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Mechanisms structuring arboreal ant communities along ecological gradients in New Guinea rainforests

Ph.D Thesis

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Annotation

The thesis explores the community ecology and diversity of tropical ants, with a strong focus on the arboreal ants of Papua New Guinea rainforests. The aim was to investigate the drivers of community structure in these diverse communities, and how these change along the ecological gradients of elevation and forest succession. Using unique datasets from censuses of whole forest plots at low, mid and high elevation forests, the effects of vegetation structure and nest microhabitat use on ant community structure and diversity are compared across elevations. Furthermore, a community study of an understorey ant-plant mutualism was undertaken to explore elevational changes in interaction networks and the costs and benefits of mutualistic interaction. For lowland primary and secondary forest, taxonomic, functional and phylogenetic diversity of arboreal ant communities were quantified. In addition, aspects of the methodology of community functional diversity studies are examined, and suggested improvements to data handling in cases where full datasets are not available are discussed.

Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem 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 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, 26 July 2018

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Nichola S Plowman

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Cover image: Polyrhachis sp. on vegetation. Photograph by Nichola Plowman.

List of papers and authors' contribution

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

I. **Plowman, N.S**., Mottl, O., Novotny, V., Rimandai, M., Kassar, F., Idigel, C., Klimes, P. Impacts of nest microhabitats and tree size on arboreal ant community structure across elevation in tropical rainforest, 2018. (Manuscript)
[*NSP, PK, MR, FK collected data in the field, NSP, PK and OM identified ant material, NSP and OM analysed data. NSP wrote the manuscript with input from PK and OM. Study was designed & financed by VN.]*

II. **Plowman, N.S.**, Hood, A.S.C, Moses, J., Redmond, C., Novotny, V., Klimes, P., and Fayle, T.M., 2017. Network reorganization and breakdown of an ant–plant protection mutualism with elevation. Proceedings of the Royal Society B 284 (1850), 20162564. (IF = 4.8)
[NSP, ASCH, JM, CR collected data, NSP and PK identified ant material, NSP analysed the data, and wrote the manuscript with input from co-authors. TMF conceived the study and VN provided financial support.]

III. **Plowman, N.S**, Matos-Maravi, P., de Bello, F., Libra, M., Bishop, T.R., Parr, C.L., and Klimes, P. Functional and phylogenetic diversity of arboreal ants in primary and secondary rainforest, 2018. (Manuscript)
[*NSP and ML collected ant trait data, NSP, PK, and PM assembled molecular data, PK identified ant material, NSP and PM analysed data, FdB, TRB and CL consulted on R code and appropriate methods. NSP wrote the manuscript and conceived the study, with input from co-authors.*]

IV. Májeková, M., Paal, T., **Plowman, N.S.**, Bryndová, M., Kasari, L., Norberg, A., Weiss, M., Bishop, T.R., Luke, S.H., Sam, K.S, Le Bagousse-Pinguet, Y., Lepš, J., Götzenberger, L., and de Bello, F. Evaluating functional diversity: missing trait data and the importance of species abundance structure and data transformation, 2016. PLOS One 11(2): e0149270. (IF = 2.8)
[NSP is corresponding author, and joint first author with MM and TP. NSP and MM wrote the manuscript, with input from co-authors. TRB, SHL, KSM, JL contributed data. NSP, TP, and MM analysed the data. FdB and LG designed the R code. The study was conceived by FdB, LG, MM, TP, NSP, MB, LK, AN, MW.

Co-author agreement

Petr Klimeš, the supervisor of this Ph.D thesis and co-author of chapters I - III, fully acknowledges the major contribution of Nichola S Plowman to these manuscripts.

Petr Klimeš

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Petr Klimes, Ph.D

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Francesco de Bello, Ph.D

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Introduction

Introduction

Mechanisms structuring arboreal ant communities along ecological gradients in New Guinea rainforests

Overview

The study of biodiversity patterns across ecological gradients can provide insights into the biotic and abiotic factors that influence the structure of whole communities. Understanding these processes may allow us to predict the effects of environmental change on the functioning of entire ecosystems. Invertebrates are an incredibly diverse and abundant component of most ecosystems, and play an important role in their functioning (Wilson, 1987). Tropical rainforests host an estimated 6 million invertebrate species (Hamilton et al., 2010), and for the overwhelming majority their ecology is unknown. Understanding the diversity and ecology of these functionally important taxa will allow us to better predict the consequences of habitat change and global warming on tropical rainforest ecosystems. This thesis focuses on the ecology of arboreal ant species in New Guinea rainforests, investigating how their communities are structured over gradients of elevation and succession.

A brief introduction to New Guinea and its ant fauna

The island of New Guinea (NG), politically divided into West Papua (Indonesia) and Papua New Guinea (PNG) in the east, is a biodiversity hotspot with high levels of endemism (Mittermeier et al., 1998; Myers et al., 2000). This biodiversity is due in part to the island's wide variety of habitats, climate zones and high mountain ranges, but also its biogeographical complexity and recent uplifting of mountains (Toussaint et al., 2014). Moreover, NG rainforests are amongst the three largest remaining wilderness areas on earth and they support the majority of the island's diversity (Mittermeier et al., 2003; Brooks et al., 2006).

Like many taxa in NG, the NG ant fauna is diverse. Represented mainly by a combination of Australian and Oriental taxa (Wilson, 1959), there are 10 Subfamilies, 96 Genera, and 892 described species of NG ants of which 60% are endemic (AntWiki, 2018). As for arboreal ant species, they account for a large portion of this diversity: less than a third of a hectare of lowland primary forest in PNG yielded over 100 species nesting and foraging in trees and perhaps half of those represented novel species (Klimes et al., 2015). Indeed, work from just this single locality resulted in the discovery of 13 new species of arboreal *Camponotus* and *Colobopsis* for NG, four of which were newly described (Klimes & MacArthur, 2014).

Thus, further studies of arboreal ant communities in the region promise to be equally fruitful in terms of species discovery, and in describing the unknown ecology of many species.

Importance of arboreal ants in tropical rainforests

While tropical rainforests are strongholds of global biological diversity (Ozanne et al., 2003), much of this diversity is hidden in the forest canopy, hosting up to 40% of all extant species, mostly arthropods (Basset et al., 2003). Ants, with over 15,500 described species and subspecies (antwiki.org), are a major component of the rainforest ecosystem, terrestrially and arboreally (Wilson & Hölldobler, 1990). With the diversification of the Angiosperms, they radiated and they adapted from primarily ground dwelling species to exploit arboreal food and nesting resources (Moreau et al., 2006). Arboreal ants now constitute an important part of ant diversity: about half of all ant species in Malaysian rainforest are active in trees (Floren et al., 2014) and a quarter are found exclusively in the crowns of trees (Brühl et al., 1998). Moreover, arboreal ants can comprise up to 70% of arthropod biomass in tropical trees (Tobin, 1995).

As one might expect from their numerical dominance, they are an ecologically important group in trees. In tropical rainforests they are important predators of arboreal arthropods (Floren et al., 2002), as well as being scavengers and generalist foragers (Wilson & Hölldobler, 1990). While ants are often thought of as a mainly predatory guild, in the canopy they can often play the role of primary consumers, by feeding on floral nectar and extra-floral nectar (EFN), or indirectly feeding on plant sugars excreted by other insects (mainly Homopterans). Indeed, this mode of feeding can account for the extraordinary abundance and biomass of many dominant arboreal ant species – by exploiting a high carbohydrate diet from a stable resource, more energy can be expended on territoriality and aggression (Davidson, 1998).

Largely due to their predisposition for homopteran honeydew and plant nectar, and often aggressive defence of food and nest resources, ants can also play an important role in the biotic defence of plants. In some cases, ants will aggressively defend their host trees from herbivores to the substantial fitness benefit to the plants (Rosumek et al., 2009). To some extent they can shape plant communities with this behaviour. For instance, 'Devil's Gardens' in the Neotropics are ant-created monocultures of *Duroia hirsuta* ant-plants. Not only are the plants aggressively protected by their ant-partners, the ants poison all other competing plant species in the 'garden' with their formic acid. Another example of this ecosystem engineering occurs in the rainforest canopy itself: ants are the major seed dispersers of certain myrmecophytic epiphyte species in the Neotropics, creating so-called 'Ant Gardens' in the canopy by planting epiphyte seeds in their nutrient rich carton nests (Orivel & Leroy, 2011). In NG, there are also numerous examples of myrmecophytic epiphytes which rely on ants for dispersal, nutrients and defence (e.g. Benzing & Clements, 1991; Maeyama & Matsumoto, 2000). Even outside of these specialised mutualistic interactions, ant nest constructions can also provide a microclimate suitable for a whole host of other symbiotic organisms (e.g. Cushing, 2012; Perez-Lachaud and Lachaud, 2014).

The ecological and numerical importance of arboreal ants in tropical rainforest marks them as an excellent model group for community ecologists. Moreover, their relatively well-described taxonomy makes it possible to test hypotheses on biodiversity and community assembly rules. Furthermore, ant communities are sensitive to shifts in habitat and climate (Hoffmann & Andersen, 2003; Diamond et al., 2012), so they are suitable model to investigate the role of ecological gradients in community assembly, especially the relative importance of abiotic and biotic factors.

Drivers of ant community structure with elevation & succession

The main drivers structuring arboreal ant communities are still widely debated, so studying patterns over ecological gradients can help to elucidate the most important factors. Abiotic factors important for ants, such as temperature, vary with elevation and succession (Sanders et al., 2007). However many biotic factors are also correlated with elevation and succession, especially plant diversity and vegetation structure (McAlpine et al., 1983), which provide the keystone habitat and food resources for arboreal ants. For tropical rainforest communities, however, there is rather low specificity of arboreal ant species to particular tree species (Longino & Colwell, 1997; Floren & Linsenmair, 2005; Klimes et al., 2012). Vegetation structure plays a more important role, especially the presence of large trees, canopy connectivity, and epiphytes (Stuntz et al., 2003; Campos et al., 2006; Powell et al., 2011) which can provide nesting and feeding resources. Intraspecific competition is also thought to be a major force in shaping arboreal communities via competitive exclusion, especially by dominant ants (Dejean et al., 2007). The relative balance of these abiotic and biotic factors may shift across landscapes and thereby influence species composition and structure.

Since ants are ectothermic, temperature is one of the most important factors shaping ant communities, especially across elevation where temperature varies predictably

(Sanders et al., 2007; Warren & Chick, 2013). Ant abundance and diversity declines with elevation in tropical forests either monotonically or with a mid-elevation peak (e.g. Brühl, Mohamed and Linsenmair, 1999; Colwell *et al.*, 2016). With succession, ant community composition changes can be indicative of the level of disturbance. The vegetation changes that occur with selective logging do not always result in a major change on total number of species or abundance of ants but have more important influence on species composition and dominance structure of communities (Widodo et al., 2004). However, more pronounced differences are found when forest is clear-felled and natural succession takes over. Primary forests typically host more ant species per tree than secondary forests (Schulz et al., 2002; Klimes et al., 2012), and it is likely that vegetation structure plays a major role. There is evidence that in highly disturbed habitats like plantations nest site limitation results in more competitive interactions (Philpott & Foster, 2005; Fayle et al., 2013). The structure of communities in disturbed habitats can be more influenced by competition, whereas in primary forests communities tend to be more randomly structured (Floren et al., 2001; Fayle et al., 2013).

Functional and phylogenetic diversity of ants with succession

Examining the functional diversity (FD) and phylogenetic diversity (PD) of ants can provide complementary ecological information in addition to traditional species diversity indices. One approach to ant functional diversity is to categorise ants into functional groups, equivalent to those of plants, and record their response to disturbance and elevation (Andersen, 1995; Hoffmann & Andersen, 2003; Bharti et al., 2013). However, these groupings based on Australian ants are broad and may not be universal, especially since the ecology of most ant species assemblages is not well known.

A trait-based approach to ant community ecology promises new insights into how communities are structured (McGill et al., 2006). By measuring ecologically relevant traits of individuals, related to their feeding and nesting ecology for example, we can obtain a higher resolution of species' potential roles in a community. Indices of functional diversity have been shown to be a good surrogate for actual ecosystem function, with higher FD able to predict changes in ecosystem function better than species richness measures (Cadotte et al., 2011). PD and FD do not always have the same trends as species richness, since the traits of species, determine whether or not a species can cope with a particular set of environmental or biotic constraints. This approach can therefore help to infer some community assembly processes (Cavender-Bares et al., 2009). For instance, if the species present at a particular location are clustered in the phylogeny, and traits are phylogenetically conserved, this can be an indication of niche-based habitat filtering, since only species with traits suitable to the environment at that location can pass through the filter (Kraft et al., 2015). If PD or FD is overdispersed this can be an indication of competitive exclusion or limiting similarity, which leads to the assembly of species that are dissimilar in their traits, and thus exploit different niches. However, this approach must be interpreted with caution since other larger scale processes such as speciation, extinction and dispersal limitation can play a role, as well as density-dependent processes like prevalence of diseases, predators and parasites (Cavender-Bares et al., 2009).

For ants, the study of trait-based FD is beginning to gain traction. The most recent advance is the advent of the Global Ants Database (GLAD; Gibb *et al.*, 2015) which provides information on how to measure ant traits and which to prioritise, whilst providing an online repository of trait values of ant species worldwide. As yet, only few studies have investigated how ant functional traits vary with succession and typically have focused on ground-dwelling ants, which are easier to sample (Bihn et al., 2010).

Ant-plant mutualisms along environmental gradients

Although most arboreal ants in rainforests are generalists in terms of what plant species they occupy (Klimes et al., 2012), many plants offer food rewards or specialised housing or 'domatia' to encourage ants to live and forage on them in return for biotic defence. However, the benefits of hosting ants can be context dependent (Pringle & Gordon, 2013). Abiotic factors such as sunlight and rainfall (Kersch & Fonseca, 2005) and biotic factors such as the identity of partner species (Feldhaar et al., 2003) can affect the outcomes of the mutualism.

The strength of the mutualism is expected to depend on the selective pressures facing the plant, which can be affected by the environment and the availability of mutualistic partners. However relatively little is known about the structure of whole communities of ant-plant mutualists, in particular the changes in costs and benefits in relation to the effects of environmental drivers (although see Passmore et al. 2012; Fayle et al. 2015). While ant-plant mutualisms have been reasonably well-documented, less is known about shifts in these relationships with elevation. Ants decrease in abundance with elevation and become less important as predators of herbivores (Sam et al., 2015). This is expected to result in them being less effective mutualist partners. The decreased importance of ants in cooler habitats is already known to affect the diversity of animals relying on their protection; for example, the diversity of myrmecophilous treehoppers and butterflies declines towards higher elevations (Olmstead & Wood, 1990; Pellissier et al., 2012). Ants can also be poorer at providing biotic defence at higher elevations: for example, ants protecting *Inga* (Leguminosae), a Neotropical EFN-bearing plant, are less active and less effective in the uplands, resulting in greater herbivore damage (Koptur, 1985). In contrast, *Piper immutatum*, a Neotropical domatia-bearing plant, experiences similar levels of herbivory throughout its elevational range from 400 – 1600 m a.s.l (Rodriguez-Castaneda et al., 2011). However, it is not known how whole communities of domatia bearing plant-ants and ant-plants change with elevation, as previous work has typically focused only on single plant species and/or ant species interaction (e.g. Koptur, 1985; Trimble & Sagers, 2004).

Aims and scope of this thesis

Despite the importance of ants in rainforest ecosystems, the ecology of whole communities of arboreal ants remains poorly understood, especially in the remote forests of Papua New Guinea. This thesis aims to provide further ecological insight into these communities, building on pioneering work by Klimes and colleagues in whole-forest plots in the lowlands, by investigating the patterns and potential drivers of diversity along ecological gradients of succession and elevation in Papua New Guinea. To do this I used various methods exploring the taxonomic, functional, and phylogenetic diversity of arboreal communities, considering differences in forest structure, nesting habits and behaviour of individual species, and the distribution of ant-plant mutualistic interactions over elevation.

In **Chapter I** we examined arboreal ant communities in lowland, mid-elevation and high elevation primary rainforests. Using data from felling whole forest plots (trees ≥5cm DBH), we investigated how much of the variation in ant communities with elevation can be explained by vegetation structure and nest sites. We hypothesised that intraspecific competition may also be a powerful mechanism influencing diversity patterns with elevation, testing this by modelling within-tree species co-occurrence at each elevation. We found a strong mid-elevation peak in diversity and abundance of arboreal ants and that vegetation structure and nest microhabitat (i.e. tree size and nest site) were important explanatory factors. Differences in species-co-occurrence patterns in different size classes of trees varied over elevation, suggesting that competitive effects also change with elevation, with large aggressive colonies being a feature of low elevation while mid and high elevation had smaller nests.

Continuing to explore the effect of elevation on ant communities, **Chapter II** takes a detailed look at an ant-plant mutualism in the rainforest understorey, and how mutualistic interactions, and ant and plant communities change with elevation. We sampled all ant-plant individuals found in transects from 700 – 1600 m above sea level, to quantify changes in the mutualistic interaction network over elevation. To explore whether there are changes in the costs and benefits of mutualism with elevation we performed behavioural assays to assess ant protective behaviour in the most common and wide-ranging species *Myristica subalulata*. We found that ant-plants and ant partner species became rarer and less species rich at high elevations, and as a result interaction networks became less complex and less specialised with elevation. Ant partners provided less benefit at higher elevations and ant-plants suffered higher herbivory damage, likely driving the breakdown of the mutualism at high elevations.

In **Chapter III.** While quantifying taxonomic diversity remains a key aspect of ecology, there is a need to move beyond traditional species diversity estimates if we are to predict the impacts of anthropogenic global climate change and habitat alteration on the natural world. Functional and phylogenetic diversity (FD & PD) can provide complementary insight into how communities are structured. Functional diversity studies of arboreal ants are rare, and to our knowledge there has been no whole community study investigating the phylogenetic diversity of arboreal ant communities with succession. Here we examined the functional and phylogenetic diversity of arboreal ant communities in whole plots of primary and secondary lowland rainforest, and found that while there were large differences in species richness, there were smaller difference in FD and PD. However, we found that primary forests had more functional redundancy than secondary forest, and hosted larger ants than were present in secondary forest.

Finally, **Chapter IV** continues with the theme of functional diversity, this time investigating how robust functional diversity indices are under different data-handling scenarios, using real datasets of ant, bird and plant communities. We explore the effects of incomplete trait datasets, and how different transformations of trait and abundance data affect the robustness of FD indices. We found that species abundance structure and trait data distribution can affect the robustness of functional diversity indices, especially in cases where trait data for all species are unavailable or species are under-sampled, and in some cases these effects can be mitigated by appropriate data transformation.

An overview of the datasets used

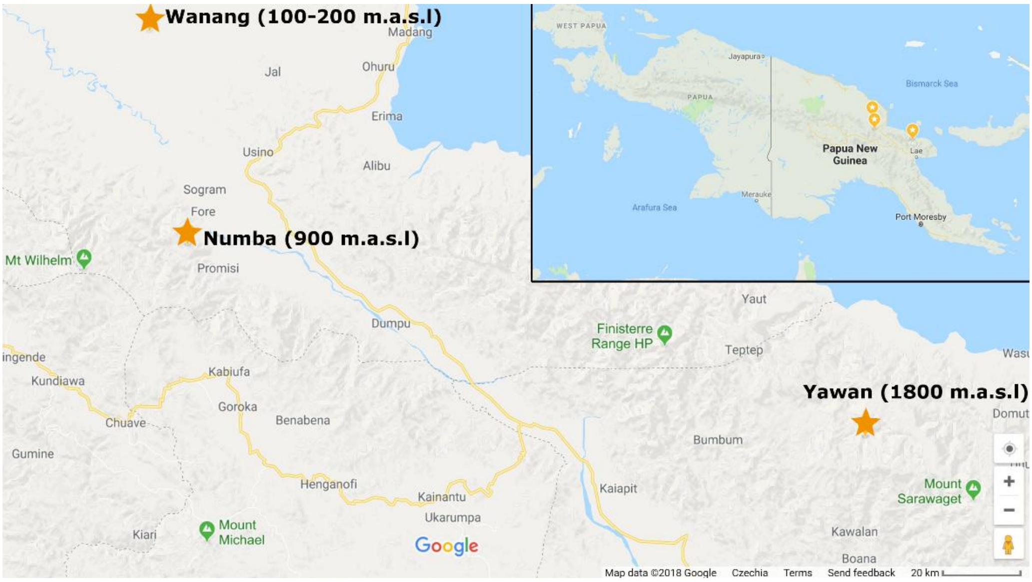

Figure 1. Map indicating location of study sites in north-eastern Papua New Guinea. Stars mark the location of our lowland plots in Wanang (200 m.a.s.l), our mid-elevation plot (900 m.a.s.l) and ant-plant transect in Numba (700 - 1600 m.a.s.l) and high elevation plots in Yawan (1800 m.a.s.l). Chapter IV used data on bird communities from the slopes of Mt. Wilhelm, also seen here. Image adapted from Map data © 2018 Google.

This first three chapters of the thesis use several datasets from different localities in Papua New Guinea (Table 1; Map 1). The datasets used in Chapter I and III were collected during a larger project on rainforest foodwebs and succession, where trees ≥5cm DBH were felled and sampled for ants and herbivores (Klimes et al., 2012; Whitfeld et al., 2012; Miller et al., 2013; Fibich et al., 2016). First, two unreplicated 1 ha plots of a primary and a secondary lowland forest were felled (of which 0.4 ha were sampled for ants) and later the same sampling method was applied at mid and high elevation localities but with replicated 0.2 ha plots (Table 1).

The final chapter focuses on testing the robustness of functional diversity indices under different methodological conditions, to improve the methods that are often used to assess functional changes of communities along various environmental gradients. Here we used real datasets of ant, bird and plant communities from Borneo, New Guinea, and Czech Republic respectively (details in Chapter IV supplementary materials).

Table 1. A summary of the localities, sampling area & methods used in each chapter.

Chapter	Locality	Habitat	Sampling method	Sample Area	Sample year
Chapter I	Wanang, PNG	Primary rainforest (200 m.a.s.l)	Felling of whole plot	0.4 ha (100 x 40 m plot)	2006 - 2007
	Numba, PNG	Primary rainforest (900 m.a.s.l)	Felling of whole plot	0.2 ha (44.5 x 44.5m plot)	2013
	Yawan, PNG	Primary rainforest (1800 m.a.s.l)	Felling of whole plot	0.4 ha (two 44.5 x 44.5m plots)	2010 – 2012
Chapter II	Numba, PNG	Primary rainforest (700 – 1600 m.a.s.l)	Transect based census	1.5 ha (ten 150 x 10 m transects)	2013
Chapter III	Wanang, PNG	Primary & Secondary rainforest (200 m.a.s.l)	Felling of whole plot	0.8 ha (two 0.4 ha plots)	2006 – 2007
Chapter IV	Mt. Wilhelm, PNG	Montane forest gradient (100 - 3700 m.a.s.l)	Transects (point counts, mist netting for birds)	80 ha transects every 500m in elevation	2010 & 2012
	Sabah, Malaysia	Primary & secondary rainforest, oil palm plantation	Litter quadrats (ants)	59 plots (4x4m quadrats)	2010
	South Bohemia, Czech Republic	Central European wet meadow	Quadrats & mowing (plants)	12 2x2m plots	2006

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Chapter I

Impacts of nest microhabitats and tree size on arboreal ant community structure across elevation in tropical rainforest

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Chapter I.

Impacts of nest microhabitats and tree size on arboreal ant community structure across elevation in tropical rainforest

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Abstract

Invertebrate communities change in diversity and species composition along elevational gradients, usually declining towards summits monotonically or with a mid-elevation peak. Most current hypotheses relate these patterns to temperature and geometric constraints. However, other mechanisms such as vegetation structure, niche availability and competitive interactions may also be important. For tree-dwelling ants, which are highly diverse and abundant in the tropics, changes in vegetation and nest microhabitats and their utilisation may influence species diversity and community structure. To understand how these factors affect arboreal ant communities over elevation, we censused all ant nests in trees within continuous plots in low, mid and high elevation rainforest in Papua New Guinea. We found a mid-elevation peak in nest abundance and per-tree and per-plot ant species diversity. Occupancy of trees was also highest in mid elevation, while nest size decreased linearly with elevation. Turnover of ant species between trees was high at all elevations. Differences in the vegetation structure, i.e. utilisation of different nest microhabitats and tree sizes, explained twice as much species variability in trees than elevation itself. Species richness per tree increased with tree size within each elevation, and across all tree sizes, trees at mid elevation supported more species than those at low or high elevation. Patterns of species co-occurrence within trees differed both between elevations and between different tree size classes, suggesting shifts in competition for nesting resources. Notably, ant communities were over-dispersed in all sites across all trees, but in mid elevation co-occurred positively in large trees or randomly in small and medium-sized trees. We conclude that turnover in nesting resources (tree size and microhabitats) and how these resources are utilised across different elevations, accompanied by decreased interspecies competition, drive community structure and the mid-elevation diversity peak in tree-dwelling tropical ants.

Chapter II

Network reorganization and breakdown of an ant-plant protection mutualism with elevation

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Research

Network reorganization and breakdown of an ant–plant protection mutualism with elevation

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Both the abiotic environment and the composition of animal and plant communities change with elevation. For mutualistic species, these changes are expected to result in altered partner availability, and shifts in context-dependent benefits for partners. To test these predictions, we assessed the network structure of terrestrial ant-plant mutualists and how the benefits to plants of ant inhabitation changed with elevation in tropical forest in Papua New Guinea. At higher elevations, ant-plants were rarer, species richness of both ants and plants decreased, and the average ant or plant species interacted with fewer partners. However, networks became increasingly connected and less specialized, more than could be accounted for by reductions in ant-plant abundance. On the most common ant-plant, ants recruited less and spent less time attacking a surrogate herbivore at higher elevations, and herbivory damage increased. These changes were driven by turnover of ant species rather than by within-species shifts in protective behaviour. We speculate that reduced partner availability at higher elevations results in less specialized networks, while lower temperatures mean that even for ant-inhabited plants, benefits are reduced. Under increased abiotic stress, mutualistic networks can break down, owing to a combination of lower population sizes, and a reduction in context-dependent mutualistic benefits.

1. Introduction

The structure and composition of plant and animal communities are affected by both the biotic and the abiotic environment [1]. Every species is involved in a myriad of beneficial, antagonistic and neutral interactions with multiple other species, and the strength and direction of these interactions is often dependent on the environmental context [1]. Beneficial interactions are widespread, abundant and important in the structuring of communities [2] to the extent that they can determine the geographical ranges of species [3] owing to the context-dependent costs and benefits for the species involved [4]. Climatic context may be particularly important in determining the strength of these mutualistic interactions [5]. Shifts in mutualistic interaction networks in relation to latitude are well known, with interactions being less specialized in the tropics owing to high diversity of plant partner species, which in turn may be related to climate [6]. Examining the distribution of mutualistic species over natural temperature gradients on mountains is the next step towards understanding how climate can shape these networks, and potentially allows comparisons in responses between latitudinal and altitudinal gradients [7,8].

Among the best-studied mutualistic networks are ant–plant mutualisms [9], the outcomes of which can be highly context-dependent [10]. Hence, these interactions are particularly interesting to study in relation to shifts in the abiotic

One contribution to a special feature ‘Ant interactions with their biotic environments’.

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environment. Ant-plants, or myrmecophytes, provide ants with nesting space or food rewards such as extra-floral nectaries (EFNs) and protein-rich food bodies, in return for protection against herbivores or trimming of encroaching vegetation [9]. In some cases, symbiotic ants can also provide nitrogen for plants through absorption of ant waste [11,12]. In the tropics, where ants are most diverse and numerous, these mutualisms become more common, with greater incidence of both EFN-bearing plants [13] and those with structures to house ant colonies (domatia) [14]. The costs and benefits to plants of hosting ants or providing food rewards can depend on biotic factors such as herbivory pressure, or the identity of the colonizing ant species, which can vary in their effectiveness of protection [15], and on abiotic factors such as light or nutrient limitation [16,17]. As such, the strength of the mutualism is expected to depend on the selective pressures facing the plant, which are affected by the environment. If costs outweigh benefits for at least one partner, then this can result in the breakdown of the mutualism, with one partner becoming parasitic, or with the interaction being abandoned [18].

Although network structure in ant–plant mutualisms has been reasonably well documented, much less is known about shifts in these interactions with elevation, and how this might affect partner benefits (but see [19–21]). Partner availability may play an important role in such shifts. With elevation, ants decrease in abundance and may be less important as predators of herbivores [22]. In addition to decreased ant-partner availability, there can be changes in the effectiveness of persisting ant partners. For example, ants protecting Neotropical EFN-bearing *Inga* species are less active and less effective in the uplands, resulting in greater herbivore damage [19]. By contrast, *Piper immutatum,* a Neotropical domatia-bearing plant, experiences similar levels of herbivory throughout its elevational range [21]. As previous work has focused on single ant or plant species [19,20], it is not known how whole networks change with elevation, and the associated effects on plant benefits. If abundance and species richness of plant–ants and ant–plants declines with temperature at higher elevations, the structure of mutualistic networks will also change. At a network level, decreased specialization could occur as a result of reductions in population sizes of some partner species, and complete loss of others, reducing possibilities for partner choice. This would result in a greater degree of connectance (a greater proportion of possible links between species are realized) [23], and a corresponding lower modularity (the degree to which the network is divided into discrete groups of interacting species) [24]. These effects are distinct from changes in network structure that occur only as a result of changes in network size, and also from spatial turnover of networks (independent of any environmental factors), in which only a central core of generalist species persist where ant-plants are surveyed at a single elevation [25]. Furthermore, associations with the 'wrong' partner species might reduce the effectiveness of plant protection, resulting in increased herbivory damage [15]. A similar effect is also expected if there are reductions in patrolling rates within-ant species as temperatures decrease.

We studied a community of terrestrial (non-epiphytic) ant-plants and their ant inhabitants in primary forest from 700–1600 m.a.s.l. in Papua New Guinea (PNG) to investigate: metres above sea level (m.a.s.l.) (i) how ant–plant interaction networks change with elevation, (ii) how ant protective behaviour on a focal species, *Myristica subalulata*, changes with elevation, and (iii) whether there are correlated changes in plant herbivory damage.

2. Methods

(a) Study site

We censused a community of terrestrial understorey ant-plants in June–August 2013 in wet primary rainforest on the slopes of Mount. Wilhelm near Numba village in Madang Province, PNG (5° 43′ 18″ S, 145° 16′ 12″ E; electronic supplementary material, figure S1). The area experiences a mild dry season between late June and early August. Temperature drops linearly with elevation from a daily mean of 27.4°C at 200 m.a.s.l. at approximately 0.58°C 100 m^{-1} (electronic supplementary material, figure S2).

(b) How do ant–plant interaction networks change with elevation?

We established ten 0.15 ha transects (150 × 10 m), at elevational intervals of 100 m, from 700 to 1600 m.a.s.l., the highest point of the local topography. This spans the rapid decline in ant species richness observed on many tropical mountains [26,27], including Mount Wilhelm [28]. We did not sample forests below 700 m.a.s.l., which were subject to human disturbance. In each transect, we examined all understorey trees (up to 15 m height) for entrance holes and ant activity in stems, branches or other pre-formed domatia and tagged all ant-inhabited trees ($n = 386$; figure 1).

Additionally, we censused all unoccupied individuals more than or equal to 1 m in height ($n = 102$) of the three most commonly inhabited species *Ryparosa amplifolia* (Achariaceae), *Myristica subalulata* (Myristicaceae) and *Chisocheton lasiocarpus* (Meliaceae). We identified each tree to species, recording height and diameter at breast height (DBH). Plant vouchers are deposited at New Guinea Binatang Research Center, Madang, PNG. We conducted transects every 100 m in elevation, rather than at fewer elevations with more replications, and since we tested elevational trends, local irregularities owing to unreplicated transects should manifest as outliers. The unimodal abundance of most ant-plants across elevations (see Results) indicates that we captured shifts in distributions with elevation reasonably well. However, because our results relate to only a single mountain, we are cautious in their interpretation.

Where possible without causing damage to the plant (and hence compromising plant-benefit assays; see below), we collected 1–15 ants in absolute ethanol from each inhabited tree (355 of 386 individuals). Where ants were resident but not collected the species was assigned as 'uncertain'. Ants were identified to morphospecies and species where possible, with species delineations refined using existing reference collections and DNA barcoding (electronic supplementary material, appendix S3).

All statistical analyses were performed in R [29]. We generated bipartite networks for each elevation and calculated the metrics *Connectance* (realized proportion of possible links), *generality* (plant species per ant species), *vulnerability* (ant species per plant species), *modularity* (see [30]) and *network specialization* (H_2'; deviation from random partner choice [31]) with the function 'networklevel' in the R package bipartite [32]. Observed H_2' was compared with randomly expected values (Monte Carlo statistics; electronic supplementary material, table S3). Connectance and generality metrics, respectively, were square root and log transformed to meet normality assumptions before testing their relationship with elevation (linear or quadratic regressions depending on fit as measured using Akaike information criterion (AIC)). Vulnerability, H_2' and modularity residuals were not improved by transformation so their relationship with elevation

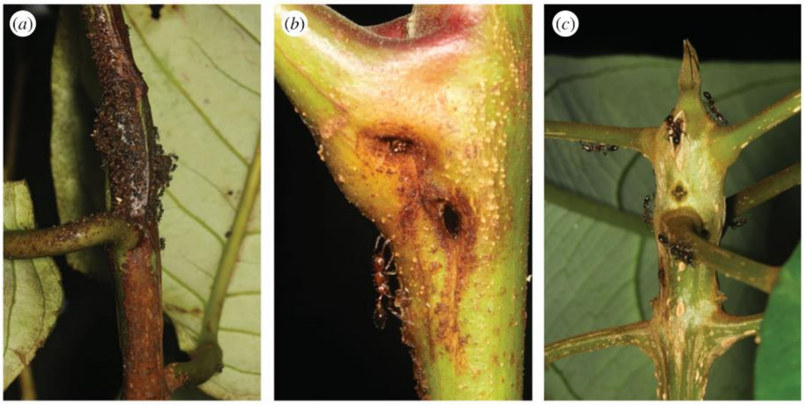

Figure 1. Domatia of the three most abundant ant-plant species in our study. (*a*) Swollen stem domatia of *Myristica subalulata* being excavated by *Anonychomyrma* ants, (*b*) entrance holes of a *Chisocheton lasiocarpus* domatium occupied by *Podomyrma* sp. 3, and (*c*) *Podomyrma* sp. 3 patrolling the swollen stem domatia of *Ryparosa amplifolia*.

was tested using the non-parametric Hoeffding's D statistic [33]. Variation in abundance can account for changes in network metrics [34]. To account for the effects of decreasing ant-plant abundance with elevation, we calculated 95% prediction intervals for all metrics based on repeated rarefaction of a pooled low-elevation community (700 and 800 m combined) to match abundances at higher elevations (1000 replications per elevation).

(c) How do ant patrolling, herbivore detection, recruitment and attack change with elevation?

To understand how ant protective behaviour changes with elevation, we focused on the interaction between the most common myrmecophyte, *M. subalulata*, and the most common genus of resident ants, *Anonychomyrma*, both spanning the entire elevational range. *Myristica subalulata* is a widespread understorey tree in New Guinea [35]. Although lacking EFNs and food bodies, when occupied by ants *M. subalulata* frequently has honeydew producing coccids inside its pre-formed domatia [35,36].

Ant behaviour was assessed from 700 to 1400 m.a.s.l. ($n = 80$), because ant occupancy was rare above 1400 m (figure 2; electronic supplementary material, figure S3). We surveyed 10 trees per elevation between 10.30 and 15.00, when ants were most active. Trees 0.4–9.0 m in height were selected at random within transects, supplemented from the surrounding area when necessary. To assess active leaf patrolling by resident ants, we randomly selected two mature and two young leaves per tree to control for leaf age (not all trees had both; $n = 74$ and 28 trees respectively), and instantaneously recorded the number of ants. Young leaves were defined as smaller, paler and fleshier than mature leaves, and were selected only when fully expanded. The relationship of elevation and ant species with active leaf patrolling was tested with repeated measures ANOVA, using leaf age as a within-subject variable.

Following previous work [37], we assessed ant responses to simulated herbivory. A single live worker termite, *Microcerotermes* sp. (not a natural herbivore of *M. subalulata*, but uniform in size, smell and lack of defences), hereafter referred to as the surrogate 'herbivore', and a paper control (0.5 × 0.5 cm) were pinned on 10 plants per elevation. The position was standardized to the second pair of leaves from the base of a randomly selected branch more than 1.5 m from ground level, 5 cm from the petiole along the midrib of different leaves. As only a minority of plants had young leaves (see above), at this position most leaves were mature and thus reasonably uniform in size. The control and treatment were alternated between left and right for every trial. We observed for 10 min to record:

(i) time until first discovery (ant touching paper/herbivore with antennae or mandibles);
(ii) time until arrival of first recruit (the second ant to locate the paper/herbivore);
(iii) time spent by any ants actively attacking the paper/herbivore; and
(iv) maximum number of ants on the leaf simultaneously.

Each metric was modelled as a function of elevation, tree height and ant species using repeated measures ANOVA to account for control and herbivore treatments on each tree. As the explanatory variables ant species and elevation are co-dependent, we present models for each predictor individually, and with all predictors present (electronic supplementary material, tables S4–14). Additionally, we individually modelled the two ant species that were most widespread across elevations to test separately for within-ant species effects (see the electronic supplementary material, S16–17).

(d) Are there changes in herbivory damage with elevation that might be driven by changes in ant protection?

Herbivory was estimated visually for all trees less than or equal to 5 m in height by assigning each leaf to a damage category (0%, less than 5%, 5–33%, more than 33% missing leaf area). On trees with less than or equal to 50 leaves, estimates were based on all leaves, and on trees with more than 50 leaves, approximately every third leaf. For plotting herbivory and for testing repeatability (but not for the main analysis, see below), we estimated mean percentage herbivory per tree by using an abundance-weighted average of the midpoint of each herbivory category. N.S.P. performed estimates for the census data, and C.R. for the behavioural assay data, with 45 trees in common. Estimates were highly correlated between observers (Pearson's product–moment correlation; $t_{43} = 5.11$, $p < 0.001$). Though this method only provides a 'snapshot' measure of

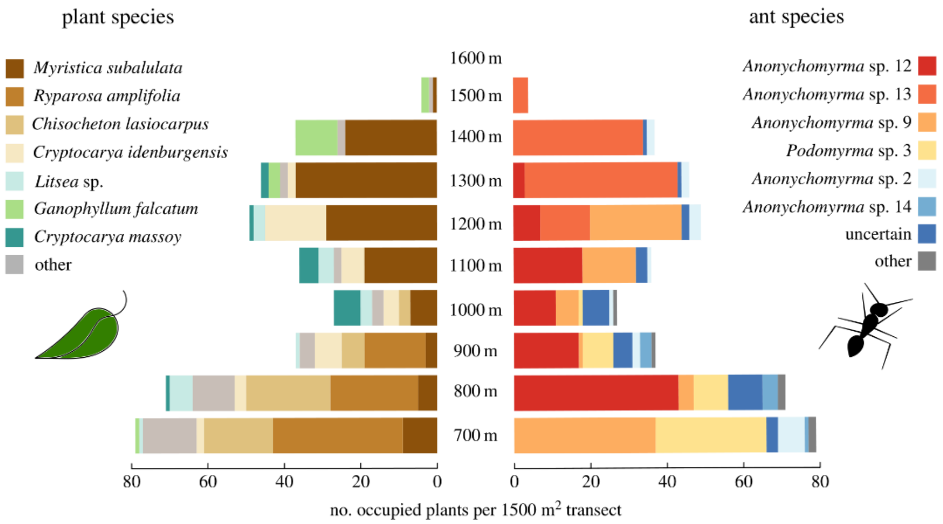

Figure 2. Distribution of ant-inhabited plant species ($n = 386$ trees) and their ant occupants from 700 to 1600 m.a.s.l. No ant-inhabited trees were found at 1600 m. Bars on the left indicate the number of ant-occupied individuals per tree species. Bars on the right indicate the number of occupied trees per ant species. Individual plants were only ever occupied by one species of ant, but most plant species were inhabited by multiple species of ant across multiple plant individuals. Where ants were observed in domatia, but could not be collected, they were recorded as 'uncertain'.

herbivory, and could not capture leaves that were completely missing, it reflects the damage accrued to leaves over their lifetime, and is appropriate for comparison over a landscape scale.

For the most common species (more than 10 occurrences: seven plant species, five ant species), herbivory was modelled as a function of elevation, tree height, ant species and tree species using ordinal logistical regression (clmm function, package 'ordinal'), with leaf as a random factor and data as counts in ordered categories. Again, because ant species, tree species and elevation are co-dependent, we present models for each predictor individually, and with all predictors present (electronic supplementary material, tables S18–S26). Models were selected using AIC.

3. Results

(a) How do ant–plant interaction networks change with elevation?

We found 23 species of ant-inhabited plants belonging to six families, and 10 species of ant inhabitants in five genera (figures 2 and 3; electronic supplementary material, tables S1 and S2). Ant inhabitation ranged from 700 to 1500 m.a.s.l., with no evidence of inhabited plants at 1600 m. Each individual plant was occupied by only one species of ant, presumably representing a single colony (no within-plant aggression was observed). Occupancy of the three most abundant plant species was high; 72%, 60% and 68% for *M. subalulata*, *C. lasiocarpus* and *R. amplifolia* respectively, though this varied with elevation, with some evidence for reduced partner availability at higher elevations (electronic supplementary material, figure S3*a–c*). Five species of *Anonychomyrma* (Dolichoderinae) and one species of *Podomyrma* (Myrmicinae) were the most common plant inhabitants. Ants from the genera *Colobopsis*, *Pheidole* and *Tetramorium* were also found inhabiting plants, but only rarely (less than three occurrences per ant species; electronic supplementary material, table S2).

With increasing elevation, the species richness of both plants and their ant inhabitants decreased (linear regressions; plants: $p < 0.001$, $r^2 = 0.80$, $F = 36.3$; ants: $p < 0.001$, $r^2 = 0.85$, $F = 52.5$; figure 4*a*,*b*). Although generality (quadratic regression; $p = 0.001$, $r^2 = 0.85$, d.f. = 6; figure 4*c*) and vulnerability (Hoeffding's D; $p = 0.006$, $D = 0.23$, $n = 9$; figure 4*d*) decreased with elevation, there was an increase in connectance with elevation (quadratic regression; $p = 0.003$, $r^2 = 0.81$, d.f. = 6; figure 4*e*). Network specialization (H_2') (Hoeffding's D; $p = 0.031$, $D = 0.15$, $n = 8$; figure 4*f*) and modularity (Hoeffding's D; $p = 0.002$, $D = 0.34$, $n = 8$; figure 4*g*) both declined with elevation. The decrease in modularity at higher elevations corresponds to an increase in the dominance of *Anonychomyrma* sp. 13, which interacted with all plant species present at those elevations. At 700 m, 1000 m and 1100 m, network specialization (H_2') was greater than would be expected at random ($p < 0.02$). All network metrics changed more than would be expected from rarefaction of lowland ant-plant communities (grey bars in figure 4*a–g*) although results were less consistent at 1500 m owing to small sample size. When 1500 m data were excluded, the effect of elevation on generality and connectance remained significant ($p = 0.001$ and 0.008 respectively), but not on vulnerability ($p = 0.136$). Networks lacked discrete compartments, i.e. there were no groups of species that were entirely disconnected with the rest of the network (figure 3), and the overall network specialization was low compared with other myrmecophytic (i.e. domatia-bearing) networks ($H_2' \leq 0.5$) [23].

(b) How do ant patrolling, herbivore detection, recruitment and attack change with elevation?

More ants patrolled young *M. subalulata* leaves than mature leaves (repeated measures ANOVA; $F_{24} = 15.1$, $p < 0.001$; electronic supplementary material, figure S4). There was no effect

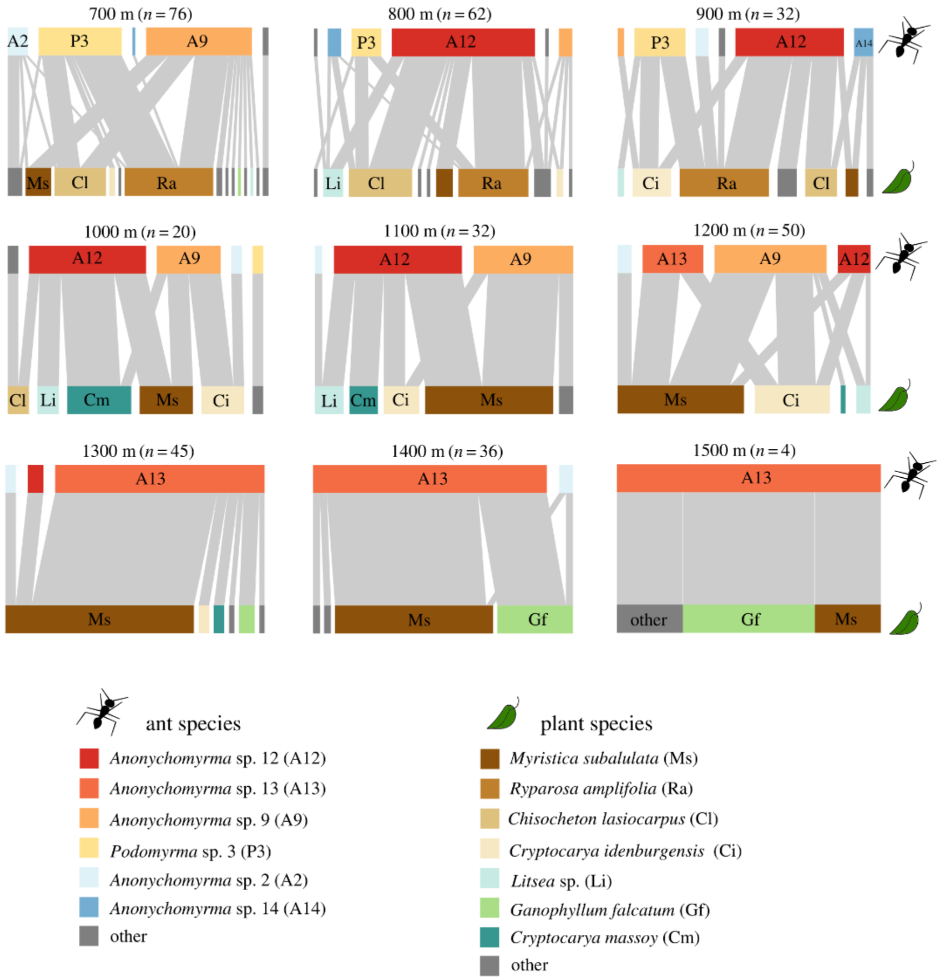

Figure 3. Bipartite interaction networks of ant-inhabited plants and their ant occupants from 700 to 1500 m.a.s.l. Upper blocks represent ant species, lower blocks represent plant species and connecting light grey bars indicate species interactions. Species with fewer than 10 occurrences are assigned as 'other' (grey blocks), but are not combined (i.e. the foodweb is fully resolved). Width of bars represents the proportion of the total community of ants or plants interacting at a given elevation. Note that the total abundance of ant-plants varied between elevations (sample size in brackets).

of elevation on patrolling of mature leaves (ANOVA; $F_{74} = 0.18$, $p = 0.673$) or young leaves (ANOVA; $F_{28} = 1.03$, $p = 0.32$) but patrolling numbers differed between ant species ($p < 0.001$, $F_{74} = 5.70$).

On *M. subalulata* 76% of controls and 79% of herbivores were detected by ant inhabitants (all were species in the genus *Anonychomyrma*; $n = 140$; electronic supplementary material, figure S5). For the three most abundant ant species (sp. 9, sp. 12 and sp. 13, inhabiting $n = 20$, 34, and 21 trees, respectively), there was no effect of elevation (repeated measures ANOVA; $p = 0.078$, $F_{54} = 1.96$) or of treatment ($p = 0.758$, $F_{54} = 0.10$) on detection time (figure 5*a*). However, detection time differed between ant species ($p = 0.028$, $F_{58} = 3.81$; electronic supplementary material, figure S6*a*).

Of those detected, all herbivores and 89% of controls were attacked. Time spent attacking declined with elevation (figure 5*b*, $p < 0.001$, $F_{66} = 4.75$), although attack times were variable at middle elevations. Ants spent more time attacking the herbivore than the control ($p < 0.001$, $F_{74} = 18.38$; figure 5*b*). Time spent attacking varied between ant species ($p < 0.001$, $F_{71} = 11.09$), with *Anonychomyrma* sp. 9 spending longest attacking both herbivores and controls (electronic supplementary material, figure S6*b*).

Of those that were attacked, further workers were recruited to 84% of herbivores and 77% to controls. Recruitment time differed among elevations ($p = 0.021$, $F_{42} = 2.71$) but did not differ between species or treatments (figure 5*c*; electronic supplementary material, figure S4*c*). The maximum abundance of ants simultaneously present on the leaf declined with elevation ($p < 0.001$, $F_{66} = 3.09$), with more ants on leaves in the herbivore treatment than the controls ($p < 0.001$, $F_{74} = 69.0$) (figure 5*d*). Maximum abundance

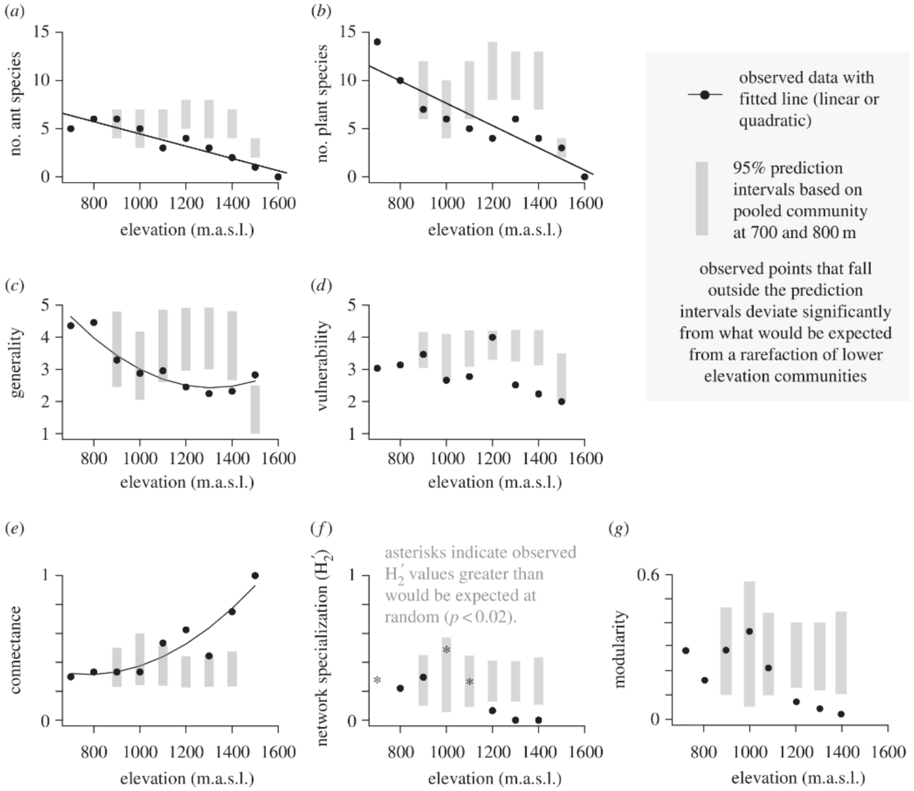

Figure 4. (*a*) Species richness of plant-inhabiting ants, (*b*) species richness of ant-inhabited plants, (*c*) generality, (*d*) vulnerability, (*e*) connectance, (*f*) network specialization (H_2') and (*g*) modularity of networks from 700 to 1600 m.a.s.l. Grey bars indicate the 95% prediction intervals based on rarefying a pooled community from 700 and 800 m, and black circles indicate observed data fitted with linear (*a*,*b*) and quadratic regressions (*c*,*e*). In panels (*d*), (*f*) and (*g*) non-parametric analyses were used, hence no line of best fit is presented, although significant relationships with elevation were detected. Asterisks in panel (*f*) indicate observed H_2' values which differed significantly from what would be expected if ant species colonized plant species at random within elevations.

also differed between ant species ($p < 0.001$, $F_{71} = 9.60$) with *Anonychomyrma* sp. 9 being the most abundant, (electronic supplementary material, figure S6*d*). When testing *Anonychomyrma* sp. 9 and 12, which were present at five or more elevations, we found no evidence for any within-species changes in any of the measured protective behaviours with elevation (electronic supplementary material, tables S16–S17).

(c) Are there changes in herbivory damage with elevation that might be driven by changes in ant protection?

For the entire plant community, herbivory increased with elevation (ordinal logistic regression; $p < 0.001$, $z = 54.6$, $n = 7584$ leaves; 507 trees; figure 6), and differed between ant species ($p < 0.001$; electronic supplementary material, table S18) and tree species ($p < 0.001$; electronic supplementary material, table S19), although the significant effect of elevation was reduced when included in the same model as tree species ($p = 0.002$, $z = 3.05$), and disappeared when included in the same model with ant species ($p = 0.177$, $z = 1.35$). This does not mean that elevation is unimportant; more likely is that elevation drives species composition and ant abundance, which in turn affects herbivory. For *M. subalulata*, herbivory increased with elevation ($p < 0.001$, $z = 3.61$; figure 6*b*,*c*), and this effect disappeared when ant species was included in the model ($p = 0.076$, $z = 1.78$), probably owing to ant species occurrence being co-dependent with elevation. There was no effect of plant occupation by ants on herbivory damage ($p = 0.103$, $z = 1.63$), but when elevation was excluded from the model unoccupied plants showed more herbivory ($p = 0.027$, $z = 2.21$; electronic supplementary material, figure S7).

4. Discussion

Few studies have investigated quantitative interaction networks along elevational gradients [38,39], and none, to our knowledge, have studied ant–plant networks in this context. We found that network structure changed with elevation and benefits for plants of ant inhabitation may have been reduced owing to decreased ant recruitment and increased herbivory. Ants of 10 different species inhabited 23 species of terrestrial ant-plants from six families, many of which have not

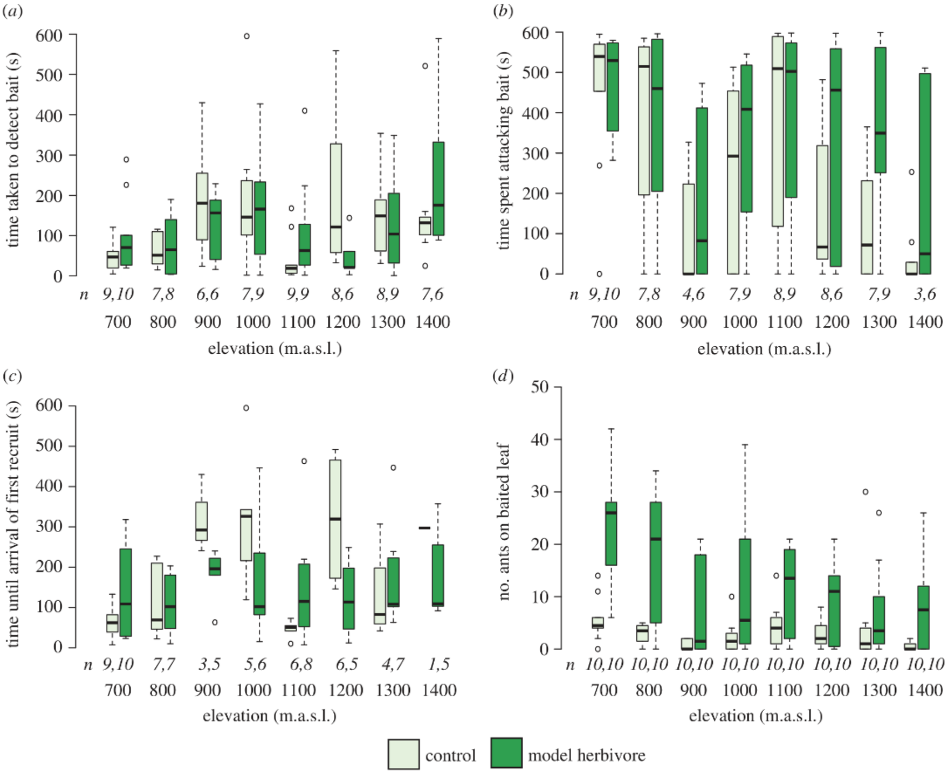

Figure 5. Responses of *Anonychomyrma* ants inhabiting *Myristica subalulata* trees to a surrogate herbivore and control treatment from 700 to 1400 m.a.s.l. (*a*) Time taken to detect, (*b*) time spent attacking, (*c*) time until the first recruit contacted the control/herbivore, and (*d*) maximum number of ants observed on experimental leaf at one time. Sample size in italics. (Online version in colour.)

previously been recorded as myrmecophytes (electronic supplementary material, table S1). The high local diversity of ant-hosting plants was not constrained to a particular clade, in common with global patterns [14], and the high ant-plant density indicates significant advantages of ant protection (533 ha^{-1} at 700 m, compared with up to 380 ha^{-1} in central Amazonia [40]). Most plant species hosted multiple species and genera of ants (see also [9]), with numbers of both ant and plant species declining with elevation. As a result, at higher elevations both ants and plants interacted with fewer partners, and networks became more connected, and less specialized. In addition, patterns of plant inhabitation for the most common species suggest reduced partner availability at higher elevations (electronic supplementary material, figure S3*a*–*c*). The most abundant ant-plant, *M. subalulata*, benefitted less at higher elevations, with slower ant recruitment, and higher levels of herbivory. This is, to our knowledge, the first time that context-dependent benefits for domatia-bearing plants have been documented in a whole-community context along an environmental gradient.

Incidence and species richness of both plants and their ant inhabitants declined with elevation, probably owing to lower temperatures and increased precipitation which can limit plant–ant interactions [41]. The upper limit we observed (1600 m) is similar to that for myrmecophytes worldwide [21,42], indicating some fundamental limitation for myrmecophily in plants. Ant communities in general are very strongly limited by elevation, with decreases in ant activity [27,43,44], and in plant-ant colony size [21], presumably owing to thermal limitations [45]. However, the reduction in species richness that we observed was greater than would be expected from reductions in abundance alone (figure 3*a*,*b*), indicating that communities at upper elevations were not just rarefied versions of communities from lower elevations.

The change in ant and plant communities with elevation was accompanied by changes in ant–plant network structure, with the average ant or plant species interacting with fewer partners. However, this apparent increase in partner selectiveness is owing to reduced species richness at higher elevations, as connectance increased, and both network modularity and network specialization (H_2') decreased (although the latter result should be treated with caution because specialization only differed from the null expectation at three elevations). This indicates that with fewer partner species to choose from at higher elevations, ants and plants may be less selective in their associations (although note that active partner choice in this system has not been demonstrated). This contrasts with patterns found for seed dispersal and pollinator networks across latitudinal gradients, in which reduced partner availability results in greater specialization [6]. However,

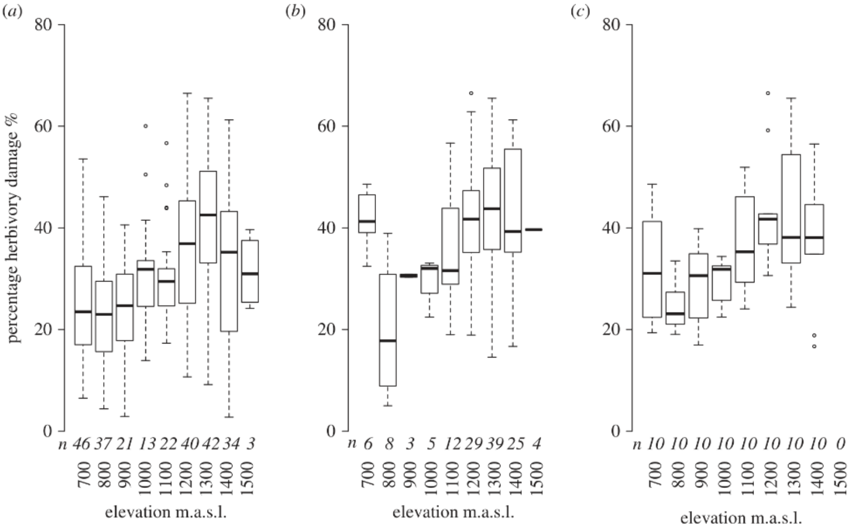

Figure 6. Percentage herbivory damage of (*a*) all ant-inhabited plants, (*b*) all inhabited individuals of *M. subalulata* in the transects, and (*c*) all individuals of *M. subalulata* at the time of baiting experiment from 700 to 1400 m.a.s.l. Leaves were assessed on all trees more than or equal to 5 m and categorized from 1 to 4 (0%, less than 5%, 5–33%, more than 33% damage, respectively). Counts across categories were converted into means for the purposes of plotting, using the midpoint for each category. Sample size in italics.

decreased specialization with elevation has been observed for leaf miner–parasitoid interaction networks [38]. In contrast with our results, these antagonistic networks showed no change in connectance. The more connected networks that we observed at higher elevations (see [46] for similar results from plant–pollinator networks in the Andes) are probably less sensitive to the loss of species than the less connected networks at lower elevations, because lost species are more likely to be replaced [47], unless core species are lost (e.g. *Anonychomyrma* sp. 13). We found turnover of interactions with elevation, with particular interactions becoming dominant, rather than networks at higher elevations comprising a subset of those from lower elevations (as is the case for cavity nesting hymenoptera and their parasitoids and kleptoparasites [39]). This contrasts with the expected spatial turnover of ant–plant networks, in which the central core of generalist species remains the same [25], supporting the idea that the observed changes are elevation-driven. It is likely that climate plays a key role in these changes, as observed for other mutualistic networks, which are affected by temperature and precipitation [48]. For plants, fewer ant-partner options could result in a suboptimal biotic defence, because with a smaller selection of hosts, it is less likely that a suitable partner will be present. This might in turn reduce plant fitness at certain elevations and ultimately define elevational ranges.

We found some evidence for this reduction in plant protection by ant partners with increasing elevation, accompanied by increased herbivory damage. Although patterns of ant patrolling did not consistently change with elevation, recruitment metrics (first worker recruited, time spent attacking, maximum number of workers observed) indicated a decreased investment in protective behaviour. Similar patterns have been observed for ant predation more broadly at high elevations, with ants becoming less important natural enemies of caterpillars than birds, parasitoid wasps and parasitoid flies [19,22]. Overall, the outcome of ant–plant symbioses is expected to be context-dependent, with our findings indicating that ants provide greater benefits at higher temperatures (within the range that we studied). At lower elevations ants spent similar lengths of time attacking the paper control as they did attacking the surrogate herbivore, but at higher elevations they spent less time on controls and more on surrogate herbivores. This may indicate that only plants at lower elevations receive the benefits of ants removing detritus, vines or other encroaching vegetation (e.g. [49]). The response to the surrogate herbivore also differed between ant species. *Anonychomyrma* sp. 9 was the fastest to detect paper/herbivores, spent longer attacking, and was more abundant on leaves with surrogate herbivores. However, owing to limited overlap in ant elevational ranges, we could not distinguish effects of elevation from effects of species turnover on ant protection of host plants. Yet it is clear that overall, plants were equally well-patrolled, but less well defended at higher elevations. This could partially explain the increase in herbivory damage with elevation, both for the ant-plant community as a whole, and for the species *M. subalulata*.

Such changes in the overall benefits for plants might relate to network structure in two different ways: (i) the smaller number of available ant partners at higher elevations (figure 3*a*) are less likely to include a more beneficial partner (c.f. the ‘sampling effect’ in biodiversity–ecosystem function relationships [50]), or (ii) higher elevation ants in general are less likely to be good partners. Regardless of the driver, these reduced benefits might then cause the breakdown of the mutualism [18], owing to the parallel changes in costs with elevation. Given our findings, it is likely that future anthropogenic-driven changes in the environmental context for these mutualistic networks will alter both interaction network structure, and the balance of costs and benefits for mutualistic partners.

Data accessibility. Data and R code available from the Dryad Digital Repository (http://dx.doi.org/10.5061/dryad.r9q18) [51].

Authors' contributions. T.M.F., N.S.P., P.K., V.N. and A.S.C.H. conceived the study. N.S.P., A.S.C.H., J.M., C.R. and T.M.F. conducted field work. N.S.P., A.S.C.H. and T.M.F. conducted statistical analyses. N.S.P. and P.K. identified ants. V.N. and P.K. provided resources and materials. N.S.P. wrote a first manuscript draft and all authors contributed to revisions.

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Supplementary materials for Chapter II. Network reorganization and breakdown of an ant–plant protection mutualism with elevation – Proc. Roy. Soc. B

Appendix 1 - Supporting figures

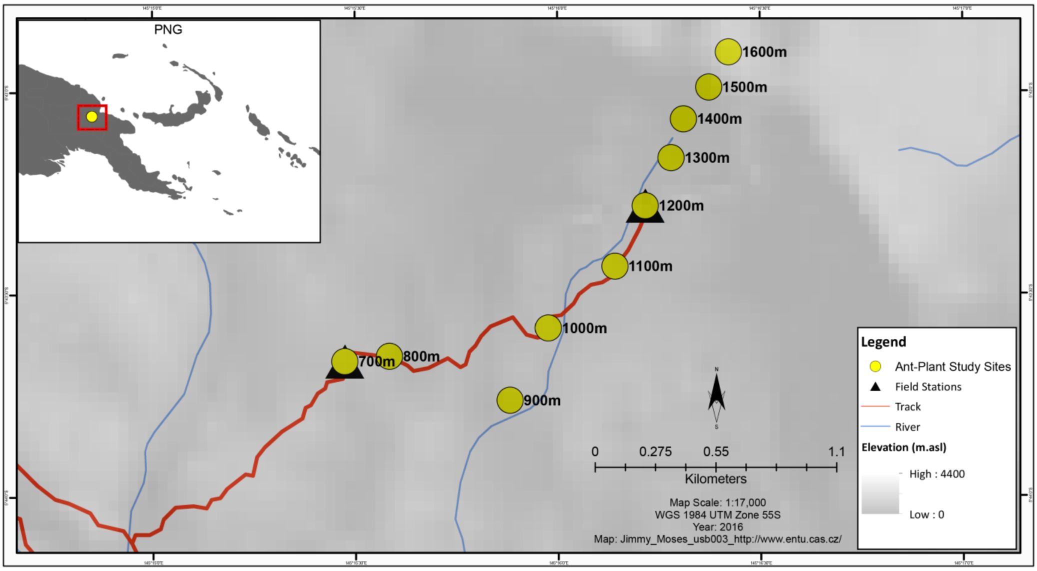

Figure S1. Schematic map of our study site and sampling points in Madang Province, Papua New Guinea. Yellow circles denote each elevation site in this study, while black triangles are locations of field camps.

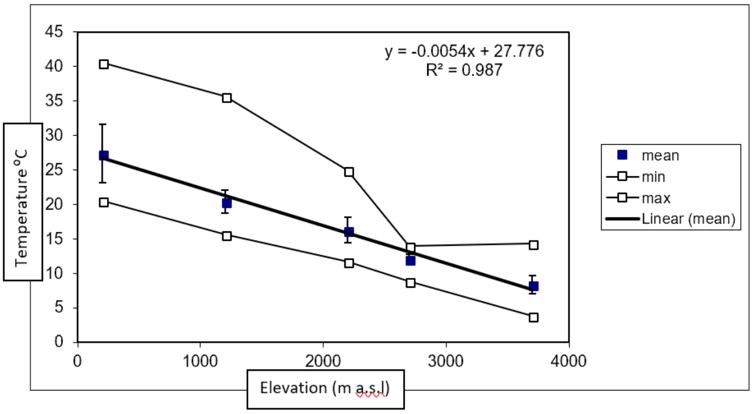

Figure S2. Mean temperatures on Mt. Wilhelm gradient over the course of a year, recorded as part of the IBISCA project “Our Planet Reviewed – Papua New Guinea” (http://www.laplaneterevisitee.org/en; data provided by K. Sam). Data loggers (Comet R3120) were placed in standardised vegetation plots at 200, 700, 1200, 1700, 2200, 2700, 3200 and 3700 metres above sea level, and recorded temperature hourly from 30th March 2010 until 1st September 2011.

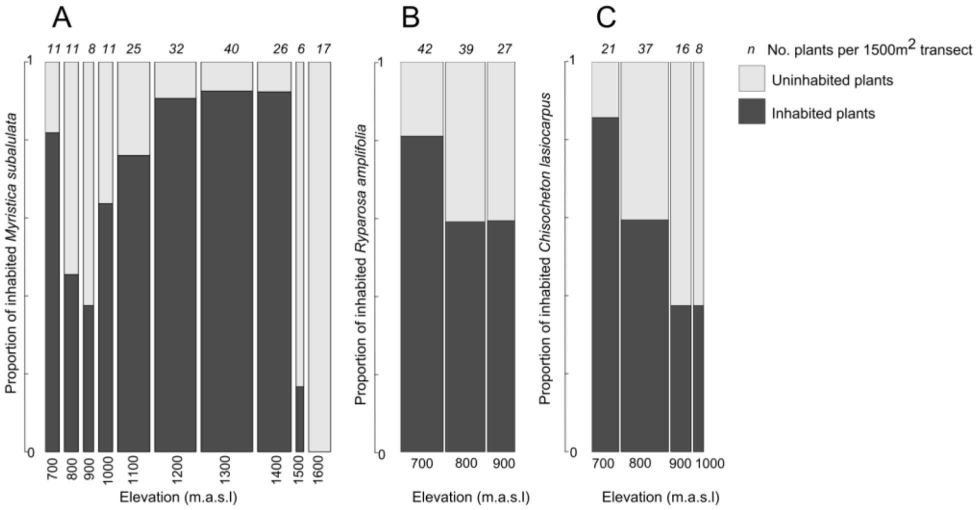

Figure S3. Proportion of inhabited and uninhabited plants of the three most common species, A) *Myristica subalulata* (n=187), B) *Ryparosa amplifolia* (n=108) and C) *Chisocheton lasiocarpus* (n=82), across their elevational ranges. Note that bar width scales with *M. subalulata* population size.

Figure S4. Mean number of ants patrolling was greater on new leaves (n=28) than on old leaves (n=78) of *M. subalulata (*repeated measures ANOVA; F=15.1, *P*<0.001, df=24).

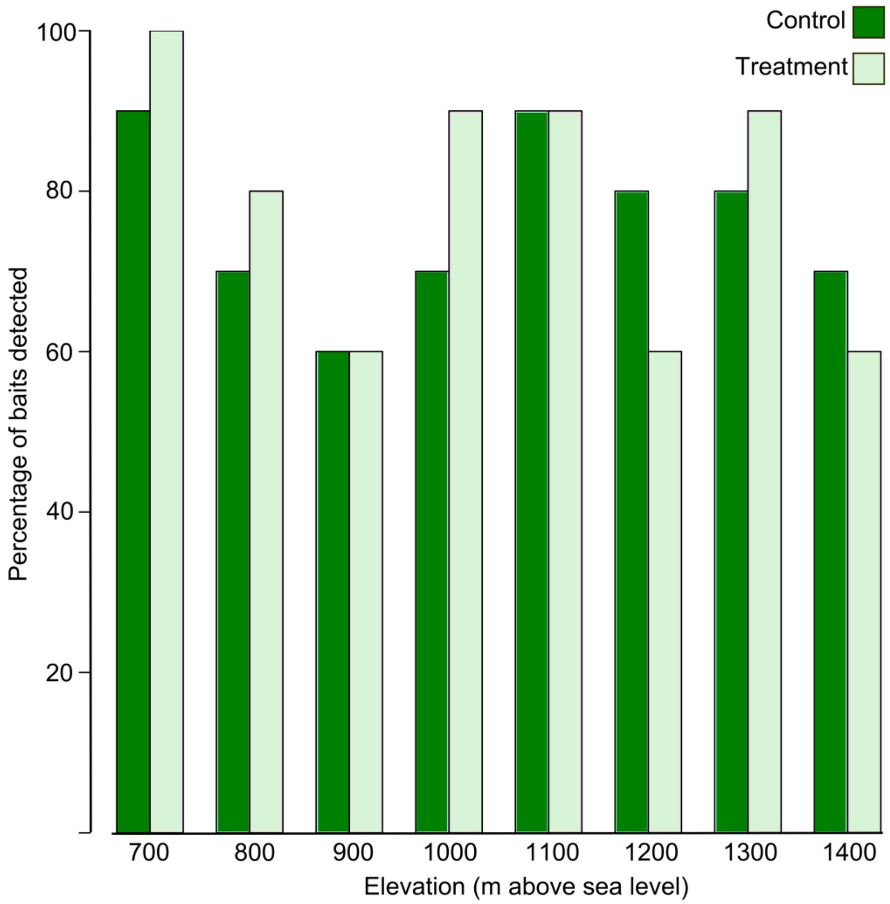

Figure S5. Percentage of baits (control=paper square, treatment=worker termite) detected by ants on *Myristica subalulata* leaves from 700 to 1400 metres above sea level (n=10 trees per treatment per elevation).

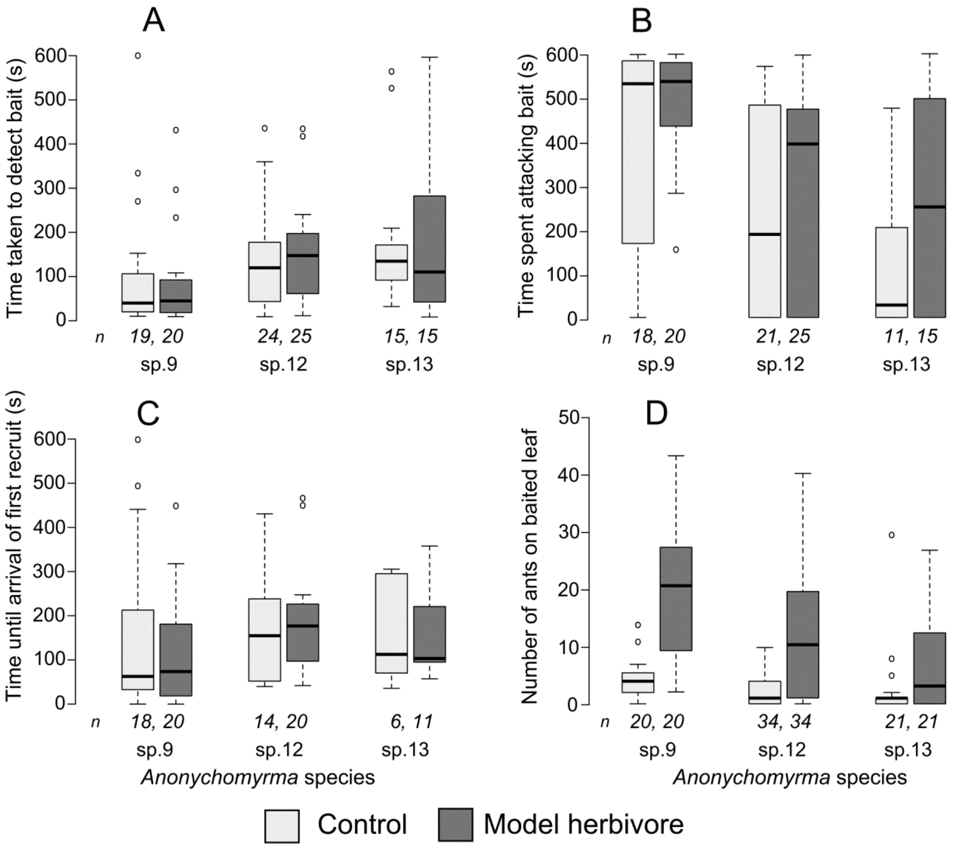

Figure S6. Detection, attack and recruitment of different *Anonychomyrma* ant species to a model herbivore on *Myristica subalulata* host trees. During a ten minute observation period we recorded the following ant responses to a piece of paper (control, light grey) and a live termite (model herbivore, dark grey) pinned to opposing leaves on each plant: A) time taken to first detect paper/model herbivore, B) time spent attacking, C) time taken for the first recruit to contact the paper/model herbivore, D) maximum number of ants observed at one time on experimental leaf. Sample sizes are indicated in italics under each bar (See Baiting ANOVA result tables in Appendix 2).

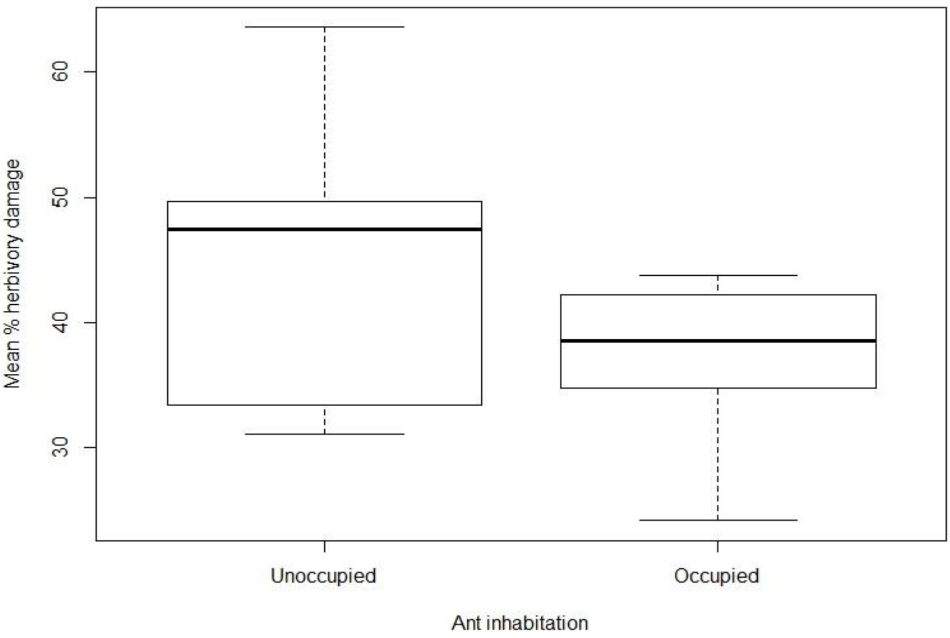

Figure S7. Mean percentage herbivory damage of unoccupied (n=89) and occupied plants (n=288) of *Myristica subalulata* (n=187), *Ryparosa amplifolia* (n=108) and *Chisocheton lasiocarpus* (n=82). Unoccupied plants showed more herbivory than those inhabited by ants (ordinal logistic regression; *P*=0.027, z=2.21).

Appendix 2 - Supporting Tables

Table S1. The abundance and species identity of all twenty-three species of ant-inhabited plants (n=386) found in our census.

Family	Species	N. ant-inhabited	Previous literature of ant inhabitation
Myristicaceae	*Myristica subalulata* Miq.	134	(Gullan, Buckley & Ward 1993; de Wilde 1998)
Achariaceae	*Ryparosa amplifolia* Mildbr.	73	(Webber *et al.* 2007)
Meliaceae	*Chisocheton lasiocarpus* (Miq.) Valeton	49	
Lauraceae	*Cryptocarya idenburgensis* C.K.Allen	40	
Lauraceae	*Litsea* sp.	18	
Sapindaceae	*Ganophyllum falcatum* Blume	17	
Lauraceae	*Cryptocarya massoy* (Oken) Kosterm.	16	
Lauraceae	*Cryptocarya viridiflora* Kosterm.	8	
Sapindaceae	*Harpullia longipetala* Leenh.	6	
Myristicaceae	*Myristica* sp.	4	
Lauraceae	*Cryptocarya albifrons* Kosterm.	3	
Lauraceae	*Cryptocarya depressa* Warb.	3	
Meliaceae	*Aglaia* sp.	2	
Euphorbiaceae	*Endospermum moluccanum* (Teijsm&Binn.) Kurz.	2	(Letourneau, Arias & Jebb 1993; Letourneau & Barbosa 1999)*
Sapindaceae	*Mischocarpus* sp.	2	
Sapindaceae	*Allophylus cobbe* (L.) Raeusch	2	(Moog *et al.* 2009)**
Meliaceae	*Chisocheton ceramicus* Miq.	1	
Meliaceae	*Chisocheton montanus* P.F. Stevens	1	
Meliaceae	*Dysoxylum excelsum* Blume	1	
Meliaceae	*Dysoxylum parasiticum* (Osbeck) Kosterm.	1	
Myristicaceae	*Horsfieldia* sp.	1	
Euphorbiaceae	*Macaranga* sp.	1	
Euphorbiaceae	*Neoscortechinia forbesii* (Hook.f.) S. Moore	1	

* Published under the synonym *E. labios* **Published under the synonym *Pometia pinnata* (Source: theplantlist.org)

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Table S2. The abundance, species identity, and accession number of all ten species of plant-inhabiting ants found in our census. Sequence data is available on BOLD/GenBank, and DNA and ant vouchers are deposited at the Institute of Entomology, Czech Academy of Sciences, České Budějovice. For more details on identification methods see Appendix 3.

Subfamily	Genus	Species	Species Code	No. trees occupied	Accession no.	Genbank/BOLD BIN code
Dolichoderinae	*Anonychomyrma*	sp. 12	ANON012	99	NA0006	KY095829
Dolichoderinae	*Anonychomyrma*	sp. 13	ANON013	91	NA0008	KY095830
Dolichoderinae	*Anonychomyrma*	sp. 9	ANON009	86	AP700-0250	KY095831
Myrmicinae	*Podomyrma*	sp. 3 aff. *laevifrons* Smith F., 1859	PODO003	47	ASPNA690-09	BOLD:AAG3969
Dolichoderinae	*Anonychomyrma*	*minuta* Donisthorpe, 1943	ANON002	18	ASPNA764-10	BOLD:AAK0675
Dolichoderinae	*Anonychomyrma*	sp. 14	ANON014	8	NA0065	N/A
Formicinae	*Colobopsis*	*quadriceps* Smith, 1859	CAMP013	2	ASPNA686-09	BOLD:AAG4070
Myrmicinae	*Tetramorium*	*bicolor* Viehmyer, 1914	TETR010	2	ASPNA1533-10	BOLD:AAP2923
Formicinae	*Colobopsis*	*vitreus* Smith, 1860	CAMP001	1	ASPNA2352-11	partial sequence
Myrmicinae	*Pheidole*	*amber* Donisthorpe, 1941	PHEI046	1	ASPNA566-09	BOLD:AAG4220

Table S3. Observed and expected specialisation (H2') of networks at each elevation. P values in bold indicate elevation s at which observed H2' was significantly different to the expected value. Randomisations were generated using Montec arlo statistics (at http://rxc.sys-bio.net/).

Elevation (m)	H2 observed	H2 randomised (mean)	P	H2 min	H2 max	H2' observed	H2' randomised (mean)
700	2.479	2.680	**<0.001**	1.825	2.807	0.334	0.129
800	2.461	2.517	0.134	1.819	2.642	0.220	0.152
900	2.401	2.496	0.103	1.683	2.704	0.297	0.204
1000	2.125	2.347	**0.014**	1.779	2.528	0.537	0.241
1100	1.843	1.982	**0.017**	1.340	2.076	0.317	0.128
1200	2.022	2.004	0.629	1.307	2.072	0.066	0.088
1300	1.202	1.166	0.688	0.805	1.202	0.000	0.092
1400	1.055	1.031	0.599	0.848	1.055	0.000	0.113
1500	NA	NA	NA	NA	NA	NA	NA
1600	NA	NA	NA	NA	NA	NA	NA

Baiting ANOVAs

We provide all model summaries to allow the readers to compare the results of models where covarying factors are included or excluded.

Table S4. Repeated measures ANOVA testing the effect of elevation, tree height and treatment on detection rate.

Coefficient	Df	SS	MS	F	P
Elevation	7	573.6	81.94	1.96	0.078
Treatment	1	4.0	4.00	0.096	0.758
Height	1	2.2	2.20	0.053	0.819
Residuals	53	2214.2	41.78		
Treatment	1	0.3	0.314	0.019	0.89
Residuals	54	884.8	16.385		

Table S5. Repeated measures ANOVA testing the effect of ant species, tree height and treatment on detection rate.

Coefficient	Df	SS	MS	F	P
Species	2	323.3	161.52	3.807	**0.028***
Treatment	1	6.0	6.0	0.142	0.707
Height	1	3.9	3.94	0.093	0.762
Residuals	58	2460.9	42.43		
Treatment	1	0.3	0.314	0.019	0.89
Residuals	54	884.8	16.385		

Table S6. Repeated measures ANOVA testing the effect of elevation, ant species, tree height and treatment on detection rate.

Coefficient	Df	SS	MS	F	P
Elevation	7	573.6	81.94	1.994	0.074
Species	2	120.2	60.12	1.463	0.241
Treatment	1	3.3	3.26	0.079	0.779
Height	1	1.0	0.96	0.023	0.879
Residuals	51	2095.9	41.10		
Treatment	1	0.3	0.314	0.019	0.89
Residuals	54	884.8	16.385		

Table S7. Repeated measures ANOVA testing the effect of elevation, tree height and treatment on attack rate.

Coefficient	Df	SS	MS	F	P
Elevation	7	2104113	300588	4.75	**<0.001*****
Height	1	29348	29348	0.464	0.498
Residuals	66	4174243	63246		
Treatment	1	373402	373402	18.38	**<0.001*****
Residuals	74	1503030	20311		

Table S8. Repeated measures ANOVA testing the effect of ant species, tree height and treatment on attack rate.

Coefficient	Df	SS	MS	F	P
Species	2	1493554	74677	11.09	**<0.001*****
Height	1	32310	32310	0.48	0.491
Residuals	71	4781840	67350		
Treatment	1	373402	373402	18.38	**<0.001*****
Residuals	74	1503030	20311		

Table S9. Repeated measures ANOVA testing the effect of elevation, ant species, tree height and treatment on attack rate.

Coefficient	Df	SS	MS	F	P
Elevation	7	2104113	300588	4.795	**<0.001*****
Species	2	184888	92444	1.475	0.237
Height	1	6776	6776	0.108	0.743
Residuals	64	4011927	62686		
Treatment	1	373402	373402	18.38	**<0.001*****
Residuals	74	1503030	20311		

Table S10. Repeated measures ANOVA testing the effect of elevation, tree height and treatment on maximum abundance.

Coefficient	Df	SS	MS	F	P
Elevation	7	73.74	10.535	3.091	**<0.001*****
Height	1	0.57	0.567	0.166	0.685
Residuals	66	224.94	3.408		
Treatment	1	91.98	91.38	69.03	**<0.001*****
Residuals	74	97.97	1.32		

Table S11. Repeated measures ANOVA testing the effect of ant species, tree height and treatment on maximum abundance.

Coefficient	Df	SS	MS	F	P
Species	2	63.44	31.72	9.598	**<0.001*** **
Height	1	1.18	1.18	0.356	0.553
Residuals	71	234.64	3.30		
Treatment	1	91.38	91.38	69.03	**<0.001*** **
Residuals	74	97.97	1.32		

Table S12. Repeated measures ANOVA testing the effect of elevation, ant species, tree height and treatment on maximum abundance.

Coefficient	Df	SS	MS	F	P
Elevation	7	73.74	10.535	3.160	**0.006** **
Species	2	12.10	6.049	1.814	0.171
Height	1	0.04	0.043	0.013	0.910
Residuals	64	213.37	3.334		
Treatment	1	91.38	91.38	69.03	**<0.001*** **
Residuals	74	97.97	1.32		

Table S13. Repeated measures ANOVA testing the effect of elevation, tree height and treatment on time until first recruit.

Coefficient	Df	SS	MS	F	P
Elevation	7	463.9	66.27	2.708	**0.021***
Treatment	1	58.7	58.71	2.399	0.129
Height	1	4.8	4.85	0.198	0.659
Residuals	42	1028.0	24.48		
Treatment	1	21.0	20.96	1.066	0.309
Residuals	36	708.1	19.67		

Table S14. Repeated measures ANOVA testing the effect of ant species, tree height and treatment on time until first recruit.

Coefficient	Df	SS	MS	F	P
Species	2	150.0	74.98	2.659	0.081
Treatment	1	74.9	74.87	2.655	0.110
Height	1	5.2	5.18	0.184	0.670
Residuals	47	1325.4	28.20		
Treatment	1	21.0	20.96	1.066	0.309
Residuals	36	708.1	19.67		

Table S15. Repeated measures ANOVA testing the effect of elevation, ants species, tree height and treatment on time until first recruit.

Coefficient	Df	SS	MS	F	P
Elevation	7	463.9	66.27	2.769	**<0.019***
Treatment	1	58.7	58.71	2.453	0.125
Species	2	75.4	37.70	1.575	0.220
Height	1	0.0	0.04	0.001	0.970
Residuals	40	957.4	23.93		
Treatment	1	21.0	20.96	1.066	0.309
Residuals	36	708.1	19.67		

Table S16. Repeated measures ANOVA testing the effect of elevation, tree height and treatment on the detection time of *Anonychomyrma* sp.9 to check for within species effects of elevation.

Coefficient	Df	SS	MS	F	P
Elevation	4	349.4	87.34	2.110	0.138
Treatment	1	6.8	6.77	0.164	0.692
Height	1	19.8	19.84	0.479	0.501
Residuals	13	538.1	41.40		
Treatment	1	2.12	2.115	0.129	0.724
Residuals	18	295.51	16.417		

Table S17. Repeated measures ANOVA testing the effect of elevation, tree height and treatment on the detection time of *Anonychomyrma* sp.12 to check for within species effects of elevation.

Coefficient	Df	SS	MS	F	P
Elevation	5	153.8	30.76	1.174	0.360
Treatment	1	31.2	31.16	1.190	0.290
Height	1	49.9	49.92	1.906	0.184
Residuals	18	471.4	26.19		
Treatment	1	6.1	6.119	0.353	0.558
Residuals	22	381.0	17.317		

Ordinal Logistic Regression results

We provide all model summaries to allow the readers to compare the results of models where covarying factors are included or excluded.

Table S18. Results of ordinal linear regression model (*clmm* function, *ordinal* package) testing the effect of elevation and tree height on herbivory damage of all ant-inhabited trees ≤ 5 m in height (n=7584 leaves; 507 trees).

Coefficients	Estimate	Std Error	Z value	P
Elevation	0.14	0.003	54.6	**<0.001 *****
Height	0.13	0.003	37.7	**<0.001 *****

Table S19. Results of ordinal linear regression model (*clmm* function, *ordinal* package) testing the effect of ant species and tree height on herbivory damage of all ant-inhabited trees ≤ 5 m in height (n=7584 leaves; 507 trees).

Coefficients	Estimate	Std Error	Z value	P
ANON009	-0.98	0.40	-2.44	**0.015***
ANON012	-1.13	0.40	-2.87	**0.004****
ANON013	-0.17	0.39	-0.44	0.658
PODO003	-0.86	0.42	-2.04	**0.042***
Height	0.17	0.07	2.39	**0.017***

Table S20. Results of ordinal linear regression model (*clmm* function, *ordinal* package) testing the effect of tree species on herbivory damage of all ant-inhabited trees ≤ 5 m in height (n=7584 leaves; 507 trees).

Coefficients	Estimate	Std Error	Z value	P
Cryptocarya idenburgensis	-0.76	0.27	-2.81	**0.005 ****
Cryptocarya massoy	-0.43	0.40	-1.06	0.289
Ganophyllum falcatum	-1.68	0.31	-5.42	**<0.001*****
Litsea sp.	-0.09	0.34	-0.27	0.789
Myristica subalulata	0.87	0.22	3.97	**<0.001 *****
Ryparosa amplifolia	-0.60	0.24	-2.47	**0.013 ***

Table S21. Results of ordinal linear regression model (*clmm* function, *ordinal* package) testing the effect of elevation, ant species and tree species on herbivory damage of all ant-inhabited trees ≤ 5 m in height (n=7584 leaves; 507 trees).

Coefficients	Estimate	Std Error	Z value	P
Elevation	0.00	0.00	0.77	0.441
ANON009	-0.83	0.37	-2.27	**0.023 ***
ANON012	-0.97	0.36	-2.66	**0.008 ****
ANON013	-0.18	0.36	-0.49	0.621
PODO003	-0.37	0.41	-0.89	0.372
Cryptocarya idenburgensis	-0.89	0.33	-2.70	**0.007 ****
Cryptocarya massoy	-0.55	0.46	-1.18	0.237
Ganophyllum falcatum	-2.01	0.42	-4.74	**<0.001 *****
Litsea sp.	-0.09	0.38	-0.24	0.812
Myristica subalulata	0.28	0.31	0.90	0.368
Ryparosa amplifolia	-0.64	0.26	-2.46	**0.014 ***

Table S22. Results of ordinal linear regression model (*clmm* function, *ordinal* package) testing the effect of elevation and tree species on herbivory damage of all ant-inhabited trees ≤ 5 m in height (n=7584 leaves; 507 trees).

Coefficients	Estimate	Std Error	Z value	P
Elevation	0.13	0.04	3.05	**0.002 ****
Cryptocarya idenburgensis	-1.13	0.29	-3.87	**<0.001 *****
Cryptocarya massoy	-0.83	0.42	-2.00	**0.045 ***
Ganophyllum falcatum	-2.39	0.38	-6.23	**<0.001 *****
Litsea sp.	-0.33	0.35	-0.94	0.346
Myristica subalulata	0.32	0.28	1.13	0.360
Ryparosa amplifolia	-0.59	0.24	-2.47	**0.013 ***

Table S23. Results of ordinal linear regression model (*clmm* function, *ordinal* package) testing the effect of elevation, tree height and ant species on herbivory damage of all ant-inhabited trees ≤ 5 m in height (n=7584 leaves; 507 trees).

Coefficients	Estimate	Std Error	Z value	P
Elevation	0.07	0.05	1.35	0.177
Height	0.16	0.07	2.29	**0.022 ***
ANON009	-0.86	0.41	-2.09	**0.036 ***
ANON012	-0.99	0.41	-2.45	**0.014 ***
ANON013	-0.31	0.40	-0.78	0.437
PODO003	-0.59	0.46	-1.29	0.198

Table S24. Results of ordinal linear regression model (*clmm* function, *ordinal* package) testing the effect of elevation, tree height and ant species on herbivory damage of *Myristica subalulata* in our baiting experiment.

	Estimate	Std. Error	Z value	P
Elevation	0.11	0.06	1.78	0.076
Height	-0.02	0.08	-0.27	0.786
ANON012	-0.17	0.23	-0.77	0.441
ANON013	0.24	0.36	0.67	0.504

Table S25. Results of ordinal linear regression model (*clmm* function, *ordinal* package) testing the effect of elevation and tree height on herbivory damage of *Myristica subalulata* in our baiting experiment.

	Estimate	Std. Error	Z value	P
Elevation	0.15	0.04	3.61	**<0.001 *****
Height	-0.04	0.08	-0.53	0.598

Table S26. Results of ordinal linear regression model (*clmm* function, *ordinal* package) testing the effect of ant species and tree height on herbivory damage of *Myristica subalulata* in our baiting experiment.

	Estimate	Std. Error	Z value	P
ANON012	-0.03	0.22	-0.15	0.877
ANON013	0.73	0.25	2.96	**0.003****
Height	-0.07	0.08	-0.85	0.397

Appendix 3 - Supplementary Methods & Ant Photos

Ant identification and barcoding

Ants were stored in 99% ethanol and examined under Olympus SZX7 microscope at magnification 12-84x. Since taxonomic knowledge of ants from the region is poor at the species level, ants were first sorted to genera and morphospecies. We compared them with reference collections from Klimes et al. (2015) and from the international expedition *Our Planet Reviewed* at Mt. Wilhelm (Leponce et al, 2017), which sampled ants in the same or nearby sites. All morphospecies were also compared with the ant image databases New Guinea Ants and Antweb (www.newguineants.org, www.antweb.org). Some species within the *Anonychomyrma* genus, which is in need of taxonomic revision, were only possible to identify to morphospecies level. For each (morpho)species, a representative worker was sequenced for the mitochondrial gene cytochrome c oxidase I (COI), either previously as part of the Barcode of the life project (sequence deposited in BOLD database, http://www.barcodinglife.org) or newly for the purpose of this study (deposited in Genbank, See Table S2). The BOLD sequences were obtained at the Biodiversity Institute of Ontario, University of Guelph using a standard protocol described in Smith et al. (2005), with respective primers used listed for each sample in the BOLD database. The rest of the samples were obtained at the Institute of Entomology, where total genomic DNA was extracted using the GenomicDNAKit Tissue (Geneaid Biotech Ltd., New Taipei City, Taiwan) following the manufacturer's protocol. COI was then amplified using published primers and polymerase chain reaction conditions (Folmer et al, 1994). DNA sequences were assembled, edited and aligned in Geneious version 6.1. The *Anonychomyrma* sequences were also compared with sequences from previously collected specimens of the genus from New Guinea and Australia (M. Janda, P. Matos-Maraví unpublished data; and available data in Genbank and BOLD). DNA and ant vouchers are deposited at the Institute of Entomology, Biology Centre of Czech Academy of Sciences, České Budějovice.

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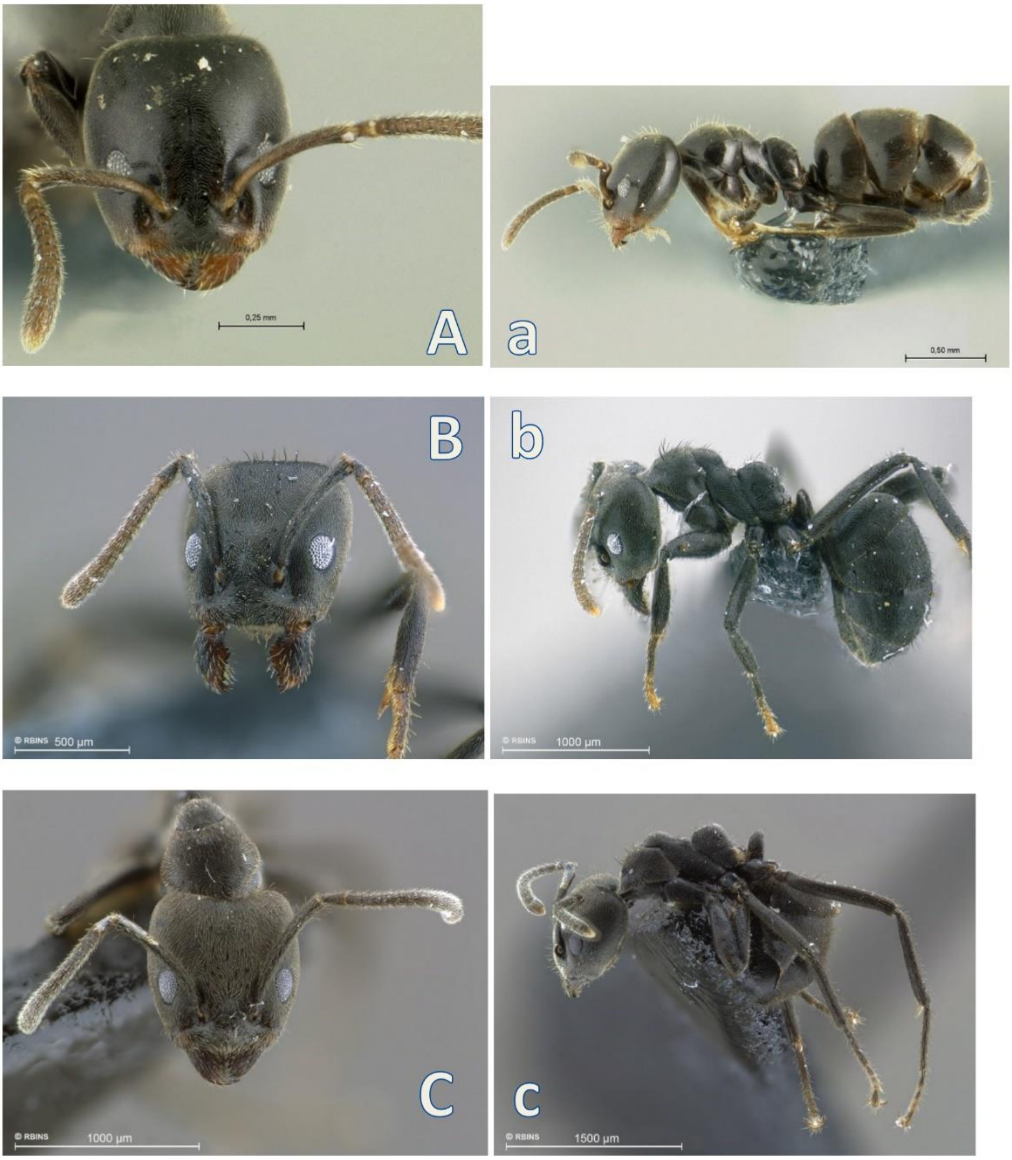
Frontal and lateral view of ant species

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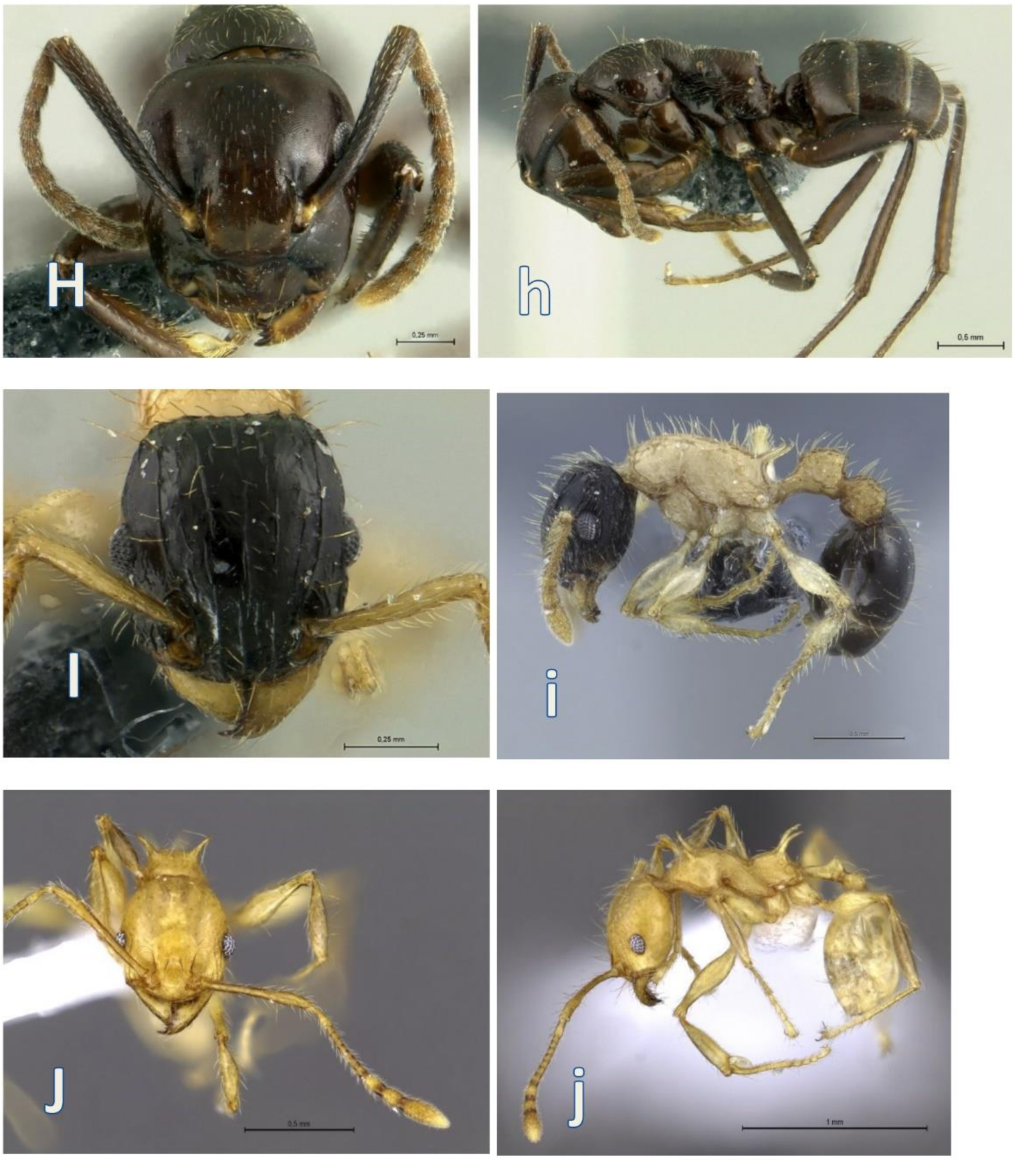

Photos Aa-Jj: Head (uppercase letter) and lateral (lowercase) views of *Anonychomyrma* sp.2 (Aa), *Anonychomyrma* sp. 9 (Bb), *Anonychomyrma* sp.12 (Cc), *Anonychomyrma* sp. 13 (Dd), *Anonychomyrma* sp.14 (Ee), *Podomyrma* sp.3 (Ff), *Colobopsis vitrea (Gg)*, *Colobopsis quadriceps (*Hh), *Tetramorium bicolor* (Ii), *Pheidole amber* (Jj). See table S2 for further details. Photos courtesy of the Institute of Entomology, Biology Centre of the Czech Academy of Sciences (A, D-J)) and the Royal Belgian Institute of Natural Sciences (B-C). Ant specimens are deposited at the Institute of Entomology, Biology Centre CAS, České Budějovice.

Chapter III

Functional and phylogenetic diversity of arboreal ants in primary and secondary rainforest

(manuscript)

Functional and phylogenetic diversity of arboreal ant communities in primary and secondary rainforest in New Guinea

Nichola S. Plowman, Pavel F. Matos-Maravi, Francesco de Bello, Martin Libra, Tom R. Bishop, Catherine L. Parr, Petr Klimes

Abstract

Tropical rainforest canopies host a high abundance and diversity of ant species, but the ways in which this diversity changes with forest disturbance is little known. Investigating not only taxonomic diversity (TD) but also phylogenetic diversity (PD) and functional diversity (FD) of communities can give further insights into how succession affects community structure. Here we studied the effect of succession on arboreal ant communities in two continuous primary and secondary lowland rainforest plots in New Guinea, where all trees ≥5cm DBH were censused for ants. We then measured their functional traits and constructed their community phylogeny to test how FD and PD change between the two forest stages. In addition, we assessed the influence of visiting species which were not nesting in the trees sampled, on overall diversity in each habitat.

Primary forest had higher TD, PD and FD than secondary forest, but only for measures that are highly correlated with species richness. When using abundance and distance-weighted indices, there was little difference between the two forests in most diversity measures. Both forests had random phylogenetic structure, and secondary forest had random functional structure. In contrast, the primary forest community was slightly more functionally clustered than expected by chance, usually an indication of niche-based habitat filtering. However, the effect was small and excluding visiting species from analysis found that the functional structure of the nesting community was random. Excluding visiting species resulted in higher FD in primary forest, but lower FD in secondary forest. This suggests that in primary forest visiting species were functionally similar to nesting species, whereas in secondary forest they were functionally different to the nesting community. PD was more robust to excluding visiting species, differing little between primary and secondary forest. The main driver of functional differences was body size: ant species were on average larger in primary than in secondary forest.

We conclude that while there was little difference in PD and phylogenetic structure with succession, there were some important functional differences. Primary forest communities were slightly more functionally clustered than secondary forest communities, which were randomly structured. While visiting species boosted FD

in species-impoverished secondary forests, they were functionally redundant in primary forest. However, primary forest hosted the largest ant species, which were altogether missing in secondary forest, and such species may provide ecological functions unique to pristine habitats.

Chapter IV

Evaluating functional diversity: missing trait data and the importance of species abundance structure and data transformation

(in PLOS ONE, 2016)

PLOS | ONE

RESEARCH ARTICLE

Evaluating Functional Diversity: Missing Trait Data and the Importance of Species Abundance Structure and Data Transformation

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Abstract

Functional diversity (FD) is an important component of biodiversity that quantifies the difference in functional traits between organisms. However, FD studies are often limited by the availability of trait data and FD indices are sensitive to data gaps. The distribution of species abundance and trait data, and its transformation, may further affect the accuracy of indices when data is incomplete. Using an existing approach, we simulated the effects of missing trait data by gradually removing data from a plant, an ant and a bird community dataset (12, 59, and 8 plots containing 62, 297 and 238 species respectively). We ranked plots by FD values calculated from full datasets and then from our increasingly incomplete datasets and compared the ranking between the original and virtually reduced datasets to assess the accuracy of FD indices when used on datasets with increasingly missing data. Finally, we tested the accuracy of FD indices with and without data transformation, and the effect of missing trait data per plot or per the whole pool of species. FD indices became less accurate as the amount of missing data increased, with the loss of accuracy depending on the index. But, where transformation improved the normality of the trait data, FD values from incomplete datasets were more accurate than before transformation. The distribution of data and

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its transformation are therefore as important as data completeness and can even mitigate the effect of missing data. Since the effect of missing trait values pool-wise or plot-wise depends on the data distribution, the method should be decided case by case. Data distribution and data transformation should be given more careful consideration when designing, analysing and interpreting FD studies, especially where trait data are missing. To this end, we provide the R package “traitor” to facilitate assessments of missing trait data.

Introduction

Functional trait-based approaches are increasingly used in ecology for understanding the environmental and evolutionary processes underlying biological diversity [1,2]. While traditional measures of biodiversity encompass the richness and abundance of organisms in an ecosystem, trait-based studies can provide additional information on their functions. Where taxonomic diversity categorises organisms by relatedness, functional diversity (FD) captures the extent of the differences between organisms in terms of functional traits, i.e. the measurable characteristics associated with their fitness and ecological function [3]. A functional approach can therefore allow generalizations beyond taxa and biogeographical regions, and can reveal both how species coexist together and how they might affect multiple ecosystem processes [4,5].

Functional diversity can be measured with different FD indices, which capture different aspects of diversity: functional richness, functional evenness and functional divergence (for full review see [6] and references therein). All indices are calculated using both trait and species composition data. Intuitively, the more species for which trait data are available, the more FD indices will reflect the real community values [7]. However, complete trait data is often not available for all species because it might be difficult to measure certain traits for particular species, time and resources are usually limited, and species might be very rare. It is particularly common to be missing trait data for rare species, and they are generally the first to be omitted in incomplete datasets [7]. The omission of the rare species first is mainly because the most abundant species are expected to have the most functional influence on ecosystem functioning (see 'mass ratio hypothesis'[8] and [9]) and are therefore sampled with higher priority.

It also remains unclear what proportion of species one needs to measure to make a reliable assessment of functional diversity, and whether it is possible to generalize this across study systems, different taxa or different sampling methods. This is an increasingly important issue to address with the advent of trait databases, of which even the largest are still plagued by missing data [10] reducing their power for larger-scale comparative studies. Many trait-based studies use an 80% relative abundance threshold as a rule of thumb, i.e. sampling trait information for the most common species that comprise at least 80% of the total abundance in a community [11]. This measure was proposed for and is adequate for community weighted mean of traits (CWM), which is not sensitive to outliers and focuses on the most dominant species in a community [9]. In contrast to CWM, FD indices such as functional richness, evenness and divergence encompass the variability of both rare and dominant species and are therefore more sensitive to missing trait information [7]. In general FD values decline in reliability with missing trait data [6], however, how this decline affects the biological conclusions drawn from such indices still remains to be tested.

Since FD indices are sensitive to missing trait data, it is important to make an *a priori* decision on how to deal with potential data gaps. One approach is to fill in the missing trait data by imputation [12,13]. Another option is to set ‘safe’ trait data completeness thresholds, i.e. the minimum proportion of species for which trait data must be obtained, usually prioritizing the most dominant [11]. Data completeness thresholds can be applied either on the whole assessed pool of species or in each plot separately [7]. The choice of applying either a pool-wise or plot-wise threshold also influences the way researchers conduct trait sampling, and depends on whether the focus is on species abundant in the whole dataset (pool-wise) or locally (plot-wise) [14], as well as on the rate of species turnover between plots and/or habitat heterogeneity (ie. beta diversity). For example, along ecological gradients with high replacement of dominants across locations, setting the trait sampling thresholds for each plot can reveal more relevant information than setting it for the whole pool [15].

FD indices are calculated using both species composition and trait data, therefore the structure and the choice of transformation of both types of data can strongly influence their computation. Abundance structure reflects the dominance patterns of a community [16,17], with the most abundant species expected to have the most functional influence (see 'mass ratio hypothesis'[8]). Abundance data can spread over several orders of magnitude [18–20]. Therefore, abundance data are often transformed to avoid relatively small differences between species, which are often biologically relevant, being overshadowed by larger ones. Trait data present the same problem, with traits varying on different orders of magnitude, but with small biological differences at low values of a trait being often as biologically relevant as bigger differences at greater values. As such, traits can also be transformed, which can influence the measures of functional differences between species [20]. For example, traits related to size or weight are mostly skewed in distribution and thus routinely transformed to meet normality criteria. In such cases, log-scaled values rather than absolute values may better reflect the study system in question [21,22]. Both abundance and trait transformation changes the relative influence of rare species as well as affecting features of the distribution (namely variance, skewness and kurtosis), and effectively decreases the differences between rare and abundant species. In synthesis, both species abundance and trait structure and data transformation could affect the resulting FD indices and their interpretation. Although some studies have been conducted on the effect of missing data [7,11,12,23], the additional effects of abundance and trait structure and data transformation on missing trait data are not yet resolved. This is a crucial issue to address, since FD indices of communities with different trait and abundance distributions may differ in their sensitivity to the same amount of missing data and may not be directly comparable.

Here we test the specific questions: (1) How do missing trait data influence the robustness and reliability of FD indices; (2) How does defining a safety threshold for missing trait data either pool-wise or plot-wise influence the robustness of FD indices to missing trait data; (3) How do abundance structure, abundance measures and abundance transformation affect the robustness of FD indices to missing trait data; (4) How do trait distribution and transformation affect the robustness of FD indices to missing trait data?

Material and Methods

Datasets

We selected three datasets that represent (1) different groups of organisms (i.e. plants, invertebrates, and vertebrates), and for which we have complete information on species abundance and trait data; (2) different types of abundance sampling methods; (3) different dominance-diversity structure. For each of the datasets we selected traits that were available for all sampled species, are commonly used, and represent important dimensions of species ecological strategies([2,22,24] and references therein). These traits represented both continuous and categorical traits. More details on all study systems and traits are given in S1 Appendix. Taking the lead from previous studies on the effects of missing data [6, 7, 8, 19] we focus on real communities that reflect the nuances that are important for the structuring of species assemblages. At the same time, to expand upon previous work, we decided to test different types of communities and sampling patterns to assess consistency in the patterns detected.

Plant data were collected from an oligotrophic species-rich wet meadow in the south-western Czech Republic with 12 plots differing in their management [25,26]. Three methods of measuring plant abundance were adopted: (1) species frequency in quadrats in each plot (henceforth "frequency"), (2) percentage cover estimates performed visually from the centre of each plot (henceforth "cover"), and (3) biomass, where plants were clipped, sorted into species, oven-dried and weighed. We assessed two categorical (growth form and position of leaves along the stem) and three continuous traits (canopy height, specific leaf area and seed mass) of 62 species.

Ant data were collected at the Stability of Altered Forest Ecosystems (SAFE) project in Sabah, Malaysia [27,28]. In total the survey included 59 plots in different habitat types; oil palm, logged forest, and old growth forest. Ants were hand collected from soil pits and dead wood in quadrats in each survey point. The abundance of each species was expressed as number of individuals. We assessed two categorical (pilosity and sculpturing) and two continuous traits (head length and the ratio of leg length to body size) of 297 species.

A repeated survey of rainforest birds was carried out along an elevation gradient on the slopes of Mt. Wilhelm in the Central Range of Papua New Guinea [29,30]. The study was completed along a 30 km long transect with eight sites, spanning from lowland floodplains at 200 m to the timberline at 3700 m. The sampling method was individual counts, which comprised surveying the bird communities at each site by point counts, mist-netting and random walks through the area. We assessed one categorical (trophic guild) and two continuous traits (body length and weight) of 238 species.

Missing trait data simulations and data collection scenarios

We simulated different degrees of trait data availability by progressively removing trait data, starting by omitting trait data for the least abundant species. Removing trait data generally results in some species not being included in the calculation of CWM and FD, i.e. part of the total community abundance is not considered. Removing the least abundant species first, mimics a frequently encountered sampling condition, where rarer species are often those with missing trait information [6]. We then followed the approach of Pakeman [6] to uniformly decrease the total abundance in a community by small steps (0.5%; Fig 1), in order to obtain a comparable continuous measure for hypothesis testing and comparison between communities. If a species accounts for less than 0.5% of total abundance, the whole species is removed in one step. If a species accounts for more than 0.5% of total abundance, the entire species is removed in several steps. The alternative to this approach is removing one species at a time, but this would result in unpredictable changes in relative abundance, since abundance distributions differ from community to community and from dataset to dataset. As an example, assume a plot with the following abundances (e.g. number of individuals), sorted by increasing abundance: {1, 2, 3, 4, 4, 7, 9, 14, 33, 89}. Expressed in relative abundances, rounded for simplicity to two decimals, this becomes {0.01, 0.01, 0.02, 0.02, 0.02, 0.04, 0.05, 0.08, 0.19, 0.54}. If we now remove 5% (i.e. 0.05) from that plot, we remove the first 3 species and 0.01 of the fourth species. In the second step, we would remove the remaining 0.01 of the fourth species, the fifth species, and 0.02 of the sixth species, and so forth for each step. Hence, for each of these steps we omit species, simulating that we do not consider them and their accompanied trait values when calculating measures of functional composition and diversity.

As in Pakeman [6] we used two missing trait scenarios, pool-wise and plot-wise. For the pool-wise scenario (Fig 2), we first ranked species in the whole pool (i.e. species from all

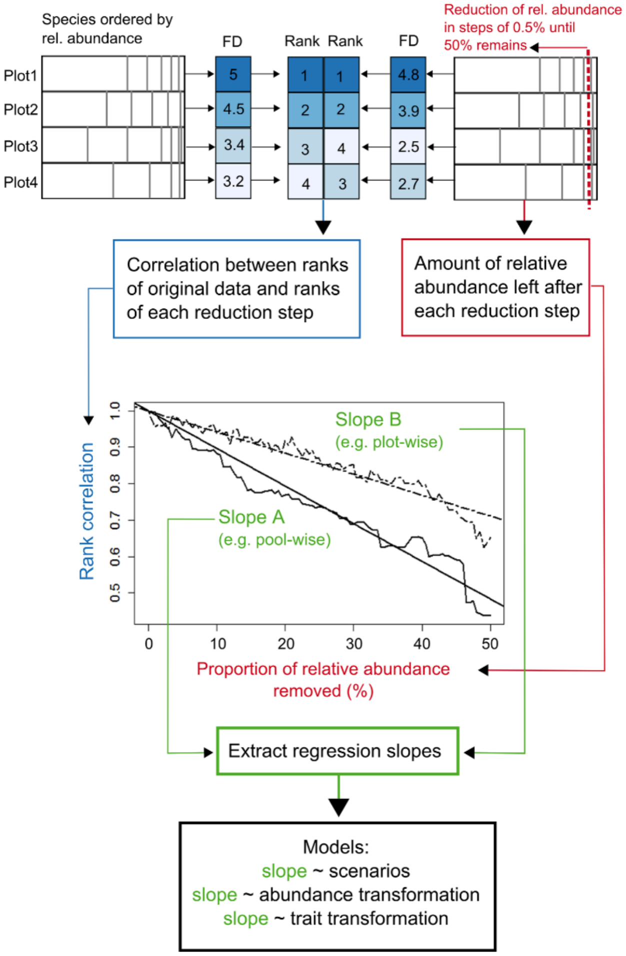

Fig 1. Flow diagram of the consecutive methodological steps. Upper left corner—in each plot species are ordered by their relative abundance and FD index is calculated for each plot of a community. Upper right corner—0.5% of the species relative abundance is removed in consecutive steps, starting with the least abundant species and FD index is then calculated again for each plot at each reduction step. Upper middle columns—plots are ranked based on the values of the FD index and the ranks of original data and data at each reduction step are correlated. Figure in the middle—regression slopes from fitting the linear model represent the robustness of FD index to missing trait data; in this example FD index is (A) less robust and (B) more robust to missing trait data (example RaoQ on head length of ants).

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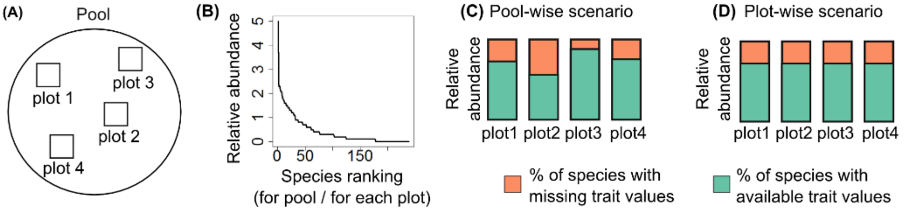

Fig 2. Plot-wise and pool-wise trait data thresholds. Schematic figure depicting plot-wise and pool-wise scenarios for setting the thresholds for trait data sampling. (A) species from all plots make up the pool of species; (B) species can be ordered by their abundance in each plot or in the whole pool; (C) the least abundant species in the whole pool of species are removed until reaching the desired threshold for trait sampling; (D) the least abundant species in each plot are removed until reaching the desired threshold for trait sampling.

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communities in a dataset) by abundance. We calculated the relative abundance of each species in the entire species pool and then we progressively removed the rarest species from the species pool, as demonstrated above. This resulted in differing availability of trait information between plots since some individual plots contain the removed species, and are affected, while others are not. Moreover, communities have different abundance structure (i.e. dominance-diversity curves) so that removing the same number of species in different communities can have different results. For the plot-wise scenario, we first ranked species in each plot by their abundance. We then calculated the relative abundance of each species for each plot and progressively removed the rarest species from each plot, as demonstrated above. In both scenarios, we removed 0.5% of the relative abundance at a time (Fig 1) until only 50% of the total abundance remained. We provide functions to apply this removal procedure to any community dataset in the R package "traitor" (see Data Accessibility for details).

Transformation of abundance and trait data

We used different combinations of transformations of abundance and trait data for both scenarios (plot-wise and pool-wise, see previous section) in order to reveal how the two scenarios combined with different forms of data transformation influence the FD indices. For continuous traits we applied square root and $\log_{10}$ transformations (henceforth “log”). For abundance data we applied log (x + 1) and log (x / min(x) + 1) transformations where x is the abundance of a species and min(x) stands for the minimum positive abundance value of the species in the dataset. The log (x / min(x) + 1) transformation is a special case of a general form log (a × x + b), which is usually applied for data containing zeroes. Similar to the often used log (x + 1) it keeps the absence (i.e. original zero) to be zero after transformation, but unlike the log (x + 1) it also keeps the logarithmic character of the transformation when the values are rather low. For example, if the values are between zero and two, the character of log (x + 1) transformation is close to linear, whereas log (x / 0.1 + 1) keeps the typical log shape, therefore equalizing the species abundances. In the case of individual counts and frequency log (x + 1) equals log (x / min (x) + 1), therefore we calculated the latter only for the plant datasets with biomass and cover.

Functional diversity indices

We assessed three FD indices using the *FD* package [31]. We first computed the trait dissimilarity matrices with the Gower distance for both single and multiple traits [32]. Then we calculated the FD indices: (1) functional richness (FRic), which reflects the range of functional trait variability in a given species assemblage; (2) functional evenness (FEve), which represents the evenness of abundance distribution across species traits; and (3) Rao's quadratic entropy index (RaoQ), which captures the degree of divergence in the abundance distribution of species functional traits. In addition, we computed community weighted mean (CWM) which expresses the structure of trait values in the community. We computed all four indices for 100% of species and individuals in each plot and then repeated this approach (including recalculation of the Gower distance) for each reduction step.

Robustness of FD indices to missing trait data

The values of FD indices calculated for each plot within a dataset can change when missing different amounts of trait data. As a result, plots can change in how functionally diverse they are relative to each other, ie. they may end up ranked in a different order by their values of FD indices (Fig 1). Here we assessed whether the ranking of FD values across plots was conserved when trait data were removed in order to evaluate how missing trait information would affect biological conclusions. To do this we calculated Spearman's rank correlations between the FD index values of the original data (100% of species) and those of every step of the simulated reduction sequence (Fig 1). When the rank correlations are high, the order of samples according to their FD values is maintained between the original and the reduced data, even if FD values themselves change. In contrast to a focus on FD *per se* [7], using rank correlations allows us to assess how data availability could affect possible biological interpretations, as this represents the amount of biological information retained in the reduced datasets. We produced these simulations for each organism, FD index, sampling scenario (pool-wise and plot-wise), trait (separately and combined together), and abundance and trait transformation type.

We used linear regression models for combinations of all cases described in the previous section, where the log-transformed rank correlations were considered as a response variable and the amount of relative abundance remaining in the plot as an explanatory variable (as shown in the example in Fig 1). We then extracted slopes from each regression model in order to estimate the decline of rank correlations, and therefore robustness of FD indices to missing trait data, for all possible combinations of cases considered. The less negative a slope estimate was, the lower the decrease in rank correlations with data reduction, and therefore the more robust the FD index was to missing trait data. The intercept for these regressions was forced through 1 to account for the theoretical starting point of the slopes and to make the slopes comparable. In further analyses, we refer to the regression slopes fitted with linear models as "robustness", as it represents the rate of decline of rank correlations with missing trait values.

Data analyses

Testing the effects of sampling scenarios, abundance distribution, abundance measures and their transformation. We tested which of the different predictors had a significant effect on the slope values extracted in the previous step, therefore indicating which variables affect the robustness of FD indices to missing trait data. We used linear mixed effects models with maximum likelihood estimation (*nlme* package, [33]) with regression slope values (from linear regression models described in previous section) as the response variable, and scenario (pool-wise or plot-wise), abundance transformation (transformation or no transformation), index (CWM, FRic, FEve, RaoQ), and their two-way and three-way interactions as fixed effects. Because the traits selected for each organism represent just a subset of all possible traits that can be measured, we used trait as a random factor in the linear mixed effect models. The models were performed for each organism type separately. In the case of plants, where three different abundance measures were applied (biomass, cover, and frequency) we also used the abundance measures (i.e biomass, cover, and frequency) as another fixed explanatory variable.

Because we could not calculate slopes for all combinations of our explanatory variables (e.g. CWM of categorical traits) we used a mixed effects model, which allows an unbalanced design of the data [34].

Testing the effects of trait data structure and its transformation. In a separate model, we also tested the effect of trait transformation on the robustness of FD indices to missing trait values. For that we first calculated the skewness of all continuous trait distributions before and after each transformation type (square-root transformed, $\log_{10}$ transformed). Categorical traits were not included in this analysis. Trait values were always positively skewed. The difference in skewness between untransformed and transformed data indicated how much data transformation improved the normality of the traits. Small differences in skewness indicated that the trait was already close to normality and transformation was not necessary. We then tested with linear mixed effects models whether trait transformations improved the effect of missing trait data on the FD indices. In the models, regression slope values (from linear regression models described in the "Robustness of FD indices to missing trait data") were used as the response variable and improvement in skewness, scenario (plot-wise or pool-wise), index (CWM, FRic, FEve, RaoQ) and their interactions were used as variables with fixed effects. Again, because traits considered in our analyses represent only a subset of all potential traits that can be measured for each organism, we used trait as a random factor in the models. The models were done for each organism type separately.

Results

The effect of missing trait data and the effect of sampling scenarios on FD indices

All assessed indices were, as expected, sensitive to missing trait data (Table 1). Community weighted mean (CWM) was less sensitive to missing trait data than the FD indices for all three datasets. Within the FD indices Rao's quadratic entropy index (RaoQ) was the least sensitive, followed by functional richness (FRic), and functional evenness (FEve) (Fig 3). The effects differed for the different community types. For plant data, FEve was more sensitive to missing trait information for the pool-wise scenario than for the plot-wise scenario. The other three indices were equally sensitive to missing trait information in both scenarios (Fig 3A). For the ant data, in the plot-wise scenario RaoQ was less sensitive to missing trait information, and CWM, FEve, and FRic were more similar in both sampling scenarios (Fig 3B). For the bird data, none of the interaction terms were statistically significant (Table 1), indicating that all indices were similarly sensitive to missing trait information for both scenarios (Fig 3C).

The effect of abundance distribution, abundance measures and their transformation

For plant data, abundance transformation greatly decreased the sensitivity of FEve and FRic to missing trait data, but only slightly decreased RaoQ and CWM sensitivity (Fig 4A), which were however the least sensitive to missing trait data. When considering the three different abundance measures available for the plant dataset (biomass, frequency, and cover), the frequency measure was the least sensitive to missing trait data (Fig 5). The interaction between abundance transformation and abundance measure was significant ($F_{(2, 461)} = 6.3$; $P = 0.002$; see also S2 Appendix in Supplementary material); the differences between these measures were equalized after transformation (Fig 5). For ants, the interaction between abundance transformation and index indicated that FD indices computed from transformed species abundances were less sensitive to missing trait data than FD indices computed from original species abundances (Fig

Table 1. Effect of sampling scenario and abundance transformation on FD index sensitivity.

Community	Predictors	Df	F	*P*
Plants	Scenario	1, 493	13.28	<0.001
	Index	3, 493	236.22	<0.001
	Abun.Transf.	1, 493	12.24	<0.001
	Scenario × Index	3, 493	21.10	<0.001
	Scenario × Abun.Transf.	1, 493	0.03	n.s.
	Index × Abun.Transf.	3, 493	27.20	<0.001
	Scenario × Index × Abun.Transf.	3, 493	1.66	n.s.
Ants	Scenario	1, 110	101.68	<0.001
	Index	3, 110	170.13	<0.001
	Abun.Transf.	1, 110	189.39	<0.001
	Scenario × Index	3, 110	24.84	<0.001
	Scenario × Abun.Transf.	1, 110	7.65	0.007
	Index × Abun.Transf.	3, 110	14.89	<0.001
	Scenario × Index × Abun.Transf.	3, 110	0.93	n.s.
Birds	Scenario	1, 84	0.29	n.s.
	Index	3, 84	20.25	<0.001
	Abun.Transf.	1, 84	0.48	n.s.
	Scenario × Index	3, 84	0.09	n.s.
	Scenario × Abun.Transf.	1, 84	0.005	n.s.
	Index × Abun.Transf.	3, 84	5.10	0.003
	Scenario × Index × Abun.Transf.	3, 84	0.09	n.s.

Results of general linear mixed effects models showing the effect of the two scenarios (pool-wise or plot-wise; "Scenario"), FD indices (functional richness, functional evenness, Rao's Quadratic entropy index, and community weighted mean; "Index"), and abundance transformation ("Abun.Transf.") on robustness of FD indices to missing trait data (the regression slopes).

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4B). For birds, abundance transformation did not significantly change the sensitivity of FD indices (Fig 4C).

The effect of trait distribution and transformation

The more normal the distribution of traits before transformation, the less sensitive the indices became to missing trait information (Fig 6). There was a significant interaction between the change in skewness (from untransformed to log-transformed trait data) and index ($F_{(3,\ 274)}$ = 6.78; $P > 0.001$; see also S2 Appendix in Supplementary material). Trait data transformation most improved the accuracy of RaoQ, followed by FEve, FRic, and there was no improvement for CWM, as expected since trait transformation did not alter the ranking in species trait values (Fig 6).

Discussion

The effect of missing trait data and the effect of sampling scenarios on FD indices

The number of species for which trait information is available can influence not only the accuracy of functional diversity indices [7], but also the possible biological interpretation. We demonstrated this by showing that the ranking of FD values changes across communities with an

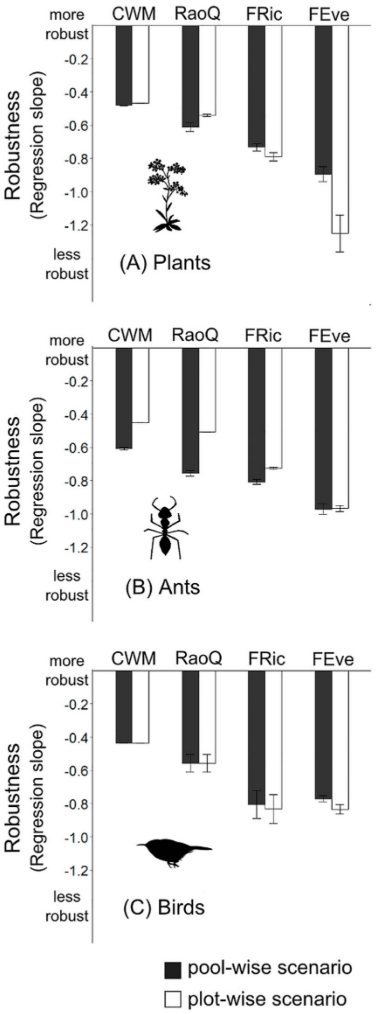

Fig 3. Effect of sampling scenario on FD index sensitivity. Barplots showing the results of linear mixed effects model, specifically the effect of the two sampling scenarios on the sensitivity of indices for three different types of organisms. The more negative the regression slope, the more sensitive the particular index is to missing trait information. The error bars denote the 95% confidence intervals. (A) plant community (n = 12 plots), (B) ant community (n = 58 plots), and (C) bird community (n = 8 plots).

doi:10.1371/journal.pone.0149270.g003

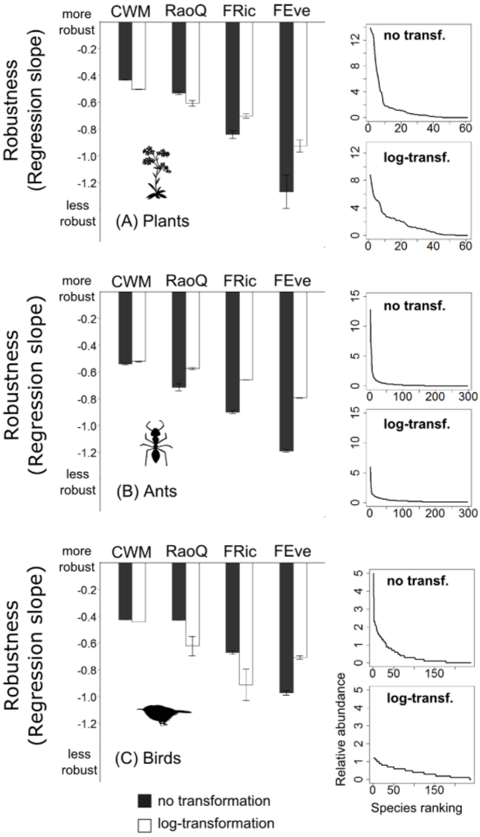

Fig 4. Effect of abundance transformation on FD index sensitivity. Barplots showing the results of linear mixed effects models, specifically the effect of the abundance transformation on the slopes for the three different types of organisms. The more negative the regression slope, the more sensitive the particular index is to missing trait information. The error bars denote the 95% confidence intervals. (A) plant community (n = 12 plots), (B) ant community (n = 58 plots), and (C) bird community (n = 8 plots). The right panels depict dominance-diversity curves for the respective organism dataset before and after log-transformation.

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increasing amount of missing trait data compared to a complete dataset. Our results also confirmed the previous finding that some FD indices are more sensitive than others to missing trait information [7,11]. FD indices were not as robust as CWM, which is heavily influenced by the most abundant species. Functional evenness was the most sensitive index to missing data, perhaps because the missing trait information for rare species removed extreme or outlier trait values.

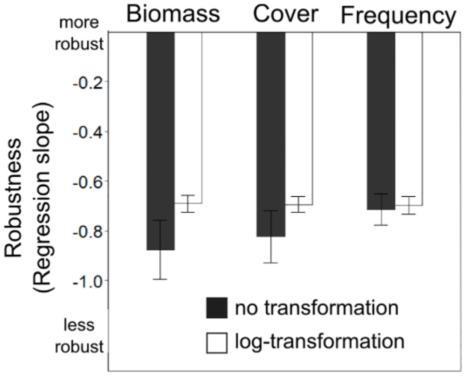

Fig 5. Effect of sampling method and abundance transformation on FD index sensitivity. Barplot depicting the results of linear mixed effects models, specifically the interaction between abundance transformation and the different abundance measures used in plant ecology (all three abundance measures were used for the same plant dataset in order to make their effects comparable). The effect of down-weighting the dominant species by log-transformation of their abundance was most pronounced in the biomass abundance measure. When log transformed, all three sampling methods have a very similar effect on the sensitivity of indices to missing trait data. Error bars denote the 95% confidence intervals.

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Our results suggest that neither pool-wise nor plot-wise scenario should ultimately be considered as "best" when setting data completeness thresholds, and that the decision is contingent upon the particular study system, FD index, and research question. Previous studies have suggested that plot-wise sampling is more appropriate because it produces values closest to the FD values obtained with a complete dataset [7] and can be performed with less sampling effort [14]. However, our approach of testing the ranking of FD rather than the actual values showed no consistent trend in this direction. When considering the effect of missing data under the two sampling scenarios indices behaved differently between the different communities.

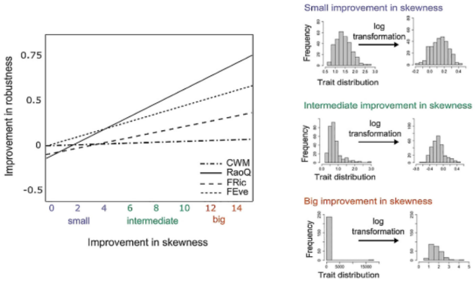

Fig 6. Effect of trait transformation on FD index sensitivity. The effect of trait transformation on the improvement in slope (transformed—untransformed trait data)—the bigger the improvement in slope, the more robust the index becomes to missing trait data (y axis). The right panels illustrate the different improvements in trait skewness, depicting examples of trait distribution before and after transformation, which correspond to the x axis of the main figure (matching colours).

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The effect of abundance distribution, abundance measures and their transformation

Abundance distribution, abundance measures (biomass, cover estimate, frequency) and abundance transformation influenced the robustness of FD indices to the amount of missing trait information. Transforming the abundance leads to a shift of the abundance distribution in a community, thereby changing the relative abundance. In our simulation approach this implies that fewer species are removed at a given level of relative abundance compared to untransformed data. The different indices are designed to capture different aspects of diversity, and they thus responded differently to transformation. We found that functional evenness always improved with transformation because species were given more equal weighting, but functional divergence (RaoQ) improved less or not at all since transformation essentially shrinks the range of values. CWM remained unaffected as it focuses on the dominants even before transformation. Care should be taken in studies comparing functional diversity between communities with different abundance distribution and with the use of different FD indices, since transformation may have different effects on the accuracy of FD indices.

Abundance transformation should not be considered as a purely technical matter, because it reflects our understanding of the community structure. Transforming abundance changes the community structure by flattening the dominance-diversity curves [17]. And therefore the question whether to transform abundance mainly depends on the biological question behind the particular analysis. If, for example, we followed the mass-ratio hypothesis [8] focusing on the effect of the dominant species in a community, then dominant species should be given more weight, in which case abundance transformation would not be the most appropriate option. On the other hand, if the scope of our study included coexistence mechanisms, abundance transformation could better reveal the structure of our community giving biological relevance not only to the few dominant species.

Different abundance measures also influence the number of species for which traits are needed in order to achieve a desired amount of information for a given community. This trend is visible when we consider three different abundance measures available for the plant community in our study (biomass, cover, frequency). For example, the biomass measure after transformation resembled the frequency measure without transformation. The difference between the abundance measures can be explained by how evenly spread the abundance values are [35,36]. Using plant biomass as a measure typically produces the most uneven abundance values. In our plant community for example, the least abundant species accounted for as little as 0.001 g (generally, the precision of weighing), and the dominants exceeded 10g, so the potential difference is several orders of magnitude. Frequency, on the other hand, will have smaller differences, as in our data where it was measured in 25 subplots, and so the possible maximum was only 25 fold higher than the possible minimum. Transforming the abundance data therefore equalizes the effects of different abundance measures, which can be used as a way of standardization in comparative studies and meta-analyses.

The effect of trait distribution and transformation

Our results show that the more the transformation improves the trait data normality (i.e. from very skewed to normal), the more it improves the robustness of the FD indices to missing trait information. FD indices were sensitive to changes in the distribution of trait values (see also [20]), as the changes in distribution altered the functional differences between species in the communities. This was true mainly for indices that comprise the variance of trait distribution, i.e. functional richness and functional divergence (RaoQ). CWM, on the other hand, focuses on the dominant species, and therefore its robustness to missing trait information was not affected by trait transformation (e.g., big species are still big species after transformation).

Trait distribution is of a great importance, as trait data in natural communities vary in the extent that they deviate from the normal distribution, which also influences FD calculations [19,20]. Outlying values or values spreading across several orders of magnitude manifest as the skewness of the data. Some traits are by their nature more normally distributed in a community (e.g. specific leaf area in plants), whereas some are usually highly skewed (e.g. seed weight in plants, body size in ants and body weight in birds; Fig 6 "Big improvement in skewness") and are therefore routinely transformed to meet normality assumptions. As the data are on a ratio scale, the outliers are usually positive, and so the high skewness means that there are either more positive outliers, or there is one highly positive outlier. The most extreme case is when a species with the most different trait value is the rarest one. An example can be found in each of our communities: (1) the plant dataset is dominated by small-seeded grasses and forbs but has a big-seeded and rare legume species (*Lathyrus pratensis* L.), (2) the ant dataset is dominated by small species but the giant forest ant (*Camponotus gigas* Latreille) is very rare, and (3) the bird dataset is dominated by small flying species but also contains the much larger and flightless cassowary (*Casuarius bennetti* Gould, 1857), which is very rare.

Our results empirically support previous suggestions that trait values should be log-transformed, as the relationship between any two species is best characterized by the difference in logs, i.e. by the ratio of traits, rather than by the absolute difference between them [21,22]. Like abundance transformation, the choice of transforming the traits is also dependent on the particular research question. When upscaling for the questions regarding ecosystem functioning, the absolute values of a trait are more important. For example, sometimes it is more interesting to know strictly how tall a species is (absolute value of a trait), rather than how tall a species is compared to its neighbours (transformed value of a trait). On the other hand, it is important to transform trait data when detecting the processes behind community assembly and/or species co-existence, for example how tall a species is compared to its neighbours.

Practical implications

Since functional diversity indices are sensitive to missing trait data, and this sensitivity is further affected by abundance structure, different abundance measures and the transformation of abundance and trait data, all should be given careful consideration during experimental design, analyses and interpretation of functional diversity. One can obtain trait values for all species in the desired study system by: (1) measuring the traits, (2) using trait data from trait databases available, and/or (3) inferring trait data from phylogeny [12]. Even with these tools it is often not possible to have trait data for all species in a community, due to limited time and resources, incomplete or unavailable trait databases and/or constraints of imputation approaches. In this case, one needs to work with a number of traits that are feasible to measure and to make an *a priori* decision for how many and for which species the trait data is essential and whether to set this particular missing trait data safety threshold for the whole pool of species or per each plot separately. As shown and discussed above, in some cases abundance and/or trait transformation can increase the robustness of FD indices, which in practical terms means that for a given amount of effort one could increase the amount of replication of sampling for species abundances at the expense of measuring traits.

To assist researchers in assessing effects of missing trait data and planning trait sampling campaigns we provide the R package "traitor" (see Data Accessibility). The functions in this package help ecologists to estimate trait data availability for their datasets and provide a list of species for which traits need to be obtained to increase data availability and as a result improve the accuracy of FD calculations. Given an existing community data set and information for which species trait data is available, the function within the package estimates how much of the relative species abundance is covered by the available trait data. This is either done on a pool-wise (across all plots) or plot-wise (for each single plot) basis to account for our findings that neither the pool-wise nor plot-wise scenario produce systematically less reliable results. Additionally, the package contains functions to assess how omitting trait data in a given data set can bias FD measures, i.e. it provides the tools to conduct the analyses in this study, and those in [11] and [7]. It can also be used to assess the impact of species loss on functional diversity, i.e. the vulnerability of communities to the extinction of rare species (e.g. [23]).

Conclusions

Our study demonstrates that not only the amount of trait data available, but also the species abundance structure and distribution of trait values have a significant effect on the calculation of FD indices. Consequently, their transformation greatly affects the evaluation of functional diversity. Even though the details about data structure and its transformation often appear a trivial part of FD analyses, we show that they are as important as the amount of available species trait information. Thus the careful treatment of both abundance and trait data is essential to interpret functional diversity and can, to a certain degree, even compensate for the lack of trait data. Such methodological choices are crucial for a faithful evaluation of functional diversity.

Supporting Information

S1 Appendix. Study sites and sampling methods. Detailed description of the sampling and trait collection in the three communities.
(DOCX)

S2 Appendix. Results of the linear mixed effect models. Tables A1 –A5 presenting results of all linear mixed effects models.
(DOCX)

S1 Dataset. Data used for the analysis. Abundance and trait data for our plant, ant, and bird communities.
(ZIP)

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Data Accessibility

Data for this paper is available in S1 Dataset. We also provide the R package "traitor" to help ecologists estimate trait data availability for their datasets and generate a list of species that need to be sampled to increase data availability. The R package is stored on https://github.com/larsito/traitor and can be installed through R using the following lines of code:

```
install.packages (devtools)
library (devtools)
install_github ("larsito/traitor")
```

Author Contributions

Conceived and designed the experiments: MM TP NSP MB LK AN MW LG FdB. Analyzed the data: TP MM LG. Wrote the paper: MM NSP TP MB LK AN MW YLBP LG FdB. Contributed data: SHL TRB KS JL. Commented on manuscript drafts: MM TP NSP MB LK AN MW TRB SHL KS YLBP JL LG FdB.

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Supplementary materials for Chapter IV. Evaluating functional diversity: missing trait data and the importance of species abundance structure and data transformation. – PLOS One.

S1 Appendix: Detailed description of the study systems

Plant data

Plant data consists of 12 plots and 62 species. Plants were surveyed in a species-rich wet meadow located 10 km south-east of České Budějovice, Czech Republic (48°57’N, 14°36’E, altitude 510 m). Plants were surveyed in June 2006 as a part of a long term experiment (1994-2014). The plant community is an oligotrophic wet species-rich meadow (around 30 spp. of vascular plants per m^2) dominated by a tussock grass, *Molinia caerulea* (L.) Moench. Other more abundant species included mostly grasses and over 10 species of sedges (*Carex* L. spp.). The meadow, as those in the region around, has been traditionally extensively mown for centuries.

In 1994, 12 permanent mown plots were established, each 2 × 2 m. Fertilization and dominant removal were applied as treatments in a factorial design. The four treatment combinations (fertilization and removal; fertilization and no removal; no fertilization and removal; no treatment) were repeated three times. The fertilization treatment consisted of annual application of a commercial NPK fertilizer (12% N, as nitrate and ammonium, 19% P, as P_2O_5, and 19% K, as K_2O). *Molinia caerulea* tillers were manually removed with a screwdriver in April 1995 with a minimum of soil disturbance and new individuals of *M. caerulea* were removed annually. More details about the experimental setup are given in ([1–3].

We chose traits that represent the L-H-S scheme proposed by Westoby (1998)[4]: specific leaf area (SLA), plant height, and seed mass. These traits represent independent dimensions of plant ecological strategies [4–7]. We measured plant height, specific leaf area and seed mass values for 42 species following protocols in [8] for a minimum of 10 individuals collected in the different treatments where the species occurred [9]. Trait values were then averaged to allow comparisons with values taken from LEDA trait database [10] for the remaining species (approximately 20 depending on the sampling method). Specific leaf area represents the ratio of leaf area to dry weight (m^2 kg^{-1}). The two categorical traits, growth form and position of leaves along the stem, were also taken from LEDA trait database and from local floras.

Ant data

Ant data consists of 59 plots and 297 species. Ant data were collected in April and May 2010 as part of the Stability of Altered Forest Ecosystems (SAFE) project in Sabah, Malaysia [11,12]. In total the survey included 59 plots in different habitat types; 9 plots in a single block of oil palm, 32 plots in two blocks of secondary forest and 18 plots in two blocks of primary forest. Ant sampling was conducted at 2nd order survey points arranged in equilateral triangles with sides of 178 m. Oil palm and secondary forest sites are located in Benta Wawasan, a matrix of oil palm *Elaeis guineensis* Jacq. monocultures planted in the year 2000 and twice-logged forest of varied quality. Primary forest sites are in Maliau Basin Conservation area, and not subject to anthropogenic disturbance.

For each sampling point, as close to the 2nd order point as possible, a 4 × 4 m quadrat was set out. In each square meter of the quadrat a 12 × 12 cm soil sample with a depth of 10 cm was extracted (16 soil pits per quadrat) and the soil searched for ants for 10 person-minutes. In addition, dead wood within the quadrat was examined for ants by removing the bark and investigating any holes or crevices. All specimens were preserved in 80% ethanol. Ants were identified to species level using published keys and online resources (see references in [13]), with the majority of specimens being assigned to morphospecies due the inaccessibility of a reference collection and lack of adequate keys for certain rare or hyperdiverse Bornean genera.

Morphological traits related to the species' ecology were measured to the nearest 0.01 mm using an ocular micrometer attached to a Lecia MS5 stereo-microscope (Leica Microsystems, Heerbrugg, Switzerland). The following traits were measured for the worker caste only: head length, the ratio of leg length to body size, pilosity, sculpturing. Since the reproductive castes have a non-foraging role and major castes are not sampled effectively using the above protocol they were not included in the study. Head length is highly correlated, and thus a good surrogate of body length, which relates to many life history traits such as prey selection [14] and thermal tolerance [15]. Leg length may be an adaptation to carrying different loads of foraging items [16], and the spatial niche in which the species forages [17]. As it is highly correlated with body size we chose to express leg length as a ratio of body size: the sum of the hind femur and hind tibia length/head length. Pilosity and sculpturing may be related to the ant thermal tolerance. See [18]for a summary of ant traits and their hypothesised ecological relevance.

Bird data

Bird data comprise 8 plots and 238 species. A repeated survey of forest birds was carried out along an elevational rainforest gradient on the slopes of Mt. Wilhelm (4509 m a.s.l.) in the Central Range of Papua New Guinea between 9th April 2010 and 15th October 2012 [19,20]. The study was completed along a 30 km long transect with eight study sites, spanning from lowland floodplains of the Ramu river (200 m) to the timberline at 3700 m, evenly spaced at 500 m elevational increments. At each elevation, bird communities were surveyed by point counts (n=14), mist-netting (n=11) and random walks (20 hrs total) through the area (80 ha). Point counts were conducted at 16 points regularly spaced along a 2250 m transects (successive points 150 ± 5 m apart to avoid overlap). Transects at each site were directed through the representative and diverse microhabitats in the area (e.g. ridges, valleys, streams). All birds seen or heard within 50 m of the point were recorded. Censuses began 15 min before sunrise, at a randomly selected point, and each count lasted 15 min with all 16 points surveyed before 11am. At each study site, we mist-netted birds along a 200 m line (using nets 2.5 m high × 12-18 m long, mesh 16 mm) from 5:30am till 5:30pm daily. We identified all mist-netted birds, marked them with colour bands, and released them within 10 min. Finally, we walked along existing trails and along point-count transects, surveying the area of 80 ha evenly, and noted all species identified during these random walks (within a 50 m radius). Walks lasted 2-3 h per day (starting at 3pm, walking distance = 3-4 km/d), and were standardized to 20 h per site. All surveys were conducted by three people, all of whom had previous experience with bird surveys in Papua New Guinea. We also recorded vocalizations at each survey point during surveys and any unrecognized bird vocalizations during our random walks (using a Marantz PMD 620 recorder and a Seinnheiser ME67 microphone) to allow later identification.

Birds were identified using the species-level taxonomy of Handbook of the birds of the world [21] and updated according to IOC Bird World List 5.1. All species were categorized into three broad trophic guilds: insectivores, herbivores (granivores + frugivores) and omnivores (with equal intake of different items), based on dietary information from the field and in standard references [19, 21, 22, 23]. Only forest species were included in the analyses and all raptors and swifts were excluded (68 individuals of 15 species) since it was difficult to sample them in a standardized manner from the forest interior. We assessed three ecologically relevant traits; one categorical (trophic guild) and two continuous traits (body length and weight) of 238 species. The body measurements (body length and weight) for most of the birds in analysis (238 species, 87.5% of total) were obtained as averages from mist-netted birds. Data on body measurements for the other 34 bird species were obtained from Museum collections (Natural History Museum of Denmark, Queensland museum) and from literature [21].

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S2 Appendix: Results of the linear mixed effect models

Table A1. Full results of the general linear mixed effects models for plant data showing the effect of the two scenarios (pool-wise and plot-wise; “Scenario”), FD indices (functional richness, functional evenness, Rao’s Quadratic entropy index, and community weighted mean; “Index”), and abundance transformation (“Abun.Transf.”) on robustness of FD indices to missing trait data (the regression slopes).

Predictors	Df	F	*P*
Intercept	1, 493	110.8	<0.001
Scenario	1, 493	13.28	<0.001
Index	3, 493	236.22	<0.001
Abun.Transf.	1, 493	12.24	<0.001
Scenario × Index	3, 493	21.10	<0.001
Scenario × Abun.Transf.	1, 493	0.03	n.s.
Index × Abun.Transf.	3, 493	27.20	<0.001
Scenario × Index × Abun.Transf.	3, 493	1.66	n.s.

Table A2. Full results of the general linear mixed effects models for ant data showing the effect of the two scenarios (pool-wise and plot-wise; “Scenario”), FD indices (functional richness, functional evenness, Rao’s Quadratic entropy index, and community weighted mean; “Index”), and abundance transformation (“Abun.Transf.”) on robustness of FD indices to missing trait data (the regression slopes).

Predictors	Df	F	*P*
Intercept	1, 110	311.7	<0.001
Scenario	1, 110	101.68	<0.001
Index	3, 110	170.13	<0.001
Abun.Transf.	1, 110	189.39	<0.001
Scenario × Index	3, 110	24.84	<0.001
Scenario × Abun.Transf.	1, 110	7.65	0.007
Index × Abun.Transf.	3, 110	14.89	<0.001
Scenario × Index × Abun.Transf.	3, 110	0.93	n.s.

Table A3. Full results of the general linear mixed effects models for bird data showing the effect of the two scenarios (pool-wise and plot-wise; "Scenario"), FD indices (functional richness, functional evenness, Rao's Quadratic entropy index, and community weighted mean; "Index"), and abundance transformation ("Abun.Transf.") on robustness of FD indices to missing trait data (the regression slopes).

Predictors	Df	F	*P*
Intercept	1, 84	37.1	<0.001
Scenario	1, 84	0.29	n.s.
Index	3, 84	20.25	<0.001
Abun.Transf.	1, 84	0.48	n.s.
Scenario × Index	3, 84	0.09	n.s.
Scenario × Abun.Transf.	1, 84	0.005	n.s.
Index × Abun.Transf.	3, 84	5.10	0.003
Scenario × Index × Abun.Transf.	3, 84	0.09	n.s.

Table A4. Full results of the general linear mixed effects models for plant data showing the effect of the two scenarios (pool-wise and plot-wise; "Scenario"), FD indices (functional richness, functional evenness, Rao's Quadratic entropy index, and community weighted mean; "Index"), abundance transformation ("Abun.Transf."), and abundance measure ("Measure") on robustness of FD indices to missing trait data (the regression slopes).

Predictors	Df	F	*P*
Intercept	1, 461	104.1	<0.001
Scenario	1, 461	14.1	<0.001
Index	3, 461	251.6	<0.001
Abun.Transf.	1, 461	13.1	<0.001
Measure	2, 461	3.8	0.02
Scenario × Index	3, 461	22.5	<0.001
Scenario × Abun.Transf.	1, 461	0.03	n.s.
Scenario × Measure	2, 461	0.5	n.s.
Index × Abun.Transf.	3, 461	28.9	<0.001
Index × Measure	6, 461	2.8	0.01
Measure × Abun.Transf.	2, 461	6.3	0.002
Scenario × Index × Abun.Tranf.	3, 461	1.7	n.s.
Scenario × Index × Measure	6, 461	2.3	0.04
Scenario × Abun.Transf. × Measure	2, 461	2.1	n.s.
Index × Abun.Transf. × Measure	6, 461	1.2	n.s.
Scenario × Index × Abun.Transf. × Measure	6, 461	0.2	n.s.

Table A5. Full results of the general linear mixed effects models showing the effect of trait data transformation ("Trait Transf."), difference in skewness of trait data before and after trasformation ("Skewness"), the two scenarios (pool-wise and plot-wise; "Scenario"), and FD indices (functional richness, functional evenness, Rao's Quadratic entropy index, and community weighted mean; "Index") on robustness of FD indices to missing trait data (the regression slopes).

Predictors	Df	F	*P*
Intercept	1, 274	12.0	0.001
Scenario	1, 274	0.6	n.s.
Index	3, 274	3.5	0.02
Trait	1, 274	1.1	n.s.
Skewness	1, 274	47.4	<0.001
Scenario × Index	3, 274	0.1	n.s.
Scenario × Trait	1, 274	0.3	n.s.
Scenario × Skewness	1, 274	2.0	n.s.
Index × Trait	3, 274	1.3	n.s.
Index × Skewness	3, 274	6.8	<0.001
Trait × Skewness	1, 274	1.8	n.s.
Scenario × Index × Trait	3, 274	0.1	n.s.
Scenario × Index × Skewness	3, 274	1.8	n.s.
Scenario × Trait × Skewness	1, 274	<0.01	n.s.
Index × Trait × Skewness	3, 274	1.3	n.s.
Scenario × Index × Trait × Skewness	3, 274	0.1	n.s.

Summary

Thesis Summary

This thesis explored the diversity and ecology of arboreal ants of tropical rainforests in Papua New Guinea (PNG) over gradients of elevation and succession. Our datasets, from the most extensive arboreal sampling to date, use tree dissections and a census of understorey ant plants to provide a unique view of whole communities of tree-dwelling ants and the factors influencing their diversity and species composition along ecological gradients.

The thesis used data from 1.4 ha of continous rainforest (Introduction -Table 1), where over 1600 trees ≥5cm DBH were felled and exhaustively sampled for ants and their nests. In just this area, we recorded 195 ant species living and/or foraging in trees. The total number of recorded ant species in New Guinea is 892 (AntWiki, 2018), so our findings further highlight the extraordinary diversity of arboreal ants in this region. Additionally, smaller ant-plants in the rainforest understorey were surveyed from 700 – 1600 m above sea level to quantify for the first time whole communities of ant-plant mutualists over an elevational gradient. Here we found 10 different species of ants living in domatia of 23 different tree species, many of which had not previously been recorded as myrmecophytes.

This thesis goes far beyond providing inventories of ant biological diversity in the remote PNG rainforests we sampled. With our unique datasets from felling whole forest plots, we explored the effects of vegetation structure and nest microhabitats on the community structure of arboreally nesting ants over elevation, using modern multivariate statistics and null modelling approaches. Moreover, after quantifying the taxonomic diversity of arboreal ants with elevation and succession, we measured functional traits of 127 lowland ant species, and constructed their community phylogeny to investigate for the first time the effects of succession on both functional and phylogenetic diversity of arboreal ant communities. Further to this study, using datasets from ants, birds and plants, we considered how functional diversity indices stand up to different data-handling techniques, and how robust these indices are to a common problem faced by ecologists – incomplete species trait datasets.

Below I summarise our main findings from each chapter and discuss some future directions for arboreal ant community ecology research.

Main findings and conclusions

In Chapter I we investigated nesting arboreal ant communities over an elevational gradient, and the potential drivers of differences in community structure and diversity. We found a strong mid-elevation peak in diversity and nest abundance of arboreal ants, in contrast to findings from understorey and terrestrial ants in the PNG region (Colwell et al., 2016; Orivel et al., 2018), and terrestrial ants from a comparable gradient in Borneo (Brühl et al., 1999) which saw rather monotonic declines in ant diversity. While tree size and nest site were important explanatory factors for the differences in communities over elevation, we found that differences in nesting microhabitats across elevation explained more of the variation than tree DBH or elevation itself. Indeed, in mid-elevation forest trees of all sizes had a greater diversity of ant species than at other elevations. Our findings suggest that the smaller, and more cryptic nests in trapped aerial soil and under epiphytes most common in the more humid mid and high elevation forests may contribute to this trend. Furthermore, smaller colonies of less territorial species may have contributed to the mid-elevation peak in diversity, by enhancing positive or neutral species coexistence in trees, while in high elevation the cooler temperatures are likely a strong limiting factor to these ectothermic animals. In contrast, in the lowlands the prevalence of highly aggressive territorial species nesting in carton or silk nests on leaves in the canopy may act to limit biodiversity via competitive exclusion.

While in the canopy ants tend to be generalists in which tree species they nest in (Klimes et al., 2012, 2015), in the understorey some ant species exclusively nest in domatia-bearing ant-plants. In Chapter II we focused on these interactions in the rainforest understorey and quantified ant-plant mutualistic interactions over elevation. We found a diverse community of ant-plant mutualists, with elevational shifts in species composition and abundance, with the ant genus *Anonychomyrma* as the dominant partner in these networks, and the ant-plant *Myristica subalulata* as the most common wide-ranging ant-plant species. At higher elevations ant-plants were rarer and species richness of both groups declined, resulting in less complex interaction networks and decreased network specialisation. We found that ant behaviour also changed with elevation, signalling a change in costs and benefits of the interaction. As elevation increased, ant partners recruited less to an experimentally placed surrogate herbivore, and spent less time attacking it. Moreover, plants suffered higher herbivory damage at high elevation. However, the shift in ant behaviour was driven by turnover of ant species, suggesting that reduced availability of effective ant partners at high elevations results in less specialised networks and may ultimately lead to the breakdown of the mutualism due to increased fitness costs to the plant.

In Chapter III we investigated the effect of succession on the functional and phylogenetic diversity (FD and PD respectively) of arboreal ant communities and their structure in primary and secondary lowland forests in PNG, exploring the contribution of visiting species (i.e. species not nesting in the trees sampled) to both FD and PD in each habitat. We found that unlike taxonomic diversity, there were smaller differences in FD and PD between the two forest types. Interestingly, while both communities had neutral phylogenetic structure (i.e. species were not over or underdispersed compared to a null model), in primary forest (but not secondary forest), species were slightly more functionally clustered than expected by chance, indicating an effect of niche-based habitat filtering. However, this small effect disappeared when only nesting species were considered, they too were randomly structured. The effect of removing the visiting species revealed some interesting patterns in the communities. While PD was relatively robust, in our rarefactions of FD we found that excluding non-nesting species increased overall species dissimilarity in primary forest, whereas in secondary forest species dissimilarity decreased. This indicated that in primary forest the visiting species were functionally redundant but in secondary forest they boosted the functional diversity of the more species impoverished forest. In terms of individual functional traits, primary forest hosted on average larger ant species and the largest species were altogether missing in secondary forest. This agrees with previous findings that the largest ant species can be lost after forest disturbance (Gibb et al., 2017b).

Unusually for studies of FD, our data included traits of all species in the community. However, often FD studies are limited by the availability of trait data, and often use instead only the most common species for which traits are available. In Chapter IV we explore the effects of missing data on the robustness of FD indices and how this can be affected by the distribution of trait and abundance data. We used data from real ant, plant and bird communities. Unsurprisingly we found that FD indices became less accurate the more data was missing, but this loss of accuracy was dependent on the index. Interestingly, in cases where data transformation improved the normality of the trait data, the effect was to improve the accuracy of the indices in the face of missing data. Our results highlighted that the common practice of *de facto* selecting only the species that compose 80% of the community in FD studies needs more careful consideration since selecting your most common species by how common they are per plot or how common they are in the whole community makes a difference to the accuracy of FD indices since the trait and abundance data distributions can vary from plot to plot. We provided an R function which can help ecologists to make these decisions.

Future directions

The field of arboreal ant ecology still holds many mysteries, since the ecology of most ant species remains unknown, despite the numerical and ecological importance of the group (Wilson & Hölldobler, 1990; Lach et al., 2010). While this thesis is unique in its scope and details of the ecological drivers of arboreal ant diversity, it remains limited to a few forest plots in one tropical region. For community ecologists, the priority of research in ant diversity should move from single locality studies to comparative studies across habitats and regions. Such studies promise to reveal if our conclusions about the mechanisms responsible for structuring ant communities hold across the regions, or if they differ, a topic still widely debated. Importantly during this era of widespread anthropogenic habitat modification and global warming, advances in this area will help in predicting the effects of species loss on arboreal communities and ecosystems.

This will require a global effort to standardise sampling techniques, and extensive international collaboration and data sharing. Vojtech Novotny and colleagues are leading the way by conducting further whole forest sampling across the world, felling whole forest plots and conducting canopy crane studies in Czech Republic, USA, Panama, PNG and Japan with the aim to compare latitudinal diversity trends but also to test predictions of biodiversity based on initial plant species richness (Novotný et al., 2015). In addition, it would be helpful to bring together studies of different scales to better understand how arboreal ant communities are structured from the immediate intraspecific interactions in trees, to local communities and regional scales. The global network of 50h forest plots (the Smithsonian's oForestGEO network, formerly CTFS; https://forestgeo.si.edu/), currently used to monitor plant community dynamics, would be an excellent resource for ant ecologists to this end. These long-term plots would also be ideal for the study of arboreal ant community dynamics over the longer term, especially to monitor the effects of climate change.

Similarly, ant functional diversity studies would also benefit from an increasingly comparative approach. FD studies in ants are still in their infancy, but are increasingly growing in popularity (e.g. Blaimer et al., 2015; Liu et al., 2016; Rocha-Ortega et al., 2018). The recent introduction of global databases for ant traits and species abundances (Gibb et al., 2017a; Parr et al., 2017) is promising for global comparative studies of ant communities. However there are a jungle of different methods and indices in functional ecology, so developing standard methodology for trait measurement like plant ecologists (Cornelissen et al., 2003), and identifying the most ecologically important ant traits with experimental studies will become increasingly important for such global comparisons.

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Wilson, E.O. & Hölldobler, B. (1990) The ants. Harvard University Press.

Curriculum vitae

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Research & Education

Researcher | 2018 – Present | Czech Academy of Sciences

Ant-plant mutualisms in degraded & fragmented forests in Borneo. Fieldwork, research & analysis of ant – *Macaranga* interactions. The effect of invasive ants on the costs & benefits of the mutualism, and long-term dynamics of ant-*Macaranga* communities.

PhD in Entomology | 2012 – Present | University of South Bohemia

Community ecology of ants & plants with elevation & succession in New Guinea rainforest. Extensive fieldwork in remote parts of Papua New Guinea, ant mounting & identification, DNA barcoding, data management and analysis of large ecological datasets.

MSc in Entomology | 2011 – 2012 | Imperial College London

Taxonomy, ecology, conservation and applied ecology of insects. 3 months fieldwork in Malaysian Borneo. Research on effects of fungal and invertebrate exclusion on leaf litter decomposition rates across a gradient of forest disturbance.

Internship | Sep – Dec 2011| Centre National de la Recherche Scientifique

Cell culture, DNA extraction, data management in molecular laboratory of French national research body, supported by the EU funded Leonardo da Vinci Programme.

BSc Biology with French | 2005 – 2010 | University of St Andrews

General biology with French language modules & integrated year as a teacher in Lille, France.

Additional courses

Ant Course 2014, Maliau Basin, Borneo (California Academy of Sciences)
Bugshot 2014, Cairns, Australia (Alex Wild's insect macrophotography course)
Tropical Biology Association field course 2010, Kibale, Uganda.

Communication & outreach

Languages: English, French, Czech, Malay, Tok pisin (Papua New Guinea pidgin).
Guest scientist: CatCave science improvisational comedy show in Lausanne, Switzerland (2016).
Summer school Science Demonstrator at Cambridge University & Botanic gardens, 2016.
Science communicator at Edinburgh International Science Festival 2012.

Grants and awards

Varley Gradwell Travelling Fellowship in Insect Ecology 2018 (£2,500).
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Best poster at Royal Entomological Society (RES) Postgraduate forum 2015.
Travel & training grants from IUSSI (£940), BES (£500), and RES (£500) in 2014.
RES scholarship for Entomology MSc. tuition in 2011 (£4000).

Conference Presentations

Plowman, N.S. 2018. The effect of invasive ants on ant-Macaranga mutualisms & long-term dynamics of ant-Macaranga communities in degraded forests. Insect-plant mutualistic interactions workshop 2018. Ceske Budejovice, Czech Republic. 2nd June 2018. (Talk)

Plowman, N.S., 2016. Arboreal ant ecology and the role of research in conserving PNG rainforest. Invited speaker, Cambridge Conservation Initiative & University of Cambridge. 13th July 2016. Cambridge, UK. (Talk)

Plowman, N.S., Hood, A.H., Moses, J., Redmond, C., Novotny, V., Klimes, P., Fayle, T.M. An uphill struggle: network simplification of a montane ant-plant network in Papua New Guinea. Ants 2016: Ants and their biotic environment. Munich, Germany. 5 – 7th May 2016. (Poster)

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