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Spatial structure and community dynamics of arboreal ants in tropical rainforests

Ph.D. Thesis

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Annotation

This thesis explores the drivers of distribution patterns and community structure of ant communities in tropical rainforest. The aim is to test how diverse arboreal ant communities are shaped by ecological gradients such as latitude, succession or forest strata, on both small and large spatial scales. This work represents a compilation of studies based on observational patterns as well as field experiments. Plot-based sampling and advanced statistics (e.g. multivariate analyses, rarefactions, and null-models) are used throughout the thesis to explore ant distribution in tropical forests. Manipulative experiments with artificial nests, and inter- and intra-behavioural trials, are used to test the hypotheses of non-random vertical species distribution (community stratification) and segregation within- and across- individual trees (*ant mosaics*). The effect of secondary succession on canopy ants is disentangled using unique montane forest datasets and the results are compared with previous lowland-based studies. On large geographical scale, general patterns of ant abundances are described along a latitudinal gradient and used to test the hypothesis of spatial segregation of ants with other arthropods on individual trees. The thesis results suggest that the behaviour of individual species and microhabitats are more important factors at a local scale, while forest structure and climate influence ants over larger scales.

Declaration [in Czech]

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

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Ondřej Mottl

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List of papers and author's contributions

The thesis is based on the following papers:

- I. **O. Mottl**, J. Yombai, T. M. Fayle, V. Novotny, and P. Klimes. 2019. Experiments with artificial nests provide evidence for ant community stratification and nest site limitation in a tropical forest.

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OM and PK designed the study. OM and JY performed the experiments. OM analysed and interpreted data, and led manuscript writing. OM, JY and PK identified ant specimens. PK and TMF contributed significantly to the text, the ideas and interpretation of the results. VN provided financial resources and co-supervised the work with PK.

- II. **O. Mottl O.**, J. Yombai, V. Novotny M. Leponce, G. D. Weiblen, P. Klimeš. Ant mosaics in primary forest canopies are driven by interspecies behaviour. (Manuscript)

OM conceived the study, led the fieldwork, identification of ant specimens, manuscript writing, analysed and interpreted the data. OM and JY collected the data and conducted the experiments. GDW contributed plant data from the CTFS database. PK contributed to specimen identification, designing the study, and writing of the first draft. PK, VN, and ML contributed the conceptualisation of the methods and interpretation of the results. All authors critically commented on drafts and gave final approval for publication.

- III. **O. Mottl**, N.S. Plowman, V. Novotny, B., Gewa, M, Rimadai, P. Klimes. 2019. Secondary succession has surprisingly low impact on arboreal ant communities in tropical montane rainforest.

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OM led the manuscript writing, analysed and interpreted the data. PK and VN designed the study and contributed financial resources. BG and MR led the field work and collected the data; OM, NSP and PK identified ant specimens; PK, VN and NSP contributed to text and interpretation of the results.

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OM led the manuscript writing, analysed and interpreted the data. OM, VN, GW, PF, PK, and MV conceived the ideas and designed the study. OM, PK, MV, JA, TB, GC, EG, AG, GL, ML, MM, KM, GN, NSP, CR, CS, and JV led the data collection for the arthropods guilds in different sites. OM, PK, MV, KAT, PB, PD, OK, GL, SEM, NSP, CR, and CS contributed to data management. PF contributed to data analysis. VN, PF, PK, MV, and RT contributed to text and interpretation of the results.

Co-author agreement

Petr Klimeš, the supervisor of this Ph.D. thesis and co-author of chapters I - IV, fully acknowledges the major contribution of Ondřej Mottl to these manuscripts.



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RNDr. Petr Klimeš, Ph.D

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~ Introduction ~

Thesis introduction

Spatial structure and community dynamics of arboreal ants in tropical rainforests

~ Overview ~

Tropical rainforest is one of the most diverse ecosystems on the planet (Fisher A. G. 1960, Hamilton et al. 2010). Organisms in this system interact with each other in many ways, representing an incalculable number of theoretical ecological links, which together, affect the entire ecosystem (Chapin et al. 1997). This creates a very complex structure, where one organism can have ecological links with many others, and therefore participate in a cascade of effects (Pace et al. 1999). The study of these connections is at the forefront in modern tropical ecology, with research focusing on species assembly rules (Diamond 1975, Weiher and Keddy 1999) and food webs (May 1973, Paine 1980, Novotny et al. 2010). Arthropod communities are not only very species-rich and numerous in terms of abundances and biomass in tropical rainforests (Hamilton et al. 2010), but also occupy all forest strata and fulfil many ecological niches (Wilson 1988, Collins 2012). Studying species assembly rules of those communities and their interactions is crucial for understanding the functioning of these extraordinarily complex tropical rainforests, which are rapidly vanishing from this planet, with deforestation estimated at 5.5 M ha of tropical forest per year (Keenan et al. 2015).

The main focus of this thesis is the community structure of ants in tropical rainforests of New Guinea, with an emphasis on ant species abundances (individuals and/or nests), and their changes across different spatial scales. The aim is to uncover various drivers of their distribution patterns, such as stratification, succession, or effects of ant dominance by combining observational studies of the ant patterns in tropical vegetation, and experimental manipulations of their nests and live workers. Finally, the effect of ants on the spatial distribution of other arthropod herbivores is tested in both tropical and temperate forest canopies. Overall, this thesis uses datasets collected from ‘whole-forest’ plots, rather than focusing only on individual trees of a particular

size, as has been typical for most previous studies (e.g. Schulz and Wagner 2002, Salazar and Marquis 2012, Fayle et al. 2013).

~ *Tropical rainforests of Papua New Guinea and the main ecological gradients affecting their structure* ~

One of the most diverse tropical regions in the world is Papua New Guinea (PNG hereafter), a large tropical island hosting about 5% of the world's biodiversity. It is one of the world diversity hotspots with plant diversity of 20,000 vascular plants (Swartzendruber et al. 1993). In addition, it is one of the five places on the planet with the status of '*wilderness with high biodiversity*' (Mittermeier et al. 2003). The '*tropical rainforest*' of PNG is used rather as an umbrella term since rainforests are represented by various types of forest habitats, with large biogeographical complexity and different climate zones, mainly due to mountain ranges, with relatively recent origin (Toussaint et al. 2014).

Tropical rainforests of PNG are not homogenous habitats. In contrast, they are ever-changing ecosystems, due to many simultaneous processes, and their responses to various gradients. The gradient with one of the highest impacts on rainforest vegetation structure is elevational, including the presence of a complete rainforest gradient ranging from coastline to the tree line at 3700 m asl at Mt. Wilhelm, the highest peak of the island (Leponce et al. 2016). The different forest habitats across elevations can be described in a simplified classification as '*lowland alluvial forest*' (200-700 m asl), '*foothill forest*' (700-2200 m asl) and '*montane forest*' (above 2200 m asl) (Paijmans 1976). There are several important changes in forest structure with increasing altitude that can affect associated arthropod fauna: increase number of stems and decrease of tree size (Paijmans 1976), decrease in plant diversity, and canopy becoming more open with a higher amount of epiphytes (Ding et al. 2016). Mean annual temperature at Mt. Wilhelm decreases from 25.8 °C at 100 m asl with a constant rate of 0.42 °C per 100 vertical meters (McAlpine et al. 1983). This is crucial as the change of temperature is considered to be the main abiotic driver for many different organisms, especially ectotherms (Fisher A. G. 1960, Lister and Garcia 2018).

Disturbance of tropical rainforest creates mosaics of secondary succession patches surrounded by old-growth primary forests. In contrast to other tropical regions, the majority of people in PNG have customary ownership of land and perform '*slash-and-burn*' swidden agriculture. This practice is, in addition to selective logging, the main cause of human-caused forest disturbance in the region (Shearman et al. 2009). However, the swidden agriculture occurs in rather small forest patches (typically 0.2 to 1 ha), which are left for natural regrowth after several harvests. In addition to human-caused disturbances, the natural disturbance is also common, caused by tree falls and/or landslides, which are common especially at a higher elevation of PNG. All these disturbances lead to the process of forest regeneration to the original state after destruction, i.e. ecological succession. Succession is one of the few complex community-level processes that are relatively well-understood by ecologists (Glenn-Lewin et al. 1992, Palmer et al. 1997, Shugart 2003). It is associated with considerable shifts in vegetation structure, species composition and plant functional traits: fast-growing pioneer species are very light-demanding plants, with short-lived leaves that recruit from a limited number of lineages, later replaced by slow-growing shade resistant forest trees in a locally predictable chronological sequence (Glenn-Lewin et al. 1992, Chazdon 2014). This all leads to very dynamic forests in PNG with a tree mortality rate of nearly 4%, which is higher than in other tropical forests in South-East Asia or Neotropics (Vincent et al. 2018).

In addition to elevation and succession, the tropical rainforests can be also divided into different stratification layers, which are dependent on many abiotic factors, thus creating a large number of different habitats. For example, tree canopies are affected by fluctuations in daily temperature, wind speed, and humidity (Parker 1995). The strength of variation of those abiotic factors then diminishes from the top of the canopies into deeper layers. Therefore, the undergrowth is a much more stable habitat but does not get as much light. Despite the differences in the height of the individual layers (the ground, low vegetation, and crowns), the vertical stratification of the forest is not definitive, with lianas or old broken branches connecting the different strata (Baker and Wilson 2000), and the homogenous canopy being disrupted by tree gaps and emerging trees.

To summarise, we can understand tropical rainforest as a very complicated system with many biotic and abiotic factors creating various gradients. Thus, studying how this ecosystem is functioning should be one of the main pillars of modern ecology. New Guinea represents a suitable region for such studies, with well-preserved rainforests and diverse flora and fauna.

~ *Tropical ants and drivers of their distribution* ~

Ants (Hymenoptera: Formicidae) represent one of the most diverse, abundant and ecologically important animal groups on this planet (Holldobler and Wilson 1990, Lach et al. 2010). Currently, there are more than 15,000 described species (antwiki.org), making up nearly 15% of all animal biomass in tropical lowland forest (Fittkau and Klinge 1973). In addition, ants are often characterised as ecosystem engineers, due to many ecological niches they fill: e.g. scavengers, predators, seed-dispersal agents, decomposers, and, through their trophobiosis with hemipteran insects, also secondary herbivores (Holldobler and Wilson 1990, Lach et al. 2010). This variety of associations makes ants a ‘keystone’ animal group, not only, in tropical rainforests (Lach et al. 2010).

Diversity of ants, similarly to many other organisms, is highest at the Equator (Fisher A. G. 1960). Rainforests of PNG, with a more than 900 described ant species, and perhaps a similar number still formally undescribed (Janda et al. 2016), are ant diversity hotspot. Species richness can be more than 120 ant species per only 0.04 ha area of a lowland forest across multiple strata (Janda and Konecna 2011), and over a hundred species have been found nesting in trees in less than a 1-ha of the forest (Klimes et al. 2015). Despite this high richness and the ant essential role in the functioning of ecosystems, many species remain unknown to science (Snelling 1998). Furthermore, there is a relative lack of knowledge of ecological characteristics and interactions of the ant species with each other, as well as with other organisms, in species already described. This is especially true for tropical canopies, where ants represent 20–70% of the arthropod biomass and a high proportion of individuals (Hammond 1992, Tobin 1995), but, similarly to other arthropods, remain relatively unknown (Basset 2003, Novotny et al. 2006, Hamilton et al. 2010). In terms of the distribution of

ant communities across forest strata, ants play major role in canopy ecosystem with estimates of half of all ant species of a forest being active in trees (Floren et al. 2014) and at least a quarter found exclusively in the tree crowns (Bruhl et al. 1998). Therefore, the species composition of ants is very different among strata of tropical rainforest (Ryder Wilkie et al. 2010).

The general ecological success of ants seems to be connected to their highly organized eusocial colony lives and to their great ability to adapt to various environments (Holldobler and Wilson 1990, Lach et al. 2010). Thus, the adaptability to the various environment makes the ants a great model organism for studying the effects of various gradients on the species assembly rules (Arnan et al. 2018). Prominently, diversity and species composition of arboreal ants communities are known to change rapidly along various environmental gradients, i.e. latitude, altitude, succession and stratification (Lach et al. 2010).

Ant diversity and abundances are very good indicators for testing the effects of various ecological gradients and their combination. For example, the latitudinal gradient of ant diversity is well known with ant species richness strongly decreasing from tropics to temperate, with only small differences between the main subfamilies (Dunn et al. 2010). There are four times fewer ant species in the canopies of the temperate forests compared to the tropical ones (Jaffe et al. 2007). Ant abundance is following a similar pattern to the species richness, as there are more ant individuals in tropical canopies than in temperate (Floren et al. 2014). However, studies based on abundances and/or biomass of ants are often conducted by canopy-fogging of several big trees, neglecting the potential differences between tree sizes, and therefore differences in total numbers of ants in the whole ecosystem. Ants, as ectotherms organisms, are also strongly affected by temperature (Cerdá et al. 2013) and mean temperature declines not only with latitude but also with elevation. Studies of ground ants communities with changing altitude had shown, how ant activity, abundances and species richness decline with increasing elevation (Fisher 1996, Samson et al. 1997, Bruhl et al. 1999). However, since strata could respond to elevation changes differently (Scheffers et al. 2013), there is a relative lack knowledge about changes of arboreal ant communities with elevation (but see Samson et al. 1997, Orivel et al. 2018). Since species composition of ants is different among

strata of tropical rainforest, there is potential that those communities respond to other ecological gradients differently. However, some ecological gradients have very predictable effect, as secondary succession, with species composition and diversity of ants being affected usually in a similar way in lowland rainforest, disregard of forest strata: ant diversity increases with forest age and species composition changes between successional stages with less complex vegetation hosting less of the ant species (Schulz and Wagner 2002, Osorio-Perez et al. 2007, Klimes et al. 2012).

~ *Ants and their effects on other organisms* ~

The arboreal ants developed connections to the trees they nest in, on both evolutionary and ecological scales (Ness et al. 2010, Klimes 2017). There is a whole spectrum of tree-ant interactions ranging from ant species, which nest in trees very non-specifically (Klimes 2017), to very close mutualistic relationships (i.e. myrmecophytic tree species used by one or few specialised ant species). The latter is commonly entitled '*ant-plant interactions*' (Holldobler and Wilson 1990, Ness et al. 2010). In addition, many of those arboreal-nesting species are active on not only trees they are nesting in but also across multiple canopies, where they forage for food resources (Klimes et al. 2015, Adams et al. 2017).

Ants are also interacting with many animals in rainforest canopies, helping the trees as predators of herbivorous arthropods, and damaging the trees as secondary herbivores. The most know interactions between ants and other arthropods are part of a symbiosis between ants and hemipteran insects (Holldobler and Lumsden 1980), where ants tend those sap-sucking species for sugar-rich honeydew in exchange for protection from predators. This interactions are very evolutionary old, and they are happening among several suborders of sap-sucking insects and many genera of ants (Ness et al. 2010, Klimes et al. 2018). It is suggested that ants are, together with birds and spiders, one of the main top-down controls of herbivore arthropod abundance in forest tropical canopies (Holmes et al. 1979, Riechert and Lockley 1984, Floren et al. 2002). Even though there is some ambiguous evidence for birds having a higher effect on the herbivorous arthropods (Philpott et al. 2004), ants have been repeatedly shown as the source of predation pressure on herbivorous insect and

herbivory damage in temperate forest canopies (Mooney 2007, Piñol et al. 2010). A similar phenomenon is also known from tropics, although it has been usually demonstrated in particular species of myrmecophytes (Letourneau et al. 1993), or rather indirectly via observations on baits (Novotny et al. 1999, Plowman et al. 2017). This ant predation effect is considered to have an effect on trophic cascades and other interactions (Cardinale et al. 2003, Finke and Denno 2005), affecting the whole ecosystem. In addition, ants have to compete with other arthropods for potential prey, and there is evidence for intra-guild competition between ants and spiders (Halaj et al. 1997, Mestre et al. 2012).

~ *Competition in arboreal ant communities* ~

Since ants have high species diversity and hyper-abundance in such a complex ecosystem of tropical canopies, competition, and other interspecific interactions are thought to be more important in structuring ant assemblages at the local scale (Arnan et al. 2018). As most ant species are generalist and/or scavengers (Davidson 1998), the main competitor is in most cases another ant (Holldobler and Wilson 1990). Those competitive interactions between species can consequently affect their distribution.

Three main types of competition between ants can be described as ‘*interference competition*’ (i.e. contest competition; direct), ‘*exploitation competition*’ (i.e. scramble competition; indirect), and ‘*apparent competition*’ (indirect) (Parr and Gibb 2010). The direct competition takes part, when two ant species/colonies physically compete over resources, with one colony interfering with foraging, reproduction, or survival of another. Indirect competition, on the other hand, takes place when species/colonies do not physically interact with each other but share either limiting resources (exploitation competition) or natural enemy (apparent competition). Considering processes, which are affecting the distribution of species in space, such as habitat filtering, as a type of competition, can shed new light to understanding factors shaping distribution patterns of ants via resource-limitation factors.

Due to the high abundance and diversity of ant species in the tropics, food and space are the most common resources, which are worth competing for

because they are limiting ant distribution. (Holldobler and Lumsden 1980, Bluthgen et al. 2000, Stanton et al. 2002). Limitation by nesting space is a general phenomenon in ant community ecology and it was documented in both tropical (Byrne 1994, Sagata et al. 2010) and temperate ecosystems (Herbers 1986, Foitzik and Heinze 1998). However, nest-site limitation of ants in tropics was only confirmed in a single stratum and there is a possibility that communities in various strata could be limited by different factors. Despite the high vegetation complexity in tropical rainforest (due to trees of different sizes, lianas, aerial trapped soil and leaf litter), there is still a high demand for nesting places, and their availability is postulated to be a limiting factor for arboreal ant communities (Armbrecht et al. 2006, Powell 2009). However, there is still a lack of studies that would compare the effect of nest-site limitation between different strata.

The second most common limiting factor is food, with ants commonly using multiple resources to maintain large colonies. (Bluthgen and Stork 2007, Lach et al. 2010). In this way, the high-energy requirements can be met via multiple feeding approaches. From those provided by plants (bottom-up effects) two seem to be most common in ants: utilisation of honeydew of attended hemipterans (Holldobler and Lumsden 1980) and extrafloral nectar (EFN) in certain trees (Bluthgen et al. 2000). In a tropical forest, both resources are present mostly on trees (i.e. in the upper canopy) and therefore the ground forest level has less rewarding and stable resources for ants to compete for, than the upper canopy, based on a plant diet. That could be the reason for changes in species composition between strata (Ryder Wilkie et al. 2010), and a more profound spatial distribution of arboreal ants than ground ones (Bluthgen and Stork 2007). Since tropical trees have a higher amount of stable limiting supply (food, nest space, etc.) that is worth spending energy for, species and their colonies often create an area, which can be defended from others: a territory (Holldobler and Lumsden 1980).

~ Territoriality and dominance of arboreal ants ~

Territorial behaviour is surprisingly common in most ant communities (Holldobler and Lumsden 1980). Ant territoriality is usually correlated with the size of the colony (Elton 1932, Tschinkel et al. 2016), and it might explain why

are many invasive species, which are generally very abundant, also very territorial (LeBrun 2005, Dejean et al. 2007, 2015). The main cause of territoriality being common among ant species might be, that living in a colony with the advanced organisation (eusocial) helps to deal with predators and different environmental struggles, allowing ants to focus on different ecological function, such as gathering different food resources. Consequently, species with a rich carbohydrate diet can spend more energy on aggression and territorial encounters, than species that rely on less predictive resources (Davidson 1998). Thus, territorial behaviour probably gives ant species an evolutionary advantage, because its origin can be traced to the early part of ant phylogeny (Dejean et al. 2007). However, it is worth mentioning, that it was the co-evolution with plants (nesting on vegetation, new food sources), which helps the diversification of most recent ant lineages (Moreau et al. 2006, Lucky et al. 2013).

There is common determination between ‘*dominant*’ and ‘*sub-dominant*’ ant species. This is caused by the unbalanced distribution of ant assemblages (log-skewed shape of ranked species abundances), which is a common pattern observed in community ecology. The term ‘*dominant*’ ant species is used mostly in two scenarios: a) ‘*numerical dominance*’: species exceed others in terms of biomass, abundance and/or frequency of occurrence (Leston 1978, Gilbert 1980, Holldobler and Lumsden 1980, Vepsalainen 1982, Davidson 1997); or b) ‘*behavioural dominance*’: species is more likely to win direct competition due to higher aggression, better combat skills or stronger recruitment (Schoener 1983, Fellers 1987, Morrison 1996). It is not an uncommon phenomenon that some species in communities possesses exceed in both numerical numbers (colony size) and behavioural (aggression). Those are typically regarded to be ‘*ecologically dominant*’ species, with presumably the highest ecological impact on the whole ecosystem (Davidson 1998). However, it is usually only one or two species, which numerically dominate a local diverse community at the local scale (Klimes et al. 2015). Surprisingly, those dominants are able to co-exist with a high richness of sub-dominant species in pristine forest. Although, dominant species could also negatively affect the rest of the community, in particular, in disturbed habitats (Bos et al. 2008, Arnan et al. 2018). The ecological interactions between dominant and sub-dominant species might create

hierarchies (layers), with species affecting each other, resembling multi-trophic food webs (Room 1971, Arnan et al. 2011).

The special phenomenon of arboreal ant territoriality is '*ant mosaic*'. Since space is a limiting factor in forest canopies, and tropical ants are very territorial, the spatial distribution of arboreal ants and their colonies could be the focus of studying co-existence of diverse insect species communities. The *ant mosaic* theory was established to describe the spatially segregated structure of dominant arboreal ant communities in forest canopies, built upon the concept of dominance hierarchies and the existence of negative and positive associations between ant species, or between colonies of the same species (Leston 1973, Bluthgen and Stork 2007). The *ant mosaic* is associated with the presence of several dominant species accompanied by several subdominant, shaping the structure of the overall community of ants (Room 1971, Majer 1976b) and/or other insects (Dejean et al. 1997, Liere et al. 2012, Lourenço et al. 2015). However, it is still relatively unknown if the spatial distribution of dominant ants is more affected by direct or indirect competition (indirect competition is primarily driven by an environmental filter). For example, ants are strongly affected by temperature (Cerdá et al. 2013), but changes in temperature could also affect behavioural of dominant species (Bestelmeyer 2000), and that can have a cascade effect on species composition. Disentangling these complicated relationships could bring more insights to general ecological processes in tropical canopies that maintain insect abundances, using the ants as an example. However, separating the direct effect of ecological gradients on ant communities from non-random, and identifying the key drivers of those patterns in ant community distribution is only possible with acknowledging the non-random distribution of species in the space (Legendre and Fortin 1989, Liebhold and Gurevitch 2002). In addition, connecting the observational spatial patterns with experiments, such as intra-species and inter-species behaviour, would make great progress in current research but it is rarely included (Mathis et al. 2016).

Presence of *ant mosaic* has been confirmed, not only in degraded habitats, such as plantation and secondary forest (Room 1971, Majer 1972, Leston 1978, Jackson 1984), but also in pristine lowland forest of Africa (Dejean et al. 2007, 2015, 2016), Neotropics (Sanders et al. 2007a, Dejean et al. 2019), and Borneo

(Yusah et al. 2018). However, there is still ambiguity of description of *ant mosaic* and drivers of this spatial distribution. Apart from species behavioural, other biotic factors thought to maintain the mosaic distributions of ants in trees including food resources (Bluthgen and Stork 2007), host-tree taxonomy (Dejean et al. 2015, 2016, 2018, 2019) and disturbance by invasive species (Dejean et al. 2010). Abiotic factors such as climate and microhabitats might also play an important role in those spatial processes (Farnon Ellwood et al. 2009). In addition, most of those studies in pristine tropical rainforest are based on a small study area up to 1-ha of forest (Bluthgen and Stork 2007, Klimes et al. 2015), or individual trees sampled randomly (Fayle et al. 2013, Yusah et al. 2018), with some rare exceptions (Dejean et al. 2015). This is mostly due to the difficulties of sampling in very inaccessible habitat, such as the canopy of the tropical rainforest. The spatial segregation of species can be missed as it could be happening on a different scale (Legendre and Fortin 1989). Hence, to what extent is the pattern of spatial segregation of dominant species shaped by biotic interactions (interference competition, behavioural traits) or abiotic factors (exploitation competition, habitat filtering) is still unknown.

~ *Aims and scopes of this thesis* ~

We aim to disentangle the effects of various drivers on distribution patterns of hyper-diverse ant communities in tropical rainforests, using pattern observation and experiments with the fauna of New Guinea.

In **Chapter I**, we explore the effect of vertical stratification gradient on the community of tropical forest ants, and test how different is the limitation by space between the forest strata. We were using artificial bamboo nests and twig-nesting ant communities. Although similar methods of artificial nests have been used previously in ground stratum of tropical forests (Byrne 1994, Sagata et al. 2010) or on trees in structurally simpler habitats as plantations (Philpott and Foster 2005, Powell et al. 2011), here for the first time we compared occupation rates and species composition between all three main strata of primary lowland forest: ground, understorey, and canopy. In addition, we test the utility of translocation of whole ant colonies among the paired-forest plots in this pilot study to assess if it might be a useful method for experimental spatial

manipulations of the ants in rainforests (e.g. manipulations of nests among different ant species territories in space or different forest successional stages). We predict that ground and arboreal ant communities would be different in the species composition and that there is a higher nest site limitation in higher strata compared to the forest ground level.

In **Chapter II**, we demonstrate a finer-scale stratification of arboreal ant communities along tree trunks of high-canopy trees, and we test for the presence of *ant mosaic* (territorial segregation of dominant arboreal ants) across the canopies over larger scale of lowland primary forest. To achieve this, we studied a large area of the forest (9-ha plot) at the site of 50-ha CTFS forest inventory plot in Wanang in PNG (Anderson-Teixeira et al. 2015, Vincent et al. 2018), that allowed us to utilise the detailed data on vegetation structure on the numerically dominant ants. We explore how competition between those communities affect their species assembly rules in primary lowland forest. We used tuna-baits to map ant communities on big trees in 9 ha of the forest and gather information about the position and size of each colony. The presence of *ant mosaic* has been tested by a novel null model ‘*Canopy Area Null Modelling Algorithm*’. This method is using the position of whole ant colony territories in space, i.e. canopy area used by a colony extending to multiple trees, rather than using standard methods that allow co-occurrence tests only within a tree (C-scores method), which is current standard in *ant mosaic* studies (Sanders et al. 2007a, Fayle et al. 2013). We hypothesised that spatial segregation of dominant arboreal ants is more driven by behavioural of species (direct competition) than by habitat filtering (indirect competition), which has been suggested by previous studies (Bluthgen and Stork 2007, Dejean et al. 2019). We tested if behavioural traits (inter-species aggression) of certain species are correlated with their body size and/or with their interspecies spatial segregation, to answer if behavioural and morphological traits of dominant species could be the main driver of their spatial segregation.

Due to non-random spatial distribution, the effects of ecological gradients on plant community are mostly studied using whole-forest plot approach, where all individuals above a certain stem size are mapped within plots (Fibich et al. 2016, Vincent et al. 2018). Arboreal ants are rarely sampled by the whole-forest

census, primarily due to practical reasons such as accessibility to the forest canopy. However, studying the response of those communities of arboreal arthropods to changes on an ecological gradient on a level of a whole-forest patch should be the modern way in ecology. While effect of secondary succession on arboreal ant communities has been well examined and demonstrated by multiple studies, in the lowland communities, i.e. high changes in species diversity and composition with succession (Schulz and Wagner 2002, Osorio-Perez et al. 2007, Klimes et al. 2012), there is a big knowledge gap for effect of succession in montane rainforest. Previous studies in the montane tropical forest found a rather weak effect of succession on species richness, but strong effects on species composition and turnover among different successional stages in butterflies (Spitzer et al. 1997), birds (Soh et al. 2006), and spiders (Yanoviak et al. 2003a). However, information about the effect of succession from a whole-forest study on the distribution of arboreal ant communities was missing. In **Chapter III**, we test the effect of succession on foraging and nesting arboreal ants in a mountain tropical forest of PNG (1800 m a.s.l.). We sampled 1249 trees in a successional series and test changes of ant species diversity and composition. In addition, we use rarefaction-based models manipulating vegetation structure and multivariate randomisations to assess the effects of tree density, tree size, and nesting microhabitats on the ant communities to test the importance of forest structure (tree size and availability of microhabitat) and succession itself on species diversities of arboreal ants.

Using a whole-forest approach for detailed information about the spatial distribution of arboreal arthropods could help us not only to understand better to community structure and its drivers of the ants themselves but to uncover links between multiple ecological guilds in forest canopies and the ants. Those canopies are complex ecosystems, with previous studies suggested effect of competition between both herbivorous (Bird et al. 2019) and predatory (Halaj et al. 1997, Mestre et al. 2012) arthropods. In addition, the abundances of arboreal ants, as well as their predation pressure towards herbivorous insects, is decreasing with latitude (Salazar and Marquis 2012, Floren et al. 2014). However, how is the abundance of ants, on a scale of the whole-forest plots, affecting the number of other arthropods in the canopy is relatively unknown. In

Chapter IV, we used detailed datasets of nine 0.1 ha plots in two ecological gradients: latitude (CZ - USA - PNG) and altitude (PNG: 150 - 900 - 1900 m asl); 1001 trees in total. We test how abundances of arboreal arthropods are changing with the increase of mean temperature (as a combination of the latitudinal and the altitudinal gradient). We predicted increasing arthropod numbers with higher temperature (Salazar and Marquis 2012, Bird et al. 2019). Furthermore, we study different herbivores (caterpillars and leaf miners) and potential predation effects of ants and spiders on their abundances, and the spatial competition effects among ants-spiders, and caterpillars-miners. This is done indirectly via spatial segregation of their varying abundances in tree canopies. We developed a statistical approach to test segregation between guilds within each tree while filtering out the size of a tree, and spatial auto-correlation of tree stems in each of the plots. We predict that the spatial predation, and competition within guilds, should lead to their spatial segregation within trees. We also expect that this effect should be stronger in the tropics than in temperate, due to the higher abundances of the arboreal arthropods in tropical canopies.

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~ Chapter I ~

Experiments with artificial nests provide evidence for ant community stratification and nest site limitation in a tropical forest

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Experiments with artificial nests provide evidence for ant community stratification and nest site limitation in a tropical forest

Ondrej Mottl, Jacob Yombai, Tom M. Fayle, Vojtech Novotny, Petr Klimes

~ Abstract ~

Ants are dominant in tropical forests and many species nest in hollow cavities. The manner in which species are vertically stratified in these complex habitats is not known, with lack of nest sites being proposed to limit ant populations. Here, we assess ant community stratification and nest site limitation in a lowland rainforest in New Guinea using experimental addition of artificial bamboo nests of two cavity sizes (small: ~12 mm large: ~32 mm diameter) placed at ground level, in the understorey, and in the canopy. We also conducted a pilot experiment to test the utility of nest translocation. Nests were checked for occupancy after 10 weeks and half of the occupied nests were then translocated between forest plots, while keeping same vertical position. Occupancy of small nests was much higher in the understorey and canopy than at ground level (~75% vs. ~25%). Translocation was successful, as a majority of nests was inhabited by the same species before and after translocation and there was no impact of translocation to a different plot compared to the control, except for a reduction in colony size at ground level. Our experiment demonstrates a vertical stratification in community composition of ants nesting in hollow dead cavities and shows that these ants are more nest site limited in the higher strata than at ground level. Use of small artificial cavities has great potential for future experimental studies, especially for those focused on arboreal ants, as occupancy is high and translocation does not negatively affect their colony size.

~ Chapter II ~

**Ant mosaics in primary forest canopies are driven by interspecies
behaviour**

Manuscript

Ant mosaics in primary forest canopies are driven by interspecies behaviour

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~ Abstract ~

1. The ant mosaic theory describes the patchy spatial distribution of arboreal communities in tropical forest canopies. It is built upon the concept of behavioural hierarchies of ant species and the negative and positive interspecies associations, but these assumptions are rarely tested experimentally.

2. It is not clear if ant mosaics are structured by species behaviour as the theory predicts, or by habitat filtering. Furthermore, the evidence for ant mosaics in pristine habitats varies among studies.

3. We sampled arboreal ants from a 9-ha of primary lowland forest in Papua New Guinea, the largest forest area ever studied for ant mosaics. In total, 225 high-canopy trees randomly distributed across the plot were sampled, using tuna-honey baits installed along tree trunks up to the canopy. We performed behavioural tests to determine individual colonies boundaries and experimentally assessed the rate of interspecies aggression among the four most dominant species. We created a novel statistical method to test for the non-random spatial structure of ant communities across the plot via spatial randomisation of individual colony territories and compared it to the C-scores. Using detailed data on the plot vegetation and topography, we also tested the environmental effects on the communities and their spatial distribution.

4. We found 57 ant species, which varied greatly in their abundance and vertical stratification. Ant community composition in trees was spatially dependent, but it was not affected by tree species composition or canopy connectivity. Only the local elevation had a significant but small effect, suggesting a rather weak influence of habitat filtering. Individual colony territories ranged from one tree to 0.7-ha and they were more likely not to overlap with each other. The level of aggression between the most common four species correlated with their spatial segregation, but not with their body size.

5. We stress the importance of studying over larger spatial scales to detect the ant mosaics in primary forest canopies. Our study suggests that individual species behaviour is the main driver of the non-random spatial distribution of canopy ants, while vegetation structure has rather a small effect linked to the microclimate variability.

~ *Key-words* ~

ant mosaic, arboreal ants, community ecology, competition, Papua New Guinea

~ *Introduction* ~

All biological processes in ecology are spatially correlated to some degree, due to a spatially structured environment. In ecology, the spatial distribution of species is a result of assembly rules caused by segregation of these species in space (Gotelli and Mccabe 2002) but the detectability of spatial autocorrelation and spatial processes is often influenced by the scale of observation (Legendre and Fortin 1989). All living organisms are affected by their spatial distribution, which is moderated by two main causes: intra/inter-specific interactions (e.g. behavioural competition, dispersal limitation) and/or resource limitation (habitat filtering). Organisms with a sedentary lifestyle are easier to observe and have been therefore objects of spatial research for a long time, with focal groups being corals (Reiswig 1973, Jackson 1977, Álvarez-Noriega et al. 2018) and particularly plants (Watt 1947, Fibich et al. 2016, Vincent et al. 2018). Tropical rainforest, as a plant-based ecosystem with the highest species diversity in terms of the number of vascular plants and insect species (Swartzendruber et al. 1993, Mittermeier et al. 2003), is crucial for studying the spatial distribution of species and our understanding of what is affecting it. Various theories were suggested to explain the high species diversity in tropical forest (Wright 2002) but, to some degree, limitation of space is affecting them all.

In tropical rainforests, ants make up a significant part of the overall biomass, compared to other animal taxa, especially in the forest canopy (Hammond 1992, Floren and Linsenmair 1997, Davidson et al. 2003). The spatial distribution of ant species and their colonies in tree canopies had been therefore often used as a model to study co-existence of diverse insect species communities in tropical forests (Floren and Linsenmair 2000, Janda and Konecna 2011, Fayle et al. 2015). The arboreal ants can be seen as mobile organisms that forage in all strata of tropical forest as their workers search for resources (Holldobler and Wilson 1990); but also, due to the sedentary life of ant nests and pheromone-based hierarchical communication, as sessile organisms that are moving over a longer temporal scale, and, therefore, more similar to a plant. While spatial ecology of tropical trees had received considerable attention and it is relatively well-studied subject (Condit et al. 2000, Bagchi et al. 2011, Fibich et al. 2016), spatially

explicit analyses of insect distribution are rarely conducted in pristine forests (Basset 1991, Ribas et al. 2005).

Since most ant species are generalist and/or scavengers (Davidson 1998), the main competitor is in most cases another ant (Holldobler and Wilson 1990). Those competitive interactions between colonies can consequently affect the species distribution within a site. Currently accepted hypothesis is, that spatial distribution of dominant ant species is caused by intraspecific/interspecific competition and territorial behaviour (Holldobler and Wilson 1990, Ribas and Schoereder 2002). The term “dominant ant species” is used to describe the imbalanced species structure in ant community ecology, where dominant species exceed others in terms of biomass, abundance and/or frequency of occurrence (numerical dominance) (Leston 1978, Gilbert 1980, Holldobler and Lumsden 1980, Vepsalainen 1982, Davidson 1997), or by winning direct competition due to higher aggression, better combat skills or stronger recruitment (behavioural dominance) (Schoener 1983, Fellers 1987, Morrison 1996, Dejean et al. 2007).

Spatially non-overlapping territories of dominant ant species were first described as *ant mosaic* in African cocoa plantation tree canopies (Room 1971). The *ant mosaic* theory was established to describe the observed patterns of segregated structure of arboreal ant communities in forest systems, built upon the concept of dominance hierarchies and the existence of negative and positive associations between ant species, or between colonies of the same species (Leston 1973, Bluthgen and Stork 2007). The *ant mosaics* are associated with presence of several dominant species accompanied by several subdominant species (i.e. either less abundant or behaviourally submissive to the dominants), shaping the structure of overall community of ants (Room 1971, Majer 1976b) and/or other insects (Dejean et al. 1997, Liere et al. 2012, Lourenço et al. 2015). Due to those behavioural traits and the relationships between ants and trees they nest in (Klimes 2017), studying the ants and their spatial distribution in pristine forests could serve as a suitable model to not only test the *mosaic theory* but help us understand the processes shaping complex insect-insect and insect-plant interactions in a tropical ecosystem over different spatial scales.

Not only the inter- and intra-species interactions among ant species can drive spatially non-random distribution of ant communities in trees: e.g. the spatially dependent patterns could suggest that there is a limited supply (food, nest space, etc.), worth spending energy to defend it (Holldobler and Lumsden 1980, Bluthgen et al. 2004). In addition, various abiotic factors are simultaneously affecting the assembly of communities. For ants, as ectotherms organisms, temperature and microhabitat variability is also crucial (Cerdá et al. 2013). Temperature strongly declines with elevation, as well as ant activity, abundances and species richness (Fisher 1996, Samson et al. 1997, Bruhl et al. 1999). Temperature changes could have an indirect effect on *ant mosaics* through changes in behavioural (Bestelmeyer 2000) or through day/night cycles in species activity (Yusah et al. 2018). Other studies indicate that limitation by food availability, through scale insects and extrafloral nectaries can be also an important factor that drives *ant mosaics*, at least at smaller scales of individual tree branches (Bluthgen et al. 2000, 2006, Bluthgen and Stork 2007). However, all these studies were conducted on a smaller spatial scale that usually allowed testing only for species segregation within each tree. Limitation by nest sites was also suggested to affect the arboreal ant structure (Philpott and Foster 2005, Dejean et al. 2008b, Fayle et al. 2013), but those studies were either conducted in a disturbed ecosystem or again at a small spatial scale.

The few experimental studies available to date in pristine forests are also limited to small scales, focusing on manipulation of a single nesting microhabitat within trees (Fayle et al. 2015), or only on baits exposed in understorey stratum (Davidson et al. 2007). Those studies highlighted species behaviour and/or their morphological traits as a key driver for species assembly of the ant communities. Similarly, the experimental manipulations of dominant ants in cocoa plantation suggested the main role of species dominant behaviour in shaping the distribution of arboreal ants (Majer 1976a, 1976c). However, those studies did not include a formal analysis of the behaviour and environmental factors. In contrast, most recent plot-based studies suggested that tree taxa and other environmental factors like tree size are possibly also driving *ant mosaic* distribution in primary forests (apart of pre-assumed interspecies competition) (Dejean et al. 2015, 2019); but their analyses neglected the spatial distribution

of trees, and hence the core of the original theory (Majer 1972, Leston 1973). All those examples show that disentangling individual effects from each other is not trivial in the tropical forest ecosystem, due to the high complexity of all processes and number of species. Therefore, there is a need for a unified methodology for studying the spatial distribution of ant community that would consider the spatial effects. Furthermore, we need to include both the experimental (e.g. behavioural intra- and interspecies tests) and forest structure components, to disentangle the individual drivers of the canopy *ant mosaic* distribution.

Current research on the ant canopy distribution have shown results, that are often not conclusive and researchers used very different methodologies and rather various definitions of the *ant mosaic* (Bluthgen and Stork 2007, Sanders et al. 2007a, Dejean et al. 2008a, 2015, 2016, 2019, Fayle et al. 2013). Variability between study scales and approaches can strongly affect the results of studies trying to detect the *ant mosaics*. Despite the original concept of *ant mosaic* being focused on spatial segregation of whole territories of ants in space (Majer 1972), some current studies consider segregation of ant species within each tree as evidence for its presence (Sanders et al. 2007a, Fayle et al. 2013). This high difference in the methods between the studies affects the way the hypothesis is tested, and may result to the presence/absence of *the mosaics* in a forest. Those differences are caused in particular by a) usage of different statistical test (Sanders et al. 2007a) and/or b) scale of observation (Bellier et al. 2007, Sanders et al. 2007b) and/or c) different sampling Materials and Methods (e.g. ant census at baits or fogging). Research on *ant mosaics* or arboreal ant territories is popular (157 results found on search “*ant mosaic*” on Web of Science between 2010-2019) but it is unfortunately often based on comparisons of individual trees rather than the comparison of entire territories and whole forest plots, as it is a case in the plant research (Fibich et al. 2016, Vincent et al. 2018, Pescador et al. 2019).

Here, we focus on the spatial structure of arboreal ants of lowland primary forest in Papua New Guinea (PNG hereafter). A rapid slingshot baiting method and inter-colonial aggression tests within ant species (Leponce and Dejean 2011) allowed us to map the spatial distribution of colonies of dominant species in a

9-ha area of the forest, a largest ant spatial dataset for the tropical tree canopies to date. For the first time, we developed a test of *ant mosaics*, which explicitly randomise the territories of the ant colonies in space. We used the detailed data on the plot vegetation and topography and conducted additional behavioural interspecies behavioural aggression experiments to disentangle the drivers of the observed spatial species distributions. We predict that: (i) there is a non-random spatial distribution of arboreal ants (i.e. *ant mosaic*) in primary forest tree canopies; (ii) spatial structure of ant community will be driven more by species behavioural (competition) than resource limitation (habitat filtering); (iii) Spatial segregation of species will correlate with behavioural traits of species (aggression).

~ *Materials and Methods* ~

Study site and ant sampling

All fieldwork was conducted in Center for Tropical Forest Science (CTFS hereafter) (Anderson-Teixeira et al. 2015) lowland primary forest plot in Wanang Conservation Area, Madang Province, PNG, between 14th of April and 23rd of September 2016. The plot is of 50-ha size with all trees with a diameter at breast height (DBH) >1 cm tagged, their DBH measured, identified to species level and their stem coordinates mapped (Vincent et al. 2018). The area has total plant richness of 351 tree species, average basal area of 32.0±13.7 cm per ha, and the average elevation of 131.3±25.6 (min=80.3, max=189.7) m asl. During our fieldwork, we measured mean day temperatures between 23.5 °C and 31.2 °C, and mean precipitation of 381 mm per month. Seasonality in the regions is rather low (McAlpine et al. 1983) and the sampling was targeted in the driest period of the year. We chose 9-ha (300 x 300 m) within the CTFS plot as our focal area for ant sampling. We randomly selected 25 high-canopy trees (DBH≥ 40 cm) in each hectare. This has been done to cover canopy area evenly, while also maximizing the sampling effort over this large scale. In total, 225 trees were studied from 428 trees available within the size-threshold. In several cases, the tree, randomly selected from CTFS plot database of 2009 census, was found dead in the field. In that case, we selected a nearby tree of a similar size (for the map of all big trees and selection of individuals for the sampling see Fig. S1A).

We used the slingshot bait-line protocol for sampling trees (Leponce and Dejean 2011). Tuna-honey baits (circa 5 g of the mixture of canned tuna in oil and honey, in ratio 5:1, placed into paper towel) were placed on a rope along the tree trunk from the ground level to the canopy, with the distance of 5 m between baits, starting at ground level. Top bait was always touching the highest branch in the canopy, which was accessible to shoot the rope over it in a loop. The number of baits and the position of the highest bait slightly varied depending on tree height and the accessibility of the top canopy branch (mean n of baits per tree \pm SD: 5.0 ± 0.9 , mean reached height \pm SD: 20.0 ± 4.7). The rope was twisted around a tree so all baits touched the tree. We set up baits in the morning and left them 4-5h for exposure (minimum 4h). We then moved the rope down, checked the baits for occupancy and the estimated number of individuals of all the ant species found on each bait. We collected several individuals of each species to vials with absolute ethanol for later species confirmation. Baits were placed only during sunny weather without rain. If rain came during the experiment, the tree has been resampled to ensure the similar condition of ant activity. All samples were later sorted to species/morphospecies level using key (Bolton 1995), online pictures (antweb.com) and morphological traits, and compared with reference collections of New Guinea ants in Wanang available at Institute of Entomology, Biology Centre, Czech Academy of Science (Klimes et al. 2015). All specimens from this study are stored at this institution.

Data analysis

All following analyses were done using R 3.5.2 (R Core Team 2016) and various packages (see below), except for multivariate analyses where Canoco 5.04 (Braak and Smilauer 2012a) was used. The scripts with R code can be found in Supporting Information Code Appendix I-II.

We explored the vertical distribution of individual species along the tree trunks graphically for those present in at least 1% baits and ranked them by their stratified ranges (Fig. 1). Furthermore, we tested if the species composition differed between near ground level and the canopies. Therefore, we divided all data into two subsets: a) below canopy fauna (baits with height <10m) and b) canopy fauna (baits with height \geq 10m). This division was supported by

multivariate analysis with a highly significant species difference between the two strata (Canonical Correspondence Analysis (CCA), see Results and Supporting Information S1 for more information). Only the canopy fauna was then used for all following analyses (i.e. *Canopy Ant Community*) (Table 1), as our study focus was primarily on the arboreal ant fauna and tree canopies.

To test if arboreal ant communities were spatially segregated at the level of individual trees and to compare the results to previous studies, we performed co-occurrence test using Checkerboard Score (C-Score)(Stone and Roberts 1990). This simple and commonly used method is a measure of spatial species segregation between individual samples (here trees), but it does not consider the spatial distance among the samples. For detailed information about the calculation of C-score, see Supporting Information S2. Standardize Effect Size (SES) was then calculated as:

$$SES = \frac{\text{Observed index} - \text{Mean of Simulated index}}{\text{Standard deviation of Simulated index}}$$

Habitat filtering effect: the multivariate analysis of the multiple environmental effects on the Canopy Ant Community

First, we tested if the ant community composition was affected by the spatial positions of the sampled trees (space effect). We used Principal Coordinates of Neighbour Matrices (PCNM) with distance-based Moran's Eigenvector Map (dbMEM) as a measure of the spatial effect.

We created five CCAs with interactive forward selection method to test the role of habitat filtering and its possible correlation with the spatial distribution of trees on the ant species composition. We tested the effects of the following predictors on the species distribution of canopy ant community for each tree as a sample. First, we tested the effect of tree species identity on the distribution of arboreal ants. We used tree family/genus/species as predictors and only included those taxa with more than two tree individuals. CCA_F tested **tree family** (18 families), CCA_G **tree genera** (21 genera) and CCA_s **tree species** (17 species), respectively. Next, CCA_{ENV} covers tree sampled attributes and forest plot topography with the tested variables: **tree size** (DBH of sampled tree in cm), **elevation** (in m.a.s.l.), **slope of surrounding area** (in %), **topography**

(convex/concave, calculated from height of surrounding subplots), and **canopy connectivity** (defined as estimation of number of canopy neighbouring trees, connected to the sampled tree by their branches or lianas). All topography variables were measured for subplots at a grid of 20x20m. For details about the measured plot-variables see (Vincent et al. 2018).

Since individual ant colonies can occupy multiple trees and an ant foragers often seek the food also on surrounding vegetation (Bluthgen et al. 2004, Dejean et al. 2015), it is possible that it is not a sampled tree species, which is affecting the ant species distribution in a tree, but rather some tree species from the whole forest site surrounding it. We have therefore created yet a fifth CCA_{PLOT} to test if species distribution of ants is affected by tree species composition and frequencies of trees in 10 x 10 m area around each sampled tree (89 common tree species based on their basal area).

Finally, to assess how much were the effects of environmental variables spatially auto-correlated (i.e. if the ant spatial distribution in trees is driven by environment), we created CCA_{VAR} with variation partitioning to test for the separate effect of the significant variables (i.e. only elevation of tree from the above five CCA, Table S1) from the spatial effect (dbMEM).

For graphical interpretation, we also calculated ant species response curves using GLM for the variable that showed a significant effect on ant species composition (here only elevation). We also plotted the position of each species on the first axis of CCA_{ENV} with and without spatial autocorrelation.

See Supporting Information S3, for detailed information about all multivariable tests and used matrices.

Territorial effect: Spatial segregation of Dominant Ant Community colonies

We have excluded subdominant species in each tree for drawing of territories of the dominant arboreal species. The subdominant and dominant species were defined using numerical dominance approach and was based on both abundances of workers at baits and the number of baits, occupied by each species, in a given tree (see Supporting Information S4 for more information about the species

selection). This *Dominant Ant Community* dataset was further used for experimental tests of territories boundaries within the 9-ha plot.

First, we re-analyse the same habitat filtering analysis (CCA_{ENV} , CCA_F , CCA_G , CCA_S) with the *Dominant Ant Community* dataset to compare if the same trends hold for the dominant and the canopy communities.

Next, after mapping the distribution of the dominant species observed in the area, we resampled some of the trees, using the bait-line protocol, to gather enough living workers for intraspecies behavioural tests. We sampled ants from a tree dominated by a species within the forest area it occupied (e.g. *Crematogaster polita*) and repeated this for the same species from a neighbouring tree. We then performed a simple behavioural test: we placed live workers from the two trees together into a ziplock bag, and observed the interactions of individuals for 10 minutes (attacking / not attacking each other). All workers at a bait were typically used for the confrontation, choosing the bait with the highest ant abundance for that tree, and the whole paper tissue with bait and workers were placed in the bag. The result of the test was used to determine, if ant species workers of the two adjacent trees came from the same colony or not, with the assumption that no aggression indicates a single colony that extends to multiple tree canopies. Finally, when all local (continuous) territories have been mapped, we repeated such confrontation among the two distant (discontinuous) canopy areas in the plot occupied by the same species, using the same methods (with minimum one tree from each continuous area used as the source of living workers; 184 behavioural test in total, see example in Fig. S2). Based on these subsequent tests, we were able to determine the boundaries of each dominant ant species colony (i.e. its canopy territory) in the whole 9-ha area. We used polygons to draw the individual territories and their overlaps while excluding the areas, where no-overlap was confirmed or sampled trees did not host dominant ant species.

As the C-scores analysis does not consider the spatial effects across the 9-ha plot, we created a novel algorithm for statistical testing of the *ant mosaic* exclusion between the territories of spatially dominant arboreal ant species: *Colony Area Null Modelling Algorithm* (CANMA). The aim was to develop a

method, which would a) allow spatial reshuffling the position of the whole territories (individual canopy areas occupied by species) within the forest plot, and b) measure the amount of the overlap among the colony territories compared to a random simulation. The overlaps of territories between different species are represented by the Overlap Index (OI, the ratio between the sum of overlaps and the total area of territories). We test if the observed value of OI is significantly smaller than would be at random. Model corrects for a density of sampled trees, so there is a higher possibility that a colony will be found in the areas where the sampled trees occur. It also corrects for the edge and mid-domain effect to avoid the lower colony density at borders of the observed area in the randomised maps. For information about the territory-polygons creation, OI calculation, and randomisation process see Supporting Information S5, and Supporting Information Code Appendix I for the full script.

In addition to testing the whole *Dominant Ant Community*, we compared the overlaps among the most dominant species, and the rest of the fauna, using CAMNA with the same randomisation settings as above. We selected the four species that occupied the largest colony area when summed across the whole 9-ha plot (Fig. S3), *Spatially Dominant Species* hereafter: *Crematogaster polita* (CREM 003), *Anonychomyrma cf. scrutator* (ANON 001), *Oecophylla smaragdina* (OECO 001), and *Podomyrma laevifrons* (PODO 001). We recalculated OI but focusing only on single species (CANMA_s) and its territory overlaps with all other species in the plot, and comparing the OI observed value to randomised values (see Supporting Information S5). We then calculated standardised effect size (SES) as a standardised measure of the species segregation from the rest of the canopy fauna for each of the four species. The positive SES values indicate species aggregation; negative values the segregation, and values close to zero the spatially random occurrence of the species in the forest plot.

Aggression effect: Behavioural segregation of Spatially Dominant Species

We conducted a series of interspecies behaviour tests, using pair-wise interactions among the top four *Spatially Dominant Species*. The limitation to those species was necessary to perform the tests in all interspecies combinations

with enough statistical power due to time constraints and need for a sufficient amount of living workers from different colonies to perform standardised tests.

We have selected nine independent colonies for three focal species (*CREM 003*, *ANON 001* and *OECO 001*) and three colonies for *PODO 001* (maximum possible). One colony of each species was used in up to three tests with colonies of other species. Each colony was used in each species-species combination only once (see Fig. S4). For each test, we sampled about 10-20 live specimens from each colony and leave them in 100 ml clean plastic vial with cotton soaked in honey-water overnight to habituate.

Five randomly selected individuals from each colony were then kept inside each vial, removing carefully the rest of the individuals. Next, the two vials were joined via their opening, creating an arena, which was used to perform the paired-test between the two species (Fig. S5). During each confrontation, we observed the type and frequency of all reactions between all individuals of the species-pair for two minutes. Preliminary tests showed that such scoring was feasible by an observer with five workers at the same time. We aimed rather to confront multiple individuals of the two species, as the species built large colonies with many foragers that cooperate, and single-single worker interaction might thus obtain biased results.

Aggression Index (AI) was then calculated for each species in each of the tests as:

$$AI = \frac{\sum a_i b_i}{T}$$

Where a is the category of interaction (1-4 categories based on aggression, 4 being the most aggressive, see Supporting Information S6 for their definition), b is the frequency of that action, i is the type of interaction and T is the total number of interactions. This is a novel index inspired by other behavioural studies (Dejean et al. 2010, Wittman and Gotelli 2011). Result values were standardised to range between 1 (really aggressive) and 0 (mostly run away).

To determine differences between aggressions of species, we created Generalized Linear Mixed Models via Template Model Builder (glmmTMB;

glmmTMB package (Brooks et al. 2017)) with AI as a dependent variable with beta distribution(link=logit, no zero-inflation), and Species as an independent variable. We compare Species levels using post-hoc Tukey test. See Supporting Information S6 for more information about behavioural tests.

We tested the correlation between behavioural aggression of focused species (values of AI) and their spatial segregation (SES values from CANMA_s). We created *glmmTMB* model, with AI as a dependent variable with beta distribution (link=logit, no zero-inflation) and SES as an independent variable. Next, we created a second *glmmTMB* model with AI as dependent variable and AntBodySize as an independent variable to test if species aggression varies with worker body size. The mean head length of each of the four ant species in mm was used as the morphological trait that is regarded as a good proxy of ant body size (Gibb et al. 2018). The mean values were taken from the GLAD database (Parr et al. 2017) and rescaled to range between 0 and 1.

~ Results ~

We have sampled 225 big canopy trees in 9-ha of the lowland primary forest. In total, 57 ant species in 20 genera were found on a total of 1127 baits (i.e. *Whole Ant Community*, Fig. 1A). 221 trees were occupied with ants (98% occupancy rate, see Table I). There was significantly different species composition between below canopy (< 10 m baits) and canopy strata (> 10 m baits) (pseudo-F = 6.5, P = 0.001) and individual species distribution showed strong vertical stratification from the trunk bottoms towards the top of the canopies (Fig. 1B).

Arboreal ant species co-occurred on trees less than they would be expected at random (C-score 39.2, p<0.01, SES = 6.27; Fig. S6), indicating a strong segregation of species in the community at the level of individual trees.

Habitat filtering: the multivariate analysis of the multiple environmental effects on the Canopy Ant Community

Spatial distribution of trees (dbMEM) explained 3.5 % of the overall variance (28.5% efficiency of the first axes, Table S1) in *Canopy Ant Community* composition, indicating spatial auto-correlation of the ant species distributions

in the trees across the 9-ha plot. In contrast, our tested environmental variables in CCA_{ENV} showed no significant effect on the composition of this ant dataset, except for elevational changes within the plot (**elevation**, pseudo-F =4.9, Padj=0.005, Table S2). CCA_{ENV} analysis explained 2.0% adjusted variability with 24.3% efficiency of the first axis.

Tree taxonomy (CCA_F , CCA_G , CCA_S), as well as vegetation structure surrounding the sampled tree (CCA_{PLOT}), did not show any significant effect on the ant species composition in the canopies (all predictors were not significant, see Table S3-S5).

CCA_{VAR} explained 4.4% variability in total with both elevation and space (dbMEM). Variation partitioning among the two variables showed that elevation solely was responsible only for 20.5% of all explained variability while the space for 55.2%, with 24.3% overlap among the two (see Fig. S7). Elevation was therefore highly correlated with space, but it still explained an independent part of the species composition after the reduction of spatial autocorrelation (0.9% of all variation).

Species response curves to elevation showed a significant effect on seven of the 13 species tested (see Fig. 2, Table S6).

Territorial effect: Spatial segregation of Dominant Ant Community colonies

Dominant Ant Community has been significantly affected only by elevation (CCA_{ENV} , pseudo-F = 5.4, Padj=0.005), while other environmental were not significant (i.e. the same result as for the communities that included not dominant species).

Based on the mapping and the intraspecies aggression tests, we found 127 individual ant colonies of 23 ant species in the tree canopies, with the average size of 288.2 m² (smallest is 29.3 m² and largest 6612.6 m²) (Fig. 3A and Fig. S3).

CAMNA showed significant spatial segregation of all colonies, with observed overlap index (OI) smaller than its value generated by randomisation (CANMA: OI= 8.2, p<0.05, Fig. 3B). This result was stable with the change of the number of randomisation and usage of different tree density maps (Table S4).

Aggression effect: Behavioural segregation of Spatially Dominant Species

We have performed a total of 36 aggression tests between the top four *Spatially Dominant Species* and found a significant difference in aggression index (AI) between those species (glmmTMB: Chisq= 13.99; DF=8; $p < 0.01$; Fig. 4A). Mean AI per species ranged from the most aggressive *Crematogaster polita* (AI = 0.66) to the least aggressive *Podomyrma laevifrons* (AI = 0.22).

We have found a significant relationship between AI and SES from CANMA_s spatial segregation (glmmTMB: Chisq= 3.99; DF=3; $p < 0.05$), with more aggressive species being more spatially segregated from others (Fig. 4B). However, there was no significant correlation between AI and ant body size (glmmTMB: Chisq= 1.92; DF=3; $p = 0.166$, Fig. 4C).

~ Discussion ~

Presence of *ant mosaic* in pristine lowland forest has been confirmed in Africa (Dejean et al. 2007, 2015, 2016), Neotropics (Sanders et al. 2007a, Dejean et al. 2019), Borneo (Yusah et al. 2018), Australia (Bluthgen and Stork 2007) and we confirmed its presence in lowland forest of Papua New Guinea. Hence, there is increasing evidence that a highly non-random distribution of the ant species in tree canopies is typical not only for the less complex canopies of the secondary forests and plantations but also for pristine forests.

Separating stochastic processes from non-random, and identifying the key drivers of those patterns in the distribution of any community is only possible with the right methodological approach and appropriate statistical analysis. The currently most commonly used statistical test for detecting *ant mosaic* patterns is C-Score, a metric based purely on the coexistence of species in selected space (single tree); hence not taking into account the spatial distribution of trees in a sampled site and the fact that ant colony can also occupy multiple trees. This is problematic, as the same C-Score value can be same for the community of two species occupying opposite sides of a plot (with almost no opportunity to compete) and two adjacent territories in two nearby trees, as soon as they do not occupy the same trees. Therefore, the use of this analytical approach is not correct for spatially not-independent datasets, as it assumes the spatial

independence of samples (trees). A similar bias also exists in older methods, such as Chi-square or ranked-based method, which do not perform a randomization (Majer 1972).

Here, we looked at a larger spatial scale of the forest, considering also the extension of ant territories across the tree canopies, and developed a new randomisation algorithm that allowed us to use a null modelling approach to test for presence of *ant mosaics* using the spatially extending ant colony territories, and their randomisation in 2-D space. The bait-line protocol provides information on the vertical distribution of colonies along trees. However, it remains challenging to develop such approach to 3-D space, as it is possible that some of the species might mix their territories between tree trunks and canopies, or between small and large trees within the same forest local site (while we focused explicitly on the largest trees). Yet, our CANMA approach, combined with multivariable analyses, is a big step further to understand the ant mosaics and their drivers. It, accompanied by behavioural intra- and interspecies tests, allowed to test the larger-scale patterns (9-ha) with various environmental drivers (plot topography and vegetation) of species distribution, which is unique not only in the ants, but for other canopy invertebrates.

Acknowledging effect of spatial auto-correlation in any test of species assemblage should become standard in insect community ecology, as it is in plants (Legendre and Fortin 1989, Perry et al. 2002). Detection of spatial segregation of species distribution (whole colonies) is an approach used in plant biology (Pescador et al. 2019) but very rarely used in ant ecology (Boulay, R.Cerdá et al. 2007). While mapping all dominant ant species and their territories can be very time consuming, it is crucial to perform behavioural confirmation of colony boundaries. We are aware of imperfect sampling using bait-line on only selected big trees in our study, as only part of the ant communities is being sampled with likely under-representation of their behaviourally subdominant and/or rare taxa (Klimes et al. 2015). However, this relatively rapid sampling technique using baits, allowed us to detect the dominant ant species and colony segregation on a larger-scale of the observation. This could not be achieved otherwise. It also demonstrates the importance to study ant mosaics over a much larger spatial scale, with some territories of near 0.6 ha size. Therefore, sampling

smaller-scale plots or few individual trees, might just randomly be placed into one or multiple ant species territories (and thus find “a mosaic” or not). In addition, this method enables the use of behavioural tests with live workers, which is not feasible with other methods, where ants are typically killed (e.g. fogging, pitfall trapping), and hence only the patterns can be studied (Fayle et al. 2013, Camarota et al. 2016). Consequently, most studies on arboreal ant species in pristine tropical forest are based on a small study area up to 1-ha of forest (Bluthgen and Stork 2007, Klimes et al. 2015, Dejean et al. 2019), or individual trees sampled randomly (Fayle et al. 2013, Yusah et al. 2018), with some rare exceptions (see Dejean *et al* (2015)). This is mostly due to the difficulties of sampling in a very inaccessible canopy of tropical forest. While we acknowledge that our study might miss positive relationships between behaviourally subordinate species that avoid baits (e.g. parabiosis between *Colobopsis* and *Crematogaster* (Menzel et al. 2008)), it is a useful method to detect high spatial segregation between the canopy ant, particular the dominant ones.

The log-skewed shape of the ranked distribution of arboreal ant assemblages indicates a hierarchy in ant. However, the area occupied by ant territories can be also used to define dominancy of ant species in the community, in addition to their abundances (numerical dominance). We have found that spatial segregation of those territories correlates with species aggression. Moreover, species that built the largest colonies (territories) and occupied most of the canopies, were also the most aggressive. This was interestingly independent of ant body size. However, we observed a slightly negative trend of the smaller-bodied ant dominant species being those the most aggressive. This is relatively surprising, as e.g. *Oecophylla smaragdina* is one of the most studied and most aggressive ant species in the world (Holldobler 1983, Bluthgen and Fiedler 2002). Yet, it seems to be less aggressive than *Crematogaster polita* and *Anonychomyrma scrutator* in the New Guinea region. The latter two species probably also yield higher worker densities and larger nests, not only the overall territories. The aggression of species has been linked to individual body size interspecifically (Nowbahari et al. 1999) but not in intraspecifically (Retana and Cerdá 1994). However, interspecific aggression is known to be affected by competitor familiarity, habitat type and resource value (Tanner and Adler 2009). Although

our interspecies tests are limited to only four most common species in the communities, this is, to our knowledge, the first evidence using both spatial patterns and behavioural experiments showing that canopy ant mosaic are driven mainly by species aggressive behaviour, as proposed by *ant mosaic* theory and the evidences from the patterns-only based studies (Majer 1972, 1993, Camarota et al. 2016). As we studied primary forest site and all four most dominant species are native in the region, these results are not biased by an invasive species or habitat degradation, which may affect the ant species distributions in the forests, and thus the *ant mosaics* (Sanders et al. 2003, Pfeiffer et al. 2008, Fayle et al. 2013).

Despite a strong agreement among our behavioural experiments and the spatial patterns observed in the canopies, one might suggest that these findings could have been driven by the habitat filtering altogether. For instance, a most dominant ant species might be the most aggressive, but it could be spatially not occurring with other species, due to an environmental filter. Even though trees themselves are non-randomly distributed in our study area, and their species and growth forms follow environmental gradients (Vincent et al. 2018), we have not found surprisingly a strong effect of habitat filtering in our study. The only environmental variable affecting the composition of ants in our study was elevation. Effect of habitat filtering through changes in elevation is well-studied (Fisher 1996, Bruhl et al. 1999, Staab et al. 2014) but rarely on such a small scale (elevation change in our system is only ~100m). Even though our results show only a small effect of elevation, those results are relatively robust, with elevation being significant for both *Canopy Ant Community* and *Dominant Ant Community*, and remained present even after subtracting the effect of spatial auto-correlation. While other studies reported the effect of habitat filtering on the composition of dominant ants, in particular through ant preference for certain tree families or species (Dejean et al. 2015, 2016, 2018, 2019), we were not able to detect any effect of tree taxonomy on our community of arboreal ants. However, as those previous studies made conclusions without any statistical randomisation test (only observation of clustering of samples based on similarities), and they often include rare taxa to their conclusions (e.g. rare ant species in a singleton tree species), this may bias their results and make direct

comparisons to our study difficult. However, it is possible that other regions studied (Neotropics, Africa) might have a stronger relationship between canopy trees and the ant species (Dejean et al. 2008a, 2019), than it is the case in New Guinea (Klimes 2017). More studies using the same methods and statistical approaches are hence needed to reveal the possible multi-continental differences in ant distribution and relation to vegetation parameters, to test if our findings from one region are general.

There is a possibility that other drivers could also shape arboreal ant distribution and one of them is species dispersal possibilities, which we were not able to include. This is because a long term census with detailed life-history data on the ant species is needed to model such effects in ants and trees (Bruna et al. 2011). Sedimentary ant colonies, similarly to plants, have limitation distance of active reproduction and dominant species often start new nest using budding (Holldobler and Wilson 1990), which leads to clustering of one species in place (spatial-autocorrelation). Effect of elevation might be thus auto-correlated with the history of colony growth: e.g. the largest territory of *C. polita* based in lower elevations might be a “past effect” of colony establishment. Alternatively, *C. polita* might migrate more easily and hence prefer lower elevation (flatter terrain), than other dominant species. However, the effect of terrain slope was not significant in our multivariable analysis. Therefore, studies of more plots and sampling across more varying elevational gradients are needed to disentangle those possibilities.

Another aspect of ant reproduction is relatedness of colonies, with two colonies being potentially so genetically close, that the workers cannot be distinguished, if it is the same colony or not. We probably have this example in our results with *Podomyrma laevifrons* being not aggressive but having the second biggest territory in our plot. This colony has different nests far from each other with a low possibility of exchange individuals between the distant trees, yet workers were still unable to distinguish between those populations. As the species prefers the nesting in living tree tissues (Klimes 2017), its queens might perhaps establish new colonies over longer distances in new trees, compared to the other dominant species. More information about the ecology of species and/or cuticular hydrocarbons (Mathis et al. 2016) is therefore needed to test this

hypothesis. To achieve this is not trivial, due to high species richness in tropics, with relatively scarce ecological knowledge about them (Snelling 1998).

In summary, *ant mosaic* seems to be present in pristine lowland rainforest all over the world, and it can be detected on a larger scale of observation. Species assembly of those arboreal ant communities is always a combination of behavioural traits, habitat filtering and dispersal possibilities, that are difficult to disentangle. Our study brings evidence of the aggression behavioural of several dominant species being the key factor, accompanied by additional effect of habitat filtering of microclimate mediated by elevational variance.

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Author contributions

OM conceived the study, led the fieldwork, identification of ant specimens, manuscript writing, analysed and interpreted the data. OM and JY collected the data and conducted the experiments. GDW contributed plant data from the CTFS database. PK contributed to specimen identification, designing the study, and

writing of the first draft. PK, VN, and ML contributed the conceptualisation of the methods and interpretation of the results. All authors critically commented on drafts and gave final approval for publication.

Data Availability Statement

Data available from the Dryad Digital Repository with DOI after acceptance.

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~ *Figures and Tables* ~

Table 1. Description of the ant community datasets sampled in 225 high canopy trees of primary forest in New Guinea within the 9-ha area used in this study. *Whole Ant Community:* All ant fauna sampled with tuna-honey baits on trees from 0 to 35 m vertical height. *Canopy Ant Community:* As above but all samples from trees with height < 10 are excluded. *Dominant Ant Community:* We selected only ecologically dominant ant species from Canopy Ant Community, based on the worker abundance observed at baits and number of occupied baits by each species in each tree (see Materials and Methods, and Supporting Information S4).

	Whole Ant Community	Canopy Ant Community	Dominant Ant Community
Number of ant Species	57	31	23
Number of samples	1127 baits	677 baits	677 baits
Number of samples with ants	819 baits	457 baits	197 baits
Ant Species per tree \pm SD	3.87 \pm 1.51	2.42 \pm 1.10	0.88 \pm 0.03
Number of trees with ants of 225 sampled	221	204	190

Figure 1. Distributions of Whole Ant Community fauna sampled with tuna-honey baits on trees within 9-ha area of lowland primary forest in Papua New Guinea (225 trees). Individual species are named by species codes, for full names see Table S7. Those marked as Dominant Species (see Materials and Methods) are color-coded. A: Number of baits occupied by each ant species, with species ranked from the most common to the rarest (57 species). B: Vertical distribution of baits in the trees for ant species that occur on more than 1% of the baits (15 most common species). A circle represents mean height and line represents SD.

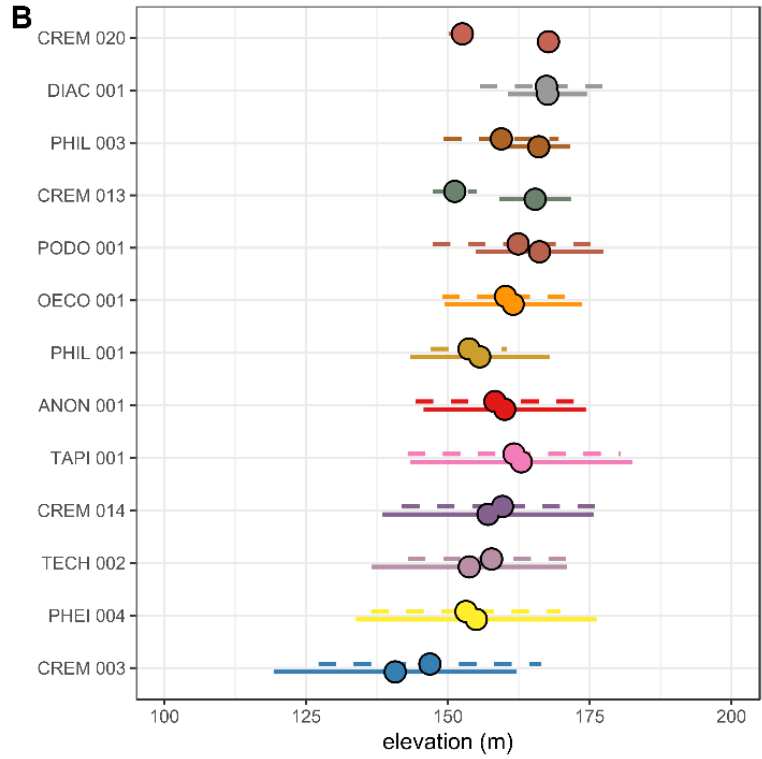
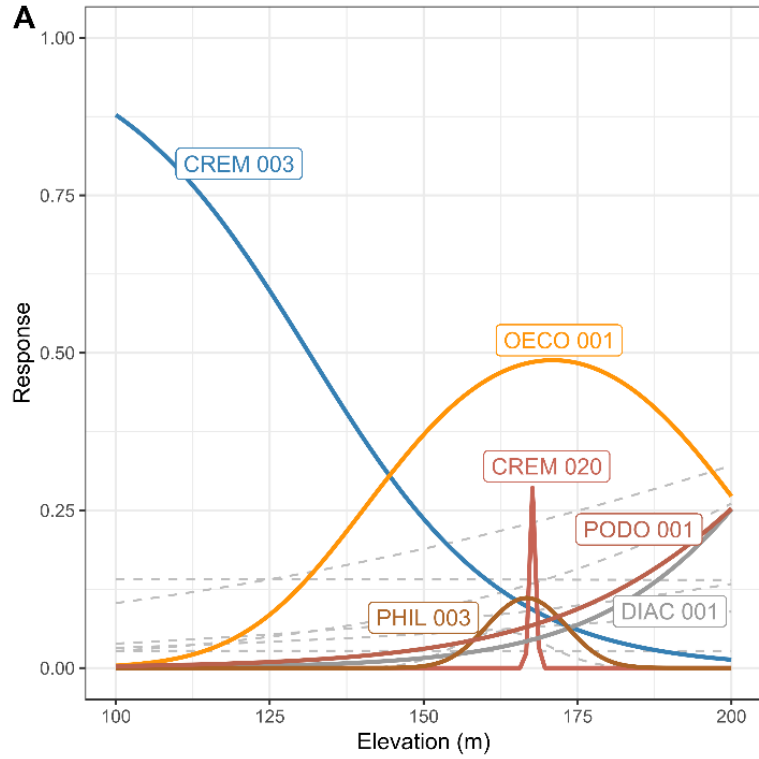


Figure 2. *The response of Canopy Ant Community to elevation across the 9-ha primary forest plot in New Guinea lowlands. Species that were presented on less than three trees were excluded as rare. Species names are represented by codes, for full names see Table S7. A) Species response curves to elevation. Each species was fit with a linear/polynomial GLM model. Species without significant response are showed with dashed lines. For a full list of used models for each species and their significance, see Table S6. B) Species optima (position on first CCA axis) and tolerance in multivariate analysis of Canopy Ant Community constrained by elevation (values are rescaled back to meters). For each species, two values are shown: Solid line – Uncorrected; Dashed line – Corrected for spatial autocorrelation (see Supporting Information S3)*

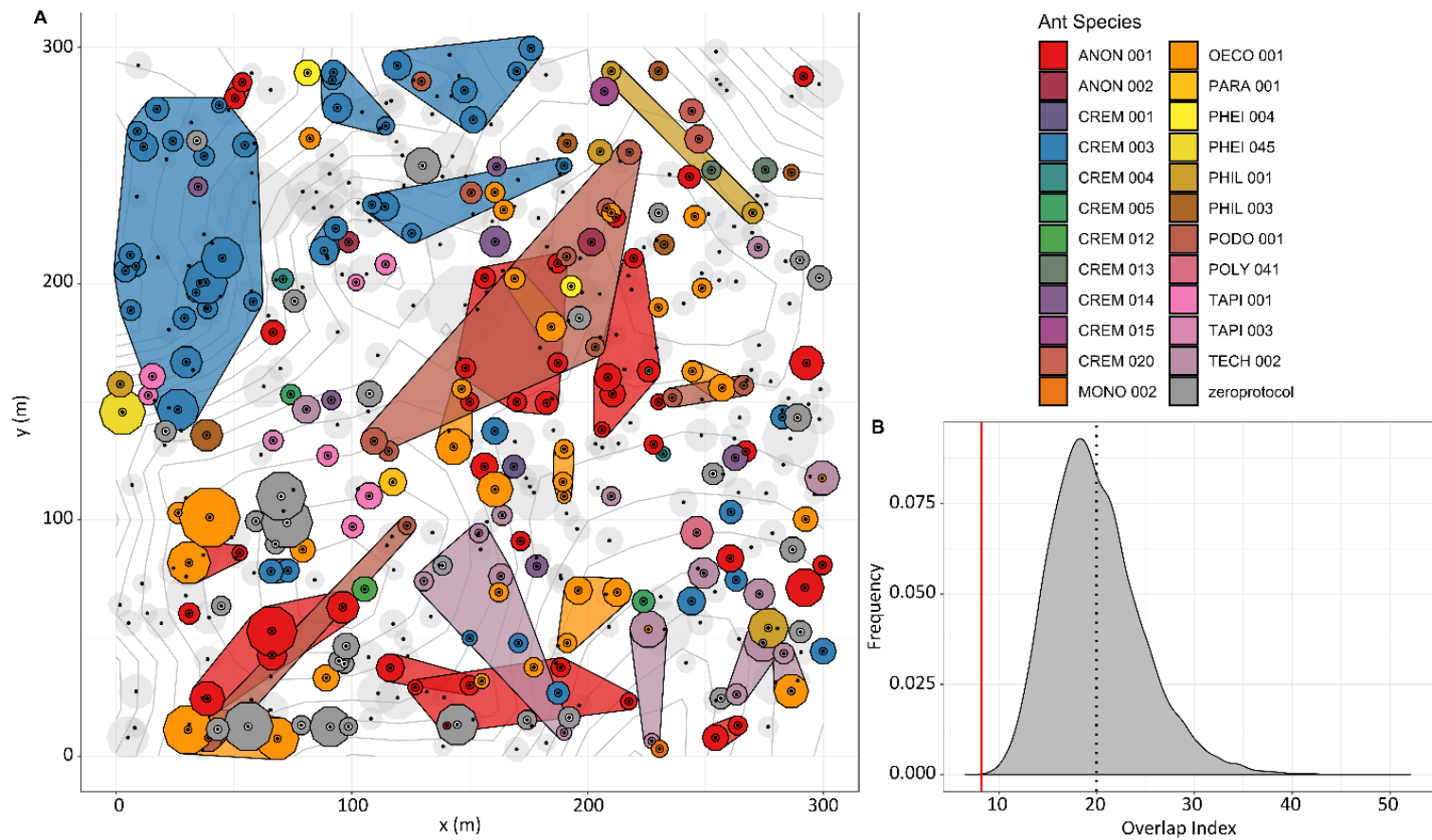


Figure 3. A) Distribution of territories of dominant canopy ant species in 9-ha primary forest area in Papua New Guinea. Each dot is a high canopy tree (DBH ≥ 40 cm). Circle around each tree indicates its canopy width, which has been estimated as perimeter (in m) based on DBH and species of the tree (see Supporting Information S7). Perimeters marked by black lines represent the tree individuals sampled for canopy ants. Colony territory is drawn as a connection of canopy of trees occupied with the same colony. We made an assumption, that area between two trees, which are occupied by the same colony, is also occupied by that colony (for details of colony boundary estimation, see Supporting Information S5). Grey contour lines indicate topography of area (for detailed figure see Fig. S1B). For full species names, see Tables S7. B) Distribution of values of Overlap Index generated by Canopy Arena Null Modelling Algorithm (CANMA) with 10.000 spatial randomisations of the territories. Dotted line shows mean simulated Overlap Index and red lines shows observed Overlap Index value, which is significantly smaller than simulated values (CANMA: OI= 8.2, $p < 0.05$). The spatial overlap of the individual colony territories is hence much smaller than it would be expected at random. For a full description of CANMA, see Materials and Methods, and Supporting Information S5.

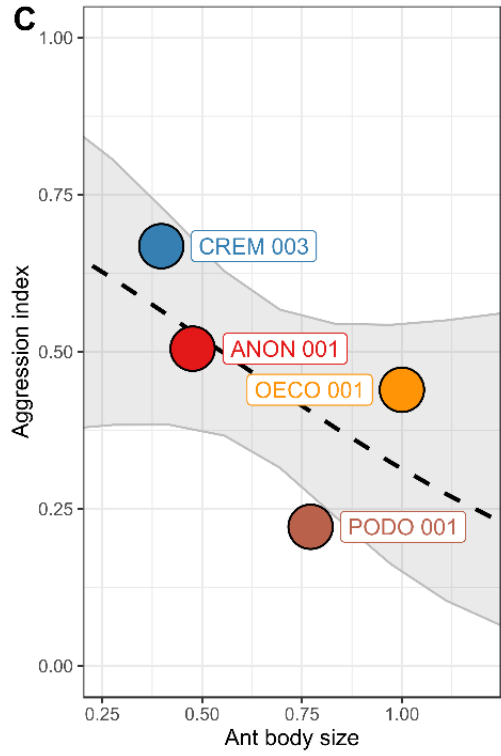
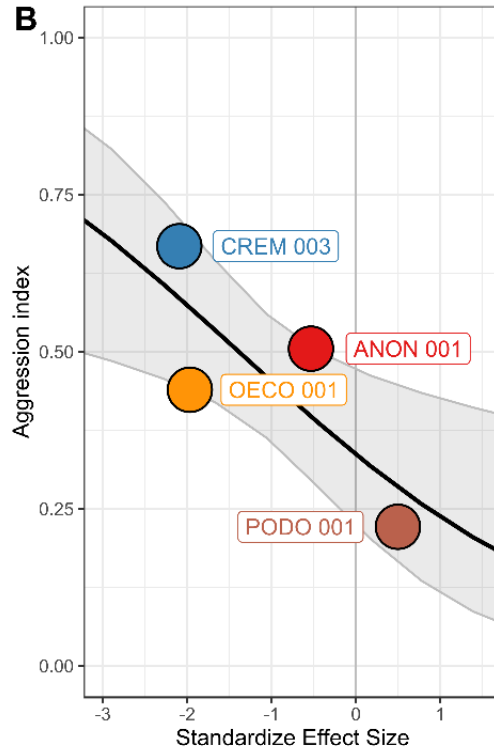
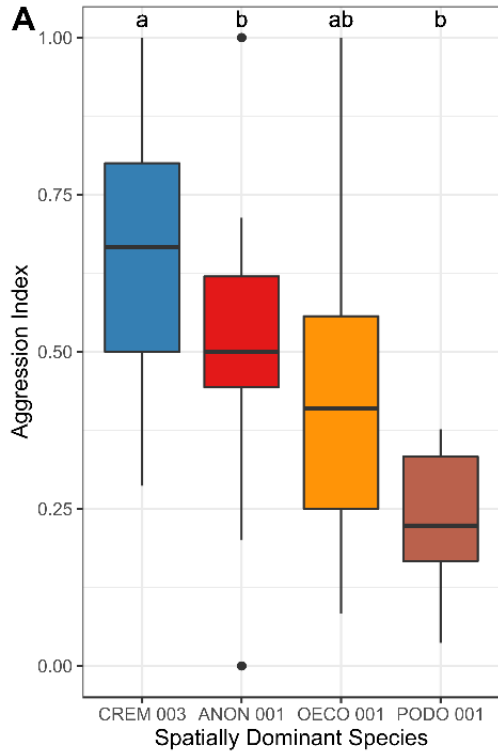


Figure 4. Aggression Index (AI) based on a behavioural test between the top four Spatially Dominant Species. Aggression Index ranges from 0 (behavioural avoidance of contact with another species in an arena) to 1 (direct attacking another species). A) Comparison of variability in AI between the four species (central bold lines indicate medians, boxes the 1st and 3rd quartiles of the data, and whiskers 1.5 times the interquartile range. Outliers beyond the range of the whiskers are plotted as circular points.). There is significant effect of the species (glmmTMB: $\text{Chisq} = 13.99$; $\text{DF} = 8$; $p < 0.01$). Differences between individual species are shown by different letters above the charts. B) Relationship between AI of the four species and their spatial segregation, represented by Standardized Effect Size (SES) of Canopy Arena Null Modelling Algorithm (CANMA_s, see Materials and Methods). There is a significant effect of a decline in species aggression with their decreasing spatial segregation (glmmTMB: $\text{Chisq} = 3.99$; $\text{DF} = 3$; $p < 0.05$). Negative SES shows strong species segregation from other species; values around zero represent random spatial overlapping, and positive values show aggregation. C) Relationship between AI of the four species and their body size (measured as the mean head length and rescaled to range between 0 and 1). There is no significant relationship between species aggression and their body size (glmmTMB: $\text{Chisq} = 1.92$; $\text{DF} = 3$; $p = 0.166$).

~ *Supplementary information* ~

Supplementary Materials and Methods

Supporting Information S1: Stratification of ant fauna between the canopy and bellow-canopy strata

We used Canonical Correspondence Analysis (CCA) and the whole ant fauna sampled on baits to test if species composition varied among ground + understorey level (“ground & understorey”: baits bellow canopy at height < 10 m baits m) and the canopy (“arboreal”: baits at > 10 m). Data matrix of 28 ants species x 778 baits with presence/absence was used and all species present in less than two baits were excluded as rare species. Stratification position was then used as an explanatory variable (arboreal x ground) and TreeID as a covariate.

Supporting Information S2: C-score

C-score was calculated using *EcosimR* packaged (Gotelli et al. 2015). *Canopy Ant Community* dataset was entered as a matrix (57 ant species x 225 trees) with presence/absent data. The algorithm was set as “sim2” (fixed-equiprobable; a sum of species distributions is fixed and sums of trees are equiprobable) with the number of simulation as 10 000. We tested if species co-occur less or more than they would do at random. We, therefore, marked the difference as significant if observed C-score was in 2.5 or 97.5% quantile (two-tail test).

Supporting Information S3: Multivariate analysis of the multiple environmental effects on the Canopy Ant Community

Canopy Ant Community dataset was used in multivariable analysis but species that occurred in less than three trees (defined here as a rare species) were not included. Data were used as a binominal matrix (presence-absence) of each ant species in a tree (ant species x trees). Trees without any ants were also excluded from the analysis. The final data matrix was 119 trees x 13 ant species.

In all CCA analyses, the explanatory variables were selected using forward selection (i.e. canonical correspondence limited ordination) with the corrected p

values (false-discovery rate correction, 999 randomisation, $\alpha = 0.05$). The efficiency of the axis was calculated as variability explained by constrained axis divided by variability explained by unconstrained axis.

To test if tree species surrounding the tree, where ants were sampled, is significantly affecting the composition of ants (i.e. if plant community has a direct impact of *Canopy Ant Community*), we created vegetation plots for each tree as 10x10 m quadrat, with that sampled tree in the middle. We include all trees in these vegetation plots with DBH>10cm from the Wanang CTFS forest plot database (Vincent et al. 2018). We created a matrix of vegetation plots x all tree species in them, with numbers representing a summary of basal area of that tree species, and divided by total BA of that vegetation plot. We excluded from calculation those tree species as rare, which sum of BA was less than 1 m² (before standardization) across all the plots. We then performed interactive forward selection CCA_{PLOT} (with correction for false-positive p) of the tree species (N=89) to see if any tree species affect species distribution of ants.

To test for the effect of space on the ant community composition and the spatial autocorrelation with the effects of the environmental predictors, we used Principal Coordinates of Neighbour Matrices (PCNM). The method uses distance-based Moran's Eigenvector Map analysis (dbMEM) as a measurement of spatial effect. It creates PCO axes from a Euclidian-distance matrix of trees, with a threshold of only including one nearest neighbour (60 PCO axis in total). Elevation (i.e. solely significant factor) was used as one variable set for the variation partitioning (Table S2), while significant Principal Coordinates Ordination (PCO) axes from dbMEM as the second set (Table S1). This analysis was used to test how much the effect of elevation is inter-correlated with the spatial distribution of arboreal ants in the 9-ha plot.

To visualize species response to elevation, we plotted optimum and tolerance on the first CCA axis of each Ant Arboreal Community species. We calculate this for a) CCA_{ENV} analysis constrained only by elevation and b) CCA axis constrained by elevation, with space as covariate (calculated by dbMEM). We rescaled all values back to meters.

Supporting Information S4: Definition of dominant species in each tree

We have determined which ant species was dominant in each tree individually, using numeric dominance criteria. First, we marked bait as occupied if there were at least 10 individuals on it. Next, we determine dominant species based on their total abundances on the tree and number of occupied baits. In cases, where two or more species occupied a similar proportion of baits, and where were presented in similar abundances, we included them all as dominants in such tree. We used this quantitative approach, as we did not wish to define the dominants using the behavioural observational or literature data that are scarce for most of the species. Hence, it is possible that in some cases, the species might be not behaviourally dominant (e.g. *Tapinoma melanocephalum*, or *Polyrhachis* spp.), but in most cases, the behavioural dominance at baits can be also assumed as it is highly correlated with numeric dominance (Arnan et al. 2018).

Supporting Information S5: Colony Area Null Modelling Algorithm

Preparing dataset for CANMA

Entry data structure is matrix with rows as samples (trees) and columns as set variables: X and Y coordinates (in meters), unique individual tree number code, Dominant ant species code, unique code of colony, and tree crown radius for each tree (in meters, calculated from DBH, see Supporting Information S7).

Colony polygons

To create a spatial representation of species colonies in space, the algorithm first creates selected number of points (default = 10) around each tree point in selected distance (default set as the crown radius of tree). All edge points were then connected to polygons by their unique colony code, each representing total space occupied by the given colony. We made an assumption, that all area between two trees, which are occupied by the same colony, is also occupied by that colony. If there is a tree present inside of the colony polygon area, that was sampled and we are sure that the dominant species was not present there, we exclude the area from the polygon.

Correction for edge effect in the randomisation

To prevent edge effect with randomisation, we included only the subsample of observed polygons excluding border of the observation. Hence, the algorithm includes only data with coordinates in a rectangle (bordered area), with distance from the edge of the observed area as the average radius of all colonies.

Calculation of the Overlap Index (OI)

The algorithm creates all combinations between all polygons (colonies) and then calculates overlapping area between them, inside of the bordered area.

Overlap Index (OI) was calculated as $OI = \frac{\sum \text{area of all overlaps}}{\sum \text{area of all polygons}} * 100$, representing the percentage fraction of the area of overlaps to the total area of polygons inside of the bordered area.

Note that compared the C-scores (i.e. high C-scores = segregation), the lower values of OI indicate segregation of the colonies (and species) from each other.

Randomisation process

To create a null model of colony distribution, we had to randomise the position of each colony, calculate OI across all colonies (i.e. all pairwise overlaps between colonies) and compare it to the observed value.

Position of each polygon was randomised and adjusted by the density of sampled trees in the focused area. The algorithm created a new position for each centroid of each polygon skewed by the given density matrix. If no density matrix was given, then the position of centroid would have been random (note default setting uses the density matrix of sampled trees). Polygon was rotated randomly (1-360 degree) around the centroid. Each centroid was placed so the polygon fits whole to the observed area. This was repeated for all polygons.

After each randomisation (reshuffling and replacing of all polygons), model separates the forest plot into two pieces of the same area (Center and Trim, see Fig. S8) and calculates the sum of all area of all polygons in each area. To prevent mid-domain effect, the model only considers the randomisation as valid if the sum of the area of all polygons in Trim is up to 1.5x of Sum of the area of all

polygons in Centre. If this criterion is not fulfilled, the randomisation process is repeated (e.g. there were 150,741 runs for 10,000 used randomised maps used for default model OI calculations).

OI of randomised polygons was calculated with border cutting being the same as for the observed OI values.

Significance of test

Randomisation process was run 10,000 times as default, and the test was scored as significant if observed OI was in 5% quantile (we were testing if randomised OI is bigger than observed, one-tail test).

Effect of number of randomisation and tree density map

To investigate the stability of results we also run the algorithm with a) a different number of randomisations (100, 1,000, 10,000) and b) different tree density maps (no density map, map of all big trees, map of only sampled trees). Note that we did not run 10,000 randomisations on all combinations due to a long computation time (3.1 days in the default model). We then compare the p values (see Table S8).

Supporting Information S6: Aggression tests

For three focal most common species (*CREM 003*, *ANON 001* and *OEKO 001*, for full names, see Table S7) we have selected nine independent colonies (maximum possible). We took a sample of living workers from each colony of one species and conducted a test with one colony of each other species (18 tests per one species, each colony in two tests), making it total of 27 tests. In addition, we selected three colonies of *PODO 001* (maximum possible) and each colony was tested against one colony of each dominant species (9 tests available in total). The total number of tests was therefore 36 (Fig. S4).

For each test, every interaction was scored as one of the four categories (1: individual starts to run away after interaction, 2: individual does not change direction or speed after interaction, 3: individual does warning position (*Crematogaster* with raised abdomen, *Oecophylla* with front legs, etc.), 4: individual directly attacks the other species worker(s) (bite, sting)). Interactions

were marked for each species separately by two observers (each observer monitored one species). Observers changed the species they observed after each test to avoid the effect of the observer. We also measured temperature in the room during each test to account for the possible effect of change in weather on the worker behaviour/activity.

We standardized the AI to range between 0 and 1 as

$$x' = (x - 1)/3$$

We have created Generalized Linear Mixed Models via Template Model Builder (glmmTMB; *glmmTMB package* (Brooks et al. 2017)) with AI as dependent variable with beta distribution (link=logit) and no zero-inflation, **Species** as fixed categorical variable and three random effects: **interaction type** (categorical variable: Anon-Crem, Oeco-Crem, Anon-Oeco, Podo-Crem, Podo-Oeco, Podo-Anon), **ID of observer** (N=2) and **temperature** in room during test (continuous variable). Differences between individual species were tested using a post-hoc Tukey test.

Supporting Information S7: Calculating canopy width

Since we did not have information about canopy size (width), we extrapolated it from DBH using another dataset from Wanang, a nearby location in the same forest type and a similar elevation (1321 trees), that have measured information about DBH, tree species and canopy crown width (Whitfeld et al. 2012). Crown width was calculated from DBH values using a linear model with correction by species/genus/family of tree based on the availability of that information for each tree species. See Supporting Information Code Appendix II for used function.

Supplementary Figures and Tables

Table S1. Result of Principal Coordinates of Neighbour Matrices (PCNM) with distance-based Moran's Eigenvector Map (dbMEM) as a measure of the spatial effect affecting the Canopy Ant Community composition.

Name	Explains %	Contribution %	pseudo-F	P	P(adj)
PCO.2	2.1	5.8	4.1	0.001	0.03
PCO.5	1.6	4.3	3.1	0.001	0.03
PCO.3	1.4	3.9	2.8	0.003	0.045
rest 57 PCO axis	-	-	-	-	n.s.

Table S2. Result of CCA_{VAR} Interactive Forward Selection of variables tested in multivariable analysis for the effects of environmental variables on the Canopy Ant Community species composition (see Materials and Methods). Variables tested in the analysis were: **tree size** (DHB of sampled tree in cm), **elevation of tree** (in m.a.s.l.), **slope of surrounding area of tree** (in %), **topography** (convex/concave, calculated from height of surrounding plots) and **canopy connectivity** (defined as number of canopy trees connecting the sampled tree).

Name	Explains %	Contribution %	pseudo-F	P	P(adj)
Elevation	2.5	51.6	4.9	0.001	0.002
slope of surrounding area	-	-	-	-	n.s.
canopy connectivity	-	-	-	-	n.s.
tree size	-	-	-	-	n.s.
canopy connectivity	-	-	-	-	n.s.

Table S3. Result of CCA_F Interactive Forward Selection of variables tested in multivariable analysis for the effects of tree family ID (18 tree families) on the Canopy Ant Community species composition (see Materials and Methods). We included only families that have more than 2 individuals.

Name	Explains %	Contribution %	pseudo-F	P	P(adj)
Sapindaceae	-	-	-	-	n.s.
Fabaceae	-	-	-	-	n.s.
Meliaceae	-	-	-	-	n.s.
Rubiaceae	-	-	-	-	n.s.
Cannabaceae	-	-	-	-	n.s.
Lamiaceae	-	-	-	-	n.s.
Myristicaceae	-	-	-	-	n.s.
Polygalaceae	-	-	-	-	n.s.
Moraceae	-	-	-	-	n.s.
Malvaceae	-	-	-	-	n.s.
Apocynaceae	-	-	-	-	n.s.
Combretaceae	-	-	-	-	n.s.
Burseraceae	-	-	-	-	n.s.
Sapotaceae	-	-	-	-	n.s.
Achariaceae	-	-	-	-	n.s.
Chrysobalanaceae	-	-	-	-	n.s.
Annonaceae	-	-	-	-	n.s.
Euphorbiaceae	-	-	-	-	n.s.

Table S4. Result of CCA_G Interactive Forward Selection of variables tested in multivariable analysis for the effects of tree genus ID (21 tree genera) on the Canopy Ant Community species composition (see Materials and Methods). We included only genera that have more than 2 individuals.

Name	Explains %	Contribution %	pseudo-F	P	P(adj)
<i>Pometia</i>	-	-	-	-	n.s.
<i>Intsia</i>	-	-	-	-	n.s.
<i>Celtis</i>	-	-	-	-	n.s.
<i>Mastixiodendron</i>	-	-	-	-	n.s.
<i>Chisocheton</i>	-	-	-	-	n.s.
<i>Vitex</i>	-	-	-	-	n.s.
<i>Xanthophyllum</i>	-	-	-	-	n.s.
<i>Terminalia</i>	-	-	-	-	n.s.
<i>Dysoxylum</i>	-	-	-	-	n.s.
<i>Ficus</i>	-	-	-	-	n.s.
<i>Myristica</i>	-	-	-	-	n.s.
<i>Aglaiia</i>	-	-	-	-	n.s.
<i>Alstonia</i>	-	-	-	-	n.s.
<i>Neonauclea</i>	-	-	-	-	n.s.
<i>Pangium</i>	-	-	-	-	n.s.
<i>Microcos</i>	-	-	-	-	n.s.
<i>Pterocymbium</i>	-	-	-	-	n.s.
<i>Polyalthia</i>	-	-	-	-	n.s.
<i>Tristiropsis</i>	-	-	-	-	n.s.
<i>Pimelodendron</i>	-	-	-	-	n.s.
<i>Pterocarpus</i>	-	-	-	-	n.s.

Table S5. Result of CCAs Interactive Forward Selection of variables tested in multivariable analysis for the effects of tree species ID (17 tree species) on Canopy Ant Community species composition (see Materials and Methods). We included only species that have more than 2 individuals.

Name	Explains %	Contribution %	pseudo-F	P	P(adj)
<i>Alstonia scholaris</i>	-	-	-	-	n.s.
<i>Celtis latifolia</i>	-	-	-	-	n.s.
<i>Celtis philippensis</i>	-	-	-	-	n.s.
<i>Chisocheton cumingianus</i>	-	-	-	-	n.s.
<i>Intsia bijuga</i>	-	-	-	-	n.s.
<i>Kingiodendron novoguineense</i>	-	-	-	-	n.s.
<i>Mastixiodendron pachyclados</i>	-	-	-	-	n.s.
<i>Myristica fatua</i>	-	-	-	-	n.s.
<i>Neonauclea obversifolia</i>	-	-	-	-	n.s.
<i>Pangium edule</i>	-	-	-	-	n.s.
<i>Pimelodendron amboinicum</i>	-	-	-	-	n.s.
<i>Pometia pinnata</i>	-	-	-	-	n.s.
<i>Pterocymbium beccarii</i>	-	-	-	-	n.s.
<i>Pterocarpus indicus</i>	-	-	-	-	n.s.
<i>Tristiropsis acutangula</i>	-	-	-	-	n.s.
<i>Vitex cofassus</i>	-	-	-	-	n.s.
<i>Xanthophyllum papuanum</i>	-	-	-	-	n.s.

Table S6. Results of Canopy Ant Community response to elevation of a sampled tree. Species that we not presented on at least three trees were excluded as rare. For each species, we created GLM, and we run both binomial and polynomial error distribution. Polynomial was selected if it explained the relationship with elevation better than linear, based on deviance test. Significant species are in bold and the best model (linear or polynomial) is shown.

Species	Model	Deviance	Resid. Deviance	DF	DF Resid	P
ANON 001	Linear	2.17	223.91	1	219	0.141
CREM 003	Linear	46.41	182.31	1	219	0.000
CREM 013	polynomial	5.41	49.71	2	218	0.067
CREM 014	Linear	0.00	55.112	1	219	0.996
CREM 020	polynomial	14.40	17.36	2	218	0.001
DIAC 001	linear	4.85	63.95	1	219	0.028
OECO 001	polynomial	23.58	270.91	2	218	0.000
PHEI 004	linear	0.86	123.92	1	219	0.355
PHIL 001	linear	0.43	99.46	1	219	0.514
PHIL 003	polynomial	11.49	75.60	2	218	0.003
PODO 001	linear	5.03	88.23	1	219	0.025
TAPI 001	linear	3.69	152.33	1	219	0.055
TECH 002	linear	0.00	179.21	1	219	0.991

Table S7. Species list of all ant species sampled in this study and number of baits they have been found on from 1127 baits exposed in total (57 ant species in 20 genera). Species are ordered by their species codes and followed by their taxonomic identification (i.e. species or morphospecies, see Materials and Methods).

Code	Number of occupied baits	Number of occupied Trees	Scored as dominant species	Latin name
ANON 001	122	46	True	<i>Anonychomyrma</i> cf. <i>scrutator</i> (Smith F., 1859)
ANON 002	3	3	True	<i>Anonychomyrma minuta</i> (Donisthorpe, 1943)
APHA 001	1	1	False	<i>Aphaenogaster</i> sp. aff. <i>dromedaria</i> (Emery, 1900)
CALY 001	1	1	False	<i>Calyptomyrmex beccarii</i> (Emery 1887)
CAMP 001	3	3	False	<i>Colobopsis vitrea</i> (Smith F., 1860)
CAMP 004	2	1	False	<i>Colobopsis aruensis</i> (Karavaiev, 1933)
CARE 001	1	1	False	<i>Carebara minima</i> (Emery, 1900)
CARE 007	2	2	False	<i>Carebara melanocephala</i> (Donisthorpe, 1948)
CREM 001	4	1	True	<i>Crematogaster paradoxa</i> (Emery, 1894)
CREM 003	178	47	True	<i>Crematogaster polita</i> (Smith F., 1865)
CREM 004	9	6	True	<i>Crematogaster</i> cf. <i>pythia</i> (Forel, 1915)
CREM 005	2	2	True	<i>Crematogaster flavitarsis</i> (Emery, 1900)
CREM 010	2	2	False	<i>Crematogaster emeryi</i> (Forel, 1907)

CREM 012	1	1	True	<i>Crematogaster</i> sp. 12 aff. <i>flavitarsis</i> (Emery, 1900)
CREM 013	7	6	True	<i>Crematogaster tarsata</i> (Smith, F. 1865)
CREM 014	24	6	True	<i>Crematogaster</i> cf. <i>irritabilis</i> (Smith, F., 1860)
CREM 015	1	1	True	<i>Crematogaster</i> sp. 15 aff. <i>flavicornis</i> (Emery, 1897)
CREM 020	12	3	True	<i>Crematogaster meijerei</i> (Emery, 1911)
DIAC 001	9	8	False	<i>Diacamma rugosum</i> (Le Guillou, 1842)
LEPM 002	1	1	False	<i>Leptomyrmex flavitarsus</i>
MONO 002	5	3	True	<i>Monomorium intrudens</i> (Smith F., 1894)
MONO 003	4	3	False	<i>Monomorium</i> sp. 3
ODON 001	2	2	False	<i>Odontomachus simillimus</i> (Smith F., 1858)
OECO 001	182	85	True	<i>Oecophylla smaragdina</i> (Fabricius, 1775)
PARA 001	5	4	True	<i>Paraparatrechina pallida</i> (Donisthorpe, 1947)
PARA 005	5	4	False	<i>Nylanderia</i> aff. <i>vaga</i> (Forel, 1901)
PARA 006	3	3	False	<i>Paraparatrechina</i> sp. 6
PARA 007	1	1	False	<i>Nylanderia nuggeti</i> (Donisthorpe, 1941)
PHEI 001	2	2	False	<i>Pheidole</i> sp. 1
PHEI 002	1	1	False	<i>Pheidole</i> sp. 2 aff. <i>sexspinosa biroi</i> (Emery, 1900)
PHEI 003	10	9	False	<i>Pheidole fuscula</i> (Emery, 1900)

PHEI 004	18	18	True	<i>Pheidole hospes</i> (Smith, F. 1865)
PHEI 006	1	1	False	<i>Pheidole</i> sp. 6
PHEI 007	1	1	False	<i>Pheidole</i> sp. 7 aff. <i>gambogia</i> (Donisthorpe, 1948)
PHEI 013	2	1	False	<i>Pheidole</i> sp. 13 aff. <i>tricolor</i> (Donisthorpe, 1949)
PHEI 014	2	2	False	<i>Pheidole</i> sp. 14 aff. <i>gambogia</i> (Donisthorpe, 1948)
PHEI 015	1	1	False	<i>Pheidole</i> sp. 15 "bifurca clade"
PHEI 017	1	1	False	<i>Pheidole</i> sp. 17 aff. sp. 19
PHEI 018	10	10	False	<i>Pheidole</i> cf. <i>distincta</i> (Donisthorpe, 1943)
PHEI 026	8	7	False	<i>Pheidole</i> sp. 26
PHEI 035	1	1	False	<i>Pheidole tricolor</i> (Donisthorpe, 1949)
PHEI 045	1	1	True	<i>Pheidole</i> sp. 45 cf. <i>lobulata</i> (Emery, 1900)
PHEI 051	2	2	False	<i>Pheidole</i> sp. 51 aff. <i>distincta</i> (Donisthorpe, 1943)
PHIL 001	34	13	True	<i>Philidris</i> cf. <i>cordata</i> (Smith F., 1859)
PHIL 003	25	11	True	<i>Philidris</i> sp. 3 aff. 1
PODO 001	26	12	True	<i>Podomyrma laevifrons</i> (Smith F., 1859)
PODO 011	1	1	False	<i>Podomyrma</i> sp. 11
POLY 002	1	1	False	<i>Polyrhachis (Myrma) sericata</i> (Guérin- Méneville, 1838) (relucens-group)
POLY 006	1	1	False	<i>Polyrhachis (Myrmhopla) bubastes</i> (Smith F., 1863) (sexspinosa-group)
POLY 015	1	1	False	<i>Polyrhachis (Myrmhopla) waigeuensis</i> (Donisthorpe, 1943) (sexspinosa-group)

POLY 041	8	5	True	<i>Polyrhachis (Polyrhachis) sp. 41</i>
RHYT 001	1	1	False	<i>Rhytidoponera cf. aenescens</i> (Emery, 1900)
RHYT 002	3	3	False	<i>Rhytidoponera strigosa</i> (Emery, 1887)
STRU 001	1	1	False	<i>Strumigenys cf. loriae</i> (Emery, 1897)
TAPI 001	28	25	True	<i>Tapinoma melanocephalum</i> (Fabricius, 1793)
TAPI 003	1	1	True	<i>Tapinoma sp. 3 aff. williamsi</i> (Wheeler 1935)
TECH 002	72	31	True	<i>Technomyrmex albipes</i> (Smith F., 1861)

Table S8. Significance values for different tests of spatial segregation of Canopy Ant Community in the observed area in big canopy trees ($DBH \geq 40$ cm) using the CANMA randomisation (see Materials and Methods) and C-scores. Asterisk indicated $p < 0.05$. “X” mark not tested combinations due to long computation time.

Model	Number of randomisations		
	100	1000	10 000
CANMA			
Density of trees in randomisation process	p values (* < 0.05)		
<i>No density map</i>	< 0.001*	< 0.0001*	X
<i>Tree density based on all trees</i>	< 0.001*	< 0.0001*	X
<i>Tree density based on sampled trees</i>	< 0.001*	< 0.0001*	0.0002*
C-score	0.01*	0.001*	0.0001*

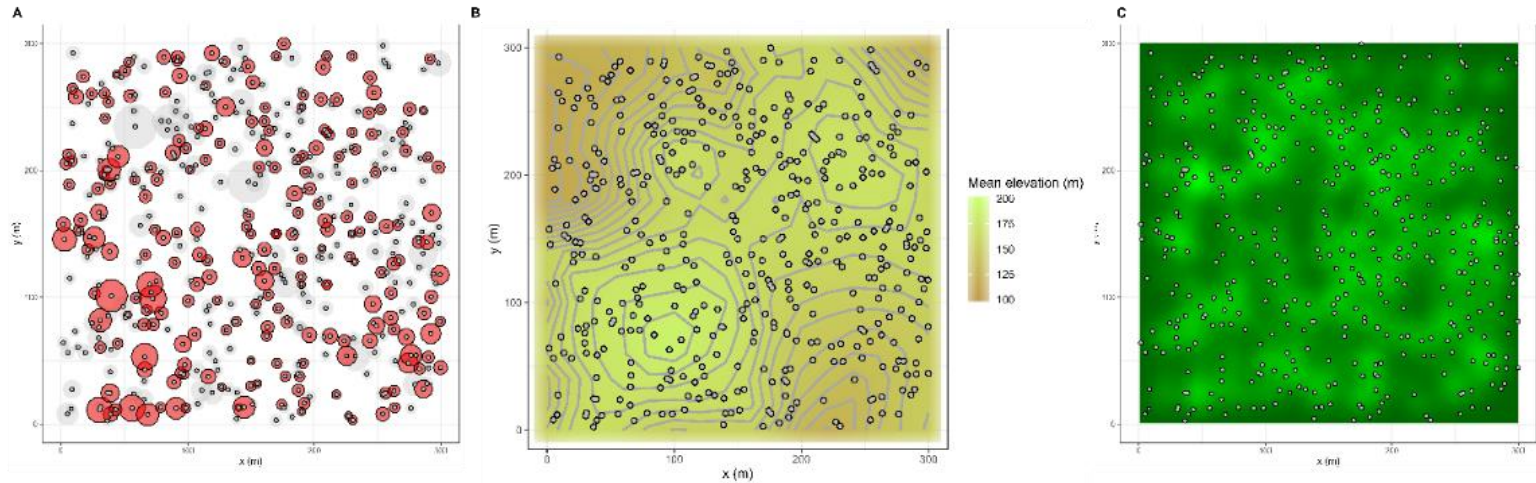


Figure S1. Supplementary information for the spatial distribution of high canopy trees ($DBH \geq 40cm$) within 9-ha area sampled for the ants in Wanang CTFs plot lowland forest, Papua New Guinea, and the terrain in that area. Each dot is a tree. A) Map of all sampled trees. Circle around the tree shows tree canopy perimeters. Width of each tree canopy is calculated from DBH, for more information see Supporting Information S7. Red colour indicates trees sampled for ants (see Method). B) Topography map of the plot. Grey contour lines indicate topography of the area. C) Density map of all big trees. Lighter colour represents higher density of big trees.

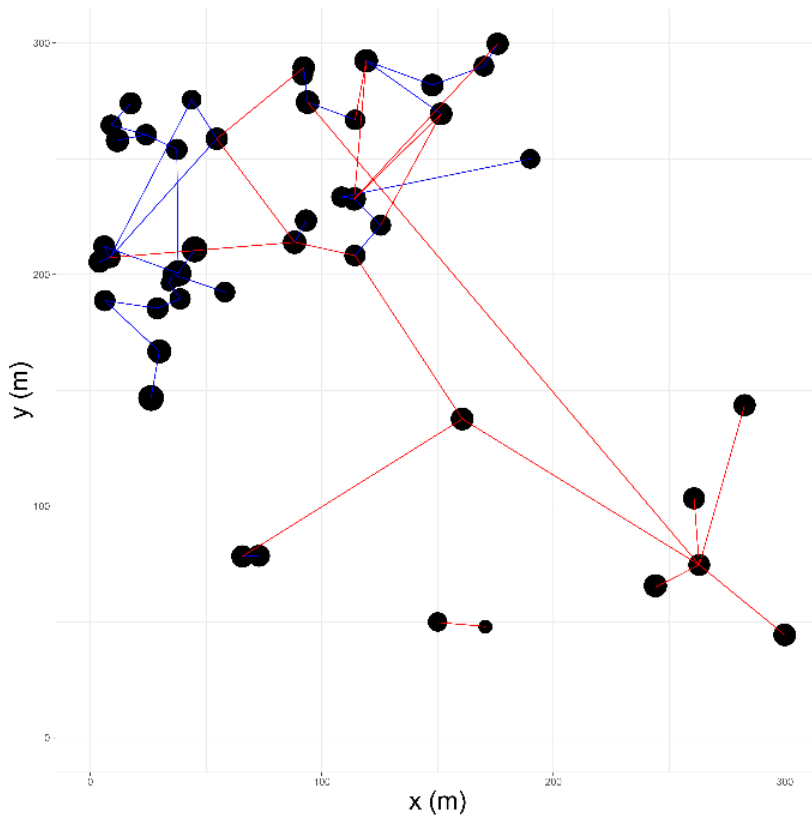


Figure S2. Diagram of behavioural tests conducted between trees occupied by a dominant ant species, here using the most common species *Crematogaster polita* (CREM 003) as an example. The tests were conducted to measure territory boundaries of individual colonies of each species within the Canopy Ant Community (see Materials and Methods). Each black circle represent a single tree and each line represent a single behavioural test. Red colour indicates a negative result (workers were attacking each other) and blue represent a positive result of the test (workers were not hostile against each other).

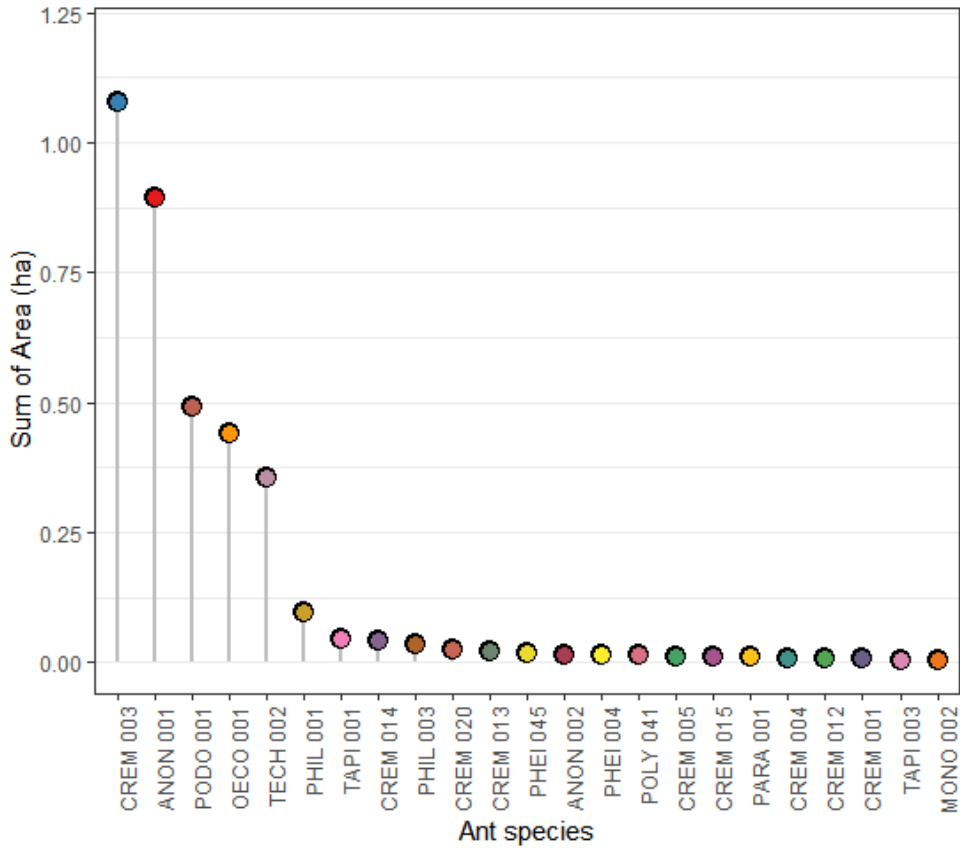


Figure S3. Summary of the total area occupied by all colonies of each of the species from the Dominant Ant Community, calculated from the map of their territories (Fig. 3A) in the observed area. The top four species were selected as Spatially Dominant Species for subsequent interspecies aggression tests (Fig. S4-S5 and Materials and Methods).

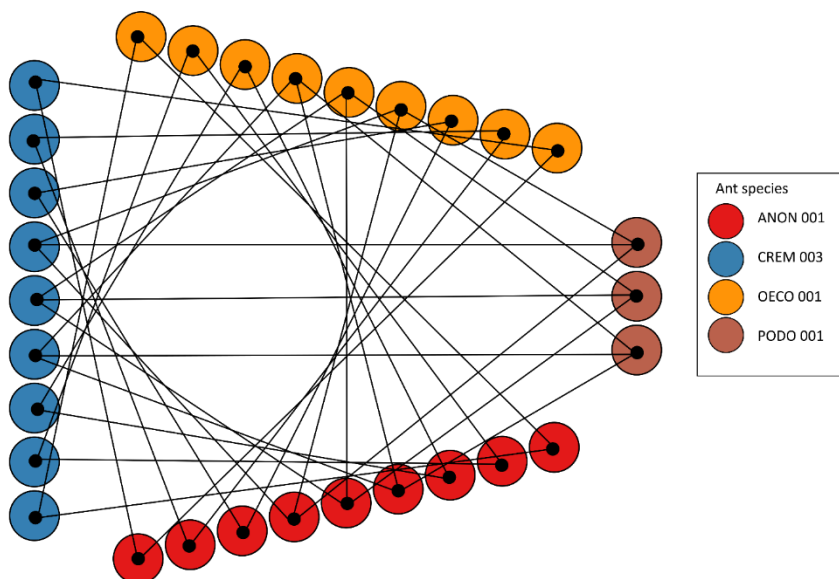


Figure S4. Scheme of aggression tests conducted between canopy ant colonies of the top four Spatially Dominant Species. Each circle represents an independent colony tested by behavioural tests with other species (2-3 tests per colony, see Materials and Methods). Each line between colonies represents one behavioural test.



Figure S5. Photo of an arena used in field lab to run an aggression test between the pair of ant species (see Method). Arena was constructed from two plastic vials (100ml each) connected together by their openings using sticky tape. Tubes were used before tests for over-night habituation of the ants, honey-water infused cotton was placed in each tube to provide food and moisture. New tube clean sets were used for each test.

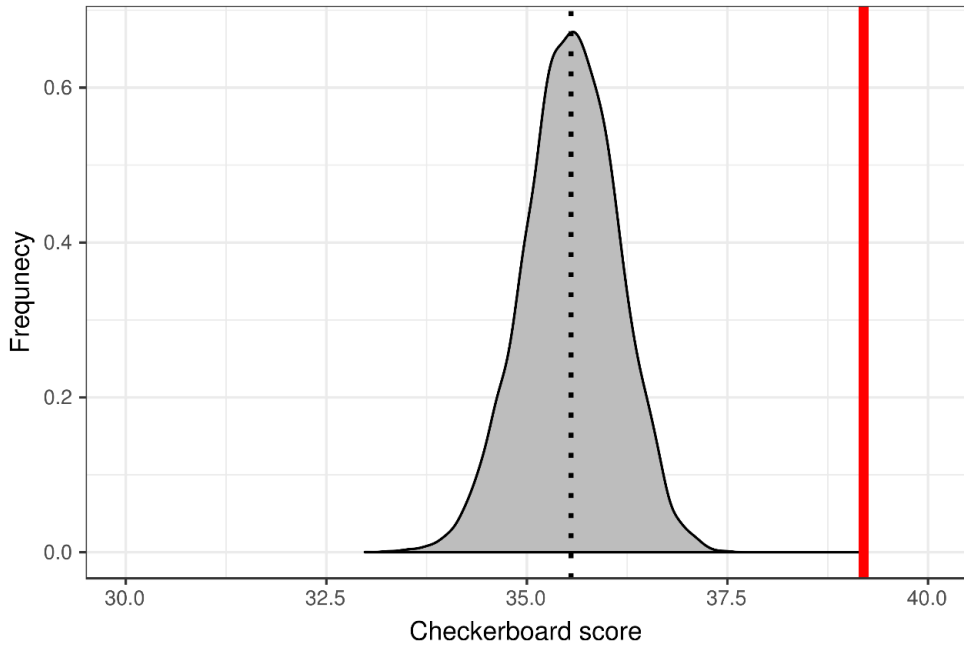


Figure S6. Distribution of 10 000 randomised values of Checkerboard score (C-score) for Canopy Ant Community in the 9-ha lowland primary forest plot. The randomised matrix consisted of 57 ant species x 225 sampled trees with binominal distribution (species presence-absence). The algorithm was set as “sim2” (fixed-equiprobable; i.e. the sum of species distributions is fixed and sums of trees are equiprobable). The dotted line shows the mean C-score value and the red line shows observed C-score, that is significantly bigger than randomises values ($p < 0.01$).

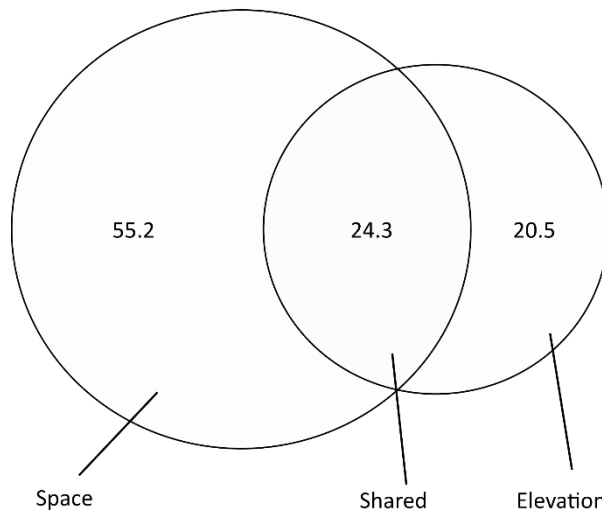


Figure S7. Venn diagram of variation partitioning explained by the significant environmental variables (elevation) and space, using canonical correspondence analysis (CCA_{VAR}) on Canopy Ant community in the 9-ha forest plot (presences of ant species in trees). Effect of space is expressed as the amount of variance explained by the significant Principal Coordinates Ordination (PCO) axes from distance-based Moran's Eigenvector Map (dbMEM) (see Supporting Information S3 and Table S1-S5 for calculation).

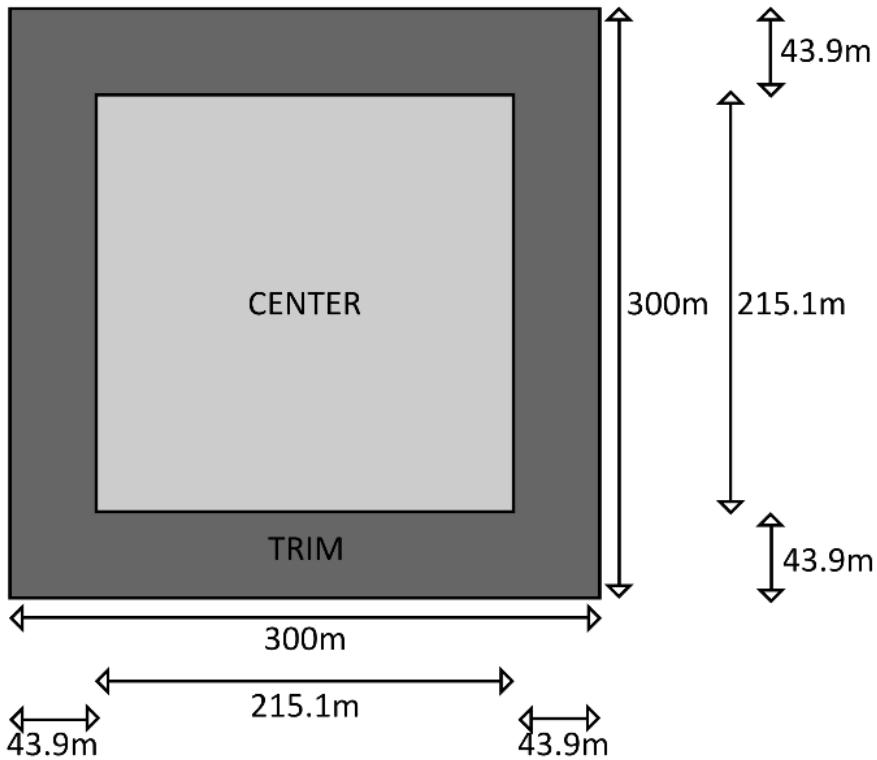


Figure S8. Diagram of the 9-ha forest plot sampled divided into two subsections: *CENTER* and *TRIM*. Both subsections have exactly the same area.

Supplementary Code Appendix I & II

R scripts will be available online after manuscript acceptance or by request from author (email:ondrej.mottl@gmail.com).

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~ Chapter III ~

Secondary succession has surprisingly low impact on arboreal ant communities in tropical montane rainforest

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Secondary succession has surprisingly low impact on arboreal ant communities in tropical montane rainforest

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Abstract. Understanding forest regeneration processes is increasingly important as disturbed forests spread rapidly in tropical landscapes. While successional dynamics are relatively well-understood for plants, they remain poorly known for tropical insects, particularly in montane rainforests and tree canopies. We studied the effect of montane forest succession on arboreal ant communities in New Guinea near their natural elevational limit (1800 m a.s.l.). We censused ant species and nests in replicated 0.1-ha plots of primary, old and young secondary forests, destined to be felled for gardens by the local landowners. Overall, 1249 felled trees were dissected for all ant-associated microhabitats. We used rarefaction-based models manipulating vegetation structure and multivariate randomizations to assess the effects of tree density, tree size, and nesting microhabitats on the ant communities. We expected increased ant species diversity, microhabitat specialization, and species turnover among trees throughout the succession, with distinct community composition among the stages. In contrast to our expectations, ant species composition and diversity per plot did not change during succession, and species similarity among trees was significantly higher only in old secondary forest. The number of ant species per tree increased with forest age, but trees of similar size hosted only slightly more species in primary than secondary forests. Many ant species were associated with particular nesting microhabitats; however, the same microhabitat types occurred in all stages and thus did not generate successional trends in ant communities. Secondary succession had an unexpectedly low impact on tree-dwelling ant communities in this montane ecosystem, in contrast with our previous findings from lowland rainforests. These results highlight the need to study successional processes along entire elevational gradients, as montane taxa may react differently to vegetation changes than their lowland counterparts.

Key words: ants; canopy; cloud forest; community ecology; Formicidae; mountains; Papua New Guinea; successional gradient.

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INTRODUCTION

Ecological succession of vegetation is one of the few complex community-level processes that is relatively well-understood by ecologists (Glenn-Lewin et al. 1992, Palmer et al. 1997,

Shugart 2003). Forest successional dynamics begin with a disturbance event, either natural (e.g., tree falls, landslides) or human-caused disturbance (e.g., selective logging, swidden agriculture), where plants typically colonize empty space after the disturbance. Secondary

succession is thus associated with considerable shifts in vegetation structure, species composition, and plant functional traits: Pioneer species are usually fast-growing, light-demanding plants with short-lived leaves that recruit from a limited number of lineages, later replaced by slow-growing shade-resistant mature forest trees in a locally predictable chronological sequence (Glenn-Lewin et al. 1992, Chazdon 2014). The concept of ecological succession, and our ability to understand it, is becoming increasingly important in tropical landscapes, where areas of secondary vegetation and regenerating forests have been spreading extensively over recent decades (Wright 2005, Malhi et al. 2014).

Secondary vegetation of tropical forest is characterized by lower plant biomass and species richness relative to primary forests (Whitfeld et al. 2014), where the fast-growing pioneer plants are more attractive to herbivores as their leaves have higher nitrogen and water content, lower specific leaf area, and lower anti-herbivore defenses than primary forest species (Chazdon 2014, Whitfeld et al. 2014). Therefore, successional processes affect arthropod composition, and young vegetation tends to host a higher density of insect herbivores (Leps et al. 2001). This effect can be propagated along trophic cascades to predators and scavengers (Bowman et al. 1990, Basset et al. 2012). However, it is also possible that generalist predators, such as spiders and ants, do not respond primarily to species richness of herbivores but rather to the vegetation structure. For instance, old-growth vegetation offers a higher epiphytic load and microhabitat variability, and feeding and nesting resources are more stratified and diverse compared to early-successional stages (Schulze et al. 2004, Yanoviak et al. 2007, Klimes et al. 2015, del Pliego et al. 2016).

Succession is a key factor influencing the composition of arboreal insect communities (Leps et al. 2001, Villa-Galaviz et al. 2012, Redmond et al. 2018). While most studies find correlations of insect and plant diversity, the causal mechanisms driving successional change in insect arboreal communities remain poorly known (Ribas et al. 2003, Basset et al. 2012). There is a lack of spatially explicit data allowing exploration of plant–insect interactions at different scales and linking vegetation structure and composition

with the structure of insect communities. Moreover, an overwhelming majority of successional studies in tropical forests have focused on the lowlands (Glenn-Lewin et al. 1992, Chazdon 2014, but see Axmacher et al. 2004, Gomes et al. 2008, Redmond et al. 2018), while studies of elevation gradients tend to restrict their attention to primary forests (Colwell et al. 2008). This is an important knowledge gap because successional trends in montane forests do not necessarily follow the same trends as those in the lowlands (Whitfeld et al. 2014, Redmond et al. 2018). Montane forests are also characterized by high levels of endemism and are diversity hotspots of high conservation value (Korner and Spehn 2002, Gradstein et al. 2008), and their communities are predicted to be at the greatest risk from global temperature changes (Colwell et al. 2008).

In tropical canopies, ants are one of the most abundant and ecologically important groups, representing 20–60% of the arthropod biomass and a high proportion of individuals (Hammond 1992). They play important roles in forest ecosystems, comprising predators, scavengers, and secondary herbivores (Holldobler and Wilson 1990, Folgarait 1998). In lowland forest, ant species diversity usually increases throughout forest succession, with a high turnover of species, as secondary forest species are replaced by primary forest species (Schulz and Wagner 2002, Osorio-Perez et al. 2007, Klimes et al. 2012). It is well-known that ant communities also change with elevation, with maximum species diversity at lower elevations (Fisher 1996, Bruhl et al. 1999, Staab et al. 2014) or at mid-elevations (Samson et al. 1997, Longino et al. 2014). However, species diversity in montane communities is still expected to increase over the course of succession (Schulze et al. 2004, Colwell et al. 2008), with significant changes in species composition from early- to late-successional stages (Fisher and Robertson 2002).

The effects of succession on plant communities are typically studied using a whole-forest plot-based approach, where all individuals above a certain stem size are mapped within plots (Fibich et al. 2016, Vincent et al. 2018). While this approach usually lacks or has low plot replication at the local scale, it allows sampling of the full range of tree sizes and tree species within the area (Whitfeld et al. 2012). Insect communities

are rarely sampled by whole-forest census, primarily due to practical reasons such as the sheer abundance of samples to process, and poor accessibility to the forest canopy (Whitfeld et al. 2012). However, sampling only the most accessible trees or branches of a few preselected tree taxa within an unsampled forest matrix (Schowalter 1994, Schulz and Wagner 2002, Campos et al. 2006, Sam et al. 2015) offers a low-resolution view of insect communities and a biased view of the entire forest community. Plot-based sampling of non-flying canopy insects, using canopy cranes or tree felling and dissecting, yields high-resolution data on where insects are found in trees (Whitfeld et al. 2012, Redmond et al. 2018). In the case of ants, these methods make it possible to sample and distinguish between nesting and foraging individuals. Tree dissection, although laborious, is the most exhaustive method as it allows collection of the many species that nest cryptically inside epiphytes and twigs (Klimes 2017). With standard methods, such as fogging with insecticide or tree-climbing, it is not possible to access all tree sizes and all microhabitats (Yanoviak et al. 2003b, Adams et al. 2017). This is especially problematic at higher elevations, where epiphytes become more common (Ding et al. 2016). In addition, epiphytes also have to be dissected to successfully cover their invertebrate diversity (Yanoviak et al. 2003b).

Ground-dwelling ant communities have been studied at high elevation (Bustos and Ulloa-Chacon 1996, Estrada and Fernandez 1999), but less is known about montane arboreal ants and their nesting habits. Current knowledge of arboreal ant communities at high elevations is limited to a single study of a few individual high-canopy trees in Costa Rica (Schonberg et al. 2004). This study showed about ~50% increase in total species richness from secondary to primary forest, but it did not explore ant nests. Hence, no data for ant nests from montane forest tree canopies are available. In lowland rainforest, a plot-based study also found significant increases in ant diversity (including their nests), and high species turnover throughout succession driven by changes in tree density, tree size, and microhabitat diversity (Klimes et al. 2012, 2015). There is no a priori reason why similar trends should not also be expected in montane forest succession.

Here, we sampled whole-forest plots in a tropical montane forest to quantify the effect of succession on ant species diversity, species composition, and nesting microhabitat use. To achieve this, a census of all arboreal ant species and their nests was conducted on >1200 felled trees in nearly 1 ha of montane forest across three successional stages. We asked the following questions: (1) Does montane forest succession affect species diversity and turnover of arboreal ant communities? We predicted increases in ant species richness and species turnover among trees as successional progresses (Floren and Linsenmair 2001, Klimes et al. 2012). (2) Does variation in forest structure (i.e., shifts in tree densities and tree sizes) drive changes in ant species diversity throughout succession? We tested this with models that mimic younger successional stages by rarefying primary forest plots to test for separate effects of forest structural changes on ant species diversity. We expected increases in tree size and microhabitat diversity to drive increasing ant diversity during forest succession (Klimes et al. 2012). (3) Is ant community composition affected by successional change and/or nesting microhabitats? We predicted that both the ant community and nesting microhabitat composition will vary among the forest stages (Klimes 2017). (4) Does succession affect arboreal ant communities in montane forest as much as in the lowlands? We compared the successional changes in ant richness and turnover in montane forest to previous results from lowland forest in New Guinea (Klimes et al. 2012, 2015).

METHODS

Field sites and experimental design

The study was conducted in a montane tropical forest in the Saruwaged Mountains, near Yawan village, Papua New Guinea (S6.16291° E146.83957°, ~1800 m a.s.l., Appendix S2: Fig. S1). The average daily temperature in the region is 17.5°C, humidity 95%, and total rainfall approximately 3000 mm/yr (McAlpine et al. 1983). Seasonality is mild with max 2°C change in daily mean temperature and a mild dry season from July to August.

The area supports indigenous migratory slash-and-burn agriculture on small patches (0.2–1.0 ha) of primary forests. Patches are selected by

locals randomly without any preference to a specific soil type or quality, and then clear-cut and burned. They are then planted with a mix of crops, and after a few harvests, they are abandoned and left to undergo natural succession. Our study area consisted of secondary forest patches regenerating after food gardens, as well as primary forest patches destined to be cleared for new gardens by the landowning community. Our research therefore did not contribute to net deforestation. Trees and associated arthropods were surveyed in nine selected 0.2-ha patches (45 × 45 m, distanced 45–500 m apart) from March 2010 to November 2012. In this study, we use the data for trees and ant communities that were censused in half of each of the patches, that is, plots of 0.1 ha hereafter (22.5 × 40 m, see Appendix S2: Fig. S1). These plots formed a successional series consisting of (1) 13-yr-old regrowth (young secondary forest), two plots; (2) 20- to 30-yr-old regrowth (old secondary forest), three plots; and (3) old-growth vegetation of at least 50 yr without human disturbance (primary forest), four plots. The plots were in the same locality, spread across a narrow range of elevations (1720–1860 m a.s.l.), and spatially mixed as far as possible to avoid pseudoreplication of individual successional stages, allowing us to study a time series by “substituting space for time” (Pickett 1989).

While we have low replication of plots across the different stages of succession, our sampling within them is exceptionally detailed, allowing us a representative view of the whole ant and tree community. This trade-off between replication and sampling area is particularly important in studies of highly diverse rainforest communities and often results in either non-replicated but sufficiently large samples, for example, ForestGEO forest dynamics plots (Global Earth Observatory Network, <https://forestgeo.si.edu>), or well-replicated samples that are too small to be representative of the whole forest (e.g., replicated tree individuals but of certain size and species across a site). In this study, vegetation structure varied little among plots from the same successional stage, with far greater differences among plots of different stages (Fig. 1, Table 1), suggesting that our 0.1-ha plots were representative of their successional stage.

Sampling methods

All trees with a diameter at breast height (dbh) ≥ 5 cm in each plot were mapped and their dbh, total height, and canopy height and width measured (Whitfeld et al. 2012). Trees in each plot were gradually felled, starting in one plot corner and advancing systematically through the plot. Lianas connecting trees were cut to facilitate tree felling and also sampled. The felling took approximately 3 months per plot with a team of 15 local workers and one supervising researcher. Each tree was searched for ants immediately after felling, following Klimes et al. (2015). Representatives of all ant species were sampled, including foraging and nesting species from the trunk up to the high canopy. Entire trees, as well as lianas and epiphytes (including mosses, ferns, and orchids), were thoroughly searched for ants, including cryptically living species. Branches and twigs were broken open to check for ant nests, and the trunk was cut in several pieces to search for cavities. For the analysis, ants from large lianas (dbh ≥ 5 cm) were assigned as present on the liana's host tree. The height above the ground was measured (in meters) for each nest before being classified as one of 10 microhabitat types: Under epiphyte, In trunk, On leaves, Liana, Under bark, Myrmecophyte, Live branch, Live twig, Dead branch, and Dead twig (detailed descriptions in Appendix S1a). An advantage of this detailed inspection of entire trees is that it allows a full census of ant nests that would not be possible by other the non-destructive methods such as baiting, fogging, or hand collection using single rope access to the canopy. Although trees suffer some damage when felled, it introduces only minimal bias to ant sampling, as loss of colonies and influx of foragers from the ground after felling are rare (Klimes et al. 2015). Several ant individuals from all recorded castes were sampled for species identification (Appendix S1b, Appendix S2: Table S1).

Data analysis

Records for all ant species found either nesting or foraging on each tree were included in the analysis, except for a few foragers, which were not found nesting in any sampled trees and thus probably do not belong to tree-dwelling fauna (five species and <0.02% of the records).

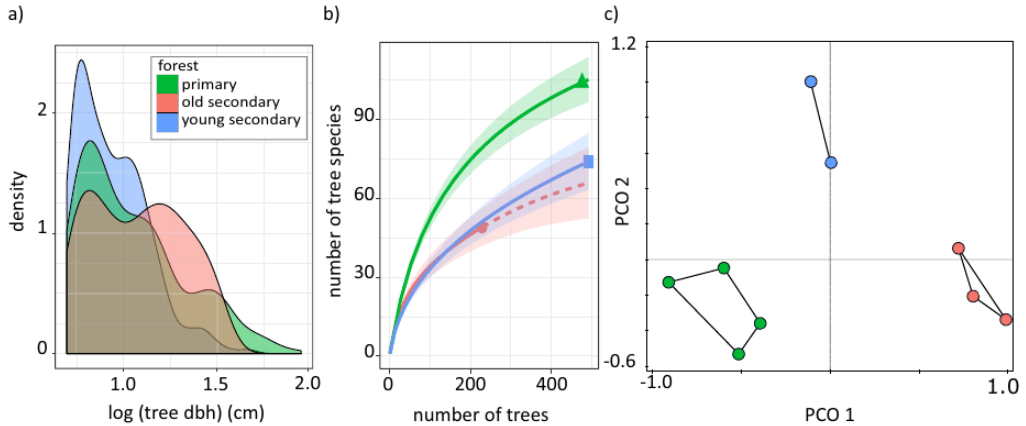


Fig. 1. Description of forest structure in different successional stages of New Guinea montane forest (green = primary forest, red = old secondary, blue = young secondary). See Table 1 and *Methods* for details. (a) Density diagram of distribution of trees, by their sizes (diameter at breast height [dbh]) in the three successional stages of montane rainforest in New Guinea. Note that the colored area below each density curve sums to a unit area to allow a relative comparison among successional stages that differ in their overall tree numbers per plot (Table 1). (b) Tree species accumulation curves using observed species richness (Chao et al. 2014). Points represent observed values, dashed lines show extrapolation to 500 trees, and colored fields represent 95% confidence intervals. (c) Plot-based ordination diagram of principal coordinates analysis (PCoA) based on tree species composition, each circle represents one 0.1-ha plot.

Diameter at breast height was used as a metric for tree size. All univariate analyses and rarefaction models were conducted in R 3.5.2 (R Core Team 2016) and the multivariate analyses of species composition in CANOCO 5.04 (Braak and Smilauer 2012a). All tests were performed at $\alpha = 0.05$ significance threshold. The common logarithmic transformation was applied to the data. For all analyses, presence–absence data were considered for each ant species on a tree, except for multivariate analysis of nesting ant species, where nest abundances were used.

We first tested how the vegetation structure itself differs in tree species richness and composition among successional stages using species accumulation curves and a multivariate analysis. The accumulation curves were calculated with the *iNEXT* package using trees as individual samples and excluding unidentified trees (diversity observed in samples \pm confidence intervals, with 999 bootstrap replications; Chao et al. 2014, Hsieh et al. 2016). We tested the differences in tree species between successional stages using

principal coordinates analysis (PCoA): A matrix of tree species \times individual plots (145 tree species \times 9 plots) was used as the community dataset for the analysis, with number of trees of each species in each plot used as a measure of abundance. Unidentified trees and species that occurred in just one plot were excluded (see Table 1 for tree richness and number of excluded trees per stage). We ran the PCoA based on Bray–Curtis distance and created ordination diagrams of plots to visualize the differences in the species composition among plots and forest stages.

Does montane forest succession affect species diversity and turnover of arboreal ant communities?

We tested for differences in ant species richness per plot (SPP) and ant species richness per tree (SPT) among successional stages (as categorical factors) using a general linear model (GLM (SPP \sim succession), $N = 9$) and generalized estimating equations model for non-normal distribution with random effect using *GeeGLM* package

Table 1. Plot vegetation characteristics of the three montane forest successional stages (0.1-ha each, all trees with dbh \geq 5 cm included).

Characteristic	Young secondary	Old secondary	Primary
Forest age (yr)	<13	20–30	>50
Number of plots	2	3	4
No. of trees (estimated per ha)	2515 (2440–2590)	830 (680–930)	1243 (1130–1390)
Basal area (m ² ; estimated per ha)	24.3 (20.7–28.0)	16.9 (15.3–19.9)	32.5 (27.1–39.7)
Tree dbh (cm)	9.8 (5–47.0)	14.0 (5–36.0)	14.0 (5–88.4)
Tree height (m)	11.0 (3.4–29.8)	11.3 (1.1–33.2)	14.1 (2.38–40.1)
No. of tree genera per plot	29.0 (22–36)	17.3 (15–22)	30.8 (30–32)
No. of tree species per plot	47.0 (45–49)	26.3 (20–33)	48.8 (46–53)
Percentage of unidentified tree individuals per plot	2.2 (1.6–2.7)	8.3 (6.5–11.8)	4.1 (0.9–10.3)
Most common tree genera	<i>Homalanthus</i> , <i>Piper</i> , <i>Saurauia</i> , <i>Cyathea</i> , <i>Ficus</i>	<i>Ficus</i> , <i>Saurauia</i> , <i>Piper</i> , <i>Pipturus</i>	<i>Pandanus</i> , <i>Elaeocarpus</i> , <i>Ficus</i> , <i>Saurauia</i> , <i>Sloanea</i>

Notes: dbh, diameter at breast height. Values are means with minimum and maximum ranges in parentheses.

((GeeGLM(SPT ~ succession + 1|plot), $N = 1249$; Halekoh et al. 2006). We also explored how SPT was affected by tree size (dbh) and successional stage, taking into account the interaction of dbh and successional stage (GeeGLM(SPT ~ dbh + succession + dbh:succession + 1|plot), $N = 1249$). The same analysis was performed to test the effect of dbh and successional stage on nesting microhabitat diversity (the number of different nesting microhabitats used by ants per tree).

Species accumulation curves of the number of observed arboreal ant species in trees (as individual samples) were generated using the iNEXT package for individual plots and for each successional stage with plots pooled together (Hsieh et al. 2016).

Species similarity between trees (1 – beta diversity) was quantified using Sørensen similarity (S_o) between all pairs of individual trees within each forest plot and compared between successional stages (GLM($S_o \sim$ succession), $N = 9$). We evaluated the spatial dependence of ant communities on trees using the relationships between species similarity (S_o) and physical distance of trees within each plot. Further, we tested the magnitude of variation of Sørensen similarity at a larger scale by comparing mean Sørensen similarity calculated as above (i.e., for all pairs of trees within each plot) with the mean similarity calculated for all trees from the same successional stage. See Appendix S1c for more information on the regression models, accumulation curves, and spatial model used.

Does variation in forest structure drive changes in ant species diversity throughout succession?

We used a rarefaction model adapted from Klimes et al. (2012) to test the effect of successional changes in vegetation structure (tree density and size distribution) on species diversity of ants. In the model, primary forest vegetation from the four plots combined was repeatedly randomly subsampled to obtain datasets that match the number and size distribution of trees found in each young and old secondary forest plot. We then compared the ant diversity (SPP, species diversity per tree) and similarity (S_o) observed in the plots with the modeled (rarefied) values that represent predictions based on primary forest communities with the vegetation structure matching the two secondary successional stages (Appendix S1d, Appendix S2: Table S2). We did not model the taxonomic composition of trees due to the lack of plant species identifications for some of the trees (Table 1) and insufficient number of trees in each dbh category needed to mimic the taxonomic diversity and distribution.

Is ant community composition affected by successional change and/or nesting microhabitats?

First, we tested the variation in the species composition of whole ant communities (i.e., both foraging and nesting records included) using multivariate canonical correspondence analysis (CCA, constrained) with individual trees as samples. Tree dbh and the approximate successional

age (young secondary forest = 10 yr, old secondary = 25 yr, primary = 50 yr) were tested using forward selection as explanatory variables.

Second, we performed the CCA using only nesting species, while also including nest parameters (nest height and nesting microhabitat category) for each tree alongside tree dbh, height, and the successional stage. We used forward selection to test for significance of each predictor. Finally, we used variation partitioning to assess the relative contributions to the explained variance in the nesting communities by (1) the successional age, (2) the other predictors (i.e., tree parameters and microhabitats), and the inter-correlation of the two groups (Braak and Smilauer 2012b). For more information on the multivariate analyses, see Appendix S1e and Appendix S2: Table S3.

Does succession affect arboreal ant communities in montane forest as much as in the lowlands?

We combined young and old secondary plots together to compare ant community species richness and species overlap between primary and secondary forest stages. We used data from the montane forest studied here and data from the same two forest stages in Wanang lowland forest (~100 m.a.s.l.) previously sampled (Klimes et al. 2015, Klimes 2017). The lowland site is in Madang Province, 210 km from our study plots in Yawan. Mean species richness and standard deviation per 0.1-ha plot were calculated and visually compared between the primary and secondary forests using Venn diagram (Appendix S1f).

RESULTS

In total, 1249 trees from nine plots (0.1-ha each) in three successional stages were sampled and 654 (52.4%) of those were occupied by ants. When occupancy was considered per plot, trees were occupied in all successional stages in a similar proportion (Appendix S2: Table S4). In total, we found 24 species of arboreal ants in 13 genera and 4 subfamilies, and we recorded 1374 ant species occurrences (i.e., presences) in trees (Appendix S2: Table S1).

The forest structure varied greatly among the three successional forest ages, but was relatively similar among the replicated plots within each

stage (Fig. 1, Table 1). Tree density varied from 680 (old secondary forest) to 2590 (young secondary forest) trees per ha. The forest stages differed in size distribution of trees: The young secondary forest comprised many small trees and consequently had a lower mean dbh (9.8 cm) compared to the other two stages (both mean dbh = 14.0 cm; Table 1, Fig. 1a). The number of tree genera and species per plot was similar in young secondary and primary forest, but lower in old secondary forest (Table 1). However, tree species richness accumulated similarly in both secondary forest stages with number of trees sampled (Fig. 1b). Tree species composition was distinct between successional stages but homogenous within each stage (Fig. 1c).

Does montane forest succession affect species diversity and turnover of arboreal ant communities?

Mean SPP did not differ significantly among successional stages (deviance test, $df = 2,6$; $P = 0.149$; Fig. 2a). Accumulation curves of ant species richness with the number of sampled trees showed an increase in both SPP and per tree with forest age (Fig. 3a). However, the differences among the stages were not significant as the accumulation trends varied between individual plots, particularly in secondary stages (Fig. 3b).

Mean SPT grew gradually with increasing forest age and differed significantly between all successional stages (mean number of species per tree increased from 0.8 to 1.5 along the successional gradient; $\chi^2 = 31.21$, $P < 0.001$; Fig. 2b). Species richness per tree also varied with tree size (Fig. 4, from 0 to max. 9 species per tree). The rate of increase of SPT with dbh was uniform among successional stages ($\chi^2 = 1.1$, $P = 0.55$), and the same relationship with dbh was found for the number of different nesting microhabitats used by ants per tree (Appendix S2: Tables S5, S6). However, primary forest trees had on average slightly higher ant and nesting microhabitat richness for a given tree size relative to the other two stages (Fig. 4).

Mean Sørensen similarity was significantly higher in the old secondary forest than in the other two successional stages (deviance test, $df = 6,2$; $P < 0.01$), while that of young secondary and primary forests did not differ from

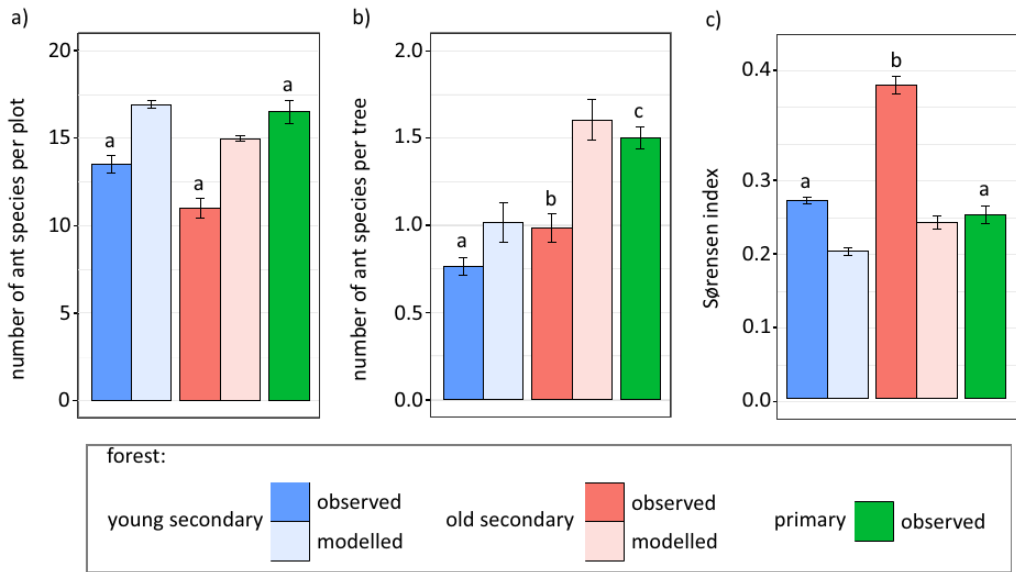


Fig. 2. Diversity of arboreal ants observed in the three successional stages of New Guinea montane rainforest compared with modeled values. Models are based on primary forest trees rarefied to the same forest structure (tree density and size) as observed, respectively, in the young and old secondary successional stages. Error bars represent standard errors. Different letters above bars mark significant observed differences among the forest stages. (a) Number of ant species per 0.1-ha plot. (b) Number of ant species per tree. (c) Similarity in ant species composition between trees calculated for all pairs of occupied trees in each plot using Sørensen index. For detailed information about the models, see *Methods*.

one another (Fig. 2c). There was no correlation between physical distance of pairs of trees within each plot and ant species similarity (S_o) of those communities (Appendix S2: Fig. S2). However, the mean Sørensen similarity was lower when calculated for all pairs of trees within the same successional stage and compared to within-plot values (Appendix S2: Fig. S3).

Does variation in forest structure drive changes in ant species diversity throughout succession?

Our rarefaction model predicted values of SPP and Sørensen similarity for both early and late secondary successional plots similar to the observed values in the primary forest (Fig. 2a, c). This suggested that changing vegetation structure throughout the succession has an unexpectedly weak effect on these ant diversity parameters at the plot scale.

The model predicted values of SPT lower in young secondary forest than in primary forest

(modeled decrease from 1.5 to 1.0 species on average). In contrast, old secondary forest was predicted to reach values of species diversity per tree similar to those of primary forest, but the observed values were in fact lower (Fig. 2b). In the case of Sørensen similarity, old secondary vegetation departed from the prediction, with much higher similarity values than expected (Fig. 2c).

Is ant community composition affected by successional change and/or nesting microhabitats?

The species composition of ant communities in trees changed only slightly along the successional gradient (Fig. 5). Notably, all successional stages shared the most abundant ant species and in similar proportions (Fig. 5c; Appendix S2: Table S1). This was also the case among individual plots of the same stage, except for a change in the frequency of dominant species between two old secondary plots, H and J (Appendix S2: Fig. S4).

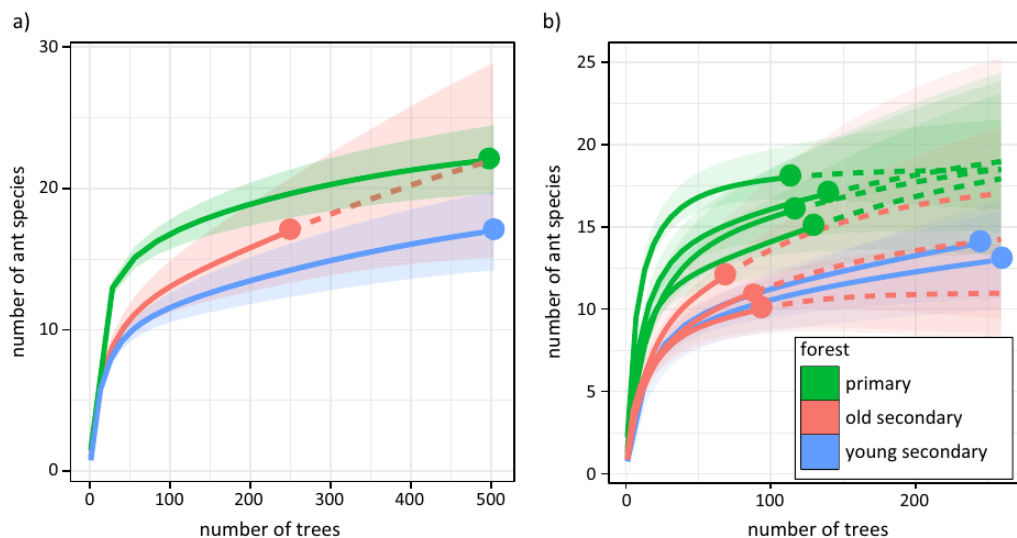


Fig. 3. Species accumulation curves for the observed species richness of ants in mountain forest trees in New Guinea. Points represent observed values, and dashed lines show extrapolation to 500 and 250 trees, respectively. Colored fields represent 95% confidence intervals. (a) Three successional stages of montane forest pooled together (primary forest green: 497 trees in four plots), old secondary (red: 250 trees in three plots), and young secondary (blue: 503 in two plots). (b) Each of the 0.1-ha plots plotted separately (nine plots).

The CCA showed that both dbh and successional stage affected ant species composition (Fig. 5a; pseudo- $F = 9.4$; $P = 0.001$), but the model explained only 2.8% (2.5% adjusted) of the overall variability in communities. Although the successional gradient corresponded to the first (strongest) canonical axis, its efficiency (proportion of the explainable variability) was only 18%.

Nesting microhabitat use by ants also varied only slightly during succession (Fig. 5d). The two most common nest habitats overall were Under epiphytes and Live twig in all successional stages. The CCA on nesting species showed a significant effect of environmental variables on the species composition of arboreal ant nests (stepwise forward selection, pseudo- $F = 7.0$; $P = 0.001$; Fig. 5b). The selected variables explained 18.3% (16.2% adjusted) variability (Appendix S2: Table S3), and the axis efficiency was high compared to the previous analysis (69.2% and 62.8% of explainable variability in the first and second axis, respectively). Most of the ant nest variability was driven by the effect of the individual nesting microhabitats being used

by certain ant taxa, not by the successional gradient itself. Variation partitioning revealed that successional stage contributed only 8.2% to the total explained variability, while the selected environmental variables (microhabitat variables and tree dbh) contributed 91.8%, with a null overlap among the two sets of variables.

Does succession affect arboreal ant communities in montane forest as much as in the lowlands?

Visualization of species diversity and its overlap between primary and secondary forests showed that montane forests have fewer ant species (as expected), but also that both overall species diversity and composition varied little in the course of secondary succession in montane forests (Fig. 6). This pattern is in contrast with arboreal ants in lowland forests that had distinct successional communities characterized by lower diversity in secondary compared to primary forests (Fig. 6). On average, there were 76.3 species per 0.1-ha plot in the lowlands but only 17.6 species in the same area in montane forest (i.e., 77% decrease). Primary and secondary lowland

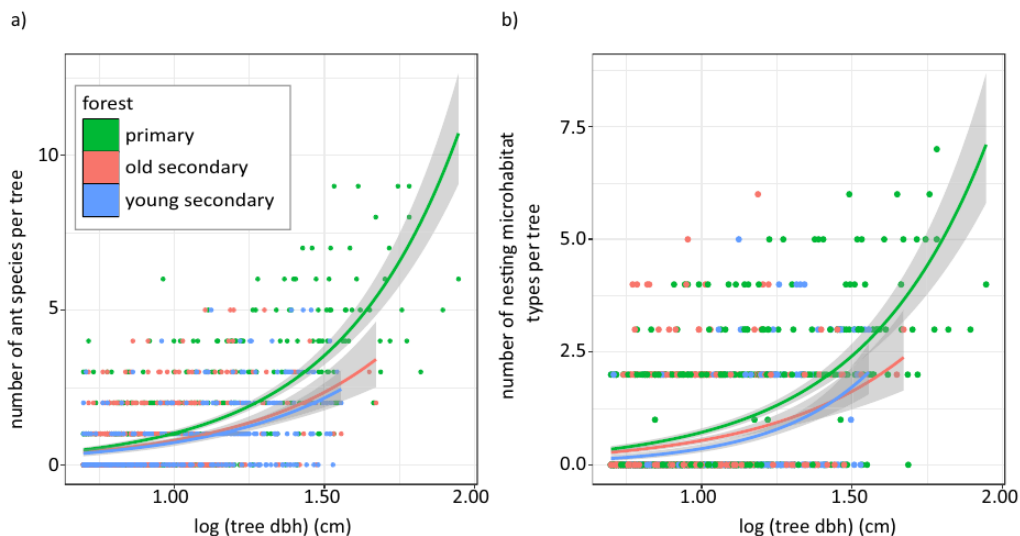


Fig. 4. Relationship between tree size (diameter at breast height [dbh]) and ant species richness (a) and ant nesting microhabitat diversity (b) in three montane forest successional stages in New Guinea. Lines represent general linear model (GLM) fitted with Poisson distribution, and gray areas represent the 95% confidence intervals. There is no statistical difference in the curve slopes among the three stages in both analyses (GeeGLM, interaction effects: $P > 0.2$), but both measures of ant diversity are higher for primary than secondary forest trees across the tree size gradient (forest stage effects: $P < 0.01$).

forests shared only 16% of species, and species diversity of the primary forest was about two times higher than that of secondary forest. In contrast, montane ant communities shared 64% of the species and montane primary forest only had 1.3 times higher species richness than that of the secondary forest.

DISCUSSION

Our study highlights unexpected trends in the successional dynamics of arboreal ant communities in montane rainforest, an ecosystem that remains severely understudied for invertebrate communities. Our census data from over 1200 trees represent the most comprehensive sampling of montane arboreal ants to date. Contrary to our expectations, the effects of succession on arboreal ants were rather small despite large changes in vegetation structure and diversity. This contrasts with previous findings in the lowlands where structural changes of vegetation have significant

impacts on ant communities, and primary forests have much higher ant species richness, relatively distinct communities, and a greater variety of nesting resources than secondary forests (Floren and Linsenmair 2001, Bihn et al. 2010, Klimes et al. 2012).

The arboreal ant community in this montane rainforest was relatively species-poor and a significant proportion of trees (over 40%) lacked any ants. This pattern was expected and could be attributed to colder climatic conditions (McAlpine et al. 1983, Colwell et al. 2008). Tropical ants exhibit some of the fastest declines in abundance, species richness, and predation activity with elevation recorded in insect taxa, and this pattern is well documented in New Guinea, where ants disappear above 2200 m a.s.l. (Sam et al. 2015, Plowman et al. 2017, Orivel et al. 2018). While ants were relatively common in the forest canopy at our study sites at 1800 m asl, just 400 m below their local elevational limit (Orivel et al. 2018), a large proportion of colonies were nesting cryptically underneath epiphytes and

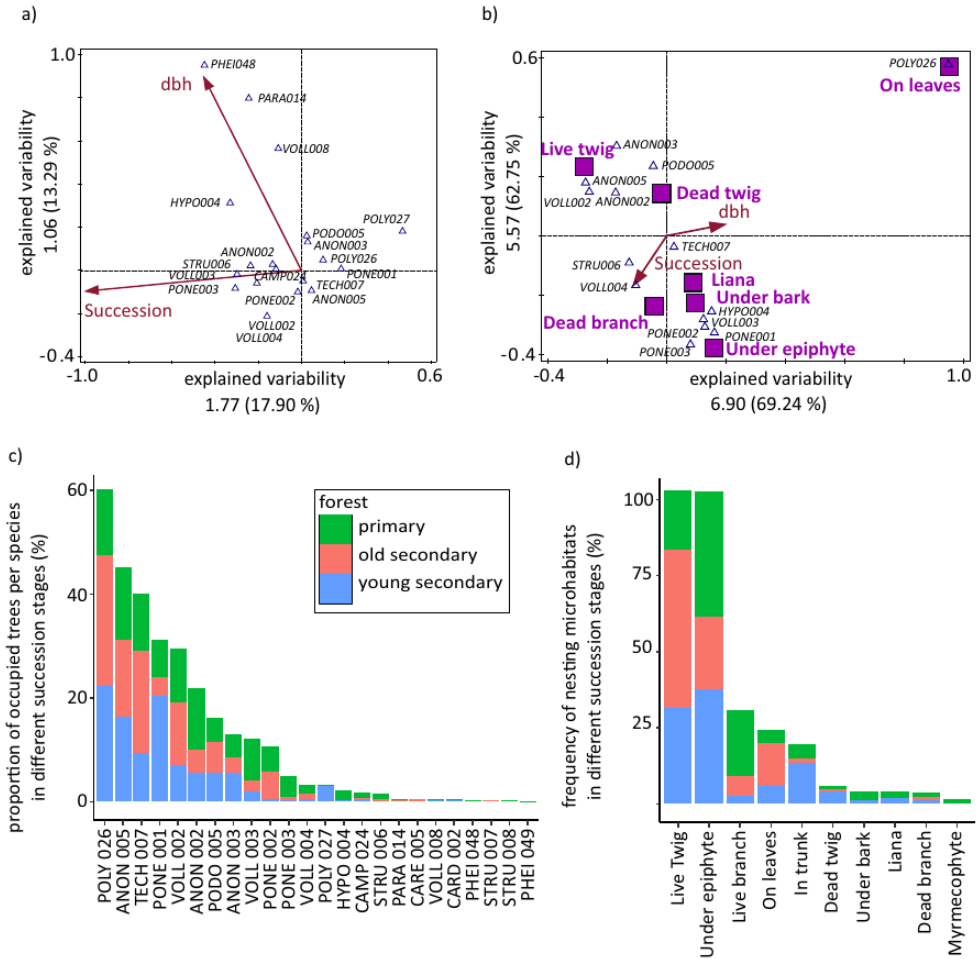


Fig. 5. Ant species and their nest composition in New Guinea tropical montane forest. (a) Canonical correspondence analysis (CCA) of variability in ant communities explained by the effects of tree size (diameter at breast height [dbh]) and succession (forest plot age) on the composition of all common ant species ($n = 19$, >2 occurrences). Species presence-absence on each tree with $\text{dbh} \geq 5$ cm was considered. (b) CCA showing the effects of all environmental variables (i.e., including nesting microhabitats) on the composition of common tree-nesting ant species ($n = 14$; >2 nests per species). Triangles represent the ant species (for full names see Appendix S2: Table S1). Squares represent different nesting microhabitats. Only variables with significant effects are included. (c) Arboreal ant species distribution among successional stages expressed as percentages of trees occupied by ant species in each successional stage. (d) Composition of ant nesting microhabitats used by ants in successional forest stages expressed as percentages of sampled nests in each category.

inside tree branches and would not have been found without dissecting the trees.

Structural changes of the forest coupled with successional processes did not affect overall ant

species composition, ant diversity per plot, or nesting resources utilized by the ants, and ant species similarity among trees did not decrease along the successional gradient. These results are

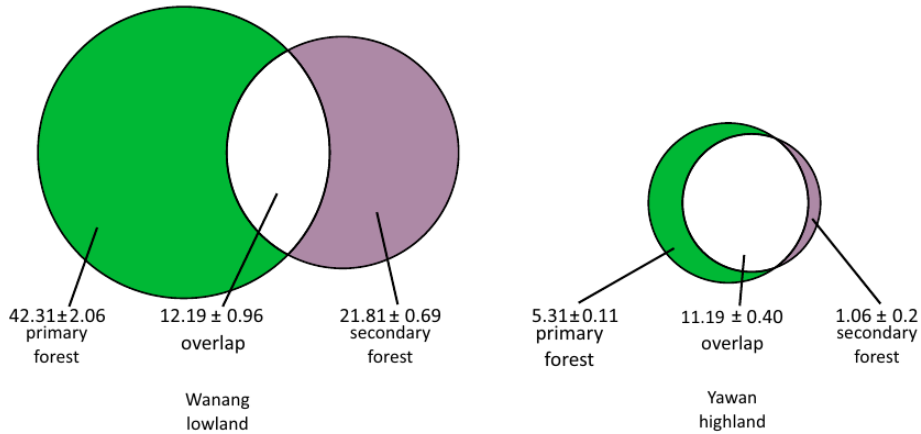


Fig. 6. Mean species diversity per 0.1-ha plot (\pm standard error) and species overlap of arboreal ant communities between primary (old growth) and secondary forests in the lowland and the highland tropical forests in New Guinea. The Venn diagrams, calculated from four 0.1-ha plots per forest type and elevation, are proportional to species diversity values. The lowland data are from Klimes et al. (2015) and Klimes (2017), and the highland data from this study (young and old secondary forests pooled, see *Methods*).

in contrast with those for lowland canopy ants (Floren and Linsenmair 2001, Klimes et al. 2012, 2015), as well as other animal taxa that respond strongly and predictably to successional gradients in montane forest (Spitzer et al. 1997, Axmacher et al. 2004, Gomes et al. 2008). Only ant SPT increased with forest age, which is surprising because this was not the case in the lowlands where SPT was similar between primary and secondary forests (Klimes et al. 2015). The increase in SPT could be partly attributed to differences in density and tree size among our montane stages (as indicated by the rarefaction model). Young montane forest had more than double the stem density and a much higher proportion of small trees compared to the same forest age in the PNG lowlands (Klimes et al. 2012, Whitfeld et al. 2014).

The increased similarity of ant species among trees in old secondary forest has several possible explanations: (1) Changes of vegetation connectivity could play some role, as the influx of ants foraging from surrounding trees influences ant species composition and richness (Klimes et al. 2015). However, as we did not observe a relationship between ant species similarity and distance between pairs of trees, this effect was probably

negligible in our system. (2) Ant species turnover might be related to the observed decrease in plant taxonomic richness in the old secondary stage (Table 1). Although high ant specificity for certain host plants in our sites is unlikely, even for smaller myrmecophytic trees (Plowman et al. 2017), we cannot completely exclude plant-taxa effects. (3) Different distribution (patchiness) of dominant ants across canopies may influence species composition and turnover (Bluthgen and Stork 2007, Dejean et al. 2007, Yusah et al. 2018). This partially affected the higher species similarity in our old secondary plots. However, given only two of the nine plots differed in their most common ant species, and we observed no spatial dependency of our data on the plot scale, spatial patchiness is an unlikely source of bias for other parameters we studied. While spatially clumped super-colonies and behaviorally aggressive invasive ants are abundant in the lowlands (Dejean et al. 2010), their occurrence was not observed at our high elevation site.

The weak successional effect on ant composition contrasts strongly with results on canopy ants studied elsewhere at higher and lower altitudes: for example, in mid-elevational forests in Africa at 1200 m a.s.l. (Schulz and Wagner 2002)

and in the lowlands (Floren and Linsenmair 2001, Klimes et al. 2012). The exception is one study from a montane forest in Central America at 1500 m a.s.l. (Schonberg et al. 2004), which found that secondary forest species were a subset of primary forest community. However, the direct comparison to our study is difficult, as this study was limited to small number of trees and its results could be also affected by geographical and altitudinal differences. In addition, the elevations might not be comparable as the ant elevational distribution in Neotropical cloud forests is about five hundred meters higher than in New Guinea (Van der Hammen and Ward 2005, Orivel et al. 2018). For ground and litter montane ant communities, the successional trends in species diversity and composition remain equivocal as some studies documented succession having a strong effect on ant communities (Estrada and Fernandez 1999, Fisher and Robertson 2002), and others a weak effect (Bustos and Ulloa-Chacon 1996). Studies of butterflies (Spitzer et al. 1997), birds (Soh et al. 2006), and spiders (Yanoviak et al. 2003a) at high elevations also found variable or weak effects of succession on species richness, but strong effects on species composition and turnover among different successional stages.

The low impact of succession on ant diversity (also predicted by the rarefaction model) is quite surprising given the relatively high differences in the vegetation structure between the forest stages. For instance, Garcia-Martinez et al. (2016) showed that the diversity of ground-dwelling ant communities increases with increasing complexity and fragment size of forest vegetation in Mexico. Similar trends of increasing diversity with vegetation complexity were found in other animal taxa including birds (Soh et al. 2006, Gomes et al. 2008) and moths (Axmacher et al. 2004). Although Redmond et al. (2018) showed a lack of successional changes in the structure of caterpillar-plant food webs in the same study site, they reported differences in species richness with large changes in caterpillar species composition throughout the succession, likely mediated by changes in host tree composition. We did not observe such species turnover in ant communities.

However, our multivariate models suggest that microhabitat composition is a more important driver of these relatively homogenous ant communities than forest succession. The

importance of nesting microhabitats and tree size for ant diversity and species composition has been found in simpler habitats such as plantations and savannahs (Philpott and Foster 2005, Powell et al. 2011). Successional changes in microhabitat use by canopy ants also drove changes in ant communities in a lowland rainforest in New Guinea (Klimes et al. 2012, Klimes 2017). In our montane forest system, we found a high effect of the utilization of different nesting microhabitats on ant community composition. However, the same microhabitat types occurred in all successional stages, and therefore, this effect was not generated by succession itself. Nevertheless, the changes in availability and diversity of nesting microhabitats with succession (del Pliego et al. 2016) could still play some role in the increase of ant richness in primary forest trees that was not fully explainable by variation in tree density and size (Figs. 2, 4). Although we do not have independent measure of microhabitat availability in our study, as nest sites are limited resource to arboreal ants (Philpott and Foster 2005, Mottl et al. 2019), we assume their variability in utilization by ants can be used as proxy for their availability in our forests.

Our results on canopy ant diversity can be compared directly with a lowland forest study from Papua New Guinea comprising old secondary and primary forest, using the same census methods from felled trees (Klimes et al. 2012, 2015). The montane communities were very distinct from those in the lowlands: Only one of the 24 species in this study, *Anonychomyrma minuta*, was found to nest in trees both in montane and lowland regions of New Guinea (Klimes et al. 2015, Plowman et al. 2017). The montane ant species pool is also small as it was likely filtered by cooler environmental conditions and more extreme low temperatures in the past, during the relatively recent evolution of arthropod species in the montane region in New Guinea (Toussaint et al. 2014). It is possible that the competition driving habitat specialization is limited near the abiotic limits of ant distributions as there are too few competing species (Machac et al. 2011). The species that are able to live at high elevations are hence free to colonize both primary and secondary forest habitats.

While we acknowledge that this study is limited by low replication of plots, we are convinced

that the relatively large-scale whole-forest sampling carried out here provided suitable and sufficiently detailed data to test the successional trends and their environmental drivers in ant communities. Insufficient sampling in highly diverse communities tends to exaggerate, rather than conceal, differences among the sampled communities (Cardoso et al. 2009, Jost et al. 2011), further supporting our finding that the effects of succession are minimal in montane arboreal ant communities. Moreover, our species accumulation curves suggest that our conclusions on successional dynamics in species diversity would remain identical even if we sampled more trees. In addition, species-turnover measures suggested that variability in species similarity among successional stages would even decrease if individual trees were chosen independently of plots (e.g., ants sampled in trees randomly across the landscape).

In conclusion, arboreal ants are not good indicators of successional processes in highland forests compared to the lowlands (Floren and Linsenmair 2001, Klimes et al. 2015), where they are greatly affected by forest structure and successional dynamics. While tropical montane forests host less diverse ant communities, the species there are often endemic, and thus, like other montane fauna, of high conservation priority.

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DATA ACCESSIBILITY

Data are available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.js48f56>

SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2848/full>

~ *Supporting information* ~

Appendix S1

Appendix S1a - Nesting microhabitat categories

We defined 10 nesting microhabitats, utilized by ants in trees: ‘Under epiphyte’ (under the roots and in aerial soil of epiphytes, including moss, orchids and ferns), ‘In trunk’ (in the hollow trunk or trunk cavity), ‘On leaves’ (carton and other nests on the leaf), ‘Liana’ (inside or underneath a climber stem), ‘Under bark’, ‘Myrmecophyte’ (inside of a myrmecophytic epiphyte; *Hydnophytum* and *Myrmecodia* genera), ‘Live branch’ (in a living hollow branch), ‘Live twig’ (in a living hollow twig), ‘Dead branch’ (in a dead hollow branch) and ‘Dead twig’ (in a hollow dead twig). See also Klimes (2017) for more details on the scoring of the categories. Note that although in some cases the individual nesting microhabitats might be correlated also to the ant species traits (nest types), for instance, *Polyrhachis* genus builds its carton nests on leaves, nesting microhabitats were defined a priori as nest sites where nests were placed. Under null expectation, every ant species could nest in every nesting microhabitat in a tree. Indeed, e.g. common species *Polyrhachis* sp. 026, nesting typically on leaves, also nested in some cases under epiphytes and in hollow branches. Different ant species exhibited various levels of specificity to individual nesting microhabitat, as has been demonstrated also for the lowland communities (Klimes et al. 2012, Klimes 2017).

Appendix S1b - Ant species determination

Ant genera and species were determined using keys (Bolton 1995, Andersen 2000), online picture databases (e.g. antweb.org, newguineants.org) and morphological characters to species or morphospecies (Appendix S2: Table S1). For a few genera with polymorphy in workers and poorly known taxonomy, molecular data (i.e. variation in COI gene sequences) was also considered to define species boundaries (formicidaebol.org). Representatives of all the species and available castes were mounted and the reference collections of both dry specimens and the remaining individuals in ethanol is stored at the Institute of

Entomology, Biological Centre, Academy of Science CZ (sample codes YA0001-YA0098).

Appendix S1c – Ant diversity models information (Fig. 2-4; Fig. S2-S4):

For the analysis of diversity per plot, we ran a General Linear Model (GLM) with the number of species per plot as a dependent variable fitted with Poisson distribution (N=9) and successional stage as a categorical variable. The average number of ant species per tree (SPT) was analyzed using Generalized estimating Equations model (*GeeGLM* package (Halekoh et al. 2006)), which accounted for the non-normal distribution and random effect. The species diversity per tree (SPT) was the dependent variable with Poisson distribution (N=1249), successional stage as a fixed categorical factor and plot as a random categorical factor.

Accumulation curves were calculated with *iNEXT* package using diversity observed in samples (with $q=0$; \pm confident intervals made by 999 bootstrap replications, Fig. 3) (Chao et al. 2014, Hsieh et al. 2016).

For the test of the relationship between species diversity per tree (SPT) and DBH in succession, and interaction of DBH with successional stage, the *GeeGLM* was created with species diversity per tree as a dependent variable with Poisson distribution (N=1249), plot as a random categorical factor, successional stage as a fixed categorical factor and log (DBH) as continuous predictor.

Sørensen index (So) value ranges between 0 (two trees do not share any identical species) and 1 (two samples shared the same species pool). We calculated Sørensen similarity as 1-Bray-Curtis dissimilarity index with binary values (species presences) using the *vegan* package (Oksanen et al. 2007). Trees without ants were excluded from the analysis because the Sørensen index is not defined for the empty samples. A GLM was used to compare Sørensen similarity among the forest stages, where average Sørensen index for the plot was included as a dependent variable with quasibinomial distribution (N=9) and successional stage as a fixed categorical factor. Note, we deliberately did not employ any corrections for rare species, as we assume that we censused the communities

sampled across all trees (i.e. rarity of some ant species is likely natural in our plots).

The effect of spatial non-independence of trees on Sørensen similarity was explored using the following approaches: (i) Exploring relationship of Sørensen similarity and physical distance between trees (in meters); note that ~1.5% of trees are missing coordinates, so only a subset of the data was used for this analysis. The relationship was visualized by the “loess” smoothing method (span=0.75), which fits a polynomial surface using local fitting (Appendix S2: Fig. S2). (ii) Comparing mean Sørensen similarity calculated within plots (see above) with those calculated for all trees in successional stage pooled together (Appendix S2: Fig. S3). (iii) Exploring individual ant species frequencies per plot (Appendix S2: Fig. S4)

Appendix S1d - Rarefaction model (Fig. 2):

We subsampled the pool of our primary forest trees to mimic the observed density and size (DBH) distribution of the young secondary and old secondary forest stages. All trees from the four primary forest plots (A, B, C, and D) were pooled and used as a source of trees drawn to match the forest parameters in individual 0.1 ha plots of secondary stages (F, G, H, J, K) that served as templates. For each template, the rarefaction model tried to replicate the following characteristics of its observed forest structure using primary forest trees: (i) the number of trees per plot as well as (ii) the number of trees in different tree-size categories. Those ranged from the smallest trees to the largest trees (eight categories based on log(DBH); see Appendix S2: Table S7). The model then randomly selected trees from the primary forest pool that fulfilled required structural characteristics of each template and placed the trees into the modeled forest one by one, until it reached (i) the total observed number of trees and (ii) the number of trees by their DBH-classes Appendix S2: Table S7). The rarefaction algorithm repeated this 1000 times for each template and calculates species richness per plot, species diversity per tree and Sørensen similarity values for each selection. The means of these parameters for each modeled forest plot were used as the output values. The modeled ant community parameters were then plotted for secondary forest stages (young secondary and old

secondary forest) using mean and SE per plot, and compared with observed values (see Fig. 2 and Appendix S2: Table S2). This comparison was done only qualitatively (visually) without statistical tests because modeled values are not count data (integer) and therefore cannot be fitted into the same model as observed values. Furthermore, the rarefaction algorithm uses the same data pool repeatedly for all modeled values, so a direct statistical comparison to primary forest and among the modeled forests would also violate statistical assumptions (Klimes et al. 2012).

Appendix S1e - Multivariate analysis (Fig. 5):

Species matrix

A matrix of presence-absence of each ant species in a tree (individual trees as samples) was used as the community dataset for ordination analyses (652 trees x 19 ant species). We excluded species that occurred < 3 times across all plots, and trees without ants. We ran both unconstrained and constrained gradient analyses and calculated the percentage of variance explained by our variables in total and by the first two axes, including their efficiency (the proportion of variability explained by the constrained axis of the total variability explainable) (Braak and Smilauer 2012b).

Nest matrix

A matrix of ant nesting species x trees was used, with the number of nests each ant species had in each tree (individual trees as samples, 371 trees with ant nests x 14 ant species). In the cases where multiple nests were found in a tree, the proportions of nest occurrences per nesting microhabitat category and mean nest position (in m) were used as explanatory variable values. This approach allowed us to use a tree as a sample, as multiple nests within a tree are not independent (Klimes 2017), and our aim was to compare this analysis to above CCA conducted on all species occurrences at the tree level. Explanatory variables were selected using a forward selection in CCA with the corrected p-values (false discovery rate, 999 randomizations, alpha = 0.05; see Appendix S2: Table S3 for the list of significant predictors and their individual contributions).

Appendix S1f - Venn diagram analysis (Fig. 6):

We visualized the ant community richness and species overlap between forest successional stages in montane forest studied here, and between stages in the lowland forest available from previous studies. The data from Klimes et al. (2015, 2017) for the lowland forest were used for comparison. They had been sampled by identical methods but from one 0.4 ha primary and one 0.4 ha secondary plots. These plots were therefore subdivided into 0.1 ha subplots to allow for comparison based on the same area with the montane data presented here. For full standardization, we selected the same number of plots from our montane dataset: four primary plots (A, B, C, and D) and four secondary plots (E, F, G, H; randomly including four of the five plots). Young and old secondary plots were pooled as 'secondary forest stage'. We then compared the overall species diversity and species turnover between primary and secondary forests in both elevations. Species overlap between one primary and one secondary plot was calculated for each combination within elevation (16 combinations for the montane dataset and 16 for lowland dataset). Then the average number of species overlap was plotted in a Venn diagram for each elevation separately using the *VennDiagram* package (Chen and Boutros 2011) (Fig. 6).

Appendix S1e - Abstract in Tok Pisin

Yumi mas traim long luksave long how ol nupla bus i save kirap bekgen bihain long taim bus i kisim bagarap bikos displa em i save kamap long planti hap long ol bus blong yumi. Yumi gat sampla kilia long how ol bus na diwai i save kirap bekgen. Tasol long how ol binatang i save kirap bekgen bihain long taim bus i kisim bagarap, yumi ino gat gutpla save tumas. Na em i hat moa long save long ol binatang we i save stap long ol maunten bus na antap long kuru blong ol diwai bikos ino gat planti study i bin kamap pinis long displa.

Long displa study, mipla traim long skelim: taim ol bus long maunten i kisim bagarap na i wok long kirap bekgen, wanem samting tru i save kamap long ol anis we i save stap antap long ol bus na diwai? (Displa study i kamap long arere mak we ol anis i ken stap long maunten ples, long mak 1800 metre abrusim solwara igo antap). Mipla kauntim ol kainkain anis na ol haus blong ol insait long 0.1 hectare mak long olpla bus, na wankain long ol nupla bus we i bin kisim

bagarap na i wok long kirap bekgen. Mak long 1249 pla as diwai mipla i bin katim pundaunim na sekim gut long ol haus blong anis. Behain mipla yusim ol komputa program long traim skelim sapos strong blong diwai, sais blong diwai, na ol kain haus na ples we ol anis i stap, i ken tokim yumi how ol anis i save laik stap insait long bus.

Mipla i bin ting olsem taim bus i kirap bekgen na i wok long kamap olpla, namba blong ol kainkain anis na ol kainkain haus ol i save wokim long ol diwai bai igo antap. Na tu, mipla i bin ting olsem ol kain anis i stap long olpla bus, bai ino inap wankain tumas olsem ol anis i stap long nupla bus we i wok long kirap bekgen. Tasol em ino bin olsem. Study blong mipla i painim aut olsem namba blong ol kainkain anis ino bin gat bikpla senis namel long ol olpla na nupla bus. Long ol nupla bus we i wok long laik kamap olpla tasol, ol kain anis we i stap long displa ol diwai i bin wankain liklik. Tu, mipla painim aut olsem taim bus em i wok long kamap olpla, namba blong ol kainkain anis i stap long wanwan ol diwai tu i save go antap. Na taim mipla skelim ol wankain sais diwai namel long olpa na nupla bus, namba blong ol kainkain anis i antap liklik tasol long olpla bus, tasol ino winim tumas nupla bus. Mipla tu painim aut olsem planti wanwan kain anis i save laikim ol wanwan kain haus long stap insait. Tasol displa kain ol haus blong anis i wankain tasol long olpla bus na nupla bus, na ino bin gat bikpla senis tumas namel long tupla kain bus.

Long pasim het tok blong study blong mipla, mipla i painim aut olsem taim bus long ol maunten ples i kisim bagarap na i wok long kirap bekgen, displa ino save kamapim bikpla senis tumas long ol anis we i save stap long ol diwai. Tasol tu, mipla lukim olsem displa risalt ino wankain olsem pastaim wankain study mipla i bin wokim long bus blong nambis. Displa em i showim olsem bus blong maunten na nambis ino wankain tumas long taim ol kisim bagarap na i wok long kirap bekgen. Long displa as, yumi mas wokim planti moa study long luksave gut long displa ol senis i save kamap namel long maunten na ol nambis bus.

Appendix S2

Appendix Tables

Table S1. Table of arboreal ant species in three successional stages of tropical montane forest in Papua New Guinea. Crosses indicate that a species was found at least once in that successional phase. For full ant and forest plot data, see Dryad DOI: 0.5061/dryad.js48f56.

Code	Species	Subfamily	Number of occupied trees	Primary forest	Old secondary forest	Young secondary forest
anon 003	<i>Anonychomyrma dimorpha</i> (Viehmeyer, 1912)	Dolichoderinae	59	x	x	X
anon 002	<i>Anonychomyrma minuta</i> (Donisthorpe, 1943)	Dolichoderinae	115	x	x	X
anon 005	<i>Anonychomyrma</i> sp. 005	Dolichoderinae	206	x	x	X
camp 024	<i>Camponotus</i> sp.024 aff. <i>Aruensis</i>	Formicinae	10	x	x	X
card 002	<i>Cardiocondyla</i> sp. 002	Myrmicinae	2		x	X
care 005	<i>Carebara</i> cf. <i>armata</i> (Donisthorpe, 1948)	Myrmicinae	1	x		
hypo 004	<i>Hypoponera</i> sp.4	Ponerinae	16		x	X
para 014	<i>Paraparatrechina</i> sp. 014	Formicinae	3	x	x	
phei 048	<i>Pheidole</i> sp. 048	Myrmicinae	3		x	
phei 049	<i>Pheidole</i> sp. 049	Myrmicinae	1		x	

podo 005	<i>Podomyrma keysseri</i> (Viehmeyer, 1914)	Myrmicinae	67	x	x	x
poly 026	<i>Polyrhachis</i> (<i>Cyrtomyrma</i>) <i>mondoi</i> (Donisthorpe, 1938)	Formicinae	252	x	x	x
poly 027	<i>Polyrhachis</i> (<i>Chariomyrma</i>) sp. 27 (sp.nov.)	Formicinae	13		x	x
pone 001	<i>Ponera</i> sp. 001	Ponerinae	140	x	x	x
pone 002	<i>Ponera</i> sp.002	Ponerinae	52	x	x	x
pone 003	<i>Ponera</i> sp.003	Ponerinae	35	x	x	x
stru 006	<i>Strumigenys</i> sp. 006 aff. <i>szalayi</i> (Emery, 1897)	Myrmicinae	10	x	x	
stru 007	<i>Strumigenys</i> <i>tigris</i>	Myrmicinae	1	x		
stru 008	<i>Strumigenys</i> sp. 008 aff. <i>szalayi</i> (Emery, 1897)	Myrmicinae	2		x	
tech 007	<i>Technomyrmex</i> <i>mixtus</i> (Bolton, 2007)	Dolichoderina e	158	x	x	x
voll 002	<i>Vollenhovia</i> sp. 002	Myrmicinae	130	x	x	x
voll 003	<i>Vollenhovia</i> sp. 003	Myrmicinae	75	x	x	x
voll 004	<i>Vollenhovia</i> sp. 004	Myrmicinae	20	x	x	x
voll 008	<i>Vollenhovia</i> sp. 008	Myrmicinae	3		x	x

Table S2. Summary of different forest stages attributes (young secondary, old secondary, primary forest) in Papua New Guinea as observed or modeled using the rarefaction-based model method and 0.1 ha forest plots

Forest stage	Species per plot	Species per plot SE	Species per plot – regression model estimate 95% interval (upp)	Species per plot– regression model estimate 95% interval (down)	Species per tree	Species per tree SE	Species per tree – regression model estimate 95% interval (upp)	Species per tree – regression model estimate 95% interval (down)	Sørensen index	Sørensen index SE	Sørensen index – regression model estimate 95% interval (upp)	Sørensen index – regression model estimate 95% interval (down)
Young secondary observed	13.50	0.5000	19.25	9.03	0.76	0.0510	0.78	0.76	0.24	0.0039	0.31	0.21
Old secondary observed	11.00	0.5774	15.19	7.66	0.98	0.0818	1.15	0.93	0.38	0.0105	0.45	0.36
Primary observed	16.50	0.6455	20.80	12.83	1.50	0.0629	2.00	1.37	0.22	0.0105	0.26	0.19
Old secondary rarefaction model -tree abundance	16.21	0.2679	NA	NA	1.50	0.0028	NA	NA	0.20	0.0003	NA	NA
Young secondary rarefaction model -tree abundance	19.69	0.1135	NA	NA	1.50	0.0017	NA	NA	0.20	0.0002	NA	NA
Old secondary rarefaction model -tree abundance + DBH	14.99	0.1373	NA	NA	1.60	0.1162	NA	NA	0.21	0.0084	NA	NA
Young secondary rarefaction model -tree abundance + DBH	16.90	0.2320	NA	NA	1.01	0.1155	NA	NA	0.18	0.0051	NA	NA

Table S3. Result of a forward selection of variables explaining ant species composition of the communities that nested in the studied plots using permutation test in CCA multivariate analysis (Fig. 5B). Only significant variables (*adj. p*<0.05) are shown.

Name	Explained variability (%)	Contribution (%)	pseudo-F	P	P(adj)
Nesting microhabitat – Carton on leaves	6.5	33.9	25.5	0.001	0.00186
Nesting microhabitat – Under epiphytes	5.5	29.1	23.2	0.001	0.00186
DBH	1.7	9.1	7.4	0.001	0.02600
Succession (i.e. Forest age)	1.4	7.2	5.9	0.001	0.00325
Nesting microhabitat – Dead hollow twig	0.8	4.0	3.3	0.002	0.00433
Nesting microhabitat – Dead hollow branch	0.7	3.7	3.0	0.010	0.01625
Nesting microhabitat – Living hollow twig	0.6	3.1	2.6	0.002	0.00433
Nesting microhabitat – Under the bark	0.6	3.1	2.6	0.015	0.02438
Nesting microhabitat – Liana	0.5	2.8	2.3	0.018	0.02600
Total variability	18.3	-	-	-	-

Table S4. Percentage of trees ($DBH \geq 5\text{ cm}$) occupied by ants in different plots of tropical montane forest in New Guinea.

Forest	Plot	Occupied trees (percentage)
Young secondary	F	46.72
	G	43.85
Old secondary	H	48.53
	J	53.76
	K	56.82
Primary	A	66.37
	B	42.24
	C	64.34
	D	61.87

Table S5. GLM model testing the relationship of the number of ant species per tree with DBH in three successional stages of New Guinea montane forest (Fig. 3A).

Variable	DF	X2	p
DBH	1	175.1	<0.01
Successional stage	2	10.3	<0.01
Interaction	2	1.2	0.5537

Table S6. GLM model testing relationship of the number of different nesting microhabitat categories (i.e. nest diversity) per tree with DBH in three successional stages of New Guinea montane forest (Fig. 3B).

Variable	DF	X2	p
DBH	1	314.1	<0.01
Successional stage	2	19.1	<0.01
Interaction	2	2.7	0.26

Table S7. DBH categories used for modeling forest structure using the rarefaction-based approach (see Methods).

Category	range of Log(DBH)
A	0.69 - 0.78
B	0.78 - 0.95
C	0.95 - 1.11
D	1.11 - 1.28
E	1.28 - 1.45
F	1.45 - 1.62
G	1.62 - 1.78
H	1.78 - 1.94

Appendix Figures

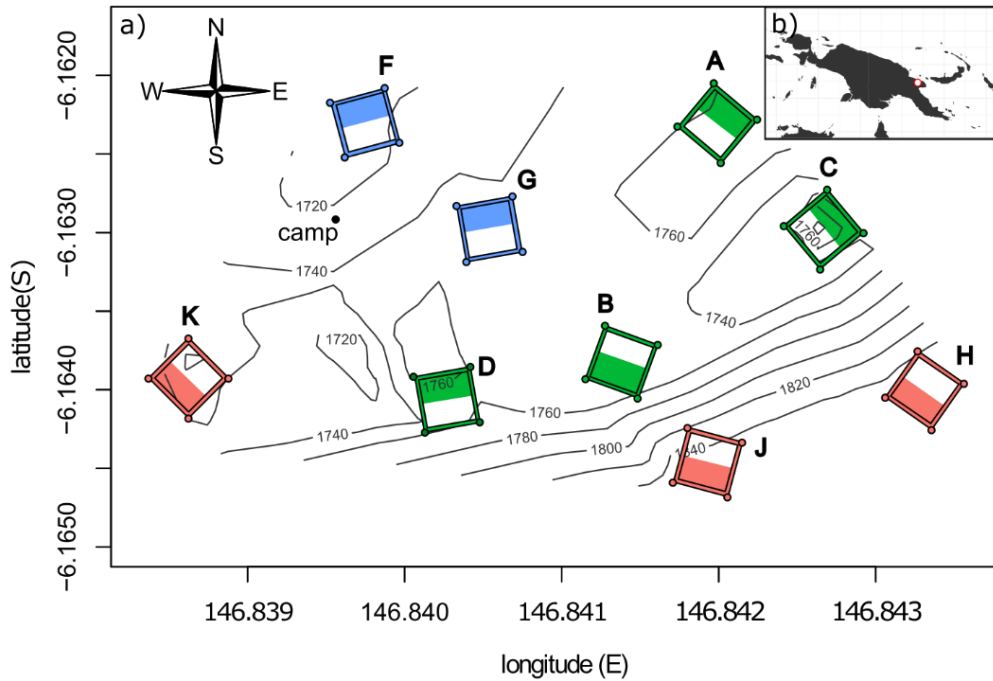


Figure S1. a) Map of the 0.2 ha forest patches sampled by felling; colored halves indicate the 0.1 ha plots sampled for ants in this study (22.5 x 45 m). Successional stages are marked by different colors: primary (A, B, C, D - green), old secondary (J, K, H - red) and young secondary (F, G - blue) forest. Contour lines show elevation (m). b) Study site (Yawan village) in the Saruwaged Mountains, Papua New Guinea.

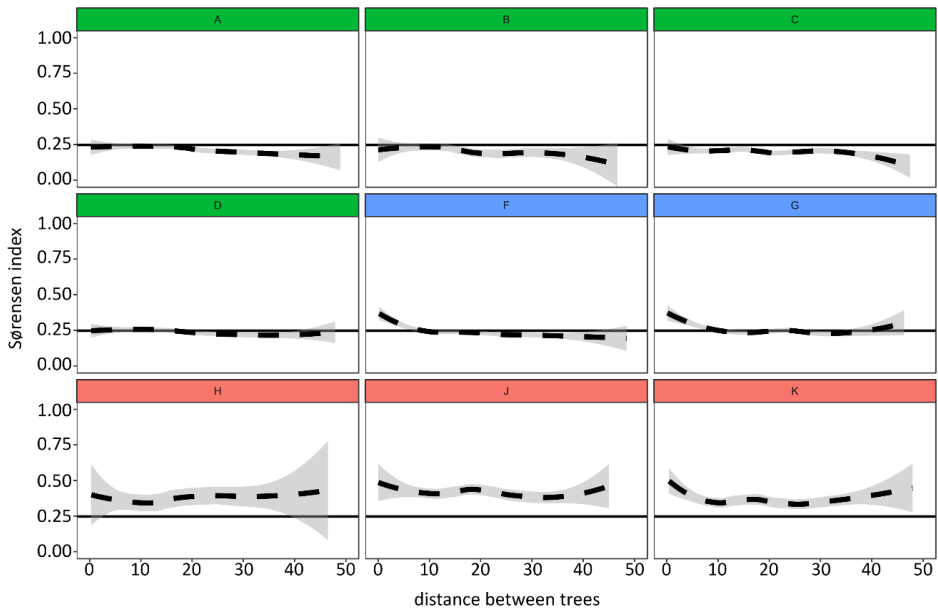


Figure S2. Relationship between ant species similarity (Sørensen index; S_o) and physical distance between trees. Each box represents a single forest plot (see Methods). The solid black line represents average S_o in the entire dataset, the black dashed line shows “loess” smoothing method with grey 95% confidence interval (See Supplementary Material Appendix 1c).

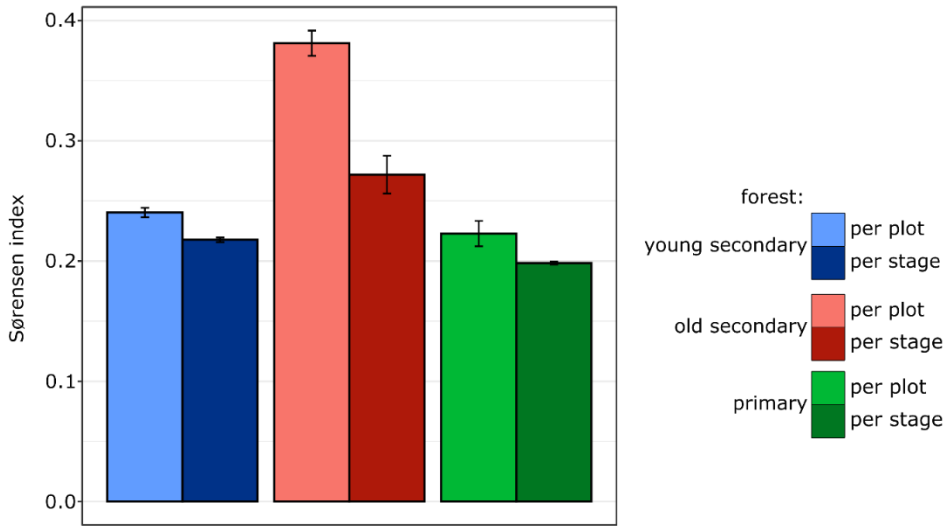


Figure S3. Similarity in arboreal ant species composition between trees in three successional stages of tropical montane forest for all pairs of occupied trees using Sørensen index. The index is calculated for each 0.1 ha plot separately and then averaged (per plot: left bars, i.e. as in the model in Fig. 2), or calculated between all trees in the same successional stage (per stage: right bars).

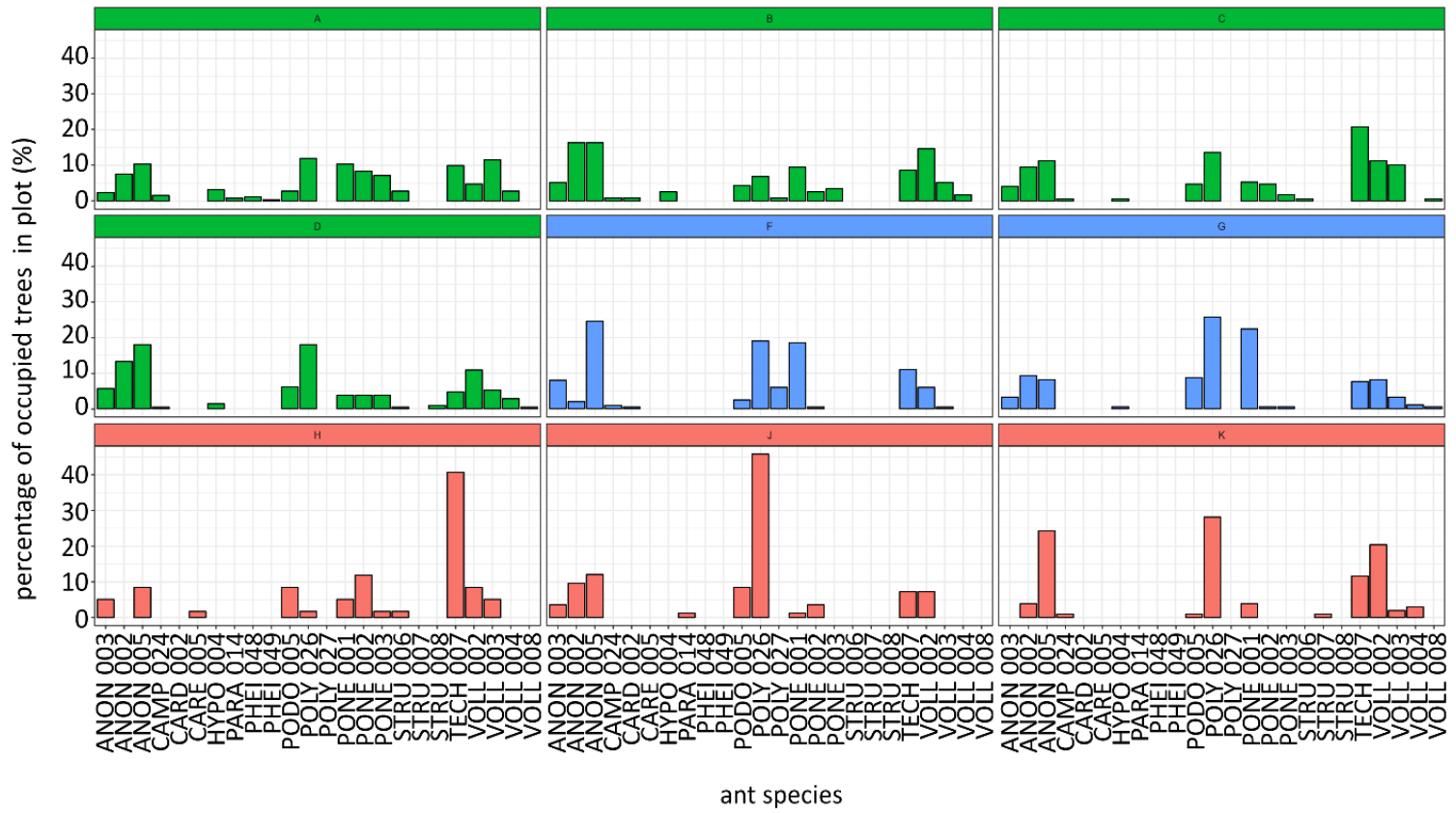


Figure S4. *Ant species distribution in each 0.1 ha plot. Bars represent the percentage of trees occupied by each ant species (for full names see Appendix S2: Tab. S1). Primary forest plots are green (A, B, C, D), young secondary blue (F, G) and old secondary red (H, J, K). Note that species frequencies are very similar among plots and stages except for the most common species differing between plot H and J.*

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~ Chapter IV ~

**Are guilds of arthropod communities spatially segregated in forests
canopies?**

Manuscript

Are guilds of arthropod communities spatially segregated in forest canopies?

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~ *Abstract* ~

Forest canopies host highly diverse and abundant arthropod communities. Density distribution of those communities is affected by abiotic factors, such as temperature, on the global scale and by biotic interactions, such as predation and competition, on the local scale. The importance of biotic factors should become higher in tropical regions, due to the supposedly higher abundances of arthropods there. Here, we conducted a census of abundances of several

arthropod guilds (leaf chewers and miners, ants and spiders) from all trees (with DBH \geq 5 cm) in nine replicated 0.1 ha plots in various latitudes and altitudes. The abundances of ants increased while the abundances of herbivores and spiders decreased with the increasing mean annual temperature. We did not detect any spatial segregation between the focal taxa that would suggest the effect of predation or competition in individual trees, and this pattern did not change in a temperature gradient. On a local scale, resource limitation seems to be much more important in determining densities of arboreal arthropods.

~ *Key words* ~

community ecology, Lepidoptera, Formicidae, guilds, spatial interactions, competition, predation

~ *Introduction* ~

Forest canopies harbour an astonishing number of arthropod species (Basset et al. 2015). This rich habitat has attracted the attention of numerous ecologists over the years, who sought to disentangle the factors driving and maintaining such high diversity. Despite great effort (Hammond 1992, Ozanne et al. 2003, Hamilton et al. 2010), studies comparing arthropod abundance across large geographic regions are relatively scarce. Since arthropod biomass accounts for a significant proportion in forest canopies (Basset et al. 2015), and the fact that canopy arthropods fulfil many important ecological roles (e.g. in herbivory, predation, pollination, and seed dispersal), arthropod abundance is undoubtedly a key factor affecting the entire ecosystem (Wilson 1987, Basset 2003).

Abiotic factors, such as temperature, have strong effects on the abundances of arboreal arthropods at large geographical scales (Speight et al. 1999). Arthropod abundance generally increases with mean annual temperature (Lister and Garcia 2018) and, thus, arthropod density on foliage tends to be higher in tropical than in temperate forests (Basset et al. 1992). However, quantifying arboreal arthropod abundance is problematic, especially in the tropics, and many studies estimate it instead. For example, studies focused on herbivores commonly use herbivore damage as a proxy for abundance, which is justified by herbivorous arthropods having both density and species richness increase with

latitude (Salazar and Marquis 2012, Bird et al. 2019), a similar pattern to herbivory (Dyer and Coley 2002, Lim et al. 2015).

The overall trends in arthropod abundance are further complicated by the fact that individual arthropod groups do not show a unidirectional response to latitude (Andrew and Hughes 2005a). There are pronounced differences even among individual herbivore guilds. For example, damage by leaf miners and external feeders exhibit contrasting trends with increasing latitude (Andrew and Hughes 2005b). In several guilds, such differential trends can be directly linked to host specialization, with abundances of generalists showing a weaker response to the latitudinal gradient than abundances of specialists (Salazar and Marquis 2012). Therefore, changes in arthropod density need to be studied from a broader perspective, ideally including multi-guild comparisons and a wide range of plant hosts.

At local scales, interactions such as predation and competition are most likely to limit populations in a density-dependent manner. Since competition increases with population density (Belovsky 1986), there should be high within-guild competition in the tropics, where both density and species richness are the highest for most groups. However, a meta-analysis showed that competition between herbivorous guilds tends to be higher than within a guild (Bird et al. 2019). In addition, interspecific interactions could change within the latitudinal gradient. For example, tropical herbivores are thought to be more adversely affected by within-guild competition, while temperate herbivores suffer more from environmental pressure (Schemske et al. 2009). However, *The Green World Theory* posits that herbivorous arthropod populations should be under such strong natural enemy pressure that intraspecific competition is relatively uncommon (Hairston et al. 1960).

Herbivore densities, therefore, are most likely regulated by a combination of biotic factors (predation, pathogens, and parasites/parasitoids) and abiotic factors (temperature, humidity, i.e. weather). Arthropod predation pressure was observed to be higher in the tropics than in temperate regions (Dyer and Coley 2002, Novotny et al. 2006). In addition, predation rates can be affected by the type of herbivore feeding guild. For example, endophytic herbivores are better

protected, and therefore less predated, than exophytic herbivores. However, leaf miners and gallers suffer more from parasitoid-induced mortality (Hawkins et al. 1997). Furthermore, manipulative experiments with plasticine caterpillars also confirm that predation pressure is higher in the tropics (Roslin et al. 2017).

Predation of herbivores by other arthropods is well known to have potentially significant effects on food webs by, for example, modifying the strength of trophic cascades (Cardinale et al. 2003, Finke and Denno 2005). The main predators of herbivorous arthropods worldwide are considered to be ants, spiders, and also birds (Holmes et al. 1979, Riechert and Lockley 1984, Floren et al. 2002). The relative importance of these groups in predation, however, is unclear, with some studies observing a greater effect of birds (Philpott et al. 2004), and others of ants (Mooney 2007, Piñol et al. 2010). High predation pressure by ants in the tropics relative to the temperate zone (Jeanne 1979), though, suggests that they exert the predominant top-down control of herbivore arthropods in the tropics, at least (Lach et al. 2010, Roslin et al. 2017). Lourenço *et al.* (2015) confirmed the importance of ants in canopy ecosystems of montane forest, observing an increase in potential herbivorous prey in individual trees where ant abundance was lower. Further studies comparing the larger-scale patterns, however, found no such effect of ants as the main arthropod predator along elevational gradients (Supriya et al. 2019). Furthermore, like the competition between herbivore guilds, there is competition between predatory arthropod groups. For example, spiders compete with ants for prey (Halaj et al. 1997, Mestre et al. 2012), and negative co-occurrence of ants and spiders abundances had been observed in the high-canopy of a lowland rainforest (Katayama et al. 2015). Nevertheless, in tropical forests of lower diversity or in the case of ant-mimicking spiders, positive spatial associations of both predatory groups were also observed (Marín et al. 2015, Schuldt and Staab 2015, Hashimoto et al. 2016).

The density distribution of arthropod predators has been shown to follow the distribution of their prey (Holt 1984), which often has a non-random spatial structure. We may speculate that, because herbivorous species are relatively specialized (Novotny et al. 2006, 2010) and tree species are distributed non-randomly in space (Fibich et al. 2016, Vincent et al. 2018), the distribution of

herbivorous arthropods should be also spatially dependent. The density of arthropod predators has been confirmed to have an effect on the distribution of their prey in agro-ecosystems (Room 1975, Hooks et al. 2003, Tobin and Bjørnstad 2003, Liere et al. 2012) and temperate forests (Mooney 2007), but such an effect has not been demonstrated in pristine tropical forests (but see Floren et al. 2002).

In this study, we gather a unique abundance dataset of two predacious arthropod groups (ants and spiders) and two guilds of insect herbivores (external feeding caterpillars and leaf miners) from the forest canopies in five geographic regions, representing both tropical and temperate areas. Arthropods were sampled in a standardized way from 0.1 ha forest plots using appropriate collection methods (see Methods). Sampling yielded abundance information of the arboreal arthropods in each tree, but also at the whole-forest level, incorporating a wide range of tree sizes. To account for our plots being situated at various latitudes and altitudes, we used mean annual temperature in each plot as a combination of those ecological gradients. We hypothesise that: (i) arthropod abundance will increase with increasing mean annual temperature, (ii) there will be within-tree segregation between arthropod groups (due to predation and competition), and (iii) the level of segregation between arthropod groups will increase with temperature, due to increased densities of arthropods.

~ Methods ~

Field sites and experimental design

We studied lowland temperate forests in the **Mikulcice** (Czech Republic; 1 plot) and **Toms Brook** (Virginia, USA; 2 plots), and tropical forests in Papua New Guinea (PNG hereafter): lowland forests in **Wanang** (2 plots), mid-elevation forest in **Numba** (2 plots), and montane forest in **Yawan** (2 plots). For detailed information on each site see Table 1.

Sampling methods

Each plot was 0.1 ha in size, rectangle-shaped, and carefully positioned so that its vegetation structure and species composition were typical of the local broadleaf forests. The plot was surveyed for vegetation, with all stems with a

diameter at breast height (DBH) ≥ 5 tagged and identified to species. Further, trees were completely surveyed for focal taxa of non-flying arthropods by manual searching of the foliage made possible by the felling of all stems in the plot (see detailed protocols in Volf et al. 2019). Focal taxa included all live leaf-chewing insect larvae (free feeding and semi-concealed), leaf mines, spiders, and ants. We used only information about the number of live leaf miners since abandoned mines do not reflect the current population size. All recorded live mines, caterpillar and spider individuals were collected. For ants, we have sampled foraging ants by forceps during a standardised time immediately upon the felling from the bottom trunk up to the top canopy (Klimes et al. 2015). For nests, we used a visual estimate of individuals for each nest on a categorical scale and an arbitrary number of individuals was set for each category (in brackets): 1) <100 (50). 2) 100-1000 (500), 3) >1000 (1500). We calculated the total number of ant individuals sampled outside the nest in each tree (freely foraging on a tree; *Foraging ants* hereafter), as well as estimated the total ant abundances for each tree (abundances for foraging and nests summed; *Ants* hereafter). Unlike abundances of other arthropods, the overall abundances for ants are more likely to be underestimated, as some nests and foraging events from them are likely to have more individuals than our conservative estimates. However, compared to other methods (beating, fogging), they well reflect the overall representation of the group in trees, as nest sizes and the cryptic nests (from inside tree tissues and epiphytes) are considered, unlike in the standard methods (Yanoviak et al. 2003b). The felled trees were stripped of leaves and the total leaf area and leaf biomass of the foliage was estimated (see detailed in Volf et al. 2019).

At all locations, we took advantage either of ongoing logging operations (CZ, USA) or subsistence shifting agriculture (PNG) so that we did not contribute to net deforestation. In PNG, the surveys were conducted in collaboration with local communities of indigenous land owners, improving their livelihoods by bringing employment and income.

Data Analysis

All analyses were conducted using R 3.5.2 (R Core Team 2016) and the *nlme* package (Pinheiro et al. 2014).

Datasets preparation

We generated tree-based and plot-based datasets. In the *Tree dataset* each of the 1001 trees was characterized by DBH, cut-down date, species identity, total leaf biomass, total leaf area, spatial coordinates in the plot, and the number of arthropod individuals from individual focal taxa; for ants we used the numbers of all *Ants* and *Foraging ants* separately (see above). All arthropods found on epiphytes or lianas were included into the host tree for the analysis. Note that in two plots (USA A and Numba B) the coordinates for trees were not available. Season of sampling was determined as the date of sampling for each tree, with disregard of the year.

The *Plot dataset* comprised nine plots characterized by the number of tree individuals and tree species, mean DBH, total basal area (BA), total leaf area, and abundance of individual focal taxa of arthropods (Table 2).

Arthropod abundances in the temperature gradient

We aimed to explore patterns of arthropod abundance from temperate to tropical latitudes. However, since our tropical locations had very different abiotic properties (e.g. altitude), we decided to use mean annual temperature as a main ecological gradient. The relationship between arthropod abundance and mean annual temperature was analysed using plots as data points. Arthropod abundance was expressed as a) total number of individuals in each plot, i.e. per 1,000 m² of land area (N_{tot}); b) average number of individuals per tree (N_{tree}); c) average number of individuals in plot per 1m² of foliage (N_{leaf}); and d) average number of individuals per 1m² of BA (N_{BA}). For each arthropod group from *Plot dataset* separately, we follow the same Model building guideline (see below).

Model building guideline:

1) Create GLM (arthropod abundance ~ temperature) with selected error distribution. Select Poisson error distribution for any count data and Gamma error distribution for data of species abundances, which were transformed if not an integer. Gamma was selected over Gaussian because all values were positive non-zero values, and we wanted to restrict the model to only predict positive non-zero values. 2) Test if a polynomial relationship is not significantly better

than linear using the deviance test, and refit accordingly. 3) Check model with Poisson error distribution for overdispersion and refit with Quasipoisson if necessary. 4) Perform Shapiro-Wilk test of normality on residuals. If residuals do not have normal distribution refit the model with values transformed as $\ln(y+1)$ and return to step 2. 5) Perform significance test based on deviance between null ($y \sim 1$) and final model.

Standardization by tree size and sampling season

The abundance of arthropods depends on the amount of resources (e.g. foliage biomass) available as well as physical structure and microclimate in each tree, and as such can vary non-linearly with tree size, expressed by either DBH or foliage biomass (see Fig S1-S2). Further, arthropod abundance can also vary seasonally (see Fig. S3). We have therefore developed a protocol to standardize arthropod abundances across trees of different sizes sampled at different seasons (Fig. 1) and used it to calculate *Standardized Abundances*, which are independent of tree size and season.

Arthropod abundance correlation

The relationships in abundance between the focal taxa were tested within each plot, using individual trees as units of analyses. We tested all pair-wise combination of the focal taxa, except the *Ants vs Foraging ants* comparison (as the later groups is a subset of the first, see above). We used linear regression with LM ($N_{\text{taxon1}} \sim N_{\text{taxon2}}$) and estimated beta regression coefficients (RC) if there was a significant relationship between taxa (significance was corrected using Šidák correction of significance (Sidak 1967)). We expressed arthropod abundance as a) a transformed number of individuals per tree $\ln(N+1)$, and b) as the *Standardized Abundances* (see Fig. 1).

Next, the analyses were also repeated using least-squares model, as GLS ($N_{\text{taxon1}} \sim N_{\text{taxon2}}$) with spatial coordinates of trees as rational quadratic spatial correlation structure, taking into account that individual trees within the plot may not be independent data points. We could calculate this correction for only seven plots where tree coordinates were available.

Finally, to reduce the possible noise of non-predacious ant individuals, we assigned all ant individuals from four genera (*Polyrhachis*, *Camponotus*, *Calomyrmex*, and *Echinopla*) as herbivorous (based on the literature and isotope analysis that highlights a nectar- and honeydew- based diet in those taxa (Davidson et al. 2003, Bluthgen et al. 2003)). We subset both *Ant* and *Foraging ant* datasets excluding those individuals and repeat the analysis of linear regression between arthropod taxa.

Number of interactions in the temperature gradient

We calculated the number of significant pair-wise combination of the focal taxa in each plot and divided it by the number of all tested combinations, as Interaction index, ranging between 0 (there were no significant correlations between taxa) to 1 (all possible interactions were significant). We calculated Interaction index from normal abundances and also *Standardized Abundances*, and tested the relationship between Interaction index and mean annual temperature of each plot. The approach followed the Model building guideline (see above) and created a GLM (Interaction index ~ temperature).

~ Results ~

In total, we sampled 1001 trees in 9 replicated plots (each 0.1ha) in 5 locations. These plots comprised 293 tree species in 70 families, with a total basal area of 29.7 m² and 2.6 ha of leaf foliage (Fig. 2A-C). The forest structure changed dramatically among the plots: the number of trees per plot (from 53 in Mikulcice to 157 in Wanang A); total leaf area (150.3 m² in Mikulcice to 395.4 m² in Wanang A); and mean DBH (from 11.7m in Wanang A to 28.2 in Mikulcice). This variation reflected a high number of relatively big trees in Mikulcice and an increasing number of smaller stems with annual temperature. In total, we sampled 14,333 caterpillars, 5,123 leaf miners, 6,037 spiders, and 1849 nests (with estimated 418,700 ants) and 22,476 foraging ants (Table 2, Fig. 2D).

Arthropod abundance in the temperature gradient

N_{tot} of ants increased and leaf miners and caterpillars decreased with increasing mean annual temperature (Fig 3A, Table S1). N_{tree} decreased with the

temperature in caterpillars, leaf miners, and spiders, and increased in *Ants* and *Foraging ants* (Fig. 3B, Table S1). The same trends were found for N_{leaf} (Fig. 3C, Table S1). N_{BA} significantly increased for *Ants* and *Foraging ants* and decreased for leaf miners with increasing temperature (Fig.3D, Table S1).

Arthropod abundance correlation

We have found 51 out of 78 (65%) possible pair-wise relationships between focal arthropod taxa to be significant when using the number of individuals on each tree as a measure of observed abundance, but only 13 out of 78 (17%) such relationships significant when using Standardized Abundances (Fig. 4). However, all relationships were positive with $RC > 0$.

Similar trends were observed when taking the spatial position of the trees into account: 60% relationship showed significant relationship for abundances and 19% for Standardized Abundances, with all significant relationship having $RC > 0$, and rather small differences among the significant pairs (Fig S4).

Excluding herbivorous ant species leads again to only minor changes, and 62% relationships were significant in the abundance-based calculation and 15% when using Standardized Abundances with all $RC > 0$ (Fig. S5).

Number of interactions in the temperature gradient

There is not a relationship between Interaction index and mean annual temperature, disregard if Interaction index was calculated from normal arthropod abundance (deviance test; $DF=1,7$; $p= 0.793$) or *Standardized Abundance* (deviance test; $DF= 2,6$; $p= 0.912$).

~ Discussion ~

This is the first study to analyse the abundances of arboreal arthropods from a standardized whole-forest sampling across several altitudes and latitudes. This approach enabled us to look at both large geographical scale (per plot; including vegetational parameters) and local co-occurrences (per tree). We showed that abundances of predators have contradictory trends, where spiders decreased and ants increased their abundance with mean annual temperature. The observed pattern for ants has been expected, as other studies showed the increasing trend

when comparing temperate and tropical forests (Jaffe et al. 2007, Floren et al. 2014), or different altitudes (Samson et al. 1997, Sam et al. 2015). Despite a considerable change in forest structure across the temperature gradient, the pattern for ants was consistent even after standardization to the same leaf area or basal area. Spiders did not always have a significant relationship, which could be caused by spiders not being sampled in three out of the nine plots and/or spiders being the taxa, which is perhaps more probable to escape while the trees were felled (i.e. spiders might have the most under-counted numbers).

In contrast to other studies (Salazar and Marquis 2012) and our prediction, we observed a negative correlation between the herbivore abundance and increasing temperature in all models, except for caterpillar abundances standardized to the same basal area. This could be due to several factors. One possibility is that tree species at lower latitudes have been under selective pressure to evolve more effective chemical defences because they generally experience more intense biotic interactions than do species at higher latitudes (but see Moles et al. 2011). Another explanation is that the lower abundance of host trees and higher herbivore specialization in the tropics results in relatively scarce resources on which large populations of specialized herbivores are difficult to maintain (Dixon et al. 1987). Several studies even reported insignificant changes in herbivore abundance along latitudinal gradients for leaf chewers (Andrew and Hughes 2005a) and leaf miners (Sinclair and Hughes 2008).

We were not able to detect segregation between any focal taxa in our study, contrary to our prediction. We did, however, observe a strong aggregation of abundances of arthropods. This could be explained by the differences in tree sizes. Big trees host many more arthropod individuals than small trees (Fig. S1-2), a pattern that can be explained as aggregation by resources (tree species, microhabitat), caused by arthropod species that are highly specialized to their host trees. This has been shown in other studies, with specialist herbivores feeding only on a single tree genus or species (Novotny et al. 2006, 2010), or by arboreal ants limited by nest-site diversity and availability (Klimes 2017). This pattern mostly disappeared, however, when abundances were standardized for tree size. Some relationships were still significant, but there was not a consistent

pattern in predation or competition between taxa. Moreover, all relationships had small but positive regression coefficients, suggesting that we still detected some aggregation of individuals in resource-rich trees. Another possibility for a lack of segregation between studied taxa could be the scale of observation. Arthropods may segregate on individual branches, but the pattern does not scale up to the whole tree. This was the case, for example, in Australia, where dominant arboreal ants were spatially segregated on individual branches of trees in a tropical forest plot in Australia (Bluthgen et al. 2004).

Trees are often spatially non-independent, with aggregations of tree species, tree sizes and/or tree biomass (Condit et al. 2000, Law et al. 2009, Fibich et al. 2016, Vincent et al. 2018). Even so, the inclusion of the tree plot position in our model did not reveal any differences in the results, highlighting the robustness of our result (Fig. S4). However, at smaller spatial scales, resource distribution is more likely to be uniform and tree spatial pattern can be indistinguishable from the random distribution due to the small sample size (Legendre and Fortin 1989). This phenomenon may be reflected in our results since our analysis was based on 0.1 ha plots.

Several studies have suggested that predation pressure is higher in the tropics either by observing the disappearance of dead arthropods (Novotny et al. 2006) or by experiments with plasticine caterpillars (Roslin et al. 2017). In light of this, we expected negative relationships to be stronger in the tropics, but it was not the case. The cited studies (Novotny *et al.* 2006, Roslin *et al.* 2017) used methods that focused on ant behaviour at different latitudes but concentrated more on the attack rates of ants rather than their real success rate, where the impact on the whole ecosystem could be different. While it has been demonstrated that bait methods might reflect the ant protection to be higher on specialised myrmecophytic ant-plants with a higher ant activity via decreasing their leaf herbivory (Plowman et al. 2017), the same might be not true at the level of a whole rainforest. At this level, it is possible that arboreal ants are not such important predators, but rather function more like omnivores in forest canopies, gathering nutrients from hemipteran symbionts and extrafloral nectaries in order to supplement their diet (Davidson 1997, Bluthgen et al. 2003). Furthermore, it is possible that we were not able to detect predation by spiders, because spiders

are more focused on other arthropod groups, which we did not sample (Birkhofer and Wolters 2012).

We acknowledge that our results are based on correlations between abundances of individuals and that manipulative experimentation would be required to disentangle the effects of individual taxa (see e.g. Klimes et al. 2011). However, we believe that the relatively large-scale plot-based sampling carried out in this study yielded sufficient data to provide insight into patterns of arthropod abundance in forest canopies. In this respect, we hope that this study can be a stepping stone to inspire further research into an interesting and perhaps understudied area of arthropod ecology.

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Author contribution

OM led the manuscript writing, analysed and interpreted the data. OM, VN, GW, PF, PK, and MV conceived the ideas and designed the study. OM, PK, MV, JA, TB, GC, EG, AG, GL, ML, MM, KM, GN, NSP, CR, CS, and JV led the data collection for the arthropods guilds in different sites. OM, PK, MV, KAT, PB, PD, OK, GL, SEM, NSP, CR, and CS contributed to data management. PF contributed to data analysis. VN, PF, PK, MV, and RT contributed to text and interpretation of the results.

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~ *Figures and Tables* ~

Table 1. Description of study sites. Mean annual values are reported for temperature and rainfall (McAlpine et al. 1983, Anderson-Teixeira et al. 2015, Vincent et al. 2015).

Locality	Miculcice	Toms Brook	Yawan	Numba	Wanang
Country	CZ	USA	PNG	PNG	PNG
Forest type	Lowland temp.	Lowland temp.	Highland trop.	Middle elev. trop.	Lowland trop.
Latitude	48°41' N	38°55' N	6° 9' S	5°44' S	5° 14' S
Longitude	16°56'E	78°25' W	146° 50' E	145°16' E	145° 4' E
Altitude (m asl)	164	230	1900	900	150
Temperature (°C)	9.0	12.7	16.2	22.3	25.8
Rainfall (mm)	526	970	3000	3000	4000
Number of plots	1	2	2	2	2

Table 2. Description of individual plots with total numbers of arthropod individuals recorded (herbivores and spiders) or quantitatively estimated (ants). Note that spiders were not sampled in 3 plots (see Methods for details).

Locality	Miculcice	Toms Brook		Yawan		Numba		Wanang	
Plot	A	A	B	A	B	A	B	A	B
Caterpillars	2 370	2 910	2 305	682	1 098	1 399	1 250	833	1 486
Leaf miners	2 717	232	877	251	222	120	90	223	391
Spiders	1 230	894	1 121	-	622	1 110	1 060	-	-
Ants	8 993	8 683	9 837	27 651	9 513	86 295	73 927	102 130	114 196
- Ants in nest	8 000	8 100	9 300	25 700	8 800	84 350	72 250	97 750	104 400
- Foraging ants	993	583	537	1 951	713	1 945	1 677	4 380	9 796

Abundance standardization guideline

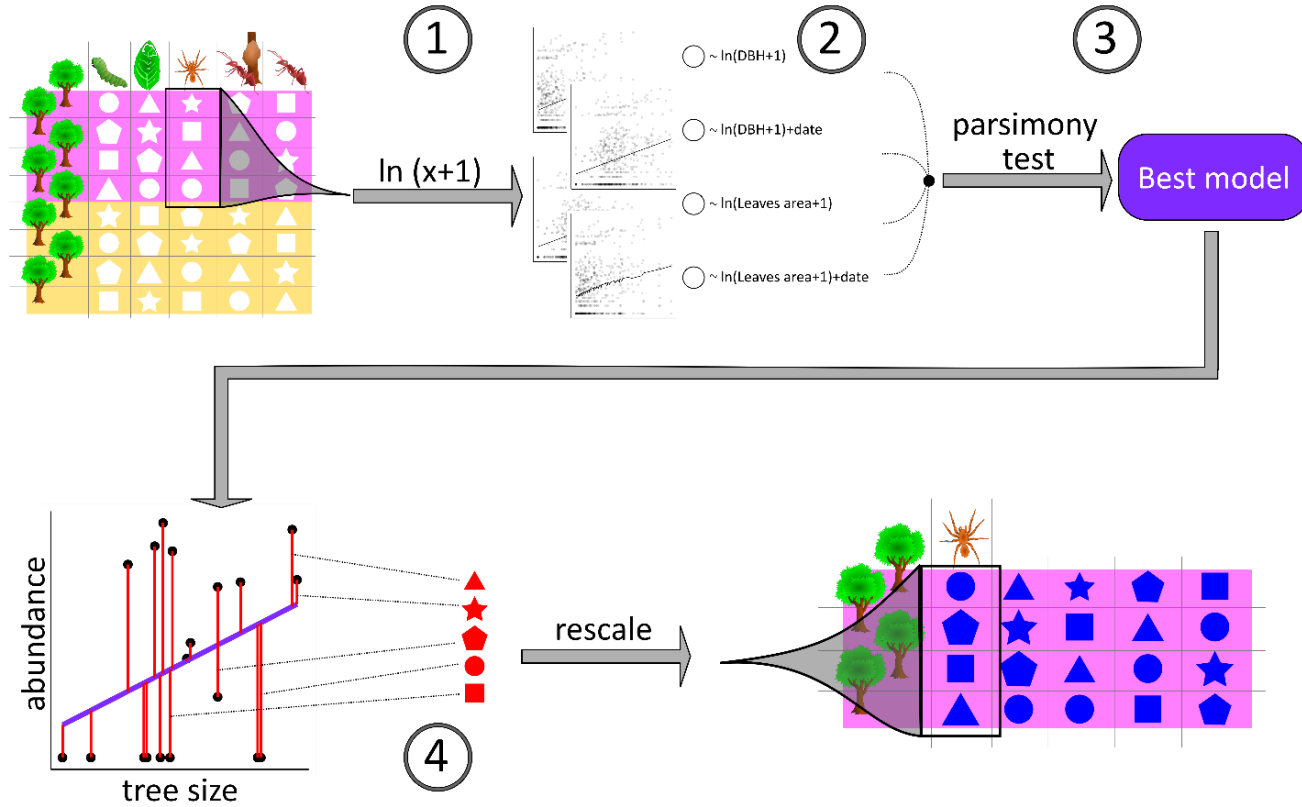


Figure 1. Diagram of abundance standardization procedure to filter out the effect of tree size (measured as DBH and/or leaf foliage biomass) and sampling season applied to the matrix of individual trees x focal arthropod taxa abundance: 1) Transform abundances as $\ln(x+1)$; 2) created 4 cubic smoothing splines with transformed abundances as dependent variable, DF set as 5 and independent variables as: a) $\sim\ln(\text{DBH}+1)$, b) $\sim\ln(\text{DBH}+1)+\text{season}$, c) $\sim\ln(\text{LeafArea}+1)$, d) $\sim\ln(\text{LeafArea}+1)+\text{season}$. 3) All 4 smoothing splines were transformed into GLMs and the best was selected based on parsimony (AIC). 4) Residuals from the best fitting GLM model were standardized to have the same range as $\ln(\text{DBH}+1)$ and those values were used as Standardized Abundance.

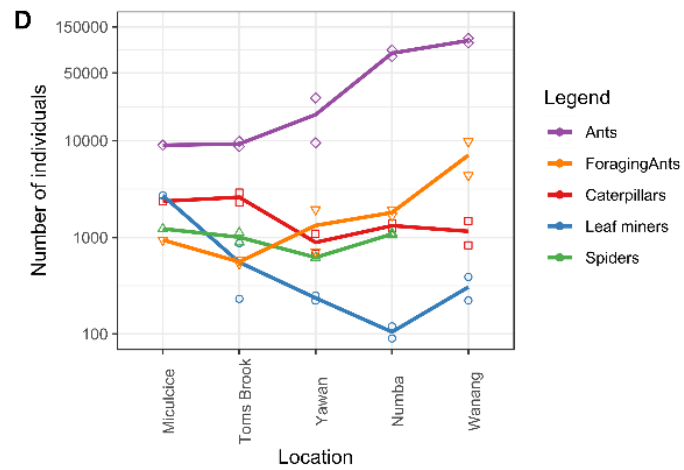
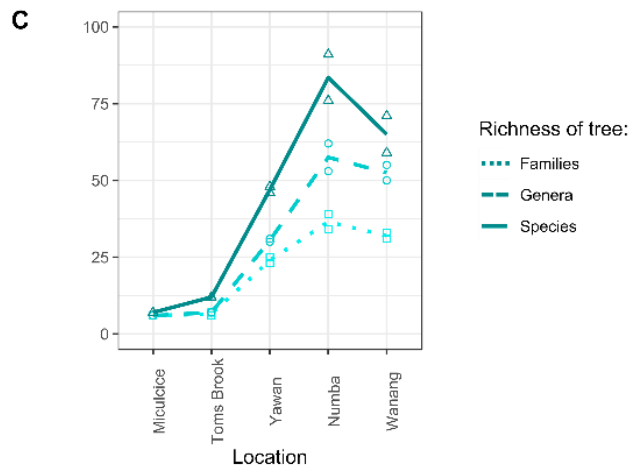
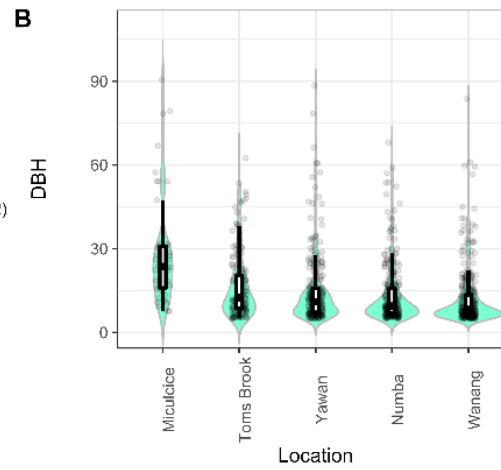
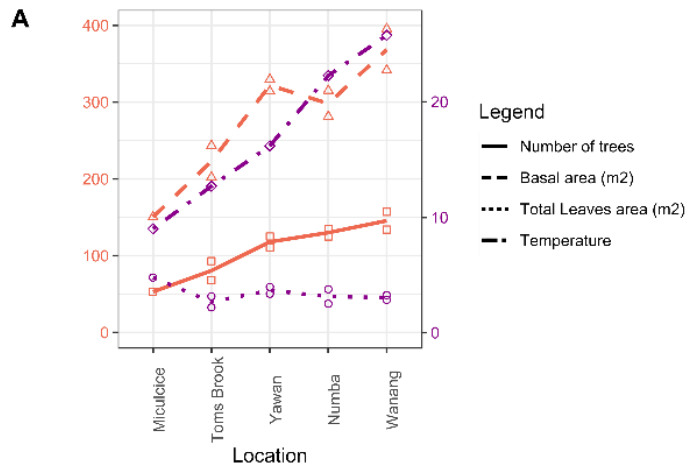


Figure 2. *Variability in plant and arthropod variables among the study plots: the total number of trees, basal area (in m²) and leaf area (in m²) per plot (A), the distribution of DBH values per plot (boxes indicate the 1st and 3rd quartile, central line the median, and whiskers 1.5 times the interquartile range) (B), the number of tree families, genera and species per plot (C), and the number of arthropods for each focal taxa per plot (D). Note that spiders were not sampled in Wanang. Individual sites are ordered by annual mean temperature from left to right.*

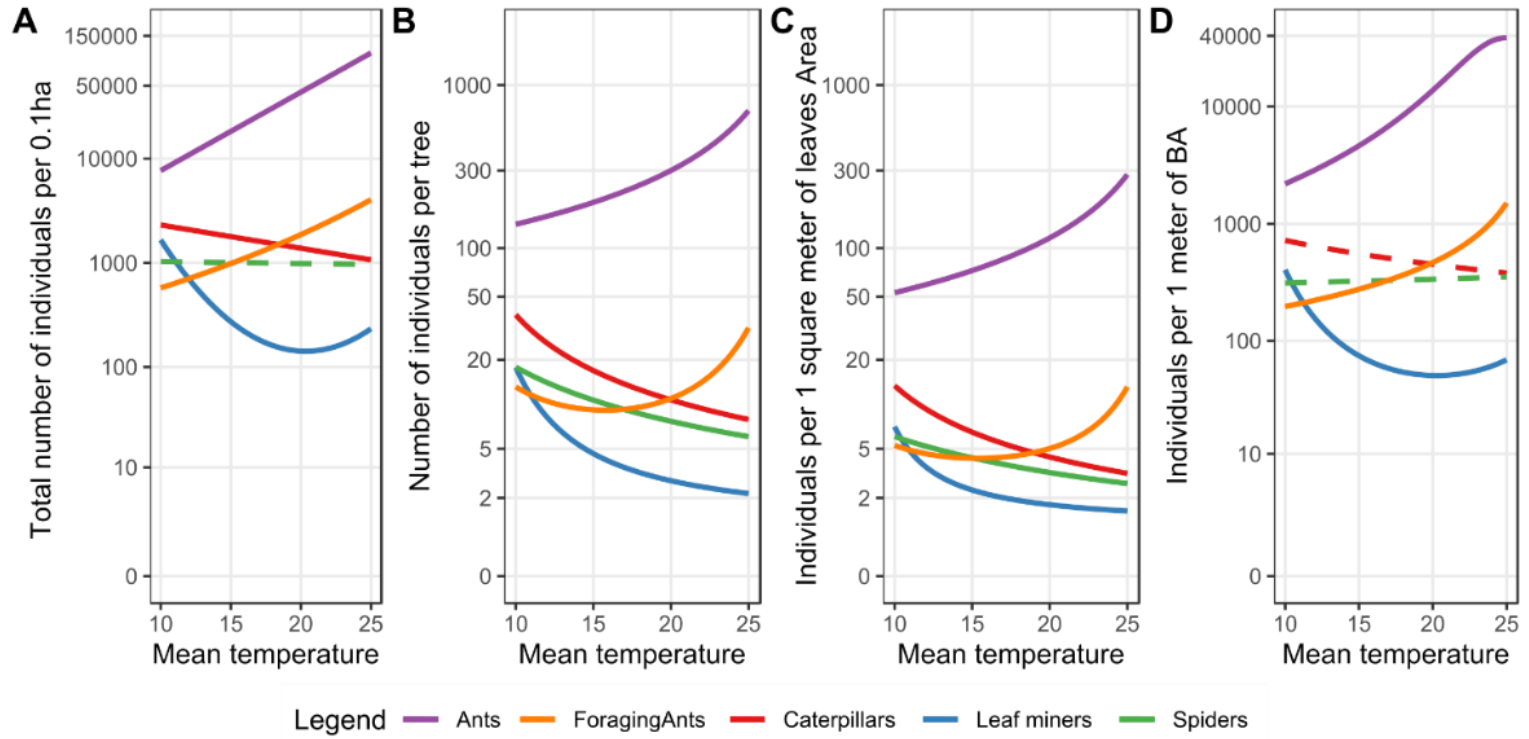


Figure 3. Relationship between mean annual temperature and the number of individuals per plot (A), per tree (B), per 1m² of foliage (C) and per 1m² of basal area (D). Each focal arthropod group is represented by the best GLM model (see Methods). Dashed lines represent non-significant relationships (see Table S2 for the models and their significances)

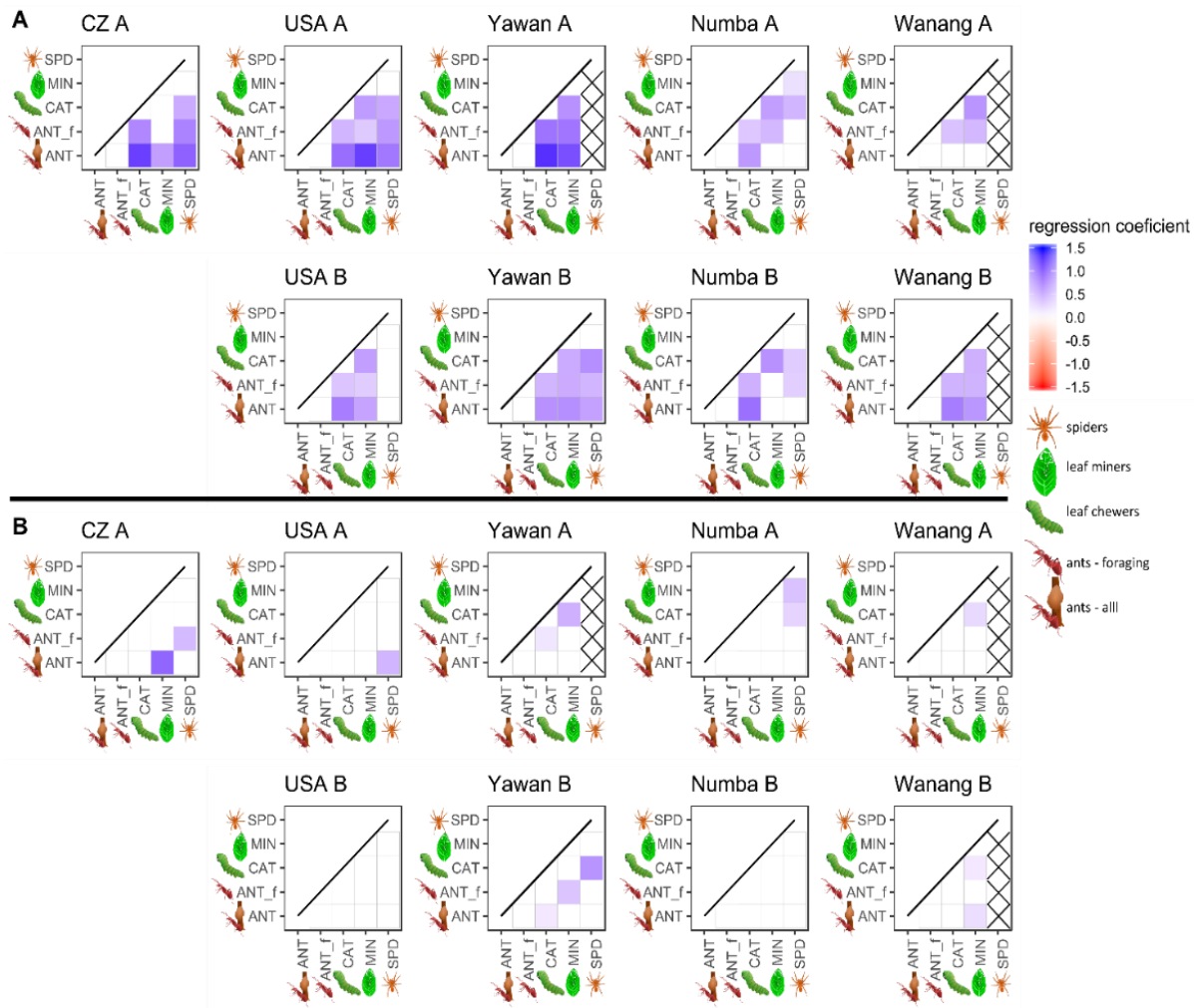


Figure 4. Results from pair-wise linear regressions between individual focal arthropod groups. Each matrix represents one plot. In each plot, each pair-wise combination of taxa was tested, except the Ants vs Foraging ants, using LM ($N_{\text{taxon1}} \sim N_{\text{taxon2}}$). Beta regression coefficient (RC) is shown in significant relationships as a colour gradient from red (negative) to blue (positive). Arthropod abundance was measured as a transformed number of individuals per tree $\ln(N+1)$ (A) and the Standardized abundance (B). Note that individual plots are ordered by annual mean temperature from left to right. No negative co-occurrence of guild abundances was found.

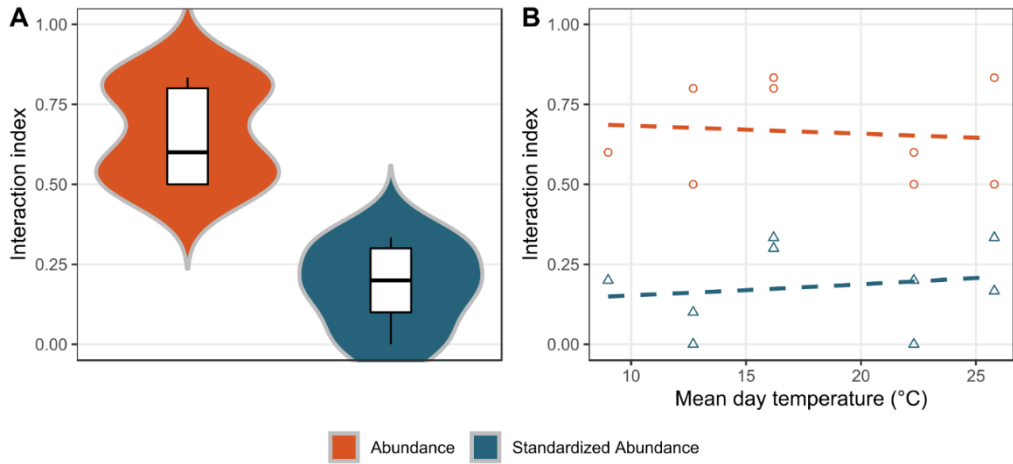


Figure 5. Interaction index calculated as the ratio of the number of significant pair-wise relationships found between focal arthropod taxa and all possible relationships in each plot. Relationships were calculated from observed abundances (Table 1) or Standardized Abundances (Fig. 1). For more details about the calculation of pair-wise relationships between focal taxa see Methods. The Interaction index variation based on the total number of relationships in each plot (boxes indicate the 1st and 3rd quartile, central line the median, and whiskers 1.5 times the interquartile range) (A). Relationship between mean temperature and the Interaction index (B). The relationship is not significant disregard if the Interaction index being calculated from normal arthropod abundance (deviance test; $DF=1,7$; $p= 0.5363$) or from Standardized Abundance (deviance test; $DF= 2,6$; $p= 0.9475$).

~ *Supplementary* ~

Supplementary Figures and Tables

Table S1. Summary table of all GLM models used in testing the plot-based relationship between arthropod abundances and mean annual temperature of 0.1 ha plots. Each model was built following Model building guideline and significance was tested using deviance against the null model (~1). Significant relationships are shown in bold.

Dependent variable	Independent variable	Error distribution	Deviance	DF	Res. Deviance	Res. DF	F	P
Caterpillars	Mean temperature	Quasipoisson	1235.8	1	1634.6	7	5.7419	0.048
Miners	Poly(Mean temperature,2)	Quasipoisson	6088.7	2	500.0	6	37.95	0.000
Spiders	Mean temperature	Quasipoisson	2.8814	1	252.42	4	0.0497	0.835
Ants	Mean temperature	Quasipoisson	322725	1	23602	7	101.6	0.000
Ln(Foraging ants+1)	Mean temperature	Gamma	0.098198	1	0.035343	7	-	0.000
Caterpillars per tree	Mean temperature	Gamma	3.6005	1	1.4386	7	-	0.000
Ln(Miners per tree+1)	Mean temperature	Gamma	2.3286	1	1.1722	7	-	0.000
Spiders per tree	Mean temperature	Gamma	0.90874	1	0.62114	4	-	0.006
Ants per tree	Mean temperature	Gamma	4.4267	1	1.6120	7	-	0.000
Ln(Foraging ants per tree+1)	Poly(Mean temperature,2)	Gamma	0.38614	2	0.16539	6	-	0.001
Caterpillars per LA	Mean temperature	Gamma	2.9153	1	1.5249	7	-	0.000
Ln(Miners per LA+1)	Mean temperature	Gamma	3.735	1	1.5766	7	-	0.000
Spiders per LA	Mean temperature	Gamma	0.51318	1	0.67318	4	-	0.044
Ants per LA	Mean temperature	Gamma	4.9468	1	1.8932	7	-	0.000
Ln(Foraging ants per LA+1)	Poly(Mean temperature,2)	Gamma	0.72888	2	0.2759	6	-	0.000

Caterpillars per BA	Mean temperature	Gamma	0.53856	1	2.0734	7	-	0.145
Ln(Miners per BA+1)	Poly(Mean temperature,2)	Gamma	0.24038	2	0.10783	6	-	0.002
Spiders per BA	Mean temperature	Gamma	0.0096295	2	0.65518	4	-	0.812
Ants per BA	Mean temperature	Gamma	9.5397	2	0.6676	6	-	0.000
Foraging ants per BA	Mean temperature	Gamma	8.3332	1	0.9397	7	-	0.000
Number of pair-wise relationships from all possible in each plot (Interaction index on Abundance)	Mean temperature	Quasibinomial	0.0086243	1	0.83950	7	0.0746	0.793
Number of pair-wise relationships from all possible in each plot (Interaction index on Standardized Abundance)	Poly(Mean temperature,2)	Quasibinomial	1.1773	2	1.2342	6	0.0939	0.912

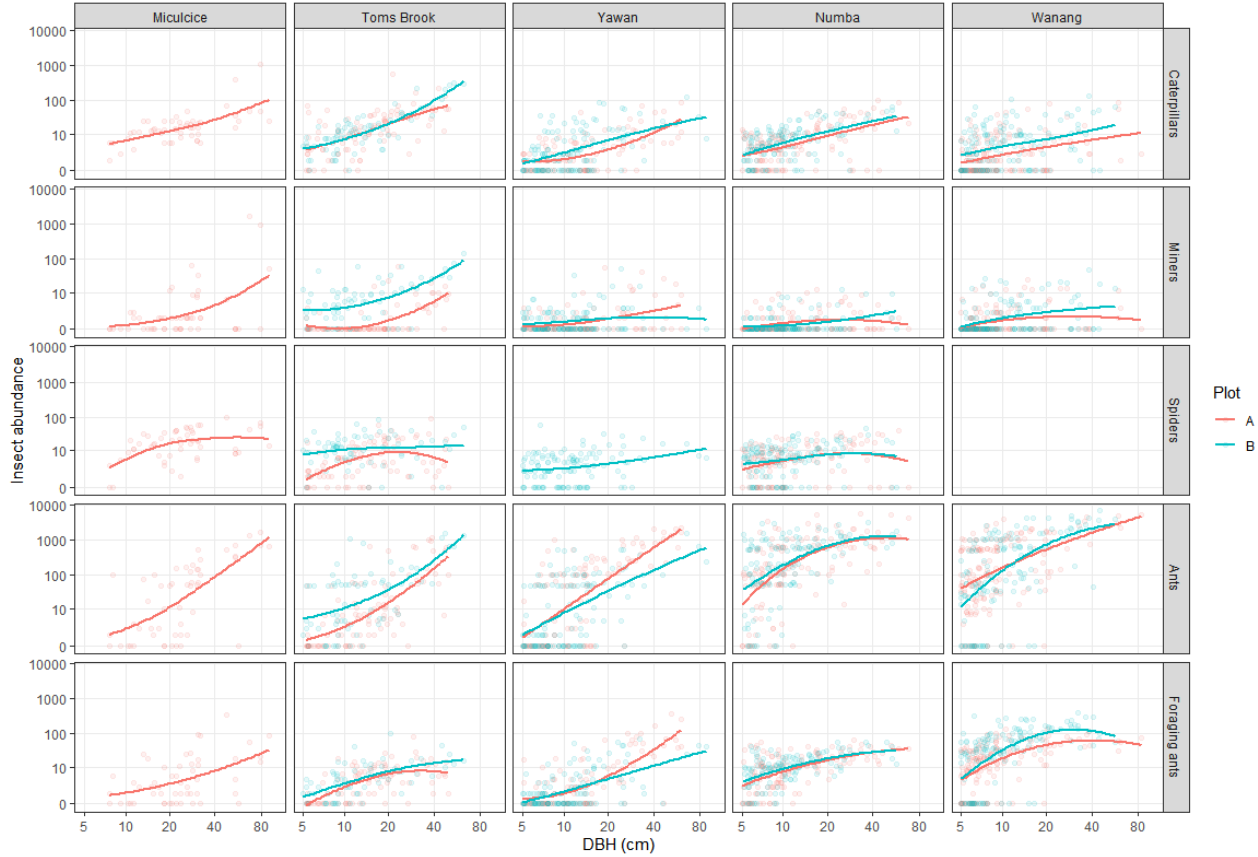


Figure S1. Relationship between the number of individuals of focal arthropod taxa and DBH of their host tree across all plots. Each point represents a single tree and line is drawn with a loess smoother (span = 2).

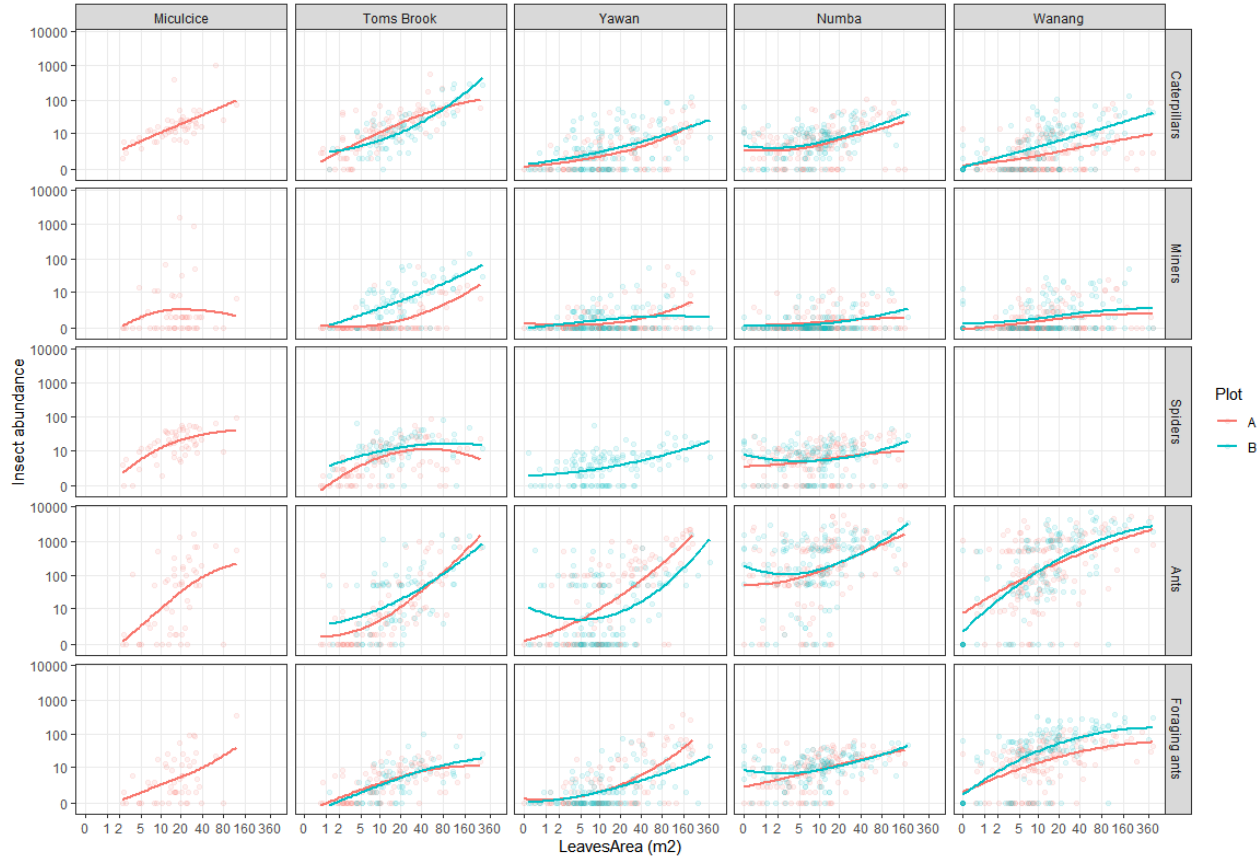


Figure S2. Relationship between numbers of individuals of focal arthropod taxa and leaf area (m^2) across all sampled plots. Each point represents a single tree and line is drawn with a loess smoother (span =2).

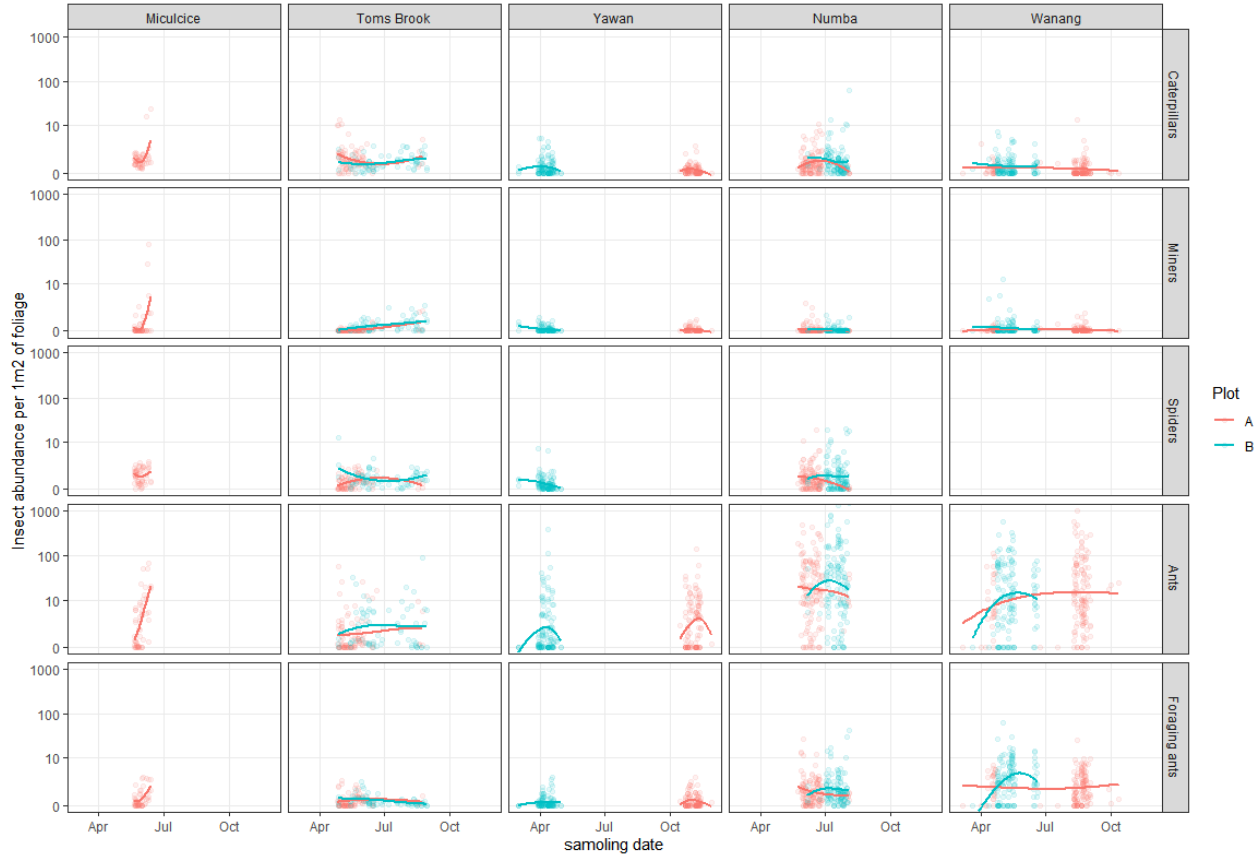


Figure S3. Relationship between the number of individuals per 1m² of foliage in focal arthropod taxa and the day of sampling across all study plots. Each point represents a single tree and the line is drawn with a loess smoother (span =2)

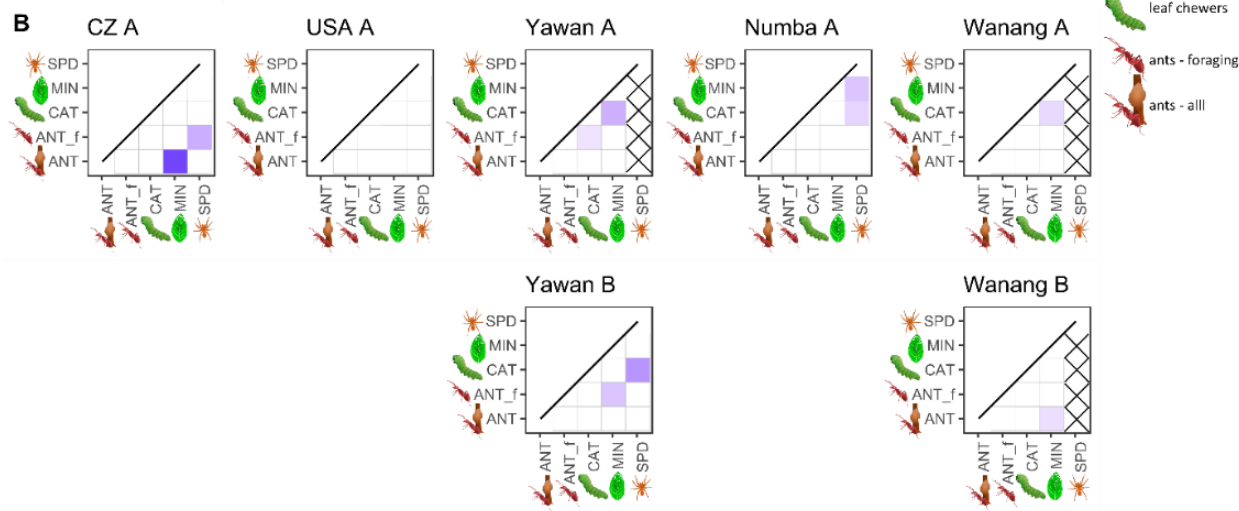
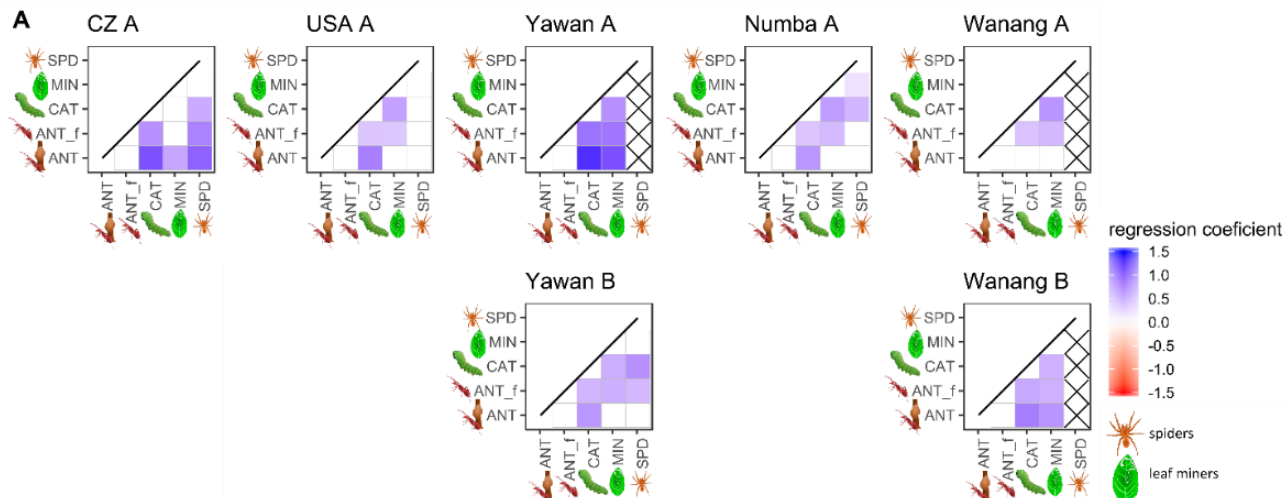
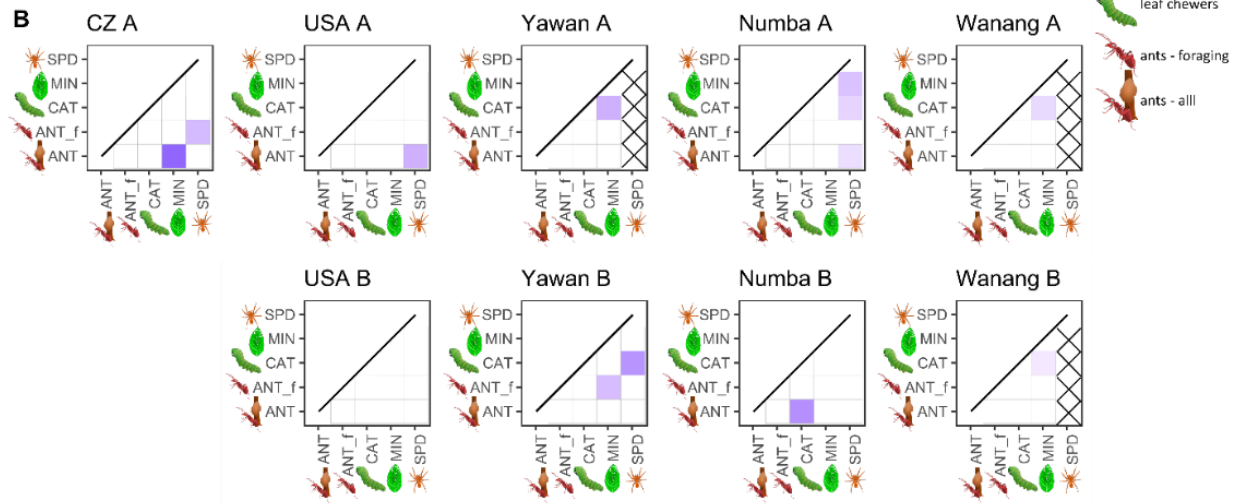
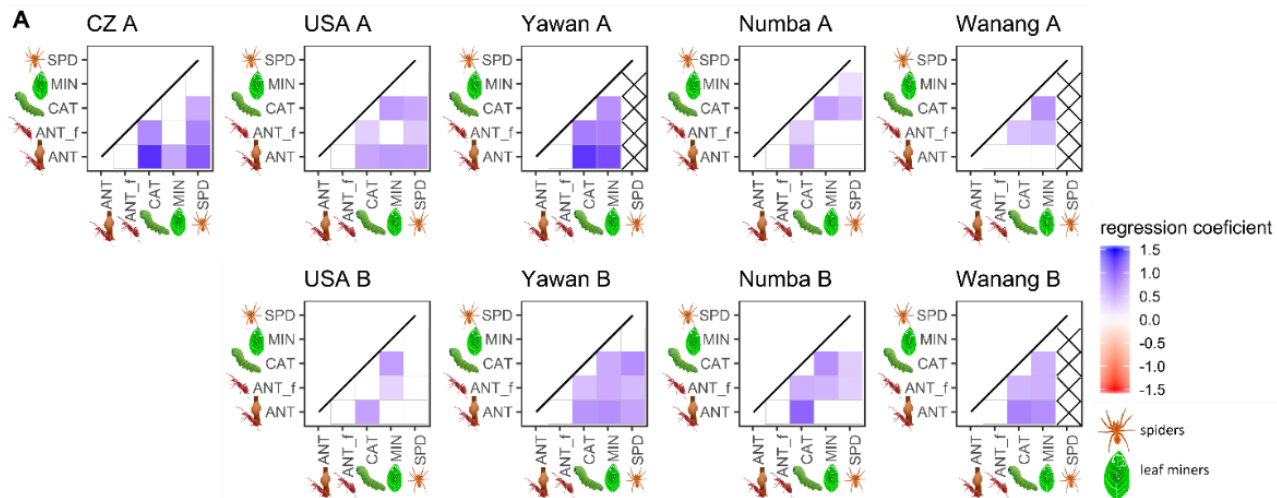


Figure S4. Visualization of pair-wise linear regressions between individual focal arthropod groups with consideration of spatial dependence of trees (i.e. the distance between their stems) in each plot in the model (see Methods for details). Each matrix represents one plot. In each plot, each combination of taxa was tested, except Ants vs Foraging ants, using the least-squares model GLS ($N_{\text{taxon1}} \sim N_{\text{taxon2}}$) with coordinates of trees as rational quadratic spatial correlation structure. Beta regression coefficient (RC) is shown in significant relationships as a colour gradient from red (negative) to blue (positive). Note that spatial coordinates of trees were not available for two plots (USA B and Numba B) and these were therefore not tested. Arthropod abundance was measured as a transformed number of individuals per tree $\ln(N+1)$ (A) and the Standardized abundance (B). Note that individual plots are ordered by annual mean temperature from left to right. No negative co-occurrence of guild abundances was found.



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Figure S5. Visualization of linear regressions between individual focal arthropod groups after excluding the herbivorous ant taxa. Each combination matrix represents one plot. In each plot, each pair-wise combination of taxa was tested, except the Ants vs Foraging ants, using LM ($N_{\text{taxon1}} \sim N_{\text{taxon2}}$). Ant individuals form species, which are known to be herbivorous (4 genera: *Polyrhachis*, *Camponotus*, *Calomyrmex*, and *Echinopla*) were excluded from the dataset. Beta regression coefficient (RC) is shown in significant relationships as a colour gradient from red (negative) to blue (positive). Arthropod abundance was measured as a transformed number of individuals per tree $\ln(N+1)$ (A) and the Standardized abundance (B). Note that individual plots are ordered by annual mean temperature from left to right. No negative co-occurrence of guild abundances was found.

~ *Summary* ~

~ *Thesis summary* ~

This thesis explored the various drivers of distribution patterns of hyper-diverse, tree-dwelling ant communities in the tropical rainforests of Papua New Guinea (PNG). Even though the datasets presented in this thesis were sampled using different methodologies, the plot-based sampling approach was used in all of them, showing the importance of whole-forest studies. Unlike previous studies that focused on the communities sampled from just a few trees, sampling whole patches of the forest makes it possible to incorporate the spatial distribution of all individual trees in tropical forest (i.e. tree-felling), or high-canopy trees (i.e. 9-ha study by baits). Furthermore, different ecological questions need a different methodology to answer them: For example, while stratification of ants can be easily demonstrated using baits and artificial nest sites, and knowing the exact position of each, tree felling is, on the other hand, a better approach to answer questions regarding a census of whole ant communities, and their natural nesting habits. The latter approach can also be used to test the effects of various vegetation structural parameters on the ant community, including rare species, which remains a challenge for experimental studies. While researchers in the past more commonly used observations, experimental studies are now increasingly implemented in these complex tropical environments. However, an experimental approach is still only rarely taken when studying arboreal insects and tropical trees. This thesis presents the aforementioned compilation of studies working with both observation patterns and experiments across multiple spatial scales.

The results of this thesis demonstrate that ant communities are strongly affected by environmental gradients, such as changes in temperature and forest strata, over both large (geographical) and local scales. However, the behaviour of individual species and microhabitats (e.g. forest topography and nest sites) are probably more important factors at the local scale. In addition, the results stress that environmental factors, e.g. succession, can change the strength of their influence on the ant communities depending on other environmental factors (e.g. elevation). Since communities of ants are distributed non-randomly in space, accounting for the spatial distribution effects in plot-based sampling should become a standard in future myrmecological studies.

~ *Main findings and conclusions* ~

In **Chapter I**, the results supported our hypothesis that vertical stratification of tropical rainforest has a strong effect on ant communities. We found that there was a significant difference between species composition of communities on the ground and the vegetation. This was confirmed by using both tuna-baits sampling and artificial bamboo nests, and the findings go in agreement with previous studies from tropical studies (Ryder Wilkie et al. 2010). In addition, the communities in different strata are affected by different limiting factors. We have found that the empty ant nests exposed on vegetation have three times higher occupancy-rates than those on the ground, suggesting a much higher nest-site limitation on vegetation. This is likely because there are much more occupied natural twigs on the ground than on the vegetation. This all suggests that changes in abiotic factors of different layers of tropical forest (such as temperature variation, microhabitat resources, etc.) have a strong impact on communities of ants. We have also demonstrated that the artificial nests are a good approach for future manipulative experiments with ants because we were able to successfully translocate whole colonies of ants between plots. That is promising, especially with arboreal communities, since the translocation had no negative effect on their colony fitness.

In **Chapter II**, we studied dominant ant species in big trees (DBH \geq 40) across 9-ha of primary lowland forest, and find out that their assembly rules are driven more by their behavioural than habitat preferences. First, we confirmed that species were vertically distributed in forest strata using multiple baits on each tree (bait-line sampling, see Leponce and Dejean 2011). We find out that communities are non-randomly spatially distributed in our study area, and that species are also segregated within each individual tree. Moreover, we were able to detect an effect of elevation of trees on ant communities sampled on them, which was rather small, but still significant after controlling for spatial-autocorrelation. Even though it is well known that ant communities response strongly to changes in elevation (Fisher 1996, Bruhl et al. 1999, Staab et al. 2014), we suggest that there is presence of some habitat filter, when communities are assembling on a fine-scale of the changes of microclimate (elevation change in our Chapter II is only \sim 100m). Overall, we found no other environmental

variable (e.g. of vegetation structure) affecting the ant communities, and we demonstrated that the uneven distribution of ant species is caused likely by the behavioural aggression of several numerically dominant species in the forest. Those species create territories up to 0.7 ha, which are spatially segregated more than they would be at random. Previous studies (Bluthgen and Stork 2007, Klimes et al. 2015, Dejean et al. 2019) also suggested that this spatial segregation of dominant species (i.e. *ant mosaic*) is mostly caused by the behaviour, but we are the first study, which has confirmed it using both spatial null models of territories across the canopies and behavioural experiments. In particular, since we were not able to find strong effect of habitat filtering (e.g. tree taxonomy, suggested by Dejean et al. 2015, 2016, 2018, 2019), and the fact that segregation of territories was correlated with aggression of the species, we could conclude that spatial pattern of segregated territories is mostly caused by their behavioural traits.

In **Chapter III**, we have focused on the effect of a well-studied ecological gradient of secondary succession on arboreal ants in unstudied conditions. We used data from a whole-plot sampling of montane tropical rainforest to test if the succession has the same effect as in the lowlands. We used a method of felling and dissecting of all trees in each plot above 5 cm DBH to gather detail information about all ant species, including cryptic ones, and their nesting habits. In contrary to our prediction, we were not able to found significant changes in species diversity or composition in successional gradient, with an exception of the number of species per tree that increased with forest age. That could be explained partly by strong changes in forest structure in succession, with young secondary forest having much more of smaller-sized trees than the other stages of forests. We used rarefaction-based models, manipulating vegetation structure, and multivariate randomisations to determine that microhabitats are much more important factor for species assembly than succession itself. It is also possible that presence of the behavioural traits, present in lowland species (e.g. colony size, behavioural dominance), is limited near the abiotic limits of ant distributions, as there are too few competing species (Machac et al. 2011), which may also lead to rather small species compositional changes (unlike in the

lowlands, Chapter II). The highland species, already filtered by temperature limitation, are then possibly free to colonize both primary and secondary forests.

In final **Chapter IV**, we investigated what effect has a higher presence of arboreal predators, including ants, on communities of herbivores in a gradient of various latitude and altitude. We used quantitative data from a plot-based sampling of replicated 0.1 ha plot and find out that abundances of ants are increasing with an increase of mean annual temperature (used as a single variable combining latitude and altitude). On the contrary to ants, abundances of spiders, leaf chewers, and leaf miners were decreasing with temperature. We have shown that the only positive correlation we can observe is between abundances, which were not corrected for tree size and season, while we did not find surprisingly any evidence for the negative correlation between arthropod guilds. This suggests that arboreal arthropods generally aggregate in bigger, resources richer, trees, while their co-occurrence after filtering out this effect is mostly neutral. We were not able to detect any spatial segregation of abundances between predators and herbivores, or within any of the group. That implies that ants and spiders might not be the main factor reducing the number of herbivores in forest canopies, which was suggested by previous studies (Novotny et al. 2006, Roslin et al. 2017), but overall it challenges the traditional view of the tropical ants exerting a high predation pressure on herbivorous insects in tree canopies (Floren et al. 2002). However, our conclusion is based only on observation patterns and correlations between individual taxa abundances. Manipulative experiments on a scale of whole-plot are still needed to confirm this hypothesis (Klimes et al. 2011).

~ *Future directions* ~

This thesis provided unique insights into the assembly rules of arboreal ants. Despite this thesis providing new knowledge about the distribution of ants in the tropical forest, the even higher impact of its results is showing the direction, which the modern ecology arboreal ants should steer in. Using the “whole-plot sampling” of the rainforest with a combination of manipulative experiments is a promising approach to disentangle the complex system of tropical communities.

Ecological gradients affect canopy community structure, as we can observe in the change of the ant species diversity, abundance and species composition along them. Even though this thesis showed that communities of dominant arboreal ants are more affected by their behavioural traits in pristine lowland forest, it is not clear if this is a general pattern for ecological gradients (e.g. elevation or succession). Biotic interactions, as competition, might be important in the realization of responses to the ecological gradient. For example, even if are montane species able to tolerate increased temperatures, it is possible that are outcompeted by species from lower elevations. Similarly, invasive species might be prevented by native species from invading pristine habitats from the secondary forest. Alternatively, the same pattern could be caused by habitat filtering, with some species having higher fitness in disturbed habitats. Since the observations of species distributions do not reveal the exclusive roles of competition and habitat filtering, the balance between biotic and abiotic drivers needs to be explored experimentally. Such manipulative experiments need to be performed outside the laboratory at a whole-plot level to include both local and spatial factors (e.g. overall forest structure and species territories, as well as microhabitats), which is challenging to set up in the field. However, we have presented a novel method of whole colony translocation, which could be used for such future manipulative studies. For instance, one could use translocation of ant species nesting in the artificial nests among higher and lower elevations, and/or primary and secondary forests, to disentangle the biotic and abiotic factors on their communities.

In addition, most past research has been limited to species diversity. This thesis stressed the importance of including behavioural traits into ecology research of tropical ants. Behavioural and morphological variability (Bishop et al. 2015) is being increasingly being used to measure life history and ecology of otherwise poorly known, highly diverse tropical taxa, and link this information to the species ecology and the effects of environmental changes on their assemblages. This is done via measurement phylogenetic and functional trait diversities within communities (Machac et al. 2011). Although these measurements have been used to study assemblage mechanisms from the observed community patterns (Donoso 2014), how they respond to experimental

manipulation and how they are affected by interspecific competition is still little understood. The manipulative translocations of colonies across different ecological gradients can be hence accompanied by measures of behavioural and morphological traits of individual species. Combining this experimental framework with the whole-plot census of ant communities and with functional and phylogenetic measures would then allow us to elucidate how, not only the species but their ecological traits and relatedness to each other, are influenced by the abiotic and biotic factors. The patterns observed in natural communities can be then compared to the experimentally manipulated.

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~ *Curriculum vitae* ~



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FIELD WORK

Extensive experience in quantitative canopy insect sampling and behavioural tests

STATISTICS

Advanced Mixed effect models, null models, multivariate analysis

IDENTIFICATION SKILLS

Good general knowledge of invertebrates, specialized in Formicidae (PNG)

MOLECULAR METHODS

DNA extraction, PCR

SOFTWARE

Statistics and community analysis: R, CANOCO, Statistica, EstimateS, Graphical tools: GIMP, Inkscape, Others: Microsoft Office, SQL database

COMMUNICATION & OUTREACH

Czech, English, German, TokPisin (Papua New Guinea pidgin)

Invited speaker at KuchaPecha (event to share personal presentations about work)

Organisation of student festival Vítání prvků (cultural festival for freshman), České Budějovice, 2014

EDUCATIONAL BACKGROUND

Ph.D. in Entomology 2015 – PRESENT

University of South Bohemia.

Spatial structure and community dynamics of arboreal ants in a tropical rainforest. Supervisor: RNDr. Petr Klimeš Ph.D. (scheduled to defend in November)

M.Sc. in Zoology 2013 – 2015

University of South Bohemia. Honours.

Structure and dynamics of arboreal ant communities along successional gradient in a mountain tropical forest of New Guinea. Awarded by Dean's prize. Supervisor: RNDr. Petr Klimeš.

B.Sc. in Biology 2010–2013

University of South Bohemia.

Detection of UV-fluorescent-marked prey in the guts of predators and scavengers, dynamics of scavenging in a model experiment. Supervisor: RNDr. Pavel Foltan Ph.D.

PROFESSIONAL EXPERIENCE

Ph.D. study 2015 – PRESENT

Institute of Entomology Biology Centre CAS

Leading field sampling of arboreal ants 2019

Nditam, Cameroon

Conducting field experiments on ERC project of V. Novotny "Ecological determinants of tropical-temperate trends in insect diversity" (6 weeks)

Data Analysis 2017 – 2018

Mobility Plus project (PIs: P. Klimes and M. Leponce)

Visit of Laboratory of Biodiversity Monitoring & Assessment at RBINS, Belgium (6 weeks)

Field experiments on tri-trophic interactions 2018

EuCFACE – WSU, Australia

Conducting field experiments on ERC project of K. Sam "Why is the world green: testing top-down control of plant-herbivore food webs by experiments with birds, bats and ants" (2 months)

Fellowship 2017

Smithsonian Institution Conservation and Research Center, Virginia, USA (2 months)

Leading field project focused on arboreal ant ecology 2016

Binatang research centre, PNG (6 months)

Student supervision 2016 – 2019

Martin Brožák (B.Sc. thesis), *Effect of succession, stratification and time on ant community in tropical lowland forest* (In Czech).

INTERNATIONAL CONFERENCES

Association for Tropical Biology and Conservation (ATBC)

Kuching, (oral): *Ant mosaics in tropical forests: Finding drivers of spatial structure*

European Conference of Tropical Ecology (GTO)

Brussels (oral): *Ant mosaics in tropical lowland forests: Take a look at a bigger scale*

European Conference of Tropical Ecology (GTO)

Göttingen (oral): *The low effect of forest succession on arboreal ant communities in a tropical mountain forest in New Guinea*

PUBLICATIONS

- 2019 *Experiments with artificial nests provide evidence for ant community stratification and nest site limitation in a tropical forest.* Biotropica (In Press). DOI: 10.1111/btp.12684.

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- 2015 *Tajemství pestrosti společenstev mravenců v korunách tropických stromů.* Živa 4: 179–182.

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Unpublished *Nest microhabitats and tree size drive ant community structure across elevation in tropical rainforest canopies.* Ecography (In review)

Plowman, N. S., O. Mottl, V. Novotny, M. Rimandai, F. Kassir, C. Idigel, and P. Klimes.

Ant mosaics in primary forest canopies are driven by interspecies behaviour. (Manuscript to be submitted to Journal of Animal Ecology)

Mottl, O., J. Yombai, V. Novotný, M. Leponce, G. D. Weiblen, P. Klimes.

Spatial segregation of guilds in arboreal arthropod communities. (Manuscript to be submitted to Ecology letters)

Mottl, O., P. Fibich, P. Klimes, M. Volf, J. Auga, K. Anderson-Teixeira, P. Butterill, T. Blair, G. Carscallen, E. Gonzalez-Akre, A. Goodman, G. Lamarre, M. Libra, M.E. Losada, M. Manumber, S.E. Miller, K. Molem, G. Nichols, N.S. Plowman, C. Redmond, C. Seifert, R. Tropek, G.D. Weiblen, V. Novotný.

Functional structure of ant assemblages along a wide elevation gradient. (Manuscript to be submitted to Journal of Biogeography)

Jacquemin J., O. Mottl, P. Klimes, N.S. Plowman, M. Leponce.

Comparing facets of avian alpha- and beta- diversity along a tropical elevational gradient provides insights into community assembly processes. (Manuscript to be submitted to Ecology and Evolution)

Hazell, R. J., O. Mottl, J. Tobias, B. Koane, S. Yama, L. Paul, K. Sam, A. J. A. Stewart, M. R. Peck.

GRANTS & AWARDS

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