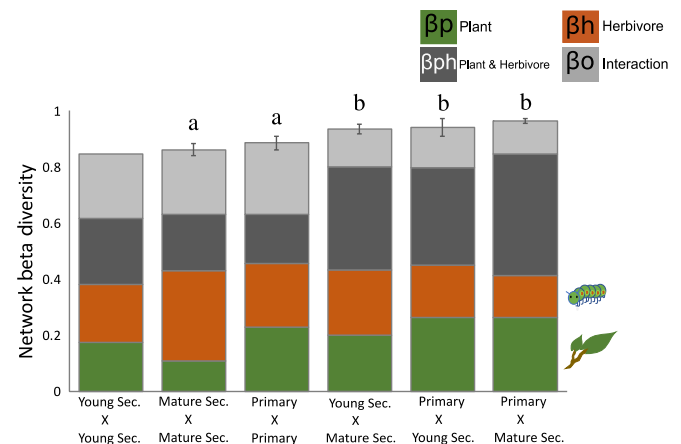


Figure 5. Beta diversity of plant-herbivore networks within and among succession stages. Beta diversity is decomposed into turnover of plants (p = green), herbivores (h = orange), both plants and herbivores (ph = dark grey) and their interactions (o = light grey). The combined total of all components represents overall network beta diversity. Different letters indicate significant differences between overall beta diversity. Error bars represent 95% confidence intervals for total network beta diversity for each pairwise comparison. The young secondary X young secondary pairwise comparison is omitted from statistical analyses due to insufficient replicates.

of these systems. This is due to greater community-wide resource overlap in secondary forest, where herbivore species are more likely to share the same host. Within primary forest, we see fewer potential niches being realized. If these primary forest hosts are indeed better defended, then we might expect this increased defensive investment would limit the number of herbivores sharing hosts by imposing some ecological costs (Poorter et al 2004, Coley et al. 2006, Zovi et al. 2008). Defensive investment should also generate more modular late succession networks. While all networks across the succession chronosequence were highly modular, quantitative analysis did not reveal a statistical increase in modularity as succession progressed, likely due to low test power. Despite this, there is a clear qualitative trend towards increasing modularity with succession which is ecologically noteworthy. This trend appears to be driven by increases in host species richness rather than changes in herbivore host use, given that phylogenetic specialisation of herbivores throughout succession remains consistently high. The network graphs reveal that many modules have a foundation in a single host species or genus. Segar et al 2017 showed that clades of herbivores tend to interact with clades of hosts in tropical forest. Exploring the changes that occur over time in these groups of strongly interacting species warrants further examination but extends beyond the scope of this study. While antagonistic networks often organise into such modules (Thébault and Fontaine 2010, Cagnolo et al. 2011), this is not always the case (Villa-Galaviz et al. 2012). Understanding modularity, and how module membership changes, is important as a modular structure increases the resilience of networks to the propagation of deleterious domino effects associated with perturbations such as species extinction or local extirpation, and species outbreaks (Stouffer and Bascompte 2011).

While herbivore outbreaks in the tropics can be less conspicuous than in temperate systems, here we encountered an outbreak of a *Leucoma spp.* complex (Dyer et al 2012). Tropical outbreaks typically arise following prolonged drought, for example, following El Nino events (Van Bael et al. 2004). Indeed, sampling took place in the wake of a moderate 2010 El Nino and during a strong 2011 La Nina event (CPC 2017). Both species within the complex were typically associated with two species of *Eleaocarpus* hosts. A severe Lepidoptera outbreak occurred in central Panama following the 1997–1998 El Nino, involving at least 12 species (Van Bael et al. 2004). Similarly, these species were also associated with one or two host plant species belonging to the same family. In PNG, coffee plantations have suffered from outbreaks of *Tiracola plagiata* in the past (Baker 1974). In our system, the outbreak species were exclusively found in primary forest however. Connectance and modularity was lowest and highest respectively in primary forest, and this may have buffered the spread of deleterious effects. Additionally, considering the narrow host-range of this species complex, community-wide effects due to direct competition with other herbivores are likely to be low.

We recognize as a study limitation that plot based network conclusions are drawn from a limited number of replicates and this may introduce biases. However, complete census of interaction networks within 0.2 ha represents a large sampling unit, which is necessary to capture the structure of complex interaction networks in rainforests. The effort needed to obtain these data equates to > 40 person years in the field. These results provide novel insights regarding large-scale community patterns that may otherwise be overlooked at smaller spatial scales or sampling intensities, where sufficient plant and insect diversity would not be captured. At smaller sampling scales, biases in the form of low within species replication will be introduced, where singleton species dominate the samples. Fayle et al 2015 argue that ‘as a guideline, manipulations should mimic the scale at which the focal process or interaction occurs’. While the authors were addressing large-scale experiments, the argument holds equally for large-scale surveys. Nevertheless, it is important to stress that biases due to low sample size may exist. The directionality of these biases for plot-based network metrics is unclear due to novelty of this sampling procedure and lack of comparable studies. However, despite the low number of replicates, clear trends emerge for metrics where variance was low, suggesting patterns are likely robust.

Lepidoptera, as a species-rich herbivore group with a relatively broad host-use spectrum, are a useful and widely-used model taxon. It remains to be seen whether the trends shown here will apply to other herbivore guilds with varying host-use patterns. However, the extremely high beta diversity both between and within all succession stages would suggest that network structure may be determined by processes which act largely independently of community composition and specific species interactions per se, where perhaps fundamental rules govern assembly of these networks (Morris et al. 2014) or replacement of species occurs between topologically similar species (Dupont et al. 2009). This idea is supported by studies of changes to networks across landscape (Kaartinen and Roslin 2011, Kemp et al. 2017), through time (Kaartinen and Roslin 2012, Kemp et al. 2017) and by comparisons of multiple independent networks across a latitudinal gradient (Morris et al. 2014). Future research directions include developing a perspective of these plant-herbivore interactions which directly accounts for differences in plant traits, and not only host species composition. Traits related to growth and defense, for example specific leaf area and C:N ratios, can vary both within and between species throughout tropical succession (Poorter et al 2004), with these likely impacting herbivore interactions also.

Promisingly, we show that not just herbivores, but also their interactions and associated ecosystem processes, recover well and rapidly post disturbance, with all stages of succession capable of hosting diverse and unique assemblages. Similarly, studies of a successional chronosequence in tropical dry forest in Mexico reported Lepidoptera herbivore and host networks recovered within six to thirteen years post-disturbance (Villa-Galaviz et al. 2012). Other animal taxa such as Coleoptera and nonvolant mammals have been shown to

recover well within 20–40 yr post-abandonment, while some, including ants and birds, tend to recover in terms of species richness, while compositional recovery takes longer (Dunn 2004). However, this recovery process will be determined by an interplay of disturbance intensity and landscape characteristics. High-intensity land use, coupled with a lack of seed sources and wildlife refugia, will inevitably slow the recovery process. While a growing list of invertebrates, birds, reptiles and mammals have been shown to recover well, most examples arise from systems well-serviced with influx sources and have experienced relatively low-intensity land use (Dent and Wright 2009). Our system is no different, as slash and burn agriculture is a low-intensity practice, and creates patches of secondary forest in a primary matrix.

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Supplementary material (Appendix ECOG-03849 at <www.ecography.org/appendix/ecog-03849>). Appendix 1–3.