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Plant-herbivore interactions along ecological gradients in tropical rainforest: Drivers of network structure and specialisation

Ph.D Thesis

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Annotation

This thesis concerns the community ecology of Lepidopteran herbivores and their host plants in rainforests of Papua New Guinea. We specifically focus on examining the drivers of plant-herbivore interaction network structure and herbivore specialisation across rainforest succession and elevation. Using one of the most comprehensive and unique datasets of its kind, gathered using a 'whole forest' approach, we investigate how networks are structured in young secondary, mature secondary and primary forest. Furthermore, we revisit a classic ecological question, exploring specialisation of herbivores and how abiotic and biotic factors might influence this. We show that an understanding of host community properties including phylogeny, physical structure and theorised defensive investment can be used to explain interaction network structure. We also find that specialisation changes with elevation, guild type and habitat use in ways which are difficult to predict. We finish by analysing and presenting our relatively novel methodological approach. It is our hope that it can gain wider adoption thus facilitating broader comparative studies.

Declaration [in Czech]

Prohlašuji, že jsem svoji disertační práci vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce, jakožto i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

České Budějovice, 04-03-2019

Conor Redmond

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Go raibh maith agat

List of papers, manuscripts and authors contribution

The thesis is based on the following papers (listed chronologically)

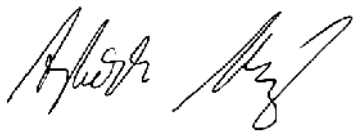
I. **Redmond CM**, Auga J, Gewa B, Segar ST, Miller S.E, Molem K, Weiblen GD, Butterill PT, Maiyah G, Hood AS, Volf M, Jorge L, Basset Y and Novotny V 2019. High specialization and limited structural change in plant-herbivore networks along a successional chronosequence in tropical montane forest. Ecography 42:162-172 (IF= 4.902). *Conor Redmond carried out data processing, species identification, constructed host phylogeny, formulated the hypotheses and wrote the first draft of the manuscript*

II. **Redmond CM**, Fayle TM, Auga J, Gewa B, Butterill P and Novotny V. "Bottom-up structuring of plant-herbivore interaction networks during rainforest succession" (Manuscript). *Conor Redmond carried out data processing, formulated the hypotheses and wrote the first draft of the manuscript*

III. **Redmond CM**, Jorge L, Auga J, Gewa B, Miller S.E, Weiblen GD, Butterill PT and Novotny V. "Phylogenetic specialisation of tropical herbivores varies with elevation, guild and habitat use" (Manuscript) *Conor Redmond carried out data processing, species identification, constructed host phylogeny, formulated the hypotheses and wrote the first draft of the manuscript*

IV. Volf M, **Redmond CM,** Klimeš P, Lamarre G, Seifert CL, Abe T, Auga J, Anderson-Teixeira K.… and Novotny V. "Quantitative assessment of arthropod-plant interactions in forest canopies: a plot-based approach" (Manuscript) *Conor Redmond carried out sampling of hosts and herbivores, data processing and contributed to the writing.*

Co-author agreement:
Vojtech Novotny, the supervisor of this Ph.D thesis and co-author of all of the papers and manuscripts included in this thesis, fully acknowledges the major contribution of Conor Redmond in all chapters



Prof. RNDr. Vojtěch Novotný, Csc.

Martin Volf, the lead author of "Quantitative assessment of arthropod-plant interactions in forest canopies: a plot-based approach" presented in Chapter IV, fully acknowledges the major contribution of Conor Redmond in this manuscript

Dr. Martin Volf Ph.D

Contents

Introduction

Tropical Forest Diversity: A Vast Interaction Network

Tropical forests are one of the most diverse systems on Earth (Whitmore 1990). The astounding insect diversity contained within these systems has long since enthralled and baffled biologists, spanning back to the days of notable naturalists such as Wallace, Darwin and Bates (Erwin 1982; Godfray et al. 1999). This diversity was a key component in the formulation of Darwin and Wallace's theory of natural selection. This fact alone illustrates the potential for garnering knowledge of extreme value through the study of these ecosystems. Despite efforts, for more than a century and a half after the pioneering biologists began contemplating the vastness of tropical diversity, surprisingly little progress was made towards quantifying this diversity, and more importantly, understanding the mechanisms which underpin it.

Key to this understanding is a knowledge of the structure and dynamics of tropical interaction networks. Price (2002) points out that of the thousands of food webs available for study not a single one is fully understood in terms of a mechanistic explanation of the distribution, abundance and dynamics of all its components. However progress is being made owing to an increase in empirical studies (for example Novotny et al. 2004; Morris et al 2014, Maunsell et al 2014, Kemp et al 2017, Plowman et al 2017). The interaction between plants and their phytophagous herbivores is the most important of all ecological interactions. It is estimated that 310,000 plant species, with 360,000 and 400,000 associated phytophagous and carnivorous insects respectively, contribute approximately 75% of global terrestrial biodiversity (Price 2002). Furthermore, herbivory has far reaching consequences for tropical forest systems. For example, herbivory maintains plant diversity through enemy mediated density dependence (Janzen 1970, Comita et al 2014) and greatly affects nutrient cycling and plant productivity (Hartley and Jones 2008). Having first arose during the early Devonian, herbivory has led to the diversification and radiation of herbivorous insects spanning across numerous insect orders (Labandeira 2007, 2013). Lepidoptera is one of the largest of these orders, forming a significant component of tropical insect herbivore assemblages. As a species-rich herbivore group with a relatively broad host use spectrum, Lepidoptera represent a useful and widely-used model taxon for investigating this crucial ecological process.

Lepidopteran Herbivores and Papua New Guinea

Lepidoptera represent possibly the largest single radiation of phytophagous insects, with 157,242 recognised extant species (Mitter et al 2017). This group is of enormous importance for both humans and numerous other species. Lepidoptera larvae are important agricultural pests (Vreysen et al 2016), a food resource for many cultures (Yen 2015) and have been used for centuries for silk production (Fedic et al 2002). In the natural world they sustain vast numbers other species through predation and parasitism, while their adult form are key mutualists to angiosperm plants through their provision of pollination services. Their importance to ecological research is equally significant. Their use as model systems within the realms of ecology, genetics, physiology and evolutionary biology has borne significant advancements in our understanding of the natural world. The sessile nature of larval Lepidoptera makes them particularly well-suited to the study of herbivory, facilitating their collection and thus testing of host interactions.

Lepidoptera have been a prominent feature of ecological research in Papua New Guinea (PNG). This biologically diverse country lies on the eastern half of the island of New Guinea, which itself is thought to harbor approximately 5% of the world's global biodiversity in less than 1% of global land area (Hoover et al 2017). Despite being one of the most poorly inventoried assemblages of Lepidoptera, they have played an important role in the advancement of our understanding of tropical forest herbivory (Basset 1996), global diversity (Novotny et al 2002) and the properties of host-herbivore interactions (Novotny et al 2004).

Interaction Networks

In order to fully understand host-herbivore ecological and evolutionary associations, we must examine the network of interactions in which they exist. One of the pioneers of this field was Elton (1927), who advanced the concept of food chains to "food cycles" with the aim of drawing food web studies closer to ecosystem functioning. Janzen (1983) spoke of tropical food webs, henceforth referred to as interaction networks, as "rich in extrapolation and conjecture, held in place by very few data points". However an upsurge in interaction network research in the last decade or so has brought about a more robust understanding of these systems (for example Tylianakis et al 2007,

Paniagua et al 2009, Morris et al 2014, see reviews by Pellsier et al 2018, Tylianakis and Morris 2017).

Interactions networks are typically placed into two broad categories; mutualistic and antagonistic. We focus here on antagonistic networks, which may essentially be described as the network of who eats whom in ecosystems. Network studies have considerable promise for shedding light on critical issues of community ecology. They often form complex architectures, providing intricate yet potentially amenable interpretations of biodiversity, species interactions, and ecosystem structure and function (Dunne et al 2002). They are thus central to an understanding of the stability and dynamics of ecological systems (Paniagua et al 2009; Stouffer and Bascompte 2010). Furthermore, these networks can reveal both fine- and large-scale trends in host specialisation, informing us of co-evolutionary relationships and processes (Segar et al 2017, Volf et al 2017).

This field saw significant advancements with the development of quantitative network studies (Memmott et al 1994, Dormann et al 2009). Unlike the previous qualitative efforts of network analysis, quantitative networks take both the abundance and interaction frequency of species into account. More recently, advancements in molecular taxonomy have provided a means to resolve interaction networks to increasingly finer detail. This has proven particularly important in systems where taxonomy is poorly known, such as tropical host-herbivore and host-parasitoid systems (Hrcek and Godfray 2015). Finally, the incorporation of network science into the analysis of interaction networks has borne significant strides forward, greatly improving our ability to describe and thus understand underlying network patterns and processes (Bascompte and Jordano 2007, Poisot et al 2016).

This analytical refinement has revealed the structure of numerous interaction networks. For antagonistic networks such as host-herbivore networks, the predominant emergent structure is modular or compartmentalized (Thebault and Fontaine 2010). Here, groups of interacting species tend to interact strongly with one another, but interact rarely or weakly with species outside of their group. These networks also tend to be of low connectance, meaning that few potential interactions are actually realized. This structure is in stark contrast with mutualistic networks, which display nested structures of high

connectance. It is believed that community stability and dynamics drive the formation of these architectures, however the precise mechanisms behind this structural dichotomy are unclear (Thebault and Fonataine 2010). For antagonistic networks, modularity should provide stability by buffering the spread of extinction effects throughout the community, promoting long term persistence (Stouffer and Bascompte 2010). For mutualistic networks, a nested structure offers redundancy for the species involved, where multiple potential mutualistic partners generates stability through a reduction in competition for resources (Bastolla et al 2009).

Specialisation

Specialisation has been a central concept of ecological studies, particularly network studies Ecological specialisation is ubiquitous throughout the natural world. All organisms specialize to some degree. They persist in certain habitat types, under particular environmental conditions, and feed on a select diet. Herbivore specialisation refers to the number or diversity of host plant species that a herbivore utilizes as a food source. Classically, insect herbivores are categorised as either specialist or generalist, however these classifications are two ends of a spectrum of host use. Herbivores may feed across many host plants (polyphagous herbivores), a single host plant (monophagous herbivores) or they may employ an intermediate strategy whereby they feed on several hosts (oligophagous herbivores). Specialisation has been central to studies attempting to, for example, disentangle the mechanisms responsible for species co-existence (Becerra 2015) and the latitudinal gradient of species diversity (Novotny et al 2006, Dyer et al 2007). It has been used to estimate global biodiversity (Novotny et al 2002a). It can inform us as to how species and communities may respond to disturbance (Büchi and Vuilleumier 2014), with specialist species facing higher extinction rates (Clavel et al 2011). The structure and stability of entire networks of interacting species is affected by the degree of specialisation of their components. This is the case for both antagonistic plant-herbivore networks and mutualistic pollination (Weiner et al 2014) and seed dispersal (Correa et al 2016) networks. Herbivore specialisation is closely linked with evolutionary relationships among resource species and between trophic levels, reflecting trait similarity and co-evolutionary processes (Ehrlich and Raven 1964; Poulin et al 2011). For example Volf et al 2017 showed that escalation and divergence of host plant

defences are reflected in the degree of specialisation of their herbivore assemblages.

Studies have repeatedly shown that the majority of insect herbivores tend to lie towards the specialized end of the host use spectrum. For example, Forister et al 2015 examined specialisation of multiple herbivore guilds along a latitudinal gradient. They showed that all guilds were relatively specialised, with highly specialised leaf miners and gallers on one end of the spectrum, and adult leaf chewers on the other. Larval leaf chewers (Lepidoptera) were intermediately positioned on this continuum (Forister et al 2015). Novotny et al (2004) showed that a randomly selected caterpillar in secondary forest will likely feed on one to three plant species and will have the great majority of their population on a single host species.

Despite the obvious importance of specialisation, much debate has existed with regards to its measurement. This has resulted in a multitude of specialisation measures, with many studies refining and reinventing the concept and its measurement (Poisot et al 2012). This problem is then further compounded by differences in methodology across studies, as differences in sampling intensities and sample size, due to the rarity of many trophic interactions, strongly impacts measures of specialisation (Lewinsohn et al 2005). A solution to quantifying specialisation in a manner which enables meaningful cross community comparisons was proposed by Jorge et al 2014,2017. This Distance Based Specialisation Index (DSI) accounts for not only host phylogeny, but also resource availability, enabling comparisons between communities where host and herbivore abundance and interaction frequency varies. It therefore provides a means to compare herbivore specialisation from geographically and ecologically distinct systems. It offers a solid framework for categorising specialist and generalist species, allowing for clear distinctions to be made between herbivores which feed on multiple but closely related congeneric species, from herbivores which feed on multiple but distantly related hosts.

Succession and Elevation Gradients

Tropical forests are a classic example of a plant-based interaction network characterized by high heterogeneity and strong bottom up effects. One major component thought to be responsible for the structure and dynamics of an

interaction network, and of species specialisation, is environmental heterogeneity (Price 2002). Sources of heterogeneity are hierarchical in their overall effect, beginning with small scale biotic interactions such as competition or herbivory, and ending with large scale environmental and climatic gradients including latitudinal, elevational and successional gradients (Price 2002). Classically, an understanding of many ecosystem processes has been developed via experimentation, however this can often be problematic. Assembling an artificial forest requires much time for it to arrive at maturity, and rarely can the complexity of natural tropical forest be attained (Feidler and Beck 2008). This issue can be overcome with the use of ecological gradients which act as a natural experimental site. Recently there has been a dramatic increase in interest concerning how interaction networks vary across such gradients, offering an avenue to further our understanding of how networks respond to their environment (see reviews by Pellsier et al 2018, Tylianakis and Morris 2017).

Succession gradients, for example, allow researchers to substitute space for time (Pickett 1989). Ecological succession is the process whereby the species composition and structure of a community shift over time. These shifts occur in response to changing biotic and abiotic conditions, for example changes to the light availability and competition, decreasing and increasing respectively as succession progresses (Chazdon 2014). Succession begins following either natural or anthropogenic disturbance events such as tree falls or swidden agriculture. This process of rainforest regeneration typically begins with a distinct set of plants possessing a suite of life history traits tailored to high resource environments (Turner 2001). In tropical rainforests, these early succession plants can be both herbaceous and woody in form. These plants typically having short lived leaves with high nitrogen and water content, high photosynthetic capacity and dark respiration rate, while retaining low mass per area. Late succession species on the other hand sit on the opposite end of the leaf economic spectrum (Wright et al 2004). This variation in host life history in turn effects their herbivore assemblages through variation in anti-herbivore defensive investment. Early succession hosts are thought to invest resources primarily into growth, however this comes at a cost to defence according to the resource availability hypothesis. In contrast, later succession species invest into physical and chemical defences, such as spines, trichomes and energy demanding C-based metabolites (Coley et al 1985). Thus herbivores should be

able to more easily utilize early succession hosts, however the impact this has on herbivore specialisation, and thus also their network structure, is poorly known (but see Villa-Galaviz et al. 2012, Leps et al 2001).

Historically, elevation gradients have proven to be an important natural experimental site for the development of major ecological theories including community assembly, niche theory, life zones, and insular biogeography (Grinnell 1917; Whittaker 1960; Whittaker 1972; Brown 1971). As with succession, environmental conditions change with elevation. For example, at higher elevations temperature decreases while solar radiation increases. This then influences numerous ecological factors such as parasitism, predation, competition and host plant quality, which in turn affect host plant choice and species distributions (Gaston 2003, Hodkinson 2005). Community changes along a local elevation gradient will reflect environmental heterogeneity as opposed to changes which may result from evolutionary and historical factors, as often is the case in latitudinal studies. As such, elevation gradients have been the target of numerous investigations into patterns of species richness and ecological interactions across various habitats and taxa, enabling an examination of ecosystem and environmental effects on biodiversity and the conservation of biodiversity (Rahbek 1995, Rahbek 1997, Tilman and Downing 1994, Austrheim 2002, Sanders et al 2003).

Networks along gradients

Interaction networks are often treated as independent systems, and are usually reconstructed at a single site or location. There is now a growing interest in exploring changes to interaction networks through space and time; along ecological gradients or across seasons (Pellsier et al 2018, Kemp et al 2017, Tylianakis and Morris 2017). This has been largely motivated by a need to understand how these systems react to their environment. This will help us predict how networks will respond to natural and anthropogenically driven environmental change. In addition, we can also begin to understand how abiotic factors shape trophic interactions and the effect they have on emergent network properties (Sanchez-Galvan et al 2017, Luviano et al 2017, Plowman et al 2017)

Variation in network structure operates through changes in community composition, frequency and strength of trophic interactions. For example,

changes in community composition arise through differential responses of species to their environment. Species which are well suited to particular conditions will increase in abundance and disproportionately impact network structure. It is difficult to predict how networks will respond to such changes, with studies producing conflicting results. Studies examining host parasitoid networks along land use gradients (Tyliankis et al 2007) and elevational gradients (Morris et al 2015) report significant changes in network properties, likely resulting from variation in species performance. Similarly, mutualistic ant-plant networks are restructured towards the upper bounds of ant elevational distribution, owing to environmentally driven changes in ant performance (Plowman et al 2017). In contrast, Kemp et al 2017 showed that network structure of host-herbivore networks in the Cape Floristic Region was unaffected by high temporal turnover of species. Similarly, Villa-Galaviz et al 2012 showed that there was little change in plant-herbivore network structure during succession in tropical dry forest, despite notable differences in species richness and composition. Continued efforts are therefore required to develop a more robust knowledge of how interaction networks respond to changing environmental conditions.

Threats and Conservation: What can networks tells us

The explosion in interest around the study of ecological gradients is a timely one. Throughout the last century, anthropogenically driven changes to the environment have been profound. It is estimated that approximately half of all tropical forests present at the beginning of the twentieth century have been destroyed or degraded due to human activity. However, much of this deforestation has occurred in recent decades, with peak deforestation occurring in the 1980s and 1990s (Wright 2005). For example, Papua New Guinea rainforest saw a marked reduction in rainforest cover between 1972-2002, with 15% of forest cleared and a further 8.8% degraded (Shearman et al 2009). Conversion of primary to secondary forest alters community composition, species richness, functional and phylogenetic diversity of hosts and higher trophic levels (Whitfeld et al 2012, Peña-Claros 2003, Dent and Wright 2009). Commercial logging, agriculture conversion and over-exploitation have been long recognized as a major threat to rainforests, however more recently the effects of climate change have come to the fore. Increasing global temperatures impacts phenological timing and species ranges, resulting is spatial and temporal dislocation of species (Montoya and

Raffaelli 2010, Walther 2010). It is likely that this decoupling of interactions will have a particularly strong impact on tropical montane species, as their narrow thermal tolerances will hinder dispersal to different altitudes (Janzen 1967).

By examining community and interaction network changes with succession and elevation, we can progress towards a full understanding of the impact of anthropogenic activities on tropical forest systems. Previous studies have shown that changes in habitat use can substantially alter host-parasitoid network structure and parasitism rates, which likely impact other trophic level and ecosystem function (Tyliankis et al 2007). Host herbivore networks have also been shown to experience substantial network change post-disturbance, however these networks recovered quickly as succession progressed (Villa-Galaviz et al 2012). Elevation studies of host parasitoid networks suggest that changing temperature may result in changes to network structure and reductions in species diversity, abundance and parasitism rate (Morris et al 2014, Maunsell et al 2014). For mutualistic networks, Plowman et al 2017 showed that networks of myrmecophytic ants and their hosts reorganize along an elevational gradient, likely driven by thermal tolerances. Responses to increasing temperatures will likely be taxa and context specific. Adedoja et al 2018 reported a breakdown of pollinator networks for bees and beetles, but not wasps and flies, along the same elevational gradient. Equivalent studies of elevational change in host herbivore networks are lacking, and therefore required given the difficulty in generalizing network responses. The trends revealed by these studies will help us to predict future change and can thus inform conservation efforts.

Scope and Aims

The interactions between host plants and their herbivores has resulted in unprecedented radiations, generating the incredible diversity of species, traits and life histories. These components of rainforest systems are thus central to their functioning. While much progress has been made in recent decades towards a mechanistic understanding of these complex systems, much work remains. Here we aim to further this endeavor by using some of the most comprehensive datasets on plant-herbivore interactions yet collected. We employ what we refer to as a '*whole forest*' approach, i.e. sampling all woody species above 5cm DBH in a series of 0.2 ha plots in primary and secondary

forest in both lowland and montane systems. From this, we attempt to scale up previous efforts at understanding drivers of tropical interaction network properties and herbivore specialisation.

In **Chapter 1** a successional chronosequence of plant-herbivore interaction networks is compiled via the sampling of distinct phases of succession (Young Secondary, Mature Secondary and Primary forest) within a humid tropical montane forest in PNG. Deriving expectations from successional theory, we examine properties of plant-herbivore interaction networks while accounting for host phylogenetic structure. We show that network structural changes throughout succession were low and specialisation metrics were more similar than expected, despite high network beta diversity. All herbivore communities were highly specialised, feeding on phylogenetically narrow set of hosts, while host phylogenetic diversity itself decreased throughout the chronosequence. We found that all succession stages harbour diverse and unique interaction networks, which together with largely similar network structures and consistent host use patterns, suggests general rules of assembly may determine the structure of these networks.

In **Chapter 2** we attempt to uncover some of these assembly rules, focusing on bottom-up structuring mechanisms. We do this by utilising a recently developed model which mimics host abundance, size classes and taxonomic structure and draws upon known interactions from a source community. Specifically, we examine whether the properties of young and mature secondary forest networks can be modelled based upon their host community properties and a complete census of an adjacent primary forest network. We found that despite the dynamic nature of rainforest succession, we could identify some constancy in shared drivers of emergent network properties. We found that changes in abundance and taxonomic structure of host trees can explain differences in network properties, while tree size distribution has little influence.

In **Chapter 3** we employ the Distance Based Specialisation Index, another recently developed methodology, to explore how elevation, habitat use and guild type affect phylogenetic specialisation of Lepidopteran communities. Species-level specialisation was calculated for herbivores in both lowland and montane forest, sampling across primary and secondary forest at each

elevation. In total, 3.8ha of tropical forest was felled and sampled. We show that lowland species are slightly less specialised than their montane counterparts, and this is driven mostly by guilds of mobile species which feed across both primary and secondary forest. This runs contrary to the idea that diversity and specialisation are necessarily tightly linked. Specialisation is also likely affected by environmental conditions which dictates resource availability in terms of both quality and quantity.

Chapter 4 deals with methodological approaches to large-scale community sampling. We compare and outline the plot-based methodology for sampling contiguous areas of rainforest exhaustively in a way which facilitates documenting trophic interactions. We compare the use of felling, canopy cranes and cherry-pickers to gain access to notoriously inaccessible forest canopies. We show that all three methods perform similarly, requiring comparable sampling effort. Cherry pickers provide access to a greater proportion of the canopy, however felling can be utilised in remote and inaccessible areas. Sampling effort and canopy accessibility were affected by forest type, total sampled leaf area, and total number of stems in a plot. We hope to promote plot-based research by providing practical and reproducible sampling guidelines for the analysis of arthropod interaction networks in forest canopies. We advocate for a global network of plot-based studies using a standardised methodology which will enable more robust comparisons across sites and biogeographical regions.

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Chapter 1

ECOGRAPHY

Research

Ecology in a human-dominated World

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-living 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 congenerics 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 understory, trees > 5 cm dbh were felled and sampled, beginning with midstory 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 complimentary beta diversity measure (βcc) was decomposed into

turnover of plants (βp), herbivores (βh), both plants and herbivores (βph) and their interactions (βo), following Novotný (2009), so that: βcc = βp + βh + βph + βo. 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 0.2 ha^{-1} ± 489 SE), followed by primary forest plots (2461 0.2 ha^{-1} ± 735 SE) and then mature secondary forest (mean 1087 0.2 ha^{-1} ± 293 SE). Herbivore abundance per kilogram foliage followed this same order; young secondary (1.29 kg^{-1} ± 0.02 SE) – primary (0.97 kg^{-1} ± 0.25 SE) – mature secondary (mean 0.49 kg^{-1} ± 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 0.2 ha^{-1} ± 142 SE). This leads to differences in total abundance (ANOVA, $F_{6,2} = 17.38$, $p = 0.003$), and abundance kg^{-1} foliage (ANOVA, $F_{6,2} = 32.7$, $p < 0.001$), 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

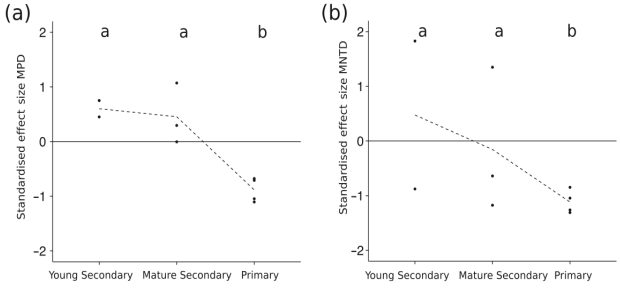

Figure 1. Standardised effect size for weighted mean pairwise distance (MPD) (a) and weighted mean nearest taxon distance (MNTD) (b) of young secondary, mature secondary and primary forest in montane forest in Papua New Guinea. The zero line represents a random structure, while values above zero tend 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.

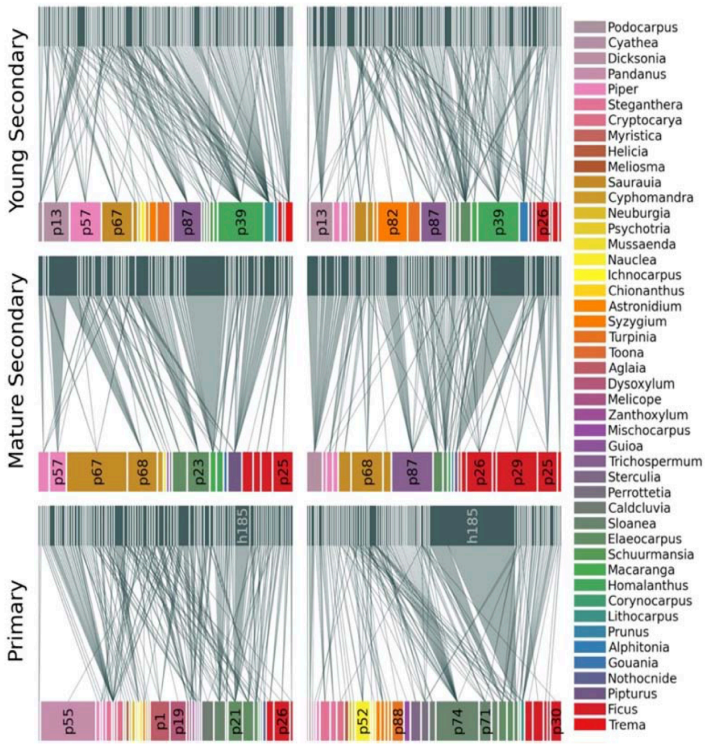

Figure 2. Representative bipartite networks from each of the three stages of succession – young secondary, mature secondary and primary forest. Only two plots per stage are shown for brevity, selected on the basis of network parameters being closest to the mean for that stage. Lower trophic level is 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,

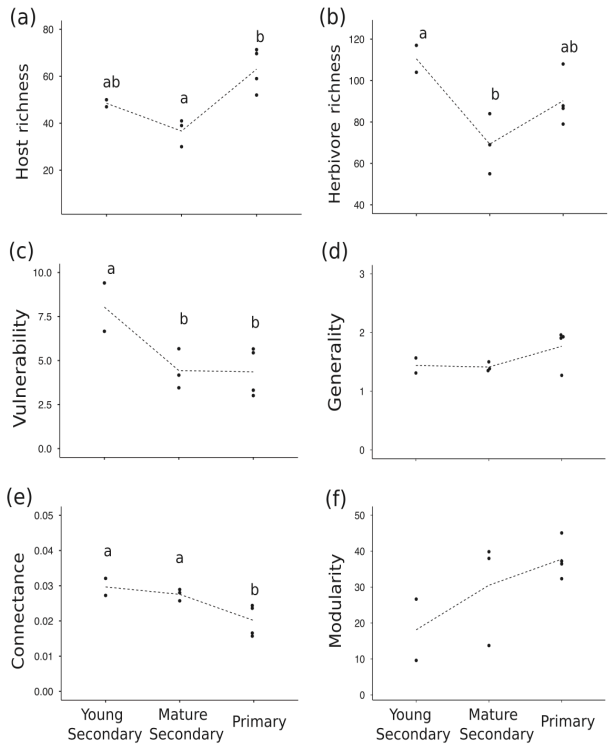

Figure 3. Network metrics for each plot along the successional chronosequence – young secondary, mature secondary and primary forest: (a) host richness, (b) herbivore richness, (c) weighted vulnerability, (d) weighted generality, (e) weighted connectance, and (f) modularity z-scores. Different letters 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 $p \leq 0.05$.

	~Succession stage		+ Network size	
Network Metric	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

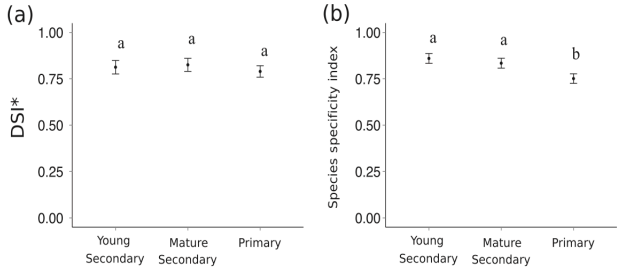

Figure 4. Mean (a) distance based specialisation (DSI*) and (b) species specificity index (SSI) of herbivores along the successional chronosequence – young secondary, mature secondary and primary forest. Error bars represent +/– standard error.

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

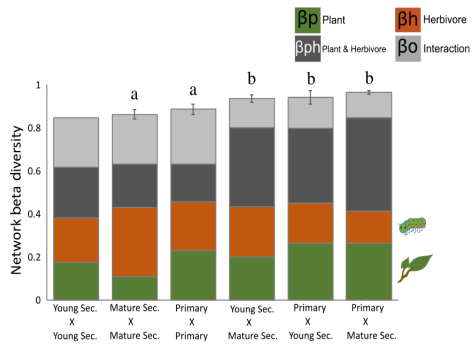

Figure 5. Beta diversity of plant-herbivore networks within and among succession stages. Beta diversity is decomposed into turnover of plants (p = green), herbivores (h = orange), both plants and herbivores (ph = dark grey) and their interactions (o = light grey). The combined total of all components 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 were 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 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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Authors contributions – CR led the writing of the manuscript; VN, YB, GW and SM conceived the ideas and designed methodology, JA, BG, PB, KM and GM collected the data; SM, BG, ASCH and CR worked on taxonomy; CR, PB, ASCH, MV, LRJ and STS analysed the data. All authors contributed critically to the drafts and gave final approval for publication.

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

Supplementary Material Appendix 1 -

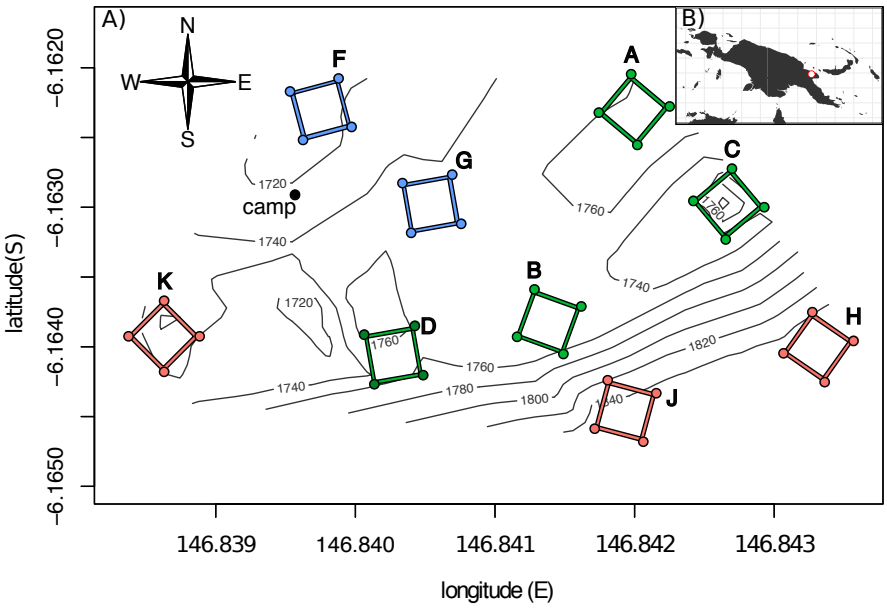

Figure A1. A- Location map of the nine 0.2ha montane forest plots sampled in Yawan, Papua New Guinea. Blue squares = Young secondary, Red squares = Mature secondary and Green squares = Primary plots.

B- Top right panel shows the location of Yawan on a map of Papua New Guinea marked as a red and white circle.

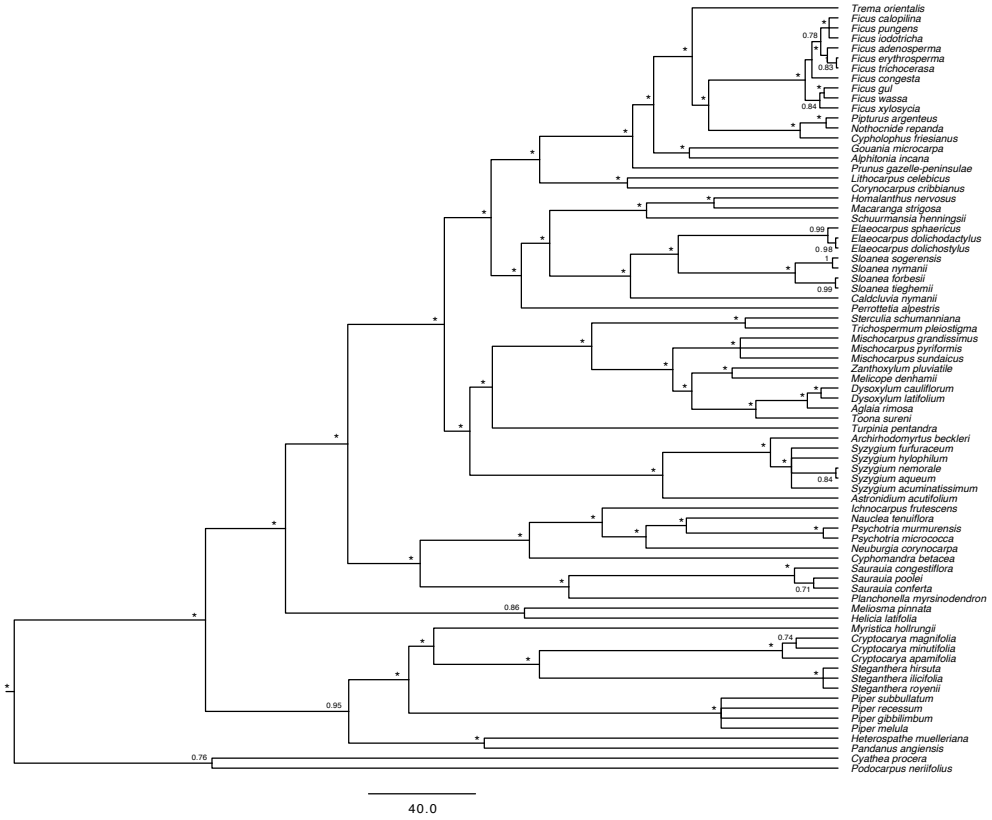

Figure A2. Reconstructed plant phylogeny of a montane rainforest community in Papua New Guinea. Host phylogeny was reconstructed using two loci: rbcL, and psbA-trnH, with these sequences located in the Barcode of Life Database (BOLD). DNA extraction, amplification and sequencing was carried out at the Canadian Centre for DNA Barcoding following standard protocols and administrated through the BOLD system. Existing sequences were sourced from online databases if available. Sequences were assembled and edited using Geneious 5.4 (Kearse et al. 2012). Host phylogeny was reconstructed using Bayesian inference as implemented in BEAST v2.4 (Drummond et al. 2012). The following substitution models were selected based on BIC computed in JModelTest 2 (Darriba et al. 2012) and were used for individual loci: rbcL: GTR+I+G, psbA-trnH: TIM1+I+G. The topology was constrained using Phylomatic 3 (Webb and Donoghue 2005). A log-normal relaxed molecular clock following Bell et al. (2010), dating based on Wikström et al. (2001) and clock rates based on Palmer (1991) were used for time-calibrating the phylogeny. Sampling was carried out every 10^3 generations for $2x10^7$ generations, the first 10% of all generations were discarded as 'burn in' and the results were summarized with a majority-rule consensus tree. All branches with posterior probability below 0.7 were treated as polytomies. Values at nodes represent posterior probabilities, nodes with a posterior probability of <0.7 were treated as polytomies. Asterisks indicate nodes that were constrained using Phylomatic 3 (Webb & Donoghue 2005). The scale represents time calibration, with dating based on Wikström, Savolainen & Chase (2001).

Table A1. Host plant taxonomic information, network host code, abundance according to number of individuals and basal area, and the number of associated herbivore interactions.

Plant Identifications	Network Code	Individuals	Basal Area (cm2)	Herbivore Interactions
Acanthaceae				
Graptophyllum pictum	na	1	27.5	0
Actinidiaceae				
Saurauia conferta	p67	156	8403.4	74
Saurauia congestiflora		4	263.1	
Saurauia poolei	p68	93	6082.2	126
Saurauia schumanniana	p69	34	1606.7	6
Anacardiaceae				
Rhus taitensis	na	4	211.7	0
Apocynaceae				
Ichnocarpus frutescens	p40	2	54.4	8
Araliaceae				
Gastonia spectabilis	na	6	869.2	0
Schefflera setulosa	na	13	480.3	0
Schefflera waterhousei	na	1	68.9	0
Arecaceae				
Heterospathe muelleriana	p38	24	1481.9	2
Asparagaceae				
Cordyline terminalis	na	1	32.5	0
Aspleniaceae				
Asplenium nidus	na	1	30.5	0
Athyriaceae				
Diplazium esculentum	na	1	58.9	0
Celastraceae				
Perrottetia alpestris	p56	19	1248.0	46
Clusiaceae				
Garcinia latissima	na	1	103.9	0
Corynocarpaceae				
Corynocarpus cribbianus	p8	2	101.4	63
Cunoniaceae				
Caldcluvia nymanii	p6	10	820.2	32
Cyatheaceae				
Cyathea auriculifera	p12	9	472.3	23
Cyathea contaminans	p13	111	7587.1	490
Cyathea procera	p14	1	32.5	2
Cyathea runensis	na	1	52.4	0
Cyathea werneri	na	1	86.4	0
Dicksoniaceae				
Dicksonia sciurus	p17	12	753.4	91

Elaeocarpaceae				
Elaeocarpus dolichodactylus	p20	33	3199.2	251
Elaeocarpus dolichostylus	p21	62	5802.9	3014
Elaeocarpus multisectus	na	2	75.4	0
Elaeocarpus sayeri	na	1	70.9	0
Elaeocarpus schlechterianus	p22	3	620.6	4
Elaeocarpus sphaericus	p23	32	2658.2	2253
Sloanea forbesii	p71	14	991.6	33
Sloanea nymanii	p72	6	840.8	43
Sloanea sogerensis	p73	10	964.7	117
Sloanea tieghemii	p74	54	5978.5	557
Euphorbiaceae				
Homalanthus nervosus	p39	224	11575.0	360
Homalanthus novoguineensis	na	6	301.1	0
Macaranga inermis	p42	2	116.8	19
Macaranga pleiostemona	p43	5	177.7	32
Macaranga polyadenia	na	1	78.4	0
Macaranga strigosa	p44	39	1950.7	760
Fabaceae				
Caesalpinia crista	na	4	115.8	0
Fagaceae				
Castanopsis acuminatissima	na	1	77.4	0
Lithocarpus celebicus	p41	10	536.7	658
Gesneriaceae				
Cyrtandra erectiloba	na	9	269.1	0
Himantandraceae				
Galbulimima belgraveana	na	1	27.5	0
Lauraceae				
Actinodaphne nitida	na	1	58.4	0
Cryptocarya apamifolia	p9	12	604.6	16
Cryptocarya magnifolia	p10	5	338.5	5
Cryptocarya minutifolia	p11	6	339.0	3
Cryptocarya multipaniculata	na	1	33.5	0
Cryptocarya pulchella	na	1	35.4	0
Cryptocarya viridiflora	na	2	52.9	0
Persea americana	na	3	393.4	0
Loganiaceae				
Neuburgia corynocarpa	p53	9	1113.4	9
Malvaceae				
Sterculia schlechteri	na	1	79.9	0
Sterculia schumanniana	p78	6	697.0	7
Trichospermum pleiostigma	na	59	6275.5	419
Melastomataceae				

Astronidium acutifolium	p4	11	787.4	16
Astronidium morobiense	p5	2	162.8	3
Meliaceae				
Aglaia brassii	na	1	65.5	0
Aglaia rimosa	p1	32	2164.0	87
Dysoxylum brevipaniculum	na	2	197.2	0
Dysoxylum cauliflorum	p18	1	69.9	14
Dysoxylum latifolium	p19	4	654.5	2
Dysoxylum parasiticum	na	2	178.7	0
Toona sureni	p85	4	143.3	4
Monimiaceae				
Kibara coriacea	na	2	70.4	0
Palmeria arfakiana	na	2	118.3	0
Steganthera hirsuta	p75	1	30.0	10
Steganthera hospitans	na	1	26.0	0
Steganthera ilicifolia	p76	3	346.1	19
Steganthera royenii	p77	7	685.8	49
Moraceae				
Artocarpus lacucha		1	31.0	0
Ficus adenosperma	p24	18	867.2	68
Ficus calopilina	p25	19	1394.5	62
Ficus congesta	p26	63	4225.9	100
Ficus copiosa	na	1	63.4	0
Ficus dammaropsis	na	5	155.3	0
Ficus erythrosperma	p27	36	1347.1	48
Ficus gul	p28	8	619.1	6
Ficus hombroniana	na	3	114.3	0
Ficus iodotricha	p29	19	1493.1	30
Ficus melinocarpa	na	1	71.9	0
Ficus morobensis	na	1	34.0	0
Ficus pachyclada	p30	1	129.3	3
Ficus pungens	p31	12	885.2	2
Ficus trichocerasa	p32	10	680.0	4
Ficus wassa	p33	31	2266.4	78
Ficus xylosycia	p34	2	649.3	2
Streblus glaber	na	1	31.5	0
Trophis philippinensis	na	1	64.9	0
Musaceae				
Musa peekelii	na	7	680.0	0
Myristicaceae				
Myristica hollrungii	p51	1	48.9	4
Myristica lancifolia	na	1	132.8	0
Myristica subalulata	na	61	2454.5	0

Myrsinaceae				
Rapanea involucrata	na	8	251.1	0
Myrtaceae				
Archirhodomyrtus beckleri	p3	1	50.9	2
Syzygium acuminatissimum	p79	22	1096.9	9
Syzygium aqueum	p80	1	231.7	10
Syzygium decipiens	p81	5	240.7	2
Syzygium furfuraceum	p82	30	2110.1	13
Syzygium hylophilum	p83	7	341.5	20
Syzygium nemorale	p84	2	138.8	4
Syzygium versteegii	na	11	847.7	0
Ochnaceae				
Schuurmansia henningsii	p70	9	438.9	2
Oleaceae				
Chionanthus ramiflora	p7	4	167.3	7
Pandanaceae				
Pandanus adinobotrys	na	18	588.2	0
Pandanus angiensis	p55	79	5253.6	12
Pandanus rostellatus	na	18	703.9	0
Piperaceae				
Piper aduncum	na	4	113.8	0
Piper gibbilimbum	p57	135	4731.7	273
Piper melula	p58	34	1055.5	27
Piper recessum	p59	73	2200.3	4
Piper subbullatum	p60	21	713.5	5
Pittosporaceae				
Pittosporum ramiflorum	na	1	30.0	0
Podocarpaceae				
Podocarpus neriifolius	p63	1	101.4	279
Proteaceae				
Helicia latifolia	p37	1	99.4	7
Rhamnaceae				
Alphitonia incana	p2	7	519.8	202
Gouania microcarpa	p35	4	136.3	148
Rosaceae				
Prunus dolichobotrys		1	29.5	0
Prunus gazelle-peninsulae	p64	3	83.4	14
Rubus diclinis		1	27.0	0
Rubiaceae				
Mussaenda ferruginea	p50	2	52.9	5
Nauclea tenuiflora	na	14	1020.3	92
Psychotria micrococca	p65	10	329.5	11
Psychotria murmurensis	p66	4	142.8	2

Timonius densiflorus	na	1	154.8	0
Uncaria nervosa	na	1	32.5	0
Rutaceae				
Melicope denhamii	p45	16	1147.8	9
Melicope elleryana	na	8	345.5	0
Zanthoxylum pluviatile	p89	10	696.0	6
Sabiaceae				
Meliosma pinnata	p46	11	870.2	4
Salicaceae				
Flacourtia zippelii	na	2	90.4	0
Sapindaceae				
Guioa subsericea	p36	1	32.0	11
Mischocarpus grandissimus	p47	1	46.4	2
Mischocarpus largifolius	na	1	44.4	0
Mischocarpus pyriformis	p48	7	379.5	72
Mischocarpus sundaicus	p49	4	141.8	26
Sapotaceae				
Planchonella myrsinodendron	p62	1	94.9	2
Solanaceae				
Cyphomandra betacea	p16	42	1521.3	20
Staphyleaceae				
Turpinia pentandra	p88	65	4271.6	131
Symplocaceae				
Symplocos cochinchinensis	na	1	43.2	0
Tetramelaceae				
Tetrameles nudiflora	na	1	79.9	0
Ulmaceae				
Trema orientalis	p86	5	453.3	382
Urticaceae				
Cypholophus friesianus	p15	7	230.7	2
Debregeasia longifolia	na	1	27.5	0
Dendrocnide cordata	na	4	402.9	0
Nothocnide melastomatifolia	na	1	31.5	0
Nothocnide repanda	p54	2	154.8	1
Pipturus argenteus	p61	30	2564.6	411
Vitaceae				
Cayratia trifolia	na	1	34.0	0
Winteraceae				
Bubbia sylvestris	na	1	37.4	0

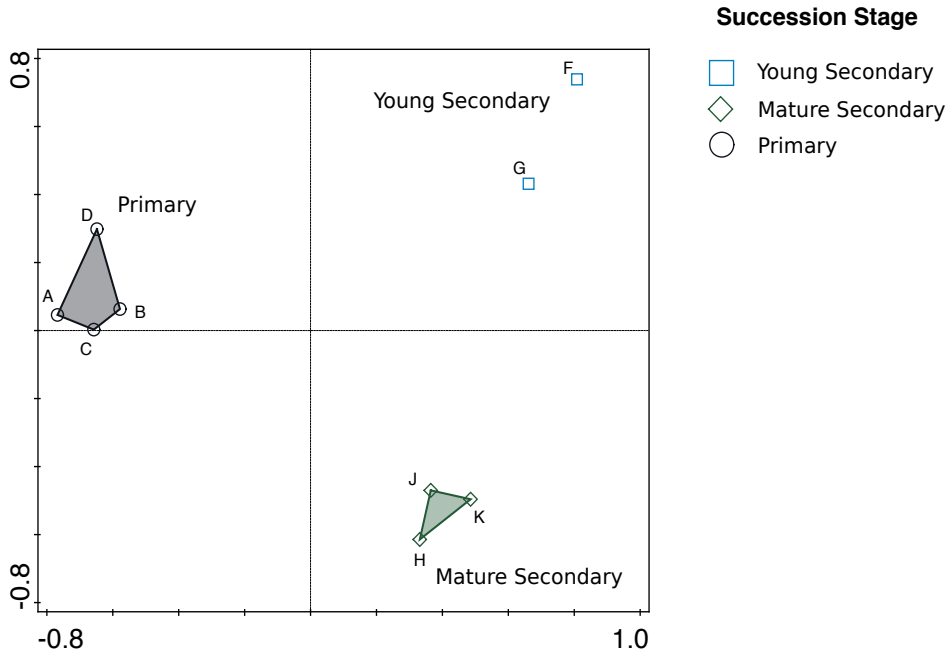

Figure A3. Principal components analysis (PCA) of host communities (>5cm DBH) and successional stage in montane rainforest in Papua New Guinea. Young secondary (~ 9 years since disturbance) plots are represented by blue squares, mature secondary (~ 25 years) plots by diamonds, and primary forest (>100 years) plots by circles. First canonical axis eigenvalue = 0.372 and the second = 0.186, with the combined variation explained = 54.85%.

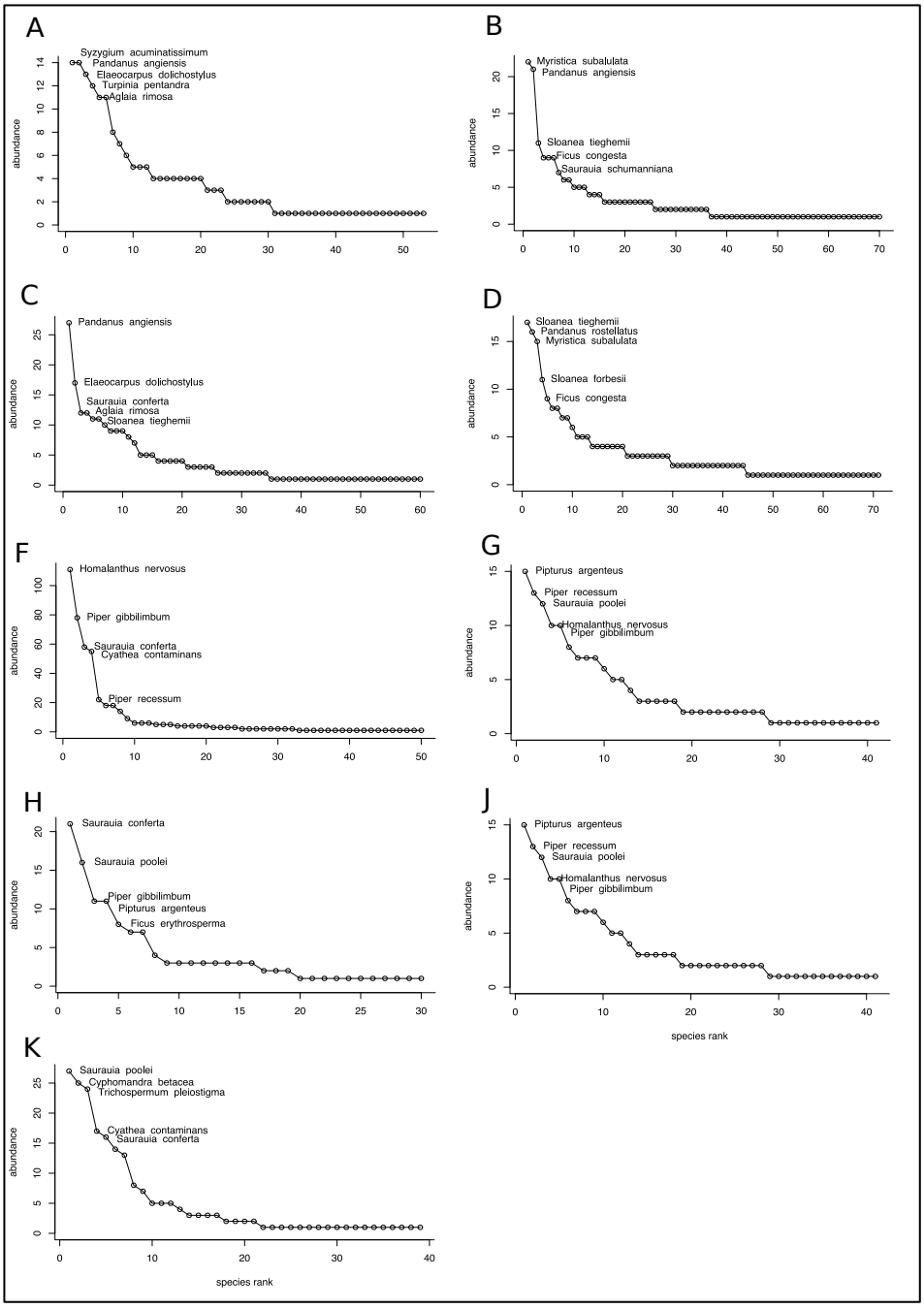

Figure A4. Dominance curves of host communities (>5cm DBH) in montane forest in Papua New Guinea. Primary forest plots (A,B,C,D), Young Secondary (F,G) and Mature Secondary (H,J,K) with labels for the 5 most dominant species in those plots.

Table A2. Herbivore (Lepidoptera) taxonomic information, total species abundance, and abundance in each succession stage. Species BIN numbers provided, further details may be found on the Barcode of Life Database. Asterisk indicate a species placement based upon phylogenetic inference.

				Abundance/Succession Stage		
Family	Species	BIN Number	Total	Young Second	Mature Second	Primary
Plutellidae	*Plutella sp. AAA1513*	BOLD:AAA1513	5	0	1	4
Crambidae	*Meekiaria sp. AAA3383*	BOLD:AAA3383	9	0	0	9
Erebidae	*Asota sp. AAA5335*	BOLD:AAA5335	2	0	2	0
Noctuidae	*Condica illecta*	BOLD:AAB2411	2	0	2	0
Noctuidae	*Tiracola sp. AAB5638*	BOLD:AAB5638	2	2	0	0
Choreutidae	*Choreutis sp. AAB5921*	BOLD:AAB5921	7	0	7	0
Choreutidae	*Choreutis cf. porphyratma*	BOLD:AAC0560	5	0	5	0
Geometridae	*Paradromulia sp. AAC1158*	BOLD:AAC1158	5	5	0	0
Choreutidae	*Choreutis niphocrypta*	BOLD:AAC1274	9	5	0	4
Tortricidae	*Adoxophyes nr. marmarygodes*	BOLD:AAC1387	246	138	8	100
Crambidae	*Herpetogramma stultalis*	BOLD:AAC2327	2	0	0	2
Geometridae	*Petelia sp. AAC2735*	BOLD:AAC2735	3	0	3	0
Choreutidae	*Choreutis sp. AAC7453*	BOLD:AAC7453	2	2	0	0
Nolidae	*Etanna brunnea*	BOLD:AAC9321	3	3	0	0
Crambidae	*Talanga exquisitalis*	BOLD:AAD8828	104	20	48	36
Sphingidae	*Gnathothlibus meeki*	BOLD:AAE7108	12	7	4	1
Geometridae	*Craspedosis aurigutta*	BOLD:AAE9296	25	6	17	2
Erebidae	*Catada apoblepta*	BOLD:AAF1549	24	0	0	24
Nolidae	*Earias uniplaga*	BOLD:AAF6217	6	6	0	0
Tortricidae	*sp. AAF9348*	BOLD:AAF9348	16	6	5	5
Tortricidae	*sp. AAF9349*	BOLD:AAF9349	26	4	14	8
Tortricidae	**Isotenes sp. AAF9350*	BOLD:AAF9350	4	4	0	0
Geometridae	*sp. AAF9464*	BOLD:AAF9464	2	0	0	2
Noctuidae	*Chasmina tibiopunctata*	BOLD:AAG6014	3	1	2	0
Erebidae	*Metaemene sp. AAI1490*	BOLD:AAI1490	4	4	0	0
Crambidae	*Meekiaria sp. AAL5545*	BOLD:AAL5545	4	0	0	4
Nolidae	*Gadirtha impingens*	BOLD:AAL6729	3	3	0	0
Notodontidae	*Chadisra striata*	BOLD:AAL8395	4	4	0	0
Geometridae	*Craspedosis sp. AAM0235*	BOLD:AAM0235	4	0	0	4
Erebidae	*Hypena gonosp.ilalis*	BOLD:AAM0874	10	0	10	0
Thyrididae	*Mellea sp. AAM5436*	BOLD:AAM5436	25	14	0	11
Geometridae	**Ascotis sp. AAM6936*	BOLD:AAM6936	6	6	0	0
Tortricidae	*sp. AAM7269*	BOLD:AAM7269	16	8	6	2
Noctuidae	*Tiracola sp. AAM9672*	BOLD:AAM9672	4	4	0	0
Crambidae	*Udea sp. AAO2713*	BOLD:AAO2713	2	2	0	0
Erebidae	*Ophyx owgarra*	BOLD:AAO3382	10	0	5	5
Thyrididae	*Mellea sp. AAO4080*	BOLD:AAO4080	16	0	16	0
Erebidae	*Axiocteta sp. AAO4116*	BOLD:AAO4116	6	0	2	4
Crambidae	*Omiodes sp. AAO4249*	BOLD:AAO4249	3	0	0	3
Tortricidae	*Rhabdotenes sp. AAP2731*	BOLD:AAP2731	7	0	7	0
Geometridae	*sp. AAP2900*	BOLD:AAP2900	102	74	10	18
Tortricidae	*Adoxophyes sp. AAP5694*	BOLD:AAP5694	92	42	4	46
Tortricidae	*sp. AAP6512*	BOLD:AAP6512	6	2	3	1
Erebidae	**Euproctis sp. AAP7433*	BOLD:AAP7433	2	0	0	2

Tortricidae	*sp. AAP7648*	BOLD:AAP7648	6	4	0	2
Erebidae	*Ophyx sp. AAQ2186*	BOLD:AAQ2186	52	9	19	24
Tortricidae	*Thaumatotibia sp. AAW6610*	BOLD:AAW6610	4	4	0	0
Pyralidae	*Faveria sp. AAY6061*	BOLD:AAY6061	37	37	0	0
Erebidae	*Lambula sp. AAY6219*	BOLD:AAY6219	24	1	5	18
Erebidae	*Calliteara sp. ABW5916*	BOLD:ABW5916	3	3	0	0
Erebidae	*Euproctis sp. ABW8356*	BOLD:ABW8356	2	0	2	0
Geometridae	*Paradromulia rufibrunnea*	BOLD:ABW8597	24	1	1	22
Geometridae	sp. ADF6011	BOLD:ADF6011	3	0	0	3
Noctuidae	*Tiracola aureata*	BOLD:ABX5542	5	5	0	0
Geometridae	*Craspedosis aurigutta*	BOLD:ABX6387	18	0	17	1
Tortricidae	*Dudua sp. ABY6340*	BOLD:ABY6340	3	3	0	0
Erebidae	*Olene sp. ABY9175*	BOLD:ABY9175	25	0	20	5
Crambidae	*Pycnarmon nr. dryocentra*	BOLD:ABZ0583	4	0	0	4
Gelechiidae	*Dichomeris sp. ABZ6084*	BOLD:ABZ6084	320	0	320	0
Geometridae	*sp. ACA3495*	BOLD:ACA3495	6	6	0	0
Geometridae	*Alcis irrufata*	BOLD:ACA8529	39	14	3	22
Geometridae	*Casbia sp. ACB0448*	BOLD:ACB0448	16	16	0	0
Geometridae	*Prasinocyma sp. ACB0527*	BOLD:ACB0527	7	0	0	7
Geometridae	*sp. ACB0687*	BOLD:ACB0687	35	32	0	3
Geometridae	*sp. ACB1815*	BOLD:ACB1815	2	0	0	2
Geometridae	*Gymnoscelis sp. ACB8931*	BOLD:ACB8931	200	200	0	0
Geometridae	*Paradromulia sp. ACB8986*	BOLD:ACB8986	35	5	9	21
Crambidae	*Agrotera semipictalis*	BOLD:ACD3447	3	3	0	0
Tortricidae	*sp. ACD3548*	BOLD:ACD3548	3	3	0	0
Tortricidae	*Diadelomorpha sp. ACD3549*	BOLD:ACD3549	27	8	10	9
Tortricidae	**Cryptoptila sp. ACD3622*	BOLD:ACD3622	2	0	0	2
Tortricidae	*sp. ACD3790*	BOLD:ACD3790	11	0	0	11
Tortricidae	*sp. ACD3861*	BOLD:ACD3861	139	136	0	3
Tortricidae	*Gatesclarkeana sp. ACE7876*	BOLD:ACE7876	5	5	0	0
Geometridae	*sp. ACK5418*	BOLD:ACK5418	47	12	0	35
Geometridae	*Casbia sp. ACK6572*	BOLD:ACK6572	52	52	0	0
Geometridae	*sp. ACK6876*	BOLD:ACK6876	2	0	2	0
Geometridae	*sp. ACK7565*	BOLD:ACK7565	5	0	5	0
Geometridae	*sp. ACK7570*	BOLD:ACK7570	26	19	0	7
Geometridae	*sp. ACK7831*	BOLD:ACK7831	4	0	0	4
Erebidae	*Arctornis sp. ACK8100*	BOLD:ACK8100	5	5	0	0
Geometridae	*sp. ACK9224*	BOLD:ACK9224	58	11	47	0
Geometridae	*Myrioblephara sp. ACK9384*	BOLD:ACK9384	54	1	1	52
Geometridae	*sp. ACL2137*	BOLD:ACL2137	96	28	19	49
Tortricidae	*sp. ACL2152*	BOLD:ACL2152	13	0	0	13
Tortricidae	*sp. ACL2211*	BOLD:ACL2211	3	1	1	1
Geometridae	*Prasinocyma sp. ACL2220*	BOLD:ACL2220	7	1	3	3
Tortricidae	*sp. ACL2255*	BOLD:ACL2255	2	0	0	2
Roeslerstammiidae	*Amphithera sp. ACL2288*	BOLD:ACL2288	24	1	6	17
Geometridae	*sp. ACL2297*	BOLD:ACL2297	15	15	0	0
Geometridae	*sp. ACL2314*	BOLD:ACL2314	32	0	4	28
Crambidae	*Palpita sp. ACL2380*	BOLD:ACL2380	4	0	0	4
Geometridae	*sp. ACL2383*	BOLD:ACL2383	88	9	29	50
Geometridae	*Casbia sp. ACL2414*	BOLD:ACL2414	32	32	0	0

Geometridae	*Paradromulia sp. ACL2441*	BOLD:ACL2441	35	9	1	25
Geometridae	*Hyposidra sp. ACL2461*	BOLD:ACL2461	9	2	0	7
Cosmopterigida	*Macrobathra sp. ACL2485*	BOLD:ACL2485	3	3	0	0
Crambidae	*Synclera sp. ACL2524*	BOLD:ACL2524	12	0	12	0
Tortricidae	*sp. ACL2557*	BOLD:ACL2557	18	18	0	0
Tortricidae	*sp. ACL2558*	BOLD:ACL2558	195	195	0	0
Geometridae	*sp. ACL2584*	BOLD:ACL2584	53	7	1	45
Geometridae	*sp. ACL2687*	BOLD:ACL2687	2	0	2	0
Geometridae	*sp. ACL2772*	BOLD:ACL2772	4	2	0	2
Geometridae	*sp. ACL2773*	BOLD:ACL2773	3	0	0	3
Geometridae	*sp. ACL2774*	BOLD:ACL2774	2	0	0	2
Tortricidae	*sp. ACL2809*	BOLD:ACL2809	5	0	5	0
Crambidae	*Herpetogramma sp. ACL2815*	BOLD:ACL2815	10	0	10	0
Geometridae	*Tolmera sp. ACL2838*	BOLD:ACL2838	3	0	0	3
Geometridae	*Tolmera sp. ACL2839*	BOLD:ACL2839	6	4	0	2
Geometridae	*sp. ACL2840*	BOLD:ACL2840	9	6	2	1
Geometridae	*sp. ACL2851*	BOLD:ACL2851	27	3	0	24
Tortricidae	*sp. ACL2861*	BOLD:ACL2861	3	0	3	0
Tortricidae	*sp. ACL2861*	BOLD:ACL2916	8	0	0	8
Geometridae	*sp. ACL2861*	BOLD:ACL2922	3	3	0	0
Tortricidae	*sp. ACL2943*	BOLD:ACL2943	13	13	0	0
Elachistidae	*Zaratha sp. ACL2964*	BOLD:ACL2964	76	0	76	0
Tortricidae	*sp. ACL2965*	BOLD:ACL2965	2	0	0	2
Tortricidae	*sp. ACL2981*	BOLD:ACL2981	22	0	0	22
Geometridae	*sp. ACL2986*	BOLD:ACL2986	7	7	0	0
Pyralidae	*sp. ACL3232*	BOLD:ACL3232	3	0	0	3
Tortricidae	*Cryptophlebia sp. ACL3303*	BOLD:ACL3303	4	0	0	4
Tortricidae	*sp. ACL3304*	BOLD:ACL3304	3	0	0	3
Tortricidae	*Zacorisca holantha*	BOLD:ACL3429	176	12	61	103
Tortricidae	*Zacorisca aptycha*	BOLD:ACL3430	15	0	0	15
Geometridae	*sp. ACL3435*	BOLD:ACL3435	101	6	0	95
Geometridae	*sp. ACL3436*	BOLD:ACL3436	8	1	0	7
Pyralidae	*Adoxophyes sp. ACL3493*	BOLD:ACL3493	2	2	0	0
Tortricidae	*sp. ACL3540*	BOLD:ACL3540	33	2	3	28
Erebidae	*sp. ACL3603*	BOLD:ACL3603	5	5	0	0
Choreutidae	*Choreutis sp. ACL3612*	BOLD:ACL3612	21	0	9	12
Geometridae	*sp. ACL3687*	BOLD:ACL3687	3	3	0	0
Alucitidae	*sp. ACL3689*	BOLD:ACL3689	2	0	2	0
Tortricidae	*Zacorisca cyprantha*	BOLD:ACL3736	39	5	17	17
Depressariidae	*sp. ACL3783*	BOLD:ACL3783	75	2	20	53
Pyralidae	*sp. ACL3835*	BOLD:ACL3835	180	36	79	65
Tineidae	*Trachycentra sp. ACL3836*	BOLD:ACL3836	12	0	0	12
Geometridae	*Scopula sp. ACL3931*	BOLD:ACL3931	10	0	0	10
Geometridae	*sp. ACL3940*	BOLD:ACL3940	5	3	2	0
Geometridae	*sp. ACL3967*	BOLD:ACL3967	6	1	0	5
Geometridae	*Parachaetolopha sp. ACL4036*	BOLD:ACL4036	36	4	32	0
Geometridae	*sp. ACL4038*	BOLD:ACL4038	4	0	0	4
Geometridae	*Myrioblephara sp. ACL4039*	BOLD:ACL4039	2	0	0	2
Tortricidae	*sp. ACL4127*	BOLD:ACL4127	14	6	1	7
Oecophoridae	*Delonoma sp. ACL4138*	BOLD:ACL4138	4	0	0	4
Noctuidae	*Rusicada bicolor*	BOLD:ACL4187	11	9	2	0

Erebidae	*Lineopalpa rufa*	BOLD:ACL4188	7	7	0	0
Erebidae	*Lemyra sp. ACL4203*	BOLD:ACL4203	238	3	220	15
Tortricidae	*sp. ACM3112*	BOLD:ACM3112	9	0	1	8
Tortricidae	*sp. ACM3119*	BOLD:ACM3119	14	0	7	7
Tortricidae	*sp. ACM3124*	BOLD:ACM3124	98	37	0	61
Tortricidae	*sp. ACM3125*	BOLD:ACM3125	12	3	4	5
Tortricidae	*sp. ACM3127*	BOLD:ACM3127	50	38	4	8
Tortricidae	*Adoxophyes sp. ACM3127*	BOLD:ACM3234	4	0	0	4
Tortricidae	*sp. ACM3250*	BOLD:ACM3250	9	0	3	6
Tortricidae	*sp. ACM3328*	BOLD:ACM3328	62	2	15	45
Tortricidae	*sp. ACM3342*	BOLD:ACM3342	7	0	3	4
Depressariidae	*Agriophara sp. ACM3388*	BOLD:ACM3388	2	0	0	2
Tortricidae	*sp. ACM3393*	BOLD:ACM3393	19	19	0	0
Tortricidae	*sp. ACM3412*	BOLD:ACM3412	16	14	0	2
Tortricidae	*sp. ACM3419*	BOLD:ACM3419	7	2	0	5
Tortricidae	*sp. ACM3440*	BOLD:ACM3440	34	34	0	0
Tortricidae	*sp. ACM3442*	BOLD:ACM3442	5	0	0	5
Tortricidae	*sp. ACM3468*	BOLD:ACM3468	5	5	0	0
Tortricidae	*sp. ACM3509*	BOLD:ACM3509	3	0	0	3
Tortricidae	*sp. ACM3510*	BOLD:ACM3510	18	0	4	14
Tortricidae	*sp. ACM3533*	BOLD:ACM3533	6	0	0	6
Tortricidae	*sp. ACM3602*	BOLD:ACM3602	4	2	0	2
Plutellidae	*sp. ACM3613*	BOLD:ACM3613	37	37	0	0
Tortricidae	*sp. ACM3694*	BOLD:ACM3694	4	4	0	0
Nolidae	*sp. ACM3703*	BOLD:ACM3703	4	0	4	0
Nolidae	*Nola sp. ACM3704*	BOLD:ACM3704	10	2	0	8
Tortricidae	*sp. ACM3711*	BOLD:ACM3711	7	1	0	6
Tortricidae	*sp. ACM3761*	BOLD:ACM3761	9	0	0	9
Nolidae	*Nola opalina*	BOLD:ACM3797	12	3	2	7
Tortricidae	*Zacorisca sp. ACM3802*	BOLD:ACM3802	34	5	6	23
Limacodidae	*sp. ACM3873*	BOLD:ACM3873	26	0	7	19
Erebidae	*Hypena poecila*	BOLD:ACM3910	2	0	2	0
Geometridae	*Sauris sp. ACM3914*	BOLD:ACM3914	22	4	7	11
Gelechiidae	*sp. ACM3945*	BOLD:ACM3945	65	20	8	37
Gelechiidae	*sp. ACM3982*	BOLD:ACM3982	2	0	0	2
Nolidae	*Nycteola avola*	BOLD:ACM4128	8	8	0	0
Nolidae	*Nycteola kebea*	BOLD:ACM4129	9	9	0	0
Tortricidae	*Cryptophlebia sp. ACM4140*	BOLD:ACM4140	4	0	0	4
Erebidae	*Somena sp. ACM4172*	BOLD:ACM4172	6	0	0	6
Erebidae	*Leucoma ACM4173 & ACM4174 complex*	BOLD:ACM4173 + ACM4174	4412	0	0	4412
Erebidae	*Euproctis kunupi*	BOLD:ACM4175	7	2	2	3
Thyrididae	*Mellea sp. ACM4185*	BOLD:ACM4185	100	44	5	51
Erebidae	*sp. ACM4197*	BOLD:ACM4197	55	7	44	4
Geometridae	*sp. ACM4248*	BOLD:ACM4248	99	0	98	1
Geometridae	*sp. ACM4260*	BOLD:ACM4260	127	67	59	1
Geometridae	*sp. ACM4261*	BOLD:ACM4261	4	4	0	0
Erebidae	*sp. ACM4272*	BOLD:ACM4272	104	21	17	66
Erebidae	*sp. ACM4273*	BOLD:ACM4273	2	2	0	0
Erebidae	*sp. ACM4274*	BOLD:ACM4274	85	0	26	59
Erebidae	*sp. ACM4274*	BOLD:ACM4275	9	0	0	9

Thyrididae	*Mellea sp. ACM4292*	BOLD:ACM4292	51	8	32	11
Immidae	*Imma sp. ACM4351*	BOLD:ACM4351	20	12	0	8
Geometridae	*sp. ACM4429*	BOLD:ACM4429	30	0	3	27
Nolidae	*sp. ACM4452*	BOLD:ACM4452	10	0	10	0
Erebidae	*Dura sp. ACM4458*	BOLD:ACM4458	13	0	0	13
Immidae	*sp. ACM4494*	BOLD:ACM4494	26	8	5	13
Nolidae	*sp. ACM4525*	BOLD:ACM4525	16	16	0	0
Erebidae	*Euproctis petasma*	BOLD:ACM4533	94	57	25	12
Geometridae	*Lobocraspeda sp. ACM4541*	BOLD:ACM4541	10	0	10	0
Erebidae	*Euproctis iseres*	BOLD:ACM4556	22	0	15	7
Erebidae	*sp. ACM4557*	BOLD:ACM4557	5	0	0	5
Nolidae	*Nycteola aroa*	BOLD:ACM4561	66	66	0	0
Geometridae	*Chloroclystis sp. ACM4629*	BOLD:ACM4629	37	37	0	0
Crambidae	*Udea sp. ACM4670*	BOLD:ACM4670	282	94	186	2
Geometridae	*Idiomilionia ventralis*	BOLD:ACM4680	38	0	0	38
Erebidae	*Somena sp. ACM4686*	BOLD:ACM4686	24	19	1	4
Erebidae	*sp. ACM4698*	BOLD:ACM4698	2	0	0	2
Eupterotidae	*Cotana nr. aroa*	BOLD:ACM8731	12	0	0	12
Erebidae	*Spilosoma sp. ACM9052*	BOLD:ACM9052	18	0	18	0
Eupterotidae	*Cotana nr. kebea*	BOLD:ACM9094	269	72	14	183
Eupterotidae	*Cotana nr. pallidipascia*	BOLD:ACM9095	76	0	0	76
Lycaenidae	*Psychonotis hebes*	BOLD:ACM9606	6	0	6	0
Hesperiidae	*Allora major*	BOLD:ACM9721	35	0	0	35
Erebidae	*Euproctis mycoides*	BOLD:ACM9751	32	24	0	8
Geometridae	*sp. ACM9942*	BOLD:ACM9942	6	0	0	6
Erebidae	*Pinacia sp. ACM9982*	BOLD:ACM9982	6	4	0	2
Erebidae	*sp. ACM9983*	BOLD:ACM9983	10	0	3	7
Crambidae	*Tyspanodes radiata*	BOLD:ACN0624	48	13	0	35
Geometridae	*sp. ACN0654*	BOLD:ACN0654	17	3	2	12
Geometridae	*Milionia sp. ACN0900*	BOLD:ACN0900	8	8	0	0
Lycaenidae	*Hypochrysops sp. ACN1400*	BOLD:ACN1400	346	264	82	0
Noctuidae	*Argyrolepidia sp. ACN1848*	BOLD:ACN1848	23	8	10	5
Thyrididae	*sp. ACN9209*	BOLD:ACN9209	7	0	0	7
Thyrididae	*sp. ACN9210*	BOLD:ACN9210	17	0	3	14
Tortricidae	*sp. ACN9347*	BOLD:ACN9347	2	0	2	0
Tortricidae	*sp. ACN9403*	BOLD:ACN9403	182	122	40	20
Tortricidae	*sp. ACN9405*	BOLD:ACN9405	38	0	0	38
Thyrididae	*sp. ACN9810*	BOLD:ACN9810	2	2	0	0
Tortricidae	*sp. ACN9885*	BOLD:ACN9885	5	5	0	0
Tortricidae	*sp. ACN9899*	BOLD:ACN9899	64	62	0	2
Pyralidae	*Salma chlorographalis*	BOLD:ACO0191	19	19	0	0
Tortricidae	*Lobesia sp. ACO0243*	BOLD:ACO0243	80	48	15	17
Thyrididae	*Mellea sp. ACO0290*	BOLD:ACO0290	14	0	6	8
Tortricidae	*sp. ACO0554*	BOLD:ACO0554	118	67	48	3
Geometridae	*sp. ACQ4822*	BOLD:ACQ4822	24	0	2	22
Lycaenidae	*sp. ACS9688*	BOLD:ACS9688	17	1	16	0
Erebidae	*sp. ACS9712*	BOLD:ACS9712	31	12	12	7
Erebidae	**Calliteara sp. ACS9712*	BOLD:ACT0038	18	0	11	7
Erebidae	*sp. ACT0909*	BOLD:ACT0909	29	7	17	5
Geometridae	*sp. ACT1506*	BOLD:ACT1506	2	0	2	0
Noctuidae	**Tiracola sp. ACT2243*	BOLD:ACT2243	2	1	1	0

Geometridae	*sp. ACT2444*	BOLD:ACT2444	7	1	0	6
Eupterotidae	**Cotana sp. ACT2703*	BOLD:ACT2703	3	0	0	3
Erebidae	*sp. ACT4130*	BOLD:ACT4130	32	9	7	16
Erebidae	*sp. ACT4598*	BOLD:ACT4598	3	0	0	3
Lasiocampidae	**Pseudophyllodes sp. ACT4640*	BOLD:ACT4640	7	0	0	7
Limacodidae	*sp. ACT5001*	BOLD:ACT5001	14	0	0	14
Erebidae	*sp. ACT5170*	BOLD:ACT5170	3	0	0	3
Erebidae	*sp. ACU4085*	BOLD:ACU4085	2	2	0	0
Erebidae	*sp. ACU4278*	BOLD:ACU4278	4	4	0	0
Crambidae	*sp. ACU4379*	BOLD:ACU4379	7	6	0	1
Erebidae	*sp. ACU4382*	BOLD:ACU4382	4	0	4	0
Thyrididae	*sp. ACU4433*	BOLD:ACU4433	2	0	0	2
Erebidae	**Spilosoma sp. ACU4479*	BOLD:ACU4479	15	0	15	0
Erebidae	**Lambula sp. ACU4513*	BOLD:ACU4513	3	3	0	0
Pyralidae	*sp. ACU4601*	BOLD:ACU4601	3	0	0	3
Crambidae	*sp. ACU4642*	BOLD:ACU4642	2	2	0	0
Erebidae	*sp. ACU4645*	BOLD:ACU4645	10	2	5	3
Noctuidae	*sp. ACU4658*	BOLD:ACU4658	2	0	1	1
Thyrididae	*sp. ACU4732*	BOLD:ACU4732	5	5	0	0
Saturniidae	*sp. ACU4765*	BOLD:ACU4765	2	0	2	0
Geometridae	**Lomographa sp. ACU4779*	BOLD:ACU4779	3	0	3	0
Geometridae	**Chorodna sp. ACU4784*	BOLD:ACU4784	2	1	0	1
Pyralidae	**Orthaga sp. ACU5090*	BOLD:ACU5090	9	0	8	1
Crambidae	**Pleuroptya sp. ACU5148*	BOLD:ACU5148	8	8	0	0
Geometridae	**Eucyclodes sp. ACU5150*	BOLD:ACU5150	19	0	3	16
Depressariidae	*sp. ACU5195*	BOLD:ACU5195	5	5	0	0
Nolidae	*sp. ACU5242*	BOLD:ACU5242	27	27	0	0
Bombycidae	**Elachyophthalmasp. ACU5409*	BOLD:ACU5409	2	1	1	0
Erebidae	**Notata sp. ACU5445*	BOLD:ACU5445	2	1	0	1
Erebidae	**Hypena sp. ACU5948*	BOLD:ACU5948	3	0	0	3
Pyralidae	*sp. ACU6119*	BOLD:ACU6119	2	0	0	2
Erebidae	*sp. ACU6207*	BOLD:ACU6207	2	0	0	2
Erebidae	*Hypena subalbida*	BOLD:ACU6882	40	0	40	0
Xyloryctidae	*sp. ACU6918*	BOLD:ACU6918	14	1	0	13
Geometridae	*sp. ACU7039*	BOLD:ACU7039	2	0	0	2
Erebidae	*sp. ACU7423*	BOLD:ACU7423	2	0	0	2
Geometridae	*sp. ACU7479*	BOLD:ACU7479	3	2	0	1
Pyralidae	*sp. ACW0938*	BOLD:ACW0938	11	11	0	0
Erebidae	*sp. ACW0964*	BOLD:ACW0964	2	0	2	0
Tortricidae	*sp. ACW0973*	BOLD:ACW0973	19	0	1	18
Anthellidae*	*sp. ACW1234*	BOLD:ACW1234	3	0	0	3
Geometridae	**Tripteridia sp. ACW1281*	BOLD:ACW1281	11	7	4	0
Erebidae	*sp. ACW1304*	BOLD:ACW1304	2	2	0	0
Gelechiidae	*sp. ACZ1730*	BOLD:ACZ1730	3	0	3	0
Thyrididae*	*sp. ACZ1731*	BOLD:ACZ1731	25	25	0	0

Table A3. Mean Distance Based Specialisation Index (DSI*) of Lepidopteran families across the successional chronosequence with SE and number species represented within each family and stage combination.

Family	Habitat	DSI*	+/-se	N Species
Choreutidae	Young Secondary	1.000	NA	1
Crambidae	Young Secondary	0.888	0.075	5
Erebidae	Young Secondary	0.593	0.099	13
Eupterotidae	Young Secondary	0.429	NA	1
Gelechiidae	Young Secondary	0.991	NA	1
Geometridae	Young Secondary	0.836	0.044	27
Immidae	Young Secondary	0.577	0.160	2
Lycaenidae	Young Secondary	0.957	NA	1
Noctuidae	Young Secondary	0.802	0.198	3
Nolidae	Young Secondary	1.000	0.000	6
Pyralidae	Young Secondary	0.822	0.162	5
Thyrididae	Young Secondary	0.924	0.076	4
Tortricidae	Young Secondary	0.693	0.070	25
Choreutidae	Mature Secondary	0.992	0.008	3
Crambidae	Mature Secondary	0.792	0.119	4
Erebidae	Mature Secondary	0.802	0.055	19
Eupterotidae	Mature Secondary	0.405	NA	1
Gelechiidae	Mature Secondary	0.986	0.014	2
Geometridae	Mature Secondary	0.701	0.097	13
Immidae	Mature Secondary	1.000	NA	1
Lycaenidae	Mature Secondary	0.922	0.078	3
Noctuidae	Mature Secondary	1.000	NA	1
Nolidae	Mature Secondary	1.000	NA	1
Pyralidae	Mature Secondary	0.931	0.069	2
Thyrididae	Mature Secondary	1.000	0.000	4
Tortricidae	Mature Secondary	0.815	0.097	15
Choreutidae	Primary	1.000	NA	1
Crambidae	Primary	0.991	0.005	3
Erebidae	Primary	0.757	0.061	21
Eupterotidae	Primary	0.681	0.183	3
Gelechiidae	Primary	0.991	NA	1
Geometridae	Primary	0.734	0.057	27
Immidae	Primary	0.610	0.085	2
Noctuidae	Primary	0.560	NA	1
Nolidae	Primary	0.990	0.002	2
Pyralidae	Primary	0.707	0.293	2
Thyrididae	Primary	0.839	0.080	6
Tortricidae	Primary	0.865	0.034	32

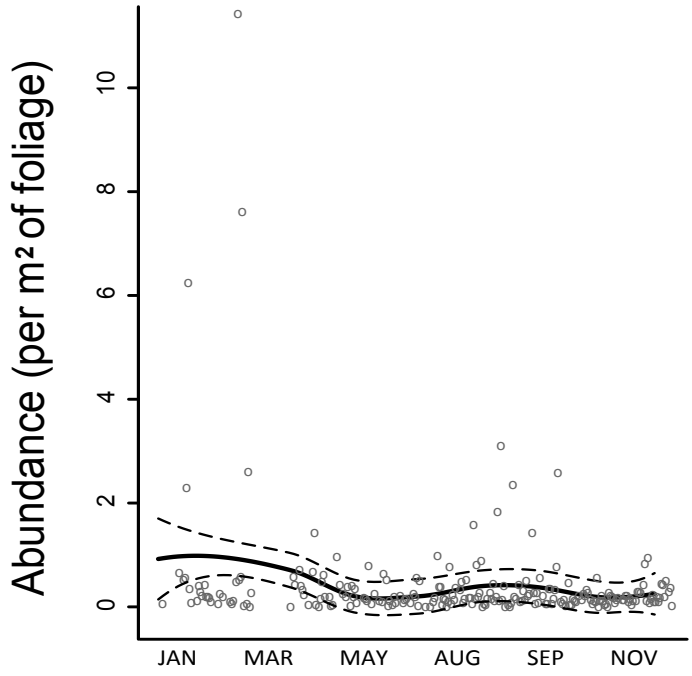

Figure A5. Seasonal trends in abundance of collected herbivores. The data points represent number of caterpillars per m2 of foliage on individual days of sampling. The seasonal trend was modelled with a loess smoother (solid line). Dashed lines show confidence intervals. The abundance was standardized by leaf area.

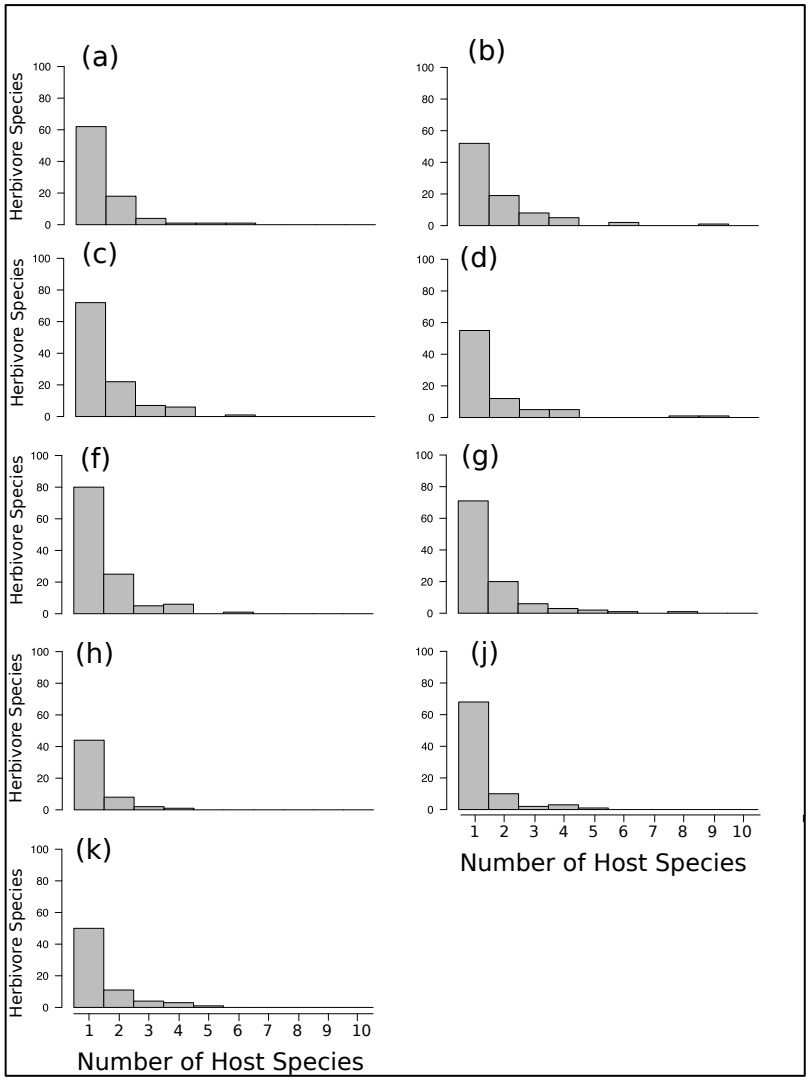

Figure A6: Degree distribution of Lepidopteran herbivores within each of the nine study plots showing the number of herbivore species that are associated with a given number of hosts. Primary forest plots (a,b,c,d), young secondary plots (f,g) and mature secondary plots (h,j,k).

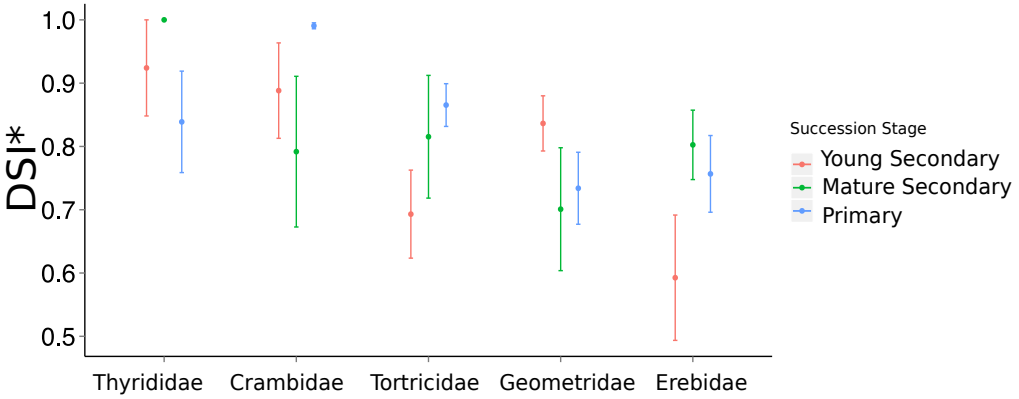

Figure A7. Mean Distance Based Specialisation Index (DSI*) +/- SE of the top five most speciose lepidopteran families (Tortricidae> Geometridae > Erebidae > Thyrididae > Crambidae), ordered by most to least specialized, across the successional chronosequence from young secondary (red) to mature secondary (green) and finally primary forest (blue).

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Supplementary Material Appendix 2 -

Identification of Lepidoptera

Adult specimens were sorted by morphospecies and confirmed using DNA barcoding and dissection of genitalia. Larval Lepidoptera were morphotyped within each host, and a subset barcoded in order to verify correct placement. Due to the large numbers of larval Lepidoptera, the lack of morphological characters in some taxa, and logistical constraints, not all individuals could be identified to species level. In total 12357 (65%) individuals, from 292 species across 29 families, were reliably identified.

Leucoma spp complex

We found two species of *Leucoma* (ACM4173 and ACM4174) that typically co-occurred on *Elaeocarpus* hosts, and were super abundant. They are two species based on male genitalia and DNA barcodes, and they are near *Leucoma sericea* Moore, but do not match any described species from New Guinea (Mackey 2016). Because they can only be distinguished by male genitalia or DNA, it was logistically impossible to sort over 4000 individuals to species.

Mackey, A. P. 2016. Two new species of *Leucoma* Hübner, 1822 (Lepidoptera: Erebidae, Lymantriinae) from Papua Indonesia.- Suara Serangga Papua **10**:8-12.

Supplementary Material Appendix 3A:

Descriptions and equations of quantitative network metrics.

The 'Bipartite' (version 2.05) R package (Dormann 2008, Dormann 2009) was used for the calculation of network metrics following and followed Bersier et al 2002, Blüthgen et al. 2006, Tylianakis, et al. 2007, and Dormann et al 2009). Hosts which had no herbivores present were accounted for using the empty.web=false argument.

The following terms are used in the equations presented below for the quantitative network metrics used in the analyses of our herbivore-host networks, namely Weighted quantitative generality (G_{qw}), Weighted quantitative vulnerability (V_{qw}), Weighted quantitative connectance (C_{qw}) and Weighted quantitative modularity (Q)

I number of species at the lower trophic level

J number of species at the higher trophic level

m total number of interactions for all species

a_{ij} number of interactions between species *i* from the lower trophic level and species *j* from the higher trophic level

A_i total number of interactions of species *i* from the lower trophic level

A_j total number of interactions of species *j* from the higher trophic level

H*i* the Shannon diversity of interactions for lower trophic level species:

$$H_i = -\sum_{j=1}^{J}\left(\frac{a_{ji}}{A_i}.\ln\frac{a_{ji}}{A_i}\right)$$

H*j* the Shannon diversity of interactions for higher trophic level species:

$$H_j = -\sum_{i=1}^{I}\left(\frac{a_{ij}}{A_j}.\ln\frac{a_{ij}}{A_j}\right)$$

Weighted quantitative generality (G_{qw})

Represents the mean effective number of hosts per herbivore species weighted by their marginal totals, calculated as:

$$G_{qw} = \sum_{j=1}^{J} \frac{A_j}{m} 2^{H_j}$$

Weighted quantitative vulnerability (V_{qw}) –

Represents mean effective number of herbivores per host plant species, weighted by their marginal totals, calculated as:

$$V_{qw} = \sum_{i=1}^{I} \frac{A_i}{m} 2^{H_i}$$

Weighted quantitative connectance (C_{qw}) –

Weighted realised proportion of all possible links, calculated as:

$$C_{qw} = \frac{LD_{qw}}{s}$$

where *LDq* is the weighted quantitative linkage density (i.e. diversity of interactions per species weighted by marginal totals), and *s* is the number of species in the network (including host species with no herbivores) (Tylianakis *et al.* 2007).

Weighted quantitative modularity (Q) –

Reflects the extent to which a quantitative network can be partitioned into distinct modules within which species interact more strongly with each other than species from outside the module. Modules are determined using an algorithm based on hierarchical random graphs (Dormann & Strauss 2014). Calculated as:

$$Q = \frac{1}{2N} \sum_{ij} \left(A_{ij} - K_{ij}\right) \delta \left(m_i, m_j\right)$$

where *N* is the total number of observed interactions in the network and A*ij* is the normalised observed number of interactions between *i* and *j*. The expected value, based on an appropriate null model, is given in the matrix *K*. The module to which a

species *i* or *j* is assigned is m_i, m_j. The indicator function δ (m_i;m_j) = 1 if $m_i = m_j$ and 0 if $m_i \neq m_j$. *Q* ranges from 0 to a maximum value of 1, where 0 represents a community with no more links within modules than expected by chance).

Modularity values were compared against a null distribution obtained from 100 runs of the r2d randomization method, which rearranges the interaction matrices keeping marginal sums fixed. These z-scores were then compared across networks.

Supplementary Material Appendix 3B:

Description of the qualitative network beta diversity methodology.

Differences in species composition between two communities X and Y can be described using three variables, namely the number of species shared in both X and Y (a), species present only in X (b) and species found only in Y (c). When applied to the complementary beta diversity (βcc) measure of the Jaccard similarity index, βcc = 1 − Jaccard index = (b + c)/ (a + b + c).

This principle can be expanded to differences in interaction networks where food webs X and Y can be described by the number of plant–herbivore interactions present in both X and Y, only in X, and only in Y (Novotny 2009).

Four additive partitions of network beta diversity can be partitioned within this framework. Considering networks b and c, plant–herbivore interactions present in only one of the two compared networks can be classified as interactions restricted to a single web due to the following four reasons (i) both the plant and the herbivore species are missing in both webs (bPH, cPH), (ii) only the plant species is absent from one of the webs (bP, cP), (iii) only the herbivore species is absent from one of the webs (bH, cH), and (iv) both the plant and the herbivore species are present in both webs, but the trophic interaction between them is not present (b0, c0) (Novotny 2009).

Beta diversity can be then partitioned into these four components as follows:

βcc = (bPH + cPH)/(a + b + c)+(bP + cP)/(a + b + c)+(bH + cH)/ (a + b + c)+(b0 + c0)/(a + b + c) = βPH + βP + βH + β0.

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Chapter 2

Bottom-up structuring of plant-herbivore interaction networks during rainforest succession

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Abstract

Studies documenting the structure of plant-herbivore interaction networks in distinct habitats and across ecological gradients are increasingly common. These studies often report rather consistent architecture and network properties which may indicate the existence of fundamental rules of network assembly. Despite this, the identification of network assembly drivers is still in its infancy. For plant-herbivore networks, the influence of host plant community properties on higher trophic levels and thus network assembly warrants closer examination. Using a dataset compiled from exhaustively sampling 1.8 ha of primary and secondary montane rainforest in Papua New Guinea, we aim to predict network structure on the basis of host community properties. We do this by applying restricted subsampling of a primary *source* plot using criteria which match the vegetation structure of young and mature secondary *target* plots (abundance of hosts, size class distribution, and host taxonomy), modelling these three factors in a hierarchical manner. From this, we attempt to identify bottom-up structuring mechanisms common to both primary and secondary forest interaction networks. We show that host abundance can be used to predict herbivore abundance and species richness. Species level network metrics were predicted with mixed success. Generality was consistently underestimated by all model iterations, while vulnerability was best modelled by matching host abundance, with the inclusion of taxonomy yielding no improvement. For metrics detailing broader network architecture, measured by connectance and the number of compartments, host taxonomy was important. Tree size distribution had little influence on network properties. Furthermore, we show that herbivore distributions are also associated with host traits related to resource quality such as SLA, C:N ratios, young leaf availability and the presence of exudates. We conclude that plant-herbivore interaction networks can largely be predicted on the basis of host properties, suggesting bottom-up structuring of these interactions and their networks. The addition of host quality traits into our subsampling procedure may reveal further drivers of network structure and should be the focus of future studies.

Introduction

Understanding network structure and its implications for interacting species is a key challenge of community ecology. These networks often illustrate complex but amenable interpretations of biodiversity, species interactions, and ecosystem structure and function (Dunne et al 2002, Dormann et al 2009). The stability of these networks depends greatly on their architecture, which also informs us of the evolutionary mechanisms that have shaped interactions (Peralta 2016, Vasquez et al 2009). Knowledge of how interactions arise, how networks of interactions are structured, and how they remain stable, will be key to predicting future trajectories in light of increasing pressures from human disturbance and climate change (Plowman et al 2017, Tyliankis et al 2007).

At the forefront of this effort has been an investigation into plant-herbivore communities, as these play a central role in natural ecosystem functioning (Price 2002, Weisser and Siemann 2008). It is well-known that insect herbivores are affected by both bottom-up (resource) and top-down (consumer) effects (Vidal and Murphy 2018). From the bottom-up, it has been shown that host plant characteristics including abundance, age and phylogenetic relatedness greatly influence herbivore host choice, fitness and distribution (Price 1991, Boege and Marquis 2005, Coley et al 2006, Futuyma and Agrawal 2009). These changes to herbivore preference and prevalence can, in turn, have cascading effects on network structure; however, little is known of how these aspects of host communities might determine the structure of entire interaction networks.

Herbivore feeding preferences for example are determined by both the quantity and quality of resources available, in turn shaping network structure (Marques et al 2000, Poorter et al 2008). While resource quantity will be determined primarily by the local abundance of host species (Marques et al 2000), resource quality will be determined by nutritive value as well as the diverse defensive adaptations of host plants, which includes morphological, phenological, physiological and chemical defences. Differences in host plant quality, in terms of both defences and nutrients, are often related with host plant age or size, and with host phylogeny (Fenner et al 1999, Boege and Marquis 2005, Coley et al 2006, Futuyma and Agrawal 2009). Invertebrates are usually negatively affected by increases in host age, where abundance and

performance are reduced (Boege and Marquis 2005, Coley et al 2006). This effect can vary between herbivore taxa however. Kearsley and Whitham 1989 showed that densities of chrysomid beetles increased 400 times on small trees compared with larger trees, but gall forming aphids followed the opposite trajectory. Host plant taxonomy is also tightly linked to herbivore feeding preferences. There is conservatism of interactions within ecological networks where closely related species, usually congenerics, will more likely share phenotypic traits and elements of the local herbivore assemblage (Thompson 2005, Fontaine and Thébault 2015, Peralta 2016). This effect is strongest in host-herbivore networks (Fontaine and Thébault 2015). In turn, there is a delineation of interacting groups of species within the entire community (Segar et al 2017). This is an outcome of evolutionary arms races whereby groups of herbivores and hosts coevolve. By influencing herbivore host choice, these bottom-up effects can thus be responsible for a community's non-random interaction network structure.

A successional gradient provides a suitable platform to assess the bottom-up effects of host plant abundance, size distribution and taxonomy on herbivore interactions. These gradients are characterised by shifting plant composition and structure, where species and phylogenetic diversity, and also structural complexity and tree size increase with time (Whitfeld et al 2012, Chazdon 2014). For example, tree species in diverse primary forest tend to occur at low densities through enemy mediated control (Janzen 1970), while plants tend to be spatially aggregated in disturbed secondary forest through dispersal limitation (Fibich et al 2016). In lowland forest in Papua New Guinea (PNG), primary forest was more phylogenetically and taxonomically diverse than secondary forest (Whitfeld et al 2012). However Redmond et al 2019 showed opposite phylogenetic trends for the montane forest studied here. Whitfeld et al 2014 quantified changes to forest structure across succession in lowland PNG, where differences in stem density varied with size class distribution. Changes to herbivore communities also occur with succession, for example herbivore abundance and density tend to be greatest in secondary forest (Whitfeld et al 2012, Redmond et al 2018). While high herbivore species richness has been associated with primary forest (Gibson et al 2011), other studies have shown that herbivore richness can be comparable between primary and secondary forest (Villa-Galaviz et al 2012, Redmond et al 2018).

Plant-herbivore network structure has also been shown to vary through succession. For example, connectance was reported to be highest (Luviano et al 2018; Redmond et al 2018) and compartmentalisation lowest in the earliest stages of succession (Villa-Galaviz et al 2012, Redmond et al 2019). Changes in species level interactions are less clear; Leps et al 2001 showed there was no change in the numbers of hosts per herbivore (generality) between succession stages on particular hosts, while Redmond et al 2019 expanded this finding to entire communities. Vulnerability, or the number of herbivores per host species, can be at its highest in the earliest stages of secondary succession (Redmond et al 2019). Despite these investigations, the mechanisms underlying change in host-herbivore interactions through succession remains unresolved.

In the present study, we exploit the relatively well-understood dynamics of plant assemblages through succession to form and test the role of plant community structure in shaping plant-herbivore networks. We hope to provide a framework for interpreting changes in host-herbivore interactions, and promote successional gradients as a promising model system for studying bottom-up community effects. We use a model-based approach to identify bottom-up structuring mechanisms common to both primary and secondary forest interaction networks. To do this, we utilise one of the largest tropical plant-herbivore datasets of its kind as a source of species interactions, and apply the TRIN model to match host community characteristics between primary and secondary forest plots. Specifically, we seek to understand the relative importance of host plant abundance, host plant size distribution, and host plant taxonomy in shaping the herbivore community (abundance, richness) and structure of the plant-herbivore network (vulnerability, generality, connectance and compartmentalisation) across a montane succession gradient in Papua New Guinea. We expect herbivore abundance and richness will be primarily determined by host abundance and size class distribution, reflecting resource quantity and quality. On the other hand we expect network metrics will be shaped by host taxonomy, as this plays an important role in delineating utilizable resources. Finally, we take first steps towards advancing the model by exploring the relationship of herbivore distribution with additional host traits related to resource quality. We expect increased host quality will promote increased herbivore abundance.

Materials and Methods

Field Site and Succession Series

Nine 0.2 hectare plots near Yawan village (-6.16388°N, 146.83833°W), Morobe Province, Papua New Guinea were sampled using felling at locations earmarked for clearance by the local community. Three distinct phases of succession were identified: primary, mature secondary and young secondary forest. Designations were made based on local accounts regarding previous land use, and plant community structure and composition, where young secondary was ~12-15 years, mature secondary ~25-30 years and primary forest >100 years 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). Further details are provided in Redmond et al 2019.

Host plant and Herbivore Sampling

Each plot was divided into four 20x20m subplots. Sampling started from the lowest subplot and proceeded in a series of steps. First, the understory was cleared of all vegetation < 1.3m in height; this included mostly herbaceous species. After this, felling and sampling of trees <5cm DBH was carried out. Next, trees >5cm DBH were felled, beginning with midstory trees. Throughout this process, care was taken to ensure minimum disturbance to other trees within the plot. Specifically, felling was directional, beginning with the shortest trees and those with the least lianas. Because trees tangled with lianas had the potential to damage other trees when felled, lianas were first cut from trees with machetes before felling, when possible. Tree felling was directed into gaps created by previous plot clearance, allowing for easier collection.

Collection of insects from all foliage of felled trees was carried out immediately after felling by a team of ~15 locally recruited collectors, and supervised by on-sight researchers. Collection involved searching for live caterpillars (Lepidoptera), both free feeding and semi-concealed, and placing them in plastic collections pots before being brought back to the field lab. Trophic links were confirmed in the lab using no-choice feeding trials, which involved supplying collected herbivores with leaves of the host they were found upon. Herbivores which did not feed were excluded from the analysis. Specimens were reared to adulthood and mounted for later taxonomic identification. Identifications were made using existing literature, COI-5P

DNA barcoding, and dissection of genitalia where necessary. See Redmond et al 2019 for further details on herbivore identification.

Host plant community traits

DBH was recorded for all plant individuals >5cm DBH. The abundance of host plants was quantified by simply counting the number of individuals. Foliage weight was measured for both mature and young leaves; this was achieved by stripping each tree of its foliage, separating mature and young leaves on the basis of rigidity, colour, damage and size, and weighing both types of leaves with a hanging scale or electronic balance. From this, percentage young leaf of each host tree could be calculated. The presence of exudates, including latex and resin, was determined in the field by damaging and examining leaf veins and petioles. Specific leaf area was ascertained using leaf discs of 2.3cm punched from fresh mature and young (where possible) leaves in the field. These discs are stored in -80°C frozen tissue collections at the University of Minnesota (St Paul, Minnesota, USA). If no leaf discs were available for an individual tree, the mean SLA of that species was used. These leaf discs were also used to assess carbon:nitrogen (C:N) ratios. For this, several dry leaf discs were pooled and milled to powder. Milled samples were analysed at UC Davis Stable Isotope Facility (Davis, California, USA) using an elemental analyser interfaced with a continuous flow isotope ratio mass spectrometer (EA-IRMS). This procedure was carried out for up to five individuals per species, and mean values were applied across all individuals of that species. Host community composition and variation was assessed by principal components analysis in Canoco v5.0 and using Whitakers beta diversity in R statistical environment ver. 3.1.3 (R Development Core Team).

TRIN Rarefication Model

The general pipeline of the TRIN model is to apply restricted subsampling to a *source* community (0.8ha whole-forest primary dataset) using criteria that match the vegetation structure of secondary *target* plots (abundance of hosts, size class distribution, and host taxonomy), modelling these three factors in a hierarchical manner; assembling interaction networks; and calculating and comparing network descriptors of both the rarefied *source* and *target* plots.

For our *sourc*e dataset, we used tree community data and the associated plant-herbivore interaction matrix from all trees >5cm DBH with recorded herbivore

interactions within 0.8ha primary forest, compiled by combining four separate 0.2 ha plots. For our *target* dataset, we used a series of ten 0.1ha secondary forest plots, including four young secondary (F1-G2) and six mature secondary plots (H1-K2). These 0.1ha secondary plots were created by combining two contiguous 0.05ha subplots in each 0.2ha plot, with the pairing combination chosen randomly. Dividing the 0.2ha plots like this was necessary to ensure target plot traits could be adequately matched in the source dataset. For each of these 10 target plots, we ascertained the following host plant community characteristics: empirical host abundance, size distribution (classified as one of 6 DBH size classes: 5-7cm, 7-10cm, 10-15cm, 15-30cm, 30-45cm and 45+cm DBH), and taxonomic placement of hosts (family, genus, and species). Finally, we performed a subsampling procedure from the *source* dataset, with the goal of replicating host characteristics of *target* plots, and evaluated similarity of network and community metrics. The subsampling procedure had three iterations of increasing complexity, which were carried out in an additive manner: (i) matching the number of tree individuals (abundance) per target plot (model 1); (ii) additionally matching the tree size distribution of the target plot (model 2); and (iii) matching abundance, size distribution, and taxonomic structure of host trees (number of species, genera and families) (model 3).

For example, under Model 1, the source dataset was randomly subsampled for *Xs* trees, where *Xs* is the number of trees in the target 0.1ha secondary plot. Under Model 2, *Xs* trees were partitioned into size classes to match the size distribution of the target plot. Under Model 3, *Xs* number of trees were once more drawn to match the size distribution of the target plot, but now the total number of species, genera and families were also matched to the numbers found in the target plot. We used an iterative process to match taxonomy under model 3, for more information on this taxonomic matching, see Supplementary Material Appendix 1. This iterative process stopped when either host taxonomy between source and target plot was matched perfectly, or when 1000 iterations had been conducted. Each of the three models was ran 1000 times.

Evaluating TRIN model predictions

First, the mean predicted value was calculated for each of the six network interaction parameters. This mean was taken from 1000 runs of the TRIN model for each of the three model iterations. Z-scores were then computed for each of these values by calculating the standard deviation of the predicted

values and using this to compare the mean predicted value with the observed value in each plot. Mean predicted values which lay inside the range of 1.96 standard deviations of the observed values were considered to be successfully predicted. Positive z-scores indicate observed values were greater than predicted values, and negative z-scores indicate observed values were lower than predicted values.

Model forecast quality across all plots was evaluated by Theil's UII (Theil 1966). This represents the root mean square deviation of the forecasting model divided by the root mean square of a no-change (random) model. This measure has a lower bound of 0, which represents perfect model predictions, and increases with decreasing model forecast quality. At values of 1, the model is approximate to a no change random model, at values higher than 1, the forecast quality of the model is worse than random.

Abundance analyses using plant traits

The relationship between herbivore abundance and host traits were analysed by a multiple linear regression in R statistical environment ver. 3.1.3 (R Development Core Team). This analysis included all trees from all succession stages. The model included factors indicative of both resource quality and quantity. Resource quality is reflected in carbon:nitrogen ratios, percentage young leaf, SLA and the presence of exudates. Resource quantity is given by DBH and the total amount of foliage of each individual host. P-values were adjusted by Bonferroni correction in order to account for multiple independent variables.

Results

830 individual trees comprising 89 species across 37 families hosted herbivores (see Redmond et al 2019 for more details). For these 830 trees, we documented a total of 12 357 interactions with Larval Lepidoptera from 292 species across 29 families (see Redmond et al 2019 for more details). We removed an outbreak *Leucoma spp* complex (Family: Erebidae) from this source dataset as it was a significant outlier, represented by 4412 individuals on two *Eleaocarpus* host species.

The properties of the source and target communities used in all TRIN models are presented in Table 1. Plant species richness was greater in young secondary

plots than mature secondary plots. This is also the case for herbivore species richness and herbivore abundance. The number of host individuals was again greater in young secondary plots, with the majority of host trees falling into smaller host size classes. On the other hand, mature secondary plots were composed primarily of trees in larger size classes. Host tree characteristics in the primary source plot were sufficiently broad to encompass the range of tree characteristics in the target secondary plots. Vegetation composition of all succession stages also differed substantially, with high mean pairwise plot beta diversity (primary-mature secondary= 0.80 ± 0.01 S.E., primary-young secondary= 0.83± 0.01 S.E.) (Figure 1). Observed values for target secondary plots are given in Table 2. Herbivore abundance, herbivore richness, vulnerability and generality were greatest in the young secondary succession stage. The primary source plots were the least connected and most compartmentalised Table 2.

Table 1: Host community taxonomical structure and physical characteristics (abundance and size class) of the primary source plot (total 0.8ha and mean 0.1ha) and secondary target plots (0.1ha) used in the TRIN models. Young Secondary plots: F1-G2, Mature Secondary plots: H1-K2.

	Primary Source 0.8ha	Primary mean 0.1ha ± S.E.	F1	F2	G1	G2	H1	H2	J1	J2	K1	K2
Host Richness: Species	122	42 ± 3	44	31	34	35	29	16	33	29	31	23
Genus	65	28 ± 2	30	26	21	20	20	9	19	20	19	13
Families	47	24 ± 2	26	23	19	18	16	8	15	19	16	13
Abundance	870	109 ± 6	248	233	232	189	77	49	89	68	112	96
Size Class I	277	35 ± 5	109	116	80	53	21	10	20	20	25	27
II	184	23 ± 2	79	66	83	34	9	18	19	11	13	15
III	180	22 ± 3	46	34	44	59	13	9	17	11	24	13
IV	164	20 ± 2	13	17	23	41	27	11	30	23	45	36
V	43	5 ± 1	2		1	2	7	1	3	3	5	5
VI	22	3 ± 1			1							

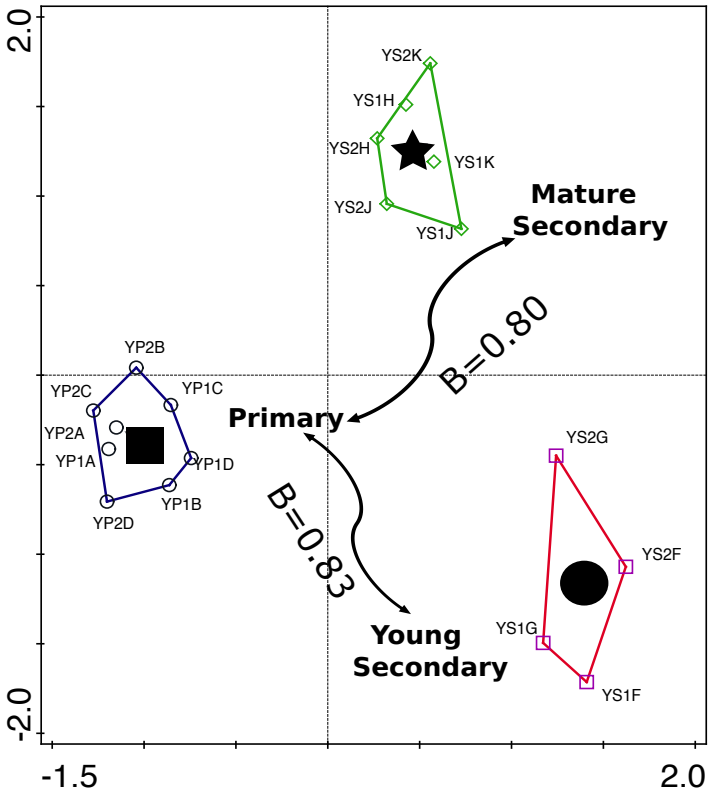

Figure 1. Principal components analysis (PCA) of host communities (>5cm DBH) and successional stage in montane rainforest in Papua New Guinea. Young secondary centroid is represented by a black circle, mature secondary by a star, and primary forest plots by a square. Lines with arrows connecting plot types show mean Beta-diversity of pairwise plot comparisons for those stages. First canonical axis eigenvalue = 0.328 and the second = 0.144, with the combined variation explained = 47.3%.

PLOT	Succession Stage	Herbivore Abundance	Herbivore Richness	Vulnerability	Generality	Connectance	Compartments
F1	Young Sec	682	72	6.67	2.02	0.078	2
F2	Young Sec	1128	95	11.91	2.6	0.098	2
G1	Young Sec	515	69	7.86	2.2	0.076	2
G2	Young Sec	688	76	6.57	2.89	0.09	1
H1	Mature Sec	371	39	5.51	2.27	0.109	4
H2	Mature Sec	142	27	3.42	1.51	0.133	3
J1	Mature Sec	584	54	4.37	1.66	0.073	4
J2	Mature Sec	383	53	4.82	2.23	0.074	5
K1	Mature Sec	340	49	6.72	2.49	0.101	2
K2	Mature Sec	468	39	4.61	1.43	0.124	1
	Young Sec	753 ± 131 S.E.	78 ± 5.8 S.E.	8.25 ± 1.2 S.E.	2.43 ±0.19 S.E.	0.085 ± 0.005 S.E.	1.75 ± 0.2 S.E.
	Mature Sec	381 ± 60 S.E.	43.5 ± 4.2 S.E.	4.91 ± 0.45 S.E.	1.93 ± 0.18 S.E.	0.102 ± 0.01 S.E.	3.1 ± 0.6 S.E.
	Primary	330.5 ± 37.01 S.E.	58.5 ± 3.20 S.E.	7.27 ± 1.28 S.E.	1.53 ± 0.10 S.E.	0.070 ± 0.01 S.E.	8 ± 0.91 S.E.

Table 2- Target values for the six metrics (Herbivore abundance, Herbivore Richness, Vulnerability, Generality, Connectance and Number of Compartments) in each of the ten 0.1ha *target* secondary plots, and their mean values in each of the three succession stages, including the primary *source* dataset (mean of 0.1ha).

Target values for herbivore abundance were predicted well under all three models and for the majority of secondary plots (Figure 2a), with model forecast accuracy, measured by Theil's UII, increasing only slightly as model complexity increased (Table 3). In a minority of plots however, the TRIN models predicted significantly lower herbivore abundance than was observed. For herbivore richness, observed values were consistently lower than predicted values, however differences were generally not significant with the exception of two young secondary plots (Figure 2b). Forecast accuracy was greatest for model 2, however differences between all three models were minor (Table 3). Vulnerability was well-predicted under model 1. Increasing the complexity of model parameters did not improve predictions (Figure 2c). This was reflected in the forecast accuracy of the models (Table 3). Generality on the other hand was predicted less well across all three models, where observed values were often significantly greater than predicted values (Figure 2d). While forecast accuracy for generality worsened with increasing model complexity, these differences were again minor (Table 3). For both measures of overall network architecture, connectance and compartmentalisation, incorporation of taxonomy improved predictive capability of the TRIN model significantly. For connectance under model 1 and model 2, observed values were for the vast majority of plots were significantly greater than predicted values. When host taxonomy was matched, observed connectance was not significantly different from predicted values in 90% of the plots (Figure 2e). In contrast, the observed number of compartments was typically lower than the predicted values across all three TRIN models. Under model 1 and 2, the number of compartments in 40% of plots was significantly overestimated. With the addition of host taxonomy, none of the ten plots were significantly overestimated (Figure 2f). For both connectance and the number of compartments, large improvements in forecast accuracy under model 3 were evident in Theil's UII (Table 3)

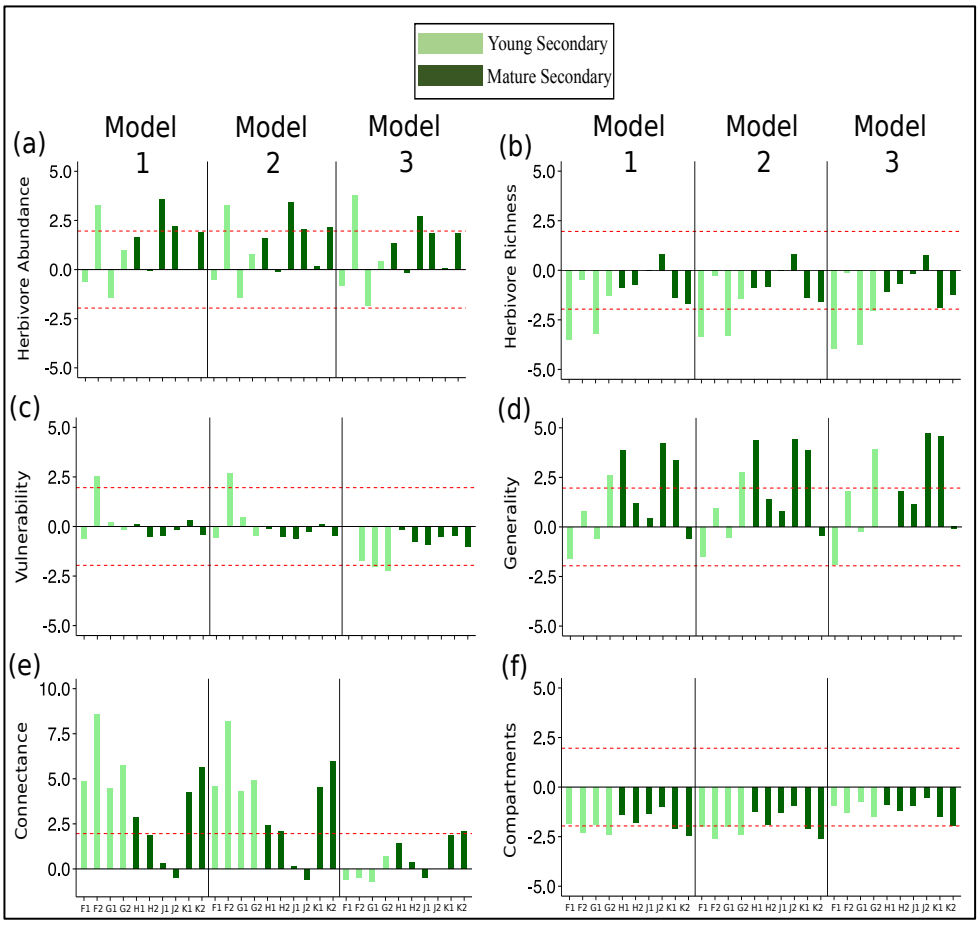

Figure 2: Z-scores comparing the observed and predicted values for each of the six network metrics a- Herbivore abundance, b-Herbivore Richness, c- Vulnerability, d-Generality, e- Connectance and f- Number of Compartments. Red dashed lines represent significance thresholds at 1.96 sd. Positive z-scores indicate observed values were greater than predicted values, and negative z-scores indicate observed values were less than predicted values. Black solid lines separate the results for each of the three models. Model 1 matches host abundance, Model 2 matches hosts abundance + size class and Model 3 matches host abundance, size class and host taxonomy.

Table 3: Theil's UII forecast quality for each of the three iterations of the TRIN model for all six network parameters (Herbivore abundance, Herbivore Richness, Vulnerability, Generality, Connectance and Number of Compartments).

	Theil's UII		
Metric	Model 1	Model 2	Model 3
Herbivore Abundance	0.348	0.340	0.327
Herbivore Sp. Richness	0.275	0.265	0.290
Vulnerability	0.241	0.251	0.480
Generality	0.248	0.259	0.271
Connectance	0.353	0.350	0.155
Compartments	1.571	1.604	0.781

A significant component of variation in herbivore abundance could be explained by host traits pertaining to both resource quantity and quality (Table 4). These traits explained a greater proportion of variation in total herbivore abundance than herbivore abundance per unit foliage. For resource quantity, DBH was significantly associated with total herbivore abundance, while total leaf weight was negatively associated with abundance per kilogram foliage. For traits pertaining to resource quality, higher specific leaf area, higher C:N content and the presence of exudates all caused reductions in total herbivore abundance. The same was true for abundance per kilogram, here however, a higher proportion of young leaves was also associated with greater herbivore abundance (Table 4).

Table 4 Multiple regression using plant traits to account for (a) Abundance of caterpillars per tree and (b) Abundance of caterpillars per kg foliage per tree.

Abundance (R^2=0.125)		
	t-value	p
DBH	16.24	<0.001
Specific Leaf Area	-3.035	0.002
Leaf Carbon:Nitrogen	-3.015	0.003
Exudates	-3.772	<0.001
Abundance per KG (R^2=0.053)		
	t-value	p
Total Leaf Weight	-2.574	<0.001
Specific Leaf Area	-4.099	<0.001
Leaf Carbon:Nitrogen	-2.91	<0.001
% Young Leaf	8.712	<0.001
Exudates	-2.983	0.003

Discussion

While tropical forest succession is characterized by large changes in host community composition, forest structure and abiotic conditions, we were able to identify some constancy in the form of shared drivers of emergent network properties between succession stages. In particular, we find that abundance of host trees can largely account for herbivore abundance, herbivore richness and vulnerability, while host taxonomy can explain changes in network structure. Interestingly, despite the pronounced shifts in tree size that accompany succession, we find that tree size distribution has little influence on network properties.

Changes in herbivore abundance with successional stage were well-predicted by shifts in host plant abundance using the TRIN model. In addition the regression analysis showed that DBH was positively associated with total herbivore abundance. Both these findings provide support for the relationship between herbivore abundance and resource availability (resource abundance hypothesis) (Marques et al 2000, Hunter 1992, Ohgushi 1992). Herbivore

abundance has been linked with resource availability across a range of diverse habitat types, including lowland tropical rainforest in Papua New Guinea (Whitfeld et al 2012), temperate grassland (Crist et al 2006) and desert in the United States (Marques et al 2000). While tree abundance was generally a good predictor of herbivore abundance, true herbivore abundance tended to be underestimated. Thus secondary forest typically contained more herbivores than primary forest when host abundance is matched across habitat types. This may be attributable to variation in host defensive investment described by the resource availability hypothesis, where earlier succession hosts represent more hospitable resource nodes, promoting herbivore growth, fitness and thus local population increases (Coley et al 2006). This may also account for the negative relationship between herbivore density and tree size uncovered by the regression analysis, as leaves of larger trees are likely more well-defended (Wright et al 2004, Boege and Marquis 2005). Changes in herbivore richness with successional stage were also relatively well accounted for by shifts in host plant abundance. Species richness in secondary plots tended to be slightly overestimated, but only significantly for the two most species-poor young secondary plots. This effect may be driven by lower resource overlap in this primary forest, where each host tree is more likely to harbour unique herbivore species (Redmond et al 2019). Thus as the model selects for higher number of host individuals, a greater diversity of herbivore species are also randomly selected.

Unlike herbivore abundance and richness, the abundance of hosts could not account for network connectance and compartmentalisation. However predictions of boarder architecture improved after host taxonomic structure was matched, particularly for connectance. This suggests that host plant taxonomic diversity – regardless of taxon identity – is a critical driver of shifts in network structure across successional stages. Low connectance and high compartmentalisation, typical of antagonistic interaction networks, result from physiological limits on herbivore host choice. These limits mean that insect herbivores typically feed on closely related host species, with highly polyphagous herbivores being relatively rare (Futuyma and Agrawal 2009). For example, in tropical forests 27% of herbivores were shown to feed on a single plant species, while 48% fed within a single genus and 58% within a

single family (Novotny and Basset 2005). The propensity for herbivores to feed within these taxonomic bounds can be utilised by ecologists when making predictions about host use. Segar et al 2017 examining host-herbivore interactions in lowland forest in Papua New Guinea, successfully predicted 79% of all Pyraloid interactions using host phylogeny and species interactions from just 15% of local woody plant diversity. Here we expand on this, and show that taxonomically driven delineation of herbivore host use can be exploited to predict larger scale network parameters such as connectance and the number of compartments. For the latter however, there was a tendency for the model to overestimate compartmentalisation in secondary forest. Thus, plant-herbivore networks in primary forest tend to be more compartmentalized than secondary forest, even when taxonomic diversity of hosts is recreated. This would again suggest that herbivores are limited in their ability to feed across multiple hosts in primary forest, perhaps owing to more effective anti-herbivore defences (Coley et al 1985, Wright et al 2004). Villa-Galaviz et al 2012 reported similar findings, where the earliest stages of succession were shown to have the lowest number of compartments.

While broad network structure could be explained by the taxonomy of the host plant community, species level metrics (vulnerability and generality) were less responsive to host species identity. Predictions for generality saw the greatest deviation from observed values across all three model iterations, typically underestimating it. As such, herbivores in primary forest appear to be restricted to fewer hosts than their secondary counterparts. For vulnerability, host abundance alone was the best predictor, where vulnerability was underestimated in only one young secondary plot. While this suggests that in some cases young secondary host species may harbour more speciose herbivore assemblages, in general there is not a substantial difference between primary and secondary hosts when sampling intensity (measured by tree individuals) is standardised. This is somewhat in conflict with the assertion that higher species richness of herbivore assemblages on earlier succession hosts is indicative of greater palatability in this succession stage (Redmond et al 2019).

It is interesting to note that there was little change in model predictive ability when host size distribution was incorporated in addition to host abundance alone. This indicates a degree of functional equivalence between hosts from different size classes within primary forest. This functional equivalence can also be found between succession stages, as evidenced by the successful prediction of herbivore abundance, richness and host vulnerability in particular, using host abundance alone. Thus, despite clear differences in the *quantity* of resources provided by individual hosts from different size classes, both small and large hosts make comparable contributions to herbivore abundance, total herbivore richness and herbivore richness per host species within the wider interaction network. This may be indicative of a preference of herbivores for smaller trees, where the advantages of greater resource abundance provided by larger trees are offset by the decreased palatability of these hosts (Boege and Marquis 2005, Endara and Coley 2011). Meanwhile, the influence of the largest host trees on the wider community interaction network will be minimal given they occur at relatively low densities. As such, these trees will be infrequently selected by the model selection procedure, particularly when host size classes are not controlled for.

A strength of the TRIN model is that it is not restricted in the number or nature of the parameters it controls for. While we focused on three iterations of the model, it can be expanded to include additional constraining factors if these constraints can be suitably matched across source and target plots. Indeed, there are a multitude of factors which are expected to impact network parameters. For example, herbivores have been shown to be greatly affected by host quality (Whitfeld et al 2012, Endara and Coley 2011, Coley et al 1985). Further evidence for this relationship has been shown here, as leaf C:N, percentage young leaf, the presence of exudates and specific leaf area are all significantly related to herbivore abundance. These factors may not only improve predictions of herbivore abundance, but may also improve predictions of other network parameters. Vulnerability or generality, for example, may be tightly related to factors reflecting host quality. Ideally, the taxonomic rarefication step of the TRIN model would fit phylogenetic relationships, rather than simply fitting taxonomic categories. Currently, reverse fitting and matching host phylogenies through rarefication is difficult and extremely

computationally demanding. Developing an accessible method to carry out this task would represent an important step forward towards the goal of uncovering rules of interaction network assembly.

Finally, these findings have some practical implications. By shedding light on assembly processes, we may be able to model post-disturbance community trajectories with ever increasing accuracy and by less resource intensive means. Classically, ecologists would be required to sample all components of interaction networks both before and after a disturbance event in order to establish disturbance effects (Luviano et al 2018, Redmond et al 2019). This process is fraught with difficulties however. This is particularly true in extremely diverse tropical forests where most target species persist within largely inaccessible forest canopies which require substantial resources to sample (Volf et al under preparation). If *source* interaction datasets are available, then we may be able determine disturbance effects by simply inventorying the post-disturbance properties of host communities. Given the unprecedented rate of forest conversion and environmental change, expediting the task of evaluating community effects will aid ecologists in making timely and cost effective assessments, allowing for the identification and prioritisation of the most threatened areas.

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Supplementary Material Appendix 1

TRIN taxonomic matching

This taxonomic matching was achieved by working through the abundances of tree species within the template (within a particular DBH class) and randomly selecting a species from the primary forest species pool that was at least that abundant. If the species was more abundant, a randomly selected subset of individuals was discarded to equilibrate abundances. In some cases there was no single species sufficiently abundant in the species pool, in which case the next most abundant species was selected. In these cases the total abundance within each DBH class was maintained by sequentially selecting other, less abundant species in the rarefied dataset, and replacing them with a species that was more abundant. In some rare cases there were no species remaining in the pool that were more abundant than any of those in the rarefied dataset, in which case it was necessary to select new singleton species to ensure that abundances within DBH classes were maintained. This matched relative abundance distributions and species richness within size classes, but did not match the overall taxonomy of the simulated datasets. To do this we used an iterative procedure that randomly selected a species occurrence from a particular size class, and replaced this occurrence with a randomly selected species from the primary source dataset. Species that already occurred within that size class were not made available for selection. The differences between the number of tree species, genera and families in the new dataset and template dataset were then compared (with equal weighting to all taxonomic levels). If the fit of the new dataset was better than that of the old, the new dataset was retained and used for the next iteration. If the fit was worse, the old dataset was used for the next iteration.

Chapter 3

Phylogenetic specialisation of tropical herbivores varies with elevation, guild and habitat use.

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Abstract

Resource specialisation is a fundamental concept in community ecology. Specialisation can inform us of evolutionary relationships, community assembly and responses of species to disturbance. Despite this, ecologists have yet to arrive at a consensus regarding how we should measure specialisation. Here we utilise the Distance Based Specialisation Index (DSI), a promising method for standardising measures of specialisation. We apply this relatively new metric to host-herbivore interactions gathered using a 'whole-forest' approach. In Papua New Guinea we felled and exhaustively sampled two hectares of lowland, and 1.8ha of montane forest, comprising both primary and secondary forest. Using Lepidoptera as the target taxon, we examine whether herbivore specialisation is affected by elevation, habitat use and guild type. We show that all three factors affect specialisation. Lowland species tend to be slightly less specialised than their montane counterparts. This difference in herbivore specialisation is driven primarily by mobile species and those which feed across both primary and secondary forest. Elevational differences in specialisation may result from environmentally driven variation in host defences, where the cost of replacing tissue is greater at higher elevations. Contrary to expectations, herbivores which fed exclusively in secondary forest were no less specialised than herbivores found only in primary forest. The least specialised species are those which feed across both primary and secondary forest. We show that accounting for phylogeny can change the measurement and interpretation of specialisation notably, where the least specialised species experience the greatest differences in this respect. We therefore suggest that ecologists should account for host relatedness and abundance when measuring specialisation, and we advocate for the use of the term *phylogenetic specialisation* in such cases.

Introduction

Ecologists have long sought to understand and measure resource specialisation as it underpins many key ecological and evolutionary processes (Futuyma and Moreno 1988). Specialisation has been the focus of studies which have, for example, aimed to estimate global biodiversity (Erwin 1982, Basset et al 1996), disentangle the mechanisms responsible for species co-existence (Becerra 2015), and the pervasive latitudinal gradient of species diversity (Novotny et al 2006, Forister et al 2015). Diet breadth can inform us as to how species and communities may respond to disturbance (Büchi and Vuilleumier 2014), with specialist species facing higher extinction rates (Clavel et al 2011). The structure and stability of entire networks of interacting species is affected by the degree of specialisation of their components. This is the case for both antagonistic plant-herbivore networks and mutualistic pollination (Weiner et al 2014) and seed dispersal (Correa et al 2016) networks. Diet breadth is tightly associated with evolutionary relationships among resource species and between trophic levels, reflecting trait similarity and co-evolutionary processes (Ehrlich and Raven 1964; Poulin et al 2011). For example, escalation and divergence of host plant defences are reflected in the degree of specialisation of their herbivore assemblages (Volf et al 2017).

Despite the obvious importance of specialisation, three decades on from the seminal review of the topic by Futuyma and Moreno (1988) it remains difficult to quantify in a manner which enables meaningful cross community comparisons. This is in part due to the multitude of specialisation measures and definitions currently in use, and to a variety of biases in underlying resource use data, specific to each of the many sampling methods in use. It is not yet feasible to consistently sample community interactions in diverse systems exhaustively. This invariably leads to differences in sampling intensities and sample size which, due to the rarity of many trophic interactions, leads to incomplete sampling affecting measures of specialisation in non-trivial ways (Lewinsohn et al 2005). Furthermore, the relatedness of host species is often not accounted for using traditional measures. This is a significant shortcoming, given host evolutionary relationships are generally considered to be the most important trait determining resource selection by herbivores (Ehrlich and Raven 1964; Jorge et al 2017). To overcome these issues, Jorge et al (2014, 2017) developed the Distance Based Specialisation Index (DSI). DSI accounts for both host phylogeny and resource availability,

and is defined as the deviation of observed phylogenetic relatedness of resource species used by a given consumer species from a null expectation of assuming random use of resources (Jorge et al 2017). As such, DSI is robust to variation in abundance, diversity and sampling intensity of host species. This facilitates comparative studies of species interactions, for example insect herbivores and their host plants from distinct geographical locations or guilds, or from distinct habitats and environmental conditions. In addition, herbivores which feed on multiple, but closely related hosts i.e. genus specialists, can be readily distinguished from generalists feeding on multiple distantly related hosts. Such distinctions are necessary to understand network structure and evolution of these complex ecological interactions.

Here we focus on interactions between larval Lepidoptera and their host plants in diverse Papua New Guinean rainforest. This group are a widely used model herbivore taxon. They form an extremely speciose assemblage with a comparatively broad host use and specialisation spectrum. Generalists however tend to be less prevalent than specialist species. Novotny et al (2005) showed that a randomly selected caterpillar in secondary forest will most likely feed on just one to three plant species and will have the great majority of their population on one host species. Lepidoptera are ubiquitous along tropical elevation gradients, often forming distinct communities with changes in elevation (Novotny et al 2005). Feeding modes are also diverse in Lepidoptera as they belong to several herbivore guilds (Novotny et al 2010). These traits make Lepidoptera a promising target taxon for the exploration of herbivore specialisation patterns and their ecological and phylogenetic correlates.

Herbivore specialisation can vary along key ecological gradients. Exploring this variation is an increasingly popular line of research (see review by Tylianakis and Morris 2017). Elevational change in particular has received much attention (Rodríguez-Castañeda et al 2010, Morris et al 2015). The shifting conditions at different elevations, for example decreasing temperature with increasing elevation or changes in solar radiation, influence numerous ecological factors such as parasitism, predation, competition and host plant quality, which in turn affect host plant choice and species distributions (Gaston 2003, Hodkinson 2005). We can advance our understanding of inter-specific associations by using increasingly sophisticated approaches to compare interaction networks within distinct elevation bands. Such approaches have

found mixed support for a link between elevational diversity trends and specialisation. For example, Pellissier et al (2012) found that herbivore specialisation decreased with elevation in Swiss Alpine Lepidoptera. Similarly Plowman et al (2017) examined mutualistic interactions of myrmecochorous plants and their ant inhabitants and showed that the system became less specialised at higher elevations. On the other hand, Rodríguez-Castañeda et al (2010) and Morris et al (2015), examining plant-herbivore and host-parasitoid interactions respectively, showed that specialisation was greatest at higher elevations.

Species interactions are also affected by habitat, reacting to both anthropogenic and natural disturbance events including agricultural conversion (Tylianakis et al 2007) and hurricanes (Luviano et al 2017). Degradation of forest is a significant issue globally, and tropical regions are the most affected. In Papua New Guinea (PNG), 15% of the native rainforest has been cleared over a thirty year period between 1972-2002 and a further 8.8% was degraded (Shearman et al 2009). The ability of herbivores to persist in the face of disturbance will dictate future community composition. Disturbance reduces plant diversity and this can favour more generalist herbivores. Pinho et al 2017 showed that forest edge herbivore communities in Brazilian Atlantic forest are notably more generalist than communities in the forest interior. Specialists may suffer disproportionately as they struggle to find resources in an increasingly homogenized landscape. However studies of PNG forest revealed little difference in specialisation between primary and secondary forest (Lepš et al 2001, Redmond et al 2018).

Host phylogenetic diversity also changes across ecological gradients. This is in turn will affect the availability of hosts. In a phylogenetically overdispersed host community, the potential for feeding on multiple hosts is reduced, increasing the prevalence of monophagous species and community level specialisation. On the other hand, in a phylogenetic clustered host community, herbivores will more likely find and feed across multiple hosts. Host phylogenetic diversity in the neotropics and lowland forest in PNG were shown to be lowest in secondary forest, increasing in primary forest (Letcher et al 2012, Whitfeld et al 2012). However montane forest in PNG and temperate forest in China followed the opposite trajectory (Redmond et al 2019, Chai et al 2016). Elevational studies are comparatively rare, but suggest

that phylogenetic diversity decreases with increasing elevation (Qian et al 2014).

While the majority of studies investigating herbivore specificity focus on leaf chewing herbivores, often Lepidoptera larvae, they generally do not distinguish between guilds (but see Novotny et al 2010, Forister et al 2015). The assignment of herbivores to guilds is determined by their feeding mode and the plant part being utilized (Novotny et al 2010). Guild type is an important factor affecting specialisation of herbivores (Forister et al 2015). In this study we distinguish external free-feeding mobile chewers from semi-concealed, relatively immobile species. Rates of predation and parasitism vary between these two guilds. Hrcek et al 2013 showed that semi-concealed larval Lepidoptera suffer higher parasitism rates, while (Tvardikova and Novotny 2012) reported higher rates of predation of exposed artificial caterpillars (4.95%) compared with semi-concealed model caterpillars (2.99%). However, it remains to be seen whether the different life histories and evolutionary pressures facing these two guilds have also driven differences in specialisation.

Here we examine how specialisation in different Lepidoptera guilds in Papua New Guinea changes with elevation and succession stage. Sampling almost four hectares of forest exhaustively, we compile species interactions within lowland and montane communities, also in both primary and secondary forest. We test the hypotheses that 1) Herbivore species in lowland communities of relatively high host species and phylogenetic diversity will be more specialised than herbivores in montane forests with lower host diversity. 2) Species within secondary forest will be less specialised than primary forest species. 3) Herbivores belonging to mobile guilds will be more generalist than immobile species.

Materials and Methods

Field Site and Succession Series

Lowland sampling was carried out near Wanang Village (145°10’55” E, 5°13’51” S), Madang Province, Papua New Guinea. Two 100 x 100 m plots were destructively sampled by felling, one plot within primary forest and one within secondary forest. These two plots were separated by a distance of 800m at 100-200m above sea level. Sampling was carried out from February 2006

to October 2007. At our montane site, nine 0.2 hectare plots near Yawan village (-6.16388ºN, 146.83833ºW), Morobe Province, Papua New Guinea were sampled destructively. Four of these plots were located in primary forest and the remaining five in secondary forest. Plots fell within a range of 1720-1860 m above sea level. Sampling was carried out between July 2010 and November 2012 (See Redmond et al 2019 for more details). At both lowland and montane sites, plot locations were subjectively chosen to avoid dangerous felling conditions. Plot locations were earmarked for clearance by the local land-owning community to facilitate swidden subsistence agriculture. Before sampling, woody plants with a diameter >5cm 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 years, allowing natural succession to take place.

Herbivore Sampling

All plots were divided into subplots of 22x22m. Sampling started from the lowest subplot and proceeded in steps. It began with clearing all understory vegetation, and woody seedlings of a height less than 1.3m. After this, felling and sampling of trees <5cm DBH was carried out. Next, trees >5cm DBH were felled, beginning with midstory trees. Trees tangled with lianas had the potential for damaging other trees when felled, and in general were difficult to fell, thus lianas were cut with machetes in order to free up trees where possible. Tree felling was directed into gaps created by previous plot clearance, allowing for easier collection. Collection from the entire foliage of the felled trees 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 before being brought back to the field lab. There, trophic links were confirmed with 24-hours no-choice feeding trials, using leaves of the host that the herbivore were found upon. 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.

Host Phylogeny

Ten leaf discs (diameter 2.3 cm) for each host individual were cut from fresh mature leaves and dried in silica gel for phylogenetic analysis. These discs are stored in -80ºC frozen tissue collections at the University of Minnesota (St Paul, Minnesota, USA). Details concerning the reconstruction of the lowland plant community phylogeny can be found in Whitfeld et al (2012). Our montane host phylogeny was reconstructed using two loci: rbcL, and psbA-trnH, with these sequences located in the Barcode of Life Database (BOLD). DNA extraction, amplification and sequencing were carried out at the Canadian Centre for DNA Barcoding following standard protocols and administrated through the BOLD system. Existing sequences were sourced from online databases if available. Sequences were assembled and edited using Geneious 5.4 (Kearse et al 2012). Host phylogeny was reconstructed using Bayesian inference as implemented in BEAST v2.4 (Drummond et al 2012). The following substitution models were selected based on BIC computed in JModelTest 2 (Darriba et al 2012) and were used for individual loci: rbcL: GTR+I+G, psbA-trnH: TIM1+I+G. The topology was constrained using Phylomatic 3 (Webb and Donoghue 2005). A log-normal relaxed molecular clock following Bell et al (2010), dating based on Wikström et al (2001) and clock rates based on Palmer (1991) were used for time-calibrating the phylogeny. Sampling was carried out every 10^3 generations for $2x10^7$ generations, the first 10% of all generations were discarded as 'burn in' and the results were summarized with a majority-rule consensus tree. All branches with posterior probability below 0.7 were treated as polytomies.

Herbivore Specialisation

Calculating DSI followed the procedure detailed in Jorge et al (2014, 2017). Here, evolutionary relationships of resources are accounted for, given they are the primary mediator of resource selection. *Analysis was carried out on a subset of the total herbivore dataset*, specifically specimens with successfully reared adults. This decision was made in order to increase robustness of the lowland dataset where larval taxonomy was unclear. The relatedness of host species was measured using mean pairwise distance (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. The rescaled upper bounds of DSI* were 1 (monophagy) and the lower

bounds -1 (maximum generalisation). DSI values close to zero represent indiscriminate feeders. 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.

Interactions within montane and lowland forest plots were pooled across habitat types, and DSI values were then calculated for all herbivore species within each elevation. Thus, DSI values were obtained for each herbivore across 2 ha and 1.8 ha of lowland and montane communities respectively. Community MPD for each elevation was calculated as outlined in Redmond et al 2019, where standardized effect size is used to compare between study sites. As undersampling can bias estimates of DSI* by inflating the number of monophages, we chose to use only species that were represented by a minimum of five individuals at each elevation. This threshold was chosen as it minimizes undersampling bias yet retains half of the reared species in the community, and 95% of reared individuals.

To assess the effect of elevation, guild and habitat use on specialisation, herbivore species were classified according to the following criteria. For elevation, herbivore species were simply defined as lowland or montane species as overlap between these two communities was extremely low. For guild type, herbivores were either assigned to mobile chewers or semi-concealed feeders. Mobile chewers are free feeding herbivores which typically consume most parts of the leaf tissue and do not construct temporary feeding enclosures. Semi-concealed feeders on the other hand are less mobile, often consume only some leaf tissues e.g. by skeletonisation, and construct feeding enclosures by leaf rolling or tying. Habitat use was divided into three categories: habitat generalists, primary forest specialists and secondary forest specialists. To avoid classifying incidental occurrences in a different habitat type as generalists, primary and secondary specialists were defined as having >90% of individuals obtained from a single habitat type. All other species were considered habitat generalists. The effect of life history traits on DSI was assessed using a series of linear mixed effect models with species as a random factor.

To assess the effect of plant phylogeny on host selection we also calculated DSI* using a completely unresolved phylogeny where all species are equally related with a single polytomy from which all species descend. We then compared these DSI* values with those accounting for phylogeny by a paired t-test and linear mixed effect models with species as a random factor.

Results

Lowland forest had a greater number of herbivore species than montane forest with the majority of these species being semi-concealed feeders. This was also the case for montane forest, however here the number of semi-concealed feeders was only marginally higher than that of mobile chewers (Table 1). Primary and secondary specialists were relatively dominant in lowland forest, while in montane forest habitat generalists were better represented, where the number of species within all three habitat preference categories was comparable (Table 1). We found no evidence of an elevation trend in phylogenetic diversity. The standardized effect size of MPD within the lowland forest = 0.372, whereas montane forest = 0.462. Thus both community structures approach a neutral host phylogenetic structure when considering both primary and secondary forest together. Bipartite networks of lowland and montane communities are presented in Figure 1.

Table 1: Number of reared Lepidopteran herbivore species and their life histories traits within lowland and montane forests. Numbers in parentheses indicates the number of species used in the Distance Based Specialisation Index (DSI) mixed effect models, defined as having n > 5 individuals.

Elevation	Life History Category	Trait	Number of Species
Lowland			
	Guild:	Mobile Chewers	124 (62)
		Semi-Concealed	236 (158)
	Habitat Preference:	Primary Specialists	158 (76)
		Secondary Specialists	130 (74)
		Habitat Generalists	72 (70)
Montane			
	Guild:	Mobile Chewers	81 (36)
		Semi-Concealed	97 (48)
	Habitat Preference:	Primary Specialists	43 (12)
		Secondary Specialists	72 (23)
		Habitat Generalists	63 (49)

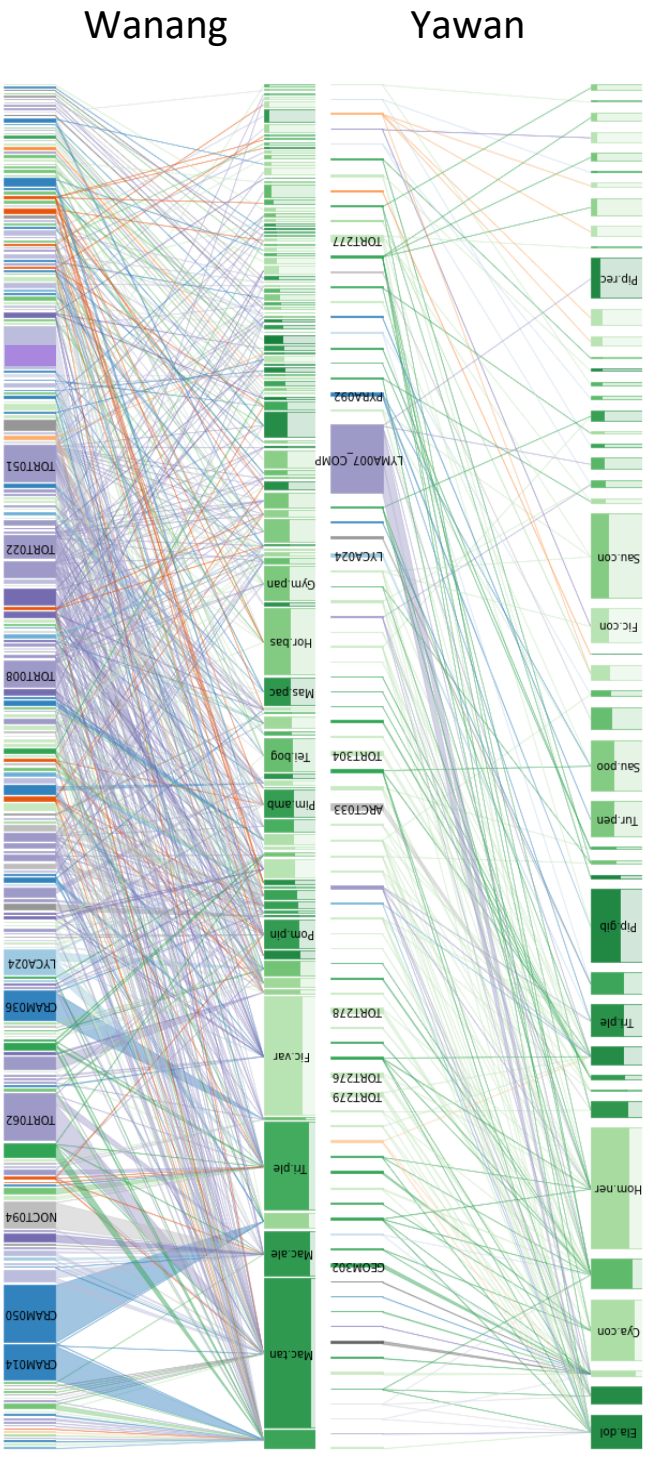

Figure 1: Representative bipartite networks from both sampling sites Wanang (lowland) and Yawan (montane). Hosts plants are represented on the lower level, the width of the bars is proportional to the abundance of hosts while the proportion of fill represents herbivore consumption .i.e. nodes with complete fill are the most consumed, and hosts are ordered by relative consumption. Labels are show for the top ten most abundant host and herbivore species. Wanang hosts: Mac.tan – *Macaranga tanarius*,Mac.ale- *Macaranga aleuritoides*, Tri.ple- *Trichospermum pleiostigma*, Pom.pin-*Pometia pinnata*,Pim.amb- *Pimelodendron_amboinicum*, Tei.bog-*Teijsmanniodendron_bogoriense*, Fic.var- *Ficus variegate*, Mas.pac-*Mastixiodendron_pachyclados*, Hor.bas-Horsfieldia_basifissa, Gym.pan- Gymnacranthera_paniculata. Yawan hosts: Ela.dol- *Elaeocarpus dolichostylus*, Cya.con- *Cyathea contaminans*, Hom.ner- *Homolanthus nervosus*, Tri.ple- *Trichospermum pleiostigma*, Pip.gib- *Piper gibbilimbum*, Tur.pen- *Turpinia pentandra*, Sau.poo-*Saurauia pooleiI*,Fic.con Ficus congesta, Sau.con *Saurauia conferta*

There were significant interactions between all pairs of explanatory variables (habitat use, guild type and elevation) (Table 2), with the best fit model given in supplementary material appendix 1. Lowland herbivore communities were typically less specialised than their montane counterparts, contrary to expectations (Figure 2a,b). These patterns mainly result from lower specialisation of lowland mobile chewers and habitat generalists. In montane forest, herbivores tend to be similarly specialised, regardless of guild and habitat preference (Figure 2a,b). Over both elevations however, guild type and habitat preference showed clear effects on host use (Figure 2c). Species found in both primary and secondary forest (habitat generalists) used a phylogenetically broader range of hosts, with this effect strongest in lowland communities. See supplementary material appendix 2 for DSI values and associated traits for all species used in the analysis.

When all hosts are treated as equally related, the overall degree of herbivore specialisation is significantly lower ($t = 9.133$, $df = 303$, p-value= <0.001) compared with the true phylogenetic distance weighting (Figure 3a). Elevation and habitat preference had a significant effect on DSI resolved and unresolved differences (Figure 3b) (Linear Mixed Effect Model, $\chi 2 = 66.6$, $p<0.001$), with the greatest differences found for montane habitat generalists (Figure 3b).

Table 2: Interaction terms and associated test values for the linear mixed effect models, taking species as a random factor.

Interaction terms:	Chi Df	Chisq	P
Elevation:Guild	1	12.60	0.001
Elevation:Habitat Use	2	18.35	0.001
Guild:Habitat Use	2	6.70	0.034

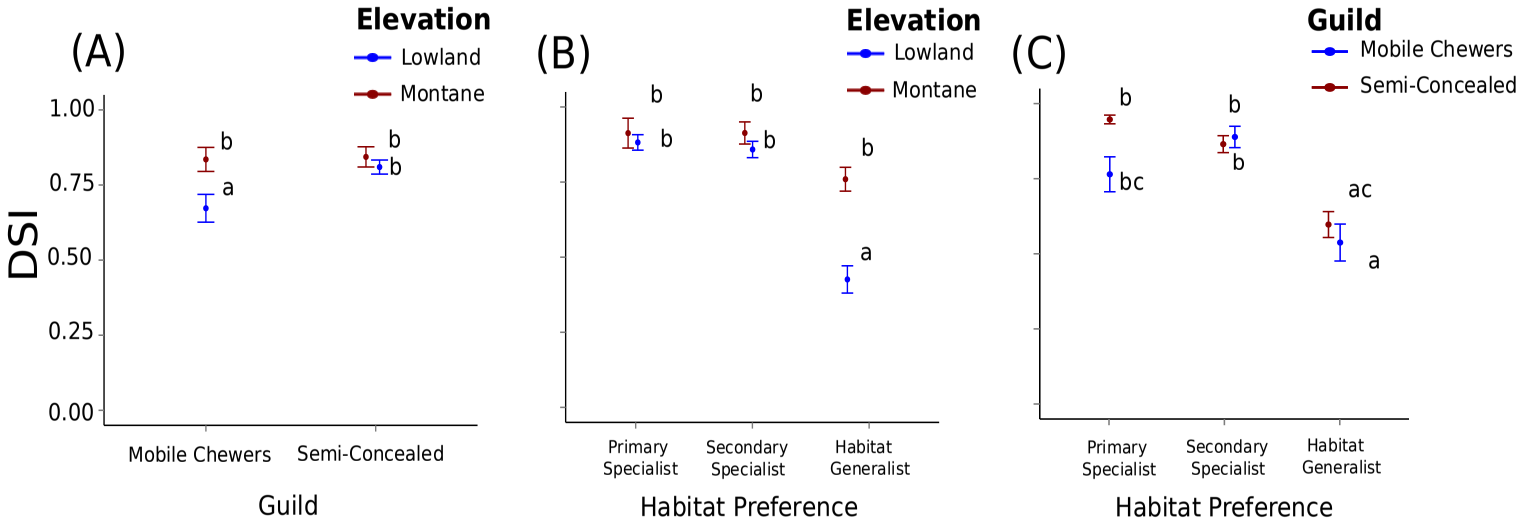

Figure 2: The effects of interactions between (a) Elevation x Guild, (b) Elevation x Habitat preference and (c) Guild x Habitat Preference on the phylogenetic Distance Based Specialisation Index (DSI) of herbivore communities.

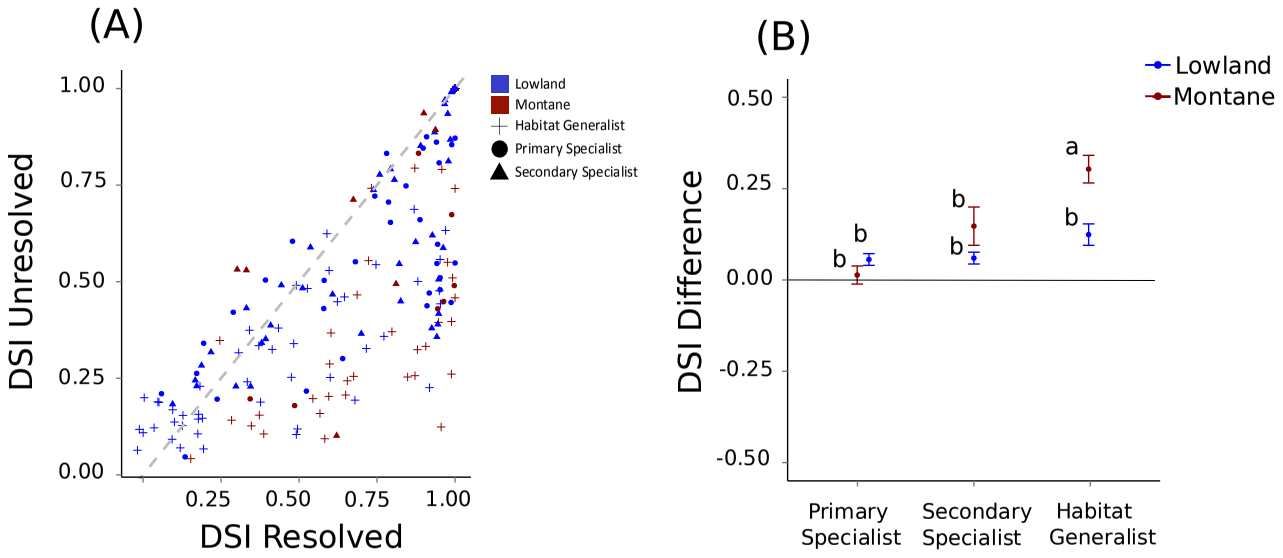

Figure 3 (A)- DSI values using the resolved Vs unresolved host phylogeny. Grey dashed line has a slope of 1, thus species below this line are more specialized when the resolved phylogeny is used to calculate DSI. Lowland species are coloured blue, montane species are red. Symbols denote habitat use: Habitat generalists-cross, Priamry specialists- circle, Secondary specialist- Triangle. (B) The effects of elevation and habitat preference on the difference between resolved and unresolved DSI values.

Discussion

High specialisation is a pervasive feature of the plant-herbivore interactions studied here and elsewhere (for example Villa-Galaviz et al 2012, Forister et al 2015, Redmond et al 2019). However, here we show that the degree of specialisation varies with guild, habitat use and elevation. Furthermore, by comparing specialisation measures accounting for true phylogenetic relationships with those assuming equal host relatedness, we show that perceived levels of herbivore specialisation can change notably. These findings have important consequences for not just understanding evolutionary and ecological mechanisms underpinning specialisation, but also for how ecologists measure and interpret resource use.

Herbivore communities in highly diverse lowland forest were less specialised when compared with less diverse montane forest. This cannot be explained by changes in community phylogenetic structure and host availability, as phylogenetic diversity of both elevations was similar. Differences in specialisation were driven primarily by mobile habitat generalists, as all other herbivore groups were similarly specialised. Other studies examining host-herbivore interactions across different elevations have reported comparable findings. For example, in the Neotropics, Rodríguez-Castañeda et al 2010 examined the Lepidopteran genus *Eois* and showed that specialisation was greater in montane forest than in lowland forest. In PNG Novotny et al 2005 showed that host specificity of Lepidopteran herbivores feeding on *Ficus* communities in lowland forest in PNG was comparable to host specificity at 1700masl. Our findings are in conflict with the widespread belief that increased diversity and specialisation are tightly linked (Dyer et al 2007), with parallels being drawn between elevational and latitudinal gradients in specialisation (Pellissier et al 2012). Futuyma and Moreno 1988 proposed that high herbivore richness and abundance, as is found in lowland tropical forests, promotes the evolution of host defences, which in turn force their herbivores to become increasingly more specialised in order to overcome them. There is however substantial evidence for the absence of a link between specialisation and diversity (Novotny et al 2006) and strong evidence to the contrary for mutualistic networks (Schleuning et al 2012). It is likely that specialisation is determined by an array of factors of which host diversity and availability are no doubt important. However, the distinct environmental conditions that communities encounter, such as those imposed by elevation through changes

in local climate (Hodkinson 2005), likely also shape specialisation non-uniformly.

Montane plant communities are exposed to conditions which limit growth. These include lower temperatures, increased drought stress on shallow soils, low nutrient supply and increased UV-B radiation which damages photosynthetic apparatus (Leuschner and Moser 2008). In contrast, lowland communities encounter high temperatures and receive sufficient rainfall to provide an excellent environment for plant growth. Plants are thought to make a greater defensive investment under conditions where tissue is more difficult to replace (Endara and Coley 2010). Fine et al 2004 showed that host plants which specialise on resource poor, white-sands substrate have more effective anti-herbivore defences than species which grow on clay soil. In the context of an elevationally driven resource gradient, host defensive investment should be greatest at higher elevations. This has been demonstrated by (Volf et al 2018 in preparation), who examined a broad range of secondary metabolites in *Ficus* communities along our study site on Mt. Wilhlem in PNG, and showed high elevation species are equipped with greater overall anti-herbivore defences. Similarly, Salgado et al 2016 showed that the expression of host defences may also change with elevation, as the production of cyanogenic glycosides in *Lotus corniculatus* increased with elevation. This increased investment in host defences may in turn drive herbivores to become more specialised in this relatively resource-poor environment.

The most generalist herbivore species overall were those with their populations spread across hosts within both primary and secondary forest i.e. habitat generalists. It has been previously shown that generalist species often feed across habitat types (Jonsen and Fahrig 1997). However, by accounting for differences in abundance and richness of host pools, we show here that species feeding across habitat types are often *true* generalists i.e. their broad host spectrum is not simply a product of being exposed to a greater number of host plants. For these habitat generalists, feeding mode did not affect their degree of specialisation, where both semi-concealed and mobile feeders fed on a comparatively diverse set of host species. However, feeding across habitat types likely imposes evolutionary costs beyond simply overcoming host defences. These herbivores must possess greater physiological tolerances to a range of environmental conditions, for instance, an ability to tolerate changes

in temperature and water availability (Schowalter 2012). Increased temperature and lower humidity in secondary forests can expose herbivores to an increased risk of desiccation (Hadley 1994). Habitat generalists should therefore possess adaptations for maintaining higher water body content, which includes behavioural adaptations such as leaf tying or the ability to locate and utilise water sources. We found some evidence of this, as 66% of all habitat generalists were semi-concealed feeders, comparable to that of secondary forest specialists (63%), with this trait being slightly less common primary specialists (58%). Biotic pressures including predation and parasitism will also vary across habitat types as herbivores encounter a broader range of enemies, although the intensity of these pressures may remain similar (Tvardikova and Novotny 2012). Additionally, foliar chemistry of host trees have been shown vary with the degree of disturbance (Hunter and Forkner 1999). However herbivores that can tolerate pressures from multiple habitat types are rewarded with an increased pool of potential resources and an implicit increase in their resilience to disturbance (Clavel et al 2011). Surprisingly, herbivores predominantly restricted to either primary or secondary forest alone, at either elevation, were similarly specialised. This finding is unexpected given the perceived differences in host quantity and quality between habitat types (Endara and Coley 2010, Coley 1987). Further, it runs contrary to the idea that disturbed habitats harbour more generalist species (Pinho et al 2017), but is corroborated by Redmond et al 2019, where a more expansive herbivore community analysis was performed along a successional gradient.

Traditionally, measures of host use have treated resource units, such as host plants, as equally distinct. Thus evolutionary history and the phylogenetic relationships of host communities have often been overlooked (for example Lepš et al 2001), although attempts at controlling for phylogeny have been made and are becoming increasingly common (Novotny et al 2006, Dyer et al 2007, Forister et al 2015). Overlooking host phylogenetic relationships can be problematic, as host plant anti-herbivore defence is often closely correlated with host phylogeny. Thus herbivores feeding on multiple, but closely related species have often been considered generalists. However the evolutionary barriers which they have overcome may be comparatively minor compared to herbivores feeding on fewer but more distantly related hosts. This concept challenges the idea of what ecologists consider specialist and generalist

herbivores. To bring clarity to this confliction of terms, we suggest the use of the term *phylogenetic specialisation* to distinguish between traditional and phylogenetic distanced based measures of host use.

By accounting for true host phylogenetic relationships, we might expect measures of phylogenetic specialisation to increase, as herbivores which feed on multiple congeneric hosts will be weighted accordingly. Indeed we found that accounting for host relatedness generally increases overall levels of specialisation, as herbivores tend to feed on a phylogenetically clustered set of hosts (Peralta 2016). This effect was greatest, as expected, in the most generalist species. Habitat generalists, and in particularly montane habitat generalists, saw the greatest changes. One such example is the crambid *Talanga sexpunctalis*, a montane habitat generalist that is typically found upon *Ficus* hosts. In our montane site, *T. sexpunctalis* is considered one of the most generalist herbivore species when host relationships are assumed to be equal. However, when taking the true host relationships into account, *T. sexpunctalis* is shown to be highly specialised. This trend is repeated for numerous genus specialists throughout the study sites. On the hand, some species were relatively more generalist when taking host relatedness into account. Geometrid larvae, which are well-known generalists, comprise seven of the ten species displaying the greatest increases in generalisation when accounting for true phylogenetic relationships. For instance, *Paradromulia nr lignifascia*, which fed across eight hosts from seven plant families (Achariaceae, Burseraceae, Cannabaceae, Fabaceae, Gnetaceae, Rubiaceae, Sapindaceae), was more generalist when accounting for the high phylogenetic diversity of its hosts. Thus, accounting for host relatedness can shift measures of specialisation in both directions. Most Lepidoteran herbivores will be shifted towards greater specialisation, given their propensity towards genus level feeding. Herbivores which feed on few species within multiple families may see the opposite shift.

Our findings have practical implications from both a methodological and conservation perspective. We have shown that incorporating true phylogenetic relationships of host plants, together with accounting for their abundance, i.e. resource availability, can change the interpretation of specialisation. Thus distinctions must be drawn between traditional specialisation measures and those which measure phylogenetic specialisation and resource availability.

The utility of both approaches will depend upon the research questions being asked. However DSI* presents a more promising methodology in respect of facilitating cross community comparisons. Host composition, abundance and phylogenetic diversity will more often than not vary greatly between study sites, particularly at larger spatial scales. By controlling for these differences, ecologists can more meaningfully explore changes along, for example, latitudinal gradients, where traditional approaches have borne conflicting findings (Novotny et al 2006, Lill et al 2007, Forister et al 2015).

From a conservation perspective, we have shown that the least specialised species are those that feed across both pristine and degraded forest. These habitat generalists should fare better than species restricted to fewer hosts or a single habitat type given the current rate of forest conversion. Studies have already demonstrated that highly specialised species are more at risk of extirpation and extinction through global biotic homogenisation (Clavel et al 2011). We have also shown that lowland herbivores are less specialised than montane herbivores species within our study sites. This suggests that lowland species may be more well equipped to deal with habitat and environmental changes than montane species. As the deleterious effects of climate change continue to mount, and as the habitable elevational range of plants, herbivores and their parasitoids shift and become desynchronised (Hance et al 2007, Telwala et al 2013), specialisation may be a key determinant of which species adapt and survive. Lower specialisation of lowland herbivore communities may facilitate upward range shifts, enabling herbivores to more easily find suitable hosts at different elevation bands. However, montane species, which are more tightly associated with their host plants, may struggle to locate suitable hosts within their habitable range.

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Supplementary Material Appendix 1

The best fit linear mixed effect model is shown below. Species are treated as random factor:

lmer(DSI.st ~ Elevation:Guild + Elevation:Habitat Preference
+ Guild:Habitat Preference
+ (1|Species)))

AIC= 33.65

Supplementary Material Appendix 2

Table S1: List of morphospecies used for the DSI analysis. Richness of hosts, sample size (numbers of reared adults), mean phylogenetic distance (MPD), Distance based specialisation (DSI), elevation, guild type (MC-mobile chewers, SC-semi-concealed) and habitat use (PS- primary specialist, SS- secondary specialist, HG- habitat generalist) are presented for each morphospecies.

Morphospecies	Richness	Sample size	MPD	DSI	Elevation	Guild	Habitat Use
ARCT002	2	8	<0.001	11.209	Lowland	MC	PS
ARCT003	2	7	168.775	3.86	Lowland	MC	PS
ARCT005	4	14	228.486	2.771	Lowland	MC	PS
ARCT015	1	6	0	4.377	Lowland	SC	SS
ARCT016	1	5	0	6.675	Lowland	MC	PS
ARCT020	1	10	0	12.207	Lowland	MC	PS
BOMB002	1	10	0	12.514	Lowland	MC	PS
CHOR001	3	11	16.669	6.145	Lowland	SC	HG
CHOR002	8	76	121.448	12.011	Lowland	SC	HG
CHOR003	5	67	73.099	11.926	Lowland	SC	SS
CHOR006	2	11	37.338	5.894	Lowland	SC	SS
CHOR008	6	68	108.187	8.548	Lowland	SC	HG
CHOR013	1	98	0	23.34	Lowland	SC	SS
CHOR014	2	36	3.867	13.319	Lowland	SC	SS
CHOR015	2	14	13.25	9.102	Lowland	SC	SS
CHOR016	2	16	10.61	8.344	Lowland	SC	HG
CHOR017	4	218	14.12	73.988	Lowland	SC	PS
CHOR021	1	22	0	22.291	Lowland	SC	PS
CRAM002	2	97	2.886	24.417	Lowland	SC	SS
CRAM003	2	8	9.763	5.394	Lowland	SC	SS
CRAM005	1	12	0	7.83	Lowland	SC	SS
CRAM006	3	51	26.224	13.85	Lowland	SC	HG
CRAM008	2	116	12.318	29.963	Lowland	SC	HG
CRAM010	1	10	0	6.366	Lowland	SC	SS
CRAM011	2	6	97.314	4.398	Lowland	SC	HG
CRAM012	1	5	0	7.418	Lowland	SC	PS
CRAM014	3	460	5.887	54.302	Lowland	SC	SS
CRAM017	1	7	0	8.673	Lowland	SC	PS
CRAM036	2	379	4.441	39.826	Lowland	SC	SS
CRAM037	2	37	43.835	25.228	Lowland	SC	PS
CRAM050	2	760	2.137	76.665	Lowland	SC	SS
CRAM051	3	30	24.05	10.308	Lowland	SC	SS
CRAM055	2	11	31.193	13.2	Lowland	SC	PS
CRAM058	1	12	0	8.05	Lowland	SC	SS
CRAM072	1	13	0	13.706	Lowland	SC	PS
CRAM076	1	6	0	4.768	Lowland	SC	SS
CRAM084	1	15	0	9.999	Lowland	SC	SS
CRAM098	1	5	0	7.022	Lowland	SC	PS
CRAM100	2	14	71.564	11.973	Lowland	SC	PS

CRAM102	2	47	0	34.561	Lowland	SC	PS
CRAM104	2	6	3.468	7.754	Lowland	SC	PS
CRAM120	1	5	0	4.878	Lowland	SC	SS
CRAM121	1	57	0	31.679	Lowland	SC	PS
CRAM124	2	10	116.265	7.183	Lowland	SC	PS
ELAC001	3	30	119.119	8.535	Lowland	SC	HG
ELAC002	1	7	0	8.889	Lowland	SC	PS
ELAC005	2	10	88.583	3.399	Lowland	SC	SS
ELAC016	1	8	0	9.991	Lowland	SC	PS
GELE005	3	43	16.126	28.751	Lowland	SC	PS
GELE006	1	41	0	29.869	Lowland	SC	PS
GELE010	1	43	0	36.793	Lowland	SC	PS
GELE011	1	13	0	9.539	Lowland	SC	SS
GELE015	1	9	0	6.447	Lowland	SC	SS
GEOM001	4	18	80.938	8.882	Lowland	MC	HG
GEOM003	3	12	69.956	11.509	Lowland	MC	HG
GEOM006	2	6	109.525	1.755	Lowland	MC	SS
GEOM012	2	24	61.054	18.784	Lowland	MC	PS
GEOM013	4	20	99.798	13.173	Lowland	MC	PS
GEOM015	8	30	256.082	1.06	Lowland	MC	HG
GEOM021	6	14	242.372	1.297	Lowland	MC	HG
GEOM023	4	16	255.005	0.045	Lowland	MC	HG
GEOM034	3	70	32.957	16.122	Lowland	MC	SS
GEOM042	5	39	261.271	1.891	Lowland	MC	PS
GEOM048	4	12	166.903	3.334	Lowland	MC	HG
GEOM053	10	37	271.028	0.01	Lowland	MC	HG
GEOM072	3	26	100.917	5.475	Lowland	MC	SS
GEOM079	3	39	142.945	14.138	Lowland	MC	PS
GEOM104	1	5	0	7.551	Lowland	MC	PS
GEOM130	2	7	33.012	3.971	Lowland	MC	SS
GEOM132	1	43	0	15.509	Lowland	MC	SS
GEOM134	8	42	223.18	6.204	Lowland	MC	PS
GEOM136	2	13	58.826	11.423	Lowland	MC	PS
GEOM151	5	97	91.861	21.21	Lowland	MC	HG
GEOM174	1	5	0	7.817	Lowland	MC	PS
GEOM176	2	10	196.904	3.61	Lowland	MC	PS
GEOM180	1	11	0	7.85	Lowland	MC	SS
GEOM181	1	6	0	8.537	Lowland	MC	PS
GEOM185	1	18	0	19.274	Lowland	MC	PS
GEOM191	2	5	149.484	2.967	Lowland	MC	HG
GEOM197	1	11	0	7.411	Lowland	MC	SS
GEOM198	1	6	0	8.273	Lowland	MC	PS
GEOM199	1	9	0	11.226	Lowland	MC	PS
GRAC001	1	6	0	5.298	Lowland	SC	SS
GRAC011	1	21	0	10.325	Lowland	SC	SS
GRAC023	1	8	0	10.885	Lowland	SC	PS
IMMI006	3	37	56.754	20.447	Lowland	MC	PS
LIMA010	1	10	0	7.765	Lowland	MC	SS
LYCA001	2	8	88.011	3.178	Lowland	MC	SS
LYCA006	2	37	6.728	12.326	Lowland	MC	SS
LYCA024	4	320	55.575	28.267	Lowland	MC	SS
LYMA003	4	11	241.845	1.141	Lowland	MC	HG
LYMA007	4	30	138.3	4.653	Lowland	MC	HG
LYMA034	3	17	150.811	4.002	Lowland	MC	HG
LYMA035	4	11	212.915	3.331	Lowland	MC	PS
LYMA038	6	22	237.761	2.444	Lowland	MC	HG
LYMA039	8	49	248.823	1.299	Lowland	MC	HG
LYMA059	1	6	0	4.763	Lowland	MC	SS
LYMA073	9	23	240.994	3.154	Lowland	MC	PS
LYMA081	1	5	0	4.564	Lowland	MC	SS
LYMA083	3	63	15.575	41.954	Lowland	MC	PS
NOCT002	1	7	0	4.881	Lowland	MC	SS
NOCT017	1	59	0	19.693	Lowland	MC	HG
NOCT020	5	25	177.286	0.956	Lowland	SC	SS

NOCT075	1	6	0	4.287	Lowland	MC	SS
NOCT094	3	346	5.93	38.123	Lowland	SC	SS
NOCT107	1	7	0	10.215	Lowland	SC	PS
NOCT109	1	5	0	7.445	Lowland	SC	PS
NOCT110	1	5	0	6.839	Lowland	MC	PS
NOCT127	1	15	0	7.951	Lowland	MC	SS
NOCT134	1	75	0	44.889	Lowland	MC	PS
NOCT154	1	24	0	24.751	Lowland	SC	PS
NOCT165	1	5	0	7.83	Lowland	MC	PS
NOLI003	1	20	0	8.528	Lowland	SC	SS
NOLI005	5	185	43.885	26.99	Lowland	SC	SS
NOLI007	4	55	82.961	9.723	Lowland	SC	HG
NOLI008	3	84	148.243	4.382	Lowland	SC	SS
NOLI011	2	16	124.57	2.861	Lowland	SC	SS
NOLI012	3	24	151.407	2.072	Lowland	SC	SS
NOLI015	1	8	0	4.862	Lowland	MC	SS
NYMP014	1	24	0	9.254	Lowland	MC	SS
OECO001	1	10	0	8.072	Lowland	SC	SS
OECO004	2	9	10.73	5.395	Lowland	SC	SS
PTER007	1	13	0	8.31	Lowland	SC	SS
PYRA002	2	8	110.014	2.091	Lowland	SC	SS
PYRA013	1	28	0	10.265	Lowland	SC	SS
PYRA018	2	41	2.95	31.753	Lowland	SC	PS
PYRA021	1	75	0	20.226	Lowland	SC	SS
PYRA022	5	13	249.281	-0.036	Lowland	SC	HG
PYRA023	3	12	116.052	8.34	Lowland	SC	PS
PYRA024	1	8	0	5.446	Lowland	SC	SS
PYRA029	1	6	0	8.427	Lowland	SC	PS
PYRA031	1	6	0	7.381	Lowland	SC	PS
PYRA047	2	69	47.27	16.155	Lowland	SC	SS
PYRA049	1	31	0	26.966	Lowland	SC	PS
PYRA051	2	33	85.878	7.968	Lowland	SC	HG
PYRA054	2	26	13.271	22.912	Lowland	SC	PS
PYRA056	1	10	0	13.08	Lowland	SC	PS
PYRA057	1	5	0	4.371	Lowland	SC	SS
PYRA061	1	7	0	9.712	Lowland	SC	PS
PYRA063	2	37	23.043	31.1	Lowland	SC	PS
PYRA065	1	9	0	11.365	Lowland	SC	PS
PYRA067	1	6	0	8.652	Lowland	SC	PS
PYRA069	1	6	0	4.654	Lowland	SC	SS
PYRA071	1	23	0	9.936	Lowland	SC	SS
PYRA082	1	8	0	10.549	Lowland	SC	PS
THYR001	3	139	12.27	25.69	Lowland	SC	SS
THYR005	1	7	0	5.044	Lowland	SC	SS
THYR010	2	26	28.343	21.24	Lowland	SC	PS
THYR012	1	8	0	7.326	Lowland	SC	SS
THYR014	1	5	0	6.948	Lowland	SC	PS
THYR016	1	8	0	5.423	Lowland	SC	SS
THYR022	1	11	0	14.247	Lowland	SC	PS
THYR026	1	86	0	45.41	Lowland	SC	PS
THYR027	1	30	0	22.693	Lowland	SC	PS
THYR029	1	5	0	4.311	Lowland	SC	SS
THYR031	2	8	25.12	10.406	Lowland	SC	PS
TORT008	16	355	163.377	7.947	Lowland	SC	HG
TORT009	2	18	13.349	20.248	Lowland	SC	PS
TORT012	1	5	0	4.452	Lowland	SC	SS
TORT015	1	16	0	11.177	Lowland	SC	SS
TORT018	1	9	0	7.619	Lowland	SC	SS
TORT022	22	309	182.257	7.584	Lowland	SC	HG
TORT034	21	208	195.435	6.663	Lowland	SC	HG
TORT039	1	8	0	11.185	Lowland	SC	PS
TORT040	14	103	229.426	0.922	Lowland	SC	HG
TORT051	31	488	186.529	9.286	Lowland	SC	HG
TORT062	4	617	13.422	53.523	Lowland	SC	SS

TORT064	1	7	0	5.608	Lowland	SC	SS
TORT065	2	32	24.842	22.055	Lowland	SC	PS
TORT066	7	25	242.062	1.997	Lowland	SC	HG
TORT067	9	84	161.256	3.498	Lowland	SC	SS
TORT071	12	63	236.306	4.231	Lowland	SC	HG
TORT075	1	12	0	7.066	Lowland	SC	SS
TORT076	3	17	137.449	7.236	Lowland	SC	HG
TORT084	1	10	0	11.233	Lowland	SC	PS
TORT085	3	10	124.192	2.905	Lowland	SC	HG
TORT086	10	38	228.584	-0.085	Lowland	SC	HG
TORT120	7	51	139.539	5.056	Lowland	SC	SS
TORT122	1	25	0	25.882	Lowland	SC	PS
TORT134	1	5	0	7.59	Lowland	SC	PS
TORT138	3	15	180.945	6.131	Lowland	SC	HG
TORT150	1	6	0	5.508	Lowland	SC	SS
TORT159	1	161	0	67.814	Lowland	SC	PS
TORT160	1	10	0	6.531	Lowland	SC	SS
TORT167	3	62	20.063	15.026	Lowland	SC	SS
TORT174	1	7	0	9.413	Lowland	SC	PS
TORT177	3	20	166.285	3.564	Lowland	SC	HG
TORT184	1	8	0	10.498	Lowland	SC	PS
TORT185	6	16	164.942	1.591	Lowland	SC	HG
TORT186	2	6	109.525	2.05	Lowland	SC	SS
TORT191	3	14	120.942	2.984	Lowland	SC	SS
TORT200	2	8	89.384	7.494	Lowland	SC	PS
TORT210	4	64	34.883	18.514	Lowland	SC	SS
TORT211	2	118	132.903	18.606	Lowland	SC	HG
TORT212	1	20	0	18.491	Lowland	SC	PS
TORT213	1	23	0	12.007	Lowland	SC	SS
TORT214	1	10	0	13.479	Lowland	SC	PS
TORT222	1	5	0	4.843	Lowland	SC	SS
TORT225	1	7	0	10.254	Lowland	SC	PS
TORT238	1	18	0	17.021	Lowland	SC	PS
URAN009	1	6	0	5.438	Lowland	SC	SS
URAN015	1	12	0	13.26	Lowland	MC	PS
XXXX021	2	57	9.463	16.388	Lowland	SC	SS
XXXX035	1	14	0	7.382	Lowland	SC	SS
XXXX048	4	56	6.004	15.456	Lowland	SC	HG
XXXX067	4	62	76.964	9.792	Lowland	SC	HG
XXXX068	2	162	9.426	27.593	Lowland	SC	HG
XXXX090	5	13	202.815	0.988	Lowland	SC	HG
XXXX093	7	53	134.355	11.411	Lowland	SC	HG
XXXX094	2	57	16.512	34.726	Lowland	SC	PS
XXXX095	1	11	0	7.207	Lowland	SC	SS
XXXX097	5	62	131.939	21.015	Lowland	SC	PS
XXXX113	4	11	199.694	1.671	Lowland	MC	HG
XXXX125	1	19	0	20.144	Lowland	SC	PS
XXXX127	3	10	61.36	8.734	Lowland	SC	HG
XXXX129	2	17	9.941	8.578	Lowland	MC	SS
XXXX135	2	560	1.776	62.344	Lowland	SC	SS
XXXX140	2	42	28.155	14.501	Lowland	SC	HG
XXXX144	2	8	142.365	1.164	Lowland	SC	SS
ARCT033	1	88	0	22.272	Montane	MC	SS
ARCT037	1	13	0	8.787	Montane	MC	SS
CHOR024	1	6	0	6.172	Montane	SC	HG
CRAM006	5	16	15.762	8.939	Montane	SC	HG
EUPT003	2	8	148.265	3.856	Montane	MC	HG
GELE022	3	24	2.843	10.635	Montane	SC	HG
GEOM224	1	5	0	4.931	Montane	MC	HG
GEOM225	7	25	220.257	4.604	Montane	MC	HG
GEOM226	1	10	0	7.762	Montane	MC	PS
GEOM228	4	35	43.004	12.407	Montane	MC	HG
GEOM229	3	8	53.387	5.314	Montane	MC	HG
GEOM230	1	21	0	10.51	Montane	MC	PS

GEOM231	4	25	149.98	7.659	Montane	MC	HG
GEOM232	5	20	128.116	6.979	Montane	MC	HG
GEOM247	1	10	0	6.033	Montane	MC	SS
GEOM248	1	23	0	9.374	Montane	MC	SS
GEOM249	1	5	0	3.829	Montane	MC	SS
GEOM250	1	7	0	4.629	Montane	MC	SS
GEOM252	3	9	123.685	5.02	Montane	MC	HG
GEOM253	2	15	0	7.634	Montane	MC	HG
GEOM255	4	11	158.465	4.482	Montane	MC	HG
GEOM258	2	22	3.462	9.481	Montane	MC	PS
GEOM271	2	5	283.814	1.237	Montane	MC	HG
GEOM277	3	7	220.683	2.075	Montane	MC	PS
GEOM281	1	25	0	11.705	Montane	MC	SS
GEOM299	1	8	0	6.492	Montane	MC	HG
GEOM301	1	13	0	9.161	Montane	MC	HG
GEOM302	1	51	0	19.577	Montane	MC	HG
GEOM305A	1	15	0	10.365	Montane	MC	SS
GEOM305B	1	5	0	4.685	Montane	MC	SS
GEOM320	2	7	65.66	4.511	Montane	MC	SS
GEOM368	1	6	0	5.725	Montane	MC	PS
GEOM392	2	8	264.031	2.062	Montane	MC	SS
HESP016	1	10	0	7.316	Montane	SC	PS
IMMI013	3	10	45.194	6.141	Montane	SC	HG
LYCA024	1	46	0	16.529	Montane	MC	SS
LYMA007	6	894	11.973	68.552	Montane	SC	PS
LYMA060	6	39	136.353	9.279	Montane	MC	SS
LYMA088	1	8	0	6.709	Montane	SC	PS
LYMA090	4	13	144.196	5.085	Montane	MC	HG
NOCT214	2	8	8.373	6.034	Montane	MC	HG
NOCT222	1	5	0	4.689	Montane	MC	SS
NOLI035	1	10	0	7.7	Montane	SC	SS
NOLI036	1	38	0	16.425	Montane	SC	SS
PYRA092	3	42	122.074	11.495	Montane	SC	SS
PYRA101	1	17	0	8.666	Montane	SC	HG
PYRA102	1	10	0	6.951	Montane	SC	SS
PYRA104	1	6	0	5.156	Montane	SC	SS
ROES002	2	7	0.854	6.254	Montane	MC	PS
THYR001	2	7	0	6.127	Montane	SC	HG
THYR046	2	23	107.387	6.499	Montane	SC	HG
THYR049	2	5	71.695	4.116	Montane	SC	HG
THYR057	2	7	18.295	5.062	Montane	SC	HG
TORT051	6	19	242.407	3.454	Montane	SC	HG
TORT275	1	29	0	14.616	Montane	SC	SS
TORT276	2	63	23.914	20.659	Montane	SC	SS
TORT277	5	86	95.033	14.802	Montane	SC	HG
TORT278	2	67	36.223	16.868	Montane	SC	SS
TORT279	3	65	3.99	20.127	Montane	SC	HG
TORT282	1	10	0	6.776	Montane	SC	PS
TORT283	7	27	154.272	7.363	Montane	SC	HG
TORT293	2	12	252.029	2.659	Montane	SC	SS
TORT295	4	10	221.845	2.592	Montane	SC	HG
TORT297	4	14	171.716	4.446	Montane	SC	PS
TORT298	5	17	252.263	2.577	Montane	SC	HG
TORT299	3	40	45.185	11.382	Montane	SC	HG
TORT301	3	12	4.101	7.899	Montane	SC	HG
TORT303	6	17	317.161	1.595	Montane	SC	HG
TORT304	1	54	0	20.314	Montane	SC	SS
TORT306	1	6	0	4.924	Montane	SC	HG
TORT313	1	8	0	5.576	Montane	SC	SS
TORT314	1	6	0	6.648	Montane	SC	PS
TORT318	1	15	0	10.288	Montane	SC	PS
TORT321	4	26	33.024	11.677	Montane	SC	HG
TORT324	3	7	157.694	3.096	Montane	SC	HG
TORT327	2	24	41.364	10.485	Montane	SC	PS

TORT352	2	5	103.785	3.265	Montane	SC	HG
TORT365	1	13	0	8.698	Montane	SC	SS
TORT375	1	6	0	5.15	Montane	SC	SS
TORT377	2	37	14.482	16.31	Montane	SC	HG
TORT384	3	13	116.382	5.397	Montane	SC	HG
TORT392	1	7	0	6.049	Montane	SC	PS
TORT396	2	6	18.565	5.134	Montane	SC	PS
XXXX068	1	28	0	13.029	Montane	SC	SS

Chapter 4

Quantitative assessment of arthropod-plant interactions in forest canopies: a plot-based approach

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Abstract

1. Studies of canopy arthropods have progressed from inventories to research of trophic interactions, aiming to explain how arthropod diversity is maintained. Methods suitable for quantitative analyses of these interactions should achieve representative sampling across large parts of the canopy, access to all plant species, and obtain quantitative information on trophic links. Therefore, large-scale, plot-based analyses represent an ideal counterpart to frequently used analyses based on stratified selection of a focal subset of plant taxa sampled by a standardized sampling effort.

2. We explore a plot-based approach to study arthropod-plant interaction networks, using three quantitative methods for sampling a continuous area of forest canopy. We focus on sampling from felled trees, or from standing trees using either a canopy crane or a cherry-picker. We compare the efficiency of these methods to access the canopies of 0.1 ha plots and census insect herbivores (leaf-chewing insect larvae, miners and gallers), and non-flying invertebrate predators (spiders and ants).

3. We quantitatively sampled arthropods from 5.3 ha of forest in five biogeographic regions, representing 6,280 trees and 167,744 m^2 of foliage. We show that all three methods required a similar sampling effort (ca 1,000-2,000 person-hours to sample a 0.1 ha plot or ca 0.5-1.1 person-hours to sample 1 m^2 of foliage). Felling and cranes facilitated access to ca 82% of the foliage. The cherry-picker, which operated in optimal conditions facilitated access to ca 89% of the foliage. Sampling effort and foliage accessibility were affected by the forest type, total sampled leaf area, and total number of stems in a plot.

4. Plot-based census is the most suitable approach for reconstructing realistic, quantitative arthropod-plant interaction networks reflecting the abundance of both plants and associated insects. It enables network comparisons across sites and regions. A global network of plot-based studies would provide important insights into the processes of interaction network assembly and dynamics. Because canopy access by cranes or cherry-pickers in most forests is limited, we also suggest tree felling in pre-existing logging concessions as a suitable method for salvage sampling, which can yield globally comparable datasets.

Introduction

Forest canopies represent one of the most diverse environments on the planet (Lowman, Schowalter and Franklin 2012). They harbour a large part of terrestrial arthropod diversity, estimated at 6.8 million species (Hamilton *et al.* 2013). Simultaneously, they belong among the least known habitats because of their low accessibility (Lowman *et al.* 2012). This combination of high diversity and inaccessibility has fascinated biologists for more than 150 years (Lowman *et al.* 2012).

The development of single-rope climbing and fogging has provided ecologists with efficient tools for researching canopy communities of arthropods, generating several influential studies (e.g. Erwin 1982; Lowman 1984; Basset, Aberlenc and Delvare 1992). These have spurred the development of new methods of canopy access that nowadays include canopy walkways, canopy rafts, balloons, cherry-pickers, or canopy cranes (Lowman *et al.* 2012). Canopy studies have contributed to our understanding of global diversity of species and biotic interactions (Novotny *et al.* 2012; Hamilton *et al.* 2013), but, as pointed out by Lowman et al. (2012), *"...the real challenge is ahead. Canopy organisms, both mobile and sessile, must be surveyed and their roles measured."*

Research of canopy arthropods has progressed from species inventories to the study of their interactions and networks, allowing us to understand how hyper-diverse communities of canopy arthropods are maintained (Godfray, Lewis and Memmott 1999). Particular sampling methods are suitable for different systems and questions (Lowman *et al.* 2012) (Table 1). Methods suitable for quantitative analysis of arthropod interaction networks need to enable access to all parts of the canopy from terminal branches, through inner canopy, to

lower branches. This is because arthropod species composition differs considerably among various parts of the canopy (Basset *et al.* 2003a), reflecting the variation in resource availability and leaf traits (Murakami *et al.* 2005). In addition, such methods should obtain live arthropods for rearing parasitoids or herbivore feeding trials (Novotny and Basset 2005). In the tropics, the transient species (i.e. species with no lasting association to the sampled plant) can comprise up to 20% of species found on a particular tree (Basset 1997). A molecular approach to identify host associations is becoming increasingly popular (Kress *et al.* 2015) but may provide low resolution in diverse host systems containing closely related or hybridizing hosts. Obtaining live herbivores is thus usually necessary. Similarly mapping ant nests, instead of sampling individual ants, is necessary as up to half of the ants foraging in a tree are tourists from surrounding vegetation (Klimes *et al.* 2015).

Most importantly, the methods needed for quantitative analysis of arthropod interaction networks should allow structured sampling across large parts of the canopy, thus including all species in proportion to their abundance (Godfray *et al.* 1999; Klimes *et al.* 2012; Volf *et al.* 2017). Previous studies often focused on sampling individual tree species, individual trees or their parts, within a forest matrix. However, such selective sampling does not facilitate quantitative analysis of interaction network structure (Godfray *et al.* 1999). We argue that for interaction network analyses, a plot-based approach where entire continuous plots are censused for plants and arthropods is much more appropriate, as it reflects the diversity and abundance of the available resources (Volf *et al.* 2017; Redmond *et al.* 2019). Plot-based approaches examining forest vegetation have greatly benefitted plant ecology research (Anderson-Teixeira *et al.* 2015); we expect the study of arthropod interaction networks would benefit in equal measure (Novotny and Miller 2014). We accessed canopies using tree felling, canopy crane, and cherry-picker techniques (Fig. 1) across biogeographic regions (Palearctic, Nearctic, Neotropical, and Australian) and forest types (tropical vs. temperate, lowland vs. montane, primary vs. secondary). We discuss the strengths and limitations of the methods used for plot-based sampling of mobile non-flying exophytic herbivores (leaf-chewing insect larvae), endophytic herbivores (miners and gallers), and non-flying invertebrate predators (spiders and ants). Our aim is to stimulate plot-based research by providing practical and reproducible

sampling guidelines for the analysis of arthropod interaction networks in forest canopies.

Figure 1. Photos from the field: measuring a felled tree in Numba (A), herbivore sampling from felled trees in Yawan and Toms Brook (B, C), sampling from canopy crane in Tomakomai (D, E), a tree climber accessing a canopy tree inaccessible from the crane in San Lorenzo (F), sampling of an understory tree by ladder in San Lorenzo (G), sampling from cherry picker in Lazhot (H, I), sample sorting and caterpillar rearing in Tomakomai (J).

Methods

Arthropods were sampled from the canopies of i) lowland temperate forests in the Czech Republic (Mikulcice, Lanzhot), Japan (Tomakomai), and USA (Toms Brook); ii) lowland tropical forests in Panama (San Lorenzo) and Papua New Guinea (hereafter PNG; Wanang); and iii) highland tropical forests in PNG (Numba, Yawan) (Table 2, Table S1). We followed a standardized protocol (Appendix 1) and workflow (Fig. 2). Note- PNG plots were larger than other plots, thus in order to facilitate comparisons between study sites, all plots were standardised to a size of 0.1ha in our analyses.

I) Setting up the plot

At each location, we selected plots with a vegetation structure and species composition typical for local forests (Table 2, Table S1). Forest edges, plantations, stands with non-native vegetation, and large gaps were all

avoided, as were steep slopes and swampy areas (for technical and safety reasons). We took GPS coordinates of all plot corners and used measuring tape or laser range finders to set up the plot and map all plants with diameter at breast height (DBH) ≥5 cm. Each stem was tagged and identified to species level. Only living plants rooted in the plot were included (Appendix 1). It took 2-12 hours for three people to set up and map a 0.1 ha plot containing 24-251 trees.

II) Timing of sampling

Seasonality is crucial for the quantitative analysis of insect-plant interactions in seasonal forests (Godfray *et al.* 1999), where arthropod abundance and species composition often strongly correlate with season. For example, leaf-chewing insects exhibit one major peak during spring leaf-flush, and a smaller peak in late summer (Murakami *et al.* 2005). Peaks in abundance may differ among arthropod guilds, for instance leaf miners, where the major peak often seems to be later than for leaf-chewers (Fig. S1, Table S3). Thus, we sampled temperate plots throughout the season, with increased effort during the spring and late summer abundance peaks if they appeared. Such variation in sampling effort tracks variable insect abundance and keeps the probability that an insect species will be sampled constant throughout the season. We spread the sampling seasonally within each target tree species. In wet tropical forests, sampling was carried out with constant effort throughout the seasons as the effects of seasonality are much less pronounced and individual species appear throughout the year (Novotny and Basset 1998). However, a variable sampling strategy would be advisable in dry tropical and subtropical forests, where seasonality asserts greater influence (Ribeiro and Freitas 2011).

III) Arthropod sampling

The requirements for accessing the forest canopy and obtaining live arthropods drastically limits the range of methods suitable for the study of quantitative arthropod interaction networks (Table 1). We sampled arthropods from felled trees, and from trees *in situ* using canopy cranes or cherry-pickers (Fig. 1). Arthropods were, as far as possible, completely sampled from all trees with DBH ≥5 cm. The percentage of the canopy accessed was visually estimated for each tree (Appendix 1). We sampled on days without strong rain or wind to mitigate safety risks and lowered arthropod activity due to harsh weather. The focal arthropod groups included all live leaf-chewing insect larvae (free

feeding and semi-concealed), all leaf mines and galls (insects and mites), spiders, and ants (foraging and nesting; Table 1). Some species of galls were extremely abundant, making the complete sampling of galls impractical. In such cases, we selected 3-5 branches each with 100-500 leaves, calculated the mean number of galls per leaf per branch, and used the resulting values to estimate the total abundance on the respective tree (Appendix 1).

Table 1. Summary characteristics for particular methods of forest canopy sampling including **Canopy accessibility** (accessibility of tree strata: T (terminal branches), U (upper canopy), L (lower canopy), I (inner canopy)); suitable **Scale** of sampling (whole canopy vs. individual branches), **Arthropod taxa** sampled (E (endophytic), T (trunk-nesting), N (non-flying exophytic herbivores and predators), F (flying). * indicates that dead insects are sampled); minimal required **Team size**; relative operational **Costs**; **Replicability** (ease and practicality of replication); **Site availability** (low - limited sites with crane or walkway access; medium – available access road for cherry picker, felling not permissible in protected forests and other situations; high - almost all forests can be sampled); and key **References**.

Method	Canopy accessibility	Scale	Arthropod taxa	Team size	Costs	Replicability	Site availability	References
Canopy crane	T,U,L	Whole canopy, branches	E,N,F	Medium	High	Low	Low	Basset *et al.* (2012); Ødegaard (2004); Wardhaugh *et al.* (2012)
Cherry-picker	T,U,L	Whole canopy, branches	E,N,F	Medium	High	High	Medium	(Corff & Marquis 1999); (Volf *et al.* 2017)
Felling	T,U,L,I	Whole canopy	E,T,N	Large	Medium	High	Medium	Whitfeld *et al.* (2012); Redmond *et al.* (2018)
Canopy rafts	T,U	Branches	E,N,F	Medium	High	Low	High	Lowman *et al.* (1996)
Canopy walks	U,L,I	Branches	E,N,F	Medium	Medium	Low	Low	Reynolds and Crossley Jr (1997)
Fogging	T,U,L,I	Whole canopy	N*,F*	Small	Low	High	High	Erwin (1982); Kitching *et al.* (1993)
Tree climbing	U,L,I	Branches	E,T,N,F	Small	Low	High	High	Lowman (1992); Schowalter and Zhang (2005)

Table 2. Sampling site characteristics: **Forest type** (Trop - tropical., Temp - temperate), lowland (90-230 m a.s.l.), highland (700-1800 m a.s.l.), primary (P), and secondary (S) forests); **Maximum tree height** (m); **Plots** (number and size of sampled plots); **Method** of sampling; mean **Number of stems** with DBH≥5 cm per 0.1 ha (±SD); mean **Sampled leaf area** (m^2) per 0.1 ha (±SD); mean number of **Leaf-chewing larvae** per 0.1 ha (±SD); mean number of **Active mines** per 0.1 ha (±SD); mean **Area-based sampling effort** per 0.1 ha (ASE, person-hours; ±SD); mean **Resource-based sampling effort** (RSE, person-hours per 1 m^2 of foliage; ±SD); mean **Accessibility** (% of foliage accessed; ±SD); average **Team size** in the field and lab combined; and **Sampling period** (month and year). See Table S2 for data by individual plots and all arthropod groups.

Site	Forest type (max tree height)	Maximum tree height (m)	Plots	Method	Number of stems	Sampled leaf area (m^2)	Leaf-chewing larvae	Active leaf mines	ASE (person-hours)	RSE (person-hours)	Accessibility (%)	Team size	Sampling period
Tomakomai (JPN)	Temp. lowland (P)	22.8	2 x 0.1ha P	Crane	92 ±16	1,219 ±116	8,300 ±825	385 ±196	1,330 ±178	1.10 ±0.25	82.0 ±0.1	7	May-Aug 14; May-Aug 15
Lanzhot (CZE)	Temp. lowland (P)	45.0	2 x 0.1ha P	Cherry-picker	29 ±6	1,208 ±194	4,891 ±576	148 ±60	1,128 ±305	0.92 ±0.10	89.3 ±6.3	8	May-Aug 13; May-Aug 14; May 15
Mikulcice (CZE)	Temp. lowland (P)	33.6	1 x 0.1ha P	Felling	53	1,137	2,352	2717	1,512	1.33	83.4	10	May - June 13
Toms Brook (USA)	Temp. lowland (P)	30.7	2 x 0.1ha* P	Felling	81 ±18	1,793 ±132	2,608 ±428	564 ±470	1,604 ±326	0.89 ±0.12	76.5 ±1.0	7	Apr-Aug 16; Apr-Aug 17
San Lorenzo (PAN)	Trop. lowland (P)	35.0	3 x 0.1ha P	Crane	91 ±6	2,023 ±303	808 ±754	1,007 ±965	2,404 ±416	1.19 ±0.03	83.3 ±5.5	5	May 16-Apr 17
Wanang (PNG)	Trop. lowland (P+S)	74.2	1 x 1.0 ha (P) 1 x 1.0 ha (S)**	Felling	120 ±30	3,377 ±1050	1,354 ±705	185 ±85	1,880 ±474	0.58 ±0.14	82.9 ±4.0	22	Jan 06-Nov 07
Numba (PNG)	Trop. highland (P+S)	49.6	2 x 0.2 ha (P) 1 x 0.2 ha (S)**	Felling	143 ±17	3,658 ±1403	1,118 ±321	60 ±32	1,800 ±642	0.52 ±0.19	81.6 ±3.5	16	May 13-Jun 14
Yawan (PNG)	Trop. highland (P+S)	65.7	4 x 0.2 ha (P) 5 x 0.2 ha (S)**	Felling	133 ±62	3,591 ±620	1,103 ±862	199 ±152	1,183 ±488	0.33 ±0.14	82.9 ±4.0	16	Jul 10-Dec 12

* one of the 0.1 ha plots consisted of a 0.06 ha plot and a 0.04 ha plot separated by a 50 m gap

** these plots were divided into 0.1 ha plots for the purpose of the analysis

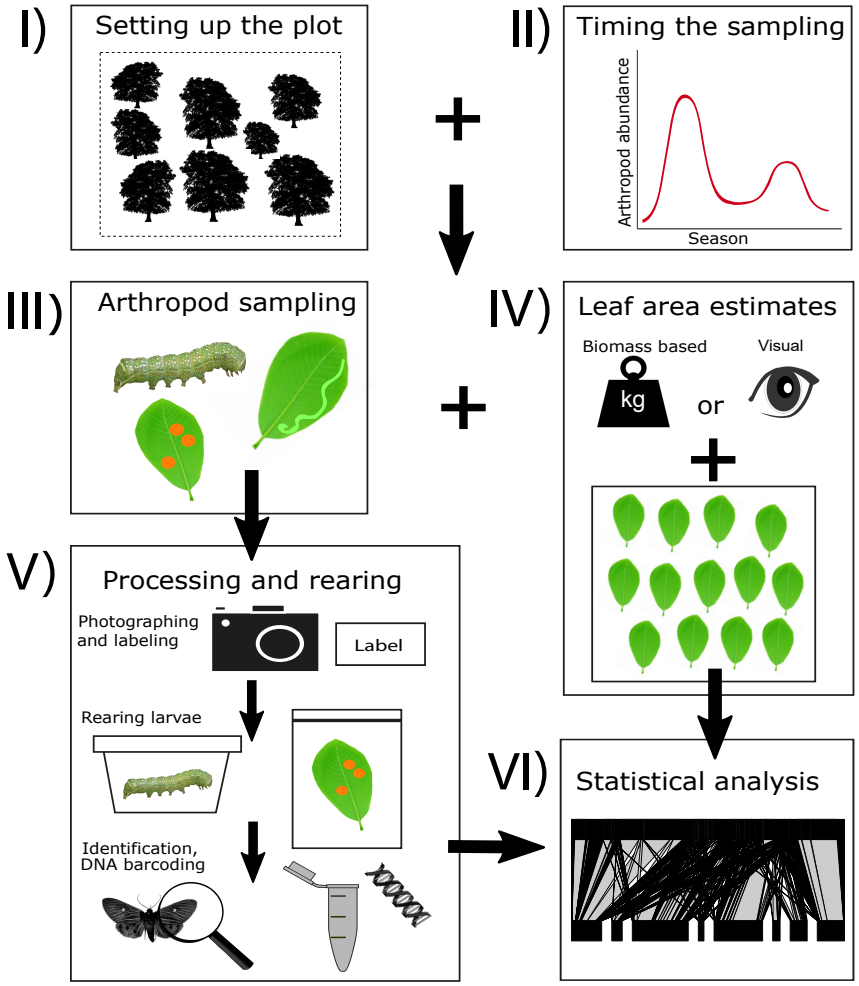

Figure 2. A workflow diagram for the proposed methods. The process starts with setting up the plot (I) and planning the sampling according to seasonality at a given site (II). The field work includes arthropod sampling (III) and estimation of leaf area (IV, including visual or biomass-based estimates and processing of leaf frames). Sampled arthropods are then processed (V), which includes labelling and photographing morphospecies, rearing, and the sending of material for taxonomic identification or DNA barcoding. Finally, the data are analysed (VI).

IIIa) Felling

Felling trees as a standardized destructive method is only suitable when it does not contribute to net deforestation. During our operations, we took advantage of ongoing logging operations (Mikulcice, Toms Brook) and shifting agriculture (PNG sites); no plot was cleared solely for sampling. All projects were conducted in close collaboration with the local community and land owners. Sampling began with the clearing of the understory, followed by the felling of trees with DBH ≥5cm. One tree was felled at a time, starting with the shortest individuals and those without lianas. Lianas on trees were cut with machetes prior to felling in order to free up the focal tree from its neighbours. Felled individuals were directed into gaps created by previous felling.

Once felled, the entire tree (trunk included) was searched and all focal arthropods hand collected, a process taking anywhere from minutes to several hours, depending on the crown size. Prompt and brisk work minimized the loss of arthropods through dispersal or predation, and the gain of foraging ants and spiders from the ground. Using division of labour, each team member focused primarily on one arthropod group, but would also contribute to the collection of secondary groups. Trees were always fully sampled on the day of felling, and necessitated teams of 7-22 members, dependent on study site and season (Table 2).

Unlike sampling from cranes and cherry-pickers, felling allows the sampling of arthropods dwelling in large branches and trunks, such as nesting ants (Table 1). At felling sites, we intensively searched every tree for ant nests and foraging ants with a team of two to three collectors, as described in Klimes *et al.* (2015). Foraging ants were collected first, before searching for ant nests by cutting branches, inspecting live and dead twigs, by dissecting parts of the trunk and bark, and by inspection of epiphytic aerial soil.

Conversely, felling is not suitable for mobile, flying herbivores (Basset, Charles and Novotny 1999). Even non-flying herbivores may become dislodged when the crown forcefully impacts the ground. If this were a serious concern, the ratio between endophytic herbivores and exophytic leaf-chewing larvae would depend on the method. However, the ratio of leaf-chewing larvae to active miners sampled in individual 0.1 ha plots did not differ among the methods (χ^2 (2) =2.57, p=0.2763) when compared by linear mixed-effect

models using the 'lmer4' R package (Bates *et al.* 2014), with site as a random effect.

IIIb) Crane

Canopy cranes were employed in Tomakomai and San Lorenzo. In Tomakomai, the crane is 25 m high, covers ca 0.5 ha of forest, and is operated by researchers from the gondola. In San Lorenzo, the crane is operated by a driver. The maximum accessible height from the gondola is 40.5 m. The crane covers almost 1.0 ha of tropical forest (Basset, Horlyck and Wright 2003b).

There were 4-7 team members working in the field, typically including 2 members sampling from the crane (canopy team), 1-2 members sorting samples on the ground (ground team), and possibly 1-2 members accessing larger mid-story trees by climbing (climbing team). The canopy team sampled branches starting at the tip and working towards the base, in order to minimize arthropod loss during sampling. Arthropods were sampled by beating onto a beating tray, followed by a visual search and hand collection of any remaining arthropods. The canopy team was assisted by an additional member during periods of peak arthropod abundance. The samples were regularly delivered to the ground team for sorting.

Sampling from the crane was augmented with other methods. The canopy team accessed understory trees from ladders. Step ladders were ideal for sampling 3-5 m tall trees. For sampling at heights up to 8 m, or on sloped terrain, modular ladder poles were more efficient and stable. In addition, more complex forest architecture, as in San Lorenzo, required the climbing team. Using a single rope technique, they accessed those mid-story trees inaccessible from the gondola or ladders (Fig.1).

IIIc) Cherry picker

A cherry picker (elevated truck-mounted work platform) was employed in Lanzhot. The 20 ton vehicle was transported by truck to the site, thus necessitating a forest access road. We used a Platform GENIE Z-135/70 JRT (Genie Industries, Redmond, WA, USA), which is equipped with a retractable arm enabling canopy access up to 43 m. The arm can be operated directly by researchers from the basket on the retractable arm tip. This four-wheel drive model can operate on gravel or clay forest roads, but not off-road terrain. Plots were set up along a forest road with a firm dirt surface (~4 m wide, and

completely covered by forest canopy) in order to provide good access to the plot from a single straight trajectory and to avoid having to manoeuvre the cherry-picker between trees. Two team members, working individually, sampled trees starting from the base and working towards the treetop. Arthropods were sampled using a beating tray combined with hand collection of remaining arthropods, before a final manual search by both workers. Samples were delivered to the ground team for processing before transportation to the laboratory. There were 2–6 people processing samples in the ground team, depending on insect abundance.

IV) Leaf area estimates

We calculated the leaf area of sampled trees in order to standardize arthropod abundance and allow cross-site comparisons (Appendix 1).

Forest felling enabled us to quantify leaf biomass directly by defoliating each tree and weighing the fresh foliage. Mature and young leaves were sampled and weighed separately by the field team immediately following herbivore sampling. Care was taken that only leaves, with no other plant parts such as twigs and flowers, were sampled. At Mikulcice and Toms Brook sites, where team size was limited, only 50% or 25% of the canopy was defoliated on the largest trees and the results extrapolated. This measure was taken to ensure the complete sampling of large trees on the day of felling. At the crane or cherry picker sites, we estimated the number of young and mature leaves visually on standing trees. Visual estimates of leaf number were conducted separately for every branch and were then compiled together to make an estimate for the entire tree. The estimates were carried out independently by two persons from the canopy team and the mean value used.

Next, a random selection of leaves from each tree were arranged on a 50 x 50 cm white background and photographed. The leaf area of the sample was then calculated using ImageJ 1.48 (Abràmoff, Magalhães and Ram 2004). For felled trees, we included the weight of the sample to obtain the area to weight ratio. Otherwise, we divided the leaf area of the sample by the number of leaves in the frame to obtain the mean area per leaf. One frame each of young and mature leaves was processed for small trees (DBH <15 cm), while at least two frames were processed for larger trees.

Finally, we calculated the total sampled leaf area for each tree using (i) the total leaf biomass and the area to weight ratio from the photographed sample

for the felled trees, or (ii) the estimated total number of leaves on the tree multiplied by the mean leaf size of the photographed sample for the crane and cherry picker trees.

V) Sample processing and insect rearing

In Tomakomai, Mikulcice, and Lanzhot, pre-sorting, photographing, and labelling of samples was done in the field by a team consisting of 1-6 members, depending on arthropod abundance (Appendix 1). This made subsequent sorting in the lab much faster. Smaller trees in Toms Brook for were also treated this way. Otherwise, samples were processed entirely in the laboratory.

We assigned all leaf-chewing insect larvae, galls, and mines to morphospecies according to their morphology (Volf *et al.* 2017). Each morphospecies was given a unique code name and was photographed. We preferred initial morphotyping to be done *de novo* per each individual tree. This approach is rapid and resistant to errors as even incorrect morphotyping does not generate false host plant records. It requires a second step where individual morphospecies are cross-referenced across all trees on completion of sampling. It is suitable for taxonomically poorly known and species diverse samples, where per-guild richness for an entire plot could reach hundreds of morphospecies.

We reared larval insect herbivores to adults or parasitoids (Appendix 1). We preserved larvae which died during rearing in 95% ethanol for later DNA barcoding. Only in Toms Brook, where insect taxonomy and host associations are well known, were leaf-chewing larvae immediately stored in ethanol due to the overwhelming logistics of rearing all. The aforementioned larvae, plus larvae that died during rearing, spiders, and representative samples of all ant castes from each nest or foraging event were stored in vials of 95% ethanol for subsequent DNA barcoding. The results of DNA barcoding along with reared adults were used to refine morphospecies concepts and assign final identifications.

VI) Statistical analysis: comparing the efficiency of different methods

We compared the efficiency of each method across the plots, expressed as i) **Foliage accessibility** per plot (the percent average of the foliage that was accessible), ii) **Area-based sampling effort** (**ASE**) required to sample each 0.1 ha plot (total time spent in the filed in person-hours), and iii) **Resource-based sampling effort** (**RSE**) required to sample 1 m^2 of foliage (in person-

hours). We modelled these components of sampling efficiency and the sampling **Method** (felling, crane, cherry picker) and **Forest type** (temperate, tropical lowland primary, tropical lowland secondary, tropical highland primary, tropical highland secondary), the **Number of stems** (DBH ≥ 5 cm) per plot, and **Sampled leaf area** using linear mixed-effect models as implemented in the R package 'lmer4' (Bates *et al.* 2014). **Foliage accessibility** was arcsine-transformed and **sampling effort** log-transformed. In addition, we modelled the correlation between both **ASE** and **RSE** and the number of **Leaf-chewing larvae** and **Active mines** (arthropod groups sampled at all sites and demanding the greatest handling effort). We used **Site** as a random factor in all mixed-effect models. Model simplification by forward selection resulted in the most parsimonious model based on the AIC criterion.

Results

In total, we sampled focal arthropod groups from 5.3 ha of forest, representing 6,280 trees and 167,744 m^2 of foliage (Table 2). We sampled 89,243 leaf-chewing larvae, 14,536 active mines, 134,783 abandoned mines, 28,698 spiders, 35,343 ant individuals, 3,487 ant nests, and sampled or estimated abundance of 2,989,808 insect and mite galls.

On average, **Foliage accessibility** was 82.5% ±3.9% (mean ±SD) foliage in felled plots, 82.7% ±3.3% foliage in plots sampled by canopy crane, and 89.3% ±6.3 foliage in plots sampled by cherry picker (Fig. S2). **Foliage accessibility** correlated with **Method** (χ^2 (2) = 6.91, p = 0.0254). The optimum model, after simplification, included the fixed effects **Forest type** (highest in lowland and highland secondary tropical forests), **Method** (highest from the cherry picker), **Number of stems** (positive correlation), and **Sampled leaf area** (negative correlation) (χ^2 (8) = 64.02, p < 0.0001) (Table S4).

The average **ASE** required to sample a 0.1ha plot was 1583 ±579 person-hours (mean± SD) for felled trees, 1867 ±673 for sampling by canopy crane, and 1128 ±305 for sampling by cherry picker. **Method** did not have a significant effect on **ASE** (χ^2 (2) = 1.49, p = 0.4740). The optimum model that explained differences in **ASE** included the fixed effects **Number of stems** (positive correlation) and **Forest type** (highest in lowland primary tropical forests) (χ^2 (5) = 95.24, p < 0.0001; Table S4).

The average **RSE** to sample 1 m^2 of foliage was 0.51 ± 0.24 (mean± SD) person-hours for sampling felled trees, 1.14 ±0.15 for sampling by canopy crane, and 0.92 ±0.10 for sampling by cherry picker. **Method** did not have a significant effect on **RSE** (χ^2 (2) = 3.52, p = 0.1722). The optimum model explaining differences in **RSE** included the fixed effects **Number of stems** (positive correlation), **Sampled leaf area** (negative correlation), and **Forest type** (highest in temperate forests) (χ^2 (6) = 80.75, p < 0.0001; Table S4).

Discussion

We propose a plot-based approach for studying arthropod interaction networks, using three methods for sampling a continuous area of forest canopy. These methods are especially suitable for large-scale sampling as they can be replicated across various forests types. They provide good access to the canopy and similar sampling efficiency. Plot-based standardisation means that frequent associations can be distinguished from those that are casual or rare (Lewis *et al.* 2002; Tylianakis, Tscharntke and Lewis 2007). Furthermore, it provides a robust description of the community structure as one can assume that the interactions are completely censused for the proportion of the canopy successfully sampled (Fig. 3). One can then test and improve the performance of models that predict trophic interactions in real communities by decomposing the effects of abundance, plant characteristics and arthropod community composition (Klimes *et al.* 2012; Segar *et al.* 2017). Food-webs obtained in this way enable us to quantify the effects of habitat type, plant traits or phylogeny in structuring arthropod communities, and to identify density-dependent processes (Whitfeld *et al.* 2012, Redmond *et al* 2019). Derived food-web metrics are comparable on a common area basis, and may identify processes shaping communities of canopy arthropods across various habitats, ecosystems, or geographic areas. For example, such data revealed that host-plant family relationships drive the structure of insect trophic networks in temperate forests of Europe and Japan (Volf *et al.* 2017).

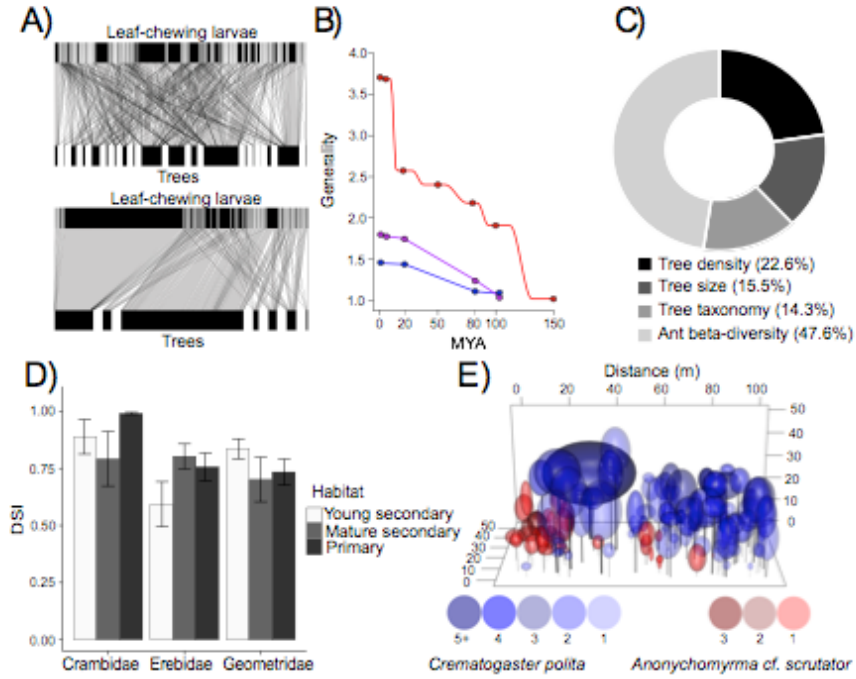

Figure 3. Example results from plot-based sampling. Construction of comparable quantitative food-webs (A: caterpillar-plant food-webs from two 0.1 ha plots with contrasting herbivore and tree diversity; adapted from Volf *et al.* (2017)). These can be used to quantify effects of plant traits or phylogeny on arthropod communities (B: effects of host phylogeny on caterpillar food-webs quantified by change in generality from herbivore data collated according to the time of divergence of their hosts (in Tomakomai (red), Lanzhot (purple), Mikulcice (blue)); adapted from Volf *et al.* (2017)). The relative contribution of such effects can be decomposed, allowing the prediction of arthropod community composition (C: the proportional difference in total ant species richness between primary and secondary forest in Wanang due to the effects of vegetation composition and species turnover; adapted from Klimes *et al.* (2012)). Furthermore, standardized measures of herbivore specialisation can be made, enabling meaningful comparisons across habitats and taxa with variable phylogenetic diversity and plant abundance (D: mean Distance Based Specialisation Index (DSI*) +/- SE for Crambidae, Erebidae, and Geometridae along a successional gradient in Yawan; adapted from (Redmond *et al.* 2019). Finally, we can analyse spatial patterns in canopy arthropod communities (E: distribution of tree canopy nest density in the two most abundant ant species in 0.4 ha of Wanang forest (only trees with nests are shown); based on Klimes and Mottl (*unpublished data*)).

Plot-based analyses represent an ideal counterpart to those based on a stratified selection of focal species sampled with an equal sampling effort. Methods using equal sampling effort are advantageous for studying host specialisation of herbivores (Novotny and Basset 2005). However, modern methods enable

the measurement of host specialisation in plot-based data also (Jorge *et al.* 2014). Furthermore, a plot-based approach can be used to investigate spatial distribution of arthropods across the forest canopy and their impact on competitors and other trophic levels. This is important, for instance, when considering competition among ants where canopy connectivity and structure play important roles in forming ant communities within trees (Klimes *et al.* 2015). Further, herbivores may have density-dependent effects on plant survival that need to be studied in a spatially explicit framework (Bagchi *et al.* 2014).

One limitation of these plot-based sampling methods is that they require relatively large effort and team size. These requirements stem from the necessity to census all parts of the canopy, including those difficult to access, in order to reconstruct truly quantitative interaction networks (Godfray *et al.* 1999; Volf *et al.* 2017). Foliage accessibility positively correlated with the number of stems in the plot, probably because many of the trees in densely vegetated plots were small and easier to access. On the other hand, the number of stems within a plot increased both types of sampling effort quantified. ASE (total effort per a 0.1ha plot) was highest in lowland primary tropical forests characterized by relatively high stem density and large trees difficult to sample. RSE (effort per 1 m^2 of foliage) was highest in temperate forests. This may be because arthropod density is generally higher in temperate forests (Basset *et al.* 1992). In our case, arthropod abundance was especially high during the spring peak of their abundance, which required increasing the team size (but note that overall numbers of leaf-chewing larvae or miners did not have any effect on their own).

High effort per site prevented a rigorous methodological comparison where the same forest is sampled by all three methods. For example, an unbalanced distribution of methods may be one reason why the cherry-picker appeared to provide better access to the canopy than felling or cranes. Similarly to Corff and Marquis (1999) we operated the cherry-picker in almost optimal conditions in temperate forest where plots were close to an access road and the trees could be accessed from a straight trajectory. Operating in less favourable conditions would dramatically decrease foliage accessibility or require employing additional methods. Sampling from cranes also had to be supplemented by other techniques at both our crane sites. While sampling by

other techniques represented a small proportion of sampling effort in the temperate Tomakomai forest, it considerably increased the sampling effort in San Lorenzo tropical rain forest. In San Lorenzo, only 49% of the trees (representing 58% of the foliage sampled) were accessed solely by crane.

Each method also has its own set of biases unrelated to its overall efficiency. For instance, felling generally requires larger teams (Whitfeld *et al.* 2012; Redmond *et al.* 2019) as felled trees need to be sampled immediately. Cranes and cherry-pickers allow proceeding at a slower pace with a smaller team (e.g.Corff and Marquis 1999; Murakami *et al.* 2005). The three methods are also not completely comparable in terms of the sampled arthropod groups. All were suitable for sampling a broad selection of arthropods from endophytic and mobile non-flying taxa. Less mobile flying herbivores, such as aphids or psyllids, were also well represented in our samples, although they were not the focus of our study. Felling was the only method which enabled sampling of nesting ants, which can represent an important proportion of the canopy arthropods (Klimes *et al.* 2015). Quantitative sampling of highly mobile macroscopic arthropods (adult beetles, flies or true bugs) was not possible by these methods, although they were better represented in crane and cherry-picker samples.

We suggest that a global network using the methods described for area-based sampling would provide important insights into the processes of food web assembly and dynamics (Novotny and Miller 2014). We propose a network of permanent plots where the canopy arthropods and their interactions would be censused by non-destructive sampling. The network of permanent plots could benefit from collaboration with the global network of ForestGEO plots (Anderson-Teixeira *et al.* 2015) which generates major insights into forest community ecology. We suggest that 0.1 ha plots sampled from cranes or cherry-pickers are suitable units, which allow for repeated censusing, while keeping the required effort manageable. A census of a single 0.1 ha plot can yield information on more than 10^5 canopy arthropods and their interactions, significantly furthering arthropod ecology research.

This network should be further supplemented by a larger network of temporal plots sampled by felling. Despite a certain revival of canopy crane construction (Nakamura *et al.* 2017), these platforms are missing from vast regions, including Africa and North America. Similarly, opportunities for the use of

cherry-pickers remain limited in many forests. Sampling plots by forest felling thus remains the only widely applicable option in many regions. These plots could be highly replicated and ideally adjacent to the ForestGeo plots. In addition, as large areas of forest are being lost (Curtis *et al.* 2018), sampling canopy arthropods by felling can become a salvage sampling strategy. There has been considerable activity in the past decade to build up the large-scale experiments, such as planting forest stands of a given richness (Grossman *et al.* 2018), or manipulation of landscape fragmentation (Ewers *et al.* 2011). But ecologists have been slow to take advantage of ongoing logging operations, urban development, or shifting agriculture for destructive arthropod and plant sampling. Yet, such data in combination with data from permanent plots would enable the exploration of trends in arthropod networks along major environmental gradients, extrapolating arthropod-plant diversity relationships over large spatial scales, or quantifying temporal changes in arthropod composition in respect to the ongoing global change.

Authors' contributions

MV, CR, PK, GL, CLS, YB, PTB, PD, ML, OK, SEM, PP, MŠ, JŠ, RT, GDW, and VN designed the protocols and general experimental approach; all co-authors lead the field sampling at least at one site or helped with the team management, MV and CR processed data, MV analysed the results and wrote the first draft of the manuscript; all co-authors critically contributed to the project and manuscript writing.

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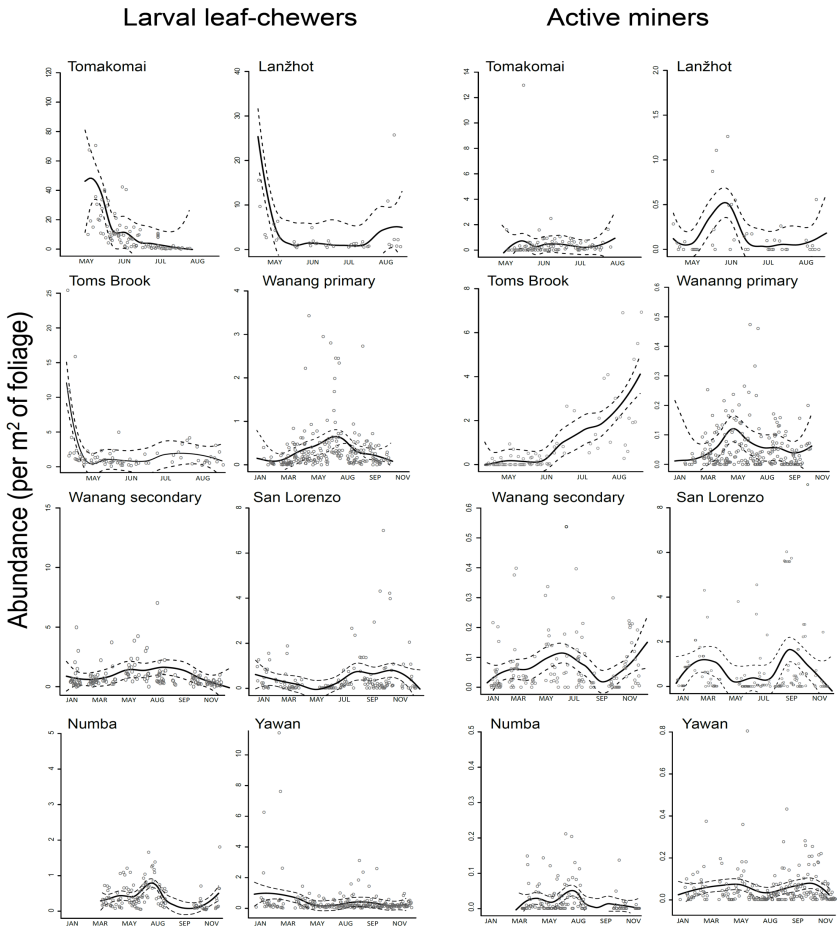

Figure S1. Seasonal trends in abundance of leaf chewing larvae and active miners across the plots sampled for multiple months (Tomakomai, Lanzhot, Toms Brook, San Lorenzo, Wanang, Numba, Yawan). The data points represent number of caterpillars and active miners per 1 m^2 of foliage on individual days of sampling. The seasonal trend was modelled with a loess smoother (solid line). Dashed lines show confidence intervals. The abundance was standardized by leaf area. Data from individual 0.1 ha plots sampled at the listed sites were combined. The data from Wanang primary and secondary forest plots were kept separate to illustrate possible differences between primary and secondary forest. Two outlier data points with leaf-chewer abundance of 226 and 18 are not shown in the case of Tomakomai and Wanang secondary, respectively.

Figure S2. Foliage accessibility (% of foliage possible to access in individual 0.1 ha plots) facilitated by individual methods. Canopy accessibility was correlated to the used method (χ^2 (2) = 6.91, p = 0.0316). The highest accessibility was achieved by the cherry-picker, which operated in optimal conditions of a temperate forest.

Table S1. Site characteristics including latitude, longitude, altitude, average temperature, and annual rainfall.

Site	Latitude	Longitude	Altitude (m asl)	T (°C)	Rainfall (mm)	Reference
Tomakomai (JPN)	42° 43' N	141° 34'E	90	5.6	1,450	[1]
Lanzhot (CZE)	48° 48' N	17° 5'E	152	9.0	525	[2]
Mikulcice (CZE)	48°41' N	16°56'E	164	9.0	525	[2]
Toms Brook (USA)	38°55' N	78°25' W	230	12.7	970	[3]
San Lorenzo (PAN)	9°16' N	79°58' W	130	26.0	3,140	[3]
Wanang (PNG)	5° 14' S	145° 4' E	150	25.8	4,000	[4]
Numba (PNG)	5°44' S	145°16' E	700	22.3	3,000	[5]
Yawan (PNG)	6° 9' S	146° 50' E	1,800	16.2	3,000	[5]

Table S3. Monthly trends in abundance of caterpillars and active miners across the plots sampled for multiple months (Tomakomai, Lanzhot, Toms Brook, San Lorenzo, Wanang, Numba, Yawan). The table shows averages (± standard deviation) of caterpillar and active miner abundance per m^2 of foliage encountered at individual days of sampling within given months.

Caterpillars	Tomakomai	Lanžhot	Toms Brook	Wanang primary	Wanang secondary	San Lorenzo	Numba primary+secondary	Yawan primary+secondary
January				0.3±0.2	0.3±0.2	0.5±0.5		0.9±1.7
February				0.1±0.1	0.5±0.3	0.3±0.1		1.3±3.2
March				0.1±0.2	0.7±0.5	0.3±0.5	0.4±0.2	0.7±1.1
April			8.8±10.1	0.3±0.4	1.1±1.3	0.2±0.2	0.3±0.2	0.4±0.4
May	41.9±51.4	11.4±15.2	1.5±2.6	0.4±0.7	1.5±0.8	0.0±0.0	0.5±0.3	0.3±0.2
June	10.4±9.1	1.1±1.1	1.0±1.1	0.6±0.8	1.6±1.3	0.1±0.1	0.5±0.4	0.2±0.2
July	1.0±0.8	0.7±0.4	2.1±1.1	0.8±0.8	1.2±1.7	0.4±0.6	0.6±0.4	0.3±0.4
August	0.5±0.1	5.3±8.8	1.4±1.2	0.2±0.2	2.1±4.9	0.8±0.7	0.4±0.2	0.4±0.7
September				0.4±0.6	0.5±0.3	0.5±1.1		0.4±0.5
October				0.1±0.1	0.9±1.2	0.9±1.7	0.1±0.2	0.2±0.1
November				0.0±0.0	0.3±0.2	0.3±0.5	0.5±0.5	0.2±0.2
December					0.4±0.4	0.5±0.7		0.3±0.2

Table S3 continued. Monthly trends in abundance of caterpillars and active miners across the plots sampled for multiple months (Tomakomai, Lanzhot, Toms Brook, San Lorenzo, Wanang, Numba, Yawan). The table shows averages (± standard deviation) of caterpillar and active miner abundance per m^2 of foliage encountered at individual days of sampling within given months.

Miners	Tomakomai	Lanžhot	Toms Brook	Wanang primary	Wanang secondary	San Lorenzo	Numba primary+secondary	Yawan primary+secondary
January				0.00±0.00	0.01±0.01	0.52±0.43		0.03±0.04
February				0.01±0.01	0.04±0.03	1.59±0.57		0.05±0.04
March				0.05±0.05	0.09±0.12	1.31±2.83	0.03±0.05	0.10±0.16
April			0.02±0.03	0.05±0.06	0.03±0.03	0.16±0.17	0.02±0.02	0.04±0.06
May	0.77±2.9	0.11±0.25	0.13±0.23	0.14±0.34	0.13±0.10	0.95±1.60	0.02±0.04	0.14±0.19
June	0.33±0.46	0.33±0.41	0.40±0.60	0.09±0.12	0.11±0.15	0.04±0.12	0.03±0.05	0.02±0.02
July	0.32±0.26	0.08±0.09	1.72±1.20	0.05±0.10	0.09±0.10	0.83±1.36	0.04±0.05	0.02±0.03
August	0.93±0.98	0.09±0.19	2.95±2.20	0.06±0.06	0.05±0.07	0.04±0.08	0.00±0.00	0.07±0.09
September				0.02±0.03	0.03±0.07	2.43±2.72		0.04±0.04
October				0.04±0.06	0.04±0.07	0.81±0.93	0.01±0.03	0.09±0.09
November				0.05±0.00	0.11±0.08	0.25±0.33	0.00±0.00	0.05±0.06

Table S4. Variables with a significant effect on **Foliage accessibility**, **Area-based sampling effort,** and **Resource-based sampling effort** as selected by forward selection in linear mixed effect models. The best model explaining differences in **Foliage accessibility** included fixed effects of the forest type, used method, number of stems with DBH≥5cm, and sampled leaf area (m^2) (χ^2 (8) =64.02, p<0.0001). Percentage data on **Foliage accessibility** were arcsine transformed. The best model explaining differences in **Area-based sampling effort** included fixed effects of number of stems with DBH≥5cm, and forest type (χ^2 (5) =95.24, p<0.0001). The best model explaining differences in **Resource-based sampling effort** included fixed effects of number of stems with DBH≥5cm, sampled leaf area, and forest type (χ^2 (6) =80.75, p<0.0001). Effort data were log-transformed. Site was used as random effect.

Foliage accessibility				
Fixed effects	**Estimate**	**Std. Error**	**t value**	**AIC**
Null model				-114.50
Selected model				-162.52
(Intercept)	0.9259	0.0290	31.94	
Forest type				
Tropical highland primary	0.0468	0.0318	1.47	
Tropical highland secondary	0.1167	0.0307	3.80	
Tropical lowland primary	0.0569	0.0323	1.76	
Tropical lowland secondary	0.1257	0.0284	4.44	
Method				
Felling	-0.0201	0.0307	-0.66	
Cherry-picker	0.2112	0.0438	4.82	
Number of stems with DBH≥5cm	0.0008	0.0002	4.59	
Sampled leaf area	0.00003	0.00001	-3.30	
Area-based sampling effort				
Fixed effects	**Estimate**	**Std. Error**	**t value**	**AIC**
Null model				56.13
Selected model				-29.11
(Intercept)	6.788	0.106	63.95	
Number of stems with DBH≥5cm	0.007	0.001	13.11	
Forest type				
Tropical highland primary	-0.294	0.165	-1.78	
Tropical highland secondary	-0.770	0.168	-4.58	
Tropical lowland primary	0.199	0.166	1.20	
Tropical lowland secondary	-0.106	0.171	-0.62	
Resource-based sampling effort				

Fixed effects	Estimate	Std. Error	t value	AIC
Null model				-70.96
Selected model				-139.71
(Intercept)	0.709	0.058	12.32	
Number of stems with DBH≥5cm	0.002	0.000	10.42	
Sampled leaf area	-0.000	0.000	-7.66	
Forest type				
Tropical highland primary	-0.268	0.097	-2.77	
Tropical highland secondary	-0.393	0.096	-4.07	
Tropical lowland primary	-0.058	0.096	-0.60	
Tropical lowland secondary	-0.108	0.096	-1.13	

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Appendix 1. Sampling protocols

1.0 Setting up a 0.1 ha plot

We propose a **standardized protocol for sampling 0.1ha forest plots to quantify interaction networks of canopy arthropods**. The choice of forest area depends on the characteristics of the forest structure and composition meeting all suitable requirements for your project and research questions. Allocate the necessary time to explore and find a suitable forest site. In particular, you should base your decision on the presence of invasive species, topography, and access to the plot (important for the removal of felled trees or a for cherry-picker access). Before you start your project, always inform yourself on all safety instructions applicable to working in the field. These are not included in this protocol. Anyone conducting the sampling is responsible for obtaining the safety instructions elsewhere and following them!

1. Select a plot, which represents a 0.1 ha with a structure and a species composition typical for the local forests. Avoid forest edges, gaps, heavily disturbed areas, sloped terrain, and plantations.
2. Set up the corner points of the plot and take GPS coordinates for reference. Use a measuring tape or a laser range finder to measure the distance between points. Use a compass to measure the angles between the corner points in order to set up the plot in the desired shape. You can use a standard or electronic compass for this. Artillery compasses, specifically designed for taking azimuth angles, are usually a good option.
3. Mark the trees with DBH $\geq$ 5 cm with labels and identify them to species level (the identifications can be improved once the canopy is accessed). Mark only the trees which are rooted in the plot. If the border of the plot goes through tree trunk, include the tree in the plot only if more than 50% of the trunk mass at breast height is within the plot perimeter.
4. Record the position of all trees within the plot. First, select a "ZERO" point within the plot from which you can see all the trees. Clear the understory vegetation to improve the visibility if necessary. You can also use brightly coloured marks (or somebody in bright clothing standing next to the trees) to further increase the visibility of individual trees. Then record the azimuth angle (using a compass) and distance (using a measuring tape or a laser range finder) of individual trees from this point. These can be later easily transformed into x and y coordinates.

5. Optional. If visibility cannot be improved by removing some of the understory vegetation, divide the plot into a grid (Fig. P1). Measure the position of their corner points and all the trees in individual sub-plots as described above. If this method is not possible, you can also take GPS coordinates of individual trees. However, this can be rather inaccurate compared to the previous method depending on the precision of your GPS.

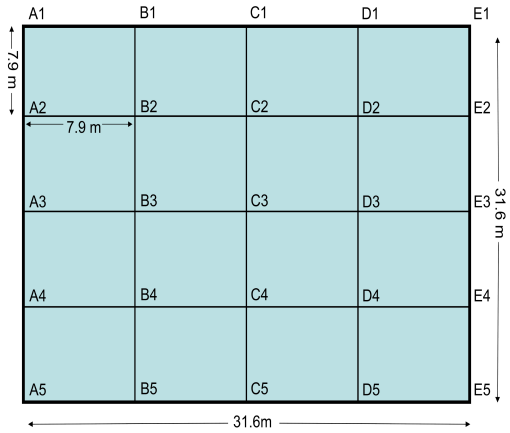

Figure P1. Example of a 0.1ha plot divided into a grid with several reference points (A1-E5). Having such a grid improves accuracy of setting up the plot in densely vegetated sites.

2.0 Arthropod sampling

In temperate (and other seasonal) forests, sampling needs to be spread seasonally within each target tree species to capture the seasonal variability in associated arthropod communities. Create a sampling plan according to the phenology in the focal region (e.g. spread your sampling across both the spring and summer peak of arthropod abundance if such peaks are typical). Avoid sampling all conspecific trees in one part of the season if possible. Spreading sampling across the season may be problematic in the case of singleton tree species. Some methods, such as forest felling, provide limited flexibility for seasonal targeting of singleton tree species as trees cannot be resampled and the data thus represent a single time-point. On the other hand, sampling from cranes or cherry-pickers provides more flexibility. If there are any singleton tree species in your crane or cherry-picker plot, sample half of their canopy during the (spring) peak of arthropod abundance, while the second half can be sampled later in the season.

2.1 Arthropod sampling from felled trees

General notes

First, prepare a sampling plan to establish an ideal sequential order from which trees should be felled. Make sure individual tree species have a similar

proportion of individuals sampled in different parts of the season. Clear the understorey. Start with felling small trees. Once enough small trees are gone and a sufficient space is opened, proceed with the larger trees. Always start with trees that are least likely to fall in a manner which may destroy other trees. This will minimize disturbance to the plot.

Trees should be felled one at a time. It is necessary to finish sampling on the same day as the tree was felled. All arthropods should be sampled as quickly as possible. This will prevent them from escaping or being predated.

Sampling should be done only during the day and when the leaves are not too wet. Avoid sampling in heavy rain, or directly after heavy rain (give the leaves some time to dry). Also avoid sampling during strong wind.

Divide sampling responsibilities within your team. If the size of your team allows, form sorting and sampling teams. Forming a sorting team, which will start pre-sorting samples in the field, will speed-up the final sorting in the lab; 2-3 team members are usually enough for pre-sorting.

There should be always skilled researchers and entomologists present in the field supervising the sampling and sample processing. Other team members should specialize primarily on a single arthropod group (leaf-chewing larvae, miners, or galls etc.) and be trained in the identification of their focal arthropod taxon prior to sampling. These specialized team members then can help other team members with assigning preliminary morphospecies and assist the skilled researcher with final morphotyping (see below).

Sampling steps

1. Select the tree to be felled according to your sampling plan. Measure its DBH (at 1.3 m).

2. Fell the tree

3. Measure its total height, trunk height, and canopy width. Trunk height is measured to the first major branch. Canopy width is measured at the widest point of the canopy. Record this into 'Plant Form'.

4. Record whether the leaves are mature or young (developing). In temperate forests, almost all leaves on a tree will be either mature or young at the time of sampling. In the tropics, this may not be the case so record mature and young leaves separately (see below in *Leaf area estimates*).

5. Sample the focal arthropod groups systematically by a manual search (see details on sampling of individual arthropod groups below). Hand the samples to the sorting team (if there is any) regularly during sampling. This is a much more efficient strategy than passing the samples all at once after the sampling is finished.

6. After the sampling, estimate what percentage of the canopy was sampled for herbivores (since part of the canopy usually gets destroyed during felling and you cannot sample herbivores from it). Record in 'Plant Form'. Estimates

should be done by two trained persons independently and the mean estimated value should be used. This provides more accurate results.

These are the following arthropod groups to be sampled:

Leaf-chewing insect larvae

Search for all free-living and semi-concealed larvae. Check all rolled, tied, or folded leaves. Sample each larva in a separate rearing container. Gregarious larvae can be placed into a single large container, record their quantity. Containers should be available in various sizes suitable for larvae of different sizes. Provide a reasonable amount of leaves based on the size of the larva. The leaves should be of the same age the larva was sampled from (i.e. mature or young). Provide the larva with both young and mature leaves if you are not sure what leaves the larva was feeding on. Do not overfill the container with leaf material and keep it in the shade.

Miners

Sample all active and record all abandoned mines. When sampling mines try to assign them to preliminary morphospecies based on their shape, size, and position on the leaf. Mainly, separate blotch and serpentine mines. Keep your preliminary morphospecies in separate bags. Your preliminary morphotyping will be later corrected by an expert during final processing, but doing preliminary morphotyping and keeping your preliminary morphospecies in separate bags will speed up the final sorting.

Active mines

- Do not sample just the leaf with the mine. Mines will last longer if the leaf is attached to a twig with a couple of other leaves (but make sure that no other mine morphospecies are on the same leaves).
- Put all active mines from one morphospecies in one bag (they will be separated later). If you are not sure whether the mine is active or abandoned, sample it (it can be checked in detail later) and put it among other active mines from the respective morphospecies. Do not overfill the bag with leaf material and keep it in a shade.
- Sample up to ca 100 active mines per morphospecies only (50 will be used for rearing, 10 will be put in ethanol, and the rest will serve as a reserve in case some mines you sampled are inactive).
- The mines exceeding 100 can be simply counted (or their abundance can be estimated if there are many of them; see below). Record the number exceeding 100 into your notebook and report it to the sorting team after sampling. Always confirm with the expert assigning mines to final

morphospecies that these mines are truly from a single morphospecies before you stop sampling them.

Abandoned mines

- Usually, you do not have to sample all abandoned mines. Just count their number or estimate their abundance visually in the event where there are too many of them (see below; but always confirm with the expert assigning mines to final morphospecies that these mines are truly from a single morphospecies). Record their number into your notebook and report it to the sorting team after finishing the sampling.
- Sample abandoned mines only if you do not have any active mine of that morphospecies available or assigning to clear morphospecies is problematic.

Gallers

- Sample all galls on all above-ground plant parts. When sampling galls, try to assign them to preliminary morphospecies. Mainly, focus on the plant part galled and shape of the gall. Your preliminary morphotyping will be later corrected by the expert doing the final processing, but doing preliminary morphotyping and keeping your preliminary morphospecies in separate bags will speed up the final sorting. It can be hard to distinguish arthropod and fungal galls. If unsure, sample all galls. Fungal galls can be identified in the laboratory and later removed from the analysis.
- Sample galled plant parts by detaching from the tree. If the galls are to be reared, and are in low numbers, galls will last longer if the plant part is attached to a twig with a couple of leaves. Otherwise, sample only the galled plant parts, preferably with active (inhabited) galls.
- Put different morphospecies in separate collecting bags. Do not overfill the bags and keep them in the shade.
- Sample enough galled material for each morphospecies to provide healthy quantities for rearing and dissection. What is considered a "healthy quantity" is dependent on the available resources (space, manpower, etc.) for rearing and dissecting, and the size of the galls. The more material reared and dissected, the better the chances of yielding insightful information to aid the species concept. Therefore, it would be ideal to rear at least 10 galled parts and retain at least 10 galls for dissection, per morphotype.

- Unsampled galls can be counted (or their abundance estimated if there are many of them; but always confirm with the expert assigning galls to final morphospecies that these galls are truly from a single morphospecies). Record the unsampled number into your notebook and report it to the sorting team after sampling.

Abundance estimates for very abundant mines and galls

Some abandoned leaf mines or gall morphospecies can be very abundant, which means counting them may take an excessive time investment. Instead of counting them individually, you can estimate their abundance in such cases. Mine and gall density can sometimes largely differ among various parts of the canopy. It is thus necessary to do the estimates repeatedly in various parts of the canopy.

- Select a reasonably large branch (ca 100-500 leaves) and count number of leaves and number of mines or galls on this branch. Divide their number by the number of leaves to calculate mine or gall per leaf average for this branch. Repeat this procedure at various parts of the canopy (at least three in the case of smaller trees and at least five in the case of larger trees). Use the averages to calculate a mean mine or gall density per individual leaf. Record this value. This can be used for estimating total mine or gall density once the total number of leaves is calculated.
- Some mite galls can be highly abundant (hundreds of galls per leaf). In such a case, pick only 20 leaves in random and calculate gall/leaf average. Repeat this procedure at various parts of the canopy (at least three in the case of smaller trees and at least five in the case of larger trees). Use the averages to calculate final mean gall density per individual leaf. Record this value. This can be used for estimating total gall density once the total number of leaves is calculated. Use this approach scarcely and only when really needed; e.g. in cases when more than 50% of leaves are galled.
- It is always better if the estimates are done by two specially trained persons using the mean estimate as a final value as it may provide more accurate results.

Spiders

Sample spiders into a vial with ethanol. All spiders from one tree can go into one vial but do not overfill it. Divide the spiders into more vials as needed to ensure a good proportion between ethanol and the sampled individuals.

Ants

Three people should be collecting ants (1 ant-trained staff member supervising 2 assistants) in tropical areas. In the temperate zone, where vegetation is less complex, two persons are enough. Sampling of foragers is done first immediately after felling. This helps to avoid contamination by ants invading the felled tree from the ground. After sampling for foragers is complete, collection continues with a search for individual nests.
Starting from the base of the tree (trunk) towards its crown, search carefully for any ants present on the fallen tree, especially those:

- foraging on the tree
- nesting on the leaves (silk or carton nest, weaved leaf nests etc.)
- living on and inside of the branches or twigs
- in the tree cavities
- under the bark
- under the lianas attached to the tree
- in the epiphytes on the tree, especially in the soil around their roots
- in any other suitable place where ants can occur

- We record several extra pieces of information for ants (such as their position on the tree, nest type etc.). This information should be recorded immediately after sampling, and recorded on both the labels and the 'Ant protocol' (see the example below). Do not wait till final processing to record this information.
- For all foragers, record their position on the tree – T (trunk below the branches) or C (crown – branches). All foraging ants (without a known nest) from one tree and similar height (T vs. C) can go together in one vial – this vial can contain a mix of different species If there is more than one vial with ants, mark each collection with a number: 1, 2, 3…
- For all nests, record their position (crown vs. trunk plus the vertical height above ground in meters), nest site type, and nest dimensions. Estimate the number of ant individuals in the nest. Record this information immediately after finding the nest. The examples of nest site types are listed below.
- Take vouchers of ant nests for photography (see *Sample processing and insect rearing*).
- Smaller colonies should be collected whole – including eggs, larvae and pupas and allates. Information as to whether the colony was collected as a whole is marked in the protocol and on labels.

- If the colony is too big (thousands of individuals), collect just part of it (20-50 individuals typically). Always try to sample all castes you can find as well as immature stages. Vials should be filled no more than halfway (1/2) with insects, the upper half should contain only ethanol to permit later molecular analysis (e.g. species barcoding). Use 2 ml vials for small samples. Use larger (e.g. 8 ml) vials for large bodied ants or larger colony samples.
- Ants from one colony (nest) should always be collected into one vial. They can be split in two, if there are too many ants for one vial – especially for big ants. In this case, each vial has to get its own label but with duplicated information. Don't mix ants from different colonies.
- Record if the host trees, or the ant-associated epiphytes, are myrmecophytes. Note if the plant contained ant domatia or nectaries (see an example of 'Ant protocol' below). Assigning plants as myrmecophytes or non-myrmecophytes can be difficult in tropical regions with poorly known flora and ant associations. Therefore, it is always crucial to record all the additional information as described above. The information on the location of the nest in dead or living tissue and trunk or branches can be especially helpful.

2.2 Arthropod sampling from cranes and cherry-pickers

General notes

First prepare a sampling plan, outlining the order in which the trees should be sampled. The primary aim here should be to account for seasonality. If the herbivore composition changes with the seasonal, ensure that you distribute sampling of conspecific tree individuals across the season. Avoid sampling all conspecific tree individuals in one part of season. If there are singleton tree species in your plot, sample 50% of their canopy in early season and the other 50% in later season.

Sampling should be done only during the day and when the leaves are not very wet. Avoid sampling in heavy rain, or directly after heavy rain (give the leaves some time to dry). Also avoid sampling during windy weather.

Divide sampling responsibilities within your team. If the size of your team allows, form sorting and sampling teams. Forming a sorting team, which will start pre-sorting samples in the field, will speed-up the final sorting in the lab; 2-3 team members are usually enough for the pre-sorting. Ideally, there should be a skilled researcher present in both teams.

Sampling steps

1. Follow your sampling plan to select the tree to be sampled.
2. Measure the tree. First, measure the DBH (at 1.3 m). Then measure total height, trunk height, canopy width using a laser range finder. Trunk height is measured to the first major branch. Canopy width is measured at the widest point of the canopy. Record these values in 'Plant Form'.
3. Record whether the leaves are mature or young (developing). In temperate forest, almost all leaves on a tree will be either mature or young at the time of sampling. In the tropics, this may not be the case so record mature and young leaves separately (see *Leaf area estimates* for more details).
4. Sample the focal arthropod groups. First, use a beating net to obtain free living arthropods. Second, do a manual search to obtain remaining caterpillars, ants and spiders and also herbivores concealed in rolled or tied leaves, galls and mines. Hand the insect samples to the ground team during the sampling regularly. This is much more efficient strategy than passing the samples all at once after finishing sampling.
5. After the sampling, estimate what percentage of the canopy was sampled for herbivores. Record it into the 'Plant Form'. This should be done by the canopy team. Estimates should be done by two trained persons independently and the mean estimated value should be used. This provides more accurate results.
6. Record the number of leaves inspected for herbivores (see the instructions below in *Leaf area estimates*). Canopy team should report this value to the ground team immediately after sampling.

Sampling low accessibility parts of the canopy

Some parts of the canopy (usually understory trees or lower branches of large trees) can be inaccessible from cranes or cherry-pickers. In such cases, you can use sampling from the ground, from ladders, or by climbing. If climbing is necessary, it usually requires forming a specialized climbing team consisting of 1-2 specially trained team members.

- Trees with height of 2-3 m can usually be sampled directly from the ground. Be careful not to break any branches or the trunk. Rather than bending such a tree by a brutal force, use a ladder.
- We used "A" shaped step ladders for sampling up to 3-5 m above ground (depending on the type, its stability, and terrain). In the case of large trees with sufficient trunk diameter, extension ladders fixed to the trunk can be also used for reaching similar heights. Always make sure the ladder is stable. During our sampling, the person on the ladder was always assisted by at least one person on the ground. We avoided using this type of ladder on sloped terrain.
- For sampling at greater heights or on sloped terrain, modular ladder poles are more efficient and stable. We used ladder poles for sampling at up to 8 m above ground. But note that this may differ depending on the type you use and its maximum load. The ladder poles should be ideally equipped with a steel fork at the basis that ensures good stability of the pole in the ground. We secured the ladder pole to the trunk of the tree with harnesses to prevent it from slipping. The person on the ladder was always assisted by at least one person on the ground.
- Trees even higher above ground, which are inaccessible from cranes or cherry-pickers, can be sampled by climbing. Descending from the gondola can ensure that even the terminal branches can be reached. But this method is usually time consuming. Also, it can only be carried out by a skilled person with proper training.
- Untrained or inexperienced team members should never sample from ladders or climb the trees.
- Always read and carefully follow safety instructions which may apply to working in the field, to working at heights, to working from ladders, or to climbing. This protocol cannot be used as a source of such information. You must obtain all the safety regulations from elsewhere and follow them!

These are the following arthropod groups to be sampled:

Leaf-chewing insect larvae

Collect all leaf-chewing larvae from the beating net. Then search for all free-living and semi-concealed larvae. Check all rolled, tied, or folded leaves. Sample each larva in a separate rearing container. Gregarious larvae can be sampled into a single large container, record their quantity. Containers should be available in various sizes suitable for larvae of different sizes. Provide a reasonable amount of leaves based on the size of the larva. The leaves provided should be of the same age as those the larva was sampled from (i.e. mature or young). Provide the larva with both young and mature leaves if you are not sure what leaves the larva was feeding on. Do not overfill the container with leaf material and keep it in a shade.

Miners

Sample all active and record all abandoned mines. When sampling mines try to assign them to preliminary morphospecies based on their shape, size, and position on the leaf. Specifically, separate blotch and serpentine mines. Keep your preliminary morphospecies in separate bags. Your preliminary morphotyping will be later corrected by an expert during final processing, but doing preliminary morphotyping and keeping your preliminary morphospecies in separate bags will speed up the final sorting.

Active mines:

- Do not sample just the leaf with the mine. Mines will last longer if the leaf is attached to a twig with a couple of other leaves (but make sure that no other mine morphospecies are on the same leaves).
- Put all active mines from one morphospecies in one bag (they will be separated later). If you are not sure whether the mine is active or abandoned, sample it (it can be checked in detail later) and put it among other active mines from the respective morphospecies. Do not overfill the bags with leaf material and keep them in a shade.
- Sample up to ca 100 of active mines per morphospecies only (50 will be used for rearing, 10 will be put in ethanol, and the rest will serve as a reserve in case some mines you had sampled are inactive).
- The mines exceeding 100 can be simply counted (or their abundance can be estimated if there are many of them). Record the number exceeding 100 into your notebook and report it to the sorting team after sampling. Always confirm with the expert assigning mines to final morphospecies that these mines are truly from a single morphospecies before you stop sampling them.

Abandoned mines:

- Usually, you do not have to sample all abandoned mines. Just count their number or estimate their abundance visually in the event where there are too many of them (see below; but always confirm with the expert assigning mines to final morphospecies that these mines are truly from a single morphospecies). Record their number into your notebook and report it to the sorting team after finishing the sampling.
- Sample abandoned mines only if you do not have any active mine of that morphospecies available or assigning to clear morphospecies is problematic.

Gallers

- Sample all galls on all above-ground plant parts. When sampling galls, try to assign them to preliminary morphospecies. Specifically, focus on the plant part galled and shape of the gall. Your preliminary morphotyping will be later corrected by an expert during the final processing, but doing preliminary morphotyping and keeping your preliminary morphospecies in separate bags will speed up the final sorting. It can be hard to distinguish arthropod and fungal galls. If unsure, sample all galls. Fungal galls can be identified in the laboratory and later removed from the analysis.
- Sample galled plant parts by detaching from the tree. If the galls are to be reared, and are in low numbers, galls will last longer if the plant part is attached to a twig with a couple of leaves. Otherwise, sample only the galled plant parts, preferably with active (inhabited) galls.
- Put different morphospecies in separate collecting bags. Do not overfill the bags and keep them in the shade.
- Sample enough galled material for each morphospecies to provide healthy quantities for rearing and dissection. What is considered a "healthy quantity" is dependent on the available resources (space, manpower, etc.) for rearing and dissecting, and the size of the galls. The more material reared and dissected, the better the chances of yielding insightful information to aid the species concept. Therefore, it would be ideal to rear at least 10 galled parts and retain at least 10 galls for dissection, per morphotype.
- Unsampled galls can be counted (or their abundance estimated if there are many of them; but always confirm with the expert assigning galls to final

morphospecies that these galls are truly from a single morphospecies). Record the unsampled number into your notebook and report it to the sorting team after sampling.

Abundance estimates for very abundant mines and galls

Some abandoned leaf mines or gall morphospecies can be very abundant, which means counting them may take an excessive time investment. Instead of counting them individually, you can estimate their abundance in such cases. Mine and gall density can sometimes largely differ among various parts of the canopy. It is thus necessary to do the estimates repeatedly in various parts of the canopy.

- Select a reasonably large branch (ca 100-500 leaves) and count number of leaves and number of mines or galls on this branch. Divide their number by the number of leaves to calculate mine or gall per leaf average for this branch. Repeat this procedure at various parts of the canopy (at least three in the case of smaller trees and at least five in the case of larger trees). Use the averages to calculate a mean mine or gall density per individual leaf. Record this value. This can be used for estimating total mine or gall density once the total number of leaves is calculated.
- Some mite galls can be highly abundant (hundreds of galls per leaf). In such a case, pick only 20 leaves in random and calculate gall/leaf average. Repeat this procedure at various parts of the canopy (at least three in the case of smaller trees and at least five in the case of larger trees). Use the averages to calculate final mean gall density per individual leaf. Record this value. This can be used for estimating total gall density once the total number of leaves is calculated. Use this approach scarcely and only when really needed; e.g. in cases when more than 50% of leaves are galled.
- It is always better if the estimates are done by two specially trained persons using the mean estimate as a final value as it may provide more accurate results.

Spiders

Sample spiders into a vial with ethanol. All spiders from one tree can go into one vial but do not overfill it. Divide the spiders into more vials as needed to ensure a good proportion between ethanol and the sampled individuals.

Ants

Sample ants foraging on the foliage and canopy branches into a vial with ethanol. All foraging ants from one tree can go into one vial but do not overfill it. Divide the ants into more vials in such a case to ensure a good proportion between ethanol and the sample. Note that while the sampling from a crane or a cherry picker allows to do a rapid assessment of ant foragers in the canopy, it is not comparable to the ant census using felling. In the case of felling, both whole trunk and canopy, as well as individual nests outside and inside the host tree tissues and the associated epiphytes and lianas can be sampled, measured, and distinguished from foragers (see 2.1).

3.0 Leaf area estimates and plant vouchers

Sample leaves for leaf area estimates as specified below. We estimate leaf area of mature and young leaves separately as they can harbour different herbivores. We define mature leaves as fully developed in terms of their size and thickness. Young leaves are still developing. We define young leaves as leaves which haven't reached their full size or are much softer than mature leaves. Usually, they are also more lightly coloured than mature leaves.

In addition to the leaf area estimates, use this step to obtain herbarium vouchers, which will help with confirming host-plant identification, or to measure herbivory damage. Follow standard protocols for sampling plant vouchers (e.g. Funk *et al.* 2017). Sampling plant vouchers is especially useful in areas with high tree diversity. To avoid wilting, sample vouchers in plastic bags and mark them with tags. A voucher should include a stem bearing multiple leaves and an apical bud. Always sample flowers or fruits if present.

Note: Although not discussed in this study, the sampled leaves can also be used for measuring leaf physical traits and nutrient content that can be relevant for structuring insect-plant interaction networks. Sampling leaves for measuring secondary metabolites usually requires special protocols and a separate sampling campaign. For example, the samples need to be cooled or frozen immediately after the sampling to avoid degradation and oxidation.

Obtain at least three vouchers from around a canopy of each tree sampled. Press and dry the vouchers on the same day they were collected. The vouchers can be later used for DNA isolation and DNA barcoding to provide additional information on species identification.

3.1 Leaf area estimates for felled trees

1. Sample foliage for biomass estimates.

i) After you have sampled the tree for arthropods, place all foliage from the canopy into bags and weigh it. For large trees (ca DBH>30 cm), you can sample 25% or 50% of the foliage and extrapolate the results if your team is small in order to speed up the process. Record the weight into the 'Plant Form'. Sample and weigh mature and young leaves separately if both young and mature leaves are present. These values will be used for separate estimates of young and mature leaf area.

ii) Avoid sampling leaves for biomass estimates when the foliage is wet and only sample leaves which have no other plants attached.

2. Sample leaves for calculating leaf area.

i) This includes obtaining individual leaves from across the canopy. A good method is to use the leaves sampled for the biomass estimate for this. Mix the leaves sampled for the biomass estimate in a bag and randomly pick some of them for calculating leaf area. Only use leaves which were not mechanically damaged during the sampling (but include those damaged by herbivores, pathogens, etc.).

ii) For small trees (ca. DBH < 15 cm), pick enough leaves (depending on their size) to fill a 50x50 cm white frame. For larger trees or trees with large leaves, pick enough leaves to fill two frames (this is to cover the variability in leaf sizes and shapes across the canopy of such trees). Sample young and mature leaves separately if there are both mature and young leaves present.

3. Take a photo of the leaves for the leaf area estimate.

i) Place the leaves for calculating leaf area into a 50x50 cm white frame. Use as many leaves as possible but make sure they do not overlap or cross the frame border line.

ii) Leaves should be flat. Use some dark heavy objects (e.g. stones or coins) to flatten the leaves if necessary (but do not cover herbivory damage).

iii) Place a paper label with the tree number, the frame number (in case you take photos of more than one frame), and the leaf stage next to the frame so it is visible in the photo.

iv) Position the camera on a tripod right above the frame so that the frame appears on the camera display as a square.

v) Avoid strong light and shade contrasts during the photographing. Try to carry out this task with same camera settings to keep light levels consistent throughout the project.
vi) Once you take the photo, weigh the leaves. Record their total weight and their total number into the 'Plant Form'.
vii) If present, repeat this procedure for young and mature leaves separately.

viii) The resulting photos will be processed in ImageJ, Photoshop or other suitable software. In summary, the measurement is based on counting the number of pixels occupied by leaves vs. the number of pixels occupied by the background within a known area (here 2500 cm^2)). Missing leaf area or the area damaged by galls and mines can also be quantified using a similar approach in order to measure herbivory damage. Do not forget to correct for lens distortion, if needed. This can be especially important if you use a wide-angle lens. See existing protocols for details on leaf processing (e.g. Bito *et al.* 2011). The total sampled leaf area will be calculated using the total leaf biomass and the area to weight ratio from the photographed sample.

3.2 Leaf area estimates for trees sampled from cranes and cherry-pickers

1. Estimate number of leaves on the tree.
i) Leaf number estimates must be done during the arthropod sampling.
ii) After you have sampled a part of the canopy for arthropods, select a reasonably large branch (with ca 500 leaves) within it and count how many leaves there are exactly (= value "A").
iii) Count how many branches of that size there are in the part of the canopy you have just sampled (= value "B"). Do this regularly. Avoid doing this across large parts of the canopy ("B" should be 5- 10, optimally).
iv) Multiply "A" with "B". Record this into your notebook as a local number of leaves ("C").
v) Repeat this procedure for each part of the canopy you sample**.**
vi) Once you finish sampling, count the sum of "C" values and report it to the ground team who will record it into 'Plant Form' as the total number of sampled leaves.
vii) Visually estimate what percentage of leaves is young and what percentage is mature if both young and mature leaves are present.
2. Sample leaves for calculating leaf area.
i) Drive the gondola all around the canopy and sample leaves in random and bring them to the ground.
ii) In the case of small trees (ca. DBH < 15 cm), pick enough leaves (depending on their size) to fill a 50x50 cm white frame. In the case of larger trees or trees with large leaves, sample enough leaves to fill two frames (this is to cover

variability in leaf sizes and shapes across canopy of such trees). Sample young and mature leaves separately if there are both mature and young leaves present.
3. Take a photo of the leaves for the leaf area estimate.
i) Place the leaves for calculating leaf area into a 50x50 cm white frame. Use as many leaves as possible but make sure they do not overlap or cross the frame border line.
ii) Leaves should be flat. Use some dark heavy objects (e.g. stones) to flatten the leaves if necessary (but do not cover herbivory damage).
iii) Place a paper label with the tree number, the frame number (in case you take photos of more than one frame), and the leaf stage next to the frame so it would be visible on the photo.
iv) Position the camera on a tripod right above the frame so that the frame appears on the camera display as a square.
v) Avoid strong light and shade contrasts during the photographing. Try to carry out this task with same camera settings to keep light levels consistent throughout the project.
vi) Once you take the photo, weigh the leaves. Record their total weight and their total number into the 'Plant Form'.
vii) If present, repeat this procedure for young and mature leaves separately.
viii) The resulting photos will be processed in ImageJ, Photoshop or other suitable software. In summary, the measurement is based on counting the number of pixels occupied by leaves vs. the number of pixels occupied by the background within a known area (here 2500 cm^2)). Missing leaf area of the area damaged by galls and mines can be also quantified using a similar approach to measure herbivory damage. Do not forget to correct for lens distortion, if needed. This can be especially important if you use a wide-angle lens. See existing protocols for details on leaf processing (e.g. Bito *et al.* 2011). The total sampled leaf area will be calculated using the estimated total number of leaves on the tree multiplied by the mean leaf size of the photographed sample.

4.0 Sample processing and insect rearing

There can be a dedicated sorting team in the field. Typically it may consist of 2-3 team members. If all team members are occupied by arthropod sampling, sample processing should be done immediately after returning from the field. The sorting team's main responsibilities are recording information into spread-sheets, sample sorting, labelling, and photographing of morphospecies and leaves.
The sorting team should include team members skilled and trained in morphotyping arthropods. The initial morphotyping is done *de novo* within each individual tree. The morphospecies will be cross-referenced across all individual trees once the sampling is finished. This reduces the amount of error

compared to using a system of creating morphospecies across all trees within the plot or even multiple plots. Make sure that all arthropod individuals from a given group are always morphotyped by the same person when sorting arthropods from a single tree. Minimize the number of persons involved in the morphotyping. Give this task only to the team members with a proper training. This will increase the consistency in morphotyping and lower the amount of errors.

General notes

1. Record all information about the host-plant into the 'Plant Form'.
2. Label and sort all arthropod specimens. When taking arthropod vouchers, follow available standard protocols (e.g. Millar, Uys & Urban 2000; Schauff 2001).

Leaf-chewing insect larvae

- Morphotype leaf-chewing larvae based on their morphology (e.g. size, coloration, descriptions of hairs/ spines etc.). Record morphological characteristics of each morphospecies in your notebook. It will help you to morphotype further larvae.
- A maximum of up to 50 larvae per morphospecies should be kept for rearing. Each larva is to be kept separately in a rearing container with the exception of gregarious larvae. Keep gregarious larvae from one nest together in one large zip-lock bag or container. Record the number of gregarious larvae on the label in this event.
- If there are more than 50 larvae per given morphotype (this happens rarely):
  - i) Larvae 51-75 should be preserved in ethanol. Each larva should be kept in a separate vial and labelled with a standard label.
  - ii) Larvae 76-x can be discarded. Fill the number of discarded larvae into the 'Plant Form'.
- Label each morphospecies or larva preserved in a vial (use only one label per nest of gregarious larvae). Record the following information on the label:
  - i) Unique Identifier (it can be pre-printed)
  - ii) Locality
  - iii) Tree ID number (unique number for each tree in the plot)
  - iv) Morphospecies
  - v) Body length (in mm)
  - vi) Feeding on the host (yes/no) – to be confirmed later in the laboratory

vii) Leaf age (record whether the larva was found on mature or young leaves)
viii) Mode of feeding (chewing, rolling, tying, skeletizing)
ix) Parasitized (yes/no) – to be filled in later based on the result of the rearing
x) Reared to adult (yes/no) – to be filled in later based on the result of the rearing
xi) Preserved in ethanol (yes/no) – to be filled in later based on the result of the rearing

- Photograph at least one larva per morphospecies. First, take a photo of the larva in detail. All important morphological characteristics (number of prolegs, setae, dorsal and lateral lines, head capsule etc.) should be visible. Take pictures from both the dorsal and lateral view. Afterwards, take a photo of the same larva together with its label including all information.

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Mines

- Morphotype mines based on their morphology. Record morphological characteristics of each morphospecies in your notebook. (Specifically, record whether it is a blotch or a serpentine mine, on what side of the leaf is it visible, and colour of the frass if there is any). It will help you with morphotyping future mines.
- Separate inactive mines and count them. Add this number to the number of inactive mines of the respective morphospecies reported by the sampling team and record their number into the 'Plant Form'. If you have only abandoned mines for some morphospecies, keep a mine of that morphospecies for labelling and photographing.
- Up to 50 active mines per morphospecies should be reared in zip-lock bags.
- Up to 10 other mines of the same morphospecies should be dissected. If there are less than 60 active mines in total, dissect every second mine out of first ten mines and every fifth mine of the rest. Put the dissected larvae (or any other larger remain, e.g. head capsules) in a vial with ethanol and a standard miner label.
- If there are more than 60 active mines, discard them. Add the number of mines you discarded to the number of active mines counted (but not sampled) by the sampling team (Sampling team should report this number to you). Record this number in the 'Plant Form'.
- Mines will last longer if the leaf is attached to a branch with a couple of other leaves. Do not separate them if you plan to rear them.

- Each mine is to be reared in a separate zip-lock bag. However, if there are several miners per one leaf, do not separate them. You may keep them in one zip-lock bag but put a corresponding number of labels inside.
- Label each morphospecies or larva preserved in a vial. Record following information on the label:
  i) Unique Identifier (it can be pre-printed)
  ii) Locality
  iii) Tree ID number (unique number for each tree in the plot)
  iv) Morphospecies
  v) Leaf type (record whether the mine was found on mature or young leaves)
  vi) Active/abandoned
  vii) Parasitized (yes/no) – to be filled in later based on the result of the rearing
  viii) Reared to adult (yes/no) – to be filled in later based on the result of the rearing
  ix) Preserved in ethanol (yes/no) – to be filled in later based on the result of the rearing

- Take a photo of one mine per morphospecies. First, take a photo of the dorsal side in detail. Second, take a photo of the ventral side of the leaf in detail. Third, take a photo of the same mine together with its label with all information filled in and visible.

Galls

- Morphotype galls based on their morphology (mainly, record the type of the gall according to literature (e.g. Yukawa 1996; Redfern & Shirley 2002), on what side of the leaf is it visible, and its colour).
- Use the available literature and reference collections to identify fungal galls. Dissecting and examining under a microscope can be necessary for identification of fungal galls. Once you are absolutely sure about the identification, remove the fungal galls from further processing. However, if still unsure, process all galls with uncertain status. Make sure you take vouchers of such galls for further identifications by specialists.
- If galls of a morphospecies are low in number (e.g. < 15), prioritise putting them in ethanol for dissection rather than rearing.
- Select plant parts with the best looking galls (i.e. fresh, mature, no exit holes) for each morphospecies and rear them in one or more large zip-lock bags. All rearings of one morphospecies can be given the same label. Do not rear mite galls.

- Select, preferably, 10-30 individual galls per morphospecies, remove excess plant tissue, and place in ethanol for future dissection. Don't forget to add a vial label.
- Record the following information for each gall morphospecies in a separate sheet:
  i) Locality
  ii) Tree ID number (unique number for each tree in the plot)
  iii) Date
  iv) Gall morphospecies code
  v) Morphospecies description or a diagram
  vi) Plant part which was galled
  vii) Number of plant parts galled and the average number of galls per plant part. (This can be made exact if all individual galls are counted). This should also include the number of galled parts left on the tree (the sampling team should tell you if there were any). Alternatively, record the average number of galls per plant part. The number of plant parts galled can be estimated as % cover of plant parts galled (this approach is used for very abundant galls, and where the total number of tree parts will be known).
  viii) Number of galled plant parts (or individual galls) used for rearing.
- Label each morphospecies or larva preserved in a vial. Record the following information on the label:
  i) Locality
  ii) Tree ID number (unique number for each tree in the plot)
  iii) Date
  iv) Gall morphospecies code
- Take a photo of one gall per morphospecies. First, take a photo of the dorsal side in detail. Second, take a photo of the ventral side in detail. Third, take a photo of the same gall together with its label with all information filled in and visible.

Spiders

All spiders from one tree can go into one vial. Divide the spiders into more vials in the event of high spider abundance, this will ensure there is a good proportion of ethanol. Label each vial with a spider label including:

i) Locality
ii) Tree ID number (unique number for each tree in the plot)
iii) Date

When sampling from felled trees, the information on foraging ants should be directly recorded during the sampling by the person responsible (see above). In the case of sampling from cranes and cherry-pickers, the information can be recorded once the sampling of the respective tree is finished. All vials with foraging ants should be labelled with an ant label including:

Foraging ants:

i) Locality
ii) Tree ID number (unique number for each tree in the plot)
iii) Date
iv) Trunk/Canopy (record whether the ants were foraging on the trunk or in the canopy).
v) Vial number (in case there are multiple vials with foraging ants from the respective tree)

Ant nests:

Ant nests are sampled only when sampling from felled trees. We record several extra pieces of information for ant nests (such as position on the tree, nest type etc.). This information should be recorded by the responsible person directly during the sampling in 'Ant protocol' and ant labels. Once the sampling of the respective tree is finished, check whether the following information was recorded for all ant nests:

i) Locality
ii) Tree ID number (unique number for each tree in the plot)
iii) Date
iv) Position on the tree (vertical height in m from the ground)
v) Type (description of nest site, see above)
vi) Dimensions of a nest (width times height in cm, where possible to measure)
vii) Number of individuals in the nest (assessment using categorical scale of number of workers, see example of the ant protocol)
viii) Vial number (in case there are multiple nests collected from the respective tree, each nest should have its own vial)

After the tree is searched and all samples collected, make sure that all the vials have the proper information written on their labels, and that all information is also described in the ant protocol for each tree (and that both the protocol, and labels match). Make sure all vials are full of ethanol. Check that vials are well closed/not leaking!

Take a photo of each different nest type for the common ant species, or their association with plant/symbiont species (see below). It is not necessary to take photos of all nests, but all common cases should be documented at least 3 times. The photograph should include the nest label (tree number + vial number), the voucher itself, and a scaler in cm.

Optional additions to the ant protocol:

Although not discussed in this study, the protocol for sampling ant nests can be also used for sampling other arthropods. Apart from ants, this protocol can be used for sampling termites, and the ant/termite associated trophobionts and symbionts (aphids, scale insects, beetles, bugs etc.). If the ant protocol is extended in this way, the same procedure is followed. In this case, mark if the sample contains ants, termites, or symbionts in the protocol (see example of the protocol and "*Ant, Ter, Sym"* mark for each vial number, and examples of the labels). A small sample of ant individuals (1-5 workers) should be always collected with the symbionts to confirm host associations.

Insect rearing

All sampled larval insect herbivores should be reared to adults or parasitoids. Always protect rearing containers and bags from direct sunlight. Appropriate temperature and humidity are key factors affecting the rearing success. Always keep your rearing containers clean. Check them frequently and remove any frass or other waste to prevent growth of fungi. When taking vouchers of the reared arthropods, follow standard protocols (e.g. Millar *et al.* 2000; Schauff 2001), unless otherwise specified (see below).

Leaf-chewers

- Leaf-chewers should be reared in either plastic containers or zip-lock bags for large nests of gregarious larvae. Write the most important information (host tree individual, morphotype number) on the container. This will serve as a back-up source of the most important information if the label gets mouldy or eaten by the larva.
- Inspect the containers every day.
- Provide larvae with fresh leaves and clean the boxes if necessary. This is usually needed every second day at least.
- Put some tissue paper into the bags or containers to absorb condensed water if needed.
- Record whether the larva feeds on mature or young leaves (mark it in the label). Record the mode of feeding if it hasn't been recorded already.
- Once the larvae pupate, clean the container. Remove any remaining old leaves, unless the pupa is directly attached to them. If this occurs, remove as much of the leaf tissue as possible without damaging the pupa. This will reduce the risk of fungal infection. Put some paper tissue or toilet paper inside the containers. This can either be used to absorb extra moisture (if you rear the pupae in a humid environment) or can be moistened if you rear the pupae in an environment with low air humidity. Separate the pupated individuals from the active larvae and check the container every day.

- Record if the larva died or was reared to an adult or a parasitoid. If it died, mark whether it was preserved in a vial with ethanol or not.
- Kill and mount every reared Lepidoptera adult. Killing by freezing will assure the best quality of DNA for barcoding. Abundant species with a known identification can be just pinned. Store adults in a dryer overnight. Place them in storage boxes once they are dry.
- Store reared parasitoids in ethanol. Label them with all of the host information as well as a unique parasitoid code.
- Note that many temperate insect species overwinter as pupae and you won't be able to rear their larvae into adults within a single season. Plan your project accordingly.

Mines and galls

- Mines and galls are reared in plastic bags. Inspect the bags every day.
- Put some paper tissue or toilet paper into the bags to absorb condensed water.
- Record if the larva died or was reared to an adult or a parasitoid. If it died, mark whether it was preserved in a vial with ethanol or not.
- Kill and immediately mount every reared Lepidoptera adult. Store adults in a dryer overnight. Place them in storage boxes once they are dry. Mining and galling Microlepidoptera may die relatively quickly after emerging. It is thus essential to check for emerging adults regularly, ideally twice a day.
- Once dead, Microlepidoptera adults dry quickly due to their small size and are hard to relax for mounting. Therefore, if they die spontaneously in the rearing bag or container they are very difficult to mount. Store such individuals dried and fixed in Eppendorf tubes (but try to avoid such a situation in general!).
- Importantly, mounting mining and galling Microlepidoptera adults requires training. Study and follow standard protocols on Microlepidoptera mounting (e.g. Landry & Landry 1994).
- Adult Hymenoptera, Diptera, and Coeloptera should be preserved in vials with ethanol.
- Store reared parasitoids in ethanol. Do not forget to add a label with all information on the original herbivore larva.
- Mines and galls which do not emerge in 30 days can usually be discarded in tropical areas. If you are working in temperate regions, inform yourself

if there are any ovewintering species associated with your focal host plants. Such species should be kept over winter. In addition, dissect a representative number of mines and galls per morhospecies before discarding. If there are any macroscopic remains of the larvae (e.g. head capsulas), preserve them in a vial and ethanol with a standard label.

Rearing rare mine or gall morphospecies

In the case of rare morphospecies of galls and mines, which were sampled as a single leaf (without sufficient other plant parts attached) follow the rearing protocol by Ohshima (2005):

- Remove the basal part of the leaf and expose the central vein.
- Prepare 1% sucrose solution and dip a piece of clean wiping paper in it.
- Wrap the petiole and exposed part of the central vein with the wiping paper.
- Store the leaf in a plastic container
- Check the container twice a day.
- Replace the wiping paper regularly (usually in two day intervals).

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Summary

Summary

This thesis examined interactions between herbivores and their host plants throughout succession and across different elevations in Papua New Guinean rainforest. It shed light on the drivers of interaction network structure and herbivore specialisation. Our 'whole forest' approach provided fresh insights into classical ecological questions, presenting a view of how whole communities of herbivores and hosts respond to their environment.

The main dataset used in this thesis (Chapter 1-3) was compiled by sampling a total of 3.8ha of primary and secondary forest exhaustively for larval Lepidoptera. We applied a series of quantitative and qualitative analytical approaches including network analyses, phylogenetic analyses and predictive models. We explored how interaction networks and herbivore specialisation change with succession in a montane forest, and whether successional theory can account for these changes. We examined this data further and attempted to uncover additional bottom up structuring mechanisms. We did this by using a rarefication based approach which matches host vegetation structure and taxonomy, drawing host-herbivore interactions from a known 'source' pool. Following this, we shifted focus towards specialisation, comparing the montane community with its lowland counterpart, and specifically addressing herbivore phylogenetic specialisation in different guilds and their response to elevation and habitat. Finally we carried out an analysis of the efficacy of various plot-based methods for large-scale sampling. We provided the detailed methodology to promote and facilitate others to pursue plot based approaches, thus allowing further meaningful cross study comparisons.

Main findings and conclusions

The thesis began with an investigation of plant-herbivore interactions across a successional gradient in montane rainforest (**Chapter 1**). We developed a predictive framework for the drivers of network structure and herbivore specialisation based on succession theory and in particular the resource availability hypothesis (Coley et al 1985, Endara and Coley 2011). Our interaction networks comprised of 12,357 interactions and formed one of the most comprehensive datasets of its kind yet compiled. We showed that plant communities across this succession gradient form distinct communities, with

these communities becoming increasingly phylogenetically clustered, contrary to expectations from lowland studies (Whitfeld et al 2012). High herbivore abundance and vulnerability in young secondary forest suggested herbivores preferentially fed on hosts in this succession stage, presumably due to lower physical and chemical defensive investment. However high host phylogenetic diversity in young secondary forest may act as a barrier to feeding across multiple hosts, while low phylogenetic diversity in primary forest may facilitate this. This change in phylogenetic structure together with consistently high herbivore specialisation may account for the similarities in generality despite increases in anti-herbivore defences. Network structure itself was typical of antagonistic networks, were low connectance and high modularity persisted across the succession gradient (Thébault and Fontaine 2010). While connectance decreased, and modularity increased with time, these changes were gradual and less than expected, with the mature stage acting as an intermediate phase. Despite the similarities in community level herbivore specialisation and network structure, we found extremely high beta diversity both within and between succession stages. Such species replacement may occur between topographically similar species, where species are functionally equivalent in their role in structuring entire networks (Kemp et al 2017). These findings may be indicative of the presence of some assembly rules which act outside of the species composition of communities.

Such assembly rules may act through bottom-up mechanisms, where host availability and identity impact herbivore fitness and feeding preference, ultimately shaping network structure (Scherber et al 2010, Futuyma and Agrawal 2009). In **Chapter 2**, we explored this possibility by utilising a modelling procedure which can match the physical and taxonomic structure of host communities. Using our montane primary forest dataset as a source of species interactions, we attempted to predict interaction network properties of target secondary plots on the basis of host community structure. We demonstrated that within the dynamism of successional change, there is some constancy in terms of shared drivers of emergent network properties. Abundance and taxonomic diversity of host trees could account for much of the variation in network properties between succession stages. Matching host abundance alone was sufficient to predict herbivore abundance, herbivore

species richness and vulnerability. On the other hand, host plant taxonomic structure emerged as an important driver of changes in network architecture i.e. network connectance and compartmentalisation. This is an outcome of coevolutionary processes which drive the delineation of interacting species pairs and subgroups (Segar et al 2017). Interestingly, despite large differences in tree size structure between succession stages, it had little influence on network properties. In this chapter we showed the potential utility of the TRIN model and also outline how we might expand upon this first effort at predicting species interactions by also accounting for host traits reflecting resource quality. Modelling networks in this manner can be a powerful tool for ecologists. It is a means to both identify drivers of network structure and to circumvent the need to carry out exhaustive and resource intensive sampling of trophic interactions in tropical forests.

In **chapter 3** we shifted our focus towards herbivore specialisation, applying modern methods to address a classic ecological line of inquiry. The question, 'how specialised are herbivores?' has been repeatedly addressed throughout the literature (Futuyma and Moreno 1988, Bassett et al 1996, Novotny et 2002, Forister et al. 2015). However the methods used to answer this question have varied to such a degree that making comparisons across studies or even habitat types is often difficult at best. A promising method for addressing this issue was developed by (Jorge et al 2014, Jorge et al 2017), in the form of the Distance Based Specialisation Index (DSI). By accounting for host relatedness and resource availability, it is possible to draw direct comparisons between study sites which vary in sampling intensity, host composition and community structure. We used our extensive dataset on host-herbivore interactions to explore variation in herbivore specialisation across elevation, habitat use and guild type. We showed that lowland herbivores are less specialised than montane species, and that this trend is driven in large part by mobile species feeding across habitat types. This finding contradicts the idea that diversity and specialisation are tightly linked (Dyer et al 2007). We speculate that this elevation difference is driven by environmental factors which impact host defensive investment. The least specialised herbivores are those which feed across both primary and secondary forest, with herbivores found exclusively in primary or secondary forest displaying similar levels of specialisation. This

runs contrary to the idea that herbivores in disturbed habitats are more generalist (Pinho et al 2017). We show that accounting for host phylogeny can change measures of specialisation notably, with the least specialised species being the most affected. We therefore recommend accounting for host phylogeny and availability in studies of specialisation and suggest using the term *phylogenetic specialisation* to distinguish these measures from traditional measures.

We completed the thesis by presenting and comparing different approaches for sampling whole forest plots. We compared the efficiency of using forest felling, canopy cranes and cherry pickers for collecting a suite of target taxa in terms of sampling effort in person hours and canopy accessibility. We showed that all three approaches required a similar input of person hours, with the optimal strategy is dependent upon the habitat being sampled. Using a cherry picker grants access to slightly more of the canopy than the other two methods, however this approach requires access roads in the sampling plot. Thus the use of a cherry picker will generally only be feasible in temperate forest where access roads are present. Tropical forest is typically much more inaccessible. Sampling in these forests therefore requires the use of a canopy crane or felling. Canopy cranes are available in numerous regions however sampling is limited to the area accessible by the crane. Felling on the other hand provides relatively flexible plot selection, but this should always be coordinated carefully with local land owners to minimise impact on the forest. Ideally this should take the form of salvage sampling, where sampling is carried out only in areas earmarked for clearance i.e. in logging concessions or areas where swidden agriculture is practised. Together these plot based methods provide the most suitable approaches to attain realistic quantitative arthropod interaction networks. A detailed methodology was also provided in this chapter supplementary section. It is our hope that this can be used by other researchers to build upon our efforts to enable global comparisons between distinct regions and habitats.

Future directions

Examining networks of interacting species has become a popular line of enquiry in recent decades. Despite this, our understanding of the mechanisms

which shape these interactions remains basic. While we provided one of the most comprehensive investigations in terms of sampling effort, the conclusions we can draw are limited to a few habitat types in a single geographical region. In order to grow our understanding, we should continue our effort, increasing plot replication and expanding the scope of our studies to broad comparative studies carried out across multiple regions and habitats. In doing so we can determine whether our findings here are applicable to other biographical regions, where distinct environmental and biotic conditions prevail.

Key to successfully achieving this will be standardising methodology in a manner which allows comparisons between these distinct regions. Our use of whole forest plots and phylogenetically controlled measures of specialisation offers an avenue for this. Efforts are currently underway to achieve this. Novotny et al have begun whole forest sampling extending across continents using both forest felling and canopy cranes to sample arboreal communities in PNG, Czech Republic, Panama, Japan and Cameroon. While this is a promising start, this effort would substantially benefit from collaborations with other research groups interested in adopting this methodology. An international collaboration of this kind could answer some fundamental ecological questions regarding community assembly, diversity drivers and specialisation. The costs of developing and executing whole forest studies are non-trivial, however the benefits can be substantial. The global network of 50ha forest plots may serve as an example of successful upscaling ecological studies. Despite the high cost of these plots, recognition of their value prompted international adoption and they continue to provide unique insights into plant community dynamics. It is our hope that similar insights can be garnered from a large-scale sampling effort of the numerous taxa closely associated with plants. Crucially, these studies will enable us to more accurately predict and prepare for changes to natural systems due to anthropogenic perturbation in the shape of habitat degradation and climate change.

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Appendix
Cirriculum Vitae

Conor Redmond
Date of birth 26.01.1988

Education and Academic Qualifications

2013-present

Ph.D in Tropical Ecology, University of South Bohemia
Supervisor: prof. RNDr. Vojtech Novotny CSc
Thesis: *Plant-herbivore interactions along ecological gradients in tropical rainforest: Drivers of network structure and specialisation*

2011-2012

M.Sc Biodiversity and Conservation, First Class Honours, Trinity College Dublin '12
Thesis: *Breeding system and pollination ecology of a potentially invasive alien Clematis vitalba L. in Ireland*

2007-2010

BA Botany, First Class Honours, Trinity College Dublin '10
Thesis: *Plant biodiversity assessment along the banks of Dublin's Grand Canal*

Specialisation

Interaction networks across ecological gradients
Invasive plant Ecology
Herbivory

Academic Courses

Tropical Biology Association Borneo Field Course, Sabah, Malaysia 2011
Molecular Analysis of Trophic Interactions (MATI), Innsbruck University, Austria 2015

Grants and Awards

Best Ph.D publications 2018, Biology Centre, Czech Academy of Sciences
Best presentation student conference 2018 (Zoology), University of South Bohemia
Grant Agency of the University of South Bohemia (GAJU) personal grant "Discrepancies in experimental feeding rates between tropical forests") 2016-2017
Highest grades in consecutive sophister years in Trinity College Dublin, Botany Department, Junior Edge Prize

Publications

Redmond, CM et al 2019. High specialization and limited structural change in plant-herbivore networks along a successional chronosequence in tropical montane forest. Ecography 42:162:172.

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Plowman, NS **Redmond, CM** et al 2017. Network reorganization and breakdown of an ant– plant protection mutualism with elevation. Proc. R. Soc. B, 284(1850), p.20162564.

Professional Appointments

2014- Present Researcher, Czech Academy of Sciences, Biology Centre

2015- Present Science Language Editor

2016- Three month stay as visiting researcher in the Smithsonian Tropical Research Institution, Panama City, Panama

2013- One year field project supervisor in Papua New Guinea

Project- Exploring community interactions using a plot-based approach on Mt Wilhelm

Conferences

Society of Conservation Biology Asia Conference, Melaka, Malaysia 2014 (Oral)- *Tropical Plant herbivore food web structure on Papua New Guinea*

Royal Entomological Society Annual National Science Meeting & International Symposium – Ento 2015 (Poster) *A slice of life: Comparing 0.2ha Tropical Forest Plant-Herbivore (Lepidoptera) Food Webs of Papua New Guinea*

Society of Tropical Ecology Annual conference, Brussels, Belgium 2017 (Oral) *Plant-Herbivore Food Webs: Successional trends along an altitudinal gradient in Papua New Guinea*

Student Conference, University of South Bohemia 2019 (Oral). *Plant-Herbivore interactions throughout succession in Papua New Guinea rainforest*

Skills

Statistics: Interaction network analysis, ordination, multivariate, data visualisation, ANOVA, GLM, LME, phylogenetic analyses

Software: R statistical software, Canoco, Geneious, Phylomatic, BEAST, BEAUTi, JModelTest 2, Inkscape, Adobe Photoshop, ArcGIS, Office suite

Field work: Extensive field work experience across a number of geographic regions and habitats (>23months). Plant and insect collection, field experiments and project co-ordination carried out in Papua New Guinea (13 months), Burren West Ireland (4 months), Cameroon (3 months), South Africa (1 month), Borneo (1 month) and Panama (1 month).

Laboratory work:

DNA extraction, PCR, sequence processing, soil sample analysis

Language:

English, Tok Pison (Papua New Guinea), Gaelic, French (basic), Czech (basic)

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