Following Bees and Wasps up Mt. Kilimanjaro:
From Diversity and Traits to hidden Interactions of Species

Auf den Spuren von Bienen und Wespen
auf den Kilimandscharo:

Eine Studie über die Diversität, Merkmale
und verborgenen Wechselwirkungen zwischen Arten

Dissertation zur Erlangung des naturwissenschaftlichen Doktorgrades
Julius-Maximilians-Universität Würzburg

vorgelegt von

Antonia Veronika Mayr

geboren in Eschwege

Würzburg, 2019

Submitted/Eingereicht 4 April 2019

Members of the doctoral committee/Mitglieder der Promotionskommission

Chairperson/Vorsitzender	Prof. Dr. Ricarda Scheiner
Referee/Gutachter	Prof. Dr. Ingolf Steffan-Dewenter
Referee/Gutachter	Prof. Dr. Alexandra-Maria Klein

Defence/Promotionskolloquium 29 May 2019

Date of Receipt of Certificates/Doktorurkunde ausgehändigt am:

Die Wissenschaft, richtig verstanden, heilt den Menschen
von seinem Stolz- denn sie zeigt ihm seine Grenzen.

Albert Schweitzer

To all the tiny creatures

who had to give their lives for science

Table of Contents

Affidavit

I hereby declare that my thesis entitled: „ **Following Bees and Wasps up Mt. Kilimanjaro: From Diversity and Traits to hidden Interactions of Species**" is the result of my own work. I did not receive any help or support from commercial consultants. All sources and / or materials applied are listed and specified in the thesis.

Furthermore I verify that the thesis has not been submitted as part of another examination process neither in identical nor in similar form.

Besides I declare that if I do not hold the copyright for figures and paragraphs, I obtained it from the rights holder and that paragraphs and figures have been marked according to law or for figures taken from the internet the hyperlink has been added accordingly.

Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, die Dissertation: „**Auf den Spuren von Bienen und Wespen auf den Kilimandscharo: Eine Studie über die Diversität, Merkmale und verborgenen Wechselwirkungen zwischen Arten**", eigenständig, d. h. insbesondere selbständig und ohne Hilfe eines kommerziellen Promotionsberaters, angefertigt und keine anderen, als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben.

Ich erkläre außerdem, dass die Dissertation weder in gleicher noch in ähnlicher Form bereits in einem anderen Prüfungsverfahren vorgelegen hat.

Weiterhin erkläre ich, dass bei allen Abbildungen und Texten bei denen die Verwertungsrechte (Copyright) nicht bei mir liegen, diese von den Rechtsinhabern eingeholt wurden und die Textstellen bzw. Abbildungen entsprechend den rechtlichen Vorgaben gekennzeichnet sind sowie bei Abbildungen, die dem Internet entnommen wurden, der entsprechende Hypertextlink angegeben wurde.

Würzburg, den

Place, Date Signature

Summary

Chapter 1 – General Introduction

One of the greatest challenges of ecological research is to predict the response of ecosystems to global change; that is to changes in climate and land use. A complex question in this context is how changing environmental conditions affect ecosystem processes at different levels of communities. To shed light on this issue, I investigate drivers of biodiversity on the level of species richness, functional traits and species interactions in cavity-nesting Hymenoptera. For this purpose, I take advantage of the steep elevational gradient of Mt. Kilimanjaro that shows strong environmental changes on a relatively small spatial scale and thus, provides a good environmental scenario for investigating drivers of diversity. In this thesis, I focus on 1) drivers of species richness at different trophic levels (Chapter 2); 2) seasonal patterns in nest-building activity, life-history traits and ecological rates in three different functional groups and at different elevations (Chapter 3) and 3) changes in cuticular hydrocarbons, pollen composition and microbiomes in *Lasioglossum* bees caused by climatic variables (Chapter 4).

Chapter 2 – Climate and food resources shape species richness and trophic interactions of cavity-nesting Hymenoptera

Drivers of species richness have been subject to research for centuries. Temperature, resource availability and top-down regulation as well as the impact of land use are considered to be important factors in determining insect diversity. Yet, the relative importance of each of these factors is unknown. Using trap nests along the elevational gradient of Mt. Kilimanjaro, we tried to disentangle drivers of species richness at different trophic levels. Temperature was the major driver of species richness across trophic levels, with increasing importance of food resources at higher trophic levels in natural antagonists. Parasitism rate was both related to temperature and trophic level, indicating that the relative importance of bottom-up and top-down forces might shift with climate change.

Chapter 3 – Seasonal variation in the ecology of tropical cavity-nesting Hymenoptera

Natural populations fluctuate with the availability of resources, presence of natural enemies and climatic variations. But tropical mountain seasonality is not yet well investigated. We investigated seasonal patterns in nest-building activity, functional traits and ecological rates in three different insect groups at lower and higher elevations separately. Insects were caught with trap nests which were checked monthly during a 17 months period that included three dry and three rainy seasons. Insects were grouped according to their functional guilds. All groups showed strong seasonality in nest-building activity which was higher and more synchronised among groups at lower elevations. Seasonality in nest building activity of caterpillar-hunting and spider-hunting wasps was linked to climate seasonality while in bees it was strongly linked to the availability of flowers, as well as for the survival rate and sex ratio of bees. Finding adaptations to environmental seasonality might imply that further changes in climatic seasonality by climate change could have an influence on life-history traits of tropical mountain species.

Chapter 4 – Cryptic species and hidden ecological interactions of halictine bees along an elevational gradient

Strong environmental gradients such as those occurring along mountain slopes are challenging for species. In this context, hidden adaptations or interactions have rarely been considered. We used bees of the genus *Lasioglossum* as model organisms because *Lasioglossum* is the only bee genus occurring with a distribution across the entire elevational gradient at Mt. Kilimanjaro. We asked if and how (a) cuticular hydrocarbons (CHC), which act as a desiccation barrier, change in composition and chain length along with changes in temperature and humidity (b), *Lasioglossum* bees change their pollen diet with changing resource availability, (c) gut microbiota change with pollen diet and climatic conditions, and surface microbiota change with CHC and climatic conditions, respectively, and if changes are rather influenced by turnover in *Lasioglossum* species along the elevational gradient. We found physiological adaptations with climate in CHC as well as changes in communities with regard to pollen diet and microbiota, which also correlated with each other. These results suggest that complex interactions and feedbacks among abiotic and biotic conditions determine the species composition in a community.

Chapter 5 – General Discussion

Abiotic and biotic factors drove species diversity, traits and interactions and they worked differently depending on the functional group that has been studied, and whether spatial or temporal units were considered. It is therefore likely, that in the light of global change, different species, traits and interactions will be affected differently. Furthermore, increasing land use intensity could have additional or interacting effects with climate change on biodiversity, even though the potential land-use effects at Mt. Kilimanjaro are still low and not impairing cavity-nesting Hymenoptera so far. Further studies should address species networks which might reveal more sensitive changes. For that purpose, trap nests provide a good model system to investigate effects of global change on multiple trophic levels and may also reveal direct effects of climate change on entire life-history traits when established under different microclimatic conditions. The non-uniform effects of abiotic and biotic conditions on multiple aspects of biodiversity revealed with this study also highlight that evaluating different aspects of biodiversity can give a more comprehensive picture than single observations.

Zusammenfassung

Kapitel 1 – Allgemeine Einführung

Eine der größten Herausforderungen der ökologischen Forschung ist es, die Reaktion der Ökosysteme auf den globalen Wandel, d.h. auf Veränderungen von Klima und Landnutzung, vorherzusagen. Eine komplexe Frage in diesem Zusammenhang ist, wie sich verändernde Umweltbedingungen auf die Ökosystemprozesse auf verschiedenen Ebenen von Gemeinschaften auswirken. Um dieses Thema näher zu beleuchten, untersuche ich die Triebkräfte der Biodiversität auf der Ebene des Artenreichtums, der funktionellen Eigenschaften und der Wechselwirkungen zwischen Arten bei Hautflüglern, die in Hohlräumen nisten. Zu diesem Zweck nutze ich den steilen Höhengradienten des Kilimandscharo, der starke Umweltveränderungen auf relativ kleinem Raum mit sich bringt und somit ein gutes System für die Untersuchung von Triebkräften der biologischen Vielfalt bietet. In dieser Arbeit konzentriere ich mich auf 1) Triebkräfte des Artenreichtums auf verschiedenen trophischen Ebenen (Kapitel 2); 2) saisonale Muster in der Nestbauaktivität, lebensgeschichtliche Merkmale und ökologische Raten in drei verschiedenen funktionellen Gruppen und in verschiedenen Höhenlagen (Kapitel 3) und 3) Veränderungen in kutikulären Kohlenwasserstoffen, Pollenzusammensetzung und Mikrobiomen bei *Lasioglossum* Bienen, die durch klimatische Faktoren verursacht werden (Kapitel 4).

Kapitel 2 – Klima und Nahrungsressourcen prägen den Artenreichtum und die trophischen Wechselwirkungen von hohlraumnistenden Hautflüglern

Die Triebkräfte des Artenreichtums werden seit Jahrhunderten erforscht. Temperatur, Ressourcenverfügbarkeit und Top-Down-Regulierung sowie die Auswirkungen der Landnutzung werden als wichtige Faktoren für die Bestimmung der Insektenvielfalt angesehen. Die relative Bedeutung jedes dieser Faktoren ist jedoch unbekannt. Mit Hilfe von Nisthilfen entlang des Höhengradienten des Kilimandscharo versuchten wir, die Triebkräfte des Artenreichtums auf verschiedenen trophischen Ebenen zu enträtseln. Die Temperatur war der

Hauptfaktor für den Artenreichtum auf allen trophischen Ebenen, wobei die Bedeutung der Nahrungsressourcen auf den höheren trophischen Ebenen der natürlichen Antagonisten zunahm. Die Parasitierungsrate wurde sowohl durch die Temperatur als auch durch die trophische Ebene bestimmt, was darauf hindeutet, dass sich die relative Bedeutung der Bottom-up- und Top-down-Kräfte mit dem Klimawandel verschieben könnte.

Kapitel 3 – Saisonale Schwankungen in der Ökologie von tropischen hohlraumnistenden Hautflüglern

Natürliche Populationen schwanken mit der Verfügbarkeit von Ressourcen, dem Vorhandensein natürlicher Feinde und klimatischen Schwankungen. Die Saisonalität ist jedoch auf tropischen Bergen noch nicht gut untersucht. Wir untersuchten saisonale Muster in der Nestbauaktivität, funktionale Merkmale und ökologische Raten bei drei verschiedenen Insektengruppen in niedrigeren und höheren Höhenlagen. Insekten wurden mit Nisthilfen gefangen, die während eines Zeitraums von 17 Monaten, der drei Trocken- und drei Regenzeiten umfasste, monatlich überprüft wurden. Die Insekten wurden nach ihren funktionalen Gilden eingeteilt. Alle Gruppen zeigten eine starke Saisonalität im Nestbau, die in niedrigeren Lagen höher war und dort zwischen den Gruppen stärker synchronisiert war. Die Saisonalität im Nestbau von Raupen- und Spinnen-jagenden Wespen war mit saisonalen Klimaschwankungen verbunden, während sie bei Bienen stark von der Verfügbarkeit von Blüten abhing, genauso wie die Überlebensrate und das Geschlechterverhältnis der Bienen von der Blütenmenge abhing. Die Anpassung an die Saisonalität der Umwelt könnte bedeuten, dass weitere Veränderungen der saisonalen Klimaschwankungen durch den Klimawandel einen Einfluss auf die lebensgeschichtlichen Merkmale tropischer Bergarten haben könnten.

Kapitel 4 – Kryptische Arten und versteckte ökologische Wechselwirkungen bei Schmalbienen entlang eines Höhengradienten

Starke Umweltgradienten, wie sie an Berghängen auftreten, stellen für Arten eine Herausforderung dar. Versteckte Anpassungen oder Interaktionen wurden in diesem Zusammenhang selten berücksichtigt. Als Modellorganismen haben wir

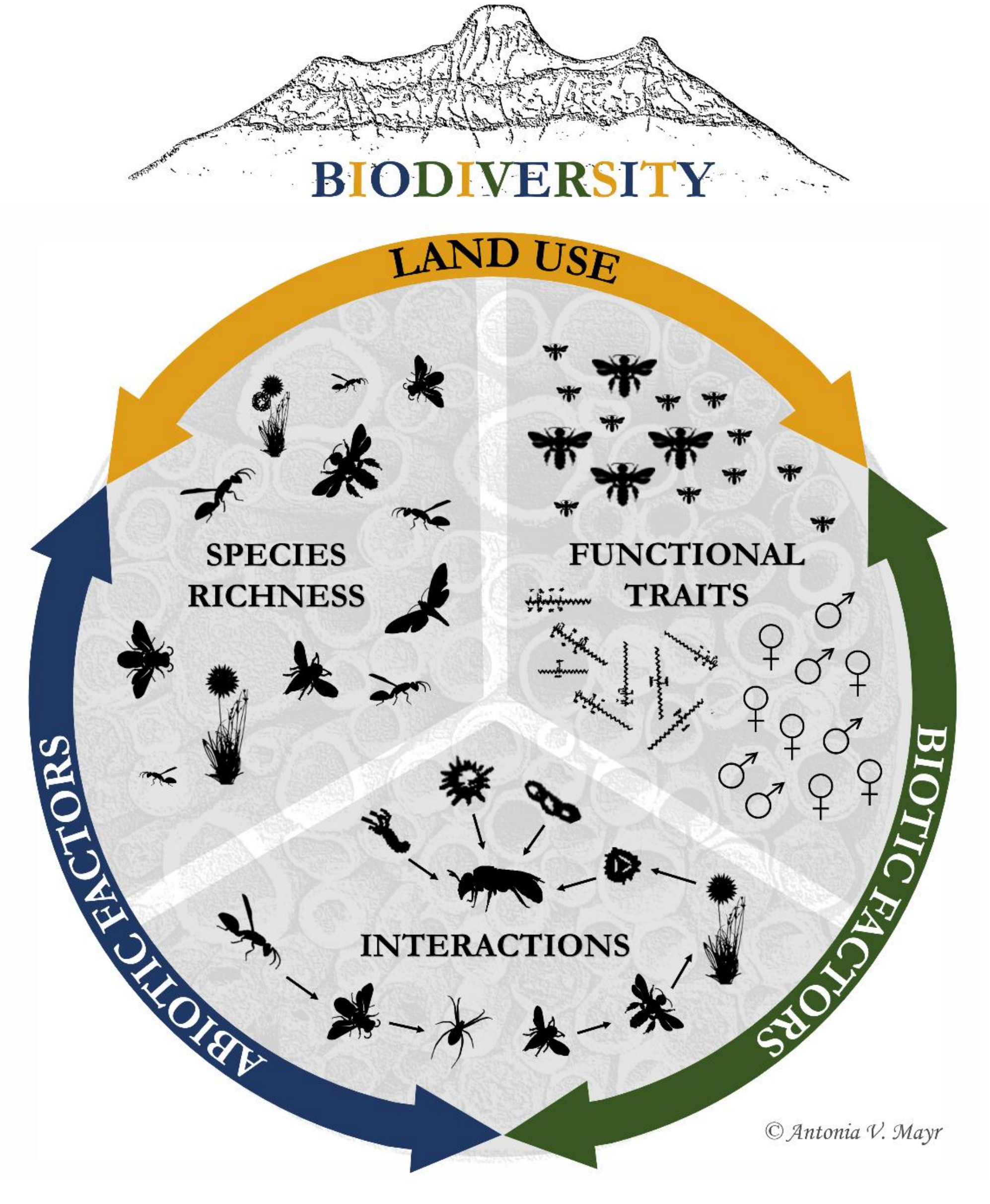
Bienen der Gattung *Lasioglossum* verwendet, da *Lasioglossum* die einzige Bienengattung ist, die über den gesamten Höhengradienten am Kilimandscharo weit verbreitet ist. Wir fragten, ob und wie (a) kutikuläre Kohlenwasserstoffe (CHC), die als Barriere gegen Austrocknung wirken, sich in ihrer Zusammensetzung und Kettenlänge entlang von Temperatur- und Feuchtigkeitsänderungen verändern; (b) *Lasioglossum* Bienen ihre Pollennahrung mit wechselnder Ressourcenverfügbarkeit ändern; (c) Änderungen von Darm-Mikrobiota mit Pollennahrung und Klimabedingungen und Änderungen von Oberflächen-Mikrobiota mit CHC und Klimabedingungen zusammen hängen, und ob die Veränderungen eher durch den Wechsel von *Lasioglossum* Arten entlang des Höhengradienten beeinflusst werden. Wir fanden physiologische Anpassungen an das Klima in CHC, sowie Veränderungen in der Zusammensetzung von Pollennahrung und Mikrobiota, die auch miteinander korrelierten. Diese Ergebnisse deuten darauf hin, dass komplexe Wechselwirkungen und Rückkopplungen zwischen abiotischen und biotischen Bedingungen die Artenzusammensetzung in einer Gemeinschaft bestimmen.

Kapitel 5 – Allgemeine Diskussion

Abiotische und biotische Faktoren förderten die Artenvielfalt, Eigenschaften und Wechselwirkungen von Arten und sie wirkten unterschiedlich, je nachdem, welche funktionelle Gruppe untersucht wurde und ob räumliche oder zeitliche Einheiten berücksichtigt worden sind. Es ist daher wahrscheinlich, dass im Lichte des globalen Wandels verschiedene Arten, Merkmale und Wechselwirkungen unterschiedlich betroffen sein werden. Darüber hinaus könnte eine zunehmende Landnutzungsintensität zusätzliche Auswirkungen oder Wechselwirkungen mit dem Klimawandel auf die Biodiversität haben, auch wenn die potenziellen Landnutzungseffekte am Kilimandscharo noch gering sind und bis jetzt die hohlraumnistenden Hautflüglern nicht beeinträchtigen. Weitere Studien sollten sich mit Nahrungsnetzwerken befassen, die empfindlichere Veränderungen aufzeigen könnten. Nisthilfen bieten dafür ein gutes Modellsystem, um die Auswirkungen des globalen Wandels auf mehreren trophischen Ebenen zu untersuchen, und können auch direkte Auswirkungen des Klimawandels auf ganze lebensgeschichtliche Merkmale aufzeigen, wenn sie unter verschiedenen mikroklimatischen Bedingungen etabliert werden. Die nicht einheitlichen Auswirkungen abiotischer und biotischer Bedingungen auf mehrere Aspekte der Biodiversität, die in dieser Studie gezeigt wurden, zeigen auch, dass die Untersuchung verschiedener Aspekte der Biodiversität ein umfassenderes Bild vermitteln kann als Einzelbetrachtungen.

Chapter 1

General Introduction


BIODIVERSITY
LAND USE
ABIOTIC FACTORS
BIOTIC FACTORS
SPECIES RICHNESS
FUNCTIONAL TRAITS
INTERACTIONS
© Antonia V. Mayr

Global change has effects across different aspects of biodiversity. Predicting biodiversity and ecosystem responses to global change is one of the greatest challenges for ecologists. Global mountain ecosystems offer centres not only for biodiversity hotspots, but also to study effects of environmental drivers on species communities. For this purpose, insects are an excellent group, because they are among the most species rich animal groups of the world, are highly threatened and fulfil important ecosystem services. Within the framework of the large-scale German-Tanzanian KiLi-project which studies the effects of global change on biodiversity and ecosystem processes on Mt. Kilimanjaro, I investigated the drivers of species richness at different trophic levels (Chapter 1), seasonal patterns of life-history traits at different elevational levels and at different trophic levels (Chapter 2), spatial variation of individual traits (Chapter 3) and drivers of species interactions in space (Chapter 1 and 3) and time (Chapter 2).

Wir leben in einem gefährlichen Zeitalter; der Mensch beherrscht die Natur bevor er gelernt hat sich selbst zu beherrschen. *Albert Schweitzer*

1.1 Global change: impact on biodiversity and ecosystem services

Global change, which includes climate change and the destruction of natural habitats, the overuse of natural resources, the introduction of alien species, pollution by synthetic pesticides and fertilisers and the intensification of land use, threatens biodiversity and biotic interactions on the one hand, and the stability of ecosystems and ecosystem processes on the other (Millennium Ecosystem Assessment, 2005; Sánchez-Bayo & Wyckhuys, 2019). Future predictions based on modelling, observations and experiments even show an increase in human-caused problems for biodiversity and related ecosystem functions (Newbold *et al.*, 2015; Pecl *et al.*, 2017). But evaluating how biodiversity, species interactions and related ecosystem functions respond to environmental changes is a major challenge in ecology (Tylianakis & Morris, 2017).

In the last century, biodiversity has been badly threatened by humankind more than ever before and extinction rates are about 1000 times higher than the background rate of extinctions (Millennium Ecosystem Assessment, 2005; Pimm *et al.*, 2014). Nonetheless, biodiversity is poorly investigated, with most species being undescribed (Pimm *et al.*, 2014). One of the major reasons for biodiversity loss is land use intensification and extension (Sánchez-Bayo & Wyckhuys, 2019). This is due to the hyper exponential growth of human populations and their associated growing need of resources (Millennium Ecosystem Assessment, 2005). Forty percent of the terrestrial surface of the earth is currently used for agriculture (Foley *et al.*, 2005). The recurrent destruction of habitats not only promotes climate change, but is also the main cause of biodiversity loss and the decline of correlated ecosystem services (Millennium Ecosystem Assessment, 2005; Bajželj *et al.*, 2014) with largely unknown consequences for ecosystems (Hooper *et al.*, 2005). How global warming and the ever increasing use of ecosystems interact with each other belongs to one of the largest uncertainties about predicting the consequences of global change (Sala *et al.*, 2000). The intensification and expansion of agriculture can have an impact on various aspects of biodiversity. On the one hand, it can influence the local species richness and composition of species communities (Wielgoss *et al.*, 2010; Rizali *et al.*, 2013). On the other hand, it can change population dynamics (Wang *et al.*, 2015) and the interactions between animals and plants, and animals and their respective antagonists, thereby also affecting ecosystem functions (Violle *et al.*, 2012).

Insects are good indicators for evaluating the consequences of global change. They are the most species-rich class of terrestrial animals. Insects alone are estimated at 5.5 million species (Stork, 2018), of ~1 million described and 2-11 million estimated animal species on earth (Chapman, 2009; Mora *et al.*, 2011; Costello *et al.*, 2013). They perform important ecosystem key functions, such as pollination or pest control. Therefore, recent insect declines are alarming and may lead to a worldwide extinction of 40 % of insect species of in the next decades, with Hymenoptera being within the most affected taxa in terrestrial ecosystems (Sánchez-Bayo & Wyckhuys, 2019). Even if there are enough moral and ethical reasons to justify their conservation (Kleijn *et al.*, 2015), the ecosystem services insects provide also have an important economic value. In the following, I give two examples of important ecosystem services with a high economic value: pollination and pest control.

The key function of pollinators for ecosystems is illustrated by the fact that 78 % to 94 % of plant species in temperate and tropical communities, respectively, and 70 % of cultivated plants worldwide are pollinated by flower-visiting animals, mainly insects and even more specifically, bees (Klein *et al.*, 2007; Garibaldi *et al.*, 2011; Ollerton *et al.*, 2011). They guarantee reproduction and genetic exchange and increase the yields of around two-thirds of all crop plants (Klein *et al.*, 2007). The economic value of pollination worldwide for direct food production is estimated at US$153 billion (Gallai *et al.*, 2009). But apart from their role in human nutrition, pollinators are also important for global health (Smith *et al.*, 2015). Regardless of their significance, pollinator diversity is threatened by a variety of anthropogenic causes. Wild pollinators such as bees, flies, beetles and butterflies, which are even more effective pollinators than honey bees (Garibaldi *et al.*, 2013), are declining and a decline of their dependent plants has already been observed as well (Biesmeijer *et al.*, 2006; Vanbergen & the Insect Pollinators Initiative, 2013). However, the degree to which the decline of pollinators will affect their services and whole ecosystems is still very speculative. Therefore, this remains a fundamental issue of environmental research. Nonetheless, there are other important functions provided by other insects (e.g. wasps, dung beetles), about which we know even less, one of them is pest control.

Even though the ecosystem function of pest control has received less attention than pollination (Jennings & Houseweart, 1984; Harris, 1994), it is an important ecosystem function associated with biodiversity (Ives *et al.*, 2000; Wilby & Thomas, 2002; Gurr *et al.*, 2003), offering the suppression of pests without the negative impacts of chemical pesticides (Östman *et al.*, 2003). In the US, the economic value of insect-mediated pest control by native predators is estimated at US$4.5 billion (Losey & Vaughan, 2006). Yet, natural enemies providing pest control are threatened by the simplification of landscapes by removal of natural habitats (Bianchi *et al.*, 2006; Tscharntke *et al.*, 2011), homogenisation of food production (Altieri, 1999; Rusch *et al.*, 2016) and pesticides, which may also affect pollinators (Brittain & Potts, 2011; Krauss *et al.*, 2011).

1.2 Spatial and temporal changes of biodiversity

Already since more than three centuries ago, patterns and drivers of biodiversity have fascinated scientists and have become object of research (Gaston, 2000; Brown, 2014; Fine, 2015). They discovered that species and their traits are not evenly distributed across the earth, but are spatially changing (Bergmann, 1847; Allen, 1877; Wallace, 1878; Dobzhansky, 1950; Lomolino *et al.*, 2006). The decline of species towards the poles (the latitudinal diversity gradient – LDG) is one of the greatest puzzles (Hillebrand, 2004; Mannion *et al.*, 2014; Pontarp *et al.*, 2018), that has led to a variety of theories, which incorporate historical, area, climatic and energetic aspects (Dobzhansky, 1950; Turner, 2004; McCain, 2007; Brown, 2014; Graham *et al.*, 2014). These theories can be further classified into three groups, depending on their emphasis on the type of drivers for the LDG: ecological limits, diversification rates and time for species accumulation, in which different processes such as selection, ecological drift, dispersal and speciation may be playing key roles

(Pontarp *et al.*, 2018). Up to now, though there is still no clear preferred theory for causing the LDG, because a mechanistic understanding on how organisms interact with the abiotic and biotic environment is still lacking (Pontarp *et al.*, 2018). Although the LDG is one of the most striking and well-studied biodiversity patterns, it is by far not the only one. It is also well known since the time of Humboldt, Darwin and Wallace that similar biodiversity gradients as from the equator to the poles can be found along elevational gradients, with decreasing species diversity towards mountain peaks (Lomolino, 2001).

However, populations and communities are naturally not only spatially variable, but also temporally dynamic (Cowles, 1899; Clements, 1936; Chesson & Huntly, 1989). Living conditions for animals change throughout the year with animals synchronising their activity to the availability of nutritious resources, low predatory and pathogen pressure and favourable climatic conditions (Tauber & Tauber, 1976; Wolda, 1988; Abrahamczyk *et al.*, 2011). Therefore, abundances, diversity and distributions of species can vary both between different years and within a year (Wolda, 1992). But, little is known about the mechanisms that shape temporal changes in species and especially temporal changes along elevational gradients are often ignored (Wardhaugh *et al.*, 2018).

For a deeper understanding of the effects of environmental changes on species and ecosystems, traits are usually more suitable than mere species. Studying traits permits that the biological properties which interact with the abiotic and biotic environment (Pontarp *et al.*, 2018) and the differences between species are taken into account (Villéger *et al.*, 2010), linking species with ecosystem functions. Therefore, environmental factors often target traits rather than species (Hodkinson, 2005; Violle *et al.*, 2012), because they are more sensitive to environmental changes and can thus be regarded as an early-warning system (Mouillot *et al.*, 2013). For this reason, functional traits are well suited for understanding the impacts of climate and land use changes (Hodkinson, 2005; Williams *et al.*, 2010; Violle *et al.*, 2012).

Patterns in species distributions along large environmental gradients are well studied (Gaston, 2000; Brown, 2014) and correlations between climatic factors, such as temperature and precipitation and species richness have been found (Hawkins *et al.*, 2003; Peters *et al.*, 2016b). However, species are surrounded by other species on which they may depend (e.g. plant-pollinator interactions), with whom they must share their resources (competition) or to which they are prey (e.g. host-parasitoid interactions). Therefore, these biotic interactions affect their distributions in complex ways (Bascompte, 2009; Tylianakis *et al.*, 2010), thereby stabilizing species communities (Bascompte & Jordano, 2007). On these grounds, it is very important to understand species interactions with respect to the evolution and preservation of biodiversity, and the functioning of species communities and ecosystems in space and time (Bascompte *et al.*, 2006; Thébault & Fontaine, 2010).

Research on latitudinal and elevation gradients can help to understand ecological mechanisms responsible for the distribution of species and composition of species communities (McCain & Grytnes, 2010). If we understand the distribution of species, predictions about the consequences of global change on species communities will be more feasible, and it will be easier to derive priorities for the protection of species and ecosystems for policy makers.

1.3 Tropical (mountain) ecosystems and elevational gradients

Tropical ecosystems cover only 40 % of the earth´s surface, but are home to the most diverse ecosystems in the world with accounting for more than 78 % of the world's biodiversity, where many previously undescribed species are discovered each year. In addition, they also host the largest proportion of human cultural diversity (85 % of all spoken languages) (Barlow *et al.*, 2016). In particular, tropical mountains are hotspots of biodiversity and endemism, due to their relative dense packing of different climate zones and ecosystems along elevational gradients (La Sorte & Jetz, 2010). They harbour not only half of the biodiversity hotspots worldwide (Körner, 2004), but also offer ecosystem services like fresh water to billions of people (Payne *et al.*, 2017). But tropical ecosystem functions are also indispensable for the global climate: 33 % of the global primary production takes place in humid tropical forests whereas 30 % takes place in in savannas. Similarly, a quarter of the world's terrestrial carbon is stored in humid tropical forests while 15 % is stored in savannas (Grace *et al.*, 2006; Bonan, 2008). Yet, tropical biodiversity is more at risk than temperate biodiversity (Dirzo *et al.*, 2014). Tropical ecosystems are rapidly changing at environmental, socio-economic and demographic levels. Therefore, losses of biodiversity are projected to be worse in economically poor countries, many of which are located in the tropics (Newbold *et al.*, 2015). Forty percent of the human population lives in tropical countries and is growing faster there than anywhere else (Edelman *et al.*, 2014; Barlow *et al.*, 2016). This leads to increasing land-use demands. In fact, some of the greatest land-use changes are already happening in the tropics, e.g. deforestation (Chapin III *et al.*, 2000; Keenan *et al.*, 2015). But the large land-use demands arise mainly from high demands of resources, which causes the expansion of agricultural land at the expense of natural habitats, the intensification of farming and increased pest load, and thereby also an increased use of pesticides (Bebber *et al.*, 2014; Lewis *et al.*, 2016). This might in turn affect animal pollination, on which food production largely depends. Developing countries are less buffered against the collapse of ecosystem services, so that these environmental impacts could also have a more dramatic impact on human well-being (Palma *et al.*, 2016). Tragically, many social and environmental problems in the tropics are caused by extra-tropical countries (Edelman *et al.*, 2014; Barlow *et al.*, 2016). On the top of this, besides of the negative impact of land use on tropical ecosystems, tropical countries will be disproportionally affected by climate change and will also be the first to experience it (Mahlstein *et al.*, 2011). Some of the warmest areas of the world are located in the tropics. Insects, which by far represent the most species rich group of terrestrial animals, and whose diversity is concentrated in the tropics, are particularly more sensitive to increases in temperature in the tropics than in temperate regions (Addo-Bediako *et al.*, 2000; Deutsch *et al.*, 2008). This, in addition, increases the risk of tropical species to extinctions (Thomas *et al.*, 2004).

In order to remain within their climatic tolerance, with climate change, species shift their distributions towards the poles and upwards along elevational gradients. This causes concomitant changes in species composition and structure, with so far unknown consequences for species interactions and ecosystem functions (e.g. plant-pollinator or host-parasitoid interactions) (Parmesan & Yohe, 2003; Williams *et al.*, 2003; Thomas *et al.*, 2004; Parmesan, 2006; Bartomeus *et al.*, 2011; Chen *et al.*, 2011; Hill *et al.*, 2011; Pecl *et al.*, 2017). Since tropical mountain species have often small geographical distributions and some species even occur only on one mountain, it appears more difficult for them than for temperate species to shift their distributional range, which could make them the most vulnerable to global change (Williams *et al.*, 2003; Thomas *et al.*, 2004; Colwell *et al.*, 2008; Wright *et al.*, 2009; Edelman *et al.*, 2014). Therefore, the investigation of species communities and their needs along elevation gradients is of great importance.

In the absence of historical data, elevational and land use gradients are used in space-for-time approaches to investigate the impact of global change on biodiversity and ecosystem services (Blois *et al.*, 2013). Species richness patterns along climate and land use gradients have received much attention in the ecological literature in recent years (Sousa *et al.*, 2006; McCain, 2007; McCain & Grytnes, 2010) and serve as model systems for global patterns. For example, the decrease in temperature towards the poles could be considered a possible driver for biodiversity along elevation gradients as well. Studies show that space-for-time approaches provide valuable data on predicted changes in species distribution (Lemoine *et al.*, 2007). Elevational gradients specifically, offer steep, small environmental gradients and ‘experiments by nature’ for the investigation of ecosystem functions and drivers of biodiversity (Körner, 2004; Payne *et al.*, 2017). Research on elevational gradients, especially in the species-rich tropics, has broadened our understanding of drivers for species distributions (McCain & Grytnes, 2010). However, it must be borne in mind that there are also significant differences between latitudinal and elevational gradients that make clear transferability impossible. Among these are, for example, increasing UV radiation, wind speed, thinner air and reduced O_2 and CO_2 levels (Hodkinson, 2005), reduction of area (Körner, 2007), stable day lengths throughout the year and a much faster decrease of temperature along elevational gradients than along latitudinal gradients, that makes upward shifts more likely than shifts towards the poles.

1.4 Study design

1.4.1 Study area - The vulnerability of Kilimanjaro´s ecosystems and the KiLi-Project

Mt. Kilimanjaro is a suitable system to study the combined effects of climate and land-use changes on species, species interactions and related ecosystem processes. Mt. Kilimanjaro has lost more than 80 % of its glaciers since 1912 with unknown consequences for the hydrosystem of the surrounding area (Thompson *et al.*, 2002). Thus, it has not only become an epitome for climate change, but it also covers a large elevational gradient since it is the highest free-standing mountain on earth with successional changing temperature and moist availability. Additionally, it is also shaped by a long history of changes in land use. Tanzania is one of the ten most populous countries in sub-Saharan Africa, with more than 70 % of the population inhabiting rural areas and mainly on subsistence farming (Kimaro & Hieronimo, 2014; GIZ, 2019). The population of Mt. Kilimanjaro is rapidly growing and almost three quarters of it depends on mountain ecosystem services (Sébastien, 2011; Misana, 2012). However, as in many other parts of the tropics (Perfecto *et al.*, 2009; Karp *et al.*, 2011), the sustainable small-holder agroforestry systems by the local tribe Chagga, who inhabit the area since more than 500 years (Odner, 2010) has been largely transformed by commercial projects in which monocultures with low biodiversity, such as coffee plantations dominate (Maghimbi, 2007). Land use at Mt. Kilimanjaro is mainly concentrated on elevations below the border of the Mount Kilimanjaro National Park, which was established in 1987. Below the national park border (~1800 m, Fig.1), natural habitats in the lowlands and submontane forest have been largely logged and land use is shaped by the long history of the Chagga people, who replaced lowland forests by diverse small-scale traditional agroforestry systems (i.e. Chagga homegardens). Besides these traditional Chagga homegardens, extensively-managed grasslands, coffee plantations, and human settlements characterise the landscape. But landscape changes are also happening in the lowlands. At the foothills which are the driest areas of the mountain (Appelhans *et al.*, 2016), natural, species-rich savannas already decreased by 85 % between 1961 and 2000, giving way to mainly maize, but also millet, sunflower and bean fields (Soini, 2005). In the interior of the national park, the ecosystems are affected by human disturbances such as illegal logging of valuable timber species or forest fires that have increased within the last decades, and have led to strong changes in forest structure and composition, or even replacement (Hemp, 2005). In the Kilimanjaro region, there are even indications of interactions between land use and climate change (Fairman *et al.*, 2011) that make the mountain an interesting study system, combining several contemporary challenges.

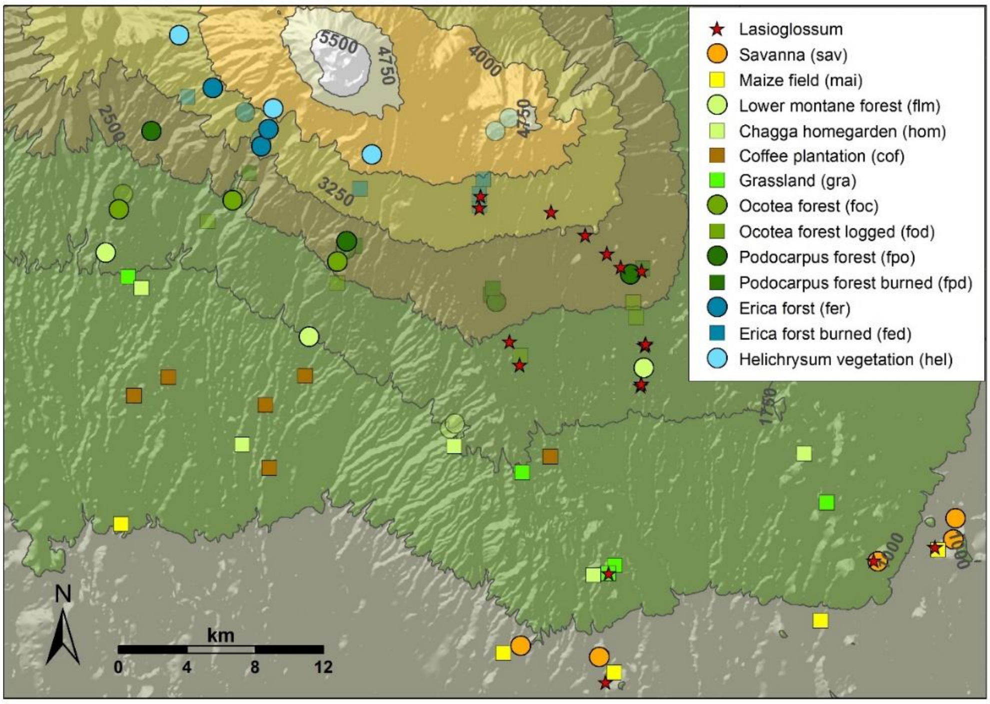


Fig. 1: Schematic representation of the experimental design of the KiLi project. Circles represent natural and squares disturbed habitats. Study sites with higher transparency were not included in the trap-nest study, which was not managable on all 66 study sites. Study sites with stars are study sites independent of the KiLi project and specifically chosen for the *Lasioglossum* study, which required exclusively natural and open habitat patches. Ecosystems from bottom to top: undisturbed ecosystems: savanna, lower montane forest, camphor forest (*Ocotea* forest), *Podocarpus* forest, heather forest (*Erica* forest), alpine strawflower vegetation (*Helichrysum*); disturbed ecosystems from bottom to top: maize field, Chagga Homegarden, coffee plantation, extensively-used grassland, camphor forest with tree felling, *Podocarpus* forest disturbed by fires, into which heather plants penetrate, heather plant forest disturbed by fires.

The present work is embedded in the large-scale KiLi project – "Kilimanjaro under global change. Linking biodiversity, biotic interactions and biogeochemical processes" (DFG Research Group 1246). This interdisciplinary project investigates the complex effects of climate and land use on biodiversity and ecosystem functions. The advantage of the project is that all sub-disciplines work on the same study sites, so that climate, biogeochemical processes such as water, carbon and nutrient cycles, plant, vertebrate (birds, mammals) and arthropod diversity and functionality can be related. The project started in 2010 and the present doctoral thesis was embedded in the second project phase, starting in 2013.

Field research was carried out from October 2013 until February 2016 on the southern slopes of Mt. Kilimanjaro (Tanzania, East Africa; 2°45’-3°25’S, 37°00’-37°43’E). Within the framework of the KiLi project 66 study sites (50 x 50 m) in natural and disturbed habitats were selected, covering an elevational gradient from 870 to 4550 m above sea level (Fig. 1). Mean annual temperature decreases from 25°C at the foothills to -8°C at the summit with an overall lapse rate of ~0.56°C per 100 m (Appelhans *et al.*, 2016). The precipitation pattern along the elevational gradient is humped-shaped with a mean annual rainfall of ~500-1000 mm at the foothills, approximately 2100-2700 mm in the forest belt at 2,200 m a.s.l. and less than 1000 mm in the Afroalpine zone (Appelhans *et al.*, 2016). The seasonal rainfall pattern is bimodal, with a long rainy season from March to May and a short rainy season from October to December and dry seasons in between (Yang *et al.*, 2015). The magnitude of seasonality in the rainfall pattern is much stronger at lower elevations outside the forest belt (Appelhans *et al.*, 2016). Temperatures are generally warmer between October and February (Appelhans *et al.*, 2016).

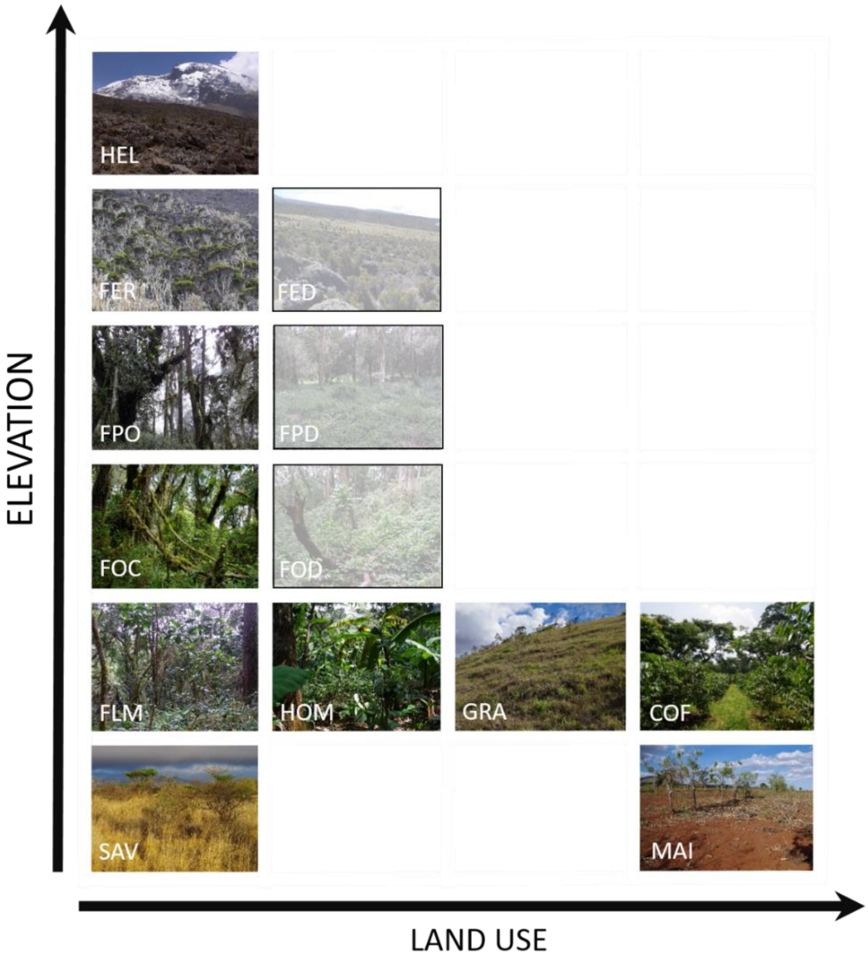


Fig. 1: Research sites along the elevational and land-use gradient of Mt. Kilimanjaro. HEL - alpine *Helichrysum* vegetation, FER – *Erica* forest, FED – disturbed *Erica* forest, FPO – *Podocarpus* forest, FPD – disturbed *Podocarpus* forest, FPO – *Ocotea* forest, FOD – disturbed *Ocotea* forest, FLM – lower montane forest, HOM – Chagga homegardens, GRA – grasslands. COF – coffee plantation, SAV – savanna, MAI – maize fields. Study sites with transparent habitat types were not used for the trap-nesting study, but for the *Lasioglossum* study. Pictures © A.V. Mayr

1.4.2 Study systems

1.4.2.1 Investigating cavity-nesting Hymenoptera with trap nests

Trap nests attract cavity-nesting Hymenoptera that naturally nest in hollow twigs, plant stems or holes in tree trunks of wood-boring insects (Krombein, 1967; Morato & Martins, 2006). These Hymenoptera provide an ideal model system for studying general questions in community ecology (Steffan-Dewenter, 2002; Steckel *et al.*, 2014). Moreover, they deepen our ecological understanding about multi-trophic interactions between bees, wasps and their antagonists, including important ecosystem functions like pollination and pest control (Losey & Vaughan, 2006; Tylianakis *et al.*, 2007; Veddeler *et al.*, 2010; Staab *et al.*, 2018). These hymenopterans further make observations of populations and about life-history traits over a long period possible (O'Neill & O'Neill, 2018), without having the problem of random observations. Moreover, the great demands of cavity-nesting Hymenoptera, with food resource, nesting sites and habitats providing nest-building materials in close proximity (Zurbuchen *et al.*, 2010a), make them good bio-indicators. Even though trap-nesting studies are increasing world-wide, there is still a large gap in Africa, with exceptions from South Africa (Staab *et al.*, 2018). In this thesis, I investigated trap-nesting communities at Mt. Kilimanjaro in Tanzania between December 2013 and February 2016. Specifically, I aimed to understand the effects of environmental factors on species, diversity, abundance, life-history traits, natural mortality and parasitism rate in space (Tscharntke *et al.*, 1998; Klein *et al.*, 2008), time (Loyola & Martins, 2006) and the interaction of both, space and time (Forrest & Thomson, 2011) (Chapter 2 and 3).

Fig. 2: Trap nests for cavity-nesting Hymenoptera. (a) Trap nests at ground level being approached by a probably *Megachile (Pseudomegachile) sinuata* (Friese) bee. (b), (c) Trap nests at canopy level. (d) *Megachile sp.* providing a brood cell with pollen and (e) cuckoo bee *Euaspis abdominalis* opening a hosts nest. (f) Opened *Hylaeus* nest with pupae in the first brood cell and following dead brood cells for unknown reason. Pictures © A.V. Mayr

Trap nests can be made out of various materials, but are commonly made out of reed internodes or wood cartridges (MacIvor, 2017). I used internodes of the common reed *Phragmites australis* (Tscharntke *et al.*, 1998) and *Fallopica japonica* (Klein *et al.*, 2004) for diameters from 1.5 mm to 25 mm. The internodes were deep-frozen twice for 2 weeks each at -20°C to avoid the introduction of foreign species. In total, I installed 328 trap nests at ground and canopy level for a total of 41 sites on natural and disturbed ecosystems from 871 m to 4,240 m a.s.l. and inspected them monthly. Because trap-nesting Hymenoptera did not occur within and above the forest belt above 1,800 m a.s.l., I limited the study to elevations up to 1,800 m a.s.l. after 9 months. For a detailed description, see the Method section in Chapter 2: For particularities working with trap nests in dry and hot tropical areas, see Chapter 5 (General Discussion).

1.4.2.2 Investigating invisible traits and interactions within *Lasioglossum*

Apart from morphological traits and host-parasitoid interactions, which, for instance, may be more obvious to perceive, hidden, invisible traits and interactions have been less often investigated, even though they might additionally contribute to the understanding of species distribution patterns and responses to environmental changes.

Although species interactions and networks have a long history in ecological research, the interest in studying interactions and networks did not abate but even increased with genetic methods (Bell *et al.*, 2016). Genetic methods, especially meta-barcoding, offer the possibility of conducting more comprehensive studies of food networks, enabling the analysis of high quantities of pollen (Sickel *et al.*, 2015). The identification of the collected pollen, instead of observational plant-pollinator networks, can reduce the sampling artefact and can make information about interactions between plants and pollinators more reliable (Dorado *et al.*, 2011). Pollen is not only the primary nutrient source for the larval development of bees, but its diversity plays an important role in bee immunity and colony health (Alaux *et al.*, 2010; Di Pasquale *et al.*, 2013). Therefore, it is expected that changes in plant communities along environmental gradients will strongly affect bee communities by affecting their health, development and the microbiota associated to the pollen.

On the other hand, insect health is strongly influenced by microbiota with regard to pathogens, dietary symbionts and facultative symbionts. In addition, microbiota are even considered to have contributed to the evolutionary success of insects (Moran *et al.*, 2008; Engel & Moran, 2013). Since domesticated and wild pollinators, together with their associated plants, are declining rapidly in many areas of the world (Potts *et al.*, 2010), it becomes imperative to study their microbial communities. But so far, the microbiota of only honeybees, bumble bees and a few solitary bee species have been studied. Moreover, the microbiome of social and solitary bees probably differs due to their different lifestyles (Keller et al., 2013). In contrast to social bees, the microbiome of solitary bees is mainly shaped by the environment in form of stored pollen and nest-building materials (Martinson *et al.*, 2012; Keller *et al.*, 2013; McFrederick & Rehan, 2016; McFrederick *et al.*, 2017) and is more diverse than the microbiome of domesticated honeybees and bumblebees (Gilliam, 1997), which share their microbes via social contact (Koch & Schmid-Hempel, 2011; Martinson *et al.*, 2012). Albeit microbiota fulfil important functions in general, the composition and specific function of the microbiome in the majority of wild bees has been rarely explored and is little known (Engel *et al.*, 2016) and even less is known how individual bee microbial associations shift due to changes in the environment.

Lastly, species interact also chemically with and adapt to their environment. The external hydrophobic layer on the insect cuticle is not only known for its function in communication between or within species (Howard & Blomquist, 2005; Leonhardt *et al.*, 2016), but plays an important role in preventing desiccation (Hadley, 1994; Gibbs & Rajpurohit, 2010) and has contributed to the success of insects. The cuticular hydrocarbons (CHC), that compose the external layer of the insect´s cuticle, reduce water loss and provide desiccation resistance (Gibbs, 2002a, 2002b). Both, the composition of CHC (Menzel *et al.*, 2017) and the chain length of the particular substances (Gibbs & Pomonis, 1995; Gibbs *et al.*, 1997; Rouault *et al.*, 2004; Rajpurohit *et al.*, 2017) determine the viscosity and thus the extent of desiccation resistance. However, reduced viscosity is accompanied by a lower fluidity which limits the property of CHC as a communication medium, thereby creating trade-offs (Menzel *et al.*, 2017), rising interesting questions about environmental limitations for species interactions which require communication abilities. Since changes in humidity and temperature occur along environmental gradients, the risk of desiccation also varies along with the gradient.

With these 'hidden' traits and interactions, I further wanted to understand so far neglected factors which might constrain bee interactions with the environment. *Lasioglossum* is the only bee genus at Mt. Kilimanjaro, which occurs from the savanna lowlands up to the alpine vegetation boundary, thereby offering an interesting model group. I caught *Lasioglossum* bees along the elevational gradient, immediately placed them on ice and froze them to -20°C for preserving them until their preparation in the lab. Cooling was necessary to avoid abrupt changes in temperature for sensitive microbiota when walking downhill to bring the samples to the field station. For details see Chapter 4. Later, they were dissected and the body parts split for different analyses: the head was used for the surface microbiome and the gut for the gut microbiome and pollen analysis; while the thorax and the abdomen for the CHC analysis.

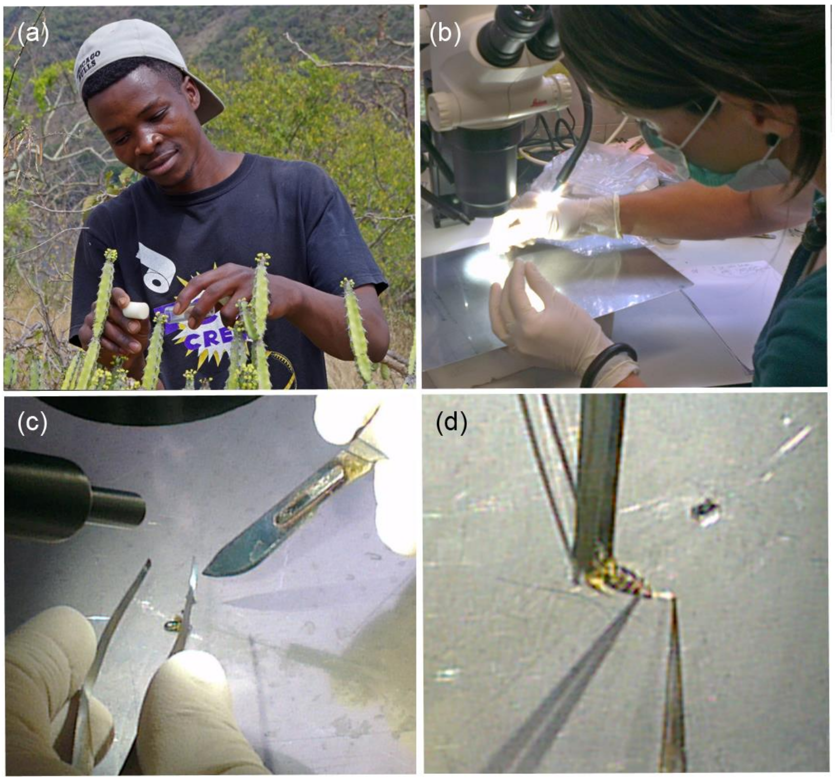


Fig. 3: *Lasioglossum* project. (a) Collection of female *Lasioglossum* bees from flowers. (b) Sterile dissection of bees in the lab under the binocular microscope. (c) Dissection in head for surface microbiome, (d) preparation of gut for gut microbiome and pollen analysis and thorax-abdomen for cuticular hydrocarbon analysis. Pictures © A.V. Mayr (a) and S. Hochrein (b)-(d)

1.4.3 Major research questions

The main aim of the thesis is to investigate the impact of environmental changes (i.e. abiotic, biotic and land use changes) on biodiversity. The thesis is divided in a trap-nesting study (Chapters 2-3) and a *Lasioglossum* study (Chapter 4) and covers different parts of the link between biodiversity and environment: species richness, functional traits, ecological rates and species interactions.

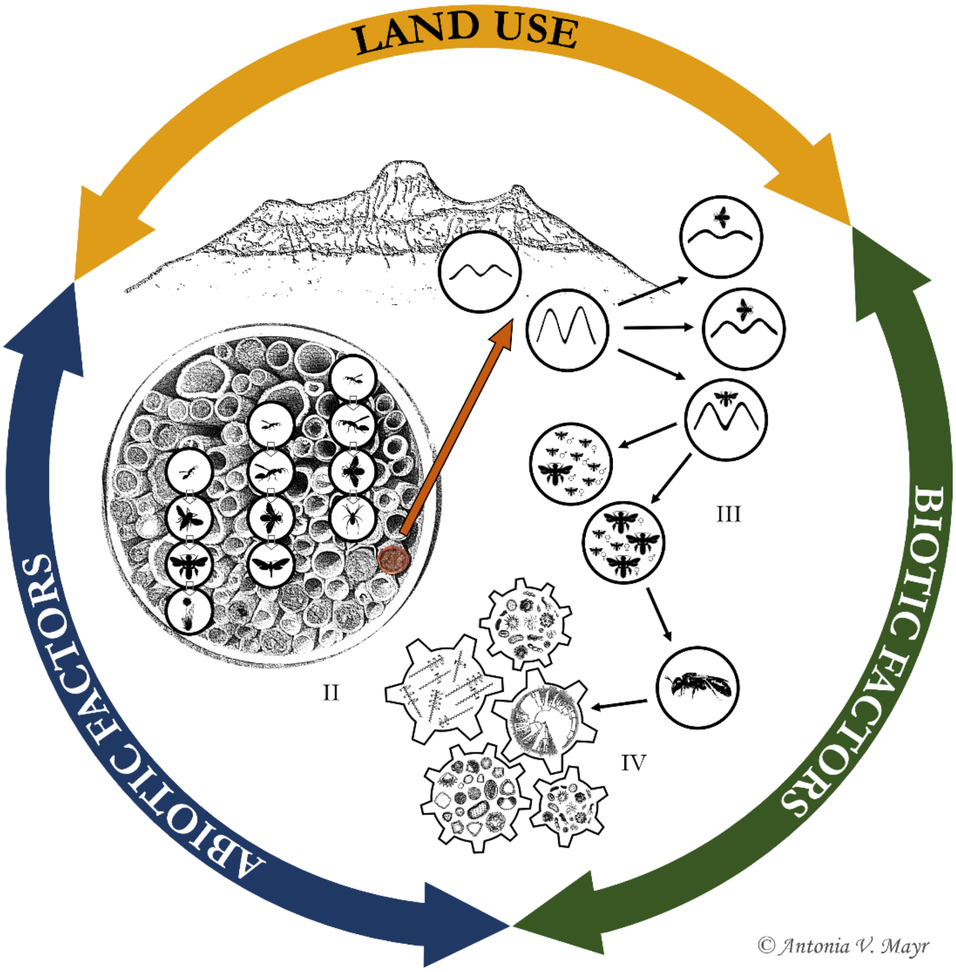


Fig. 4: Major research topics which are covered in Chapters 2-4. Chapter 2: Drivers of species richness at different trophic levels, 3: Seasonal patterns in nest-building activity, life-history traits and ecological rates in three different functional groups and at different elevations, 4: Changes in cuticular hydrocarbons, pollen composition and microbiomes of halictine bees along the elevational gradient of Mt. Kilimanjaro.

In this thesis, I systematically studied the effects of temperature, resource richness and abundance, parasitism and land use on different trophic levels in a trap-nesting community, because high trophic level react especially sensitive to environmental changes (Klein *et al.*, 2006),. I wanted to know if land use effects on species richness potentiate with trophic levels. Furthermore, I was interested in whether resources limit species richness increasingly with trophic level in resource-poor habitats, because of lower population sizes at low trophic levels (Steffan-Dewenter & Schiele, 2008). Additionally, I wanted to find out if top-down control becomes important in enhancing species richness, by reducing dominant species (Terborgh, 2015) when resources availability is not limiting. And lastly, I wanted to ascertain if temperature is driving species richness and parasitism rates across all trophic levels, by accelerating ecological and evolutionary rates in general (Peters *et al.*, 2016b). To my knowledge, this is one of the first studies which simultaneously compares drivers of species richness at different trophic levels (Chapter 2).

Environmental changes, especially in climate, do not only occur in space, but also in time and climate change is also predicted to change climate seasonality (Otte *et al.*, 2017). Therefore, I wanted to explore if insects show seasonal patterns at Mt. Kilimanjaro and if yes, compare how tightly different functional insect groups, such as pollinators and predators are linked to seasonal climate patterns with regard to life-history, development time, mortality rates and natural enemies. I was also interested to find out whether seasonality is stronger at lower elevations. There are only few studies that investigate seasonal patterns of insects at mountain ecosystems and at different elevations, and this is the first study about seasonality in trap-nesting Hymenoptera at different elevational levels (Chapter 3).

Steep environmental gradients imply strong challenges for living beings. Adaptation to these gradients may therefore not only depend on climate and macroscopic interactions, but also on interactions at a microscopic scale. In order to shed a little more light on species distributions, I studied multiple facets of bee ecology along the entire elevational gradient. *Lasioglossum* is the only bee genus which occurs along the entire elevational gradient and is therefore a suitable model group. Furthermore, it is extremely adaptable, especially in terms of social behaviour (Gibbs *et al.*, 2012).

In particular, I asked if the changes in plant diversity and composition is reflected in the bee´s nutrition. Since the bee´s gut microbiome which likely fulfils important functions for the bee, (e.g. pollen digestion (Vásquez & Olofsson, 2009)), is probably mainly shaped by pollen and other environmental factors in solitary bees (Keller *et al.*, 2013) that change along environmental gradients, it should change along the elevational gradient as well. Microbiota are also plenty on the bee cuticula. The insect cuticula evolved as a desiccation barrier and CHCs on the cuticula perform this function (Gibbs & Pomonis, 1995). With strong climatic changes, such as along the elevational gradient, I also expected changes in the CHCs composition and chain length of these molecules. But also microbiota on the cuticle might be related to the CHCs on which they grow. These factors have neither been studied in relation to each other, nor have been investigated along an elevational gradient (Chapter 4).

Chapter 2

Climate and food resources shape species richness and trophic interactions of cavity-nesting Hymenoptera

Temperature, food resources and top-down regulation by antagonists are considered as major drivers of insect diversity, but their relative importance is poorly understood. Here, we used cavity-nesting communities of bees, wasps and their antagonists to reveal the role of temperature, food resources, parasitism rate and land use as drivers of species richness at different trophic levels along a broad elevational gradient. We established trap nests on 25 study sites that were evenly distributed along an elevational gradient from 866 and 1,788 m a.s.l., including both natural and disturbed habitats. We quantified species richness and abundance of bees, wasps and antagonists, parasitism rates and flower or arthropod food resources. Data were analysed with generalised linear models within a multi-model inference framework. Elevational species richness patterns changed with trophic level from monotonically declining richness of bees to increasingly humped-shaped patterns for caterpillar-hunting wasps, spider-hunting wasps and antagonists. Parasitism rates were higher for wasps than bees and they declined with elevation. Temperature was the most important predictor of both bee and wasp host richness patterns. Antagonist richness patterns were also well predicted by temperature, but in contrast to host richness patterns also by resource abundance and diversity. Land use had no significant effects on bee and wasp communities. Our study underpins the importance of temperature as a main driver of diversity gradients in ectothermic organisms and reveals the increasingly important role of food resources at higher trophic levels. Higher parasitism rates at higher trophic levels and at higher temperatures indicated that the relative importance of bottom-up and top-down drivers of species richness change across trophic levels and may respond differently to future climate change.

This chapter is under peer review in Journal of Biogeography as: Mayr AV, Peters MK, Eardley CD, Renner ME, Röder J, Steffan-Dewenter I. Climate and food resources shape species richness and trophic interactions of cavity-nesting Hymenoptera

2.1 Introduction

A fundamental goal in ecology is to understand the origin and distribution of species. Patterns of biodiversity have been studied for more than three centuries, giving rise to a number of environmental predictors of broad-scale diversity gradients including climate, energy resources and top-down control (Gaston, 2000; Peters *et al.*, 2016b). However, the factors which determine the relative importance of one predictor over another remain scarcely understood.

In ecological theory, energy resources are often debated as important drivers of biodiversity (Hurlbert & Stegen, 2014). Ecosystems with a higher amount of available energy in the form of resources, maintain larger populations with lower extinction risks, and thus higher biodiversity (Srivastava & Lawton, 1998). Apart from the abundance of resources, the diversity of food resources may be closely linked to species diversity as a high diversity of food resources offers more distinct niches for concomitant speciation processes to take place (Forbes *et al.*, 2009). While the availability of resources has repeatedly been found to be positively correlated with species richness for endothermic taxa (Buckley *et al.*, 2012; Ferger *et al.*, 2014), its relevance for ectotherms is debated. In the latter, food resource availability is not proportional to food resource intake, since foraging is only possible within certain temperature thresholds (Willmer, 1983; Classen *et al.*, 2015).

Another approach assumes that biodiversity is primarily maintained by top-down control of natural antagonists, i.e. predators and parasitoids. The presence of antagonists restricts competition and therefore the dominance of a few species, thereby allowing coexistence (Terborgh, 2015). This hypothesis was proposed in 1960 (Hairston *et al.*, 1960). It was continued by Janzen and Connell in their theories on negative density dependence of mortality agents, which include parasites and pathogens (Connell, 1970; Janzen, 1970). Empirical evidence, however, is still mainly limited to plant communities and vertebrate top predators (Terborgh, 2015).

Ecologists have emphasised the notion of temperature as the dominating factor promoting species richness, in particular for ectothermic taxa (Buckley *et al.*, 2012). First, temperature is assumed to directly confine species richness via physiological constraints since only a few species can tolerate harsh climatic conditions at high elevations and latitudes (Currie, 1991). Second, the exploitation of resources has been argued to be temperature dependent (Brown, 2014). Third, temperature affects biodiversity indirectly through the acceleration of ecological processes such as biotic interactions (Brown, 2014) and fourth, temperature affects evolutionary processes through increasing rates of diversification (van Valen, 1973).

Human land use is regarded as an important driver of diversity loss and has reshaped mountain ecosystems. It reflects the anthropogenic impact through disturbance and conversion of natural habitats into agricultural habitats and subsequent land use intensification (Laurance *et al.*, 2014; Newbold *et al.*, 2015). Land use has been shown to have direct negative effects on the biodiversity of insect communities (Martinson & Fagan, 2014).

Past studies have given variable support to climate and resources as the major drivers of diversity. An important factor in this respect might be the trophic level of the studied organisms. Energy is lost through respiration and metabolic heat production from one trophic level to the other (Brown, 2014). Therefore, the availability of resources might have a stronger impact on higher trophic levels via cascading effects. Other cascading effects might be also driven by temperature, because temperature modulates the magnitude of the impact of higher trophic levels on lower ones (Rodríguez-Castañeda & Sykes, 2013). Moreover, higher trophic levels respond differently to land-use changes than lower trophic levels (Barnes *et al.*, 2017) and higher trophic levels seem to be most sensitive to anthropogenic disturbance (Ewers & Didham, 2006).

We installed trap nests along an elevational gradient at Mt. Kilimanjaro (Tanzania) in order to simultaneously investigate the combined effects of resources, top-down control by antagonists, climate and land use on species richness at different trophic level. Trap nests are a model system to study cavity-nesting insects. Remarkably, trap-nesting insect communities are good bioindicators for habitat quality and environmental change (Tscharntke *et al.*, 1998). They provide a valuable system to assess the influence of drivers of diversity on hymenopterans at different trophic levels. In addition to the diversity of individual trophic levels, resource diversity for the higher trophic levels can be quantified and top-down control measured; data which is often hard to quantify for other insect functional groups. Trap nesting bees and wasps provide pollen, caterpillars or spiders as food for their offspring and sustain a large number of different antagonist guilds, which predate or parasitize host larvae or use their food provisions (Wcislo & Cane, 1996). Tropical mountain ecosystems, such as Mt. Kilimanjaro, are ideal systems for studying biodiversity patterns under different environmental conditions, as they provide elevational gradients with changing climate, ecosystem structure, and availability of resources at small spatial scales (Sanders & Rahbek, 2012).

We made the following non-exclusive hypotheses:

1. Food resource quantity and diversity enhance species richness. Resource-poor habitats support a lower number of herbivores and therefore offer a lower amount of resources to sustain predator populations (Steffan-Dewenter & Schiele, 2008). Hence we assume that resources are more limited at higher trophic levels.

2. Top-down control is an important factor shaping species diversity by reducing the competitive advantage of potentially dominant species (Terborgh, 2015). Thus, we expect predation pressure, here parasitism rate, to enhance species richness if resource availability is not limiting species richness.

3. Higher temperatures accelerate ecological and evolutionary rates (Peters *et al.*, 2016b), facilitating the evolution and maintenance of a higher level of diversity in ectothermic organisms. Therefore, we expected positive correlations between temperature and species richness, as well as parasitism rates on all trophic levels in the trap-nesting community.

4. Land use has more severe negative effects on arthropod species richness at higher trophic levels (Attwood *et al.*, 2008). Therefore, we expect lepidopteran- and spider-hunting wasps and their respective antagonists to be more affected by land use than bees and their dependent antagonists.

2.2 Methods

2.2.1 Study area and design

The study was conducted at Mt. Kilimanjaro, the highest free-standing mountain of the world. It covers an elevation gradient from 700 m to 5,895 m a.s.l. and consequently a diverse number of ecosystem types in different elevational belts. Temperature decreases by ~0.56°C per 100 m; from 25°C in the lowlands to -8°C at the summit (Appelhans *et al.*, 2016). Mean annual precipitation exhibits a hump-shape elevational distribution and peaks in the forest belt at ~2,200 m a.s.l. (Appelhans *et al.*, 2016). Based on a pilot study, which showed that trap-nesting Hymenoptera were limited to elevations below 1,800 m a.s.l underneath the forest belt (see Appendix S1 in Supporting Information), we selected 25 study sites of 50 x 50 m in the lower elevations on the southern and south-eastern slopes of Mt. Kilimanjaro (3°10´-3°23´S, 37°14´-37°41´E). The study sites ranged from 866 to 1,788 m a.s.l. with a temperature gradient of 5.6°C (Fig. S2.1 in Appendix S2) and covered five ecosystem types with four to six replicates each, which constitute all major natural and disturbed ecosystem types of this elevational range. In the lowest elevations we included shrub savannahs as natural, and maize fields as disturbed ecosystems. In the mid elevations we compared three disturbed ecosystem types with different land-use intensities: diverse agroforestry systems of the local Chagga tribe (Chagga homegardens), extensively-managed grasslands and coffee plantations.

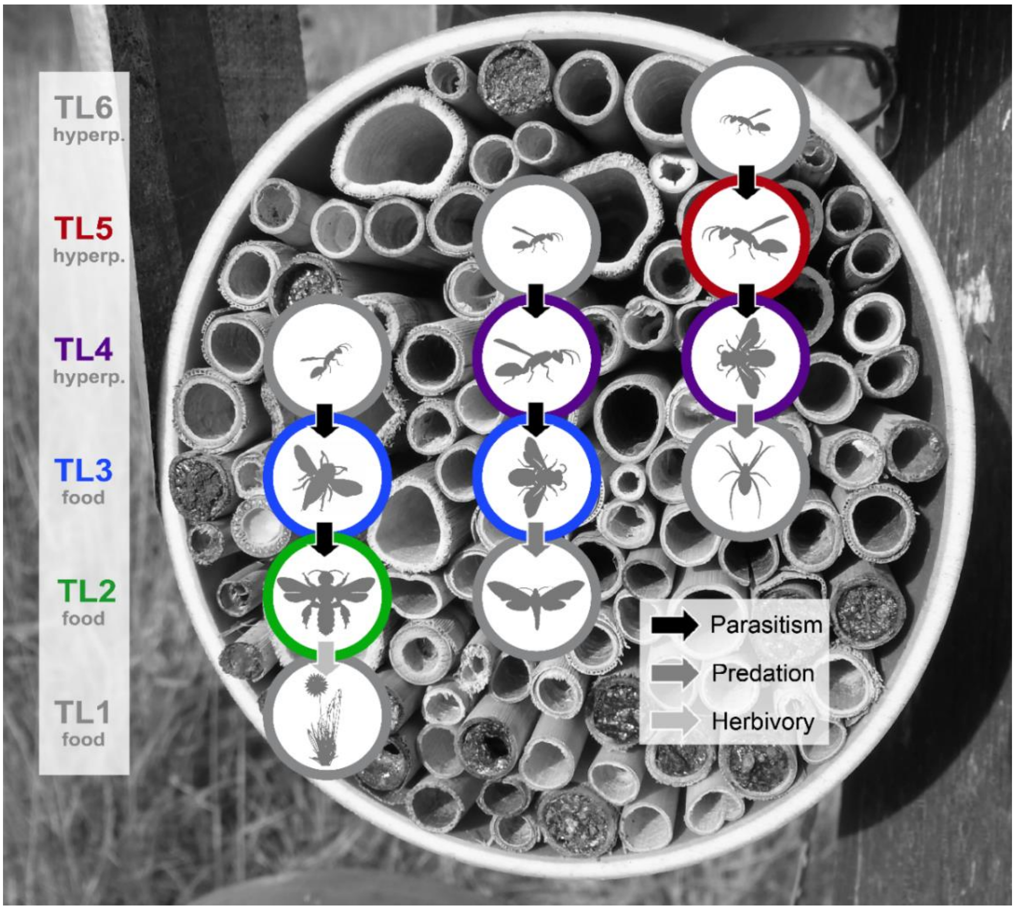


Fig. 1: Sketch showing the trap-nesting community at Mt. Kilimanjaro. Three different functional groups of hosts: pollinators (Apidae), caterpillar-hunting predators (Eumeninae) and spider-hunting predators (Pompilidae, Sphecidae and Crabronidae) with their antagonists and food resources are displayed. Parasitism is here also used for parasitoidism, which was the case for most parasitised brood cells. It was counted as all occurrences in which the host has been killed. Arrows indicate the top-down effect. Colours indicate the trophic level affiliation. Functional groups with a colourful circle display the groups of which we analysed species richness patterns. TL: trophic level.

The species richness of stem-nesting bees, eumenid, sphecid and pompilid wasps and their antagonists was assessed with trap nests (Fig. 1, Fig. S2.2d in Appendix S2). In total, we installed 208 trap nests at ground and canopy level (eight traps per site, arranged in four paired trap nests) (Fig. S2.2a,b,c and text in Appendix S2). Trap nests were operated for 15 months and checked monthly for new occupants. Internodes filled with nests were replaced by new internodes. All collected internodes filled with nests were taken out of the trap nests, closed with metal nets and reared on the study sites in hatching boxes (Fig. S2.2a in Appendix S2), so that the climatic conditions were natural during the whole development of the larvae. After hatching, nests were cut open in the laboratory in order to count the number of brood cells and measure parasitism rate (Fig. S2.2e,f in Appendix S2). All specimens were identified to the level of morphospecies or species whenever possible. We used additional taxonomic literature for the closer identification of wasp clades and bee species (identification keys for bees Tab.S3 in Appendix S3). Hosts and antagonists were identified to different taxonomic depth. Whereas the host guilds were identified to morphospecies level, some antagonist groups, like the Chrysididae could not be separated further.

2.2.2 Species richness of trap-nesting bees, wasps and their antagonists and parasitism rate

We assessed species richness of functional groups of different trophic levels: herbivores, i.e., pollen and nectar-feeders (bees: Apidae), predatory wasps (caterpillar-hunting: Eumeninae and spider-hunting wasps: Pompilidae, Sphecidae, Crabronidae) and their respective antagonists and second-order antagonists (hyperparasitoids) (Fig. 1). Species richness was calculated as the total number of species observed per study site. As the sampling of insects is rarely complete and differences in sampling completeness can lead to biases, we estimated the asymptotic species richness with the sample-based estimator Chao (Chao, 1984, 1987), using the function 'ChaoRichness' in the *iNEXT* package in R. Chao1-estimated species richness and observed species richness correlated strongly in all host groups (Pearson correlation coefficient between r = 0.90 and r = 1.00). We were able to collect 89-100% of the estimated species of local trap-nesting communities. The proportion of observed to estimated species richness showed no correlation pattern with elevation, so we can assume that the sampling was equally comprehensive at all elevations. Therefore, we used the observed species richness instead of the estimated species richness in our models. Host species with low abundances and species richness, such as aphid-hunting wasps, cicada-hunting wasps and orthopteran-hunting wasps, which were also found in trap-nests, were excluded from the analysis.

2.2.3 Temperature, land use, resource data and parasitism rate

Temperature was recorded with temperature sensors for 23 study sites in 5 minute intervals between 2011 and 2014 and the mean annual temperature (T) was calculated per study site as the average of temperature values (Appelhans *et al.*, 2016). For two study sites, where data loggers were lost, we used a co-kriging approach to estimate missing data (Appelhans *et al.*, 2016). We used a composite land-use index (LUI) to quantify the anthropogenic impact on ecosystems. The LUI is described in (Classen *et al.*, 2015) and is based on standardised measurements of land use at both the site (annual removal of plant biomass, agricultural inputs to the ecosystem, the vegetation structure in comparison to the natural habitats) and landscape level (proportion of agricultural land in the surrounding landscape of the study sites). The trends of temperature and land use along the elevational gradient are shown in Appendix S4. Resources for each functional group, i.e., flowers, moths, spiders, bee and wasp hosts, have been recorded separately, and we assessed resource quantity as well as resource diversity (Appendix S5). The parasitism rate was calculated as the number of brood cells in which the host had been killed in relation to the total number of brood cells of the respective host group. The hyperparasitism rate, used in the models as parasitism rate of the antagonists, was calculated as the number of brood cells in which the antagonists had been killed by second-order antagonists in relation to the number of parasitised brood cells.

2.2.4 Statistical analysis

We used generalised additive models (gam) as implemented in the R package *mgcv* to calculate trends in species richness of bees, caterpillar-hunting and spider-hunting wasps and their respective antagonists along the elevational gradient. In case of species richness as the response term, the data family was set to ‘poisson’ or ‘quasipoisson’ (in case of overdispersion) to account for the properties of count data. In gam models we set the basis dimension of the smoothing term k to four to avoid over-parameterisation of trend functions. Trend lines derived with gams were only plotted if the significance level of the elevation term was $p < 0.05$. In case of parasitism rates, the data family was set to ‘binomial’ and the trends in parasitism rates were calculated with a logit link-function.

We hypothesised that the species richness of a group of trap-nesting Hymenoptera is driven by temperature, land use, resource quantity and diversity, and predation pressure in the form of parasitism. We used generalised linear models (glm) with a 'poisson' or 'quasipoisson' distribution, respectively, to test the combined effects of the above-mentioned explanatory variables on species richness for each functional group (Appendix S6).

In order to test whether the magnitude of identified drivers of species richness was linked to the trophic levels, we also calculated a linear mixed effect model (lme) with study site as random factor and tested for possible interactions between the explanatory variables and the trophic level. Previously, species richness and resource richness and abundance was z-transformed per functional group:

$$z = \frac{\chi - \mu}{\sigma}$$

With: χ: raw score, μ: mean score of the functional group, σ: standard deviation of the functional group.

We used the 'dredge' function of the *MuMIn* package (Bartón, 2018) in R to evaluate the support for the full model and all nested models. Models were ranked according to their Akaike information criterion corrected for small sample sizes (Burnham & Anderson, 2004) and delta distances to the next best model were calculated. In cases of quasi models, we calculated a QAICc instead of the normal AICc (Bolker, 2017). All best supported models up to ΔAICc < 3 or ΔQAICc < 3 respectively, are given in the Supplementary Information. Before the analyses all explanatory variables were standardised by z-transformation, using the 'scale' function in R in order to facilitate the comparability of their effect strength.

Due to the fact that the parasitism rate could not be calculated for sites where the respective host guild did not exist and resource data for spider-hunting wasps was not available for all study sites, some models could not be evaluated with data from all study sites. Therefore, we recalculated the models without parasitism rate and in the last step without resource availability in order to include all study sites and therefore enhance the statistical power of the models.

For each host guild separately, we further tested with generalised linear models (glm) with (quasi)binomial distribution whether parasitism rates were affected by temperature and land use.

2.3 Results

2.3.1 Elevational patterns of species richness and parasitism rate

In total, we found 4,051 nests of stem-nesting bees and wasps, containing 14,937 brood cells of 81 morphospecies of hosts (38 bee and 43 wasp morphospecies) and 49 morphospecies of antagonists (Tab. S7.1, S7.2 in Appendix S7). Trap-nesting Hymenoptera exponentially declined with elevation and were generally limited to elevations below 1,800 m a.s.l. (Fig. S1). Species richness patterns of trap-nesting hosts differed between bees, caterpillar-hunting wasps and spider-hunting wasps and their respective antagonists. While bees and their antagonists, as well as the antagonists of the caterpillar-hunting wasps declined monotonically (Fig. 2a,b), caterpillar- and spider-hunting wasps and the antagonists of the spider-hunting wasps showed an increasingly humped-shaped elevational distribution with increasing trophic level (Fig. 2b,c).

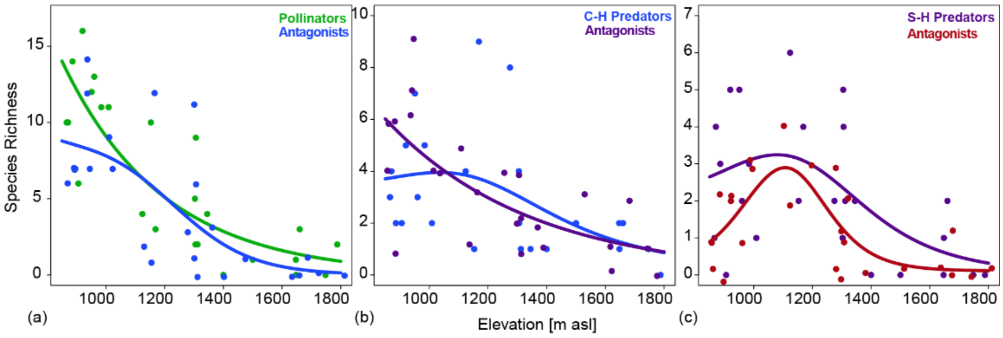


Fig. 2: Patterns of elevational species richness of three different trap nesting host guilds and their respective antagonists at Mt. Kilimanjaro. Generalised additive models were used to estimate trends of elevational richness (Poisson family, basis dimension (k) = 4). Species richness significantly changed for all insect groups with elevation (a: pollinators: explained deviance (ED) = 68.1 %, $p < 0.001$; antagonists of bees: ED = 57.9 %, $p < 0.001$; b: caterpillar-hunting wasps: ED = 31.5 %, $p = 0.01$; antagonists of caterpillar-hunting wasps: ED = 48.4 %, $p < 0.001$; c: spider-hunting wasps: ED = 34.5 %, $p = 0.01$; antagonists of spider hunting-wasps: ED = 47.4 %, $p = 0.01$). Colours indicate trophic level affiliations.

The parasitism rate differed between the different trophic levels and almost doubled from one level to another from 12 % in bee nests to 24 % in caterpillar-hunting wasp and 45 % in spider-hunting wasp nests. The hyperparasitism rate also increased with increasing trophic level (Fig. 3a). The parasitism rate decreased with elevation across all host groups, as did the ratio of antagonist to host species (Fig. 3b,c), but not the hyperparasitism rate (Fig. S8.1 in Appendix S8). The parasitism rate itself was positively correlated with temperature in case of the caterpillar-hunting predators and negatively correlated with land use in case of the pollinators (Tab. 1, see Appendix S8 Tab. S8.2 for competitive models).

Tab. 1: Best models explaining the parasitism rate at different trophic levels, derived by QAICc values.

Parasitism rate	**T**	**LUI**	**QAICc**	**Weight**	**ED**	**# Sites**
Pollinator		-0.40	22.2	0.49	0.27	22
Caterpillar-hunting predator	0.47		40.9	0.67	0.25	24
Spider-hunting predator			31.9	0.51	0	19

Standardised explanatory variables from best-fit models. Colours indicate positive significant values (blue) and negative significant values (red) from GLM models. T: mean annual temperature, LUI: land use index, ED: explained deviance, # Sites: number of sites taken into account for the model.

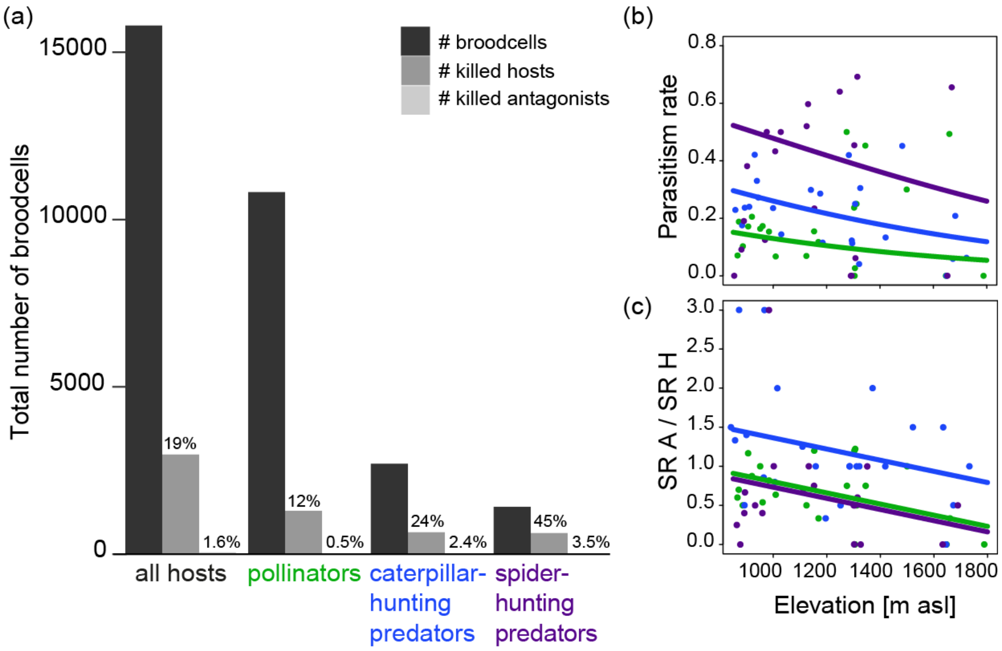


Fig. 3: Parasitism of trap nests at Mt. Kilimanjaro. (a) Parasitism and hyperparasitism rates change among functional groups. The barplots display the total number of brood cells (black), the number of brood cells in which the host was killed by the antagonist (grey, deadly parasitism also indicated above the bars as the percentage of the total number of brood cells in which the host was killed), and the number of brood cells in which the antagonist has been killed (light grey, deadly hyperparasitism also indicated above the bars as the percentage of the total number of parasitised brood cells). (b) Parasitism rates of functional groups vary along the elevational gradient (dots). The mean parasitism rate correlated significantly with elevation (GLM with quasibinomial data family, $p_{elevation} = 0.045$, lines). (c) The number of antagonist species in relation to the number of host species correlated significantly with elevation (LM $p_{elevation} = 0.013$, lines). SR A: Species richness of antagonists; SR H: Species richness of hosts. Colours indicate the trophic level affiliation.

2.3.2 Drivers of species richness

While we found no support for an effect of land use and top-down control (parasitism), both, mean annual temperature and resource quantity and diversity were important predictors of the diversity of trap-nesting host and antagonist communities (Tab. 2). The strongest positive effect of temperature was found on bee richness (ED = 0.59, $p_{temperature} < 0.001$). Caterpillar-hunting wasps and their antagonists showed a less significant correlation with temperature, whereas richness patterns of spider-hunting wasps and their antagonists were not explained by temperature (Tab. 2). Resource variables were not supported as predictors of host species richness, but their importance increased at higher trophic levels (Tab. 2). The species richness of antagonists of bees was best explained by both resource abundance and richness (ED = 0.76, $p_{temperature} = 0.053$, $p_{RES\text{-}AB} < 0.01$, $p_{RES\text{-}RI} < 0.05$). While the species richness of antagonists of caterpillar-hunting predators was predicted by resource abundance (ED = 0.62, $p_{temperature} < 0.01$, $p_{RES\text{-}AB} < 0.001$), the effects of resource richness were of higher relevance for the antagonists of spider-hunting predators (ED = 0.40, $p_{RES\text{-}RI} < 0.001$). The best models predicting species richness of hosts and their antagonists did not include land use and parasitism rate (Tab. 2).

Tab. 2: Best models explaining species richness of hosts and antagonists derived by AICc/QAICc-based model selection.

Guild	**Functional group**	**TL**	**T**	**LUI**	**RES-AB**	**RES-RI**	**ED**	**# Sites**
Host	Pollinator	2	0.77				0.59	25
Host	Caterpillar-hunting predator	3	0.30				0.14	25
Host	Spider-hunting predator	4					0.00	14
Antagonist	Pollinator	3	0.48		0.34	0.45	0.76	25
Antagonist	Caterpillar-hunting predator	4	0.43		0.31		0.62	25
Antagonist	Spider-hunting predator	5				0.69	0.40	25

Standardised parameter estimates from best-fit models. Blue indicates significant positive correlations as calculated with GLM models (no negative correlations were found). TL: trophic level, T: mean annual temperature, LUI: land use index, RES-AB: abundance of resources and RES-RI: total number of observed species/ morphospecies of resources, ED: explained deviance, # Sites: number of sites taken into account for the model.

The competitive models, differing only partially from the best model and the reduced and full models, and showing different subsets of the dataset, are presented in Appendix S9 (Tab. S9.1 in Appendix S9). Testing for possible interactions between the identified drivers of species richness and trophic levels revealed that the interactions between trophic level and resource abundance, as well as trophic level and temperature, were significant (Tab. S9.2 in Appendix S9).

2.4 Discussion

In this study elevational species richness patterns changed with trophic level from monotonically declining richness of bees to increasingly humped-shaped patterns for caterpillar-hunting wasps, spider-hunting wasps and antagonists. Furthermore, parasitism rates and antagonist-host ratios decreased with elevation and parasitism rates increased with trophic level. Our data indicate that temperature is a dominant factor in shaping diversity patterns with more intense trophic interactions in warmer climates. By systematically analysing different trophic levels, we found that the availability of resources gained more weight as a driver of species richness in antagonist groups than in bee and wasp hosts. Thus, our study provides novel insights into the relative importance of temperature, resources, trophic level and biotic interactions as drivers of elevational diversity patterns for hymenopteran taxa with broad functional relevance.

2.4.1 Decline of species richness along the elevational gradient

In total, we found a highly diverse stem-nesting insect fauna with 81 host and 49 antagonist species, in comparison to other trap-nest studies from the tropics with 5-8 bee and 7-15 wasp species (Klein *et al.*, 2006; Tylianakis *et al.*, 2007; Stangler *et al.*, 2015; Perillo *et al.*, 2017). That may be the case because the other studies did not investigate along an extensive climatic gradient as on Mt. Kilimanjaro, with the exception of (Perillo *et al.*, 2017). The decline of bee species richness at higher elevations has already been shown for Mt. Kilimanjaro (Classen *et al.*, 2015), and in other parts of the world (Hoiss *et al.*, 2012; Perillo *et al.*, 2017), whereas studies for wasp taxa are mainly lacking (Corcos *et al.*, 2018). We found a humped-shaped decline of both caterpillar-hunting and spider-hunting wasp species richness, with a peak in species richness at intermediate elevations. Depending on the taxonomic group of wasps, other studies either found a distinct decline of species richness or a mid-elevation pattern for wasps, but ultimately a decline in species richness at highest elevations (Kumar *et al.*, 2009; Perillo *et al.*, 2017). For the species richness of antagonists we found that the higher the trophic level of antagonist, the more the pattern shifts from a monotonous decline of species richness to a mid-elevation peak. Several other studies evaluated species richness of antagonists along elevational gradients and found variable patterns (Janzen *et al.*, 1976; Noyes, 1989; van Noort, 2004), but a systematic analysis of different trophic levels and the underlying drivers of richness patterns is currently lacking.

2.4.2 Temperature drives species richness across trophic levels

In our study, temperature was the most important and only common driver of species richness at all trophic levels. This result confirmed our third hypothesis that species richness at all trophic levels is correlated with temperature and is in line with the fact that all organisms are dependent on temperature (Brown *et al.*, 2004). As we were able to separate effects of resources and top-down control from temperature effects, our results suggest that ecological mechanisms such as temperature-mediated resource exploitation or evolutionary processes such as faster speciation or lower extinctions rates are the underlying temperature-mediated causes of diversity patterns (Buckley *et al.*, 2012; Classen *et al.*, 2015). We are aware that temperature is not independent of elevation and thus co-varying environmental factors, such as precipitation, atmospheric pressure, solar radiation and land area might also influence species richness (Perillo *et al.*, 2017). However, both ecological theory and empirical findings strongly support the importance of temperature instead of other environmental factors (Peters *et al.*, 2016b). Further we also found higher parasitism rates and antagonist-host species richness ratios at lower elevations, indicating temperature-mediated interaction strength as a further potential mechanism that allows the coexistence of more species in warmer climates (Terborgh, 2015; Peters *et al.*, 2016b; LaManna *et al.*, 2017).

2.4.3 Resources become increasingly important for antagonists

In addition to temperature, resource availability played a major role in explaining the species richness of antagonists. This is consistent with our first hypothesis that higher trophic levels are more limited by resources than lower trophic levels. Antagonists depend on their hosts, which have to cope with the spatially and temporally limited food resources for their offspring (Godfray, 1994). Therefore, it is very likely that there is a strong selection pressure to use their host resources very efficiently (Sanders *et al.*, 2016). We also hypothesised that the importance of resources for species richness increases with higher trophic levels of host taxa, but we found no correlation between resource availability and diversity of bees and wasp host guilds. Flower resources were repeatedly recorded in detail during the whole study phase whereas only coarse records for moths and spiders were available. Therefore, the relevance of resources for the species richness of predatory wasps might be stronger in reality. Nevertheless, the results confirm an increasing importance of resource availability for antagonists compared to host taxa. Resources are not only food resources, but also the availability of nesting sites and nest-building materials, which differ for trap-nesting bees and wasps and cover a variable range from clay, chalk, small stones, to resins, bark pieces, leaves and plant hair. Flying insects can gather these resources from the surroundings, and woody structures were always within a distance of less than 200 m from the study sites. Therefore, we do not assume that the lack of other resources could have been a limiting factor for species richness in our study.

2.4.4 Top-down control of species richness

As mentioned above, our data indicated that more intense trophic interactions occurred in ecosystems with higher temperatures, as found in (Forrest & Chisholm, 2017), supporting the notion that trophic interactions are sensitive to temperature changes (Deutsch *et al.*, 2008). However, our analyses provided no direct support for top-down control as a mechanism for the maintenance of biodiversity, as the parasitism rate was not a significant explanatory factor in any model with temperature and resources as additional factors. Thus, parasitism rate or hyperparasitism rate as a top-down effect did neither affect the species richness of hosts nor antagonists, contrasting to our second hypothesis. There are several possible explanations for the absent effect of predation pressure on species richness: First, top-down control might not be a relevant driver of diversity patterns in our study system. Second, the inclusion of temperature in the models might mask impacts of top-down control due to higher strength of trophic interactions in warmer climates. Third, other top-down factors could be important as well, such as the predation pressure from birds or insectivorous mammals, which we did not include in our evaluation. Fourth, resources might be more limiting than predation-pressure in our system as resource availability was a very important explanatory variable for the species richness of antagonists. In this case, one could assume that the effect of resource limitation on species richness was stronger than the effect of predation pressure on species richness. Since there are hardly any arthropod studies on this topic, more studies are necessary to disentangle the above-mentioned mechanisms, also testing more specifically for conspecific density-dependent abundance regulation (Forrest & Chisholm, 2017; LaManna *et al.*, 2017).

2.4.5 Limited effects of land use on species richness

We found no significant effects of land use on species richness of bees, wasps and their antagonists, in contrast to our fourth hypothesis. Previous studies in tropical agroecosystems also found no negative influence of land use on the species richness of trap-nesting Hymenoptera (Klein *et al.*, 2002; Tylianakis *et al.*, 2006). Nevertheless, there are other tropical studies which found significant negative effects of land use on the species richness of insects (Perry *et al.*, 2016). In a study with a multi-taxon approach, ranging from microbes to birds, land use had a negative effect on species richness, with increasing magnitude at higher trophic levels (Barnes *et al.*, 2017). At Mt. Kilimanjaro, land use is still moderate with manual tillage and sustainable subsistence agriculture of the local Chagga people (Fernandes *et al.*, 1984), forming a mosaic-like landscape of Chagga agroforestry systems, species-rich, extensively-used grasslands and maize fields with many flowering herbs, embedded in natural savannah areas. The distance to the nearest woody habitat has been found to have negative impacts on the number of trap-nesting species (Klein *et al.*, 2006), but it was less than 200 m for all our study sites. However, ongoing land-use change at Mt. Kilimanjaro, involving significant losses of savannah areas, replacement of Chagga agroforestry systems by coffee monocultures and agricultural intensification could become a threat to biodiversity and ecosystem services provided by bees and wasps in this area as well (Hemp, 2006b; Classen *et al.*, 2014).

2.5 Conclusions

In our study, elevational species richness patterns changed with trophic level and were best explained by temperature for host taxa, whereas antagonists were also limited by resources. Climate change driven increases of temperature will therefore affect bees, wasps and their antagonists differently. This might lead to differences in range shifts, changing the composition of communities. Importantly, changes in host species richness patterns will have impacts on whole food webs and might affect the extremely dependent antagonists most severely. Upper thermal limits will first be reached in the most diverse savannah habitats (Barlow *et al.*, 2018), leading to species losses in the lower elevations. Furthermore, future risks of land use might be reinforced by combined effects of habitat fragmentation and climate change. All in all, systematic studies on insect communities at different trophic levels along environmental gradients are still rare and provide, combined with manipulative experiments, a strong model system to better understand environmental factors and biotic interactions as drivers of species richness patterns.

2.6 Appendix

Appendix S1: Nest-building activity in the pilot study

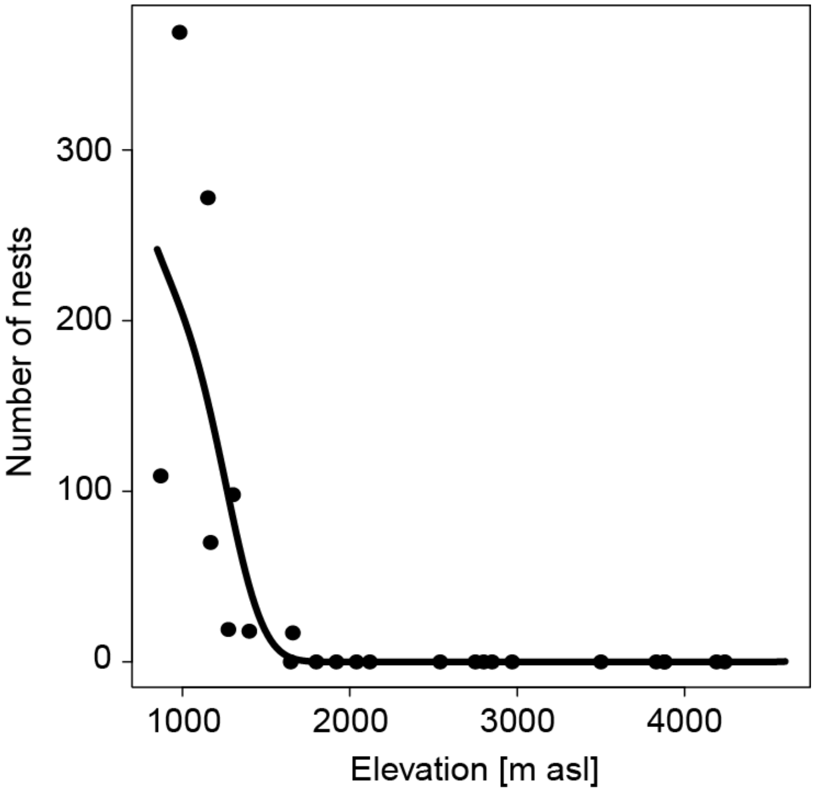


Fig. S1: Pattern of the nest-building activity along the elevational gradient at Mt. Kilimanjaro as derived in a standardised pilot study conducted over a 6 month period. A generalised additive model was used to display the elevational pattern (Poisson family, basis dimension (k) = 4). The number of nests strongly declined with elevation: ED = 84.4 %, $p < 0.0001$. The main study was restricted to the lower elevations up to roughly 1,800 m a.s.l., because no nest building activity occurred at higher elevations.

Appendix S2: Study design

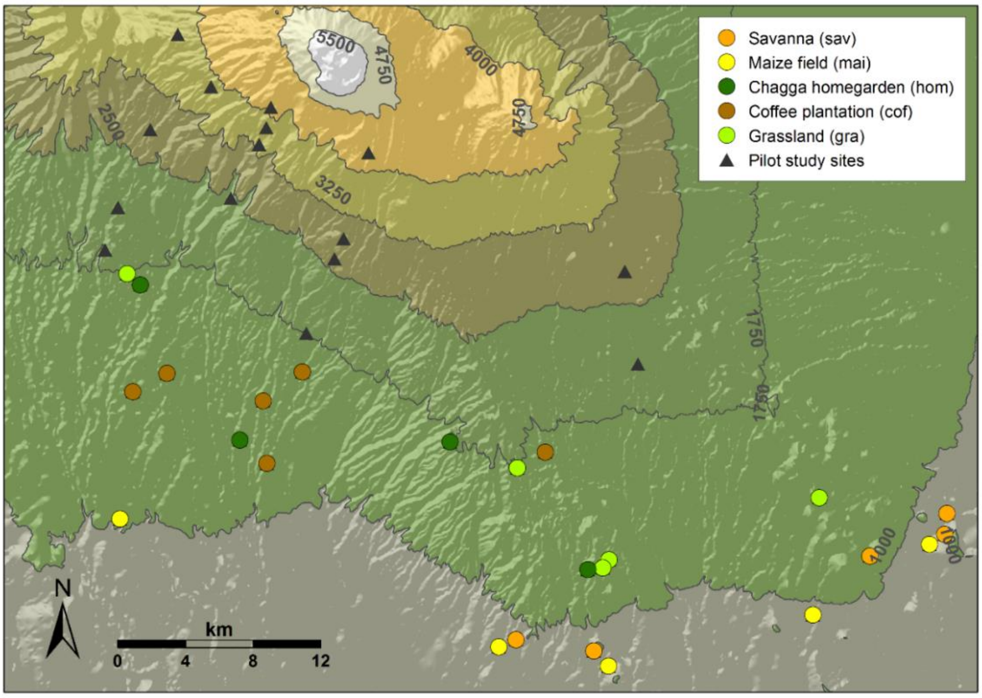


Fig. S2.1: Study area at Mt. Kilimanjaro, Tanzania. Shown are study sites in different habitat types: from low to high elevations, shrub savannahs (sav), maize fields (mai), diverse agroforestry systems of the local Chagga tribe (the so-called Chagga homegardens, hom), extensively-managed grasslands (gra) and coffee plantations (cof). At higher elevations are additional study sites of the pilot study, where no trap-nesting hymenoptera occurred.

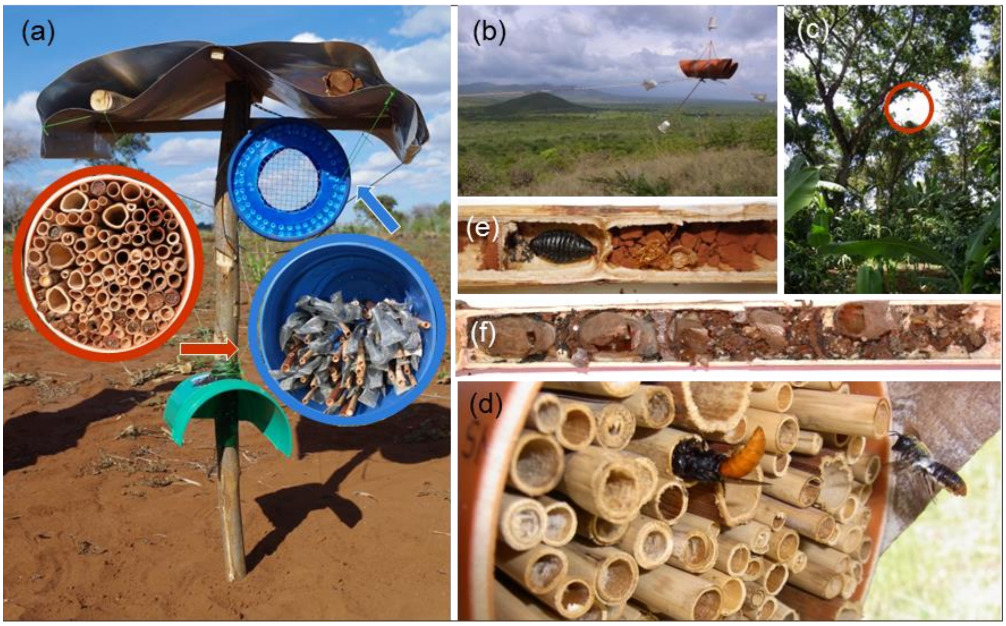


Fig. S2.2: Trap-nesting study design. (a) Trap nest ground poles with special metal roofs, allowing air to pass through against hot savannah and maize temperatures for cooling. The poles were dabbed with sticky glue to prevent ant raids (under the green plastic shield). Occupied nests were taken out of the trap nest (red), closed with a metal net and trap-nesting Hymenoptera were reared in a hatching box (blue). (b) Pair of trap nests for the canopy, in the savannah. (c) Canopy trap nests in the Chagga homegardens. (d) Trap nests approached here by host (*Megachile sp.*) and antagonist, cuckoo bee, (*Euaspis abdominalis*). (e) Wasp nests (*Rhynchium sp.1*). (f) Bee nest (*Megachile maxillosa*). Pictures © A.V. Mayr

Trap nesting

Traps at ground level were fixed to a wooden pole and placed at a height of 140 cm. The pole was dabbed with sticky glue in order to protect the trap nests from ants (Fig. S2.2a). At study sites with trees, such as savannah, chagga homegardens and coffee plantations, two pairs of traps were placed in the lower canopy (up to ~25 m height) and two pairs at ground level as for the study sites without trees. The traps in the canopy were also protected with sticky glue (Fig. S2.2b,c). The traps at ground level were covered by double-folded metal sheets approximately 5 cm apart, allowing air to pass through (Fig. S2.2a). The metal roofs were essential to protect the larvae inside the nests from extreme temperatures in the lowland habitats. Standardised trap nests were made of a mixture of common reed (*Phragmites australis*) and Japanese knotweed (*Fallopia japonica*) and contained

120 reed and knotweed internodes with variable diameters between 2-25 mm and a length of 20 cm.

Appendix S3: Identification keys for bees

Tab. S3: List of identification keys used for the genus and species identification of bees.

Taxon	Key
Hymenoptera	(Goulet & Huber, 1993)
Bee genera	(Michener, 2007)
Bee genera	(Eardley *et al.*, 2010)
Allodapini	(Michener, 1975)
Anthidiini	(Eardley, 2018)
Ctenoplectrini	(Eardley, 2003)
Megachile, dauber bees	(Eardley, 2012)
Megachile, leaf-cutter bees	(Eardley, 2013)

Appendix S4: Elevational patterns of temperature and land use

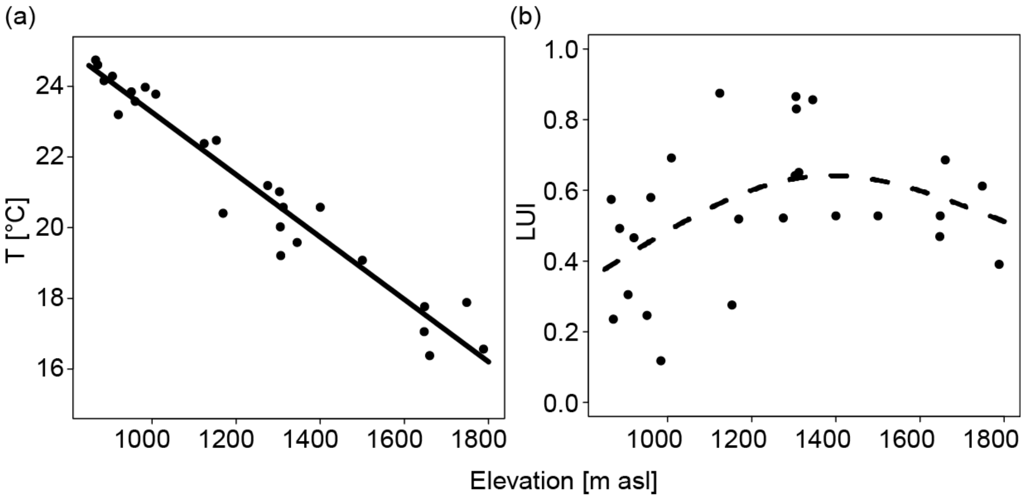


Fig. S4: Patterns of the mean annual temperature (T) and land use (LUI) along the elevational gradient at Mt. Kilimanjaro. Generalised additive models were used to display elevational patterns of temperature and land use (Gaussian family, basis dimension (k) = 4). Temperature declines with elevation (ED = 94.1 %, $p < 0.001$), while land use shows a humped-shaped distribution (ED = 27.6, $p = 0.06$).

Appendix S5: Resource data

Resource data for hosts

Mean flower abundance and flower richness: Flower abundance and richness was estimated each month along two permanent 50 m transects of 2 m width on each study site, at the same time when the trap nests were controlled. For each study site the number of flower units and flowering plant species was counted. A flower unit (hereafter flower) was defined from the perspective of a small bee (approx.. 1 cm length) as the distance the bee would rather walk than fly (Carvalheiro *et al.*, 2008). In case of very large flower numbers (>~500) flower numbers were estimated. The mean flower abundance was calculated by averaging flower abundance over all visits of the sampling site. The species richness of flowering plants was assessed by building up a herbarium and was calculated as the total number of flowering species per study site.

Mean moth abundance and moth richness: Moths were sampled with a light trap, as described in (Helbig-Bonitz *et al.*, 2015) and identified as morphospecies. Mean moth abundance was calculated as the mean number of moths per study site.

Mean spider abundance and spider richness: Spiders were sampled with pitfall traps and the mean spider abundance was calculated per study site, as described in (Röder *et al.*, 2017) and (Peters *et al.*, 2016b). Adult and subadult spiders were sorted to morphospecies.

Resource data for antagonists

Mean host abundance and host richness: The mean abundance of hosts was calculated as the mean number of brood cells of all identified host nests collected during the 15 month study period. We excluded nests where it was not possible to count the number of brood cells. Host richness was calculated as the total number of host species in the nests. Abundance and richness of hosts was calculated in the same way for all further subdivisions: mean bee abundance and bee richness, mean caterpillar-hunting wasp abundance and caterpillar-hunting wasp richness, mean spider-hunting wasp abundance and spider-hunting wasp richness.

Appendix S6: Overview about species richness models

Tab. S6: Overview of generalised linear models for species richness showing explanatory variables for each trophic level. TL: Trophic level, SR: Species richness, T: mean annual temperature, LUI: land-use index, RES-AB: abundance of resources, RES-RI: resource richness, displaying the total number of observed species of resources, 1st-predator: caterpillar-hunting predator, 2nd-predator: spider-hunting predator, para-herb: antagonist of pollinators, para-1st: antagonist of caterpillar-hunting predators, para-2nd: antagonist of spider-hunting predator.

Model	**Guild**	**Functional group**	**TL**	**SR**	**~**	**T**	**+**	**LUI**	**+**	**RES-AB**	**+**	**RES-RI**	**+**	**PARARATE**
full model	Host	Pollinator	2	SR	~	T	+	LUI	+	flowers	+	flowers	+	parasitized brood cells by antagonist of pollinators
full model - pararate	Host	Pollinator	2	SR	~	T	+	LUI	+	flowers	+	flowers		
full model - pararate, - resources	Host	Pollinator	2	SR	~	T	+	LUI						
full model	Host	Caterpillar-hunting predator	3	SR	~	T	+	LUI	+	moths	+	moths	+	parasitized brood cells by antagonist of caterpillar-hunting predator
full model - pararate	Host	Caterpillar-hunting predator	3	SR	~	T	+	LUI	+	moths	+	moths		
full model - pararate, - resources	Host	Caterpillar-hunting predator	3	SR	~	T	+	LUI						
full model	Host	Spider-hunting predator	4	SR	~	T	+	LUI	+	spiders	+	spiders	+	parasitized brood cells by antagonist of spider-hunting predator
full model - pararate	Host	Spider-hunting predator	4	SR	~	T	+	LUI	+	spiders	+	spiders		
full model - pararate, - resources	Host	Spider-hunting predator	4	SR	~	T	+	LUI						
full model	Antagonist	Pollinator	3	SR	~	T	+	LUI	+	brood cells of bees	+	bees	+	parasitized brood cells by second-order antagonist of antagonist of pollinators
full model - pararate	Antagonist	Pollinator	3	SR	~	T	+	LUI	+	brood cells of bees	+	bees		
full model - pararate, - resources	Antagonist	Pollinator	3	SR	~	T	+	LUI						
full model	Antagonist	Caterpillar-hunting predator	4	SR	~	T	+	LUI	+	brood cells of caterpillar-hunting wasps	+	caterpillar-hunting wasps	+	parasitized brood cells by second-order antagonist of antagonist of caterpillar-hunting predator
full model - pararate	Antagonist	Caterpillar-hunting predator	4	SR	~	T	+	LUI	+	brood cells of caterpillar-hunting wasps	+	caterpillar-hunting wasps		
full model - pararate, - resources	Antagonist	Caterpillar-hunting predator	4	SR	~	T	+	LUI						
full model	Antagonist	Spider-hunting predator	5	SR	~	T	+	LUI	+	brood cells of spider-hunting wasps	+	spider-hunting wasps	+	parasitized brood cells by second-order antagonist of antagonist of spider-hunting predator
full model - pararate	Antagonist	Spider-hunting predator	5	SR	~	T	+	LUI	+	brood cells of spider-hunting wasps	+	spider-hunting wasps		
full model - pararate, - resources	Antagonist	Spider-hunting predator	5	SR	~	T	+	LUI						

Appendix S7: Species list

Tab S7.1: Host bees (Apidae, Colletidae, Megachilidae), caterpillar-hunting wasps (Vespidae) and spider-hunting wasps (Crabronidae, Pompilidae, Sphecidae) reared from trap nests.

Superfamily	Family	Subfamily	Tribe	Genus	Morphospecies	Food
Apiformes	Apidae	Apinae	Ctenoplectrini	*Ctenoplectra*	*Ctenoplectra centinorii*	pollen
		Xylocopinae	Allodapini	*Macrogalea*	*Macrogalea candida*	pollen
	Colletidae	Hylaeinae	NA	*Nothylaeus*	*Nothylaeus sp.1*	pollen
					Nothylaeus sp.2	pollen
					Nothylaeus sp.4	pollen
					Nothylaeus sp.6	pollen
					Nothylaeus sp.7	pollen
					Nothylaeus sp.8	pollen
	Megachillidae	Megachilinae	Anthidiini	*Anthidiellum*	*Anthidiellum somaliense*	pollen
			Megachilini	*Megachile*	*Megachile admixta*	pollen
					Megachile demeter	pollen
					Megachile felina	pollen
					Megachile fervida	pollen
					Megachile maxillosa	pollen
					Megachile mossambica	pollen
					Megachile rufipennis	pollen
					Megachile rufiventris	pollen
					Megachile semierma	pollen
					Megachile sheppardi	pollen
					Megachile sinuate	pollen
					Megachile sp.1	pollen
					Megachile sp.2	pollen
					Megachile sp.3	pollen
					Megachile sp.5	pollen
					Megachile sp.6	pollen
					Megachile sp.7	pollen
					Megachile sp.8	pollen
					Megachile sp.9	pollen
					Megachile sp.12	pollen
					Megachile ungulata	pollen
			Osmiini	*Heriades*	*Heriades sp.1*	pollen
					Heriades sp.2	pollen
				Noteriades	*Noteriades sp.1*	pollen
					Noteriades sp.2	pollen
					Noteriades sp.3	pollen
				Pseudoheriades	*Pseudoheriades sp.1*	pollen
				Stenoheriades	*Stenoheriades sp.1*	pollen
				Wainia	*Wainia sp.1*	pollen

Superfamily	Family	Subfamily	Tribe	Genus	Morphospecies	Food
Spheciformes	Crabronidae	Crabroninae	Trypoxylini	*Pison*	*Pison sp.1*	Araneae
				Trypoxylon	*Trypoxylon sp.1*	Araneae
					Trypoxylon sp.2	Araneae
	Sphecidae	Sceliphrinae	NA	*Chalybion*	*Chalybion sp.1*	Araneae
					Chalybion sp.2	Araneae
					Chalybion sp.3	Araneae
					Chalybion sp.4	Araneae
					Chalybion sp.5	Araneae
Vespoidea	Pompilidae	Pepsinae	NA	*Auplopus*	*Auplopus sp.1*	Araneae
					Auplopus sp.2	Araneae
					Auplopus sp.3	Araneae
				Dipogon	*Dipogon sp.2*	Araneae
					Dipogon sp.3	Araneae
	Vespidae	Eumeninae	NA	*Ancistrocerus*	*Ancistrocerus sp.1*	Lepidoptera
					Ancistrocerus sp.2	Lepidoptera
				Anterhynchium	*Anterhynchium sp.1*	Lepidoptera
				Antodynerus	*Antodynerus sp.1*	Lepidoptera
					Antodynerus sp.2	Lepidoptera
					Antodynerus sp.3	Lepidoptera
					Antodynerus sp.5	Lepidoptera
					Antodynerus sp.6	Lepidoptera
					Antodynerus sp.7	Lepidoptera
					Antodynerus sp.8	Lepidoptera
					Antodynerus sp.9	Lepidoptera
					Antodynerus sp.10	Lepidoptera
					Antodynerus sp.11	Lepidoptera
					Antodynerus sp.12	Lepidoptera
					Antodynerus sp.13	Lepidoptera
					Antodynerus sp.14	Lepidoptera
					Antodynerus sp.15	Lepidoptera
					Antodynerus sp.16	Lepidoptera
				Eustenancistrocerus	*Eustenancistrocerus sp.1*	Lepidoptera
					Eustenancistrocerus sp.2	Lepidoptera
				Leptochilus	*Leptochilus sp.1*	Lepidoptera
				Pseudonotonia	*Pseudonortonia sp.1*	Lepidoptera
				Pterochilus	*Pterocheilus sp.1*	Lepidoptera
				Rhynchium	*Rhynchium sp.1*	Lepidoptera
				Subancistrocerus	*Subancistrocerus sp.1*	Lepidoptera
				Tachymenes	*Tachymenes sp.1*	Lepidoptera
					Tachymenes sp.2	Lepidoptera
				Tachymenes	*Tachymenes sp.3*	Lepidoptera
				Tricarinodynerus	*Tricarinodynerus sp.1*	Lepidoptera
					Tricarinodynerus sp.2	Lepidoptera

Tab S7.2: Antagonists (Dermestidae, Meloidae, Mordellidae, Bombylidae, Calliphoridae, Sarcophagidae, Tachinidae, Megachilidae, Eulophidae, Leucospidae, Chrysididae, Gasteruptiidae, Braconidae, Ichneumonidae, Mutilidae, Sapygidae, Acari, Fungi, Neuroptera) reared from trap nests..

Order	Suborder	Superfamily	Family	Subfamily	Tribe	Genus	Morphospecies
Acari	NA	NA	NA	NA	NA	NA	*Acari sp.*
Coleoptera	Polyphaga	Bostrichoidea	Dermestidae	NA	NA	NA	*Dermestidae sp.3*
		Tenebrionoidae	Meloidae	Meloninae	Mylabrini	*Mimesthes*	*Mimesthes sp.1*
				Nemognathinae	Nemognathini	*Zonitoschema*	*Zonitoschema sp.1*
							Zonitoschema sp.2
							Zonitoschema sp.3
			Mordellidae	NA	NA	NA	*Mordellidae sp.1*
Diptera	Brachycera	Asiloidea	Bombylidae	Anthracinae	Anthracini	*Anthrax*	*Anthrax sp.*
		Oestroidea	Calliphoridae	NA	NA	NA	*Calliphoridae sp.*
			Sarcophagidae	NA	NA	NA	*Sarcophagidae sp.*
			Tachinidae	NA	NA	NA	*Tachinidae sp.*
Fungi	NA	NA	NA	NA	NA	NA	*Fungi sp.*
Hymenoptera	Apocrita	Apiformes	Megachilidae	Megachilinae	NA	*Afrostelis*	*Afrostelis tegularis*
					NA	*Coelioxys*	*Coelioxys sp.1*
							Coelioxys sp.2
							Coelioxys sp.3
							Coelioxys sp.4.1
							Coelioxys sp.4.2
							Coelioxys sp.5
							Coelioxys sp.6
							Coelioxys sp.6.1
							Coelioxys sp.6.3
							Coelioxys sp.6.5
							Coelioxys sp.7
							Coelioxys sp.10
							Coelioxys sp.12

Order	Suborder	Superfamily	Family	Subfamily	Tribe	Genus	Morphospecies
					NA	*Euaspis*	*Euaspis abdominalis*
			NA	NA	NA	NA	*Apiformes sp.1*
		Chalcidoidea	Eulophidae	Tetrastichinae	NA	*Melittobia*	*Melittobia sp.*
			Leucospidae	NA	NA	*Leucospis*	*Leucospis sp.1*
							Leucospis sp.4
							Leucospis sp.6
			NA	NA	NA	NA	*Chalcidoidea sp.1*
			NA	NA	NA	NA	*Chalcidoidea sp.2*
			NA	NA	NA	NA	*Chalcidoidea sp.3*
			NA	NA	NA	NA	*Chalcidoidea sp.4*
			NA	NA	NA	NA	*Chalcidoidea sp.5*
			Chrysididae	NA	NA	NA	*Chrysididae sp.*
		Evanioidea	Gasteruptiidae	NA	NA	NA	*Gasteruptiidae sp.1*
		Ichneumonoidea	Braconidae	Agathidinae	NA	*Braunsia*	*Braunsia sp.1*
				Agathidinae	NA	NA	*Agathidinae sp.2*
				Microgastrinae	NA	NA	*Microgastrinae sp.2*
				Rogadinae	NA	NA	*Rogadinae sp.*
			Ichneumonidae	Cryptinae	NA	NA	*Osyprynchotus sp.1*
				Tryphoninae	NA	NA	*Tryphoninae sp.*
		Vespoidea	Mutilidae	NA	NA	NA	*Mutilidae sp.1*
			Mutilidae	NA	NA	NA	*Mutilidae sp.2*
			Sapygidae	NA	NA	*Sapygina*	*Sapygina sp.*
Neuroptera	NA	NA	NA	NA	NA	NA	*Neuroptera sp.1*

Appendix S8: Parasitism and hyperparasitism

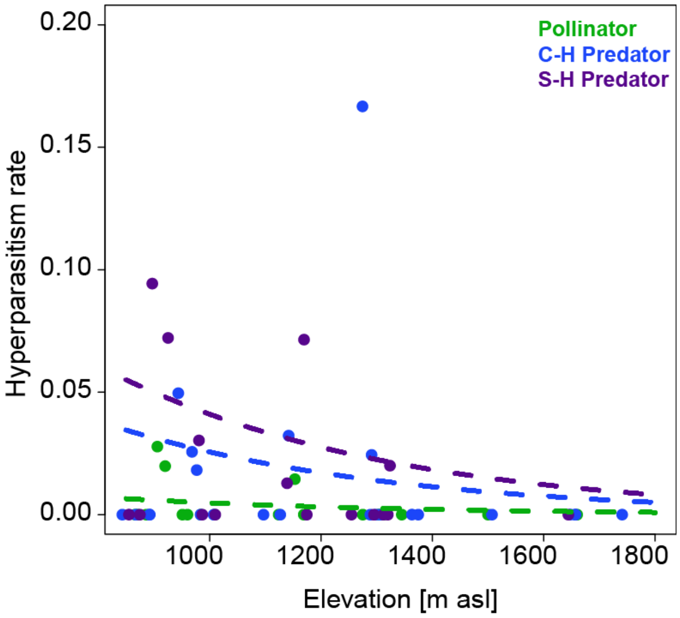


Fig. S8.1: Hyperparasitism rates showed a tendency to decline along the elevational gradient (GLM with quasibinomial data family, $p_{elevation} = 0.15$, dashed lines). C-H Predator: Caterpillar-hunting predator, S-H Predator: Spider-hunting Predator.

Tab. S8.2: Best models up to Δ QAICc < 3 explaining the parasitism rate at different trophic levels, derived from QAICc values.

Parasitism rate	T	LUI	QAICc	Weight	ED	# Sites
Pollinator		-0.40	22.2	0.49	0.27	22
	0.46		23.8	0.22		
			24.2	0.18		
	0.10	-0.35	25.1	0.11		
Caterpillar-hunting predator	0.47		40.9	0.67	0.25	24
	0.43	-0.05	43.7	0.17		
Spider-hunting predator			31.9	0.51	0	19
		0.21	33.0	0.30		
	-0.01		34.7	0.12		

Appendix S9: Species richness models

Tab. S9.1: Best models for the species richness reduced by parasitism rate up to Δ AICc < 3 explaining species richness of hosts and antagonists derived by AIC/QAICcc values. For the full models and the models reduced by parasitism rate and resources, just the best models are presented.

Model	Guild	Functional group	TL	T	LUI	RES-AB	RES-RI	PARARATE	AICc/QAICc	Weight	ED	# Sites
full model - pararate	Host	Pollinator	2	0.77				-	70.9	0.33	0.59	25
				0.66		-0.25		-	71.1	0.30		
				0.76			-0.08	-	73.2	0.10		
				0.61		-0.41	0.15	-	73.6	0.09		
				0.78	0.03			-	73.7	0.08		
full model	Host	Pollinator	2	0.63					74.4	0.35	0.61	22
full model - pararate, - resources	Host	Pollinator	2	0.77		-	-	-	69.6	0.80	0.59	25
full model - pararate	Host	Caterpillar-hunting predator	3	0.30				-	63.6	0.26	0.14	25
								-	64.2	0.19		
				0.35			-0.15	-	65.6	0.09		
					-0.16			-	65.8	0.09		
				0.34		-0.10		-	66.0	0.08		
				0.27	-0.05			-	66.4	0.06		
full model	Host	Caterpillar-hunting predator	3						63.2	0.16	0.00	24
full model - pararate, - resources	Host	Caterpillar-hunting predator	3	0.77		-	-	-	69.6	0.80	0.14	25
full model - pararate	Host	Spider-hunting predator	4					-	51.5	0.27	0.00	14
				0.42				-	51.8	0.24		
				0.72		0.40		-	53.5	0.10		
					-0.21			-	53.7	0.09		
							-0.12	-	54.4	0.06		
full model	Host	Spider-hunting predator	4						41.3	0.32	0.00	11
full model - pararate, - resources	Host	Spider-hunting predator	4	0.32		-	-	-	75.2	0.39	0.11	25

Model	Guild	Functional group	TL	T	LUI	RES-AB	RES-RI	PARARATE	AICc/QAICc	Weight	ED	# Sites
full model - pararate	Antagonist	Pollinator	3	0.48		0.34	0.45	-	89.4	0.28	0.76	25
					-0.22	0.28	0.68	-	90.5	0.16		
						0.19	0.78	-	90.9	0.14		
				0.88		0.49		-	91.0	0.13		
				0.39	-0.16	0.37	0.44	-	91.3	0.11		
							0.85	-	92.2	0.07		
				0.76	-0.16	0.52		-	92.3	0.07		
full model	Antagonist	Pollinator	3		-0.24	0.27	0.43		86.3	0.21	0.80	19
full model - pararate, - resources	Antagonist	Pollinator	3	0.80		-	-	-	42.5	0.79	0.44	25
full model - pararate	Antagonist	Caterpillar-hunting predator	4	0.43		0.31		-	91.6	0.38	0.62	25
				0.40		0.29	0.18	-	92.2	0.29		
				0.39	-0.06	0.34		-	94.4	0.10		
full model	Antagonist	Caterpillar-hunting predator	4	0.37		0.31			89.6	0.26	0.61	24
full model - pararate, - resources	Antagonist	Caterpillar-hunting predator	4	0.52		-	-	-	99.1	0.55	0.40	25
full model - pararate	Antagonist	Spider-hunting predator	5				0.69	-	65.7	0.39	0.40	25
				0.26			0.64	-	67.0	0.20		
					-0.02		0.70	-	68.3	0.11		
						-0.01	0.70	-	68.3	0.11		
full model	Antagonist	Spider-hunting predator	5						50.9	0.21	0.00	16
full model - pararate, - resources	Antagonist	Spider-hunting predator	5	0.43		-	-	-	70.5	0.43	0.13	25

Shown are standardised explanatory variables from best-fit models. Colours indicate positive significant (blue) and negative significant values (red) from GLMM models for the best model each. TL: trophic level, T: mean annual temperature, LUI: land use index, RES-AB: abundance of resources and RES-RI: total number of observed species/ morphospecies of resources, PARARATE: parasitism rate of hosts and parasitism rate of parasitised brood cells (hyperparasitism rate), respectively, ED: explained deviance, # Sites: number of sites taken into account for the model.

Tab. S9.2: Significant explanatory variables in the linear mixed effects models with test for possible interactions between the identified drivers of species richness and trophic level.

Data	**Significant drivers of species richness**	**P-value**	**# N**
Species richness of all functional groups including spider-hunting wasps	T	***	139
	T * TL	*	
	RES-AB * TL	*	
Species richness of all functional groups except from spider-hunting wasps	T	**	125
	T * TL	*	
	RES-AB * TL	*	

Shown are standardised explanatory variables from LME models: lme(SRstd~scale(T)*TL+scale(RES-ABstd)*TL+scale(RES-RIstd)*TL, random=~1|plotID). The models were calculated with and without spider-hunting wasps, because resource data of spider-hunting wasps was not available for all study sites. All significant P-values are positive. T: mean annual temperature, TL: trophic level, RES-AB: resource abundance; RES-RI: resource diversity; # N: number of data points for species richness and resource availability taken into account for the model.

Chapter 3

Seasonal variation in the ecology of tropical cavity-nesting Hymenoptera

Insect communities vary seasonally with changing climatic conditions and related changes in resource availability, strength of competition or pressure by natural antagonists. But seasonal dynamics, particularly in tropical mountain ecosystems are not well investigated. We monitored cavity-nesting Hymenoptera communities to analyse temporal patterns of nest-building activity, ecological rates and life-history traits in relation to seasonal climatic variation and elevation. We analysed nest-building activities of bees, caterpillar-hunting wasps and spider-hunting wasps by installing trap nests on 25 study sites in natural and disturbed habitat types, over a complete annual period covering two rainy and two dry seasons. Study sites covered low (<1300 m) and high elevation levels (≥1300 m a.s.l). We performed mixed generalised additive models to analyse the potential seasonality of nest-building activities, parasitism, natural mortality and survival rate, sex ratio, development time and body size. Nest-building activity showed strong seasonal trends in bees, caterpillar-hunting wasps and spider-hunting wasps. Nest-building activity was considerably higher and seasonal trends were more synchronised between functional groups at low elevations. We also detected seasonal patterns for parasitism and natural mortality rates, sex ratio and development time, which varied with functional groups and between elevation levels. Temperature, relative humidity, an interaction of both and flower abundance were important predictors for seasonal patterns in nest-building activity, ecological rates and life-history traits. Our study reveals strong seasonal patterns in the nest-building activity, life-history traits and trophic interactions of insects in tropical ecosystems. Seasonal trends in nest-building activity of lowland Hymenoptera seem to be linked to changes in climate and resource availability reflecting the seasonal patterns in plant growth and flowering known from lowland savanna ecosystems. Higher resource availability also increased the sex ratio in bees towards the more costly females and enhanced their survival rates. Finding these links between climate, resources, ecological rates and life-history traits indicate high sensitivity of plant-host-antagonist interactions to environmental changes.

3.1 Introduction

Insect communities vary spatially and temporally in their abundance, diversity and community composition. They are able to survive long periods of unfavorable conditions by reducing their mortality risk in harsh seasons and find compromises between tolerable abiotic conditions, nutritious food and low pressure of predators, parasitoids and pathogens (Wolda, 1988; Abrahamczyk *et al.*, 2011). For this purpose, they have to synchronize their activity phase with suitable environmental conditions (Tauber & Tauber, 1976).

Dynamically fluctuating population sizes of insects are known both between and within years (Wolda, 1992), and abiotic factors such as temperature and humidity are suggested to drive these temporal variations, because they are important abiotic factors for arthropod life-cycles (Chen *et al.*, 2009). Especially in temperate regions, strong seasonal variations in temperature cause distinct seasons, hereby also inducing strong seasonality in life-history traits in animals and plants (Tauber & Tauber, 1976; Denlinger, 1986). In the tropics, climatic differences between seasons are not as pronounced as in temperate regions and historically the tropics were considered to be aseasonal. Nevertheless, rainfall is highly unevenly distributed throughout the year, generating distinct rainy and dry seasons in the tropics. Rainfall variation has been considered as an important factor of seasonality in tropical insects (Frith & Frith, 1990; Novotny & Basset, 1998; Wagner, 2001; Kishimoto-Yamada & Itioka, 2013). However, climatic factors can only account for a fraction of seasonality in tropical communities and in the lowland tropics neither temperature nor precipitation seem to be limiting factors. But, the survival of individuals and species depends on so many more factors. Individuals have to tackle multiple trade-offs in synchronising with abiotic conditions and biotic factors, such as resource availability, while minimising intra- and interspecific competition and pressure by natural antagonists and pathogens (Denlinger, 1986). Resources and antagonists also face these ecological and evolutionary challenges, which should lead to feedback loops and complex seasonal patterns.

Seasonal changes in population size and species composition are presumably also reflected in the life-history traits of insect communities (Samnegård *et al.*, 2015; Osorio-Canadas *et al.*, 2018). For example, there are seasonal changes in the body size of bees (Osorio-Canadas *et al.*, 2016). Larger bees need more resources for their offspring than small species (Müller *et al.*, 2006) and therefore decline faster in abundance when resources become scarce (Bartomeus *et al.*, 2013). Moreover, seasonal variability is also known in sex ratios (Tepedino & Parker, 1988; Martins *et al.*, 1999) (for theoretical illustration, see Fig. S1 in Appendix S1 in Supporting Information). In addition, with the seasonal change of traits, the species community may also functionally change, because different species follow different strategies to solve trade-offs. Moreover, drivers of seasonality can be group-specific, with some insect orders being more abundant in the dry seasons and others more abundant in the wet seasons (Wagner, 2001). Bees, for example, can be active at lower temperatures than their parasites, so that an increase in temperature can lead to an increase in parasitism rates (Forrest & Chisholm, 2017). Furthermore, the magnitude of seasonality may vary due to the taxonomic group, showing stronger or weaker reactions to environmental seasonality, but due to the habitat type also different magnitudes within the same group (Wagner, 2001). Therefore, different seasonal patterns and different magnitudes in seasonality taken together, can be reflected in changes in the composition of the community during the year.

Since insect communities change along elevational gradients and responses of species likely vary due to different life-histories, behaviour and interactions (Boulter *et al.*, 2011), seasonal patterns of insect communities might also change with elevation. Seasonal changes in favorable conditions should directly or indirectly dictate the activity of insects (Wagner, 2001). Therefore, communities at warmer and often drier lower elevations should face stronger seasonality than those at higher elevations, because of restricted water availability, but colder temperatures might limit the activity of insects also there. The few previous studies exploring seasonality in insect communities at different elevations did not provide consensual results on the presence (Janzen *et al.*, 1976) or absence of an elevation effect (Boulter *et al.*, 2011; Lambkin *et al.*, 2011; Bishop *et al.*, 2014; Wardhaugh *et al.*, 2018). There is a critical need to study how elevation, resource availability and species interactions affect the seasonality of insect communities, especially in the tropics.

In order to explore different species strategies to solve environmental trade-off, we assessed seasonal changes in nest-building activity, ecological traits and antagonists of three functional groups of cavity-nesting Hymenoptera at elevations below and above 1,300 m a.s.l. at Mt. Kilimanjaro. Mt. Kilimanjaro, with its bimodal rainfall pattern and diverse tropical mountain ecosystems, provides a unique system to study seasonal patterns along an elevational gradient within a small spatial scale (Sanders & Rahbek, 2012). Cavity-nesting Hymenoptera fulfill important ecosystem functions like pollination and predation (Losey & Vaughan, 2006). We monitored (*i*) bees (pollinators), (*ii*) caterpillar-hunting wasps (first-order predators) and (*iii*) spider-hunting wasps (second-order predators) over a complete annual period covering two rainy and two dry seasons. For each functional group, we measured the number of nests, parasitism, natural mortality and survival rates, sex ratio, development time and body size. We also recorded local climatic conditions and the availability of flower resources given that cavity-nesting Hymenoptera react very sensitively to environmental changes (Tscharntke *et al.*, 1998). We tested the following hypotheses:

(1) Cavity-nesting Hymenoptera show seasonality in tropical mountain ecosystems in nest-building activity, ecological rates (parasitism, natural mortality and survival rate) and life-history traits (sex ratio, development time and size);

(2) Seasonal patterns differ between bees, caterpillar-hunting and spider-hunting wasps in timing and magnitude;

(3) Seasonal patterns differ between lower and higher elevations, with higher seasonality in the lowlands and lower seasonality at higher elevations;

(4) Seasonality in cavity-nesting Hymenoptera is related to changes in climatic conditions and resource availability, but responses differ among functional groups and elevational levels.

3.2 Methods

3.2.1 Study area and study design

The study was conducted on the southern slopes of Mt. Kilimanjaro (3°10´-3°23´S, 37°14´-37°41´E) between October 2014 and February 2016. The climate is generally very dry compared to other tropical land areas and has a bimodal precipitation pattern with a long rainy season from March to May and a short rainy season between October and December (Fig. 1). A long dry season spans from June to September and a short dry season from January to February (Yang *et al.*, 2015). Temperatures are generally higher between October and February (Appelhans *et al.*, 2016). The climatic seasonality is much stronger at lower elevations outside the forest belt (Appelhans *et al.*, 2016). The highest number of flowers were recorded in October both at lower and high elevations and positively correlated with relative humidity (Fig. 1). We selected 25 study sites of 50 × 50 m, ranging from 866 to 1,788 m a.s.l., which we identified as the upper limit of the distribution of cavity-nesting Hymenoptera in a previous study (AVM et al., unpublished data). Study sites covered five major ecosystem types found at the southern slopes of Mt. Kilimanjaro, each with four to six replicates, ranging from natural savannahs and maize fields at lower elevations, which are the driest areas of the mountain (Appelhans *et al.*, 2016), to diverse agroforestry systems of the local Chagga tribe (Chagga homegardens), extensively-managed grasslands and coffee plantations at mid-elevations. One study site had to be excluded, because we did record no nests. For statistic robustness of seasonal trends, (i.e. to ensure a sufficient amount of data at each elevation), we divided study sites into two elevational levels, low elevations (<1,300 m a.s.l., N = 13 study sites) and high elevations (≥1,300m a.s.l., N = 12 study sites).

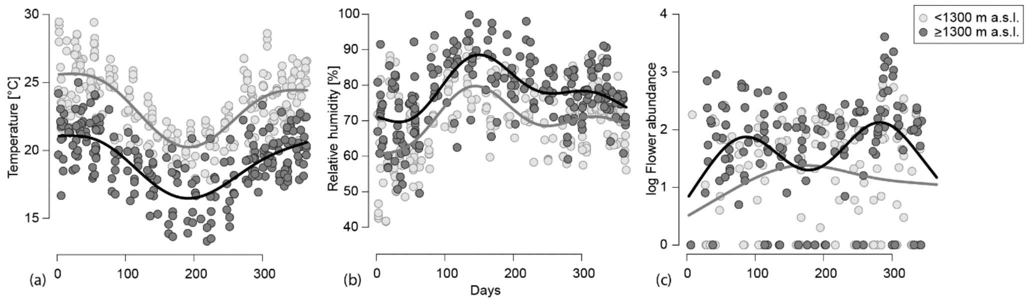


Fig. 4: Seasonal variation of temperature, relative humidity and flower abundance at elevations below and above 1,300 m a.s.l. at Mt. Kilimanjaro. Generalised additive mixed models (GAMM) were used to estimate trends of seasonality (Gaussian family, basis dimension (k) = 6 as for nest-building activity, life-history traits and ecological rates. Temperature, relative humidity and flower abundance (log-transformed), showed significant seasonality for lower and higher elevations: Lower elevations: (a) Temperature: $F_{5,735}$=309, $p<0.001$, (b) Relative humidity: $F_{5,735}$=84.79, $p<0.001$, (c) Flower abundance: $F_{3,443}$=7.31, $p<0.001$, higher elevations: (a) Temperature: $F_{5,678}$=286.6, $p<0.001$, (b) Relative humidity: $F_{5,678}$=90.72, $p<0.001$, (c) Flower abundance: $F_{5,432}$=13.2, $p<0.001$. Trend lines derived with GAMMs were plotted with continuous lines because the significance level of days of the year was $p < 0.05$ and a seasonal pattern was observed. A linear mixed effect model (LMM) with study site as random factor was used to test for possible correlations between temperature and humidity and flower abundance. Flower abundance correlated with temperature (t-value=-3.14, $p<0.01$), relative humidity (t-value=1.79, $p=0.07$) and an interaction of both, temperature*relative humidity (t-value=8.33, $p<0.001$). Days: Days of the year.

3.2.2 Sampling of cavity-nesting Hymenopter

The seasonal abundance of cavity-nesting bees, crabronid, eumenid, pompilid and sphecid wasps was assessed with 208 trap nests operated for 17 months. Eight trap nests were placed in four pairs on each study site and filled with common reed (*Phragmites australis*) and Japanese knotweed (*Fallopica japonica*) internodes. The trap nests were monthly checked for new and hatched nests, and occupied reed internodes were replaced with new ones. We reared all nests in hatching boxes on the study sites, in order not to change the climatic conditions during development. After hatching, all nests were cut open, identified and life-history traits and ecological rates recorded. All specimens were divided into functional groups following the trophic level of their food provisions, i.e., pollen- and nectar feeders (bees: Apidae), caterpillar-hunting wasps (Eumeninae) and spider-hunting wasps (Pompilidae, Sphecidae and Crabronidae). Aphid-hunting, cicada-hunting and orthopteran-hunting wasps occurred only occasionally so that we excluded them from the analysis.

3.2.3 Measurement of ecological rates and life-history traits

We measured the following data from nests and specimens: (1) we calculated the mean parasitism rate, as the number of brood cells killed by parasitoids, predators and cleptoparasites in relation to all brood cells and also used the number of brood cells killed by parasitoids, predators and cleptoparasites itself. (2) We determined the mean natural mortality, as the number of dead brood cells, which died for unknown reason (presumably pathogens), in relation to all brood cells. (3) The mean survival rate was calculated as the number of hatched brood cells in relation to all brood cells. (4) We calculated the mean sex ratio as the percentage of male brood cells to all brood cells. (5) We measured the mean development time as the number of days between the date of control, when the nests were found and the date of control when the nests were already hatched. This implies that the real development time can differ from the measured development time. It can be longer if nests have been built already 1-4 weeks before the control date and shorter if nests already hatched 1-4 weeks before the control date. The development time is therefore a coarse estimate to differentiate very short and very long development times. (6) Lastly, we measured the diameters of occupied reed internodes which correlate with body size with few exceptions (Araújo *et al.*, 2016) to estimate mean body size of the community. Different life-history traits can have a different number of data points, since it was not possible to assess all traits simultaneously. For example, host brood cells which are killed by parasitoids cannot give information about the sex of the respective host.

3.2.4 Temperature, relative humidity and flower abundance

Temperature and relative humidity were recorded by colleagues on 24 study sites in 5 minutes intervals during the whole duration of the data collection as in (Appelhans *et al.*, 2016) and averaged to daily means. We used a co-kriging approach to estimate missing temperature and humidity values for two study sites, where data loggers were not running during the whole duration of the data assessment (Appelhans *et al.*, 2016). Data for flower abundance of flowering plant species were collected in parallel to the trap-nest surveys each month along two parallel 50 m transects of 2 m width and a distance of 20 m to each other and the total number of flowers per species was assessed for each survey. Compound flowers were considered as a unit for flower distances which a small bee of approx. 1 cm length would rather walk than fly (Classen *et al.*, 2015).

3.2.5 Statistical analysis

All statistical analyses were performed using the R Project for Statistical Computing version 3.3.3 (R Development Core Team 2018). The seasonal pattern of nest-building activities, ecological rates and life-history traits were modelled as a function of the day of year, using generalised additive mixed models (GAMMs) and a Gaussian error structure, Poisson error structure or Binomial error structure depending on the variable considered (*gamm* function in the *mgcv* R-package). Nest-building activities were modelled with Poisson, while size and development time were modelled with Gaussian error structure. Sex ratio, parasitism, natural mortality and survival rate were modelled with Binomial error structure with log link function. GAMMs are modelling techniques that allow temporal spline fitting while taking account of repeated measurements on statistical units. Herein, the identities of the site were specified as a random variable. For statistical robustness of seasonal trends (i.e. to ensure a sufficient amount of data at each time period), time was rescaled on Julian dates from the first of January to show a complete annual cycle. We performed models for study sites < 1,300 m and ≥1,300 m a.s.l. and for each functional insect group separately as the inference and interpretation of statistical interactions between predictor variables is rather complicated with GAMMs. In GAMM models we set the basis dimension of the smoothing term *k* to six to account for seasonal patterns in the trend functions while limiting the complexity of the curve function. People living nearby, especially children, were moving around on the study sites and destroyed or stole some nests out of the hatching boxes. To account for the losses, we estimated the number of established nests for each functional group by multiplying the total number of primarily observed nests with the proportion for each functional group observed in the remaining nests. Because losses were random, we assumed that the relative proportion of functional groups did not change with some nests lost. In total, 13 % of the primarily observed nests were lost.

We tested whether the seasonality in nest-building activity, ecological rates and life-history traits is synchronised with temperature, relative humidity, an interaction of both, and the abundance of flowers, using generalised linear mixed effect models (GLMMs, *glmer* function in the *lme4* R-package) and a Gaussian error structure for nest-building activity, size and development time, and a Binomial error structure for sex ratio, parasitism, natural mortality and survival rate. Site was specified as a random variable to account of repeated measurements. We calculated all models first with data points covering the whole elevational gradient to reveal general seasonal patterns and then recalculated the models for lower and higher elevations separately to check for causalities which might only occur at a certain elevational level. Before the analyses, the explanatory variables were standardised by z-transformation, using the *scale* function in R. We further checked whether seasonal patterns in nest-building activity between functional groups are synchronised between each other and used linear mixed effect models (LMMs, *lmer* function in the *lme4* R-package) and study site as random factor to compare nest-building activities of different functional groups.

In order to check for migration and turnover of species in different seasons and compare species communities between seasons and elevations, we used a subset of nests in which the host could be identified to morphospecies level. We calculated Bray-Curtis distances between species communities of five different elevation zones, i.e. up to 1,000 m, from 1,000 to 1,200 m, from 1,200 to 1,400 m, from 1,400 to 1,600 m and from 1,600 to 1,800 m a.s.l. and four different seasons (long rainy season: March-May, long dry season: June-September, short rainy season: October-December and short dry season: January-February), using the *metaMDS* function in the *vegan* R-package and a community matrix with occurrence data. We applied non-metric multidimensional scaling (NMDS) along two axes.

3.3 Results

3.3.1 Seasonal patterns in nest-building activity and species turnover

Nest-building activity varied seasonally at lower and higher elevations. In total, we found 6,852 nests of cavity-nesting Hymenoptera of which 5,973 could be identified in detail for life-history traits and ecological rates and 87 % of them could be assigned to bees, caterpillar-hunting wasps and spider-hunting wasps. Overall, bees built the highest numbers of nests (3,215 nests, representing 54% of all identified nests), followed by caterpillar-hunting wasps (20%) and then spider-hunting wasps (14%). Differences in nest abundances between functional groups were less pronounced at lower elevations (2,276 bee nests, representing 60 % of the nests from the three functional groups, caterpillar-hunting wasps 23 % and spider-hunting wasps 17 %) compared to higher elevations (939 bee nests, representing 68 % of the nests from the three functional groups, caterpillar-hunting wasps 21 % and spider-hunting wasps 11 %). All three functional groups showed seasonality in nest-building activities at lower and higher elevations, with strongest seasonality at lower elevations (Fig. 2, Appendix S3, Fig. S3.2). Generally, nest-building activity was much higher at low than at high elevations. Strikingly, seasonal patterns in nest-building activity were similar between all functional groups at lower elevations, but at higher elevations only for caterpillar- and spider-hunting wasps (Fig. 2).

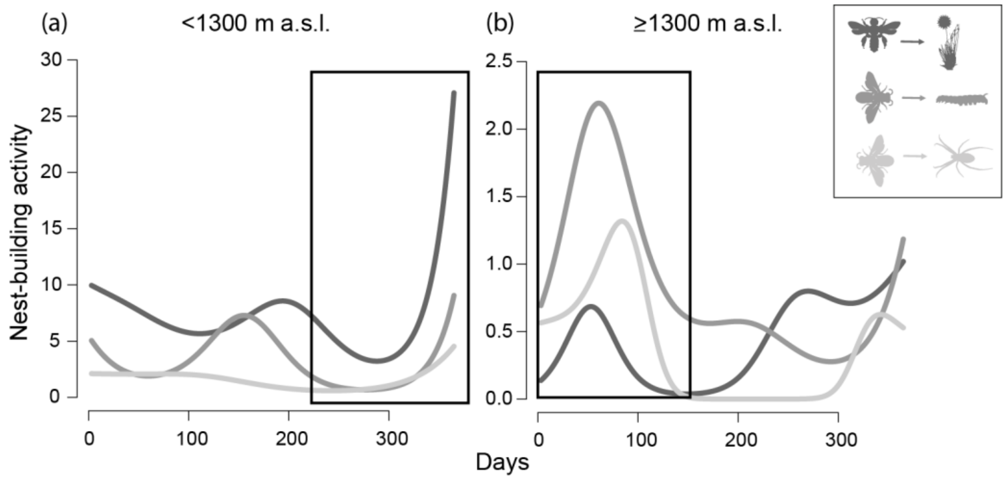


Fig. 2: Seasonal variation of nest-building activities of bees, caterpillar-hunting wasps (Wlep) and spider-hunting wasps (Wara) at elevations (a) below and (b) above 1,300 m a.s.l. at Mt. Kilimanjaro. Generalised additive mixed models (GAMM) were used to estimate trends of seasonality (Poisson family, basis dimension (k) = 6. Nest-building activities of bees, Wlep and Wara showed significant seasonality for lower and higher elevations: Lower elevations: Bees: $F_{5,206}=137.8$, $p<0.001$, Wlep: $F_{5,206}=74.64$, $p<0.001$, Wara: $F_{5,206}=33.78$, $p<0.001$, higher elevations: Bees: $F_{5,183}=37.12$, $p<0.001$, Wlep: $F_{5,183}=25.91$, $p<0.001$, Wara: $F_{5,183}=10.89$, $p<0.001$. Seasonal synchrony in nest-building activities was tested with linear mixed effect models (LME). The nest-building activity of bees, Wlep and Wara was similar at lower, but at higher elevations only for Wlep and Wara: Lower elevations: Bees-Wlep: $t=1.97$, $p=0.05$, Bees-Wara: $t=4.66$, $p<0.001$, Wlep-Wara: $t=5.48$, $p<0.001$, higher elevations: Bees-Wlep: $t=-0.21$, $p=0.84$, Bees-Wara: $t=0.91$, $p=0.37$, Wlep-Wara: $t=2.10$, $p=0.04$. Bees, caterpillar-hunting and spider-hunting wasps showed synchronised seasonality at the end of the year in the lower elevations, but, were rather synchronised at the beginning of the year at higher elevations. Only model predictions are displayed, because nest-building activity contains extreme values. Only GAMM predictions are shown for clarity, but see Fig. S3.2 for observed data Days: Days of the year.

Nest-building activity of bees increased with flower abundance, whereas nest-building activity of spider-hunting wasps increased with temperature and relative humidity and it increased in caterpillar-hunting wasps with an interaction of temperature and relative humidity (Tab. 1).

Tab. 1: Summary of the GLMM models performed to test the effects of temperature, relative humidity, flower abundance and an interaction between temperature and relative humidity on seasonal patterns of nest-building activity, ecological rates and life-history traits.

Trait	**Hym. group**	**T**		**rH**		**T*rH**		**Flower ab.**	
		t/z-value	**p-value**	**t/z-value**	**p-value**	**t/z-value**	**p-value**	**t/z-value**	**p-value**
Nest-building activity	Bees	t=0.45	p=0.65	t=-0.32	p=0.75	t=0.81	p=0.42	t=1.95	p=0.05
	Wlep	t=-0.74	p=0.46	t=-1.38	p=0.17	t=1.87	p=0.06	t=-0.31	p=0.76
	Wara	t=2.86	p<0.01	t=1.84	p=0.07	t=1.26	p=0.21	t=0.28	p=0.78
Parasitism rate	Wlep	z=-1.28	p=0.20	z=0.64	p=0.52	z=0.35	p=0.73	z=-1.56	p=0.12
	Wara	z=1.39	p=0.17	z=1.22	p=0.22	z=-0.35	p=0.73	z=-0.50	p=0.62
Natural mortality rate	Bees	z=-2.67	p<0.01	z=2.19	p=0.03	z=-0.74	p=0.46	z=1.03	p=0.30
	Wlep	z=1.10	p=0.27	z=2.40	p=0.02	z=-1.58	p=0.11	z=-1.05	p=0.29
	Wara	z=-0.26	p=0.79	z=-0.71	p=0.48	z=-2.10	p=0.04	z=-1.60	p=0.11
Survival rate	Bees	z=2.87	p<0.01	z=-1.61	p=0.11	z=-0.72	p=0.47	z=2.90	p<0.01
	Wlep	z=0.68	p=0.50	z=-2.28	p=0.02	z=1.00	p=0.32	z=2.42	p=0.02
	Wara	z=-0.93	p=0.35	z=-0.82	p=0.41	z=2.62	p<0.01	z=1.44	p=0.15
Sex ratio	Bees	z=-0.20	p=0.84	z=-0.08	p=0.94	z=-0.82	p=0.41	z=-1.74	p=0.08
Development time	Wlep	t=0.41	p=0.68	t=1.41	p=0.16	t=1.14	p=0.26	t=0.87	p=0.39
	Wara	t=0.13	p=0.90	t=0.91	p=0.37	t=0.14	p=0.89	t=1.18	p=0.25
Size	Wara	t=1.05	p=0.30	t=0.57	p=0.57	t=0.71	p=0.48	t=-0.82	p=0.42

Significant positive and negative correlations between nest-building activity and explanatory variables can be concluded from positive and negative t-values, respectively. Hym. group: Hymenoptera group, T: Temperature, rH: relative Humidity, T×rH: Interaction between Temperature and relative Humidity, Flower ab.; Flower abundance, Wlep: caterpillar-hunting wasps, Wara: spider-hunting wasps.

We further analysed seasonal migration and turnover of species. Altogether, we were able to identify 2,230 nests at species level, which comprises 33 % of all observed nests, belonging to 88 host morphospecies (Appendix S5). Seasonal turnover seemed to be low at lower elevations and higher at higher elevations, i.e. seasonal turnover in species composition was generally lowest below 1,000 and between 1,200 and 1,400 m a.s.l. (both mean distance to centroid = 0.29) and highest between 1,400 and 1,600 m a.s.l. (mean distance to centroid = 0.58). Elevational turnover was high, forming generally very distinct species communities between elevation zones (Appendix S2). Seasonal migration did not really seem to happen, otherwise wet season community at 800 m could have become dry season community at 1000 m for instance.

3.3.2 Seasonal patterns in ecological rates

Overall, we observed strong seasonal variations in natural mortality, parasitism, and survival rates and seasonal patterns were less uniform than nest-building activity, showing strong variations depending on functional group and elevation (Fig. 3, Appendix S3, Fig. S3.1, Fig. S3.2). Natural mortality rate of bees and caterpillar-hunting wasps increased with relative humidity and at the end of the warm rainy season at lower elevations (Tab. 1, Fig. 3, Appendix S3, Fig. S3.2). It then decreased again at the end of the warm dry season at low elevations, underlined for bees by a decrease of natural mortality with increasing temperature (Tab. 1, Fig. 3, Appendix S3, Fig. S3.2). The natural mortality rate of bees showed opposite trends at higher elevations (Fig. 3, Appendix S3, Fig. S3.2). In contrast, the parasitism rate of caterpillar-hunting wasps peaked at lower elevations in the transition time between short dry and long rainy and long dry and short rainy season, respectively (Fig. 3, Appendix S3, Fig. S3.2), but the highest number of parasitised brood cells were found in the transition times between rainy and dry seasons (Appendix S3, Fig. S3.3). In opposition to the caterpillar-hunting wasps, the parasitism rate of the spider-hunting wasps peaked in the transition time between long rainy and long dry season, with highest numbers of parasitised brood cells in the rainy seasons, and the parasitism rate was similar at lower and higher elevations, with lower parasitism rates at the beginning and end of the year (Fig. 3, Appendix S3, Fig. S3.2, Fig. S3.3). The survival rate of bees increased with temperature and flower abundance (Tab. 1). The survival rate of caterpillar-hunting wasps was also enhanced by flower abundance, but decreased with relative humidity. The survival rate of spider-hunting wasps was explained by an interaction between temperature and relative humidity, i.e. higher temperature in concert with low relative humidity lead to higher survival rates.

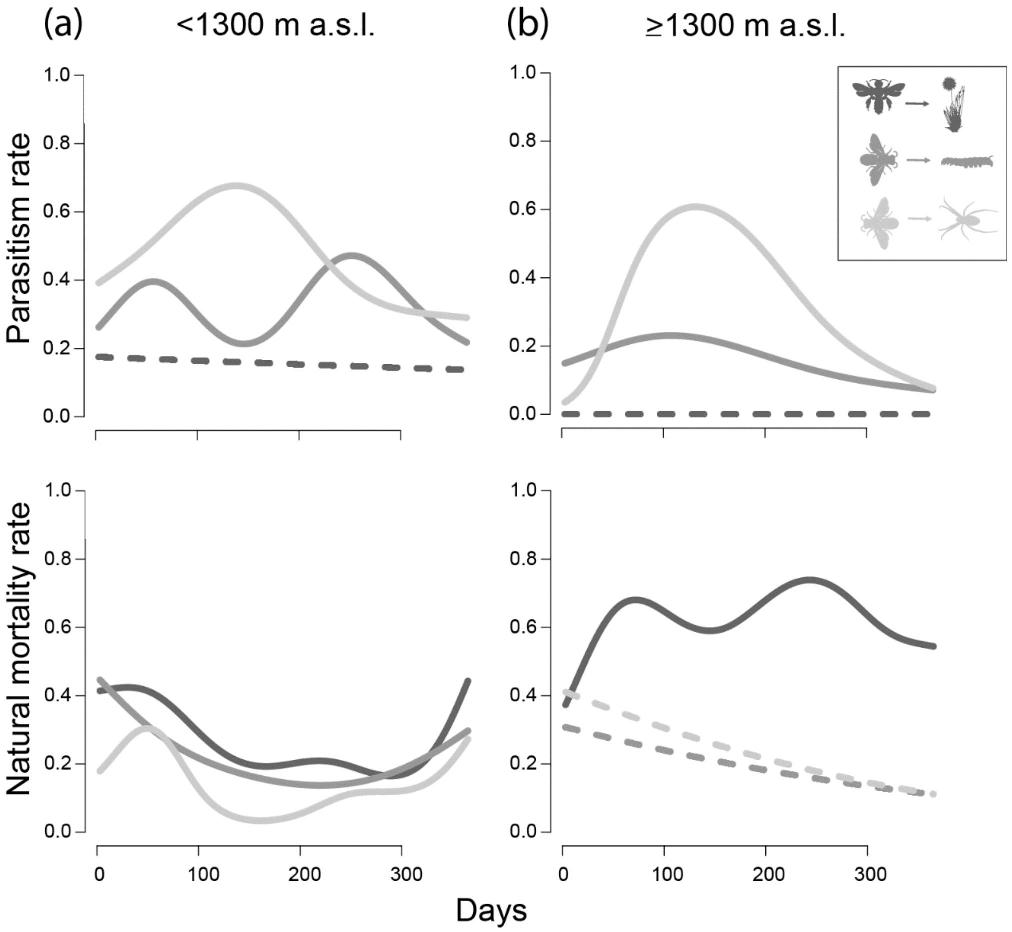


Fig. 3: Seasonal variation in parasitism and natural mortality rates of bees, caterpillar-hunting wasps and spider-hunting wasps at elevations (a) below and (b) above 1,300 m a.s.l. at Mt. Kilimanjaro. Generalised additive mixed models (GAMM) were used to estimate trends of seasonality (Binomial family for parasitism and natural mortaliy rate, basis dimension (k) = 6). Significant model GAMM estimations are shown with solid lines, not significant model estimations with dashed lines. Model statistics are given in Tab. S3. Only GAMM predictions are shown for clarity, but see Fig. S3.2 for observed data Days: Days of the year.

3.3.3 Seasonal patterns in life-history traits

We found only few significant seasonal trends for variation in sex ratio, development time and size (Fig. 4, Tab. 1, Appendix S3, Fig. S3.1, Fig. S3.2, Tab. S3). For bees at low elevations, the sex ratio increased towards more males in the transition between warm rainy and warm dry season and a lower flower abundance lead to a higher proportion of males at lower elevations (Fig. 4, Appendix S3, Fig. S3.2a, Tab. S4). The development time for caterpillar- and spider-hunting wasps slightly increased during the long rainy season at lower elevations. In general, the mean size of cavity-nesting Hymenoptera was rather similar throughout the year with only the spider-hunting wasps at higher elevations showing seasonal trends in body size, i.e. a larger size in the warmer half of the year (Appendix S3, Fig. S3.1b, Fig. S3.2c).

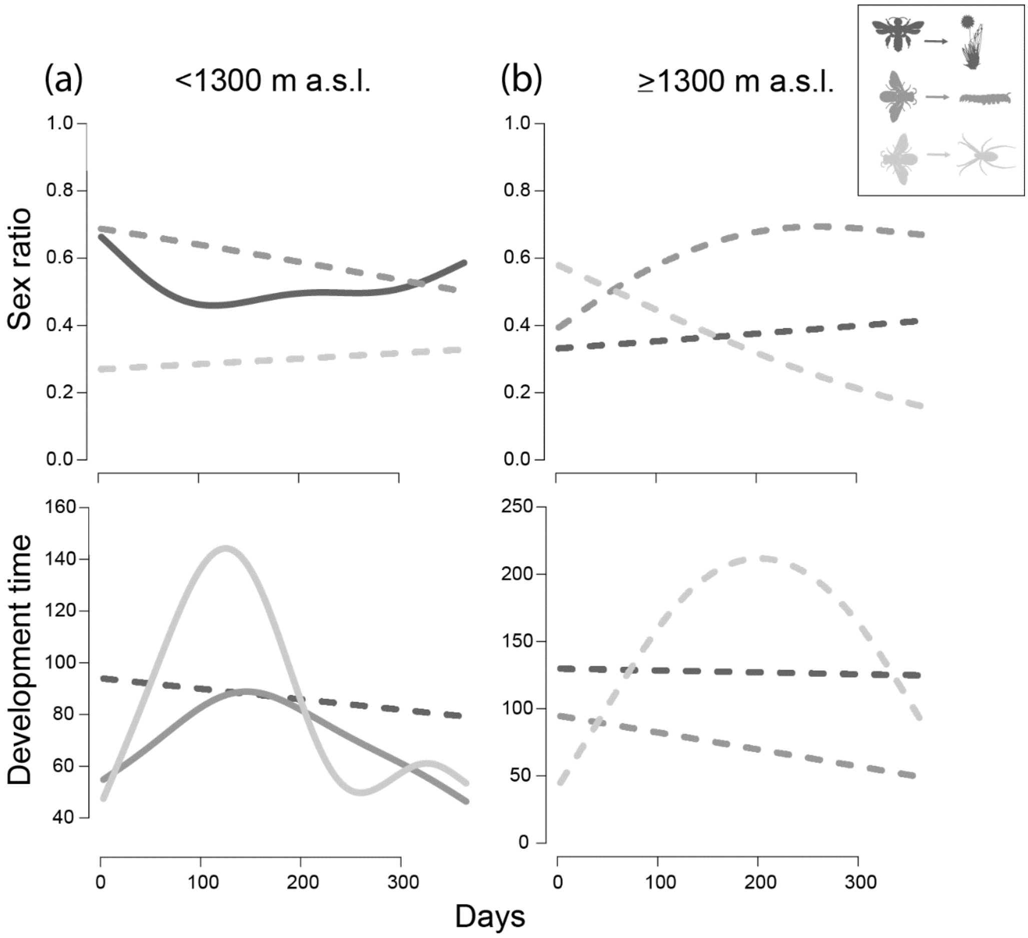


Fig. 4: Seasonal variation in sex ratio and development time of bees, caterpillar-hunting wasps and spider-hunting wasps at elevations (a) below and (b) above 1,300 m a.s.l. at Mt. Kilimanjaro. Generalised additive mixed models (GAMM) were used to estimate trends of seasonality (Binomial family for sex ratio, Gaussian family for development time, basis dimension (k) = 6). Significant model GAMM estimations are shown with solid lines, not significant model estimations with dashed lines. Model statistics are given in Tab. S3. Only GAMM predictions are shown for clarity, but see Fig. S3.2 for observed data Days: Days of the year.

3.4 Discussion

In this study, we investigated seasonal patterns of nest-building activities, species turnover, ecological rates and life-history traits at lower and higher elevations for three different functional groups of cavity-nesting Hymenoptera in a tropical mountain ecosystem. Seasonal patterns in nest-building activity occurred in all investigated groups, i.e. bees, caterpillar-hunting wasps and spider-hunting wasps at lower and higher elevations. Additionally, we found seasonal variation in parasitism and natural mortality rate, sex ratios, development time and body size. Our data indicate that seasonal patterns are often driven by temperature, relative humidity, an interaction of both variables and also flower abundance, in the case of bees. By showing seasonal patterns in different ecological rates and life-history traits, at different elevations and in different functional groups, our study provides new insights about the community composition and biotic interactions of insects under changing environmental conditions. Furthermore, it is the first study that provides seasonal patterns in cavity-nesting hymenopterans with simultaneous comparison at different elevations. So far, seasonal patterns in cavity-nesting hymenopterans have mainly been investigated in temperate regions (Tylianakis *et al.*, 2005; Matos *et al.*, 2016; Sabino & Antonini, 2017) at similar elevations and less intensive with regard to groups, ecological rates and life-history traits.

3.4.1 Synchronised nesting-activity between bees and wasps at lower elevations

In line with our prediction, the magnitude of seasonality in nest-building activity differed between functional groups, but opposing to our assumption it was synchronised at lower elevations with a strong peak at the end of the short rainy season for bees, caterpillar- and spider-hunting wasps. Seasonal patterns are believed to differ between species, families or other taxonomic groups (Wolda & Roubik, 1986; Sirohi *et al.*, 2015; Osorio-Canadas *et al.*, 2018), but except from small differences, we rather find differences in magnitude than in seasonal timing of activity. Patterns were different at higher elevations, even though nesting activity of caterpillar- and spider-hunting wasp was also synchronised there. At higher elevations, with much lower nest densities, nesting activity was less synchronised and generally less subject to strong fluctuations. Nevertheless, synchronised seasonal patterns seem to be present at both elevations. The period of nesting is the most critical life stage, because unlike social species, females spend most of their time with constructing and provisioning their nests (Morato & Martins, 2006). Therefore, it must be carefully adapted to the rhythms of nature. Warmer temperatures and the start of the short rainy season in October seemed to trigger different insect groups at lower elevations and synchronise their life-cycles. Most insect species in the tropics peak in the rainy season or in the transition time between rainy and dry season (Frith & Frith, 1990; Novotny & Basset, 1998; Tidon, 2006; da Silva *et al.*, 2011). However at higher elevations, the synchronisation of life-cycles seemed to be delayed and took place between January and March. This is the transition time (short dry season) before the start of the long rainy season. Temperatures are still high and the air humidity already slowly rises. This period seems to enable nesting by all investigated insect groups before the nest-building activity drops with the long rainy season, a period with high mean humidity between 80 and 100%. However, we observed the lowest nesting-activity during the long dry season, which follows the long rains and is colder than the short dry season (Yang *et al.*, 2015). During this period, the environmental conditions seem to be fundamentally hostile to cavity-nesting Hymenoptera, because the nesting-activity dropped in all functional groups. Decreasing abundances of different groups of flies, which are generally able to persist under extreme climate conditions, have also been observed in the coldest and driest months (Lambkin *et al.*, 2011). However, even though we find synchronisation of seasonal patterns among bees, caterpillar-hunting and spider-hunting wasps, their nutritional requirements are very different and resources may not be equally available to them (Boulter *et al.*, 2011). We found that different drivers were responsible for the similar, but still different seasonal nest-building activities in the different functional hymenopteran groups. The abundance of flowering plants, which was closely linked to relative humidity at lower elevations, was the strongest trigger for the nest-building activity of bees. Many studies show that the spatial variation in floral resource abundance and diversity is related to bee diversity, but the linkage between seasonal dynamics of flower resources and bees has been rarely achieved (e.g. Abrahamczyk *et al.* (2011)). By analysing lower and higher elevations separately, we find that the nest-building activity of bees is linked to flower abundance at lower, but less so at higher elevations. This may indicate that bees are most adapted to the onset of the rainy season at lower elevations. Temperature, which has been shown to be one of the most important factors for driving species diversity patterns at Mt. Kilimanjaro (Peters *et al.*, 2016b), was positively correlated with the nest-building activity of spider-hunting wasps, while relative humidity decreased it. Spider abundances at Mt. Kilimanjaro were found to be positively correlated with temperature (Röder *et al.*, 2017) and might therefore offer high resource abundance for their predators in habitats with higher temperatures. Indeed, high temperatures correlated with a high number of nests in *Trypoxylon*, a genus of spider-hunting wasps (Loyola & Martins, 2006). The nest-building activity of caterpillar-hunting wasps only increased when temperature and relative humidity were high, but decreased when temperature was high, but relative humidity was below 75 %. This decrease of nesting-activity with high temperature and low relative humidity is prominent at low elevations. Excessively high temperatures, especially together with low relative humidity at lower elevations might have a direct, negative impact on caterpillar abundance. This may result from caterpillars being poorly protected from excessively high temperatures due to their relatively soft cuticle. Another explanation might be that because the abundance of predators, like ants and spiders increases with temperature (Kaspari *et al.*, 2000; Röder *et al.*, 2017), the abundance of their prey, such as caterpillars, might decrease, causing decreasing nest-building activities of caterpillar-hunting wasps due to lower resource availability. On the other hand, nest-building activity of caterpillar-hunting wasps was also low when relative humidity was high, but temperatures low. Even though caterpillars prefer wet conditions, foraging of caterpillar-hunting wasps might be restricted when temperatures are too cold (temperature-mediated resource exploitation hypothesis (Classen *et al.*, 2015)). Overall, we assume that in both wasp groups temperature indirectly influences nest-building activity of wasps at lower elevations by temporarily limiting the availability of their resources.

Inconsistent seasonal patterns at different elevations have also been found in other studies of tropical insects for Hymenoptera (Boulter *et al.*, 2011; Bishop *et al.*, 2014), Coleoptera (Wardhaugh *et al.*, 2018), Diptera (Lambkin *et al.*, 2011) and Lepidoptera (Beck *et al.*, 2017). Different seasonal patterns at different elevations are linked to differences in life-history, behaviour and interactions with other organisms associated with changing environmental conditions (Hodkinson, 2005). At Mt. Kilimanjaro, different elevations harbored distinct insect communities, indicating that bees and wasps rather bridge unfavourable conditions via dormancy, than by moving up- or downwards, e.g. the dry-season community at higher elevation did not overlap with the rainy-season community at lower elevation (Appendix S2, Fig. S2). Although seasonal elevational migration is a conceivable pattern (Hunt *et al.*, 1999; Wardhaugh *et al.*, 2018), it might be rather rare for cavity-nesting Hymenopterans, because they show high nest-site fidelity. This pattern might differ for social species where migration has been found between seasons (Stein *et al.*, 2018), though not along an elevational gradient. Furthermore, the habitat types along the elevational gradient of Mt. Kilimanjaro change and along with it also the plant communities. The changing plant communities in the distinct habitats, might offer different resources and nest-building materials for insects, which make migrations difficult.

3.4.2 Seasonality in ecological rates

We also found seasonal patterns for ecological rates, but with strong variation among functional groups and elevational belts. The parasitism rate of the caterpillar-hunting wasps at lower elevations peaked in the transition times between dry and rainy seasons and was time-delayed to the nest-building activity of the caterpillar-hunting wasps at lower and higher elevations. Parasitoids are often well synchronised to their hosts (Martins *et al.*, 1999; Sabino & Antonini, 2017) and are often host density-dependent (Gazola & Garófalo, 2009; Mesquita & Augusto, 2011; Araújo *et al.*, 2016). In our study, the number of parasitised brood cells followed the seasonal pattern of the nest-building activity of the hosts with the highest number of parasitised brood cells at the beginning, middle and end of the year. But increased parasitism rates may be also correlated with decreasing host abundances (Elekcioglu & Uygun, 2013). The nest-building activity of caterpillar-hunting wasps at lower elevations dropped stronger and increased faster than the parasitism and therefore, the parasitism rate showed a time-delayed pattern in relation to the nest-building activity of the host. Parasitism rates in spider-hunting wasps were similar at lower and higher elevations. No spider-hunting nests occurred in the middle of the year. While we observed that the nest-building activity of spider-hunting wasps decreased towards the middle of the year, showing a similar pattern for lower and higher elevations, the number or parasitised cells did not drop as strong so that the parasitism rate increased as for the parasitism rate of the caterpillar-hunting wasps. An earlier study found stable parasitism rates throughout the season for bee and wasp nests, but the parasitism rates were correlated to equally stable abundances of the hosts (Osorio-Canadas *et al.*, 2018), which we only see in bee nests. On the one hand, both, the parasitoids of the caterpillar-hunting and spider-hunting wasps are closely oriented to their hosts and on the other hand temperature is very important for parasitoid activity (van Huis *et al.*, 1994; Canevazzi & Noll, 2011; Forrest & Chisholm, 2017). Activity optima are different for different organisms. Bees, for instance, can be active at lower temperatures than their parasites, so that an increase in temperature can lead to an increase in parasitism rates (Forrest & Chisholm, 2017). We further find that the parasitism rate of wasps was rather temperature-dependent, in contrast to other findings suggesting that interactions between predators and parasitoids are usually driven by precipitation (Kishimoto-Yamada & Itioka, 2015). Parasitism rates of caterpillar-hunting wasps increased with temperature at lower elevations and increased with temperature for spider-hunting wasps at higher elevations if the relative humidity was not too high. For relative humidity between 70 and 100 % the parasitism rate of spider-hunting wasps decreased with temperature, suggesting that humidity may become a limiting factor for parasitoids in combination with high temperatures.

The natural mortality, which is in our case mortality due to unknown reasons like extreme climatic conditions, pathogens or little food provisions, showed similar seasonal patterns for bees, caterpillar- and spider-hunting wasps at lower elevations. It increased with relative humidity for bees and caterpillar-hunting wasps and for spider-hunting wasps with simultaneous low temperature. Under high humidity conditions larvae and food provisions might be infected with pathogens, causing a higher mortality of the host. Simultaneously, the natural mortality rate decreased with temperature for bees and spider-hunting wasps if the relative humidity was high enough. Hot temperatures and low humidity at the same time could increase the risk of desiccation (Child, 2007). But seasonal patterns of natural mortality are difficult to link to environmental factors, as in reality, the environmental conditions during the whole development time are affecting the mortality rate and not only the conditions during the phase of nest building.

3.4.3 Seasonality in life-history traits

The sex ratio of bees changed seasonally at lower elevations, with more males occurring at both the beginning and the end of the year. However, the sex ratio did not vary seasonally for any of the studied groups at higher elevations. The strong dependence of bees on flowering plants was reflected in higher proportion of males when resource availability was low (Kümmerli & Keller, 2011). The higher proportion of males in correlation with lower amount of food resources has also been reported for wasps (Strohm & Linsenmair, 1997), but in our study resources for wasps does not seem to be limiting.

Development times tended to be longer for bee, caterpillar-hunting and spider-hunting wasp nests constructed between April and May at lower elevations. Delayed emergence has been reported for bees and spider-hunting wasps in the tropics. It is assumed to be an apparent response to avoid the most inconvenient season of the year through a state of dormancy (Martins *et al.*, 1999; Zanette *et al.*, 2004; Sabino & Antonini, 2017). Even though development times can vary between bee and wasp species and even within species, with some individuals showing direct development and others going into diapause (Martins *et al.*, 2001; Buschini, 2007), the development time seemed to be uniformly longer here. In these months, the larvae of all three groups needed between 100 to 200 days for their development to adults, even though the pattern was not seasonally significant in bees. This translated into an emergence period after the long rainy season between July to October. Humidity played an important role in the development time of both wasp groups at lower elevations, with increasing air humidity leading to longer development times. Our findings hence suggest that an increase of humidity may trigger a kind of diapause, to prevent the emergence of the brood in the long rainy season. Our results thus contrast previous findings, showing a decreasing development time with increasing relative humidity for a heteropteran host and its hymenopteran parasitoid (Cave & Gaylor, 1988). Hence, the response of insects to ambient humidity may be different for parasitoids and depend on the host order and the range of humidity, with possibly more adverse effects at very low or high humidity levels. Furthermore, emergence in tropical insects is usually triggered by rainfall or high humidity (Wolda, 1988), which should have resulted in development times bridging the long dry season at lower elevations. This seemed to be rather true for the spider-hunting wasps, but not for the caterpillar-hunting wasps, which generally had a shorter development time during the long rainy seasons. But since the development time could only be estimated within a coarse timeframe, further studies are needed to answer how the life-history timing of tropical insects is matched to environmental conditions in areas with strong climatic seasonality.

3.5 Conclusion

Insects have to adapt to variable climatic conditions and resource availability in areas with clear seasonality, such as dry and rainy seasons. That entails suitable periods for the assemblage of resources, reproduction and development, but also for associated natural enemies. The time and extent of these periods are considered to be group-specific and variable between lower and higher elevations and the drivers and mechanisms behind them are still barely understood. In this study, we investigated seasonal patterns of three functionally different Hymenoptera groups, i.e. pollinators (bees), first-order and second-order predators (caterpillar-hunting and spider-hunting wasps, respectively), at lower and higher elevations at Mt. Kilimanjaro. We found strong seasonality in nest-building activity in all groups and both areas, but seasonal patterns were also reflected in different life-history traits and parasitism rates. Seasonal patterns were more often explained by climate and resources at lower than higher elevations. Even though rainfall is regarded as the most important driver for seasonality of tropical insects, we did not find a consistent driver for seasonal patterns. Temperature, relative humidity, the interaction between temperature and humidity and resource availability all correlated with different traits and insect groups. Therefore, trade-offs between for instance high resource availability and high parasitism pressure seemed to be low for the investigated groups because they were not enhanced by the same drivers. But temperature and relative humidity interacted in complicated ways and enhanced or suppressed for example the nest-building activity of caterpillar-hunting wasps. A deeper and further study of ecological rates and life-history traits is therefore indispensable, to understand the diverse factors which shape insect communities under changing environmental conditions. The interactive effects of elevational climate gradients and seasonality might be a useful approach to perceive possible future responses of insect communities to climate warming. At Mt. Kilimanjaro, seasonality in climate has changed during the last years, especially during the long rainy season (Otte *et al.*, 2017). This may lead to immense challenges for insects that are closely adapted to seasonal changes and highlights the importance of taking a closer look when studying the seasonality in species abundances and traits, especially in a world of rapid global change.

3.6 Appendix

Appendix S1: Seasonal variation of traits

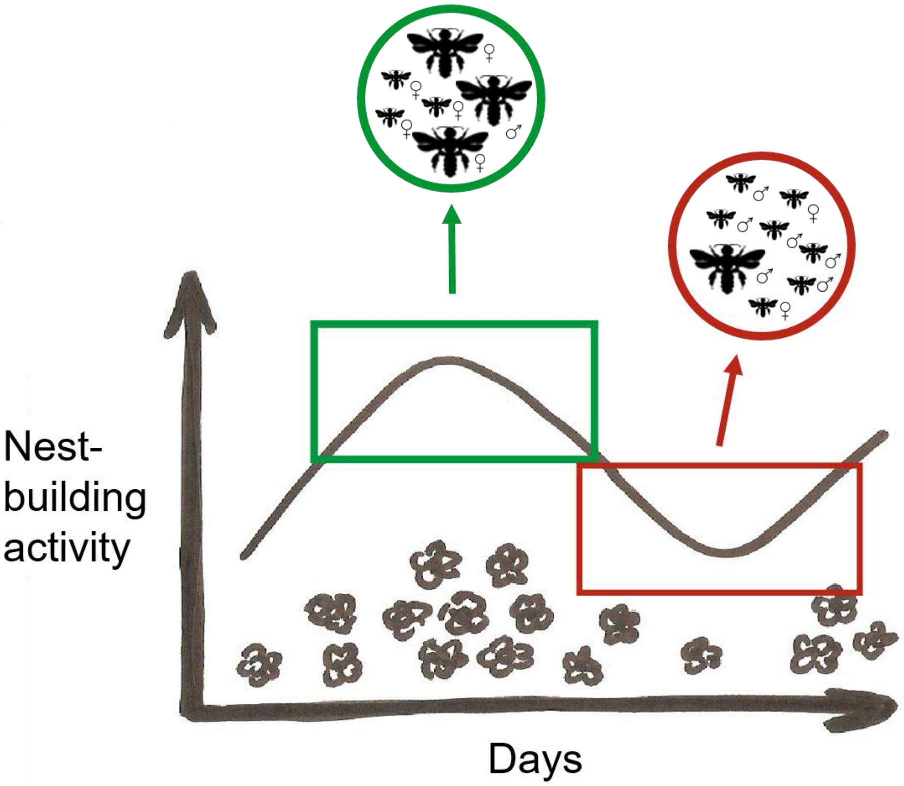


Fig. S1: Illustration of how seasonal variation in food resources, associated with seasonal variation in nest-building activity might be also reflected in seasonal variation of life-history traits, such as the percentage of male individuals in the community or the mean body size. In this example, the green box is a season with a high availability of resources and a high nest-building activity. For the traits this might be reflected into a higher proportion of females than males and high proportion of large species. The red box is a season with a low availability of resources and a low nest-building activity. For the traits this might lead to a higher proportion of males than females and high proportion of small species.

Appendix S2: Seasonal turnover in trap-nesting species compositions in different elevations

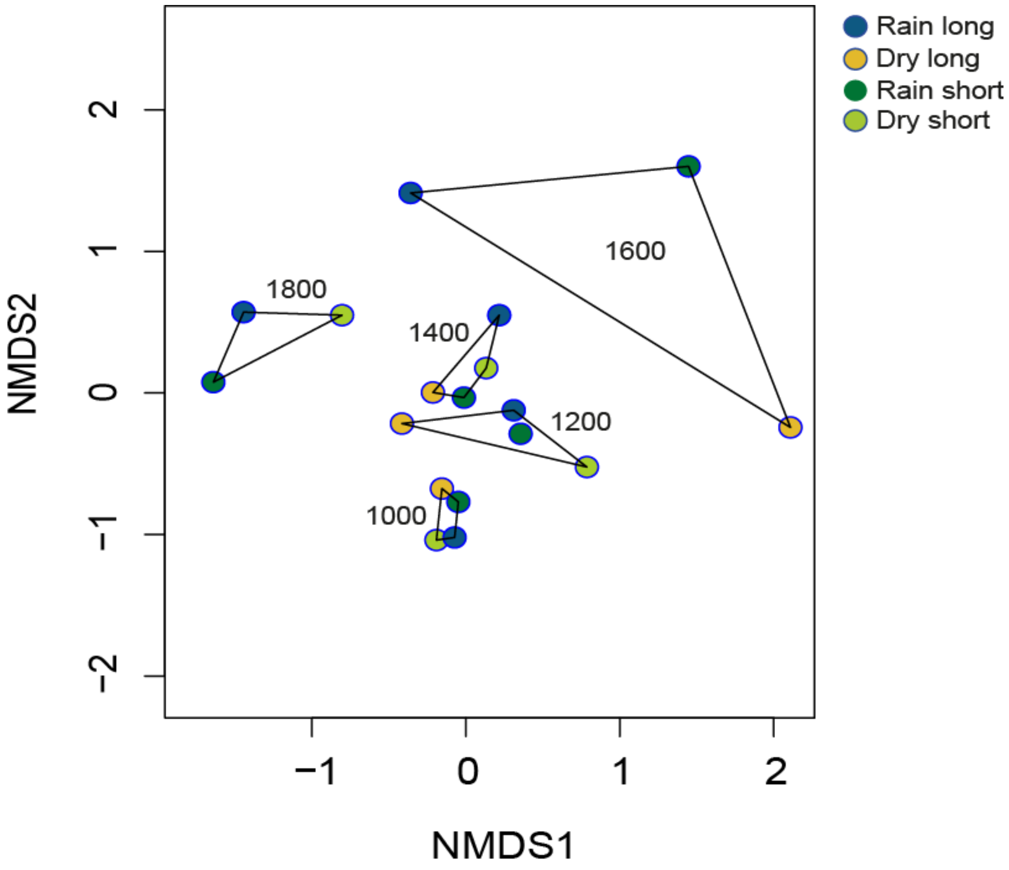


Fig. S2: Nonmetric multidimensional scaling (NMDS) two-dimensional plot of host species of cavity-nesting communities at Mt. Kilimanjaro up to 1,000, 1,200, 1,400, 1,600 and 1,800 m a.s.l. for the long rainy season (Rain long: March-May), long dry season (Dry long: June-September), short rainy season (Rain short: October-December) and short dry season (Dry short: January-February). The ordination is based on Bray-Curtis distances. A low stress values indicates a high goodness-of-fit of the ordination. Stress value = 0.10.

Appendix S3: Seasonal patterns of nest-building-activity, ecological rates and life-history traits

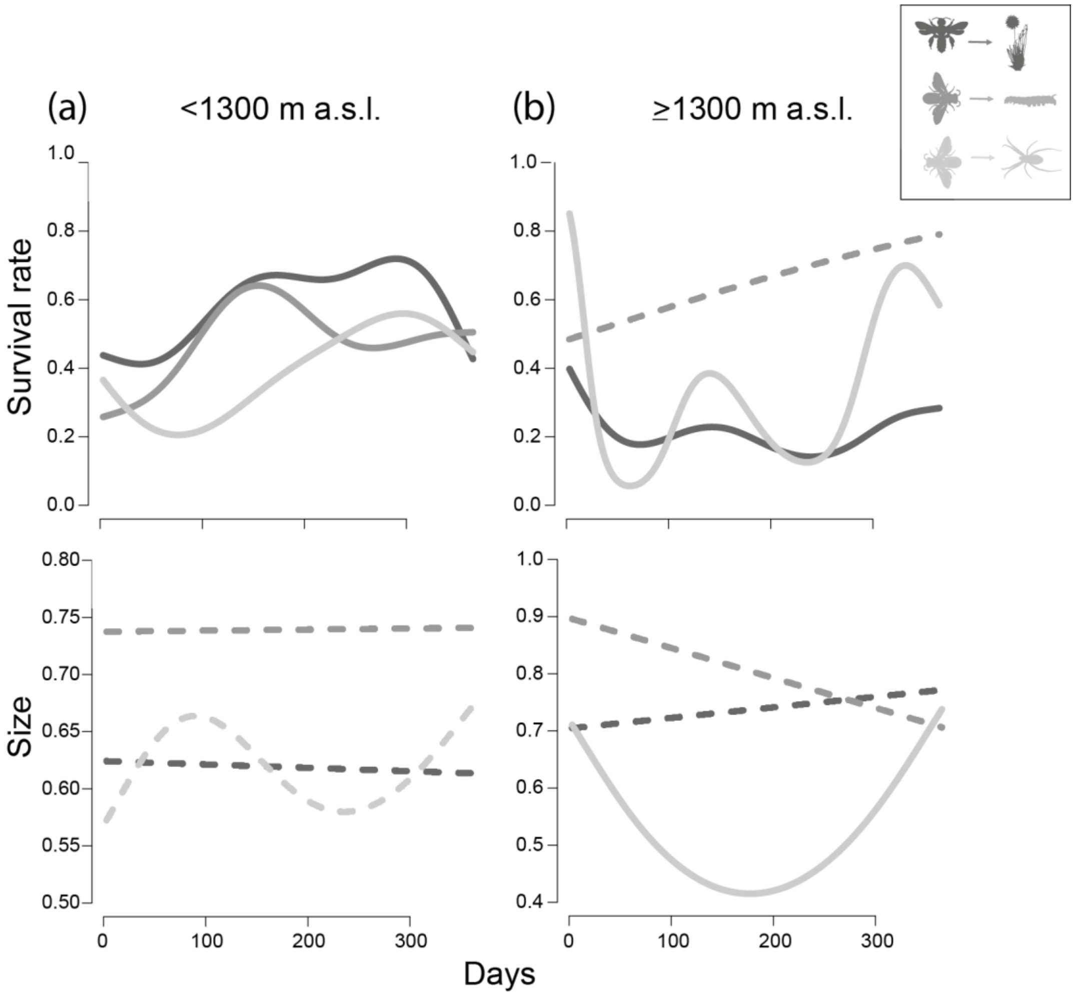


Fig. S3.1: Seasonal variation in survival rates and sex ratios of bees, caterpillar-hunting wasps and spider-hunting wasps at elevations (a) below and (b) above 1,300 m a.s.l. at Mt. Kilimanjaro. Generalised additive mixed models (GAMM) were used to estimate trends of seasonality (Binomial family for survival rate, Gaussian family for size, basis dimension (k) = 6). Significant model estimations are shown with solid lines, not significant model estimations with dashed lines. Model statistics are given in Tab. S3. Days: Days of the year.

Tab. S3: Seasonal variation of ecological rates and life-history traits of bees, caterpillar-hunting wasps (Wlep) and spider-hunting wasps (Wara) at elevations below and above 1,300 m a.s.l..

Trait	Hymenoptera group	Elevation	F-Statistics	p-values
Parasitism rate	Bees	<1300	$F_{1,152}=9.63$	$p<0.01$
		≥1300	$F_{3,48}=128.2$	$p<0.001$
	Wlep	<1300	$F_{5,100}=6.19$	$p<0.001$
		≥1300	$F_{2,48}=4.61$	$p=0.02$
	Wara	<1300	$F_{4,82}=21.07$	$p<0.001$
		≥1300	$F_{3,31}=11.1$	$p<0.001$
Parasitised brood cells	Bees	<1300	$F_{5,148}=53.65$	$p<0.001$
		≥1300	$F_{5,46}=4.27$	$p<0.001$
	Wlep	<1300	$F_{5,100}=15.75$	$p<0.001$
		≥1300	$F_{1,49}=25.4$	$p<0.001$
	Wara	<1300	$F_{5,81}=15.35$	$p<0.001$
		≥1300	$F_{3,31}=8.1$	$p<0.001$
Natural mortality rate	Bees	<1300	$F_{5,148}=68.23$	$p<0.001$
		≥1300	$F_{5,46}=17.11$	$p<0.001$
	Wlep	<1300	$F_{3,102}=21.3$	$p<0.001$
		≥1300	$F_{1,49}=14.8$	$p<0.001$
	Wara	<1300	$F_{5,82}=13.78$	$p<0.001$
		≥1300	model failed to converge	
Survival rate	Bees	<1300	$F_{5,148}=69.15$	$p<0.001$
		≥1300	$F_{5,46}=15.8$	$p<0.001$
	Wlep	<1300	$F_{4,101}=21.37$	$p<0.001$
		≥1300	$F_{1,49}=21.27$	$p<0.001$
	Wara	<1300	$F_{4,82}=21.77$	$p<0.001$
		≥1300	$F_{5,29}=10.55$	$p<0.001$

Trait	Hymenoptera group	Elevation	F-Statistics	p-values
Sex ratio	Bees	<1300	$F_{3,108}=3.50$	$p<0.01$
		≥1300	$F_{1,23}=1.74$	$p=0.20$
	Wlep	<1300	$F_{1,59}=6.53$	$p=0.01$
		≥1300	$F_{2,31}=8.38$	$p=0.02$
	Wara	<1300	$F_{1,35}=0.39$	$p=0.54$
		≥1300	$F_{1,11}=3.64$	$p=0.08$
Development time	Bees	<1300	$F_{1,132}=1.35$	$p=0.25$
		≥1300	$F_{1,29}=0.02$	$p=0.88$
	Wlep	<1300	$F_{2,71}=3.90$	$p=0.04$
		≥1300	$F_{1,32}=3.01$	$p=0.09$
	Wara	<1300	$F_{4,52}=6.81$	$p<0.001$
		≥1300	$F_{2,21}=1.29$	$p=0.22$
Size	Bees	<1300	$F_{1,154}=0.08$	$p=0.77$
		≥1300	$F_{1,51}=0.43$	$p=0.52$
	Wlep	<1300	$F_{1,105}=0.00$	$p=0.96$
		≥1300	$F_{1,49}=5.92$	$p=0.02$
	Wara	<1300	$F_{3,82}=1.86$	$p=0.10$
		≥1300	$F_{2,33}=4.31$	$p=0.01$

The effect of season on life-history traits was tested with generalised additive mixed models (GAMM).

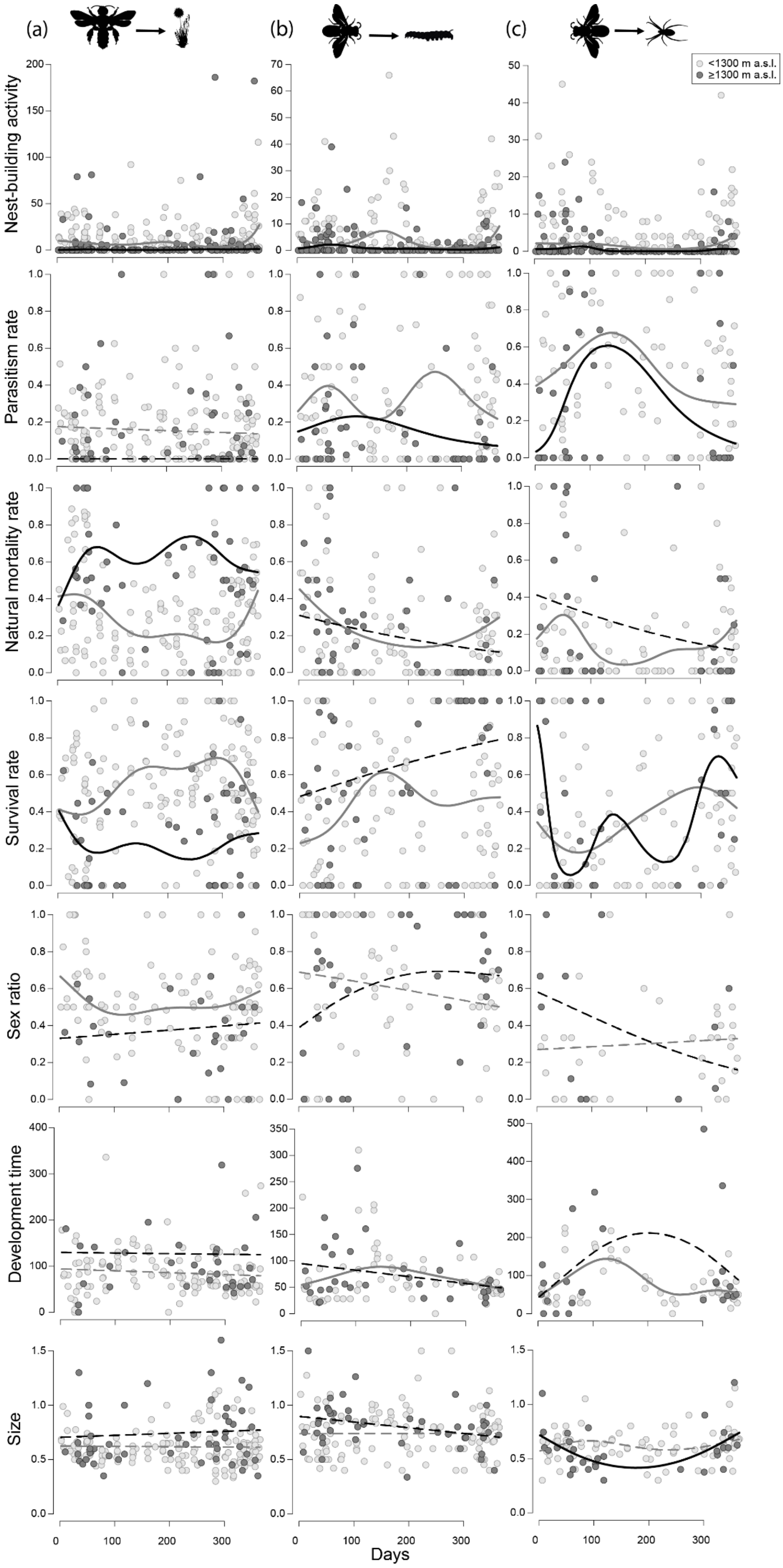


Fig. S3.2: Seasonal variation in traits of (a) bees, (b) caterpillar-hunting wasps and (c) spider-hunting wasps at elevations below and above 1,300 m a.s.l. at Mt. Kilimanjaro. Generalized additive mixed models (GAMM) were used to estimate trends of seasonality (Binomial family for sex ratio and parasitism, natural mortaliy and survival rate, otherwise Gaussian family, basis dimension (k) = 6). Significant model estimations are shown with solid lines, not significant model estimations with dashed lines. Model statistics are given in Tab. S3. Days: Days of the year.

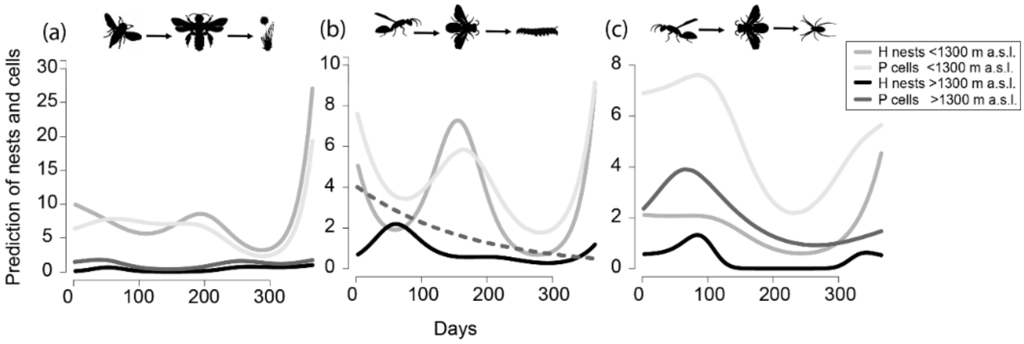


Fig. S3.3: Seasonal variation nests and parasitised brood cells of (a) bees, (b) caterpillar-hunting wasps and (c) spider-hunting wasps at elevations below and above 1,300 m a.s.l. at Mt. Kilimanjaro. Generalised additive mixed models (GAMM) were used to estimate trends of seasonality (Gaussian family, basis dimension (k) = 6). Significant model estimations are shown with solid lines, no seasonal model estimations patterns with dashed lines. Model statistics are given in Tab. S3. Days: Days of the year, H nests: predicted number of host nests, P nests: predicted number of parasitised brood cells.

Appendix S4: Synchronisation of seasonal patterns in nest-building activity, ecological rates and life-history traits and environmental variables

Tab. S4: Linear mixed effect models (LMMs) and generalised linear mixed effects models (GLMMs, family = binomial) explaining seasonal patterns of nest-building activity, ecological rates and life-history traits by temperature, relative humiditiy, and flower abundance and an interaction between temperature and relative humidity at elevations below and above 1,300 m a.s.l..

Trait	Hym. group	Ele-vation	Temperature		Rel. Humidity		T*rH		Flower abundance	
			t/z-value	p-value	t/z-value	p-value	t/z-value	p-value	t/z-value	p-value
Nest-building activity	Bees	<1300	t=0.22	p=0.83	t=-0.05	p=0.96	t=1.52	p=0.13	t=2.42	p=0.02
		≥1300	t=0.03	p=0.98	t=-0.80	p=0.43	t=-0.78	p=0.43	t=0.16	p=0.88
	Wlep	<1300	t=-2.38	p=0.02	t=-1.58	p=0.12	t=1.62	p=0.11	t=-0.17	p=0.86
		≥1300	t=1.45	p=0.15	t=-0.06	p=0.95	t=-0.27	p=0.79	t=0.16	p=0.87
	Wara	<1300	t=1.78	p=0.08	t=1.61	p=0.11	t=1.54	p=0.13	t=0.52	p=0.60
		≥1300	t=1.28	p=0.20	t=0.16	p=0.87	t=-0.49	p=0.63	t=-0.08	p=0.94
Parasitism rate	Wlep	<1300	z=-1.73	p=0.08	z=0.61	p=0.55	z=0.23	p=0.82	z=-1.30	p=0.19
		≥1300	model failed to converge							
	Wara	<1300	z=1.21	p=0.23	z=1.23	p=0.22	z=0.81	p=0.42	z=-1.23	p=0.26
		≥1300	z=1.34	p=0.17	z=1.00	p=0.32	z=-2.70	p<0.01	z=1.02	p=0.31
Natural mortality rate	Bees	<1300	z=-1.55	p=0.12	z=0.51	p=0.61	z=1.63	p=0.10	z=2.18	p=0.03
		≥1300	z=0.40	p=0.69	z=2.75	p<0.01	z=-2.63	p<0.01	z=-1.62	p=0.11
	Wlep	<1300	z=1.07	p=0.28	z=0.21	p=0.83	z=-0.40	p=0.69	z=0.32	p=0.75
	Wara	<1300	z=-0.28	p=0.78	z=-2.62	p<0.01	z=-1.08	p=0.28	z=-1.26	p=0.21
Survival rate	Bees	<1300	z=2.06	p=0.04	z=-1.57	p=0.12	z=-1.77	p=0.08	z=2.27	p=0.02
		≥1300	z=0.83	p=0.41	z=-0.99	p=0.32	z=1.21	p=0.23	z=1.85	p=0.06
	Wlep	<1300	z=1.08	p=0.28	z=-0.62	p=0.53	z=0.26	p=0.80	z=1.19	p=0.23
	Wara	<1300	z=-1.29	p=0.20	z=0.83	p=0.40	z=0.38	p=0.71	z=1.92	p=0.05
		≥1300	z=0.47	p=0.64	z=-1.68	p=0.09	z=3.38	p<0.001	z=0.18	p=0.85
Sex ratio	Bees	<1300	z=-0.80	p=0.42	z=-0.28	p=0.80	z=-1.17	p=0.24	z=-1.95	p=0.05
Development time	Wlep	<1300	t=1.18	p=0.25	t=2.68	p=0.01	t=1.54	p=0.13	t=0.95	p=0.35
	Wara	<1300	t=2.39	p=0.02	t=3.45	p<0.01	t=1.06	p=0.30	t=0.82	p=0.42
		≥1300	t=-0.59	p=0.59	t=-0.25	p=0.81	t=-0.37	p=0.73	t=0.52	p=0.63
Size	Wara	<1300	t=1.31	p=0.20	t=1.05	p=0.30	t=-0.91	p=0.37	t=-0.21	p=0.84
		≥1300	t=-0.05	p=0.96	t=-0.89	p=0.39	t=1.34	p=0.21	t=-0.65	p=0.53

Significant positive and negative correlations between nest-building activity and explanatory variables can be concluded from positive and negative t-values, respectively. Hym. group: Hymenoptera group, T: Temperature, rH: relative Humidity, T×rH: Interaction between Temperature and relative Humidity, Wlep: caterpillar-hunting wasps, Wara: spider-hunting wasps.

Appendix S5: Species table

Tab. S5: Table of host species with their larval food and their occurrences throughout the year and in different habitats. C: coffee monoculture, G: semi-natural grassland, H: homegarden, M: maize field, S: natural savanna.

Taxon	Food	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Apidae														
Anthidiellum somaliense	pollen	S	S		S	M,S	M	S	S				M	**M,S**
Ctenoplectra centinorii	pollen	C	C	C	C		M	C	C	C	C	C	C,M	**C,M**
Gronoceras felina	pollen	H,S	M,S				M	M	G,M,S	G,M,S	H,M,S	G,H,S	G,H,M	**G,H,M,S**
Heriades sp.1	pollen			G										**G**
Heriades sp.2	pollen		C					M			C,M	M	M,S	**C,M,S**
Macrogalea sp.1	pollen					M						M		**M**
Megachile admixta	pollen		C	C		M		M	G,M	G,M	C,G,M,S	C,M,S	C,M,S	**C,G,M,S**
Megachile demeter	pollen	M,S	M,S	M,S	M,S	S	S	M,S	M	M,S	S	S	M,S	**M,S**
Megachile fervida	pollen		M,S	M,S	M,S	M,S	M	M,S	M	M,S	M,S	M,S	M,S	**M,S**
Megachile maxillosa	pollen	M	M		S	S	S	M					M,S	**M,S**
Megachile mossambica	pollen	M,S	C,M,S	S		M,S	G,S	M,S	G,M,S	G,S	G,S	S	G,M,S	**C,G,M,S**
Megachile rufipennis	pollen	S												**S**
Megachile rufiventris	pollen								M					**M**
Megachile semierma	pollen	M		M		M	M		M	G,M,S	C,G,M	G,M	M,S	**C,G,M,S**
Megachile sheppardi	pollen		M		S	S	S	S						**M,S**
Megachile sinuate	pollen		S			M						M	M	**M,S**
Megachile sp.1	pollen												M	**M**
Megachile sp.2	pollen								M					**M**
Megachile sp.3	pollen								M					**M**
Megachile sp.5	pollen	S	M,S	M	M,S	M,S	S			S		M,S	M,S	**M,S**
Megachile sp.6	pollen									H	H	G,H	M	**G,H**
Megachile sp.7	pollen	M			M									**M**
Megachile sp.8	pollen	M												**M**
Megachile sp.9	pollen		M											**M**
Megachile sp.12	pollen								M					**M**
Megachile ungulata	pollen			C							C			**C**
Noteriades sp.1	pollen	S	M,S	M,S	M,S	M,S	M,S	M,S	M,S	M,S	M,S	M,S	M,S	**M,S**
Noteriades sp.2	pollen			S						M				**M,S**
Noteriades sp.3	pollen	C									S			**C,S**
Nothylaeus sp.1	pollen		C										C	**C**
Nothylaeus sp.2	pollen			H									C	**C,H**
Nothylaeus sp.4	pollen			S				M		S	S	S		**M,S**
Nothylaeus sp.6	pollen												C	**C**
Nothylaeus sp.7	pollen								M					**M**
Nothylaeus sp.8	pollen												C	**C**
Pseudoheriades sp.1	pollen	M,S	M,S	S	S	M,S	S	M,S	M,S	M,S	S	M,S	M,S	**M,S**
Stenoheriades sp.1	pollen		S	S		S								**S**
Wainia sp.1	pollen												S	**S**
Crabronidae														
Dasyproctus sp.1	NA		C											**C**
Pison sp.1	spiders	C,H		C,H				S		C			C,G	**C,G,H,S**
Polemistus sp.1	aphids	C	C	C	C,H			C	C		C		C	**C,H**
Trypoxylon sp.1	spiders	C,M									C	C	C	**C,M**
Trypoxylon sp.2	spiders	C		G					C		C	C		**C,G**

Taxon	Food	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Sphecidae														
Chalybion sp.1	spiders	G,M,S	G	C,S	C,M	M				M	C,S	C,G,M,S	C,G,M,S	**C,G,M,S**
Chalybion sp.2	spiders							M	M	M			S	**M,S**
Chalybion sp.3	spiders	M,S	M,S	M,S	S	M,S		M	M		S	M,S	M,S	**M,S**
Chalybion sp.4	spiders							C					C	**C**
Chalybion sp.5	spiders					S			M	M	S	S		**M,S**
Isodontia sp.1	locusts			H				S					M	**H,M,S**
Isodontia sp.2	locusts	S	M,S	S		S		S				S		**M,S**
Isodontia sp.3	locusts								M					**M**
Pompilidae														
Auplopus sp.1	spiders												C	**C**
Auplopus sp.2	spiders	C												**C**
Auplopus sp.3	spiders												M	**M**
Dipogon sp.2	spiders			H									C,H	**C,H**
Dipogon sp.3	spiders												H	**H**
Pepsinae sp.1	spiders			H										**H**
Eumeninae														
Ancistrocerus sp.1	caterpillars										H			**H**
Ancistrocerus sp.2	caterpillars										H	G,H		**H,G**
Anterhynchium sp.1	caterpillars							H						**H**
Antodynerus sp.1	caterpillars	H		S	S									**H,S**
Antodynerus sp.2	caterpillars		M				M		M					**M**
Antodynerus sp.3	caterpillars					S				S			M	**M,S**
Antodynerus sp.5	caterpillars											S		**S**
Antodynerus sp.6	caterpillars	C,H	C	C	C			G			C	H	C,H,M	**C,H,G,M**
Antodynerus sp.7	caterpillars	H	H	H				H				H	H	**H**
Antodynerus sp.8	caterpillars	H	H					H					H	**H**
Antodynerus sp.9	caterpillars			S										**S**
Antodynerus sp.10	caterpillars												S	**S**
Antodynerus sp.11	caterpillars												C	**C**
Antodynerus sp.12	caterpillars		H					H	S			H	H	**H,S**
Antodynerus sp.13	caterpillars			C								H	C,H	**C,H**
Antodynerus sp.14	caterpillars			H				H						**H**
Antodynerus sp.15	caterpillars											S		**S**
Antodynerus sp.16	caterpillars				M									**M**
Eustenancistrocerus sp.1	caterpillars	M												**M**
Eustenancistrocerus sp.2	caterpillars					S								**S**
Leptochilus sp.1	caterpillars	S	S		S									**S**
Pseudonortonia sp.1	caterpillars												C	**C**
Pterocheilus sp.1	caterpillars										H			**H**
Rhynchium sp.1	caterpillars	C,H,M,S	C,G,M	C,G,H,M	C,M	H,M,S	H,M	C,G,H,M,S	G,M	G,M	C	C,H,M,S	C,G,H,M,S	**C,G,H,M,S**
Subancistrocerus sp.1	caterpillars	H											H	**H**
Tachymenes sp.1	caterpillars	S	S				M					M,S		**M,S**
Tachymenes sp.2	caterpillars		C											**C**
Tachymenes sp.3	caterpillars											S		**S**
Tricarinodynerus sp.1	caterpillars	S						M						**M,S**
Tricarinodynerus sp.2	caterpillars			S	S	S						S	M	**M,S**
NA														
Aculeata sp.W1	cicads											H		**H**

Chapter 4

Cryptic species and hidden ecological interactions of halictine bees along an elevational gradient

Changes of abiotic and biotic conditions along elevational gradients represent serious challenges to organisms that require evolutionary and ecological adaptations. Information on such adaptations beyond morphological features are currently sparse for insects, and especially bees. Here, we used molecular methods to study hidden traits, biotic interactions and phylogenetic relationships of halictid bees of the genus *Lasioglossum* along a 2900 m elevational gradient at Mt. Kilimanjaro, Tanzania. We detected a strong phylogenetic species turnover of morphologically undistinguishable taxa, changes in cuticular hydrocarbon (CHC) profiles, pollen resource diversity and the gut and body surface microbiome of bees. At high elevations increased proportions of saturated compounds in CHC profiles indicate physiological adaptations to prevent desiccation. More specialised diets with higher proportions of Asteraceae pollen imply constrains by food resources. Interactive effects of climatic conditions on gut and surface microbiomes, CHC profiles and pollen diet suggest complex feedbacks among abiotic conditions, ecological interactions, physiological adaptations and phylogentic constrains as drivers of halictid bee communities at Mt. Kilimanjaro.

4.1 Introduction

Elevational gradients challenge all organisms as they are confronted with rapid abiotic and biotic changes over short geographic distances. These changes foremost, but not exclusive, include abiotic shifts in temperature, humidity, precipitation, partial pressure of atmospheric gases, UV radiation, atmospheric turbulence and wind speed (Barry, 1992). Biotic consequences are e.g. reduced suitable time periods for development, foraging and reproduction, but also changes in quality of food and pressure by natural enemies and pathogens (Hodkinson, 2005). Manifold morphological, life-history and behavioral adaptations to the changing environment along elevational gradients have been investigated, such as changes in body and wing size or number of generations. But hidden physiological adaptations, ecological interactions and phylogenetic constrains have never been linked within one study along elevational gradients.

Tropical mountain ecosystems, such as Mt. Kilimanjaro, are ideal places to study adaptations of animals to environmental conditions at regional spatial scales (Sanders & Rahbek, 2012). Pollinators, especially bees, have key functions in ecosystems and are threatened by a variety of anthropogenic causes (Vanbergen & the Insect Pollinators Initiative, 2013). Therefore, it is of particular interest to further understand factors that influence the distribution and composition of bee communities, their traits and biotic interactions. At Mt. Kilimanjaro, bee communities have been recorded, but so far mainly morphological traits and temperature influence have been studied (Classen *et al.*, 2015; Classen *et al.*, 2017). In this study, we aim to deepen our understanding of so far neglected interactive factors which shape the ecology of bees along climatic gradients. We specifically address adaptations of cuticular chemistry, plant (food resources) and microbiome interactions, as well as potential interactions of these particular factors.

These factors are expected to undergo strong changes with increasing elevation: The composition of hydrocarbons determines desiccation (Gibbs & Rajpurohit, 2010) and other qualities of the cuticula (Menzel *et al.*, 2017). Available food plant resources change in composition (Hemp, 2006a) and richness (Peters *et al.*, 2016b) with increasing elevations (Hemp, 2006a). Associations with mutualistic bacteria may be temperature dependent in ectothermic insects. To our knowledge, the response of these factors to elevational gradients are mostly uninvestigated in bees, taken for themselves as well in context with each other.

From previous studies it is known that *Lasioglossum* bees show a widespread distribution along the elevational gradient at Mt. Kilimanjaro, from savannah lowlands up to the lower alpine zone (Classen *et al.*, 2017). *Lasioglossum* is also the largest and most widely distributed bee genus on earth and is generally considered an intriguing model system known for strong inherent variations regarding ecology, behavior (Gibbs *et al.*, 2012) and resulting adaptability to different habitats.

We are thus interested how variation of traits and ecological interactions translates into the genus's widespread occurrence along the extensive elevational gradient at the Mt. Kilimanjaro and specifically investigated the following research questions:

1. How do phylogentic relationships of halictid bees change along the elvational gradient?
2. Do cuticular chemical profiles change along the elevational gradient, particularly with respect to saturated compounds, involved in the cuticula's function as a desiccation barrier?
3. How does changing plant diversity at Mt. Kilimanjaro lead to a corresponding variation of used pollen food resources?

Does the composition of the bee's associated microbiomes change due to changes in climate, diet, cuticular chemistry and species´ phylogenies.

4.2 Methods

4.2.1 Study area and bee sampling

The study was conducted in the dry season between July and September in 2015 on the south-eastern slopes of Mt. Kilimanjaro (Tanzania, East Africa, see Fig.S1 in Appendix S1 in Supporting Information), which is the highest free-standing mountain of the world. Consequently, a lot of different habitat types are present from 700 m to 5,895 m a.s.l. and cover savannah lowlands, different mountain rainforests at mid-elevations and alpine vegetation at the highest elevations. The mean annual temperature decreases about ~0.56°C per 100 m, from

25°C in the lowlands to -8°C at the summit (Appelhans *et al.*, 2016). The mean annual relative humidity is highest in the forest belt at ~2,200 m a.s.l. (Appelhans *et al.*, 2016) and shows a hump-shaped distribution along the elevational gradient (Appendix S2, Fig. S2). We caught halictine bees on 18 sites, equally distributed in elevation, between 830 m and 3780 m. The bees were caught when sitting on flowers or soil with sterile collection jars by hand and immediately placed on ice in a thermos flask in order to keep them on not more than 0°C when walking down the mountain, which could be 2-3 days. At the research station they were frozen in a freezer until they were dissected. The dissection was done under sterile conditions under a binocular microscope. The bees were dissected in head, thorax-abdomen and gut. The thorax and abdomen were used for the analysis of the cuticular hydrocarbons. Gut and head were separately used for the analysis of the microbiome, since the surface microbiome could additionally be influenced by the composition of the cuticular chemistry. We placed the head and the gut in vials with Zymo Xpedition™ Buffer and glass beads to stabilize the bacterial DNA. We only used bees of the genus *Lasioglossum* for the analysis of pollen and microbiomes and also CHCs. *Halictus* was only included for CHCs in Fig. 2 to enlarge the sample size, but it was not included in any of the statistical analyses.

4.2.2 Chemical analysis of cuticular hydrocarbons (CHC)

CHCs were extracted with hexane, in which the thorax and abdomen was submersed for 10 minutes. CHC extracts were stored at 4°C and later in Germany at -20°C. Extracts were analysed with a gas chromatograph (Agilent 7890, GC) coupled to a mass selective detector (Agilent 5975, MS). The GC (split/splitless injector in splitless mode for 1 min, injected volume: 1 µl at 300°C injector temperature) was equipped with a DB-5 Fused Silica capillary column (30 m x 0.25 mm ID, df = 0.25 µm, J&W Scientific, Folsom, USA). Helium was used as carrier gas with a constant flow of 1 ml/min. The temperature program used was: start temperature at 60°C, with an increase of 5 °C/min until 300°C and isotherm at 300°C for 10 min. An ionisation voltage of 70 eV (source temperature: 230°C) was set for the acquisition of the mass spectra by electron ionisation (EI-MS). CHCs were classified into three main substance classes: n-alkanes, methyl-branched alkanes, and unsaturated compounds, with one (*i.e.*, alkenes) or more (*i.e.*, alkadienes, alkatrienes) double bonds. n-Alkanes were confirmed by purchable standards and methyl-branched alkanes and unsaturated hydrocarbons were identified by their diagnostic ions. Retention indices were calculated and used to confirm the identification of unsaturated hydrocarbons and methyl-branched alkanes (Carlson *et al.*, 1998).

4.2.3 Pollen metabarcoding

Diversity analysis of pollen followed the method of Sickel *et al.* (2015), yet with the same extracts as for the microbial assessments: As amplifying primers we used the combination of plant barcoding primers ITS-S2F and ITS4R to amplify the ITS2 of the ribosomal cistron. PCR reaction contained 5µL 2x Phusion Master Mix (New England Biolabs, Ipswich, MA, USA), 0.33µM each of the forward and reverse primers, 3.34µL PCR grade water and 1µL DNA template. PCR conditions were as follows: initial denaturation at 95°C for 4 min, 37 cycles of denaturation at 95°C for 40 sec, annealing at 49°C for 40 sec and elongation at 72°C for 40 sec; followed by a final extension step at 72°C for 5 min. PCRs were performed similarly to the 16S assessment with dual-indexed primers, in triplicates, followed by the same quantification, normalisation and final sequencing steps as described above. Also here, forward and reverse reads were joined with fastq-join (Aronesty, 2013) and low quality (<Q20) and short (<150bp) reads removed with USEARCH v8.0 (Edgar, 2010). Sequences were classified with USEARCH by a global search with 0.97% identity against a reference database composed from all sequences available on Genbank (Benson *et al.*, 2013), which was created following Sickel et al. (2015)(Sickel *et al.*, 2015).

4.2.4 Microbiome screening

The complete laboratory workflow for 16S bacterial microbiome assessment follows the strategy of Junker & Keller (2015) (Junker & Keller, 2015): Stabilised DNA was isolated using the Xpedition™ Fungal/Bacterial DNA MiniPrep (also Zymo Research) following the manufacturer's instructions, however Proteinase K was added to also facilitate the chemical disruption of pollens alongside the standard mechanical bead disruption (Keller *et al.*, 2015; Bell *et al.*, 2016). PCR and library preparation were performed according to a previously published dual-indexing approach (Kozich *et al.*, 2013). PCR was performed in triplicate for each sample in 10µL reactions, each containing 5µL 2x Phusion High Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA), 0.33µM each of forward and reverse primer for the 16S V4 region (Eurofins MWG Operon, Huntsville, AL, USA), 3.34µL PCR grade water and 1µL template DNA. PCR conditions comprised an initial denaturation step at 95°C for 4 min, 35 cycles of denaturation at 95°C for 40 sec, annealing at 55°C for 30 sec and elongation at 72°C for 1min, followed by final extension at 72°C for 5 min. Triplicates of a sample were combined and successful amplification was verified with an agarose gel using 5µL.The remaining 25µL were cleaned up and normalised between samples in DNA amounts using the SequalPrep^{TM} Normalisation Plate Kit (Invitrogen GmbH, Carlsbad, CA, USA), eluting in 20µL. Of each sample, 5µL normalised DNA was taken for pooling 4x96 samples (together with samples of other projects and laboratory control samples with the pure extraction kit) according to Kozich *et al.* 2013. These pools were verified for library fragment size with a Bioanalyzer High Sensitivity DNA Chip (Agilent Technologies, Santa Clara, CA, USA) and quantified with the dsDNA High Sensitivity Assay (Life Technologies GmbH, Darmstadt, Germany) and merged to a final pool. This was diluted to 2nM and further prepared for sequencing following the Illumina Guide for DNA library preparation (Illumina Inc., 2013), obtaining a final library of 10pM. PhiX Control Kit V3 (Illumina Inc., San Diego, CA, USA) was added as a spike-in to ensure high quality reads (10%). Sequencing was performed on the Illumina MiSeq Platform (Illumina Inc., San Diego, CA, USA) using 2x250bp v2 MiSeq chemistry. The cartridge of the reagent kit was additionally supplied with 3µL each of the custom sequencing and index primers. Forward and reverse reads were joined with fastq-join (Aronesty, 2013). Low quality (<Q20) and short (<150bp) reads were removed, and operational taxonomic units (OTUs) clustered with USEARCH v8.0 (Edgar, 2010). Sequences were classified using the RDP classifier and a bootstrap threshold of 0.8. Microbiome and Pollen data was imported into R (R Development Core Team, 2017) and managed with the 'phyloseq' package (McMurdie & Holmes, 2014).

4.2.5 Phylogeny and species delineation

For phylogenetic reconstruction the same DNA extracts were used. The cytochrome oxidase I gene (COI) was amplified with different primer combinations (forwards: dgHCO (Meyer, 2003), RON (Danforth *et al.*, 2003); reverse: dgLCO (Meyer, 2003), MAD (Danforth *et al.*, 2003)) to successfully retrieve overlapping gene fragments merged to a consensus sequence for each specimen. These sequences were aligned using MUSCLE (Edgar, 2004) and the final alignment was 611 bp long. Supplementary reference sequences were obtained by a BLAST search against Genbank (Benson *et al.*, 2013), with picking closely related taxa, but also others to represent all major clades of the Halictine bees and three Apidae as well as three Megachilidae outgroup sequences. The tree was reconstructed using BEAST2 (Bouckaert *et al.*, 2014) and the Yule Model (Yule, 1925) with the outgroup defined as a prior. Phylospecies were delineated using the Generalised Mixed Yule Coalescent (GMYC) method (Pons *et al.*, 2006) as implemented in the R package 'splits'.

4.2.6 Climate data

The climate data for each sampling site was extracted with ArcGIS from interpolated maps of Mt. Kilimanjaro, showing the long-term mean annual temperature and relative humidity (Appelhans *et al.*, 2016).

4.2.7 Statistical anlysis

4.2.7.1 CHC

We calculated the number, Shannon diversity and evenness of CHC compounds for each sample site. Generalised additive models (gam) as implemented in the R package *mgcv* were used to correlate the number, Shannon diversity and evenness of CHC compounds with the elevational gradient. The data family was set to ‘gaussian’. In gam models we set the basis dimension of the smoothing term *k* to three to avoid over-parameterisation of trend functions. We calculated the ratio between saturated and unsaturated CHCs for each sample site. Saturated compounds are n-alkanes and methyl-branched alkanes and unsaturated compounds are n-alkenes and alkadienes:

$$y = \frac{n\text{-}alkanes\,[\%] + methyl\text{-}branched\ alkanes\,[\%]}{n\text{-}alkanes\,[\%] + methyl\text{-}branched\ alkanes\,[\%] + n\text{-}alkenes\,[\%] + dialkenes\,[\%]}.$$

We further calculated the mean chain length of CHCs for each sample site:

$$y = \frac{\sum_{i=0}^{n} chainlength\ of\ compound}{number\ of\ different\ compounds}.$$

We used linear models (lm) to calculate trends in the ratio between saturated and unsaturated CHCs and the mean chain length along the elevational gradient.

4.2.7.2 Pollen

We calculated the number, Shannon diversity and evenness of plant species for each sample site. Linear models (lm) and generalised additive models (gam), respectively, were used to correlate the number, Shannon diversity and evenness of plant species with the elevational gradient.

4.2.7.3 Gut microbiota

We calculated the number, Shannon diversity and evenness of gut OTUs for each sample site. Linear models (lm) were used to correlate the number, Shannon diversity and evenness of gut OTUs with the elevational gradient. Generalised additive models (gam) were used to calculate trends in abundances of Lactobacillaceae, Enterobacteriaceae and Rickettsiaceae along the elevational gradient. The data family was set to 'gaussian'. In gam models we set the basis dimension of the smoothing term *k* to three to avoid over-parameterisation of trend functions. Trend lines derived with gams were only plotted if the significance level of the elevation term was $p < 0.05$.

4.2.7.4 Comparative analyses

We analysed the effect of temperature and relative humidity on CHCs, pollen diet and microbiome, as well as potential effects on each other (overview about models in Appendix S6, Tab.S6). First, we calculated the differences in CHC compounds or composition of pollen species and microbiome OTUs, using the metaMDS function with Bray-Curtis distances in the R package *vegan*. We applied non-metric multidimensional scaling (NMDS) along two axes and used the axes values as explanatory and response variables in linear models. To evaluate the support for the full model and all nested models, we used the 'dredge' function of the *MuMIn* package (Bartón, 2018) in R. Models were ranked according to their Akaike information criterion corrected for small sample sizes (AICc) (Burnham & Anderson, 2004) and delta distances to the next best model were calculated. All best supported models up to ΔAICc < 3 are given in the Supplementary Information. Before the analyses all explanatory variables were standardised by z-transformation, using the 'scale' function in R in order to facilitate the comparability of their effect strength.

4.3 Results

We caught 167 individuals of halictid bees between 830 and 3,780 m a.s.l. at the southern slopes of Mt. Kilimanjaro. We dissected bees and used different body parts of the same individual for different analyses: thorax and abdomen for cuticular chemistry, gut for pollen diet and gut microbiome, head for surface microbiome, and guts, thoraxes and abdomens for DNA barcoding.

4.3.1 Elevational phylogenetics of halictid bees

The phylogenetic analysis of the COI revealed that individuals belonged to different genera: 98 *Lasioglossum,* 32 *Halictus*, 8 *Patellapis* bees (first three genera all Halictini within Halictinae), 13 *Lipotriches* and 10 *Pseudapis* (both Nomiinae), 1 *Nomioides* (Nomiodinae) and 5 bees which could not be identified at genus level). We distinguished 26 phylospecies of *Lasioglossum* (Fig. 1), 8 *Halictus,* 5 *Patellapis,* 1 *Lipotriches* and 4 *Pseudapis*. *Lasioglossum* was present along the whole gradient with highest diversity at lower elevations. At high elevations, our barcoding approach revealed four different *Lasioglossum* phylospecies, where previously only one morphospecies could be distinguished. The phylogenetic tree showed clear elevational patterns of species in their distribution range indicating strong phylogenetic clustering at high elevations and a more dispersed phylogenetic structure at low elevations (Fig. 1).

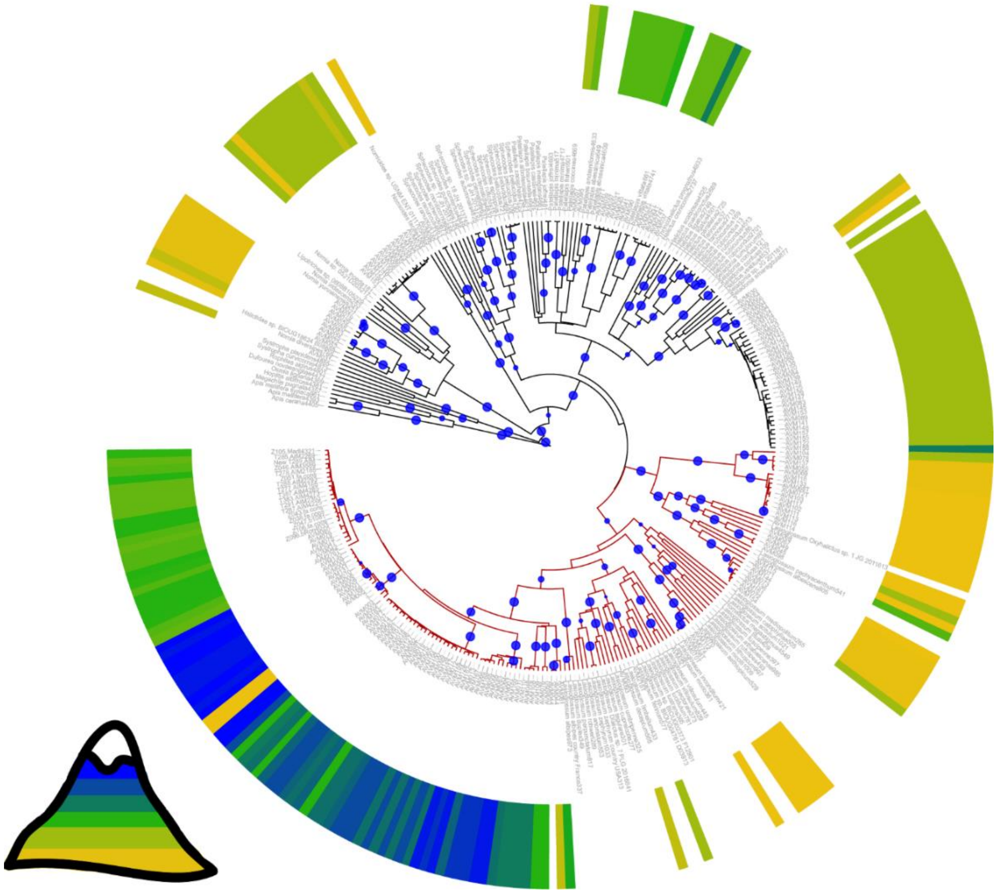


Fig. 1: Phylogenetic tree of the collected halictid bees and reference sequences from Genbank (Benson *et al.*, 2013) with representatives from all major clades of the Halictidae and three Apidae as well as three Megachilidae outgroup sequences. Red branches belong to *Lasioglossum,* black ones to other Halictidae and outgroups. Colours in the outer orbital indicate the occurrence of the phylospecies along the elevational gradient. No colours in the outer orbital means that these are reference sequences. Node bubbles size represent posterior probabilities determined by BEAST2.

4.3.2 Cuticular hydrocarbon composition and diversity

For 98 specimens of the genus *Lasioglossum*, we linked changing environmental parameters to cuticular chemistry, pollen diet and microbiomes. To test whether CHC profiles change along the elevational gradient, particular in compounds which act as a desiccation barrier, we analysed amounts of saturated compounds in relation to unsaturated compounds, mean chain length of compounds, and the compound richness. The ratio of saturated to unsaturated compounds increased with elevation ($R^2 = 0.42$, $p < 0.001$, Fig. 2a), as well as mean chain length in saturated ($R^2 = 0.13$, $p < 0.01$, Fig. 2b), but not in unsaturated compounds ($R^2 = -0.02$, $p = 0.56$). While up to 1500 m n-alkanes and olefins accounted almost equally to overall composition, n-alkanes accounted for up to seven times the amount of olefins at highest elevations. At mid-elevations, we found a high relative abundance of methyl-branched alkanes, which were of minor importance at lower and higher elevations (Appendix S3, Fig. S3.2). Highest Shannon diversity of CHC compounds (GAM: ED = 34%, $p < 0.001$, Fig. 3a) as well as their richness ($R^2 = 0.50$, $p < 0.001$, Appendix S3, Fig. S3.1a) occurred at mid-elevations.

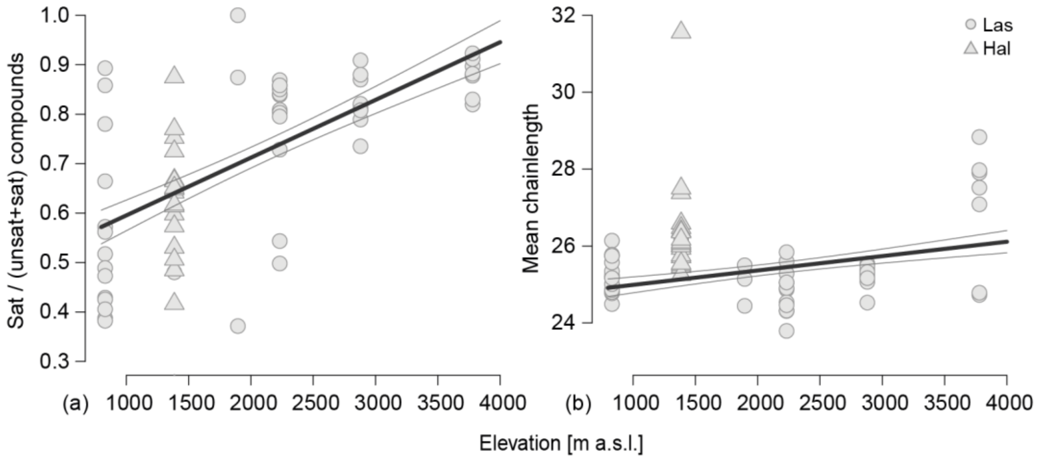


Fig. 2: CHC of *Lasioglossum* (circles) and *Halictus* (triangles) along the elevational gradient. (a) Relative amounts of saturated (sat) to unsaturated and saturated (unsat+sat) CHC compounds increased with elevation (LM: $R^2 = 0.42$, $p < 0.001$). (b) The mean chainlength of CHC increased with elevation in saturated compounds (LM: $R^2 = 0.13$, $p < 0.01$). Shown are trend lines from model fits with standard deviation. Trend lines are only given for *Lasioglossum* alone.

4.3.3 Pollen diversity

To understand bee foraging patterns along the elevational gradient, we studied the richness and composition of pollen in bee guts using plant ITS2 meta-barcoding. We obtained 615,552 sequences after merging and quality filtering. Individual samples yielded a mean of 10,433 sequences and 28 samples with less than 100 reads were removed to avoid throughput-related under-estimations in diversity. We detected in total 170 plant species from which pollen samples originated with on average 15 species per sample (range 3-26). Dominant plant families were Asteraceae, Ericacaea and Fabacaeae (Appendix S4, Fig. S4.2). Shannon diversity of collected pollen decreased along the elevational gradient ($R^2 = 0.07$, $p = 0.03$, Fig. 3c) as well as evenness ($R^2 = 0.09$, $p = 0.03$, Appendix

S4, Fig. S4.1b), but not pollen richness ($R^2 = -0.01$, $p = 0.46$, Appendix S4, Fig. S4.1a). Asteraceae were the dominant pollen resource for bees along the whole elevational gradient, but especially at highest elevations with up to 100 % of the consumed pollen (Appendix S4, Fig. S4.2, S4.3).

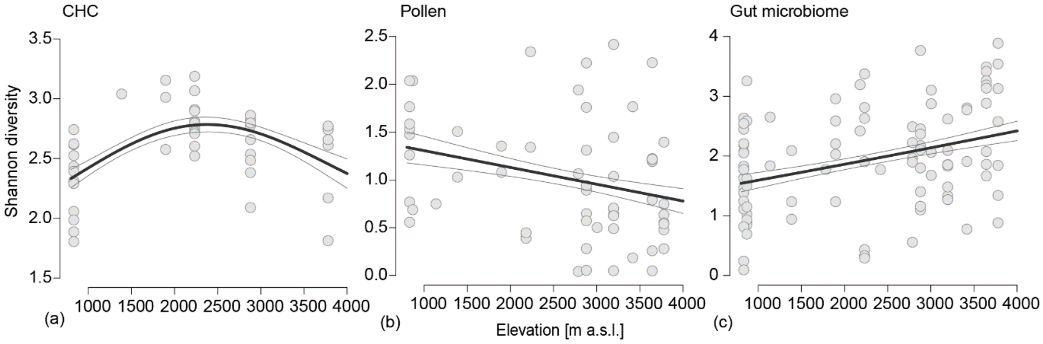


Fig. 3: Shannon diversity of CHC- compounds, pollen diet and gut microbiome along the elevational gradient. (a) CHC-compounds have the highest diversity ad mid-elevations (GAM: ED = 34%, $p < 0.001$). (b) The pollen diversity decreases with elevation (LM: $R^2 = 0.07$, $p = 0.03$). (c) The diversity of the gut microbiome increases with elevation (LM: $R^2 = 0.11$, $p < 0.001$). Shown are trend lines from model fits with standard deviation.

4.3.4 Microbial communities

To investigate whether changing environmental conditions, cuticular chemistry, diet and species along the elevational gradient at Mt. Kilimanjaro lead to a turnover in microbiome composition, we used 16S DNA meta-barcoding to analyse gut and surface microbiomes. In total, we obtained 10,092,494 sequences after merging, quality filtering as well was mitochondria and chloroplast sequences removal. Individual samples had in average 26,093 reads and samples with less than 1,000 reads were removed from the following analyses to avoid underestimations in diversity. Shannon diversity of OTUs in the gut microbiome increased with elevation ($R^2 = 0.11$, $p < 0.001$, Fig. 3c), as well as OTU richness and evenness (richness: $R^2 = 0.12$, $p < 0.001$; evenness: $R^2 = 0.09$, $p < 0.01$, Appendix S5, Fig. S5.1a, b). The community composition also varied along the elevational gradient; with Lactobacillaceae, Enterobacteriaceae and Rickettsiaceae as the three dominant groups (Fig. 4, Appendix S5, Fig. S5.2a). While Lactobacillaceae declined with elevation (ED = 21.5 %, $p < 0.001$, Fig. 4a), and Rickettsiaceae showed highest relative abundances at intermediate elevations (ED = 21.5 %, $p < 0.001$, Fig. 4c), the percentage of Enterobacteriaceae increased with elevation, mainly due to an increase in the genus *Sodalis* (ED = 12 %, $p < 0.01$, Fig. 4b). Surface microbiomes showed similar patterns, yet with general higher prevalence of Comamonadeaceae, Moraxellaceae, Oxalobacteraceae, Pseudomonaceae and Xanthomonadaceae (Appendix S5, Fig. S5.2b).

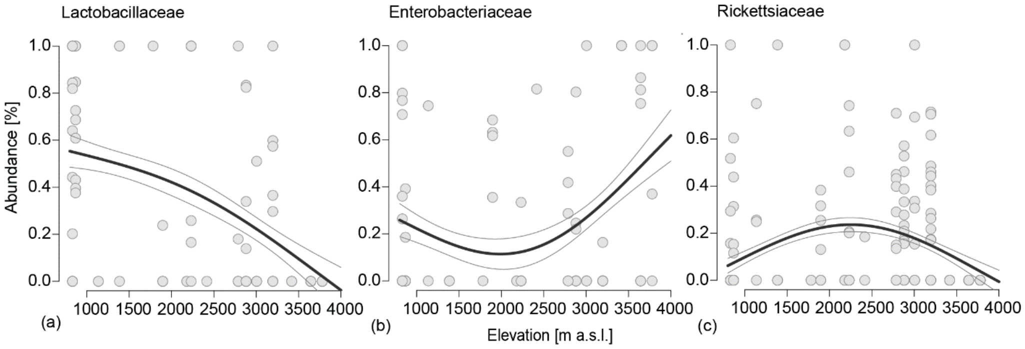


Fig. 4: Major bacterial families in the gut microbiome of *Lasioglossum* along the elevational gradient. (a) The relative abundance of Lactobacillaceae decreases with elevation (GAM: ED = 21.5 %, $p < 0.001$). (b) The relative abundance of Enterobacteriaceae increases parabolic with elevation (GAM: ED = 12 %, $p < 0.01$). (c) The relative abundance of Rickettsiaceae shows highest relative abundances at mid-elevations (GAM: ED = 21.5 %, $p < 0.001$). Generalised additive models were used to estimate trends of elevational richness (Gaussian family, basis dimension (k) = 3). Shown are trend lines from model fits with standard deviation.

4.3.5 Interactive effects of environment, phylospecies, pollen ressources, microbiomes and cuticular hydrocarbon profiles

Our results reveal that multiple traits and interactions of *Lasioglossum* bees changed along the elevational gradient. In the next step we analysed how they were shaped by environmental conditions (temperature, relative humidity) and bee phylogeny and how traits and biotic interactions were linked. First, we calculated NMDS ordinations with Bray-Curtis dissimilarities for the composition of cuticular hydrocarbons, pollen and microbiomes and then used the NMDS-axes for further analyses.

Best models, based on AICc selection, revealed that climate, CHC composition, pollen composition, gut and surface microbiomes are correlated with relative humidity, while pollen and CHC composition additionally responded to increasing temperature. Changes in pollen and gut microbiome composition correlated with each other, but also pollen and CHC, as well as CHC and surface microbiome composition were correlated. Additionally, gut microbiomes and the CHCs were mostly specific for individual bee species (Fig. 5, Appendix S6, Tab S6).

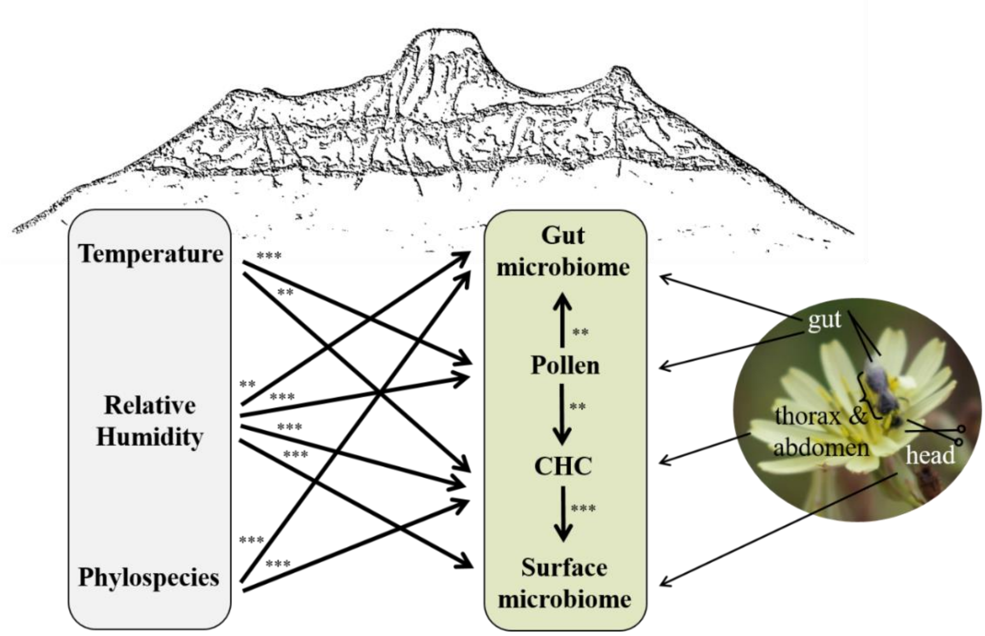


Fig. 5: Schematic analysis and significance of drivers of gut microbiome, pollen diet, CHC profile and surface microbiome. Shown are significant drivers, selected from best models, based on AICc values. Different body parts of *Lasioglossum* bees were used to obtain CHC, pollen and microbiome data. Temperature and relative humidity, as well as the phylospecies-identity were included as explanatory variables in linear models. Further details and results on the models are displayed in Appendix S6, Tab.S6. Picture © A.V. Mayr

4.4 Discussion

In this study, we revealed cryptic species with elevation-dependent phylogenetic relationships along Mt. Kilimanjaro, hidden biotic interactions and cuticular adaptations to changing environmental parameters. Above 3,000 m a.s.l., more *Lasioglossum* species were identified with DNA barcoding than previously morphologically identified (Classen *et al.*, 2015). The strong elevational zonation of species implies niche formation and clustering of species with elevation-dependent adaptations. The wide spread of *Lasioglossum* along the whole gradient supports the great evolutionary adaptability known for the genus and the consequential distribution throughout many types of habitats (Gibbs *et al.*, 2012).

4.4.1 Elevational changes shape cuticular composition and diversity

In line with the cuticula´s function as a desiccation barrier, we found higher relative amounts of saturated compared to unsaturated CHC compounds, and increased chain lengths in saturated compounds with higher elevations. Unsaturated compounds (n-alkenes), are more water-permeable than saturated CHCs (n-alkanes and methyl-branched alkanes) (Gibbs & Pomonis, 1995). However, the waterproofing effect does not only come from saturated CHC, but also from longer mean CHC chain lengths (Menzel *et al.*, 2017). Mean annual relative humidity decreases at about 2,500 m a.s.l. towards the summit at Mt. Kilimanjaro (Appendix S2, Fig. S2), and the correlation between CHC composition and relative humidity confirms our hypothesis. We also found CHC composition to be phylospecies-specific, as also shown in other bees (Pokorny *et al.*, 2014), which implies that *Lasioglossum* species are niched along the elevational gradient according to their cuticular desiccation resistance. An increase in desiccation resistance has also been found for *Drosophila* species at high elevations with very low relative humidity (Parkash *et al.*, 2008). In our study region relative humidity is however also low in savannah habitats at low elevations, and it is unclear why relative amounts of saturated CHC do not account for this. Possible explanations might be that bees at high elevations have to cope with lower relative amount of oxygen, thus keep the tracheae more open and consequently loose more water by breathing (Chown & Gaston, 1999). Secondly, when bees loose water, they also cool down through evaporation enthalpy, which might be critical in environments with low ambient temperatures. We also found a correlation between temperature and CHC composition as shown by Michelutti et al. (2018) (Michelutti *et al.*, 2018). Therefore, probably temperature and relative humidity are both important drivers with regard to water loss. This might be reflected as well in behavioural adjustments, which bees might be able to afford at low, but not high elevations. At low elevations, with generally high temperatures, bees can easily hide in microhabitats rather than forage if the weather conditions are too dry for flying and have a potentially larger foraging window. However, at higher elevations, the bees must use every opportunity to uptake energy as soon as temperatures are high enough to fly (Classen *et al.*, 2015), which coincides with low relative humidity.

4.4.2 Dominance of Asteraceae pollen in the diet of *Lasioglossum*

The turnover of the plant community along the elevational gradient (Hemp, 2006a) was reflected in the bee´s diet. We found different plant families with different abundances in the diet, as well as a decrease in the Shannon diversity along the elevational gradient. Asteraceae pollen dominated the *Lasioglossum* food throughout the entire gradient, even though there was no significant trend with elevation. Nevertheless, in some samples the Asteraceae pollen made up to 100 % of the diet at highest elevations. This reflects the dominance of Asteraceae upward from the forest and *Erica* belt and the ability of Asteraceae to grow in open and dry habitats (Judd *et al.*, 2016). Even though the Asteraceae offer an abundant pollen source (Goulson *et al.*, 2005), they are avoided by most polylectic bee species or just visited for nectar (Müller & Kuhlmann, 2008). Possible explanations are, that pollen of Asteraceae lacks essential nutrients and is hard to degrade (Praz *et al.*, 2008), has very low protein content (Roulston *et al.*, 2000) and often contains toxic compounds (Nicolson & Human, 2013). Bees foraging on such resources need special physiological adaptations, e.g. detoxification abilities (Praz *et al.*, 2008). The *Lasioglossum* species found along the whole gradient seem to be able to manage the mentioned particularities of Asteraceae pollen, which could be an advantage over other bees that are found only at lower regions. Pure Asteraceae diet has however also disadvantages for Asteraceae specialists, such as stunted growth (Williams, 2003) and delayed development (Williams, 2003; Praz *et al.*, 2008). Pollen mixing and dilution is a strategy for bees to cope with unsuitable pollen diet (Eckhardt *et al.*, 2014). A strategy, which we observed across all samples, throughout the entire elevational gradient, even though to a lesser degree at highest elevations.

The benefits for plants are not far to seek: by chemically protecting their pollen, they avoid that unspecific pollinators collect pollen and thereby maximize pollen transfer to conspecifics (Müller & Kuhlmann, 2008). However, also bees foraging on Asteraceae pollen have an advantage through their diet: Asteraceae pollen usually has antimicrobial properties (Müller & Kuhlmann, 2008) and Asteraceae specialists are known to be less often parasitised than generalists (Spear *et al.*, 2016). Beyond *Lasioglossum* however, other specialised wild bees visit Asteraceae (Müller *et al.*, 2006). At Mt. Kilimanjaro for instance, we found

Asteraceae in the diet of other halictids like *Halictus* and *Patellapis*, which we did not detect though at higher elevations. Concluding, the restricted resource diversity likely contributes to the limited bee diversity found at higher elevations, yet is not the sole explanation.

4.4.3 Drivers of microbiotic associations in *Lasioglossum* bees

We observed strong changes in the composition of bacterial microbiomes, with a general trend of diversity and evenness increasing with elevation. So far for bees, only honey bee microbiomes have been investigated along elevational gradients, whereby the microbial community changed between lower and higher elevations along a gradient of 2270 m (Sudhagar *et al.*, 2017). However, honey bees as highly eusocial insects are to a certain degree capable to compensate several factors changing with the gradient, foremost temperature and temperature-mediated food acquisition. Thus, they have greater control over shaping microbial conditions suitable for their associates. By contrast, wild bee microbiomes are generally assumed to be more influenced by environmental factors (Keller *et al.*, 2013; Rothman *et al.*, 2018; Voulgari-Kokota *et al.*, 2018), and the transitions of these factors along an elevational gradient might as well be of stronger fitness relevance than for honey bees. At Mt. Kilimanjaro we found that some bacterial families became more prominent at higher elevations, while others decreased.

Especially the relative microbiome contribution of Lactobacillaceae strongly declined with increasing elevation. The family is well known to include important bee symbionts, which fulfil functions in nutrient allocation and pollen digestion (Vásquez & Olofsson, 2009), as well as for the immune system (Servin, 2004; Vásquez *et al.*, 2012). For honey, bumble and stingless bees, as well as for the solitary genera *Osmia* and *Megachile*, previous studies place Lactobacillaceae among the most abundant bacteria (McFrederick *et al.*, 2017; Voulgari-Kokota *et al.*, 2018). This was also true in our study, but only at lower elevations. Lactobacillaceae are mostly moderately heat-tolerant, but show inhibited growth at lower temperatures, with few specialised exceptions (Niemand & Holzapfel, 1984; Matejčeková *et al.*, 2016). With decrease of their contribution at higher elevations, we suggest that the here relevant strains are unable to maintain growth under extended cold conditions.

They are thus reduced as associates at higher elevations and the functional consequences on nutrition and the immune system remain unknown. Nevertheless, if pathogens also decrease along the environmental gradient, the incremental loss of Lactobacillaceae at high elevations might not be as consequential for the host as at lower elevations. We also found that Enterobacteriaceae slightly increased with elevation, but with lowest relative abundances at mid-elevations. They include also important insect symbionts, but are more known to occur on flowers (Junker & Keller, 2015) and bee-collected pollen (Voulgari-Kokota *et al.*, 2018). High temperatures appear to be more limiting for their growth than lower temperatures (Ron, 1975; Verbarg *et al.*, 2008), which fits to the higher relative abundances at higher elevations. Rickettsiaceae showed highest relative abundances at mid-elevations. The most prominent taxon we found within this family, *Wolbachia,* is a well-known genus associated with insects, with influences on host behaviour or manipulations towards favourable host sex ratios for the bacterium (Zeh *et al.*, 2005). Temperature-dependence growth is also known for this group, reducing densities at very high and low temperatures (Jia *et al.*, 2009; Bordenstein & Bordenstein, 2011). This is consistent with the appearance of the group at mid-elevations. Concluding, our data suggests that elevation (and most likely ambient temperature as the direct driver) is a major contributor in structuring *Lasioglossum* microbiomes, with unknown consequences for symbiotic and antagonistic interactions.

4.4.4 Potential joint effects on sociality and correlations of interactions

Because in nature many factors are connected, we also tested for interdependencies of (a) pollen diet and gut microbiome, (b) pollen diet and CHCs and (c) CHCs and surface microbiome. We found correlations between the pollen diet and both, CHCs and gut microbiome. The influence of the pollen diet on CHCs has been already shown (Liang & Silverman, 2000; Otte *et al.*, 2015). As well it is known for many insects that the food influences the gut microbiome (Engel & Moran, 2013). We also found a correlation between CHCs and the surface microbiome. The correlation is plausible, because CHCs offer favourable and unfavourable substances for the growth of bacteria (Schaeffer *et al.*, 1979).

Additionally, CHCs display the first barrier to microbes and some CHCs seem to be more difficult for bacteria to assimilate, even inhibiting their growth (Pedrini *et al.*, 2013). However, so far very little is known about the quality and quantity of CHC interactions with bacteria. If, however, factors such as diet and CHCs are more related to microbiota as previously thought, their influences, e.g. on social behaviour, could be much more complex than so far assumed.

Halictids show a diverse spectrum of social behaviour. Some species change between solitary and social lifestyle depending on time of the year, geographic location, elevation and other unknown factors (Michener, 2007). CHCs are used for the communication in social insects, predominantly n-alkenes and methyl-branched alkanes. These compounds either decreased with elevation or mostly occurred at mid-elevations. The prioritisation of a strong desiccation barrier at higher elevations with a high amount of saturated instead of unsaturated and methyl-branched alkanes might therefore also interfere with or even preclude social behaviour. Because likewise to parasitism, the complexity of chemical profiles for communication is in a trade-off situation with its function for desiccation prevention, as a larger amount of saturated CHC compounds reduces the viscosity of the CHC layer and reduces the compounds which are used to encode information (Howard & Blomquist, 2005; Leonhardt *et al.*, 2016). Therefore, the results of the cuticular analysis indicate, that *Lasioglossum* might be solitary at higher elevations (Eickwort *et al.*, 1996) and social at lower elevations at Mt. Kilimanjaro.

Additional support comes from the microbiome assessment. *Sodalis* was the most dominant Enterobacteriaceae genus in our study and in a previous study identified as the most important group for distinguishing between solitary and social behaviour in halictid bee (Rubin *et al.*, 2018). At lower elevations, we found a higher diversity of *Sodalis* OTUs, which might reflect changes in social behaviour in our system as well. A third restriction of sociality might come with the low protein content of Asteraceae (Praz *et al.*, 2008), which is the main pollen source at high elevations. Low protein pollen leads to prolonged larval developmental times (Praz *et al.*, 2008). However, sociality is strongly linked to season length, i.e. the season needs to be long enough to permit two annual broods (Field *et al.*, 2010). The poor pollen quality could therefore prevent more than one annual brood. The investigated traits thus suggest that sociality is unlikely at higher elevations at Mt. Kilimanjaro. To prove this hypothesis, it would be necessary though to search for nests and queens and clarify, whether a change in sociality occurs along the gradient.

4.5 Conclusion

Insects have to adapt to physiological and biotic constraints in order to occur in habitats with different climatic conditions, such as along elevational gradients. This requires multiple adaptations on all scales of their biology, of which hidden traits and interactions are not yet well understood. In this study, we investigated multiple facets of *Lasioglossum* bee ecology along an elevational gradient at Mt. Kilimanjaro in Tanzania. We found strong changes in the surface profiles of the epicuticle, in collected food resources, as well as in associated bacterial communities, which seem to be connected. These observed patterns help us to understand not only the direct, physical impact of elevation on bees, but also indirect effects inherent with their ecology and phylogenetic constrains. The observed patterns also contribute to our ecological insights into the mechanisms behind trade-offs and prioritisation in social aggregations versus solitary life of insects, as well as the release and avoidance of parasites and pathogens. Our results provide exciting new hypotheses and elucidate the relevance of investigating biotic interactions on multiple levels to better understand species distributions and potential consequences of global climate change.

4.6 Appendix

Appendix S1: Study design

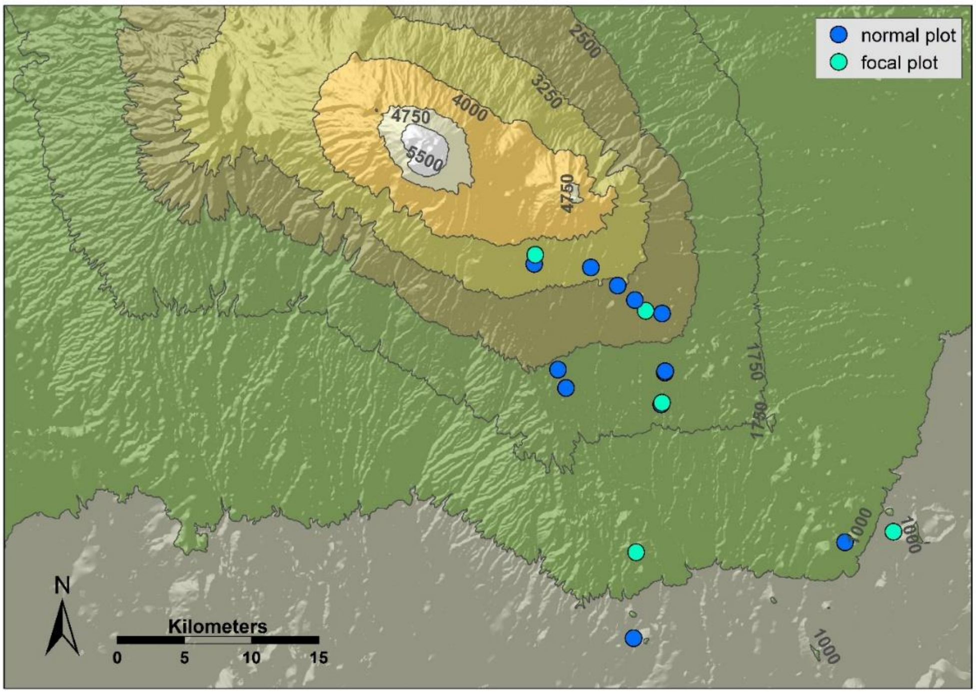


Fig. S1: Study area at Mount Kilimanjaro, Tanzania. Shown are sampling sites of *Lasioglossum* bees. Pollen and microbiota of bees were investigated on all plots, additionally CHC only on focal plots.

Appendix S2: Elevational patterns of temperature and relative humidity

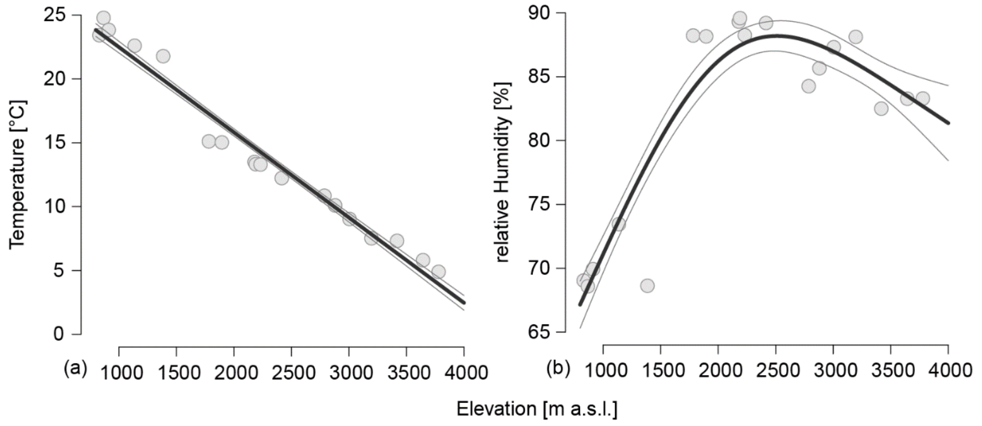


Fig. S2: Patterns of the mean annual temperature and mean relative humidity along the elevational gradient at Mt. Kilimanjaro. Linear and generalised additive models, respectively, were used to display elevational patterns of temperature and relative humidity (Gaussian family, basis dimension (*k*) = 4). (a) Temperature declines with elevation ($R^2 = 0\text{-}97$, $p < 0.001$), while (b) relative humidity shows a humped-shaped distribution (ED = 87, $p < 0.001$). Shown are trend lines from model fits with standard deviation.

Appendix S3: Elevational diversity and composition of CHC

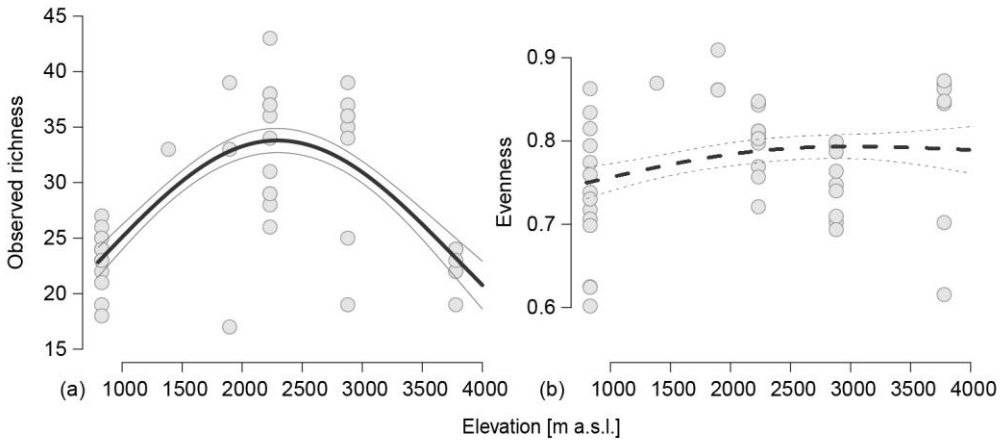


Fig. S3.1: Alpha diversity measures of CHC. Generalised additive models, were used to display elevational patterns of observed compound richness and evenness (Gaussian family, basis dimension (*k*) = 3). (a) observed compound richness shows a humped-shaped patterns with elevation (ED = 52, $p < 0.001$), while (b) evenness of compounds does not increase with elevations (ED = 8, $p = 0.21$). Shown are trend lines from model fits with standard deviation.

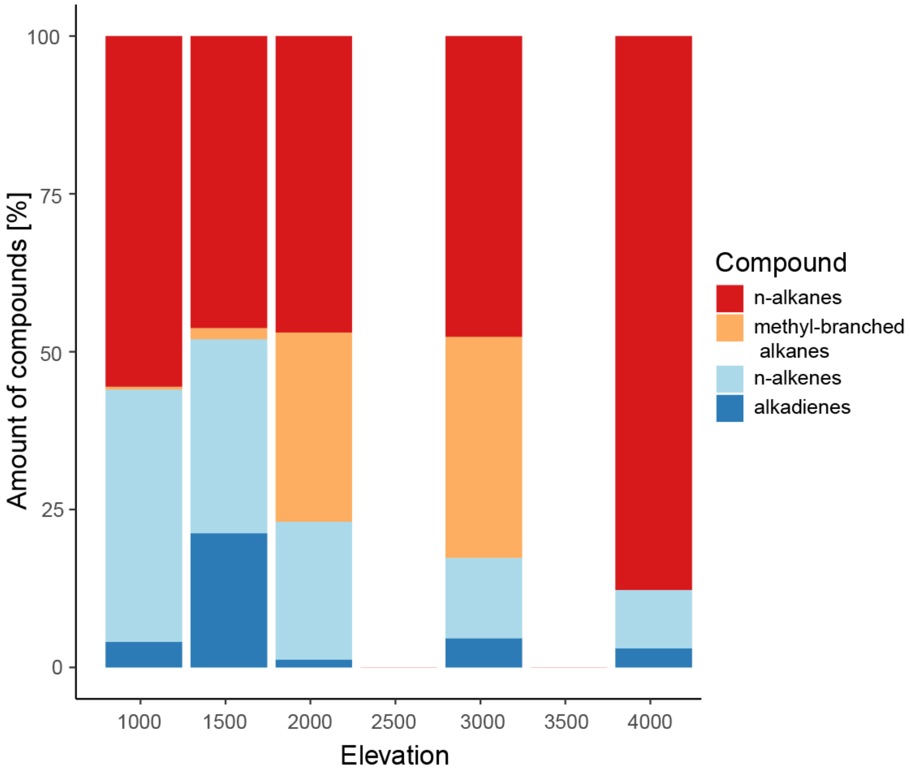


Fig. S3.2: The relative amount of compounds varies along the elevational gradient with highest proportions of n-akanes at highest elevations and highest proportions of methyl-branched alkanes at mid-elevations.

Appendix S4: Elevational richness, abundance pattern and composition of pollen

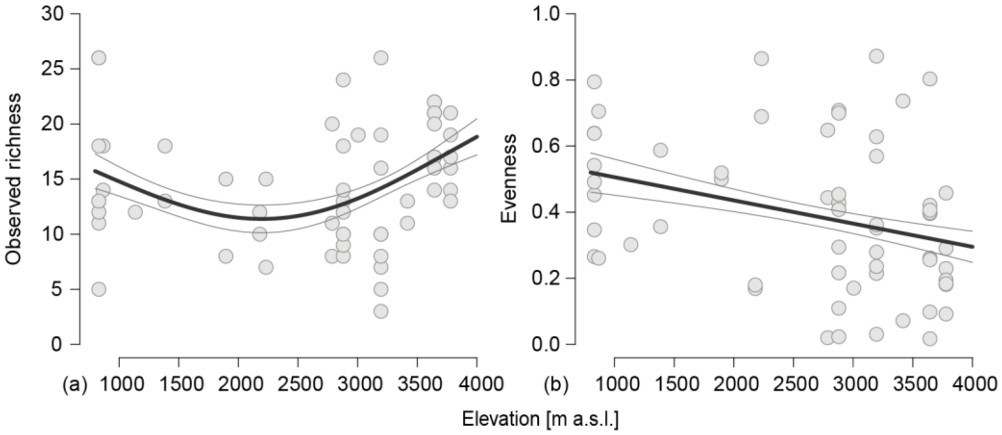


Fig. S4.1: Alpha diversity measures of pollen diet. Linear and generalised additive models, respectively, were used to display elevational patterns of observed species richness and evenness of pollen diet (Gaussian family, basis dimension (*k*) = 3). (a) observed species richness decreases towards mid-elevations and then increases towards higher elevations (ED = 15, p = 0.02), while (b) evenness of pollen diet decreases with elevation ($R^2 = 0.08$, p = 0.02). Shown are trend lines from model fits with standard deviation.

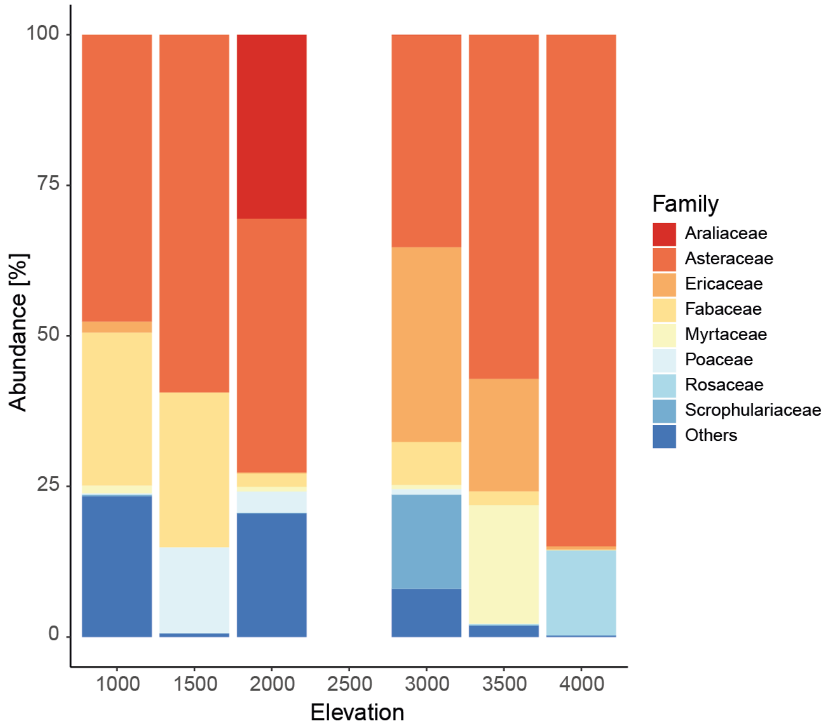


Fig. S4.2: The relative amount of plant families varies along the elevational gradient. The pollen diet generally comprises a high proportion of Asteraceae, with highest proportions of Asteraceae at highest elevations.

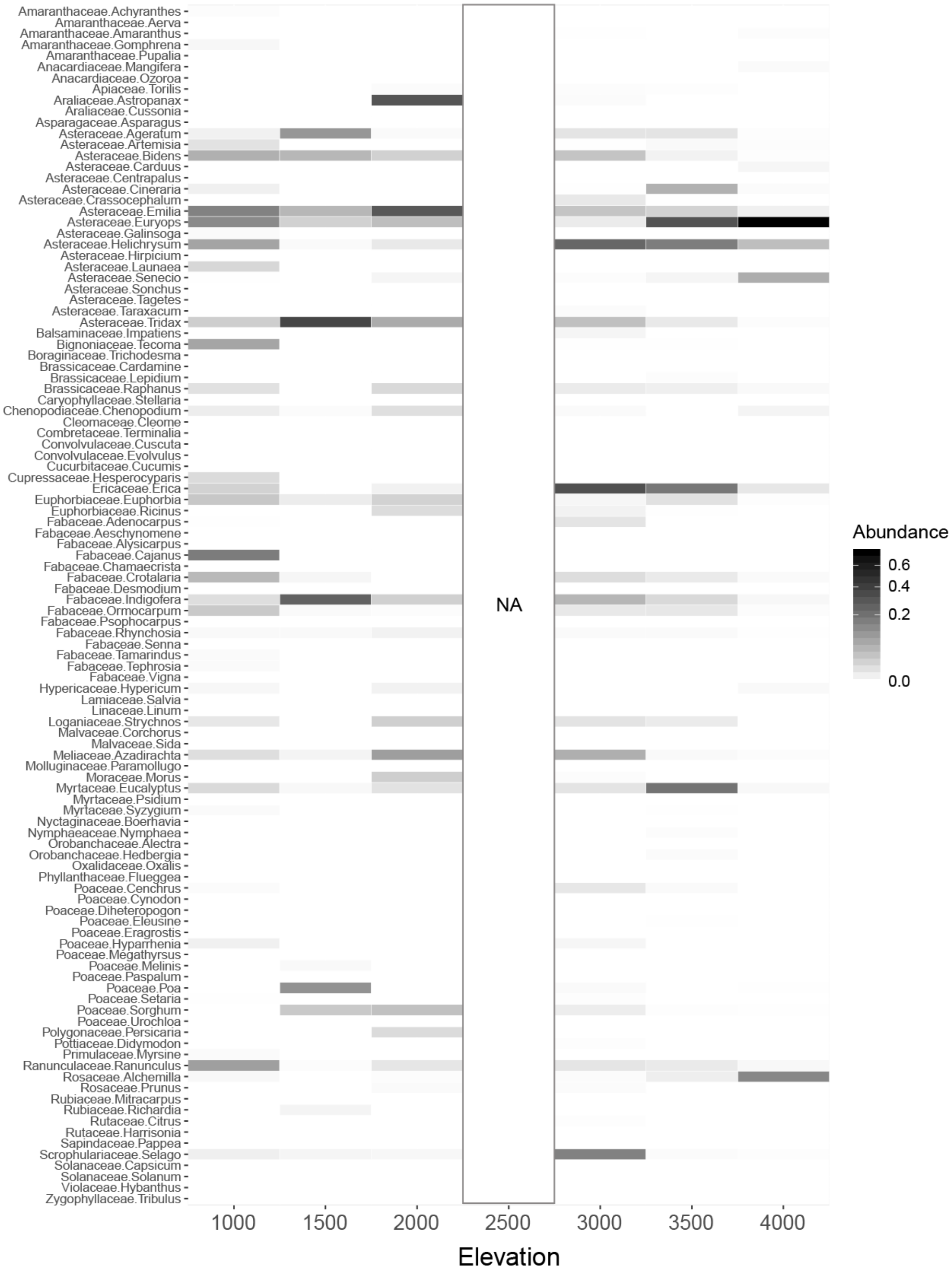


Fig. S4.3: Plant families and genera in the pollen diet of *Lasioglossum* along the elevational gradient. Shown are relative abundances of genera for each elevation. At 2,500 m a.s.l. no data was available.

Appendix S5 Elevational richness pattern of gut microbiota and composition of surface microbiome

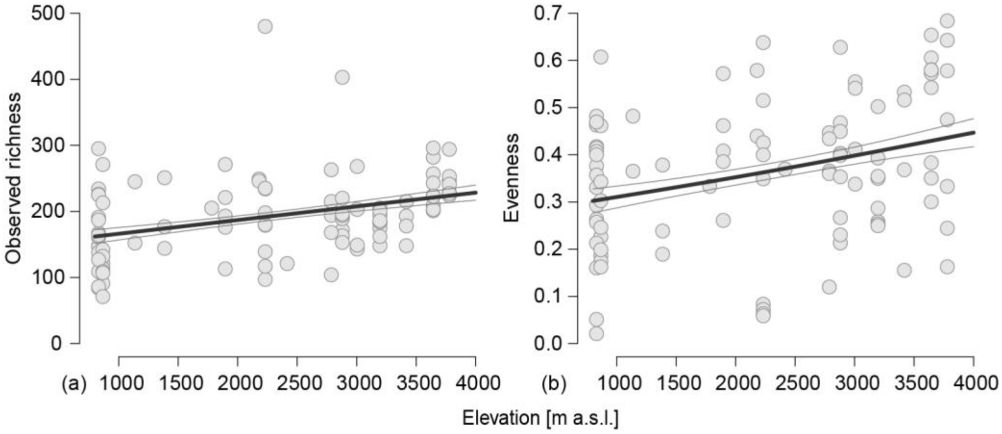


Fig. S5.1: Alpha diversity measures of the gut microbiome. Linear models were used to display elevational patterns of observed OTU richness and evenness of OTUs. (a) observed OTU richness ($R^2 = 0.12$, $p < 0.001$) and (b) evenness of OTUs both increase with elevations ($R^2 = 0.09$, $p < 0.01$). Shown are trend lines from model fits with standard deviation.

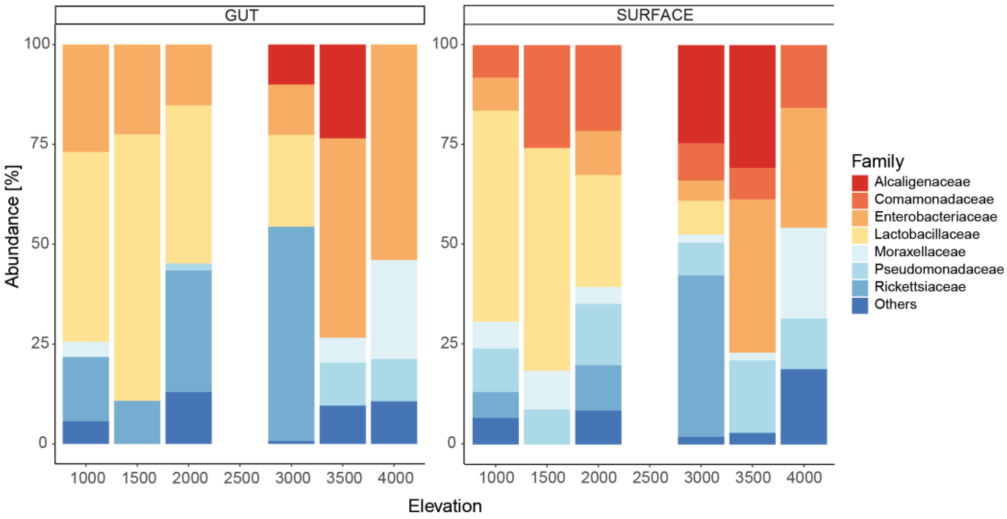


Fig. S5.2: Left panel: Gut microbiome and right panel: Surface microbiome of *Lasioglossum* varying on bacterial family level along the elevational gradient, with generally high proportions of Lactobacillaceae, Enterobacteriaceae and Rickettsiaceae. In the surface also Comamonadaceae occur in relatively high proportions along the elevational gradient, which were negligible in gut microbiomes.

Appendix S6 Comparative analyses of molecular interactions

Tab. S6: Best models for cuticular hydrocarbons (CHC), pollen diet, gut and surface microbiome up to Δ AICc < 3 by AICc values.

Molecular interaction	Axis	NMDS1 Pollen	NMDS2 Pollen	T	rH	Phylo-species	AICc	Weight	R^2	# Sample
CHC	NMDS1		-0.28	-1.68		+	14.5	0.29	0.96	28
			-0.28		-2.23	+	14.5	0.29		
			-0.28		-2.23	+	14.5	0.29		
						+	17	0.09		
CHC	NMDS2		0.13		-1.89	+	-11.6	0.24	0.98	28
			0.13		-1.89	+	-11.6	0.24		
			0.13	-1.42		+	-11.6	0.24		
				-2.11		+	-9.2	2.42		
					-2.81	+	-9.2	2.42		
					-2.81	+	-9.2	2.42		
Molecular interaction	**Axis**			**T**	**rH**	**Phylo-species**	**AICc**	**Weight**	**R^2**	**# Sample**
pollen	NMDS1			-0.77	-0.62		88.9	1.00	0.44	59
pollen	NMDS2				0.24		99.5	0.52	0.15	59
				0.17	0.38		99.8	0.46		
Molecular interaction	**Axis**	**NMDS1 Pollen**	**NMDS2 Pollen**	**T**	**rH**	**Phylo-species**	**AICc**	**Weight**	**R^2**	**# Sample**
gut microbiome	NMDS1	-0.26	0.24			+	81.1	0.83	0.81	59
gut microbiome	NMDS2				-0.30		123.8	0.29	0.15	59
				-0.21	-0.47		124.1	0.24		
		0.07			-0.30		125.4	0.13		
			-0.02		-0.29		126.1	0.09		
		-0.02		-0.23	-0.48		126.5	0.07		
			0.01	-0.21	-0.47		126.5	0.07		
Molecular interaction	**Axis**	**NMDS1 CHC**	**NMDS2 CHC**	**T**	**rH**	**Phylo-species**	**AICc**	**Weight**	**R^2**	**# Sample**
head microbiome	NMDS1	0.49			-0.43		76.2	0.43	0.30	42
		0.45	-0.08		-0.38		78.1	0.17		
		0.48		0.01	-0.42		78.8	0.12		
		0.27		0.30			79.2	0.10		
head microbiome	NMDS2						81.4	0.27		42
			-0.07				83.1	0.12		
				0.07			83.1	0.11		
		-0.07					83.1	0.11		
					-0.04		83.5	0.09		

Shown are standardised explanatory variables from best-fit models. Blue colours indicate positive significant values and red values indicate negative significant values from LM models for the best model each. The significances between phylospecies vary, turquoise means that there is at least one phylospecies correlating significantly with the response variable. T: mean annual temperature, rH: mean annual relative humidity, # Samples: number of samples taken into account for the model.

Chapter 5

General Discussion

Biodiversity is determined by a multitude of factors. In this thesis, I investigated the drivers of species richness and interactions at different trophic levels of Hymenoptera. I aimed to understand the impacts of environmental seasonality on functional traits and ecological rates; and studied how environmental factors shape hidden traits and interactions. My results show that abiotic factors such as temperature and relative humidity affect species diversity, species traits and interactions among species. However, biotic factors also played an essential role in determining patterns of species richness, especially at higher trophic levels, which can have a positive effect on the fitness of organisms. Yet, biodiversity patterns were complex; and, in addition to visible interactions between species there have been hidden interactions with other species, for examples with microorganisms. These different interactions strongly influenced each other and changed along environmental gradients. Furthermore, the associated drivers of biodiversity patterns varied according to the functional group, the ecosystem and trait being studied. This implies that environmental changes affect living beings at various levels and in different and complex ways depending on the ecosystem. Therefore, changes in bottom-up and top-down processes can be expected in the context of global change, albeit the consequences of global change are difficult to predict because they work in many ways and are system-dependent. However, I also found uniform, synchronised reactions to strong climatic changes among different functional groups of hymenopterans, especially at low elevations. Species in hot and dry ecosystems in particular are already living at the edge of their thermal limits and increasing temperatures as well as changes in climatic seasonality patterns could considerably disrupt the equilibrium established over evolutionary time. In addition, it is likely that progressive changes in land use will also affect insect communities in the future, even if at Mt. Kilimanjaro, the small-scale, well-connected habitats still seem to provide sufficient space and resources for flying insects. However, further investigations should also look at networks, other traits and microclimatic conditions that could further explain patterns of biodiversity and contribute to a deeper understanding of nature. In summary, these findings highlight the importance to study biodiversity on a community-wide level as well as in detail and on different levels of biodiversity: at the level of species diversity, functional traits and trophic interactions. Modern and well-proven techniques can complement each other and so far less studied hidden adaptations and interactions should be included in order to gain a most comprehensive understanding of species communities.

In this thesis, I aimed to understand drivers of species richness, species traits and interactions among species and I investigated changes along the elevational gradient of Mt. Kilimanjaro and during different seasons. Because of the complexity of the results of this work they are summarised in a table to keep in mind for the sake of clarity in the general discussion (Tab. 1).

Tab. 1: Summary of main objectives and results of the present dissertation.

Study	Objective	Results	Main Conclusion
Study I (Chapter 2): Climate and food resources shape species richness and trophic interactions of cavity-nesting Hymenoptera	• disentangling drivers of species richness at different trophic levels • investigating parasitism rates and antagonist to host ratios in species richness	• temperature is the major driver of species richness across all trophic levels • resources are as important or even more important as drivers of species richness in parasitoids, with increasing importance at higher trophic levels • parasitism rate decreases with elevation and increases with trophic level	• temperature is the major driver of insect species richness • resources are an important factor for dependent antagonist groups, such as parasitoids • the relative importance of bottom-up and top-down forces might shift with climate change
Study II (Chapter 3): Seasonal variation in the ecology of trap-nesting Hymenoptera at Mt. Kilimanjaro	• investigating annual dynamics at different trophic levels and different elevations • assessing nest-building activity, ecological rates and functional traits	• strong synchronisation of nest-building activity between functional groups at lower elevations • strong dependence of bees on seasonal flower availability is reflected in nest-building activity and sex ratio	• strong seasonality in savanna ecosystems leads to synchronisation of life-history across functional groups of Hymenoptera

		• nest-building activity of caterpillar- and spider-hunting wasps is linked to seasonality in climate	• strong synchronisation of life-history traits with climate seasonality shows vulnerability to climate changes in seasonality across functional groups of Hymenoptera
Study III (Chapter 4): Cryptic species and hidden ecological interactions of halictine bees along an elevational gradient	• investigating hidden traits and interactions of *Lasioglossum* which could be related to the expansion into extreme habitats • investigation of phylospecies along the elevational gradient • studying the composition of cuticular hydrocarbons with regard to the function as desiccation barrier along the elevational gradient • studying the pollen diet with changes in plant communities along the elevational gradient • studying the surface and gut microbiome along the elevational gradient	• correlation of cuticular hydrocarbons, diet and microbiome with each other, climate and phylospecies • strong turnover in phylospecies with elevation • increasing proportion of n-alkanes and chain length with elevation • changing pollen diet and overall high dominance of Asteraceae pollen • relative change of most abundant families of bacteria; i.e. decrease of Lactobacillaceae, increase of Enterobacteriaceae and humped-shaped relation of Rickettsiaceae with elevation	• complex interactions and feedbacks among abiotic and biotic conditions determine species distributions • species are adapted and limited to small elevational range • improvement of the cuticula´s function as desiccation barrier towards higher elevations • *Lasioglossum* bees are able to cope with the high proportion of Asteraceae pollen at high elevations • compositional changes of bacterial families along the elevational gradient

5.1 Drivers of biodiversity along spatial and temporal environmental gradients – species, traits and interactions

5.1.1 Abiotic drivers - temperature and humidity

At Mt. Kilimanjaro, the climate varies strongly in space along elevation, and in time between rainy and dry seasons, but also during days due to the strong tropical daytime climate. Due to these climatic changes, I observed spatial changes in species richness and interactions (Chapter 2), temporal seasonal patterns in life-history traits (Chapter 3) and I assume daily behavioural adaptations to avoid the most inconvenient times of the day (Chapter 4). Biodiversity is driven directly and indirectly by climatic conditions. Climate directly restricts the occurrence of species under spatial and temporal extreme climatic conditions for insects, such as high temperatures at low relative humidity or high relative humidity at low temperature at the same time (Chapter 2, 3 and 4). And it indirectly affects species occurrences via the availability of (food) resources (Chapter 3) and species interactions, such as top-down control by parasitism (Chapter 2).

Temperature is the dominant driver of species richness patterns of cavity-nesting bees, wasps and their respective antagonists (Chapter 2). Different theories exist trying to explain why temperature is expected to enhance species richness, especially for ectothermic taxa (Buckley *et al.*, 2012). There are physiological constraints at extreme temperatures in species that are not able to regulate their body temperature (Currie, 1991). Ecological rates can also be temperature-mediated, for example biotic interactions, such as the exploitation of food resources (Brown, 2014) or top-down control, e.g. parasitism rates are affected by temperature (Forrest & Chisholm, 2017). Increasing rates of speciation by temperature-driven metabolic rates (Allen *et al.*, 2006; Brown, 2014) may also contribute to higher species richness in warmer habitats. At Mt. Kilimanjaro, however, my favoured explanation is that temperature enhances the species richness of cavity-nesting Hymenoptera via lower extinction rates and temperature-mediated access to food resources (Buckley *et al.*, 2012; Classen *et al.*, 2015). Differences of species richness along the elevational gradient can hardly be related to differences in evolutionary rates caused by temperature because the Kilimanjaro is a very young mountain (first volcanic activity ~2.5Ma (Nonnotte *et al.*, 2008)) and there are also exceptions for places with high speciation rates under cold climatic conditions (Madriñán *et al.*, 2013). I also found a strong compositional change of cavity-nesting Hymenoptera along the elevational gradient, which was even stronger than the seasonal turnover (Chapter 3). I am aware that temperature strongly correlates with elevation and other factors co-varying with elevation could drive changes in species communities as well (Perillo *et al.*, 2017). Unfortunately I could not test the influence of relative humidity as a driver of elevational patterns, because it strongly correlated with temperature within the elevational range I investigated with trap nests. According to ecological theory, temperature is considered the most important predictor of species richness along elevational gradients and it explains most of the patterns of species richness of bees and other non-parasitoid hymenopterans at Mt. Kilimanjaro (Peters *et al.*, 2016b).

I also found effects of temperature on functional traits. Even though climatic conditions in the tropics are largely generous for insects (Denlinger, 1986), there are still exceptions such as on high mountains or during strong seasonal climatic events, like extreme dry heat or cold humidity. I found variation in functional traits associated with temperature and water regulation and concluded that insects are physiologically limited by temperature under certain conditions. The regulation of temperature and water balance are basic components of homeostasis in living organisms (Chown *et al.*, 2011). Temperature had an effect on the composition of the bees´ cuticule and bees adapted their chemical profiles at high elevations (low mean annual temperature and mean annual relative humidity) in favour of better desiccation barriers, even if this was accompanied by potential trade-offs in other functions of the insect´s cuticule (Chapter 4). Even though the mean annual relative humidity was also low at low elevations and a good desiccation barrier could be needed here as well, the bees´ cuticule was not composed in favour of a better desiccation barrier. At low elevations, high mean annual temperature seems to permit avoidance behaviour of driest conditions and enable foraging in early morning or late afternoon when the relative humidity is higher than at noon. In contrast, bees at high elevations have to restrict the foraging activity to the hottest time of the day, which usually coincides with lowest humidity values

(Willmer & Stone, 2004). Another example for physiologically borderline conditions occurred during the pre-experiment of the trap nests. The first installed trap nests with simple roofs looked like the ones used in Germany. Extremely high mortality rates were observed during the dry season in the savannas (the larval mortality was almost 100 %) because the pollen-nectar-pellet melted and the larvae either drowned in it or were grilled by the heat. This also happened in a trap-nest study in the desert and they also report the melting of the pollen pallet (Parker, 1986). The highest temperatures I measured occurred in January and November in the savanna with 50-67°C during noon and it is not surprising that eggs and young larvae die at such high temperatures. Apparently, the microhabitat conditions in the exposed trap nests were extremer than under natural conditions and they better retained heat than for example tree trunks. Living trees can better buffer temperature fluctuations (Gibbons & Lindenmayer, 1996) and the bees obviously did not foresee the risk, and eagerly used the provided easy nesting place (design of 'hot-area-adapted' trap nests in Chapter 2, Supporting Information).

Temperature is also an important driver for temporal patterns, increasing ecological rates and interactions. Temperature enhanced the nest-building activity of spider-hunting wasps (Chapter 3), possibly as an indirect link because temperature fostered spider abundances at Mt. Kilimanjaro (Röder *et al.*, 2017). I also found higher parasitism rates and antagonist host-species richness ratios at lower elevations (Chapter 2) which might indicate higher temperature-driven interactions at lower elevations, thus leading to a higher species coexistence (Terborgh, 2015; LaManna *et al.*, 2017). But I also found temporally increasing parasitism rates with temperature for caterpillar-hunting wasps; interestingly, for these groups both, along the elevational gradient and also temporally during the year (Chapter 2 and 3). However, this was more likely due to a faster increase and decrease in the number of hosts than in the number of parasitised nests, i.e. hosts fluctuated stronger in abundances than antagonists. Furthermore, temperature had a positive effect on bee survival because the natural mortality rate of the bees decreased with increasing temperature and parasitism played hardly a role as an additional mortality factor in bees. Bees are known to develop well in warm habitats. I adapted the trap nests to suit the hot lowland conditions after the pre-experiment. From this experiment I know that high temperatures are quickly reached in the savanna ecosystems, which had a negative impact on larval development and bee survival. Therefore, results might differ, if I would recalculate models with maximum instead of mean temperatures.

Temperature does not only affect insects but is also an important predictor of changing pollen resources for bees along the environmental gradient (Chapter 4). Pollen diversity declined with elevation. Even though compositional changes in plant communities are certainly not only driven by temperature but also by other factors co-varying with elevation, I was able to confirm the positive effect of temperature on the abundance of flowering plants over the course of the year (Chapter 3). At lowland and mid-elevation ecosystems, temperature only enhances the abundance of flowering plants with simultaneous high air humidity. However, if the climate is too dry, temperature has a negative effect on flower abundance. The importance of the interaction between temperature and relative humidity is also clear in other places for the community of cavity-nesting Hymenoptera, highlighting the importance of temperature and moisture for arthropod life-cycles (Chen *et al.*, 2009). The possible ability of insects to sense the interplay between temperature and relative humidity may help in their survival (Chown *et al.*, 2011).

The nest-building activity of caterpillar-hunting wasps increased only with temperature if the humidity was higher than a certain threshold and decreased with increasing humidity if it was too cold (Chapter 3). I argued that especially at low elevations, temperature and humidity might have an indirect effect on the nest-building activity of caterpillar-hunting wasps via changes in the food availability. On the other hand, at higher elevations, temperature and humidity restrict foraging and thereby the access to resources (Classen *et al.*, 2015).

Furthermore, abiotic stress has been suggested to have higher effects at higher trophic levels (Menge & Sutherland, 1987). In contrast to the survival rate of bees and caterpillar-hunting wasps, which was either affected by temperature or humidity, the survival rate of spider-hunting wasps was dependent on both abiotic factors. The survival rate of spider-hunting wasps decreased under cold and wet conditions, but also when it was too warm and dry (Chapter 3). Although I have found many effects of temperature on biodiversity, the availability of water plays a vital role for all organisms, which I have already seen in some interactions of temperature and humidity. The beginning of the rainy season acts as a trigger (Wolda, 1988) and has been shown to control, for example, the emergence, mating, dispersal and oviposition of insects (Denlinger, 1986). Therefore, most insect species in the tropics peak in the rainy season or in the transition time between rainy and dry season (Frith & Frith, 1990; Novotny & Basset, 1998; Tidon, 2006; da Silva *et al.*, 2011). Statistically, I only found a correlation between the nest-building activity of spider-hunting wasps and relative humidity, nevertheless, I found a strong synchronisation in the nesting-activity of bees, caterpillar-hunting wasps and spider-hunting wasps at lower elevations which coincides with the short (warm) rainy season (Chapter 3). After the long, cold, dry season, the vegetation is also flourishing, the temperatures are warm and the rains are moderate so that flying insects still have enough foraging windows a time which is reflected in the highest numbers of bee nests. At higher elevations, the nest-building activity is also synchronised among functional groups, even though it is less strong synchronised and the short, warm, dry season acts as a trigger for nesting activity. At lower elevations heat in combination with drought increases the risk of drying out and at higher elevations, cold in combination with moisture increases the risk of cooling down.

A synchronisation or avoidance of certain seasons was also reflected in life-history traits in the extended development time of bees, caterpillar-hunting and spider-hunting wasps during the long rainy season at lower elevations (Chapter 3). The longer development translated into an emergence after the long rainy season and partly also after the long dry season and was correlated with relative humidity which might induce diapause, preventing the brood to hatch during heavy rains and severe drought. Species, living in seasonally dry areas, are often adapted to drought with a diapause (Gill *et al.*, 2017).

Relative humidity was also an important driver of biotic interactions. The composition of the bee gut, as well as surface microbiomes changed with relative humidity (Chapter 4). The pollen composition of the bee diet was also driven by changes in the plant community along the elevational gradient and affected by relative humidity changes. These results highlight that abiotic factors such as temperature and humidity act on the level of species richness, traits, but also interactions. Nevertheless, not only abiotic, but also biotic factors drive biodiversity patterns at Mt. Kilimanjaro.

5.1.2 Biotic drivers – resources

I found restricting effects of resource availability on the level of species richness, fitness, ecological rates and interactions. Energy resources are often considered important drivers of biodiversity in ecological theory, because more resources can support larger populations and this translates into a lower extinction risk (Srivastava & Lawton, 1998; Hurlbert & Stegen, 2014). I found that both, resource abundance and richness enhanced the species richness of antagonists of bees, caterpillar-hunting and spider-hunting wasps (Chapter 2). In theory, resource richness supports species richness, because a high richness of resources leads to a high number of niches which may support a higher number of species (Forbes *et al.*, 2009). Parasitoids are usually more dependent on their hosts than on environmental factors (Steffan-Dewenter, 2003), but I suggest that at the same time temperature drives the access to the host resource (Willmer, 1983; Classen *et al.*, 2015), because temperature was a major driver for the species richness of antagonists as well. This is different to endothermic taxa which are able to forage at cold temperatures (Buckley *et al.*, 2012; Ferger *et al.*, 2014). Furthermore, with declining temperatures, the species richness of antagonists declined stronger than the species richness of their hosts (Chapter 2). In the Rocky Mountains, the activity of parasitoid wasps declined also stronger with temperature than the activity of its bee host, however this pattern was only investigated in one bee species (Forrest & Chisholm, 2017).

While I found an influence of resource availability on the higher trophic levels for species richness along the elevational gradient, during the year, resource availability played a decisive role in life-history patterns of bees. I found that the nest-building activity of bees was driven by the seasonal availability of flowers (Chapter 3, Forrest & Chisholm (2017)). This fits in with the assumption that for seasonal patterns in the tropics, biotic rather than abiotic factors are responsible (Denlinger, 1986). Nevertheless, I also found correlations with climatic seasonality. In bees, lower flower availability resulted also in a production of more 'cheap brood cells', because male brood cells are provisioned with less pollen, which fits basic theoretical assumptions (Frank, 1987). Furthermore, the survival rate of bees declined with lower resource availability. Taken these results together, low resource availability has strong consequences for bees, resulting in a lower number of nests with a shifted sex ratio and lower survival rates. If resource limitation is not just a temporary condition, it could have a big impact on bee populations.

Moreover, changes in the composition of floral resources in *Lasioglossum* bees were linked to changes in the composition of gut bacteria (Chapter 4). Microbial interactions with pollinators have hardly been investigated so far, even though insect health is strongly influenced by microbiota in relation to pathogens, nutrition symbionts and facultative symbionts (Moran *et al.*, 2008). However, at the moment it is only known that bee guts contain microbes which are also found on plants, but little is known about their functions (McFrederick *et al.*, 2017; Voulgari-Kokota *et al.*, 2018). However, the compositional linkage between resources and gut bacteria along with environmental changes implies that gut microbiota shift when resource availability changes, with unknown consequences for bees, especially because bees tend to have a species-specific microbiome (Voulgari-Kokota *et al.*, 2018).

Because abiotic and biotic factors act on such different levels of biodiversity, it is necessary for the understanding of species communities to look at them in as many different ways as possible.

5.2 Implications for Kilimanjaro´s biodiversity under global change

Predicting how biodiversity and ecosystems will respond to global change is one of the main difficulties in ecology (Tylianakis & Morris, 2017). At Mt. Kilimanjaro, biodiversity and ecosystems will most likely suffer from both, climate and land use changes.

5.2.1 Presumptions with regard to climate change

Increasing temperatures will first lead to species losses in the diverse savanna ecosystems at lower elevations (Barlow *et al.*, 2018), because hymenopteran species occurring at those elevations are already close to their thermal maxima. When the ordinary trap nests without the special roofs were used in the pre-experiment, most of the bee larvae died because their thermal maxima had been exceeded. Upward shifts are already taking place along mountain ecosystems (Chen *et al.*, 2011; Lenoir & Svenning, 2015; Freeman *et al.*, 2018), but even if savanna species shift their ranges to higher elevations, there are no other species to follow from even warmer ecosystems and provide ecosystem services (Colwell *et al.*, 2008). Furthermore, I did not find any nests above 2,000 m a.s.l., indicating that the mountain forests act as a boundary for cavity-nesting Hymenoptera. If the species are not able to cross the forest border, they will undergo extinctions far below mountaintops.

Climate change is also affecting seasonal patterns at Mt. Kilimanjaro. Seasonal climatic patterns vary between the years, whereby especially the long rainy season has changed in the last decades (Otte *et al.*, 2017). If cavity-nesting Hymenoptera at low elevations are strongly synchronised by the short rainy season and have a well-adapted diapause with prolonged development during the long rainy and subsequent dry season as suggested in Chapter 3, the question arises how fast they can shift from long-time-developed seasonality in life-history to new patterns in climate seasonality. An even bigger challenge arises when climate seasonality varies between years due to El Niño-Southern Oscillation (ENSO) and/or positive Indian Ocean dipole (IOD) events (Otte *et al.*, 2017). The strength of these climate events is even predicted to increase with climate change. Therefore, seasonal patterns will be of even greater relevance than annual mean values if populations cannot survive extreme climate periods, and are directly affected by extreme climatic conditions or indirectly by resource phenology (Potts *et al.*, 2010). For this reason, research on seasonal patterns, as well as reactions of organisms not only to rising temperatures, but also to changes in water availability, should be incorporated more often into science (Chown *et al.*, 2011). Since terrestrial arthropods are vulnerable to water loss due to their high surface-to-volume ratio and their metabolic rate is affected by temperature (Sprenger *et al.*, 2018), studying patterns of seasonality along elevational gradients with trap nests might shed light on adaptations to climatic seasonality under different temperature and humidity regimes and also provide information on how entire communities might respond to climate change (Staab *et al.*, 2018).

I found indications that climate warming might lead to shifts in bottom-up and top-down processes because temperature affects host and antagonists differently. While patterns of species richness of host taxa along elevation were best explained by temperature, resources had a strong effect on the species richness of antagonist as well. Furthermore, the parasitism rate increased towards lower elevations, implying stronger top-down effects with higher temperatures. Therefore, whole food webs might change with global warming and also the key role of solitary bees and wasps in ecosystem functioning might be threatened.

5.2.2 Presumptions with regard to land use changes

In contrast to other studies (Tylianakis *et al.*, 2005; Batista Matos *et al.*, 2013), I did not find a clear influence of land use on the community of cavity-nesting Hymenoptera. Currently, land use is still moderate at Mt. Kilimanjaro. The Chagga homegardens harbour a high species diversity and are embedded in a mosaic-like landscape, consisting of remaining islands of natural habitats and smallholder agricultural fields. Distances to well-connected woody habitats are short, which is an important factor for wasp abundances in trap nests (Holzschuh *et al.*, 2009). But changes in land use are also clearly noticeable at Mt. Kilimanjaro. Due to human influence, Kilimanjaro is becoming more and more isolated as an ecosystem. A large area of natural savanna has been converted into agricultural fields in the last decades and for this reason, the Kilimanjaro has become more isolated inhibiting the dispersal of species (Hemp & Hemp, 2018). So far, this may not be a problem for highly mobile cavity-nesting Hymenoptera, because I found similar species in natural savanna and maize fields. However, the human population at Mt. Kilimanjaro is growing (Sébastien, 2011; Misana, 2012) and if the landscape was further cleared, distances between nesting sites, sites where hymenopterans find food resources and sites where they gather nest-building materials would be too big. Moreover, most of the solitary bees have only short foraging distances of only few hundred meters and remarkably less brood cells are produced with increasing foraging distances (Zurbuchen *et al.*, 2010b; Zurbuchen *et al.*, 2010a). I observed different nest-building materials in different habitat types and changes in the species composition between extensively-used grasslands, coffee plantations and homegardens at higher elevations, indicating that foraging is restricted to certain habitats and communities differ with land-use type (but see the Outlook). Therefore, land use may have an effect on other traits that were not investigated. Moreover, usually cavity-nesting Hymenoptera account only for a small fraction of especially bee communities and land use may have a much stronger effect on ground-nesting bees. However, disturbance shows no negative effect on the abundance of epigaeic arthropods taxa so far (Röder *et al.*, 2017).

At mid-elevations, Chagga homegardens are largely transformed into coffee monocultures in some areas (Maghimbi, 2007) leading to a lower cloud formation with increasing deforestation (Fairman *et al.*, 2011) and thereby to major effects on the water balance of the mountain (Hemp, 2006b). Interactive effects between land-use changes and climate at Mt. Kilimanjaro are leading to many species losses and to a lower resilience of ecosystem functions with increasing land use in lowland arid ecosystems (Peters *et al.*, 2019). An interplay between land use and climate can be therefore expected for interactions among species and life-history traits, whose patterns are even more complicated. Moreover, in coffee plantations pollination services are performed to a higher proportion by honey bees whereas in homegardens, wild pollinators, such as wild bees, butterflies and syrphid flies, make up a large proportion (Classen *et al.*, 2014). Mass-flowering crops, such as coffee, can increase bee abundances (Holzschuh *et al.*, 2013; Diekötter *et al.*, 2014) and indeed I found the highest numbers of bee nests in one coffee plantation during the short rainy season. These nests were mostly built by one species, *Ctenoplectra centinorii*, which apparently outcompeted other species. These high abundances of either honey bees or single solitary bee species and a less balanced bee community might lead to lower stability of pollination services in more intensified coffee cultivation systems.

Unfortunately, I have no data on the amount and type of pesticides applied. Nevertheless, a questionnaire was used to investigate the pesticide application used by farmers which is mainly done for coffee plants. Their answers pointed to two issues. On the one hand, they applied pesticides in higher concentrations than suggested by the manufacturer because they did not understand the instructions on the package leaflet. On the other hand, they used some pesticides that are no longer authorised in Germany because of their high toxicity. Pesticides have already been detected in bees and bee nests (Mullin *et al.*, 2010; Botías *et al.*, 2017). They operate on different activities and stages of bees development, such as on nesting and colony growth (Whitehorn *et al.*, 2012; Rundlöf *et al.*, 2015), navigation (Balbuena *et al.*, 2015; Jin *et al.*, 2015) and pollination (Whitehorn *et al.*, 2017). Moreover, the effect of pesticides is even more pronounced when different pesticides interact synergistically (Sgolastra *et al.*, 2018). The highest proportion of pesticides stays in the soil or ground water and the lower proportion is uptaken by the plant (Goulson, 2013), with most probable physiological and ecological consequences for ground-nesting bees (Anderson & Harmon-Threatt, 2019). Also cavity-nesting bees ingest pesticides as larvae and adults, either by direct exposure or through nectar, pollen, plant materials or through the soil used as nest-building material (Kopit & Pitts-Singer, 2018). Non-negative effects of pesticides on cavity-nesting bees have been also found, but the contaminated pollen only made up a small fraction of the pollen diet in this study (Peters *et al.*, 2016a). Due to the multiple effects pesticides can have on pollinators and the locally strong pesticide use at Mt. Kilimanjaro, it would be important to investigate the amount of pesticide present in the trap nests and link them to possible unexplained mortality rates that have been observed and that were often higher than the parasitism rates in bees. Worrisome is also that pesticides can also have negative effects on higher trophic levels when they accumulate through the food chain (Teder & Knapp, 2019). For this reason, the use of pesticides should be measured more often in ecological land use studies and should be included more often in the discussion of ecological papers when it comes to the impacts of land-use change on species and ecosystems.

Despite the fact that land use is still moderate at Mt. Kilimanjaro, it is changing in various aspects, so that effects on biodiversity can soon be expected and should therefore be closely further monitored and investigated, particularly in connection with climate change.

5.2.3 Flexibility of organisms

Despite the obvious advantages of space-for-time approaches for studying abiotic drivers and possible consequences of climate change, these approaches do not account for the species´ ability to adapt to changing environments (Walther, 2010). So far, it is still unclear to what extent species can evolve adaptations in their behaviour to escape the worst consequences of global change. The highest risk of global change will occur if species are not flexible enough to adapt. Extreme climatic conditions, together with the lack of food resources and habitats and (chemical) pollution obviously set limits on adaptability. However, species also seem to be able to show flexible behavioural adaptations of which we do not know much so far. For instance, I observed fascinating nest-building behaviour in which bees and wasps used human-based materials which did not seem to harm bees and wasps. Leaf-cutter bees used duct tape, which I had used for the trap nests, instead of leaves (Fig. 1a). Duct tape has very different material properties compared to leaves, but the bees handled it well, even though one side is very sticky. In addition, duct tape is not breathable like other plastic fabrics. Nevertheless, the brood cells did not moult and all bees within the nest survived. The usage of plastic within Megachilinae has been already documented for different areas of the world (MacIvor & Moore, 2013; Gess & Roosenschoon, 2017). I also found another example for the usage of anthropogenic material. *Megachile* and other genera of bees, but also wasps, which typically used plant resins, sealed some of their nests with caterpillar glue (Fig. 1b) that I had applied to the poles of the trap nests in order to deter ants and other predators. Interestingly, the artificial, strong smelling glue did not prevent the respective antagonists to colonise these nests as usual. Hence, some hosts seem to be very flexible and not selective in using easy available nest-building materials that are in close proximity to them. Furthermore, some bees and wasps were flexible in using reed diameters which did not fit their body size and placed brood cells in all directions and angles if the diameter was too large (Fig. 1c). Even though these are just few examples, this might show ecologically adaptive traits in a world that is becoming increasingly dominated by humans (MacIvor & Moore, 2013).

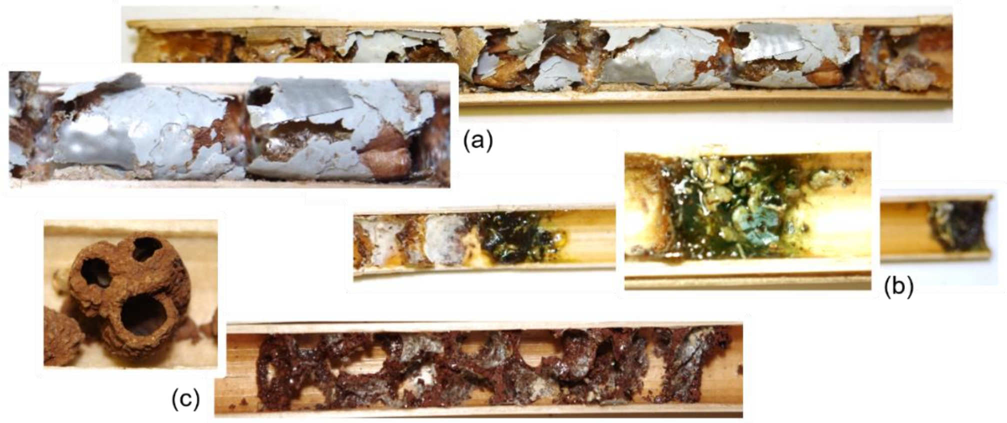


Fig. 1: Particular nest constructions. (a) *Megachile* bee which used duct tape instead of leaf discs. All six brood cells hatched. (b) Sticky caterpillar glue, which was obtained from trap-nest poles instead of natural resin, was used by bees and wasps of the genera *Megachile*, *Noteriades*, *Pseudoheriades* and *Polemistus* for nest closures and interior walls. In spite of the penetrant strong smell of the glue it did not deter antagonists and bees (*Euaspis abdominalis* and *Coelioxys*) and wasps (*Melittobia* and other Chalcidoidea and Chrysididae) parasitised those nests. (c) Sometimes even larger nest diameters than those matching the body size of the hosts precisely were used for nests and brood cells were built next to each other. Left: *Trypoxylon* nest, right: *Pseudoheriades* nest. Pictures © A.V. Mayr

5.3 Outlook

5.3.1 Thinking beyond the thesis

I found strong seasonal patterns of nest-building activity, parasitism and natural mortality rates that differed between trophic levels and elevations. The advantage of studying the effects of seasonal climatic fluctuations on the nest-building activity of species is that seasonal patterns are not masked by interactions with other species, e.g. by decline of host abundances by predators (Boulter *et al.*, 2011). Probably because nest-building activity is not masked, I found the clearest seasonal patterns here and not in other life-history traits. However, I only cover a small subset of bees and wasps with this study of solitary, cavity-nesting Hymenoptera. Thus, it would be interesting to investigate how climatic seasonality affects other hymenopterans, in particular those with social lifestyles. Social bees, for instance, might go into diapause as well as cavity-nesting bees, when resources are too limited to sustain an entire colony. On the other hand, they may be less sensitive to climatic changes due to a better regulation of the microclimate. Therefore, it would be interesting to analyse abundances of honey and stingless bees of the pantrap dataset in all study sites, including both dry and rainy seasons within the course of one year. In contrast to the trap-nest dataset, the pantrap dataset covers the whole elevational gradient and thereby provides the possibility to evaluate stronger climatic changes. Interactions between temperature and humidity should strongly limit the access to resources especially above the forest belt (Classen *et al.*, 2015). Furthermore, with this dataset it is possible to measure shifts in pollinator guilds (e.g. flies, bees, butterflies) (Abrahamczyk *et al.*, 2011) with elevation and season, and contribute to this large knowledge gap that has been rarely addressed (Boulter *et al.*, 2011; Wardhaugh *et al.*, 2018). In addition, other nesting behaviours could be strongly affected by season (Samnegård *et al.*, 2015) and the pantrap dataset allows us to compare, if ground-nesting bees even stronger fluctuate in abundances in the seasonally dry lowlands, because digging might be impossible in the dry season there.

Despite land use has negative effects on cavity-nesting Hymenoptera in other tropical regions (Tylianakis *et al.*, 2005; Batista Matos *et al.*, 2013), no effect was found in this study (except from an influence on the parasitism rate of bee nests). However, I observed a strong turnover in the nest-building materials among different habitat types. This suggests that instead of land-use intensity (measured with different variables such as vegetation dissimilarity, percentage of agricultural fields in the surrounding landscape, pesticide and fertilizer inputs and removal of biomass), it was the difference among habitat types *per se* (different habitat structure, microclimate and plant communities) what limits species with different nest-building materials, thereby changing traits of the species community. Furthermore, limitations of nest-building materials may be stronger than those of food resources. Not only food, but also the availability of nest-building materials can determine the success of nest-building in cavity-nesting Hymenoptera (Roubik, 1989; Westrich, 1996). Soil characteristics may also be important for nesting success. In fact, clay becomes increasingly rare at higher elevations (Morato & Martins, 2006; Loyola & Martins, 2008). Therefore, not only food resources, but also nest-building resources should be taken into account in trap-nest studies (Osorio-Canadas *et al.*, 2018), and it would be interesting to further analyse the species communities of the trap nests with regard to nest-building materials and habitat type, elevation and season.

Although are a large number of studies have recorded entire life-history cycles of species using trap nests, the only trap-nest studies in Africa come from

South Africa (Staab *et al.*, 2018). Trap nests capture mostly different species than other sampling methods, thus, they can often complement regional species lists (Hall & Ascher, 2010). On the other hand, tropical mountain ecosystems which are recognised as hotspots of biodiversity probably harbour many not yet described species. Therefore, I created my own collection of trap-nest information in which I summarised nest characteristics, given the absence of taxonomic literature for the region (General Appendix, Tab S1). In Germany, trap-nest identification is commonly done during the winter months and usually it is not necessary to rear every nest, because the number of species is limited. On the contrary, at Mt. Kilimanjaro, I recorded a high number of host and antagonist species and a high proportion of the nests was built by leaf-cutting *Megachile* bees and Eumeninae wasps, both are groups which have very similar nest characteristics. That is why the identification of the host species in Tanzania is impossible without the time-consuming task of rearing every nest.

The number of nests in trap nests varies (among others) due to microclimatic conditions in the location of the trap nests, both along different vegetation strata and along forest-edge effects (Stangler *et al.*, 2015, 2016). While the canopy provides more natural nesting sites, a higher humidity in the understorey could lead to higher fungal infestation (Morato & Martins, 2006). As the pre-experiment showed, small changes in temperature can lead to high mortality rates in the already warm lowlands, because hymenopterans experience temperatures close to their thermal limits in these habitats. Therefore, the analysis of the microclimatic conditions may reveal interesting explanations about development time and mortality rates. Using a thermologger (iButton®) in each trap nest, I recorded temperature every hour during 17 months of sampling. With the analysis of the microclimatic temperatures it may be possible to reveal critical temperature thresholds under natural conditions by simulating a climate change experiment, and thus it would be possible to explain the spatial variation of ecological rates within the same habitats.

Biotic interactions change naturally across space and time (e.g., within a year or a season, over years and longer periods such as decades or centuries), and with land use (Tylianakis *et al.*, 2007; Alarcón *et al.*, 2008; Olesen *et al.*, 2008; Petanidou *et al.*, 2008; Burkle *et al.*, 2013; CaraDonna *et al.*, 2014; Morris *et al.*, 2015; CaraDonna *et al.*, 2017; Osorio-Canadas *et al.*, 2018). However, the extent to which interactions are dynamic, how they react to environmental changes and what the drivers for changing interactions are, remains little understood (CaraDonna *et al.*, 2017; Tylianakis & Morris, 2017). Nevertheless, it is important to understand species interactions with regard to the functioning of communities and ecosystems in space and time (Bascompte *et al.*, 2006; Thébault & Fontaine, 2010). Networks calculated from trap nests are not biased by observational sampling (Rivera-Hutinel *et al.*, 2012), offer more complete interaction information than networks from transect walks (Bosch *et al.*, 2009; Dorado *et al.*, 2011) and can be investigated across different trophic levels (plant-pollinator networks, predator-prey networks and host-parasitoid networks). However, theses analyses are rarely done with trap-nest datasets (Tylianakis *et al.*, 2007; Staab *et al.*, 2018). The trap-nest dataset of this dissertation offers the possibility to study networks along the elevational gradient of Mt. Kilimanjaro, over different seasons and along the land-use gradient. This may provide further exciting results because the climatic changes along elevation and between seasons may be meaningful with regard to climate change (Burkle & Alarcón, 2011). Furthermore, the importance of the different drivers of species richness already varied across trophic levels and I might find an influence of land use on the level of biotic interactions and on specialist species which are usually more sensitive to change. I collected pollen samples of pollen pellets or faeces of ~700 nests at 21 study sites and during all months. A meta-barcoding analysis of the pollen collected in trap nests may provide a large number of feasible samples in terms of time and money and offers promising results (Sickel *et al.*, 2015). Moreover, meta-barcoding could be also applied for the prey of the wasps, giving also information about pest control in agricultural landscapes, or even the hosts themselves if they are difficult to identify morphologically (Turčinavičienė *et al.*, 2016).

In addition, I extracted cuticular hydrocarbon profiles from cuckoo wasps (Chrysididae) and their hosts from 200 nests. Chrysidids parasitised nests from at least 10 different species of bees, and from different wasps of the families Crabronidae, Eumeninae, Pompilidae and Sphecidae. Chrysidids are known to use chemical deception (e.g. chemical mimicry of their hosts) (Strohm *et al.*, 2008), raising interesting questions about evolutionary arms races. With the study design of the KiLi-project, similarities between chemical profiles of hosts and parasitoids might be analysed in an environmental context along the climatic elevational gradient. The advantage of trap nests here is that the appropriate host can be directly assigned to the cuckoo wasp. For a large number of parasitoid species the host identity is unknown if the nests are not known. In trap nests, within one single nests, some brood cells may be parasitised, while others are not, and in this case the host identity is known for the respective cuckoo wasp.

These examples show that the data can be evaluated in further questions and that it is advisable to build on this doctoral thesis to deepen the first findings and thus to understand the observed patterns even better.

5.3.2 Thinking about the thesis

I aimed to assess the importance of temperature and land use versus resources and top-down control on the species richness of cavity-nesting Hymenoptera. Because the investigation of different taxonomic groups was split between PhD students within the KiLi-project, the resources for the wasp groups were not monitored as continuously as I recorded flower abundances and richness and did not have the same monitoring time window. Furthermore, data on spider abundances and species richness was completely missing from locations in coffee plantations and grasslands and this could have limited the influence of resources on the species richness of spider-hunting wasps. Additionally, I used abundance and species richness data of adult moths instead of caterpillars as food resource for the caterpillar-hunting wasps. However, these abundance data might differ between caterpillars and adult moths if the mortality of caterpillars is high and if it differs among study sites. In further studies, a temporally and methodologically well-adjusted sampling-schedule could improve the synergistic effects of the different subprojects.

The colonisation of the trap nests also depends on the natural availability of nesting resources and on the habitat structure which is connected with different difficulties to detect the trap nests. Therefore, habitats with a large number of big trees, such as in homegardens, may have led to a lower occupancy rate, biasing the abundance and species richness of cavity-nesting Hymenoptera. I observed that in homegardens, trap nests were better detected in patches of open habitat than in the dense vegetation, and colonisation rates were generally low in the canopy. Yet, Stangler *et al.* (2016) recorded a larger number of nests, both at the canopy and at ground level than at an intermediate level and they justified that with a higher occurrence of natural nesting sites. But my observations suggest the opposite: a lower number of nests with higher vegetation complexity. A reason for the higher number of nests in the canopy compared to my study might be that they had higher numbers of trap nests per study site in total, and also in the canopy. Increasing the number of trap nests might have raised the chance to catch cavity-nesting Hymenoptera in forest-like habitats. But in comparison to open habitats, I assume that trap nests are not efficient in habitats which are structurally very complex and have very tall trees (up to 30 m) because they lose their attractiveness with a high abundance of natural cavities. Moreover, living trees provide a more stable microhabitat than dead trees in general (Nicolai, 1986; Tepedino & Parker, 1986; Gibbons & Lindenmayer, 1996) and are therefore preferred. For these reasons, we probably undersampled homegardens in comparison to the other, more open habitats. Yet this problem is not easy to solve. Enhancing only the number of trap nests in the homegardens and not in the other habitats would counteract standardisation. On the other hand, enhancing the number of trap nests in all study sites would lead to unmanageable amount of nests in the open lowlands.

In the analysis of patterns of species richness, I grouped chrysidid wasps into one group, even though I was aware that with ten different parasitised host species, I may have had more than one species of chrysidid wasps in my dataset. Yet, Chrysididae are a very difficult taxonomical group with many cryptic species (Paukkunen *et al.*, 2015). My motivation behind my decision was to avoid mistaken identifications which could enhance parasitoid diversity randomly on study sites, and I rather decided to underestimate their diversity at all study sites. Nevertheless, this might have slightly changed species richness patterns, because they made up ~15% of the killing in antagonist nests. Flies were also identified to only family level and for a number of nests remaining body parts of hosts or antagonists were not enough to make identification to species-level possible. In the future, barcoding of uncertain hosts and antagonists may solve these uncertainties in the identification

(Hrcek *et al.*, 2011; Turčinavičienė *et al.*, 2016), especially since molecular techniques are becoming less expensive when analysing a large number of samples.

When working with trap nests in tropical hot and dry areas, I would first suggest to carefully think about roofs which might buffer the ambient temperature, as suggested in Chapter 2 and discussed earlier in this chapter. I used double-folded metal sheets separated by a distance of few centimetres from each other, which were sprayed with white colour. Second, I would place thermologgers in each trap nest to link potential high local mortality rates to temperature values. Third, if possible, I would suggest three week intervals between sampling in the savanna habitats since the control intervals of one month were too long for some very fast developing species. And finally, I would recommend to rear every nest, because this is the only method that can provide complete information on mortality rates. Furthermore, even in very similar looking nests, I found different species of hosts and some host species just used commenced brood cells of other species, which causes identification mistakes and confusion when nests are not reared and therefore adult hosts are not available.

5.4 Conclusion

Climate showed direct and indirect effects on species richness, functional traits and interactions of hymenopterans at Mt. Kilimanjaro, both at spatial and temporal scales. This shows that spatial and temporal analyses are important because both, spatial and temporal stability is essential for ecosystem functions. Furthermore, increasing climate fluctuations across years requires long-term studies, especially because an ongoing intensification of land use in this region could even exacerbate climatic fluctuations in the future. In addition, the observed patterns were complex and as well as their drivers, were found to be variable across trophic levels, elevations, ecosystems and the investigated factors, such as species richness, life-history traits, ecological rates and biotic interactions. Therefore it is difficult to conclude global explanations at the community level, because I even found differences among species, because ecological processes take place at the individual level. Thus it is important to use the right scale to evaluate these patterns, to look from the big to the small, to study different aspects of biodiversity and to connect different factors in order to better understand the potential mechanisms that shape communities at multiple trophic levels. Only those communities that show stability at different trophic levels guarantee multi-functionality of ecosystems. New molecular techniques (e.g. faster and cheaper barcoding) may allow to investigate hidden interactions and networks at different trophic levels, whereas old, well-established observation techniques (e.g. trap nests and hand sampling of individuals) ensure that details are noticed that are essential to the understanding of patterns. Together, both old and new techniques can complement each other and help to gain a more complete picture of factors which matter for biodiversity and ecosystems. This, in turn, is necessary to derive meaningful consequences of required planning actions for conservation efforts, even though we already know enough to rethink our way of life as humans.

In this time of rapid environmental change, the many endangered species are only a drop in the flood of endangered communities

(Pimm *et al.*, 1991).

General Appendix

Tab. S1: Collection of nest parameters, describing bee and wasp morphospecies found in trap nests with indication of their occurrence in habitats, elevation and season of the year.

taxonomy					habitat (ordered by elevation)																														season												food									diameter						nest closure				interi		material										number			cocoon				faece	
family	subfamily	tribe	genus	species	mai (866-1009m)	mai1 (1009m)	mai2 (866m)	mai3 (886m)	mai4 (960m)	mai5 (920m)	sav (871-1153m)	sav1 (871m)	sav2 (906m)	sav3 (1153m)	sav4 (984m)	sav5 (951m)	cof (1124-1648m)	cof1 (1306m)	cof2 (1345m)	cof3 (1305m)	cof4 (1124m)	cof5 (1648m)	cof6 (1500m)	hom (1169-1788m)	hom1 (1647m)	hom2 (1169m)	hom3 (1788m)	hom4 (1275m)	gra (1303-1748m)	gra1 (1660m)	gra2 (1748m)	gra4 (1312m)	gra5 (1303m)	gra6 (1400m)	january	february	march	april	may	june	july	august	september	october	november	december	pollen	dry	moist	carnivore	spiders	caterpillars	locusts	aphids	cicadas	≤ 0.25	> 0.25 ≤ 0.5	> 0.5 ≤ 0.75	> 0.75 ≤ 1	> 1 ≤ 1.25	> 1.25	present	uniform	layered	mixed	interior walls	loose material	mud	lime	resin	plant material	leaves	bark	chewed plant	fibres	membrane	stones	≤ 5	> 5 ≤ 10	> 10	sturdy	fine	light	dark	faeces loose	faeces separated
Apidae	Apinae	Ctenoplectrin	*Ctenoplectra*	*Ctenoplectra centinorii*	xo	xo											x	x	xo	xo	xo			xo											x	x	x	x		x	x	x	x	x	x	x	x		x								x	x				x	x			x		x										x	x			x	x			x
	Xylocopinae	Allodapini	*Macrogalea*	*Macrogalea sp.1*	x				x																															x					x		x										x	x				x			x	x				x	x			x				x								
Colletidae	Hylaeinae		*Nothylaeus*	*Nothylaeus sp.1*													x	x																		x										x	x										x					x	x			x										x		x				x	x			
				Nothylaeus sp.2													x				x			x		x											x									x	x	x									x					x	x			x										x		x	x			x	x			
				Nothylaeus sp.3																				x		x									x												x										x					x	x			x										x		x				x	x			
				Nothylaeus sp.4	x	x					x			x	x																						x				x		x	x	x	x	x	x									x					x	x			x		xo								x		x	x			x	x			
				Nothylaeus sp.5																				x				x							x												x															xo	xo			xo										xo										
				Nothylaeus sp.6													x			x																										x	x										x					x	x			x										x		x								
				Nothylaeus sp.7	x	x																																				x					x										x					x	x			xo										x		x								
				Nothylaeus sp.8													x	x																													x										x					x	xo			x										xo		x								
Megachilidae	Megachilinae	Anthidiini	*Afranthidium*	*Afranthidium sp.1*																									x	x								x									x										x					x			x						x									x						
			Anthidiellum	*Anthidiellum somaliense*	x		xo		xo	x	x	x			x	x																			x	x		x	x	x	x	x	x			x	x	x									x					x	x		xo	x				x	xo			xo				x	x			x		x		x
			Pseudoanthidiu	*Pseudoanthidium sp.1*							x			x																											x						x															x	x				x				x				x						x			x		
Megachilidae	Megachilinae	Megachilini	*Gronoceras*	*Gronoceras felina*	x	x	x	x	xo		x	x		xo	x	x								x				x	x				x	xo	x	x				x	x	x	x	x	x	x	x		x										x	x	x	x		x	x			x		x								x	x			x	x			x
			Megachile	*Megachile admixta*	x	x	x	x		xo	xo	xo		xo	xo		x	x	xo		xo								xo				xo			xo			xo		x	x	x	x	x	x	x	x									x	x				x	x								x	x		xo				x	x	x	x			x		
				Megachile decemsignata							x				x																							x									x											x				x	x			x				x								x				x	x			x
				Megachile demeter	x		x	x	x	x	x	x	x	x	x	x																			x	x	x	x	x	xo	xo	xo	xo	xo	x	x	x		x								x	x				x	x		x	x		x		x								x	x			x	x			x
				Megachile discolor	x					x	x				x																									x						x	x	x											x	x		x	x			x					x			x				x	x							
				Megachile fervida	x		x	x	x	x	x	x	xo	x	x		xo	xo																		x	x	x	x	x	x	xo	x	x	x	x	x		x								x	x				x	xo	x		x		x		xo	x			x				x	x	x		x	x			x
				Megachile maxillosa	x			xo	xo	x	x		xo	xo	x	xo													xo			xo			x	x		x	x	x	x	xo				x	x		x									x	x	x	x	x			x	x		xo		x	xo		xo				xo	x			x			x	x	
				Megachile mossambica	x	xo	xo	x		x	x	x	xo	x	x	x	xo	xo											x			xo	x		x	x			x	x	x	x	x	x	x	x	x		x									x	x	x	x	x			x					x	x		xo	xo			xo	x	x			x		x		x
				Megachile rufipennis							x	x																							x												x											x				x	x			x				x								x							x	
				Megachile rufiventris	xo				xo		x			x																								x		x		x					x											x	x			x	x			x				x							xo	x				x		x		x
				Megachile semierma	x	x	x	x	x	xo	x	xo		x			xo		xo										x	xo			x		xo		xo		xo	x		x	x	xo	xo	x	x	x										x				x			x			x			x	x		x				xo	x	xo		x	x			
				Megachile sheppardi	xo					xo	x				x	x																			x	x		x	x	x	x						x		x								x	x				x		xo	xo	x		xo		x							x	x			x			x	x	
				Megachile sinuate	x		x			xo	x					x																				x			x						x	x	x											x	x	x	x	x	x					x										x	x			x	x			
				Megachile sp.1	x	x	x	x																																						x	x										x	x	x			x	xo								x	xo		xo					x	x						
				Megachile sp.2	x			x																														x				x					x											x				xo	xo								xo	xo	xo					x	x							
				Megachile sp.3	x				x																																	x					x											x				x	x								x	x								x						
				Megachile sp.4	x				x																																					x	x											x				x		x	x	x		x		x								x	x							
				Megachile sp.5	x		x	x	x	xo	x	x			x	x																			x	x	x	x	x	x			x		x	x	x		x								x	x	x			x	x		x	x		xo		x	xo			xo				x	x			x	x			x
				Megachile sp.6	xo			xo																x	x	xo	xo	x	x	x													x	x	x	x	x												x	x	x	x		x		x		x		x	xo							x	x			x	x			x
				Megachile sp.7	x		x			x																									x			x								x	x	x										x	x			x	x			xo					x			x				x	x							
				Megachile sp.8	x			x																											x												x										x					xo	xo	xo	xo			xo		xo	xo		xo					x								
				Megachile sp.9	x					x																										x											x											x				x		x				x			x			x					x							
				Megachile sp.10	x					x																																				x	x												x			x	x								x			x				x								
				Megachile sp.11	x		x																																							x	x		x									x				x	x								x			x				x	x							
				Megachile sp.13	x		x																																							x	x											x	x				x		x	x	x		x		x								x								x
				Megachile sp.12	x				x																																	x					x										x					xo	xo	xo	xo			xo		xo	xo	xo		xo				x	x	x						
				Megachile ungulata													x	x					x														x							x			x											x				x	x								x	x							x	x						
Megachilidae	Megachilinae	Osmiini	*Heriades*	*Heriades sp.1*																									x	x					x		x						x				x		x								x					x			x	x		x		x								x	x	x		x	x			x
				Heriades sp.2	x	x	x		xo		xo	xo		xo			xo	xo	xo																	xo		xo			xo			x	x	x	x		x							x	x					x			x	xo		x		x								x	x	x		x	x			
Megachilidae	Megachilinae	Osmiini	*Noteriades*	*Notheriades sp.1*	x	xo	xo	x	x	x	x	x	x	x	x	x																			x	xo	x	xo	x	x	xo	x	x	x	x	x	x		x							x	x					x	x			x		xo		x							xo	x	x	xo		x	x			x
				Notheriades sp.2	x					x	x					x																					x						x				x										x					x	x		xo	x				x	xo			xo					x							
				Notheriades sp.3							x			x			x	x																	x									x			x										x					x	x			x		xo		x								x								
Megachilidae	Megachilinae	Osmiini	*Pseudoheriades*	*Pseudoheriades sp.1*	x	xo	x	xo	x	x	x	x	x	x	x	x																			x	x	x	x	x	x	x	x	x	x	x	x	x		x								x	x				x			x	x		x		x							xo	x	x	x		x	x			x
Megachilidae	Megachilinae	Osmiini	*Stenoheriades*	*Stenoheriades sp.1*							x				x	x																			x	x	x		x								x	x									x	x				x			x	x		x		x								x	x	x		x	x			
Megachilidae	Megachilinae	Osmiini	*Wainia*	*Wainia sp.1*							x			x																						x		x	x		x					x	x									x	x					x	x			x					x			x				x	x			x	x		x	

taxonomy					habitat (ordered by elevation)																										season												food									diameter						nest closure				interi		material										number			cocoon				faece	
family	subfamily	tribe	genus	species	mai (866-1009m)	mai1 (1009m)	mai2 (866m)	mai3 (886m)	mai4 (960m)	mai5 (920m)	sav (871-1153m)	sav1 (871m)	sav2 (906m)	sav3 (1153m)	sav4 (984m)	sav5 (951m)	cof (1124-1648m)	cof1 (1306m)	cof2 (1345m)	cof3 (1305m)	cof4 (1124m)	cof5 (1648m)	cof6 (1500m)	hom (1169-1788m)	hom1 (1647m)	hom2 (1169m)	hom3 (1788m)	hom4 (1275m)	gra (1303-1748m)	gra1 (1660m)	gra2 (1748m)	gra4 (1312m)	gra5 (1303m)	gra6 (1400m)	january	february	march	april	may	june	july	august	september	october	november	december	pollen	dry	moist	carnivore	spiders	caterpillars	locusts	aphids	cicadas	≤ 0.25	> 0.25 ≤ 0.5	> 0.5 ≤ 0.75	> 0.75 ≤ 1	> 1 ≤ 1.25	> 1.25	present	uniform	layered	mixed	interior walls	loose material	mud	lime	resin	plant material	leaves	bark	chewed plant	fibres	membrane	stones	≤ 5	> 5 ≤ 10	> 10	sturdy	fine	light	dark	faeces loose	faeces separated
Crabronidae	Crabroninae	Trypoxylini	*Pison*	*Pison sp.1*							x	x					x	x		x	x			x		x			xo				xo		x		x				x		x			x				x	x						x	x				x	x			x		x									xo	x	x	x	x			x		x
Crabronidae	Crabroninae	Trypoxylini	*Trypoxylon*	*Trypoxylon sp. 1*	x					x							x	xo		x	x														x									x	x	x				x	x						x					x	x			x		x										x	x			x	x			x
		|*Trypoxylon*...																																																																																						

References

Abrahamczyk, S., Kluge, J., Gareca, Y., Reichle, S. & Kessler, M. (2011). The influence of climatic seasonality on the diversity of different tropical pollinator groups. *PloS one*, **6**, e27115.

Addo-Bediako, A., Chown, S.L. & Gaston, K.J. (2000). Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society B: Biological Sciences*, **267**, 739–745.

Alarcón, R., Waser, N.M. & Ollerton, J. (2008). Year-to-year variation in the topology of a plant-pollinator interaction network. *Oikos*, **117**, 1796–1807.

Alaux, C., Ducloz, F., Crauser, D. & Le Conte, Y. (2010). Diet effects on honeybee immunocompetence. *Biology letters*, **6**, 562–565.

Allen, A.P., Gillooly, J.F., van Savage, M. & Brown, J.H. (2006). Kinetic effects of temperature on rates of genetic divergence and speciation. *Proceedings of the National Academy of Sciences*, **103**, 9130–9135.

Allen, J. (1877). The influence of physical conditions in the genesis of species. *Radical Review*, 108–140.

Altieri, M.A. (1999). The ecological role of biodiversity in agroecosystems. *Agriculture, Ecosystems & Environment*, **74**, 19–31.

Anderson, N.L. & Harmon-Threatt, A.N. (2019). Chronic contact with realistic soil concentrations of imidacloprid affects the mass, immature development speed, and adult longevity of solitary bees. *Scientific reports*, **9**, 3724.

Appelhans, T., Mwangomo, E., Otte, I., Detsch, F., Nauss, T. & Hemp, A. (2016). Eco-meteorological characteristics of the southern slopes of Kilimanjaro, Tanzania. *International Journal of Climatology*, **36**, 3245–3258.

Araújo, P.C.S., Lourenço, A.P. & Raw, A. (2016). Trap-nesting bees in montane grassland (Campo Rupestre) and Cerrado in Brazil. Collecting generalist or specialist nesters. *Neotropical entomology*, **45**, 482–489.

Aronesty, E. (2013). Comparison of sequencing utility programs. *The Open Bioinformatics Journal*, **7**, 1–8.

Attwood, S.J., Maron, M., House, A.P.N. & Zammit, C. (2008). Do arthropod assemblages display globally consistent responses to intensified agricultural land use and management? *Global Ecology and Biogeography*, **17**, 585–599.

Bajželj, B., Richards, K.S., Allwood, J.M., Smith, P., Dennis, J.S., Curmi, E. & Gilligan, C.A. (2014). Importance of food-demand management for climate mitigation. *Nature Climate Change*, **4**, 924–929.

Balbuena, M.S., Tison, L., Hahn, M.-L., Greggers, U., Menzel, R. & Farina, W.M. (2015). Effects of sublethal doses of glyphosate on honeybee navigation. *The Journal of experimental biology*, **218**, 2799–2805.

Barlow, J., Lennox, G.D., Ferreira, J., Berenguer, E., Lees, A.C., Mac Nally, R., Thomson, J.R., Ferraz, S.F.d.B., Louzada, J., Oliveira, V.H.F., Parry, L., Solar, R.R.d.C., Vieira, I.C.G., Aragão, L.E.O.C., Begotti, R.A., Braga, R.F., Cardoso, T.M., Oliveira, R.C. de, Souza, C.M., Moura, N.G., Nunes, S.S., Siqueira, J.V., Pardini, R., Silveira, J.M., Vaz-de-Mello, F.Z., Veiga, R.C.S., Venturieri, A. & Gardner, T.A. (2016). Anthropogenic disturbance in tropical forests can double biodiversity loss from deforestation. *Nature*, **535**, 144–147.

Barlow, J., França, F., Gardner, T.A., Hicks, C.C., Lennox, G.D., Berenguer, E., Castello, L., Economo, E.P., Ferreira, J., Guénard, B., Leal, C.G., Isaac, V., Lees, A.C., Parr, C.L., Wilson, S.K., Young, P.J. & Graham, N.A.J. (2018). The future of hyperdiverse tropical ecosystems. *Nature*, **559**, 517.

Barnes, A.D., Allen, K., Kreft, H., Corre, M.D., Jochum, M., Veldkamp, E., Clough, Y., Daniel, R., Darras, K., Denmead, L.H., Farikhah Haneda, N., Hertel, D., Knohl, A., Kotowska, M.M., Kurniawan, S., Meijide, A., Rembold, K., Edho Prabowo, W., Schneider, D., Tscharntke, T. & Brose, U. (2017). Direct and cascading impacts of tropical land-use change on multi-trophic biodiversity. *Nature ecology & evolution*, **1**, 1511–1519.

Barry, R.G. (1992). Mountain climatology and past and potential future climatic changes in mountain regions. A review. *Mountain Research and Development*, **12**, 71.

Bartomeus, I., Ascher, J.S., Wagner, D., Danforth, B.N., Colla, S., Kornbluth, S. & Winfree, R. (2011). Climate-associated phenological advances in bee pollinators and bee-pollinated plants. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 20645–20649.

Bartomeus, I., Ascher, J.S., Gibbs, J., Danforth, B.N., Wagner, D.L., Hedtke, S.M. & Winfree, R. (2013). Historical changes in northeastern US bee pollinators related to shared ecological traits. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 4656–4660.

Bartón, K. (2018). MuMIn: multi-model interference. https://cran.r-project.org/web/packages/MuMIn/index.html.

Bascompte, J., Jordano, P. & Olesen, J.M. (2006). Asymmetric coevolutionary networks facilitate biodiversity maintenance. *Science (New York, N.Y.)*, **312**, 431–433.

Bascompte, J. & Jordano, P. (2007). Plant-animal mutualistic networks. The architecture of biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, **38**, 567–593.

Bascompte, J. (2009). Mutualistic networks. *Frontiers in Ecology and the Environment*, **7**, 429–436.

Batista Matos, M.C., Sousa-Souto, L., Almeida, R.S. & Teodoro, A.V. (2013). Contrasting patterns of species richness and composition of solitary wasps and bees (Insecta. Hymenoptera) according to land-use. *Biotropica*, **45**, 73–79.

Bebber, D.P., Holmes, T. & Gurr, S.J. (2014). The global spread of crop pests and pathogens. *Global Ecology and Biogeography*, **23**, 1398–1407.

Beck, J., McCain, C.M., Axmacher, J.C., Ashton, L.A., Bärtschi, F., Brehm, G., Choi, S.-W., Cizek, O., Colwell, R.K., Fiedler, K., Francois, C.L., Highland, S., Holloway, J.D., Intachat, J., Kadlec, T., Kitching, R.L., Maunsell, S.C., Merckx, T., Nakamura, A., Odell, E., Sang, W., Toko, P.S., Zamecnik, J., Zou, Y. & Novotny, V. (2017). Elevational species richness gradients in a hyperdiverse insect taxon. A global meta-study on geometrid moths. *Global Ecology and Biogeography*, **26**, 412–424.

Bell, K.L., Vere, N. de, Keller, A., Richardson, R.T., Gous, A., Burgess, K.S. & Brosi, B.J. (2016). Pollen DNA barcoding. Current applications and future prospects. *Genome*, **59**, 629–640.

Benson, D.A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J. & Sayers, E.W. (2013). GenBank. *Nucleic acids research*, **41**, D36-42.

Bergmann, C. (1847). *Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Größe*. Vandenhoeck und Ruprecht, Göttingen.

Bianchi, F.J.J.A., Booij, C.J.H. & Tscharntke, T. (2006). Sustainable pest regulation in agricultural landscapes. A review on landscape composition, biodiversity and natural pest control. *Proceedings of the Royal Society B: Biological Sciences*, **273**, 1715–1727.

Biesmeijer, J.C., Roberts, S.P.M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A.P., Potts, S.G., Kleukers, R., Thomas, C.D., Settele, J. & Kunin, W.E. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science (New York, N.Y.)*, **313**, 351–354.

Bishop, T.R., Robertson, M.P., van Rensburg, B.J., Parr, C.L. & Gillman, L. (2014). Elevation-diversity patterns through space and time. Ant communities of the Maloti-Drakensberg Mountains of southern Africa. *Journal of Biogeography*, **41**, 2256–2268.

Blois, J.L., Williams, J.W., Fitzpatrick, M.C., Jackson, S.T. & Ferrier, S. (2013). Space can substitute for time in predicting climate-change effects on biodiversity. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 9374–9379.

Bolker, B. (2017). Dealing with quasi-models in R. http://creativecommons.org/licenses/by-nc/3.0/.

Bonan, G.B. (2008). Forests and climate change. Forcings, feedbacks, and the climate benefits of forests. *Science (New York, N.Y.)*, **320**, 1444–1449.

Bordenstein, S.R. & Bordenstein, S.R. (2011). Temperature affects the tripartite interactions between bacteriophage WO, *Wolbachia*, and cytoplasmic incompatibility. *PloS one*, **6**, e29106.

Bosch, J., González, A.M.M., Rodrigo, A. & Navarro, D. (2009). Plant-pollinator networks. Adding the pollinator's perspective. *Ecology letters*, **12**, 409–419.

Botías, C., David, A., Hill, E.M. & Goulson, D. (2017). Quantifying exposure of wild bumblebees to mixtures of agrochemicals in agricultural and urban landscapes. *Environmental pollution (Barking, Essex : 1987)*, **222**, 73–82.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M.A., Rambaut, A. & Drummond, A.J. (2014). BEAST 2. A software platform for Bayesian evolutionary analysis. *PLoS computational biology*, **10**, e1003537.

Boulter, S.L., Lambkin, C.L. & Starick, N.T. (2011). Assessing the abundance of seven major arthropod groups along an altitudinal gradient and across seasons in subtropical rainforest. *Memoirs of the Queensland Museum*, 303–313.

Brittain, C. & Potts, S.G. (2011). The potential impacts of insecticides on the life-history traits of bees and the consequences for pollination. *Basic and Applied Ecology*, **12**, 321–331.

Brown, J.H., Gillooly, J.F., Allen, A.P., van Savage, M. & West, G.B. (2004). Toward a metabolic theory of ecology. *Ecology*, **85**, 1771–1789.

Brown, J.H. (2014). Why are there so many species in the tropics? *Journal of biogeography*, **41**, 8–22.

Buckley, L.B., Hurlbert, A.H. & Jetz, W. (2012). Broad-scale ecological implications of ectothermy and endothermy in changing environments. *Global Ecology and Biogeography*, **21**, 873–885.

Burkle, L.A. & Alarcón, R. (2011). The future of plant-pollinator diversity. Understanding interaction networks across time, space, and global change. *American journal of botany*, **98**, 528–538.

Burkle, L.A., Marlin, J.C. & Knight, T.M. (2013). Plant-pollinator interactions over 120 years. Loss of species, co-occurrence, and function. *Science (New York, N.Y.)*, **339**, 1611–1615.

Burnham, K.P. & Anderson, D.R. (2004). Multimodel inference. Understanding AIC and BIC in model selection. *Sociological Methods & Research*, **33**, 261–304.

Buschini, M.L.T. (2007). Life-history and sex allocation in *Trypoxylon* (syn. *Trypargilum*) *lactitarse* (Hymenoptera; Crabronidae). *Journal of Zoological Systematics and Evolutionary Research*, **45**, 206–213.

Canevazzi, N.C.d.S. & Noll, F.B. (2011). Environmental factors influencing foraging activity in the social wasp *Polybia paulista* (Hymenoptera. Vespidae: Epiponini). *Psyche: A Journal of Entomology*, **2011**, 1–8.

CaraDonna, P.J., Iler, A.M. & Inouye, D.W. (2014). Shifts in flowering phenology reshape a subalpine plant community. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 4916–4921.

CaraDonna, P.J., Petry, W.K., Brennan, R.M., Cunningham, J.L., Bronstein, J.L., Waser, N.M. & Sanders, N.J. (2017). Interaction rewiring and the rapid turnover of plant-pollinator networks. *Ecology letters*, **20**, 385–394.

Carlson, D.A., Bernier, U.R. & Sutton, B.D. (1998). Elution patterns from capillary GC for methyl-branched alkanes. *Journal of chemical ecology*, **24**, 1845–1865.

Carvalheiro, L.G., Barbosa, E.R.M. & Memmott, J. (2008). Pollinator networks, alien species and the conservation of rare plants. *Trinia glauca* as a case study. *Journal of Applied Ecology*, **45**, 1419–1427.

Cave, R.D. & Gaylor, M.J. (1988). Influence of temperature and humidity on the development and survival of *Telenomus reynoldsi* (Hymenoptera: Scelionidae) parasitizing *Geocoris punctipes* (Heteroptera: Lygaeidae) eggs. *Annals of the Entomological Society of America*, **81**, 278–285.

Chao, A. (1984). Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics*, 265–270.

Chao, A. (1987). Estimating the population size for capture-recapture data with unequal catchability. *Biometrics*, **43**, 783.

Chapin III, F.S., Zavaleta, E.S., Eviner, V.T., Naylor, R.L., Vitousek, P.M., Reynolds, H.L., Hooper, D.U., Lavorel, S., Sala, O.E., Hobbie, S.E., Mack, M.C. & Díaz, S. (2000). Consequences of changing biodiversity. *Nature*, **405**, 234 EP -.

Chapman, A.D. (2009). *Numbers of living species in Australia and the world*, 2nd ed. Australian Government, Department of the Environment, Water, Heritage and the Arts, Parkes, ACT.

Chen, I.-C., Shiu, H.-J., Benedick, S., Holloway, J.D., Chey, V.K., Barlow, H.S., Hill, J.K. & Thomas, C.D. (2009). Elevation increases in moth assemblages over 42 years on a tropical mountain. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 1479–1483.

Chen, I.-C., Hill, J.K., Ohlemüller, R., Roy, D.B. & Thomas, C.D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science (New York, N.Y.)*, **333**, 1024–1026.

Chesson, P. & Huntly, N. (1989). Short-term instabilities and long-term community dynamics. *Trends in Ecology & Evolution*, **4**, 293–298.

Child, R.E. (2007). Insect damage as a function of climate. *Museum microclimates* (ed. by T. Padfield and K. Borchersen). National Museum of Denmark, Copenhagen.

Chown, S.L. & Gaston, K.J. (1999). Exploring links between physiology and ecology at macro-scales. The role of respiratory metabolism in insects. *Biological Reviews*, **74**, 87–120.

Chown, S.L., Sørensen, J.G. & Terblanche, J.S. (2011). Water loss in insects. An environmental change perspective. *Journal of Insect Physiology*, **57**, 1070–1084.

Classen, A., Peters, M.K., Ferger, S.W., Helbig-Bonitz, M., Schmack, J.M., Maassen, G., Schleuning, M., Kalko, E.K.V., Böhning-Gaese, K. & Steffan-Dewenter, I. (2014). Complementary ecosystem services provided by pest predators and pollinators increase quantity and quality of coffee yields. *Proceedings. Biological sciences*, **281**, 20133148.

Classen, A., Peters, M.K., Kindeketa, W.J., Appelhans, T., Eardley, C.D., Gikungu, M.W., Hemp, A., Nauss, T. & Steffan-Dewenter, I. (2015). Temperature versus resource constraints. Which factors determine bee diversity on Mount Kilimanjaro, Tanzania? *Global Ecology and Biogeography*, **24**, 642–652.

Classen, A., Steffan-Dewenter, I., Kindeketa, W.J., Peters, M.K. & Rezende, E. (2017). Integrating intraspecific variation in community ecology unifies theories on body size shifts along climatic gradients. *Functional Ecology*, **31**, 768–777.

Clements, F.E. (1936). Nature and structure of the climax. *Journal of Ecology*, **24**, 252.

Colwell, R.K., Brehm, G., Cardelús, C.L., Gilman, A.C. & Longino, J.T. (2008). Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. *Science (New York, N.Y.)*, **322**, 258–261.

Connell, J.H. (1970). *On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees.* Pudoc, Wageningen.

Corcos, D., Cerretti, P., Mei, M., Vigna Taglianti, A., Paniccia, D., Santoiemma, G., Biase, A. de & Marini, L. (2018). Predator and parasitoid insects along elevational gradients. Role of temperature and habitat diversity. *Oecologia*.

Costello, M.J., May, R.M. & Stork, N.E. (2013). Can we name earth's species before they go extinct? *Science (New York, N.Y.)*, **339**, 413–416.

Cowles, H.C. (1899). The ecological relations of the vegetation on the sand dunes of lake michigan. Part I.-Geographical relations of the dune floras. *Botanical Gazette*, **27**, 95–117.

Currie, D.J. (1991). Energy and large-scale patterns of animal- and plant-species richness. *The American naturalist*, **137**, 27–49.

da Silva, N.A.P., Frizzas, M.R. & de Oliveira, C.M. (2011). Seasonality in insect abundance in the "Cerrado" of Goiás State, Brazil. *Revista Brasileira de Entomologia*, **55**, 79–87.

Danforth, B.N., Conway, L. & Ji, S. (2003). Phylogeny of eusocial *Lasioglossum* reveals multiple losses of eusociality within a primitively eusocial clade of bees (Hymenoptera. Halictidae). *Systematic Biology*, **52**, 23–36.

Denlinger, D.L. (1986). Dormancy in tropical insects. *Annual review of entomology*, **31**, 239–264.

Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C. & Martin, P.R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 6668–6672.

Di Pasquale, G., Salignon, M., Le Conte, Y., Belzunces, L.P., Decourtye, A., Kretzschmar, A., Suchail, S., Brunet, J.-L. & Alaux, C. (2013). Influence of pollen nutrition on honey bee health. Do pollen quality and diversity matter? *PloS one*, **8**, e72016.

Diekötter, T., Peter, F., Jauker, B., Wolters, V. & Jauker, F. (2014). Mass-flowering crops increase richness of cavity-nesting bees and wasps in modern agro-ecosystems. *GCB Bioenergy*, **6**, 219–226.

Dirzo, R., Young, H.S., Galetti, M., Ceballos, G., Isaac, N.J.B. & Collen, B. (2014). Defaunation in the Anthropocene. *Science (New York, N.Y.)*, **345**, 401–406.

Dobzhansky, T. (1950). Evolution in the tropics. *American Scientist*, 209–221.

Dorado, J., Vázquez, D.P., Stevani, E.L. & Chacoff, N.P. (2011). Rareness and specialization in plant–pollinator networks. *Ecology*, **92**, 19–25.

Eardley, C.D. (2003). Revision of the afrotropical Ctenoplectrini (Hymenoptera: Apidae). *African Plant Protection*, **9**, 5–18.

Eardley, C.D., Kuhlmann, M. & Pauly, A. (2010). The Bee Genera and Subgenera of sub-Saharan Africa. *Abc Taxa*, **7**.

Eardley, C.D. (2012). A taxonomic revision of the southern African species of dauber bees in the genus *Megachile* Latreille (Apoidea: Megachilidae). *Zootaxa*, **3601**, 1–133.

Eardley, C.D. (2013). A taxonomic revision of the southern African leaf-cutter bees, *Megachile* Latreille *sensu stricto* and *Heriadopsis* Cokerell (Hymenoptera: Apoidea: Megachilidae). *Zootaxa*, **3460**, 1–139.

Eardley, C.D. (2018). Taxonomic revision of the sub-Saharan *Anthidiellum* Cockerell (Apoidea. Megachilidae: Anthidiini). *Zootaxa*, **4402**, 201–250.

Eckhardt, M., Haider, M., Dorn, S. & Müller, A. (2014). Pollen mixing in pollen generalist solitary bees. A possible strategy to complement or mitigate unfavourable pollen properties? *The Journal of animal ecology*, **83**, 588–597.

Edelman, A., Gelding, A., Konovalov, E., McComiskie, R., Penny, A., Roberts, N., Templeman, S., Trewin, D., Ziembicki, M., Trewin, B., Cortlet, R., Hemingway, J., Isaac, J. & Turton, S. (2014). State of the tropics: 2014 report. *James Cook University Cairns.*

Edgar, R.C. (2004). MUSCLE. Multiple sequence alignment with high accuracy and high throughput. *Nucleic acids research*, **32**, 1792–1797.

Edgar, R.C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics (Oxford, England)*, **26**, 2460–2461.

Eickwort, G.C., Eickwort, J.M., Gordon, J., Eickwort, M.A. & Wcislo, W.T. (1996). Solitary behavior in a high-altitude population of the social sweat bee *Halictus rubicundus* (Hymenoptera. Halictidae). *Behavioral Ecology and Sociobiology*, **38**, 227–233.

Elekcioglu, N.Z. & Uygun, N. (2013). Population fluctuation of citrus leafminder, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) and its parasitoids in the eastern mediterranean region of Turkey. *Pakistan J. Zool.*, 1393–1403.

Engel, P. & Moran, N.A. (2013). The gut microbiota of insects - diversity in structure and function. *FEMS microbiology reviews*, **37**, 699–735.

Engel, P., Kwong, W.K., McFrederick, Q., Anderson, K.E., Barribeau, S.M., Chandler, J.A., Cornman, R.S., Dainat, J., Miranda, J.R. de, Doublet, V., Emery, O., Evans, J.D., Farinelli, L., Flenniken, M.L., Granberg, F., Grasis, J.A., Gauthier, L., Hayer, J., Koch, H., Kocher, S., Martinson, V.G., Moran, N., Munoz-Torres, M., Newton, I., Paxton, R.J., Powell, E., Sadd, B.M., Schmid-Hempel, P., Schmid-Hempel, R., Song, S.J., Schwarz, R.S., vanEngelsdorp, D. & Dainat, B. (2016). The bee microbiome. Impact on bee health and model for evolution and ecology of host-microbe interactions. *mBio*, **7**, e02164-15.

Ewers, R.M. & Didham, R.K. (2006). Confounding factors in the detection of species responses to habitat fragmentation. *Biological reviews of the Cambridge Philosophical Society*, **81**, 117–142.

Ezard, T., Tomochika, F. & Barraclough, T. (2017). splits: SPecies' LImits by Threshold Statistics. *R package version 1.0-19/r52*.

Fairman, J.G., Nair, U.S., Christopher, S.A. & Mölg, T. (2011). Land use change impacts on regional climate over Kilimanjaro. *Journal of Geophysical Research*, **116**, 32141.

Ferger, S.W., Schleuning, M., Hemp, A., Howell, K.M. & Böhning-Gaese, K. (2014). Food resources and vegetation structure mediate climatic effects on species richness of birds. *Global Ecology and Biogeography*, **23**, 541–549.

Fernandes, E.C.M., Oktingati, A. & Maghembe, J. (1984). The Chagga homegardens. A multistoried agroforestry cropping system on Mt. Kilimanjaro (Northern Tanzania). *Agroforestry Systems*, **2**, 73–86.

Field, J., Paxton, R.J., Soro, A. & Bridge, C. (2010). Cryptic plasticity underlies a major evolutionary transition. *Current biology : CB*, **20**, 2028–2031.

Fine, P.V.A. (2015). Ecological and evolutionary drivers of geographic variation in species diversity. *Annual Review of Ecology, Evolution, and Systematics*, **46**, 369–392.

Foley, J.A., Defries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., Chapin, F.S., Coe, M.T., Daily, G.C., Gibbs, H.K., Helkowski, J.H., Holloway, T., Howard, E.A., Kucharik, C.J., Monfreda, C., Patz, J.A., Prentice, I.C., Ramankutty, N. & Snyder, P.K. (2005). Global consequences of land use. *Science (New York, N.Y.)*, **309**, 570–574.

Forbes, A.A., Powell, T.H.Q., Stelinski, L.L., Smith, J.J. & Feder, J.L. (2009). Sequential sympatric speciation across trophic levels. *Science (New York, N.Y.)*, **323**, 776–779.

Forrest, J.R.K. & Thomson, J.D. (2011). An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows. *Ecological Monographs*, **81**, 469–491.

Forrest, J.R.K. & Chisholm, S.P.M. (2017). Direct benefits and indirect costs of warm temperatures for high-elevation populations of a solitary bee. *Ecology*, **98**, 359–369.

Frank, S.A. (1987). Individual and population sex allocation patterns. *Theoretical Population Biology*, **31**, 47–74.

Freeman, B.G., Scholer, M.N., Ruiz-Gutierrez, V. & Fitzpatrick, J.W. (2018). Climate change causes upslope shifts and mountaintop extirpations in a tropical bird community. *Proceedings of the National Academy of Sciences of the United States of America*, **115**, 11982–11987.

Frith, D. & Frith, C. (1990). Seasonality of litter invertebrate populations in an Australian upland tropical rain forest. *Biotropica*, **22**, 181.

Gallai, N., Salles, J.-M., Settele, J. & Vaissière, B.E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics*, **68**, 810–821.

Garibaldi, L.A., Aizen, M.A., Klein, A.M., Cunningham, S.A. & Harder, L.D. (2011). Global growth and stability of agricultural yield decrease with pollinator dependence. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 5909–5914.

Garibaldi, L.A., Steffan-Dewenter, I., Winfree, R., Aizen, M.A., Bommarco, R., Cunningham, S.A., Kremen, C., Carvalheiro, L.G., Harder, L.D., Afik, O., Bartomeus, I., Benjamin, F., Boreux, V., Cariveau, D., Chacoff, N.P., Dudenhöffer, J.H., Freitas, B.M., Ghazoul, J., Greenleaf, S., Hipólito, J., Holzschuh, A., Howlett, B., Isaacs, R., Javorek, S.K., Kennedy, C.M., Krewenka, K.M., Krishnan, S., Mandelik, Y., Mayfield, M.M., Motzke, I., Munyuli, T., Nault, B.A., Otieno, M., Petersen, J., Pisanty, G., Potts, S.G., Rader, R., Ricketts, T.H., Rundlöf, M., Seymour, C.L., Schüepp, C., Szentgyörgyi, H., Taki, H., Tscharntke, T., Vergara, C.H., Viana, B.F., Wanger, T.C., Westphal, C., Williams, N. & Klein, A.M. (2013). Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science (New York, N.Y.)*, **339**, 1608–1611.

Gaston, K.J. (2000). Global patterns in biodiversity. *Nature*, **405**, 220–227.

Gazola, A.L. & Garófalo, C.A. (2009). Trap-nesting bees (Hymenoptera. Apoidea) in forest fragments of the State of São Paulo, Brazil. *Genetics and molecular research : GMR*, **8**, 607–622.

Gess, S.K. & Roosenschoon, P.A. (2017). Notes on the nesting of three species of Megachilinae in the Dubai Desert Conservation Reserve, UAE. *Journal of Hymenoptera Research*, **54**, 43–56.

Gibbons, P. & Lindenmayer, D.B. (1996). Issues associated with the retention of hollow-bearing trees within eucalypt forests managed for wood production. *Forest Ecology and Management*, **83**, 245–279.

Gibbs, A. & Pomonis, J.G. (1995). Physical properties of insect cuticular hydrocarbons. The effects of chain length, methyl-branching and unsaturation. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, **112**, 243–249.

Gibbs, A.G., Chippindale, A.K. & Rose, M.R. (1997). Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. *Journal of Experimental Biology*, **200**, 1821–1832.

Gibbs, A.G. (2002a). Lipid melting and cuticular permeability. New insights into an old problem. *Journal of Insect Physiology*, **48**, 391–400.

Gibbs, A.G. (2002b). Water balance in desert *Drosophila*. Lessons from non-charismatic microfauna. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **133**, 781–789.

Gibbs, A.G. & Rajpurohit, S. (eds.) (2010). *Cuticular lipids and water balance.* Cambridge University Press, Cambridge.

Gibbs, J., Brady, S.G., Kanda, K. & Danforth, B.N. (2012). Phylogeny of halictine bees supports a shared origin of eusociality for *Halictus* and *Lasioglossum* (Apoidea. Anthophila: Halictidae). *Molecular phylogenetics and evolution*, **65**, 926–939.

Gill, H.K., Goyal, G. & Chahil, G. (2017). Insect diapause. A review. *Journal of Agricultural Science and Technology A*, **7**.

Gilliam, M. (1997). Identification and roles of non-pathogenic microflora associated with honey bees. *FEMS Microbiology Letters*, **155**, 1–10.

GIZ (2019). Tanzania. *https://www.giz.de/en/worldwide/347.html.*

Godfray, H.C.J. (1994). *Parasitoids. Behavioral and evolutionary ecology.* Princeton University Press, Princeton, New Jersey.

Goulet, H. & Huber, J.T. (1993). *Hymenoptera of the world. An identification guide to families,* Research Branch, Agriculture Canada.

Goulson, D., Darvill, B., Ellis, J.S. & Knight, M.E. (2005). Causes of rarity in bumblebees. *Biological Conservation*, **122**, 1–8.

Goulson, D. (2013). Neonicotinoids and bees. What's all the buzz? *Significance*, **10**, 6–11.

Grace, J., Jose, J.S., Meir, P., Miranda, H.S. & Montes, R.A. (2006). Productivity and carbon fluxes of tropical savannas. *Journal of Biogeography*, **33**, 387–400.

Graham, C.H., Carnaval, A.C., Cadena, C.D., Zamudio, K.R., Roberts, T.E., Parra, J.L., McCain, C.M., Bowie, R.C.K., Moritz, C., Baines, S.B., Schneider, C.J., VanDerWal, J., Rahbek, C., Kozak, K.H. & Sanders, N.J. (2014). The origin and maintenance of montane diversity. Integrating evolutionary and ecological processes. *Ecography*, **37**, 711–719.

Gurr, G.M., Wratten, S.D. & Luna, J.M. (2003). Multi-function agricultural biodiversity. Pest management and other benefits. *Basic and Applied Ecology*, **4**, 107–116.

Hadley, N.F. (1994). *Water relations of terrestrial arthropods. Chapter 3: Cuticular transpiration.* Acad. Press, San Diego, Calif.

Hairston, N.G., Smith, F.E. & Slobodkin, L.B. (1960). Community Structure, Population Control, and Competition. *The American naturalist*, **94**, 421–425.

Hall, H.G. & Ascher, J.S. (2010). Surveys of bees (Hymenoptera. Apoidea: Anthophila) in natural areas of Alachua County in North-Central Florida. *Florida Entomologist*, **93**, 609–629.

Harris, A.C. (1994). *Ancistrocerus gazella* (Hymenoptera. Vespoidea: Eumenidae): A potentially useful biological control agent for leafrollers *Planotortrix octo*, P. excessana, *Ctenopseustis obliquana*, *C. herana* , and *Epiphyas postvittana* (Lepidoptera: Tortricidae) in New Zealand. *New Zealand Journal of Crop and Horticultural Science*, **22**, 235–238.

Hawkins, B.A., Field, R., Cornell, H.V., Currie, D.J., Guégan, J.-F., Kaufman, D.M., Kerr, J.T., Mittelbach, G.G., Oberdorff, T., O'Brien, E.M., Porter, E.E. & Turner, J.R.G. (2003). Energy, water, and broad-scale geographic patterns of species richness. *Ecology*, **84**, 3105–3117.

Helbig-Bonitz, M., Ferger, S.W., Böhning-Gaese, K., Tschapka, M., Howell, K. & Kalko, E.K.V. (2015). Bats are not birds - different responses to human land-use on a tropical mountain. *Biotropica*, **47**, 497–508.

Hemp, A. (2005). Climate change-driven forest fires marginalize the impact of ice cap wasting on Kilimanjaro. *Global Change Biology*, **11**, 1013–1023.

Hemp, A. (2006a). Continuum or zonation? Altitudinal gradients in the forest vegetation of Mt. Kilimanjaro. *Plant Ecology*, **184**, 27–42.

Hemp, A. (2006b). The banana forests of Kilimanjaro. Biodiversity and conservation of the Chagga homegardens. *Biodiversity and Conservation*, **15**, 1193–1217.

Hemp, A. & Hemp, C. (2018). Broken bridges. The isolation of Kilimanjaro's ecosystem. *Global change biology*.

Hillebrand, H. (2004). On the generality of the latitudinal diversity gradient. *The American naturalist*, **163**, 192–211.

Hill, J.K., Griffiths, H.M. & Thomas, C.D. (2011). Climate change and evolutionary adaptations at species' range margins. *Annual review of entomology*, **56**, 143–159.

Hodkinson, I.D. (2005). Terrestrial insects along elevation gradients. Species and community responses to altitude. *Biological Reviews*, **80**, 489.

Hoiss, B., Krauss, J., Potts, S.G., Roberts, S. & Steffan-Dewenter, I. (2012). Altitude acts as an environmental filter on phylogenetic composition, traits and diversity in bee communities. *Proceedings. Biological sciences*, **279**, 4447–4456.

Holzschuh, A., Steffan-Dewenter, I. & Tscharntke, T. (2009). Grass strip corridors in agricultural landscapes enhance nest-site colonization by solitary wasps. *Ecological Applications*, **19**, 123–132.

Holzschuh, A., Dormann, C.F., Tscharntke, T. & Steffan-Dewenter, I. (2013). Mass-flowering crops enhance wild bee abundance. *Oecologia*, **172**, 477–484.

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A.J., Vandermeer, J. & Wardle, D.A. (2005). Effects of biodiversity on ecosystem functioning. A consensus of current knowledge. *Ecological Monographs*, **75**, 3–35.

Howard, R.W. & Blomquist, G.J. (2005). Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Annual review of entomology*, **50**, 371–393.

Hrcek, J., Miller, S.E., Quicke, D.L.J. & Smith, M.A. (2011). Molecular detection of trophic links in a complex insect host-parasitoid food web. *Molecular ecology resources*, **11**, 786–794.

Hunt, J.H., Brodie, R.J., Carithers, T.P., Goldstein, P.Z. & Janzen, D.H. (1999). Dry season migration by Costa Rican lowland paper wasps to high elevation cold dormancy sites. *Biotropica*, **31**, 192–196.

Hurlbert, A.H. & Stegen, J.C. (2014). When should species richness be energy limited, and how would we know? *Ecology letters*, **17**, 401–413.

Ives, A.R., Klug, J.L. & Gross, K. (2000). Stability and species richness in complex communities. *Ecology Letters*, **3**, 399–411.

Janzen, D.H. (1970). Herbivores and the number of tree species in tropical forests. *The American naturalist*, **104**, 501–528.

Janzen, D.H., Ataroff, M., Farinas, M., Reyes, S., Rincon, N., Soler, A., Soriano, P. & Vera, M. (1976). Changes in the arthropod community along an elevational transect in the Venezuelan Andes. *Biotropica*, **8**, 193.

Jennings, D.T. & Houseweart, M.W. (1984). Predation by eumenid wasps (Hymenoptera. Eumenidae) on spruce budworm (Lepidoptera: Tortricidae) and other lepidopterous larvae in spruce-fir forests of Maine. *Annals of the Entomological Society of America*, **77**, 39–45.

Jia, F.-X., Yang, M.-S., Yang, W.-J. & Wang, J.-J. (2009). Influence of continuous high temperature conditions on *Wolbachia* infection frequency and the fitness of *Liposcelis tricolor* (Psocoptera. Liposcelididae). *Environmental entomology*, **38**, 1365–1372.

Jin, N., Klein, S., Leimig, F., Bischoff, G. & Menzel, R. (2015). The neonicotinoid clothianidin interferes with navigation of the solitary bee *Osmia cornuta* in a laboratory test. *The Journal of experimental biology*, **218**, 2821–2825.

Judd, W.S., Campbell, C.S., Kellogg, E.A., Stevens, P.F. & Donoghue, M.J. (2016). *Plant systematics. A phylogenetic approach*, Fourth edition. Sinauer Associates Inc. Publishers, Sunderland, Massachusetts.

Junker, R.R. & Keller, A. (2015). Microhabitat heterogeneity across leaves and flower organs promotes bacterial diversity. *FEMS microbiology ecology*, **91**, fiv097.

Karp, D.S., Ziv, G., Zook, J., Ehrlich, P.R. & Daily, G.C. (2011). Resilience and stability in bird guilds across tropical countryside. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 21134–21139.

Kaspari, M., Alonso, L. & O'Donnell, S. (2000). Three energy variables predict ant abundance at a geographical scale. *Proceedings of the Royal Society B: Biological Sciences*, **267**, 485–489.

Keenan, R.J., Reams, G.A., Achard, F., Freitas, J.V. de, Grainger, A. & Lindquist, E. (2015). Dynamics of global forest area. Results from the FAO Global Forest Resources Assessment 2015. *Forest Ecology and Management*, **352**, 9–20.

Keller, A., Grimmer, G. & Steffan-Dewenter, I. (2013). Diverse microbiota identified in whole intact nest chambers of the red mason bee *Osmia bicornis* (Linnaeus 1758). *PloS one*, **8**, e78296.

Keller, A., Danner, N., Grimmer, G., Ankenbrand, M., Ohe, K. von der, Ohe, W. von der, Rost, S., Härtel, S. & Steffan-Dewenter, I. (2015). Evaluating multiplexed next-generation sequencing as a method in palynology for mixed pollen samples. *Plant biology (Stuttgart, Germany)*, **17**, 558–566.

Kimaro, D.N. & Hieronimo, P. (2014). Land for agriculture in Tanzania: challenges and opportunities. *Journal of Land and Society*, **1**, 91–102.

Kishimoto-Yamada, K. & Itioka, T. (2013). Seasonality in phytophagous scarabaeid (Melolonthinae and Rutelinae) abundances in an 'aseasonal' Bornean rainforest. *Insect Conservation and Diversity*, **6**, 179–188.

Kishimoto-Yamada, K. & Itioka, T. (2015). How much have we learned about seasonality in tropical insect abundance since Wolda (1988)? *Entomological Science*, **18**, 407–419.

Kleijn, D., Winfree, R., Bartomeus, I., Carvalheiro, L.G., Henry, M., Isaacs, R., Klein, A.-M., Kremen, C., M'Gonigle, L.K., Rader, R., Ricketts, T.H., Williams, N.M., Lee Adamson, N., Ascher, J.S., Báldi, A., Batáry, P., Benjamin, F., Biesmeijer, J.C., Blitzer, E.J., Bommarco, R., Brand, M.R., Bretagnolle, V., Button, L., Cariveau, D.P., Chifflet, R., Colville, J.F., Danforth, B.N., Elle, E., Garratt, M.P.D., Herzog, F., Holzschuh, A., Howlett, B.G., Jauker, F., Jha, S., Knop, E., Krewenka, K.M., Le Féon, V., Mandelik, Y., May, E.A., Park, M.G., Pisanty, G., Reemer, M., Riedinger, V., Rollin, O., Rundlöf, M., Sardiñas, H.S., Scheper, J., Sciligo, A.R., Smith, H.G., Steffan-Dewenter, I., Thorp, R., Tscharntke, T., Verhulst, J., Viana, B.F., Vaissière, B.E., Veldtman, R., Ward, K.L., Westphal, C. & Potts, S.G. (2015). Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. *Nature communications*, **6**, 7414.

Klein, A.-M., Steffan-Dewenter, I., Buchori, D. & Tscharntke, T. (2002). Effects of land-use intensity in tropical agroforestry systems on coffee flower-visiting and trap-nesting bees and wasps. *Conservation Biology*, **16**, 1003–1014.

Klein, A.-M., Steffan-Dewenter, I. & Tscharntke, T. (2004). Foraging trip duration and density of megachilid bees, eumenid wasps and pompilid wasps in tropical agroforestry systems. *Journal of Animal Ecology*, **73**, 517–525.

Klein, A.-M., Steffan-Dewenter, I. & Tscharntke, T. (2006). Rain forest promotes trophic interactions and diversity of trap-nesting Hymenoptera in adjacent agroforestry. *The Journal of animal ecology*, **75**, 315–323.

Klein, A.-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C. & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings. Biological sciences*, **274**, 303–313.

Klein, A.-M., Cunningham, S.A., Bos, M. & Steffan-Dewenter, I. (2008). Advances in pollination ecology from tropical plantation crops. *Ecology*, **89**, 935–943.

Koch, H. & Schmid-Hempel, P. (2011). Socially transmitted gut microbiota protect bumble bees against an intestinal parasite. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 19288–19292.

Kopit, A.M. & Pitts-Singer, T.L. (2018). Routes of pesticide exposure in solitary, cavity-nesting bees. *Environmental Entomology*, **47**, 499–510.

Körner, C. (2004). Mountain biodiversity, its causes and function. *Ambio*, **Spec No 13**, 11–17.

Körner, C. (2007). Climatic treelines. Conventions, global patterns, causes. *Erdkunde*, **61**, 316–324.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K. & Schloss, P.D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Applied and environmental microbiology*, **79**, 5112–5120.

Krauss, J., Gallenberger, I. & Steffan-Dewenter, I. (2011). Decreased functional diversity and biological pest control in conventional compared to organic crop fields. *PloS one*, **6**, e19502.

Krombein, K.V. (1967). *Trap-nesting wasps and bees: life histories, nests, and associates.* Smithsonian Press, Washington, D.C.

Kumar, A., Longino, J.T., Colwell, R.K. & O'Donnell, S. (2009). Elevational patterns of diversity and abundance of eusocial paper wasps (Vespidae) in Costa Rica. *Biotropica*, **41**, 338–346.

Kümmerli, R. & Keller, L. (2011). Between-year variation in population sex ratio increases with complexity of the breeding system in Hymenoptera. *The American naturalist*, **177**, 835–846.

La Sorte, F.A. & Jetz, W. (2010). Projected range contractions of montane biodiversity under global warming. *Proceedings. Biological sciences*, **277**, 3401–3410.

LaManna, J.A., Mangan, S.A., Alonso, A., Bourg, N.A., Brockelman, W.Y., Bunyavejchewin, S., Chang, L.-W., Chiang, J.-M., Chuyong, G.B., Clay, K., Condit, R., Cordell, S., Davies, S.J., Furniss, T.J., Giardina, C.P., Gunatilleke, I.A.U.N., Gunatilleke, C.V.S., He, F., Howe, R.W., Hubbell, S.P., Hsieh, C.-F., Inman-Narahari, F.M., Janík, D., Johnson, D.J., Kenfack, D., Korte, L., Král, K., Larson, A.J., Lutz, J.A., McMahon, S.M., McShea, W.J., Memiaghe, H.R., Nathalang, A., Novotny, V., Ong, P.S., Orwig, D.A., Ostertag, R., Parker, G.G., Phillips, R.P., Sack, L., Sun, I.-F., Tello, J.S., Thomas, D.W., Turner, B.L., Vela Díaz, D.M., Vrška, T., Weiblen, G.D., Wolf, A., Yap, S. & Myers, J.A. (2017). Plant diversity increases with the strength of negative density dependence at the global scale. *Science (New York, N.Y.)*, **356**, 1389–1392.

Lambkin, C.L., Boulter, S.L., Starick, N.T., Cantrell, B.K., Bickel, D.J., Wright, S.G., Power, N., Schutze, M.K., Turco, F., Nakumara, A. & Burwell, C.J. (2011). Altitudinal and seasonal variation in the family-level assemblages of flies (Diptera) in an Australian subtropical rainforest: one hundred thousand and counting! *Memoirs of the Queensland Museum*, 315–331.

Laurance, W.F., Sayer, J. & Cassman, K.G. (2014). Agricultural expansion and its impacts on tropical nature. *Trends in Ecology & Evolution*, **29**, 107–116.

Lemoine, N., Schaefer, H.-C. & Böhning-Gaese, K. (2007). Species richness of migratory birds is influenced by global climate change. *Global Ecology and Biogeography*, **16**, 55–64.

Lenoir, J. & Svenning, J.-C. (2015). Climate-related range shifts - a global multidimensional synthesis and new research directions. *Ecography*, **38**, 15–28.

Leonhardt, S.D., Menzel, F., Nehring, V. & Schmitt, T. (2016). Ecology and evolution of communication in social insects. *Cell*, **164**, 1277–1287.

Lewis, S.E., Silburn, D.M., Kookana, R.S. & Shaw, M. (2016). Pesticide behavior, fate, and effects in the tropics. An overview of the current state of knowledge. *Journal of agricultural and food chemistry*, **64**, 3917–3924.

Liang, D. & Silverman, J. (2000). "You are what you eat". Diet modifies cuticular hydrocarbons and nestmate recognition in the Argentine ant, *Linepithema humile*. *Naturwissenschaften*, **87**, 412–416.

Lomolino, M.V. (2001). Elevation gradients of species-density. Historical and prospective views. *Global Ecology and Biogeography*, **10**, 3–13.

Lomolino, M.V., Riddle, B.R. & Brown, J.H. (2006). *Biogeography*, 3rd ed. Sinauer Associates Inc., Sunderland, Massachusetts.

Losey, J.E. & Vaughan, M. (2006). The economic value of ecological services provided by insects. *BioScience*, **56**, 311.

Loyola, R.D. & Martins, R.P. (2006). Trap-nest occupation by solitary wasps and bees (Hymenoptera. Aculeata) in a forest urban remanent. *Neotropical Entomology*, **35**, 41–48.

Loyola, R.D. & Martins, R.P. (2008). Habitat structure components are effective predictors of trap-nesting Hymenoptera diversity. *Basic and Applied Ecology*, **9**, 735–742.

MacIvor, J.S. & Moore, A.E. (2013). Bees collect polyurethane and polyethylene plastics as novel nest materials. *Ecosphere*, **4**, art155.

MacIvor, J.S. (2017). Cavity-nest boxes for solitary bees. A century of design and research. *Apidologie*, **48**, 311–327.

Madriñán, S., Cortés, A.J. & Richardson, J.E. (2013). Páramo is the world's fastest evolving and coolest biodiversity hotspot. *Frontiers in genetics*, **4**, 192.

Maghimbi, S. (2007). Recent changes in crop patterns in the Kilimanjaro region of Tanzania. The decline of coffee and the rise of maize and rice. *African Study Monographs*, 73–83.

Mahlstein, I., Knutti, R., Solomon, S. & Portmann, R.W. (2011). Early onset of significant local warming in low latitude countries. *Environmental Research Letters*, **6**, 34009.

Mannion, P.D., Upchurch, P., Benson, R.B.J. & Goswami, A. (2014). The latitudinal biodiversity gradient through deep time. *Trends in Ecology & Evolution*, **29**, 42–50.

Martinson, H.M. & Fagan, W.F. (2014). Trophic disruption. A meta-analysis of how habitat fragmentation affects resource consumption in terrestrial arthropod systems. *Ecology letters*, **17**, 1178–1189.

Martinson, V.G., Moy, J. & Moran, N.A. (2012). Establishment of characteristic gut bacteria during development of the honeybee worker. *Applied and environmental microbiology*, **78**, 2830–2840.

Martins, R.P., Antonini, Y., da Silveira, F.A. & West, S.A. (1999). Seasonal variation in the sex allocation of a neotropical solitary bee. *Behavioral Ecology*, **10**, 401–408.

Martins, R.P., Guerra, S.T.M. & Barbeitos, M.S. (2001). Variability in egg-to-adult development time in the bee *Ptilothrix plumata* and its parasitoids. *Ecological Entomology*, **26**, 609–616.

Matejčeková, Z., Liptáková, D., Spodniaková, S. & Valík, Ľ. (2016). Characterization of the growth of *Lactobacillus plantarum* in milk in dependence on temperature. *Acta Chimica Slovaca*, **9**, 272.

Matos, M.C.B., Silva, S.S. & Teodoro, A.V. (2016). Seasonal population abundance of the assembly of solitary wasps and bees (Hymenoptera) according to land-use in Maranhão state, Brazil. *Revista Brasileira de Entomologia*, **60**, 171–176.

McCain, C.M. (2007). Could temperature and water availability drive elevational species richness patterns? A global case study for bats. *Global Ecology and Biogeography*, **16**, 1–13.

McCain, C.M. & Grytnes, J.-A. (2010). Elevational gradients in species richness.

McFrederick, Q.S. & Rehan, S.M. (2016). Characterization of pollen and bacterial community composition in brood provisions of a small carpenter bee. *Molecular ecology*, **25**, 2302–2311.

McFrederick, Q.S., Thomas, J.M., Neff, J.L., Vuong, H.Q., Russell, K.A., Hale, A.R. & Mueller, U.G. (2017). Flowers and wild megachilid bees share microbes. *Microbial ecology*, **73**, 188–200.

McMurdie, P.J. & Holmes, S. (2014). Waste not, want not. Why rarefying microbiome data is inadmissible. *PLoS computational biology*, **10**, e1003531.

Menge, B.A. & Sutherland, J.P. (1987). Community regulation. Variation in disturbance, competition, and predation in relation to environmental stress and recruitment. *The American naturalist*, **130**, 730–757.

Menzel, F., Blaimer, B.B. & Schmitt, T. (2017). How do cuticular hydrocarbons evolve? Physiological constraints and climatic and biotic selection pressures act on a complex functional trait. *Proceedings. Biological sciences*, **284**.

Mesquita, T.M.S. & Augusto, S.C. (2011). Diversity of trap-nesting bees and their natural enemies in the Brazilian savanna. *Tropical Zoology*, 127–144.

Meyer, C.P. (2003). Molecular systematics of cowries (Gastropoda. Cypraeidae) and diversification patterns in the tropics. *Biological Journal of the Linnean Society*, **79**, 401–459.

Michelutti, K.B., Soares, E.R.P., Sguarizi-Antonio, D., Piva, R.C., Súarez, Y.R., Cardoso, C.A.L. & Antonialli-Junior, W.F. (2018). Influence of temperature on survival and cuticular chemical profile of social wasps. *Journal of Thermal Biology*, **71**, 221–231.

Michener, C.D. (1975). A taxonomic study of African allodapine bees (Hymenoptera, Anthophoridae, Ceratini). *Bulletin of the American Museum of Natural History*, 67–240.

Michener, C.D. (2007). *The bees of the world*, 2nd ed. Johns Hopkins University Press, Baltimore.

Millennium Ecosystem Assessment (2005). *Millennium Ecosystem Assessment Ecosystems and Human Well-being:. Synthesis.* Island Press, Washington, D.C.

Misana, S.B. (2012). Land-use/cover changes and their drivers on the slopes of Mount Kilimanjaro, Tanzania. *Journal of Geography and Regional Planning*, **5**.

Mora, C., Tittensor, D.P., Adl, S., Simpson, A.G.B. & Worm, B. (2011). How many species are there on Earth and in the ocean? *PLoS biology*, **9**, e1001127.

Moran, N.A., McCutcheon, J.P. & Nakabachi, A. (2008). Genomics and evolution of heritable bacterial symbionts. *Annual review of genetics*, **42**, 165–190.

Morato, E.F. & Martins, R.P. (2006). An overview of proximate factors affecting the nesting behavior of solitary wasps and bees (Hymenoptera. Aculeata) in preexisting cavities in wood. *Neotropical Entomology*, **35**, 285–298.

Morris, R.J., Sinclair, F.H. & Burwell, C.J. (2015). Food web structure changes with elevation but not rainforest stratum. *Ecography*, **38**, 792–802.

Mouillot, D., Graham, N.A.J., Villéger, S., Mason, N.W.H. & Bellwood, D.R. (2013). A functional approach reveals community responses to disturbances. *Trends in Ecology & Evolution*, **28**, 167–177.

Müller, A., Diener, S., Schnyder, S., Stutz, K., Sedivy, C. & Dorn, S. (2006). Quantitative pollen requirements of solitary bees. Implications for bee conservation and the evolution of bee–flower relationships. *Biological Conservation*, **130**, 604–615.

Müller, A. & Kuhlmann, M. (2008). Pollen hosts of western palaearctic bees of the genus *Colletes* (Hymenoptera. Colletidae): the Asteraceae paradox, **95**, 719–733.

Mullin, C.A., Frazier, M., Frazier, J.L., Ashcraft, S., Simonds, R., vanEngelsdorp, D. & Pettis, J.S. (2010). High levels of miticides and agrochemicals in North American apiaries. Implications for honey bee health. *PloS one*, **5**, e9754.

Newbold, T., Hudson, L.N., Hill, S.L.L., Contu, S., Lysenko, I., Senior, R.A., Börger, L., Bennett, D.J., Choimes, A., Collen, B., Day, J., Palma, A. de, Díaz, S., Echeverria-Londoño, S., Edgar, M.J., Feldman, A., Garon, M., Harrison, M.L.K., Alhusseini, T., Ingram, D.J., Itescu, Y., Kattge, J., Kemp, V., Kirkpatrick, L., Kleyer, M., Correia, D.L.P., Martin, C.D., Meiri, S., Novosolov, M., Pan, Y., Phillips, H.R.P., Purves, D.W., Robinson, A., Simpson, J., Tuck, S.L., Weiher, E., White, H.J., Ewers, R.M., Mace, G.M., Scharlemann, J.P.W. & Purvis, A. (2015). Global effects of land use on local terrestrial biodiversity. *Nature*, **520**, 45–50.

Nicolai, V. (1986). The bark of trees. Thermal properties, microclimate and fauna. *Oecologia*, **69**, 148–160.

Nicolson, S.W. & Human, H. (2013). Chemical composition of the 'low quality' pollen of sunflower (*Helianthus annuus*, Asteraceae). *Apidologie*, **44**, 144–152.

Niemand, J.G. & Holzapfel, W.H. (1984). Characteristics of lactobacilli isolated from radurised meat. *International Journal of Food Microbiology*, **1**, 99–110.

Nonnotte, P., Guillou, H., Le Gall, B., Benoit, M., Cotten, J. & Scaillet, S. (2008). New K–Ar age determinations of Kilimanjaro volcano in the North Tanzanian diverging rift, East Africa. *Journal of Volcanology and Geothermal Research*, **173**, 99–112.

Novotny, V. & Basset, Y. (1998). Seasonality of sap-sucking insects (Auchenorrhyncha, Hemiptera) feeding on Ficus (Moraceae) in a lowland rain forest in New Guinea. *Oecologia*, **115**, 514–522.

Noyes, J.S. (1989). The diversity of Hymenoptera in the tropics with special reference to Parasitica in Sulawesi. *Ecological Entomology*, **14**, 197–207.

Odner, K. (2010). A preliminary report on an archaeological survey on the slopes of Kilimanjaro. *Azania: Archaeological Research in Africa*, **6**, 131–149.

Olesen, J.M., Bascompte, J., Elberling, H. & Jordano, P. (2008). Temporal dynamics in a pollination network. *Ecology*, **89**, 1573–1582.

Ollerton, J., Winfree, R. & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, **120**, 321–326.

O'Neill, K.M. & O'Neill, J.F. (2018). Cavity-nesting wasps and bees (Hymenoptera) of central New York State. Finger lakes national forest. *Proceedings of the Entomological Society of Washington*, **120**, 260–271.

Osorio-Canadas, S., Arnan, X., Rodrigo, A., Torné-Noguera, A., Molowny, R. & Bosch, J. (2016). Body size phenology in a regional bee fauna. A temporal extension of Bergmann's rule. *Ecology letters*, **19**, 1395–1402.

Osorio-Canadas, S., Arnan, X., Bassols, E., Vicens, N. & Bosch, J. (2018). Seasonal dynamics in a cavity-nesting bee-wasp community. Shifts in composition, functional diversity and host-parasitoid network structure. *PloS one*, **13**, e0205854.

Östman, Ö., Ekbom, B. & Bengtsson, J. (2003). Yield increase attributable to aphid predation by ground-living polyphagous natural enemies in spring barley in Sweden. *Ecological Economics*, **45**, 149–158.

Otte, I., Detsch, F., Mwangomo, E., Hemp, A., Appelhans, T. & Nauss, T. (2017). Multidecadal trends and interannual variability of rainfall as observed from five lowland stations at Mt. Kilimanjaro, Tanzania. *Journal of Hydrometeorology*, **18**, 349–361.

Otte, T., Hilker, M. & Geiselhardt, S. (2015). The effect of dietary fatty acids on the cuticular hydrocarbon phenotype of an herbivorous insect and consequences for mate recognition. *Journal of chemical ecology*, **41**, 32–43.

Paine 1966.

Palma, A. de, Abrahamczyk, S., Aizen, M.A., Albrecht, M., Basset, Y., Bates, A., Blake, R.J., Boutin, C., Bugter, R., Connop, S., Cruz-López, L., Cunningham, S.A., Darvill, B., Diekötter, T., Dorn, S., Downing, N., Entling, M.H., Farwig, N., Felicioli, A., Fonte, S.J., Fowler, R., Franzén, M., Goulson, D., Grass, I., Hanley, M.E., Hendrix, S.D., Herrmann, F., Herzog, F., Holzschuh, A., Jauker, B., Kessler, M., Knight, M.E., Kruess, A., Lavelle, P., Le Féon, V., Lentini, P., Malone, L.A., Marshall, J., Pachón, E.M., McFrederick, Q.S., Morales, C.L., Mudri-Stojnic, S., Nates-Parra, G., Nilsson, S.G., Öckinger, E., Osgathorpe, L., Parra-H, A., Peres, C.A., Persson, A.S., Petanidou, T., Poveda, K., Power, E.F., Quaranta, M., Quintero, C., Rader, R., Richards, M.H., Roulston, T.'a., Rousseau, L., Sadler, J.P., Samnegård, U., Schellhorn, N.A., Schüepp, C., Schweiger, O., Smith-Pardo, A.H., Steffan-Dewenter, I., Stout, J.C., Tonietto, R.K., Tscharntke, T., Tylianakis, J.M., Verboven, H.A.F., Vergara, C.H., Verhulst, J., Westphal, C., Yoon, H.J. & Purvis, A. (2016). Predicting bee community responses to land-use changes. Effects of geographic and taxonomic biases. *Scientific reports*, **6**, 31153 EP -.

Parkash, R., Rajpurohit, S. & Ramniwas, S. (2008). Changes in body melanisation and desiccation resistance in highland vs. lowland populations of *D. melanogaster*. *Journal of Insect Physiology*, **54**, 1050–1056.

Parker, F.D. (1986). Nesting, associates, and mortality of *Osmia sanrafaelae* Parker. *Journal of the Kansas Entomological Society*, **59**, 367–377.

Parmesan, C. & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, **421**, 37 EP -.

Parmesan, C. (2006). Ecological and Evolutionary Responses to Recent Climate Change. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 637–669.

Paukkunen, J., Berg, A., Soon, V., Ødegaard, F. & Rosa, P. (2015). An illustrated key to the cuckoo wasps (Hymenoptera, Chrysididae) of the Nordic and Baltic countries, with description of a new species. *ZooKeys*, 1–116.

Payne, D., Spehn, E.M., Snethlage, M. & Fischer, M. (2017). Opportunities for research on mountain biodiversity under global change. *Current Opinion in Environmental Sustainability*, **29**, 40–47.

Pecl, G.T., Araújo, M.B., Bell, J.D., Blanchard, J., Bonebrake, T.C., Chen, I.-C., Clark, T.D., Colwell, R.K., Danielsen, F., Evengård, B., Falconi, L., Ferrier, S., Frusher, S., Garcia, R.A., Griffis, R.B., Hobday, A.J., Janion-Scheepers, C., Jarzyna, M.A., Jennings, S., Lenoir, J., Linnetved, H.I., Martin, V.Y., McCormack, P.C., McDonald, J., Mitchell, N.J., Mustonen, T., Pandolfi, J.M., Pettorelli, N., Popova, E., Robinson, S.A., Scheffers, B.R., Shaw, J.D., Sorte, C.J.B., Strugnell, J.M., Sunday, J.M., Tuanmu, M.-N., Vergés, A., Villanueva, C., Wernberg, T., Wapstra, E. & Williams, S.E. (2017). Biodiversity redistribution under climate change. Impacts on ecosystems and human well-being. *Science (New York, N.Y.)*, **355**.

Pedrini, N., Ortiz-Urquiza, A., Huarte-Bonnet, C., Zhang, S. & Keyhani, N.O. (2013). Targeting of insect epicuticular lipids by the entomopathogenic fungus Beauveria bassiana. Hydrocarbon oxidation within the context of a host-pathogen interaction. *Frontiers in microbiology*, **4**, 24.

Perfecto, I., Vandermeer, J.H. & Wright, A.L. (2009). *Nature's matrix. Linking agriculture, conservation and food sovereignty*. Earthscan, London, Sterling, VA.

Perillo, L.N., Neves, F.d.S., Antonini, Y. & Martins, R.P. (2017). Compositional changes in bee and wasp communities along Neotropical mountain altitudinal gradient. *PloS one*, **12**, e0182054.

Perry, J., Lojka, B., Quinones Ruiz, L., van Damme, P., Houška, J. & Fernandez Cusimamani, E. (2016). How natural forest conversion affects insect biodiversity in the Peruvian Amazon. Can Agroforestry Help? *Forests*, **7**, 82.

Petanidou, T., Kallimanis, A.S., Tzanopoulos, J., Sgardelis, S.P. & Pantis, J.D. (2008). Long-term observation of a pollination network. Fluctuation in species and interactions, relative invariance of network structure and implications for estimates of specialization. *Ecology letters*, **11**, 564–575.

Peters, B., Gao, Z. & Zumkier, U. (2016a). Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in Northern Germany. Effects on red mason bees (*Osmia bicornis*). *Ecotoxicology (London, England)*, **25**, 1679–1690.

Peters, M.K., Hemp, A., Appelhans, T., Behler, C., Classen, A., Detsch, F., Ensslin, A., Ferger, S.W., Frederiksen, S.B., Gebert, F., Haas, M., Helbig-Bonitz, M., Hemp, C., Kindeketa, W.J., Mwangomo, E., Ngereza, C., Otte, I., Röder, J., Rutten, G., Schellenberger Costa, D., Tardanico, J., Zancolli, G., Deckert, J., Eardley, C.D., Peters, R.S., Rödel, M.-O., Schleuning, M., Ssymank, A., Kakengi, V., Zhang, J., Böhning-Gaese, K., Brandl, R., Kalko, E.K.V., Kleyer, M., Nauss, T., Tschapka, M., Fischer, M. & Steffan-Dewenter, I. (2016b). Predictors of elevational biodiversity gradients change from single taxa to the multi-taxa community level. *Nature communications*, **7**, 13736.

Peters, M.K., Hemp, A., Appelhans, T., Becker, J.N., Behler, C., Classen, A., Detsch, F., Ensslin, A., Ferger, S.W., Frederiksen, S.B., Gebert, F., Gerschlauer, F., Gütlein, A., Helbig-Bonitz, M., Hemp, C., Kindeketa, W.J., Kühnel, A., Mayr, A.V., Mwangomo, E., Ngereza, C., Njovu, H.K., Otte, I., Pabst, H., Renner, M., Röder, J., Rutten, G., Schellenberger Costa, D., Sierra-Cornejo, N., Vollstädt, M.G.R., Dulle, H.I., Eardley, C.D., Howell, K.M., Keller, A., Peters, R.S., Ssymank, A., Kakengi, V., Zhang, J., Bogner, C., Böhning-Gaese, K., Brandl, R., Hertel, D., Huwe, B., Kiese, R., Kleyer, M., Kuzyakov, Y., Nauss, T., Schleuning, M., Tschapka, M., Fischer, M. & Steffan-Dewenter, I. (2019). Climate–land-use interactions shape tropical mountain biodiversity and ecosystem functions. *Nature*.

Pimm, S.L., Lawton, J.H. & Cohen, J.E. (1991). Food web patterns and their consequences. *Nature*, **350**, 669–674.

Pimm, S.L., Jenkins, C.N., Abell, R., Brooks, T.M., Gittleman, J.L., Joppa, L.N., Raven, P.H., Roberts, C.M. & Sexton, J.O. (2014). The biodiversity of species and their rates of extinction, distribution, and protection. *Science (New York, N.Y.)*, **344**, 1246752.

Pokorny, T., Lunau, K., Quezada-Euan, J.J.G. & Eltz, T. (2014). Cuticular hydrocarbons distinguish cryptic sibling species in *Euglossa orchid* bees. *Apidologie*, **45**, 276–283.

Pons, J., Barraclough, T.G., Gomez-Zurita, J., Cardoso, A., Duran, D.P., Hazell, S., Kamoun, S., Sumlin, W.D. & Vogler, A.P. (2006). Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology*, **55**, 595–609.

Pontarp, M., Bunnefeld, L., Cabral, J.S., Etienne, R.S., Fritz, S.A., Gillespie, R., Graham, C.H., Hagen, O., Hartig, F., Huang, S., Jansson, R., Maliet, O., Münkemüller, T., Pellissier, L., Rangel, T.F., Storch, D., Wiegand, T. & Hurlbert, A.H. (2018). The latitudinal diversity gradient. Novel understanding through mechanistic eco-evolutionary models. *Trends in Ecology & Evolution*.

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O. & Kunin, W.E. (2010). Global pollinator declines. Trends, impacts and drivers. *Trends in Ecology & Evolution*, **25**, 345–353.

Praz, C.J., Müller, A. & Dorn, S. (2008). Specialized bees fail to develop on non-host pollen:. do plants chemically protect their pollen? *Ecology*, **89**, 795–804.

R Development Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria.

Rajpurohit, S., Hanus, R., Vrkoslav, V., Behrman, E.L., Bergland, A.O., Petrov, D., Cvačka, J. & Schmidt, P.S. (2017). Adaptive dynamics of cuticular hydrocarbons in *Drosophila*. *Journal of evolutionary biology*, **30**, 66–80.

Rivera-Hutinel, A., Bustamante, R.O., Marín, V.H. & Medel, R. (2012). Effects of sampling completeness on the structure of plant–pollinator networks. *Ecology*, **93**, 1593–1603.

Rizali, A., Clough, Y., Buchori, D., Hosang, M.L.A., Bos, M.M., Tscharntke, T., Didham, R. & Ewers, R. (2013). Long-term change of ant community structure in cacao agroforestry landscapes in Indonesia. *Insect Conservation and Diversity*, **6**, 328–338.

Röder, J., Detsch, F., Otte, I., Appelhans, T., Nauss, T., Peters, M.K. & Brandl, R. (2017). Heterogeneous patterns of abundance of epigeic arthropod taxa along a major elevation gradient. *Biotropica*, **49**, 217–228.

Rodríguez-Castañeda, G. & Sykes, M. (2013). The world and its shades of green. A meta-analysis on trophic cascades across temperature and precipitation gradients. *Global Ecology and Biogeography*, **22**, 118–130.

Ron, E.Z. (1975). Growth rate of Enterobacteriaceae at elevated temperatures. Limitation by methionine. *Journal of bacteriology*, **124**, 243–246.

Rothman, J.A., Andrikopoulos, C., Cox-Foster, D. & McFrederick, Q.S. (2018). Floral and foliar source affect the bee nest microbial community. *Microbial ecology*.

Rouault, J.-D., Marican, C., Wicker-Thomas, C. & Jallon, J.-M. (2004). Relations Between Cuticular Hydrocarbon (HC) Polymorphism, Resistance Against Desiccation and Breeding Temperature; A Model for HC Evolution in *D. Melanogaster* and *D. Simulans*. *Genetica*, **120**, 195–212.

Roubik, D.W. (1989). *Ecology and natural history of tropical bees.* Cambridge University Press, Cambridge.

Roulston, T.'a.H., Cane, J.H. & Buchmann, S.L. (2000). What governs protein content of pollen. Pollinator preferences, pollen-pistil interactions, or phylogeny? *Ecological Monographs*, **70**, 617–643.

Rubin, B.E.R., Sanders, J.G., Turner, K.M., Pierce, N.E. & Kocher, S.D. (2018). Social behaviour in bees influences the abundance of *Sodalis* (Enterobacteriaceae) symbionts. *Royal Society open science*, **5**, 180369.

Rundlöf, M., Andersson, G.K.S., Bommarco, R., Fries, I., Hederström, V., Herbertsson, L., Jonsson, O., Klatt, B.K., Pedersen, T.R., Yourstone, J. & Smith, H.G. (2015). Seed coating with a neonicotinoid insecticide negatively affects wild bees. *Nature*, **521**, 77–80.

Rusch, A., Chaplin-Kramer, R., Gardiner, M.M., Hawro, V., Holland, J., Landis, D., Thies, C., Tscharntke, T., Weisser, W.W., Winqvist, C., Woltz, M. & Bommarco, R. (2016). Agricultural landscape simplification reduces natural pest control. A quantitative synthesis. *Agriculture, Ecosystems & Environment*, **221**, 198–204.

Sabino, W.D.O. & Antonini, Y. (2017). Nest architecture, life cycle, and natural enemies of the neotropical leafcutting bee *Megachile* (*Moureapis*) *maculata* (Hymenoptera. Megachilidae) in a montane forest. *Apidologie*, **48**, 450–460.

Sala, O.E., Chapin III, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., LeRoy Poff, N., Sykes, M.T., Walker, B.H., Walker, M. & Wall, D.H. (2000). Global biodiversity scenarios for the year 2100. *Science*, **287**, 1770–1774.

Samnegård, U., Hambäck, P.A., Eardley, C., Nemomissa, S. & Hylander, K. (2015). Turnover in bee species composition and functional trait distributions between seasons in a tropical agricultural landscape. *Agriculture, Ecosystems & Environment*, **211**, 185–194.

Sánchez-Bayo, F. & Wyckhuys, K.A.G. (2019). Worldwide decline of the entomofauna. A review of its drivers. *Biological Conservation*, **232**, 8–27.

Sanders, D., Moser, A., Newton, J. & van Veen, F.J.F. (2016). Trophic assimilation efficiency markedly increases at higher trophic levels in four-level host-parasitoid food chain. *Proceedings. Biological sciences*, **283**, 20153043.

Sanders, N.J. & Rahbek, C. (2012). The patterns and causes of elevational diversity gradients. *Ecography*, **35**, 1–3.

Schaeffer, T.L., Cantwell, S.G., Brown, J.L., Watt, D.S. & Fall, R.R. (1979). Microbial growth on hydrocarbons. Terminal branching inhibits biodegradation. *Applied and environmental microbiology*, **38**, 742–746.

Sébastien, L. (2011). The Chagga people and environmental changes on Mount Kilimanjaro. Lessons to learn. *Climate and Development*, **2**, 364–377.

Servin, A.L. (2004). Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. *FEMS microbiology reviews*, **28**, 405–440.

Sgolastra, F., Arnan, X., Cabbri, R., Isani, G., Medrzycki, P., Teper, D. & Bosch, J. (2018). Combined exposure to sublethal concentrations of an insecticide and a fungicide affect feeding, ovary development and longevity in a solitary bee. *Proceedings. Biological sciences*, **285**.

Sickel, W., Ankenbrand, M.J., Grimmer, G., Holzschuh, A., Härtel, S., Lanzen, J., Steffan-Dewenter, I. & Keller, A. (2015). Increased efficiency in identifying mixed pollen samples by meta-barcoding with a dual-indexing approach. *BMC ecology*, **15**, 20.

Sirohi, M.H., Jackson, J., Edwards, M. & Ollerton, J. (2015). Diversity and abundance of solitary and primitively eusocial bees in an urban centre. A case study from Northampton (England). *Journal of Insect Conservation*, **19**, 487–500.

Smith, M.R., Singh, G.M., Mozaffarian, D. & Myers, S.S. (2015). Effects of decreases of animal pollinators on human nutrition and global health. A modelling analysis. *The Lancet*, **386**, 1964–1972.

Soini, E. (2005). Land use change patterns and livelihood dynamics on the slopes of Mt. Kilimanjaro, Tanzania. *Agricultural Systems*, **85**, 306–323.

Sousa, J.P., Bolger, T., da Gama, M.M., Lukkari, T., Ponge, J.-F., Simón, C., Traser, G., Vanbergen, A.J., Brennan, A., Dubs, F., Ivitis, E., Keating, A., Stofer, S. & Watt, A.D. (2006). Changes in Collembola richness and diversity along a gradient of land-use intensity. A pan European study. *Pedobiologia*, **50**, 147–156.

Spear, D.M., Silverman, S. & Forrest, J.R.K. (2016). Asteraceae pollen provisions protect *Osmia mason* bees (Hymenoptera. Megachilidae) from brood parasitism. *The American naturalist*, **187**, 797–803.

Sprenger, P.P., Burkert, L.H., Abou, B., Federle, W. & Menzel, F. (2018). Coping with the climate. Cuticular hydrocarbon acclimation of ants under constant and fluctuating conditions. *The Journal of experimental biology*, **221**.

Srivastava, D.S. & Lawton, J.H. (1998). Why more productive sites have more species. An experimental test of theory using tree-hole communities. *The American naturalist*, **152**, 510–529.

Staab, M., Pufal, G., Tscharntke, T., Klein, A.-M. & Iossa, G. (2018). Trap nests for bees and wasps to analyse trophic interactions in changing environments-A systematic overview and user guide. *Methods in Ecology and Evolution*, **21**, 477.

Stangler, E.S., Hanson, P.E. & Steffan-Dewenter, I. (2015). Interactive effects of habitat fragmentation and microclimate on trap-nesting Hymenoptera and their trophic interactions in small secondary rainforest remnants. *Biodiversity and Conservation*, **24**, 563–577.

Stangler, E.S., Hanson, P.E. & Steffan-Dewenter, I. (2016). Vertical diversity patterns and biotic interactions of trap-nesting bees along a fragmentation gradient of small secondary rainforest remnants. *Apidologie*, **47**, 527–538.

Steckel, J., Westphal, C., Peters, M.K., Bellach, M., Rothenwoehrer, C., Erasmi, S., Scherber, C., Tscharntke, T. & Steffan-Dewenter, I. (2014). Landscape composition and configuration differently affect trap-nesting bees, wasps and their antagonists. *Biological Conservation*, **172**, 56–64.

Steffan-Dewenter, I. (2002). Landscape context affects trap-nesting bees, wasps, and their natural enemies. *Ecological Entomology*, **27**, 631–637.

Steffan-Dewenter, I. (2003). Importance of habitat area and landscape context for species richness of bees and wasps in fragmented orchard meadows. *Conservation Biology*, **17**, 1036–1044.

Steffan-Dewenter, I. & Schiele, S. (2008). Do resources or natural enemies drive bee population dynamics in fragmented habitats? *Ecology*, **89**, 1375–1387.

Stein, K., Stenchly, K., Coulibaly, D., Pauly, A., Dimobe, K., Steffan-Dewenter, I., Konaté, S., Goetze, D., Porembski, S. & Linsenmair, K.E. (2018). Impact of human disturbance on bee pollinator communities in savanna and agricultural sites in Burkina Faso, West Africa. *Ecology and evolution*, **8**, 6827–6838.

Stork, N.E. (2018). How many species of insects and other terrestrial arthropods are there on earth? *Annual review of entomology*, **63**, 31–45.

Strohm, E. & Linsenmair, K.E. (1997). Low resource availability causes extremely male-biased investment ratios in the European beewolf, *Philanthus triangulum F.* (Hymenoptera, Sphecidae). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **264**, 423–429.

Strohm, E., Kroiss, J., Herzner, G., Laurien-Kehnen, C., Boland, W., Schreier, P. & Schmitt, T. (2008). A cuckoo in wolves' clothing? Chemical mimicry in a specialized cuckoo wasp of the European beewolf (Hymenoptera, Chrysididae and Crabronidae). *Frontiers in zoology*, **5**, 2.

Sudhagar, S., Rami Reddy, P.V. & Nagalakshmi, G. (2017). Influence of elevation in structuring the gut bacterial communities of *Apis cerana* Fab. *Journal of Entomology and Zoology Studies*, **5**, 434–440.

Tauber, M.J. & Tauber, C.A. (1976). Insect seasonality. Diapause maintenance, termination, and postdiapause development. *Annual review of entomology*, **21**, 81–107.

Teder, T. & Knapp, M. (2019). Sublethal effects enhance detrimental impact of insecticides on non-target organisms. A quantitative synthesis in parasitoids. *Chemosphere*, **214**, 371–378.

Tepedino, V.J. & Parker, F.D. (1986). Effect of rearing temperature on mortality, second-generation emergence, and size of adult in *Megachile rotundata* (Hymenoptera. Megachilidae). *Journal of Economic Entomology*, **79**, 974–977.

Tepedino, V.J. & Parker, F.D. (1988). Alternation of sex ratio in a partially bivoltine bee, *Megachile rotundata* (Hymenoptera. Megachilidae). *Annals of the Entomological Society of America*, **81**, 467–476.

Terborgh, J.W. (2015). Toward a trophic theory of species diversity. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, 11415–11422.

Thébault, E. & Fontaine, C. (2010). Stability of ecological communities and the architecture of mutualistic and trophic networks. *Science (New York, N.Y.)*, **329**, 853–856.

Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C., Erasmus, B.F.N., Siqueira, M.F. de, Grainger, A., Hannah, L., Hughes, L., Huntley, B., van Jaarsveld, A.S., Midgley, G.F., Miles, L., Ortega-Huerta, M.A., Peterson, A.T., Phillips, O.L. & Williams, S.E. (2004). Extinction risk from climate change. *Nature*, **427**, 145–148.

Thompson, L.G., Mosley-Thompson, E., Davis, M.E., Henderson, K.A., Brecher, H.H., Zagorodnov, V.S., Mashiotta, T.A., Lin, P.-N., Mikhalenko, V.N., Hardy, D.R. & Beer, J. (2002).

Kilimanjaro ice core records. Evidence of holocene climate change in tropical Africa. *Science (New York, N.Y.)*, **298**, 589–593.

Tidon, R. (2006). Relationships between drosophilids (Diptera, Drosophilidae) and the environment in two contrasting tropical vegetations. *Biological Journal of the Linnean Society*, **87**, 233–247.

Tscharntke, T., Gathmann, A. & Steffan-Dewenter, I. (1998). Bioindication using trap-nesting bees and wasps and their natural enemies. Community structure and interactions. *Journal of Applied Ecology*, **35**, 708–719.

Tscharntke, T., Clough, Y., Bhagwat, S.A., Buchori, D., Faust, H., Hertel, D., Hölscher, D., Juhrbandt, J., Kessler, M., Perfecto, I., Scherber, C., Schroth, G., Veldkamp, E. & Wanger, T.C. (2011). Multifunctional shade-tree management in tropical agroforestry landscapes - a review. *Journal of Applied Ecology*, **48**, 619–629.

Turčinavičienė, J., Radzevičiūtė, R., Budrienė, A. & Budrys, E. (2016). Species identification and genetic differentiation of European cavity-nesting wasps (Hymenoptera. Vespidae, Pompilidae, Crabronidae) inferred from DNA barcoding data. *Mitochondrial DNA. Part A, DNA mapping, sequencing, and analysis*, **27**, 476–482.

Turner, J.R.G. (2004). Explaining the global biodiversity gradient. Energy, area, history and natural selection. *Basic and Applied Ecology*, **5**, 435–448.

Tylianakis, J.M., Klein, A.-M. & Tscharntke, T. (2005). Spatiotemporal variation in the diversity of Hymenoptera across a tropical habitat gradient. *Ecology*, **86**, 3296–3302.

Tylianakis, J.M., Klein, A.-M., Lozada, T. & Tscharntke, T. (2006). Spatial scale of observation affects alpha, beta and gamma diversity of cavity-nesting bees and wasps across a tropical land-use gradient. *Journal of Biogeography*, **33**, 1295–1304.

Tylianakis, J.M., Tscharntke, T. & Lewis, O.T. (2007). Habitat modification alters the structure of tropical host-parasitoid food webs. *Nature*, **445**, 202–205.

Tylianakis, J.M., Laliberté, E., Nielsen, A. & Bascompte, J. (2010). Conservation of species interaction networks. *Biological Conservation*, **143**, 2270–2279.

Tylianakis, J.M. & Morris, R.J. (2017). Ecological Networks Across Environmental Gradients. *Annual Review of Ecology, Evolution, and Systematics*, **48**, 25–48.

van Huis, A., Arendse, P.W., Schilthuizen, M., Wiegers, P.P., Heering, H., Hulshof, M. & Kaashoek, N.K. (1994). *Uscana lariophaga*, egg parasitoid of bruchid beetle storage pests of cowpea in West Africa. The effect of temperature and humidity. *Entomologia Experimentalis et Applicata*, **70**, 41–53.

van Noort (2004). Ichneumonid (Hymenoptera: Ichneumonoidea) diversity across an elevational gradient on Monts Doudou in Southwestern Gabon. *California Academy of Sciences Memoir*, 187–216.

van Valen, L.M. (1973). A new evolutionary law. *Evol. Theory*, 1–30.

Vanbergen, A.J. & the Insect Pollinators Initiative (2013). Threats to an ecosystem service. Pressures on pollinators. *Frontiers in Ecology and the Environment*, **11**, 251–259.

Vásquez, A. & Olofsson, T.C. (2009). The lactic acid bacteria involved in the production of bee pollen and bee bread. *Journal of Apicultural Research*, **48**, 189–195.

Vásquez, A., Forsgren, E., Fries, I., Paxton, R.J., Flaberg, E., Szekely, L. & Olofsson, T.C. (2012). Symbionts as major modulators of insect health. Lactic acid bacteria and honeybees. *PloS one*, **7**, e33188.

Veddeler, D., Tylianakis, J., Tscharntke, T. & Klein, A.-M. (2010). Natural enemy diversity reduces temporal variability in wasp but not bee parasitism. *Oecologia*, **162**, 755–762.

Verbarg, S., Frühling, A., Cousin, S., Brambilla, E., Gronow, S., Lünsdorf, H. & Stackebrandt, E. (2008). *Biostraticola tofi* gen. nov., spec. nov., a novel member of the family Enterobacteriaceae. *Current microbiology*, **56**, 603–608.

Villéger, S., Miranda, J.R., Hernández, D.F. & Mouillot, D. (2010). Contrasting changes in taxonomic vs. functional diversity of tropical fish communities after habitat degradation. *Ecological Applications*, **20**, 1512–1522.

Violle, C., Enquist, B.J., McGill, B.J., Jiang, L., Albert, C.H., Hulshof, C., Jung, V. & Messier, J. (2012). The return of the variance. Intraspecific variability in community ecology. *Trends in Ecology & Evolution*, **27**, 244–252.

Voulgari-Kokota, A., Grimmer, G., Steffan-Dewenter, I. & Keller, A. (2018). Bacterial community structure and succession in nests of two megachilid bee genera. *FEMS microbiology ecology*.

Wagner, T. (2001). Seasonal changes in the canopy arthropod fauna in *Rinorea beniensis* in Budongo Forest, Uganda. *Plant Ecology*, **153**, 169–178.

Wallace, A. (1878). *Tropical Nature and Other Essays*. Macmillan, New York.

Walther, G.-R. (2010). Community and ecosystem responses to recent climate change. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **365**, 2019–2024.

Wang, L., Hui, C., Sandhu, H.S., Li, Z. & Zhao, Z. (2015). Population dynamics and associated factors of cereal aphids and armyworms under global change. *Scientific reports*, **5**, 18801 EP -.

Wardhaugh, C.W., Stone, M.J. & Stork, N.E. (2018). Seasonal variation in a diverse beetle assemblage along two elevational gradients in the Australian Wet Tropics. *Scientific reports*, **8**, 8559.

Wcislo, W.T. & Cane, J.H. (1996). Floral resource utilization by solitary bees (Hymenoptera. Apoidea) and exploitation of their stored foods by natural enemies. *Annual review of entomology*, **41**, 257–286.

Westrich, P. (1996). Habitat requirements of central European bees and the problems of partial habitats. *The conservation of bees* (ed. by A. Matheson, S.L. Buchmann, C. O´Toole, P. Westrich and I.H. Williams), pp. 1–16. Academic Press, London.

Whitehorn, P.R., O'Connor, S., Wackers, F.L. & Goulson, D. (2012). Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science (New York, N.Y.)*, **336**, 351–352.

Whitehorn, P.R., Wallace, C. & Vallejo-Marin, M. (2017). Neonicotinoid pesticide limits improvement in buzz pollination by bumblebees. *Scientific reports*, **7**, 15562.

Wielgoss, A., Tscharntke, T., Buchori, D., Fiala, B. & Clough, Y. (2010). Temperature and a dominant dolichoderine ant species affect ant diversity in Indonesian cacao plantations. *Agriculture, Ecosystems & Environment*, **135**, 253–259.

Wilby, A. & Thomas, M.B. (2002). Natural enemy diversity and pest control. Patterns of pest emergence with agricultural intensification. *Ecology Letters*, **5**, 353–360.

Williams, N.M. (2003). Use of novel pollen species by specialist and generalist solitary bees (Hymenoptera. Megachilidae). *Oecologia*, **134**, 228–237.

Williams, N.M., Crone, E.E., Roulston, T.'a.H., Minckley, R.L., Packer, L. & Potts, S.G. (2010). Ecological and life-history traits predict bee species responses to environmental disturbances. *Biological Conservation*, **143**, 2280–2291.

Williams, S.E., Bolitho, E.E. & Fox, S. (2003). Climate change in Australian tropical rainforests. An impending environmental catastrophe. *Proceedings of the Royal Society B: Biological Sciences*, **270**, 1887–1892.

Willmer, P.G. (1983). Thermal constraints on activity patterns in nectar-feeding insects. *Ecological Entomology*, **8**, 455–469.

Willmer, P.G. & Stone, G.N. (2004). Behavioral, ecological, and physiological determinants of the activity patterns of bees. *Advances in the Study of Behavior*, **34**, 347–466.

Wolda, H. & Roubik, D.W. (1986). Nocturnal bee abundance and seasonal bee activity in a Panamanian Forest. *Ecology*, **67**, 426–433.

Wolda, H. (1988). Insect seasonality. Why? *Annual Review of Ecology and Systematics*, **19**, 1–18.

Wolda, H. (1992). Trends in abundance of tropical forest insects. *Oecologia*, **89**, 47–52.

Wright, S.J., Muller-Landau, H.C. & Schipper, J. (2009). The future of tropical species on a warmer planet. *Conservation biology : the journal of the Society for Conservation Biology*, **23**, 1418–1426.

Yang, W., Seager, R., Cane, M.A. & Lyon, B. (2015). The annual cycle of East African precipitation. *Journal of Climate*, **28**, 2385–2404.

Yule, G.U. (1925). A mathematical theory of evolution, based on the conclusions of Dr. J. C. Willis, F.R.S. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **213**, 21–87.

Zanette, L.R.S., Soares, L.A., Pimenta, H.C., Gonçalves, A.M. & Martins, R.P. (2004). Nesting biology and sex ratios of *Auplopus militaris* (Lynch-Arribalzaga 1873) (Hymenoptera Pompilidae)*. *Tropical Zoology*, **17**, 145–154.

Zeh, D.W., Zeh, J.A. & Bonilla, M.M. (2005). *Wolbachia*, sex ratio bias and apparent male killing in the harlequin beetle riding pseudoscorpion. *Heredity*, **95**, 41–49.

Zurbuchen, A., Cheesman, S., Klaiber, J., Müller, A., Hein, S. & Dorn, S. (2010a). Long foraging distances impose high costs on offspring production in solitary bees. *The Journal of animal ecology*, **79**, 674–681.

Zurbuchen, A., Landert, L., Klaiber, J., Müller, A., Hein, S. & Dorn, S. (2010b). Maximum foraging ranges in solitary bees. Only few individuals have the capability to cover long foraging distances. *Biological Conservation*, **143**, 669–676.

Author Contributions

Chapter II has been submitted to Journal of Biogeography as: Mayr, A.V.*, Peters, M.K., Eardley, C.D., Renner, M.E., Röder, J., Steffan-Dewenter, I. (under review). Climate and food resources shape species richness and trophic interactions of cavity-nesting Hymenoptera. *Correspondence author

Participated in	Author Initials Responsibility decreasing from left to right		
Study design and methodology	AVM/MKP/ISD		
Data collection	AVM		
Identification of bees	AVM/CDE		
Identification of other Hymenoptera	AVM		
Identification of nests	AVM		
Identification of plants	AVM/MR		
Spider data	JR		
Data analysis and interpretation	AVM	MKP	ISD
First draft of manuscript	AVM		
Editing of manuscript	AVM	MKP	ISD

Acknowledgements

We thank the Tanzanian Commission for Science and Technology (COSTECH) and the Tanzanian Wildlife Research Institute (TAWIRI) for their consistent support and granting the necessary research permits (COSTECH 2013-315-ER-96-44, 2014-295-NA-96-44, 2015-177-NA-96-44). We are very thankful to Daudi Lusiba, Johannes Silvel and Raymond Vitus and all other Tanzanian field assistants for their persistent support in the field. Moreover, we especially thank Ann-Kathrin Bröger, Susanne Schiele and Sophia Hochrein for their valuable inputs and enthusiastic work on the identification of trap nests and data management. We also thank all student helpers for their help with the identification of trap nests. We are very grateful for the allocation of moth data by Maria Hellbig-Bonitz and Christina Behler, Michael Haas for the provision of spider data and David Schellenberger-Costa for his help with plant identification.

Funding

This study was funded by the Deutsche Forschungsgemeinschaft (DFG) within the framework of the Research Unit FOR1246 (Kilimanjaro ecosystems under global change: linking biodiversity, biotic interactions and biogeochemical processes, https://www.kilimanjaro.biozentrum.uni-wuerzburg.de) and partly by Scientia of the University of Würzburg.

Antonia V. Mayr	AR PD Dr. Marcell K. Peters
Dr. Connal D. Eardley	Marion E. Renner
Juliane Röder	Prof. Dr. Ingolf Steffan-Dewenter

Chapter III has been submitted to Ecography as: Mayr, A.V.*, Requier, F., Peters, M.K., Steffan-Dewenter, I. (under review). Seasonal variation in the ecology of tropical cavity-nesting Hymenoptera. *Correspondence author

Participated in	Author Initials Responsibility decreasing from left to right		
Study design and methodology	AVM/MKP/ISD		
Data collection	AVM		
Identification of traits	AVM		
Data analysis and interpretation	AVM	FR	MKP/ISD
First draft of manuscript	AVM		
Editing of manuscript	AVM	ISD/FR	MKP

Acknowledgements

We thank the Tanzanian Commission for Science and Technology (COSTECH) and the Tanzanian Wildlife Research Institute (TAWIRI) for their consistent support and granting the necessary research permits (COSTECH 2013-315-ER-96-44, 2014-295-NA-96-44, 2015-177-NA-96-44). We are very thankful to Daudi Lusiba, Johannes Silvel and Raymond Vitus and all other Tanzanian field assistants for their persistent support in the field. Moreover, we especially thank Ann-Kathrin Bröger, Susanne Schiele and Sophia Hochrein for their valuable inputs and enthusiastic work on the identification of trap nests and data management. We also thank all student helpers for their help with the collection of trap nest data.

Funding

This study was funded by the Deutsche Forschungsgemeinschaft (DFG) within the framework of the Research Unit FOR1246 (Kilimanjaro ecosystems under global change: linking biodiversity, biotic interactions and biogeochemical processes, https://www.kilimanjaro.biozentrum.uni-wuerzburg.de) and partly by Scientia of the University of Würzburg.

Antonia V. Mayr

Dr. Fabrice Requier

AR PD Dr. Marcell K. Peters

Prof. Dr. Ingolf Steffan-Dewenter

Chapter IV is yet to be submitted as: Mayr, A.V.*, Keller, A., Peters, M.K., Grimmer, G., Krischke, B., Geyer, M., Schmitt, T., Steffan-Dewenter, I.. Cryptic species and hidden interactions of halictine bees along an elevational gradient. *Correspondence author

Participated in	Author Initials Responsibility decreasing from left to right		
Study design and methodology	AVM/AK/TS	ISD	MKP
Data collection	AVM		
Processing of bees	AVM		
Genetic lab work	GG/BK		
CHC analysis	MG		
Data analysis and interpretation	AVM/AK	TS	ISD/MKP
First draft of manuscript	AVM		
Editing of manuscript	AVM	AK/ISD	TS/MKP

Acknowledgements

We thank the Tanzanian Commission for Science and Technology (COSTECH) and the Tanzanian Wildlife Research Institute (TAWIRI) for their consistent support and granting the necessary research permits (COSTECH 2015-177-NA-96-44). We are very thankful to Raymond Vitus and Zakaria Mwanga for their persistent support in the field. We are grateful to Norbert Schneider for solving practical challenges in working under field-laboratory conditions and we also thank Dr. Ruth Castillo Cajas and Doris Waffler for their support in running GC/MS samples.

Funding

This study was funded by the Deutsche Forschungsgemeinschaft (DFG) within the framework of the Research Unit FOR1246 (Kilimanjaro ecosystems under global change: linking biodiversity, biotic interactions and biogeochemical processes, https://www.kilimanjaro.biozentrum.uni-wuerzburg.de) and partly by Scientia of the University of Würzburg.

Antonia V. Mayr

AOR PD Dr. Alexander Keller

AR PD Dr. Marcell K. Peters

Gudrun Grimmer

Beate Krischke

Mareen Geyer

Prof. Dr. Thomas Schmitt

Prof. Dr. Ingolf Steffan-Dewenter

Publication List

Publications as part of the doctoral thesis

Mayr, A.V., Peters, M.K., Eardley, C.D., Renner, M.E., Röder, J., Steffan-Dewenter, I. (under review). Climate and food resources shape species richness and trophic interactions of cavity-nesting Hymenoptera. Journal of Biogeography

Mayr, A.V., Requier, F., Peters, M.K., Steffan-Dewenter, I. (under review). Seasonal variation in the ecology of trap-nesting Hymenoptera at Mt. Kilimanjaro. Ecography

Mayr, A.V., Keller, A., Peters, M.K., Grimmer, G., Krischke, B., Geyer, M., Schmitt, T., Steffan-Dewenter, I. (in preparation). Cryptic species and hidden interactions of halictine bees along an elevational gradient.

Additional publications

Peters MK, Hemp A, Appelhans T, Becker JN, Behler C, Classen A, Detsch F, Ensslin A, Ferger SW, Frederiksen SB, Gebert F, Gerschlauer F, Gütlein A, Helbig-Bonitz M, Hemp C, Kindeketa WJ, Kühnel A, **Mayr AV**, Mwangomo E, Ngereza C, Njovu HK, Otte, Pabst H, Renner M, Röder J, Rutten G, Schellenberger Costa D, Sierra-Cornejo N, Vollstädt MGR, Dulle HI, Eardley CD, Howell KM, Keller A, Peters RS, Ssymank A, Kakengi V, Zhang J, Bogner C, Böhning-Gaese K, Brandl R, Hertel D, Huwe B, Kiese R, Kleyer M, Kuzyakov Y, Nauss T, Schleuning M, Tschapka M, Fischer M, Steffan-Dewenter I (2019). Climate-land use interactions shape tropical mountain biodiversity and ecosystem functions. *Nature* 568: 88-92.

Albrecht J, Classen A, Vollstädt MGR, **Mayr A**, Mollel NP, Schellenberger Costa D, Dulle HI, Fischer M, Hemp A, Howell KM, Kleyer M, Nauss T, Peters MK, Tschapka M, Steffan-Dewenter I, Böhning-Gaese K, Schleuning M (2018) Plant and animal functional diversity drive mutualistic network assembly across an elevational gradient. *Nature Communications* 9: 3177.

Peters MK, **Mayr A**, Röder J, Sanders NJ (2014). Variation in nutrient use in ant assemblages along an extensive elevational gradient on Mt Kilimanjaro. *Journal of Biogeography* 41: 2245–2255.

Presentations and posters

03/2019 **Mayr A.**, Peters M, Steffan-Dewenter I: "Climate and food resources shape species richness and trophic interactions of cavity-nesting Hymenoptera on Mt. Kilimanjaro". Talk, Macroecology, Würzburg, Germany

03/2018 **Mayr A**, Peters M, Keller, A., Schmitt, T., Steffan-Dewenter I: "Macro-ecological determinants of halictine bee-microbiomes". Talk, Annual Meeting of the Society for Tropical Ecology (GTÖ-Conference), Paris, France

12/2017 **Mayr A**, Peters M, Steffan-Dewenter I: "Diversity and interactions in host-antagonist systems along climate and resource gradients at Mount Kilimanjaro". Talk, Ecology Across Borders (EAB conference), Gent, Belgium

06/2016 **Mayr A**, Peters M, Steffan-Dewenter I: "Bees, wasps and their antagonists along an elevational gradient at Mount Kilimanjaro: effects of climate and land use". Poster, Annual Meeting of the Association for Tropical Biology and Conservation (ATBC conference), Montpellier, France

09/2013 **Mayr A**, Steffan-Dewenter I, Peters M: "The influence of altered temperature and the intensification of coffee cultivation on the functionality on ant communities". Talk, Annual Meeting of the Ecological Society of Germany (GFÖ conference), Potsdam, Germany

04/2013 **Mayr A**, Peters M, Steffan-Dewenter I: "The influence of altered temperature and the intensification of coffee cultivation on the functionality of ant communities". Talk, Annual Meeting of the Society for Tropical Ecology (GTÖ-Conference), Vienna, Austria

09/2012 **Mayr A**, Peters M, Steffan-Dewenter I: "Influence of habitat destruction and land use intensification of coffee plantations on ant communities on Mt. Kilimanjaro". Poster, Annual Meeting of the Ecological Society of Germany (GFÖ-Conference), Lüneburg, Germany

Acknowledgements

This thesis would not have been possible with the support, trust and love of soo many people…

…I am very grateful to Ingolf Steffan-Dewenter for the opportunity to work in the tropics and on such a beautiful and exciting mountain! I thank you for giving me the freedom also to develop my own ideas, for your awareness of my pressure for the final sprint and for reading my manuscripts very quickly (and even in your free time). I thank you most of all for your support when I was sick in Tanzania and your trust that I knew best what helped me in this situation. And thank you very much that I got the chance to go again and again!

…I thank Alexandra-Maria Klein for taking the time and effort for being part of my thesis committee.

…I thank my co-supervisor Marcell, for nice time together in the field, your valuable ideas about data interpretation, advice on writing manuscripts and statistical analyses and your uncomplicated way which makes it nice to work with you.

…I am grateful to Alex and Thomas for coming up with the idea of the *Lasioglossum* project and making it possible. It was always very motivating to discuss with you about the project and your excitement was contagious, Thomas. I especially thank Alex for his reliable advice, patience and time for spending hours and hours with me, working with R, discussing different topics and analysing the different datasets!

…I thank Fabrice for taking over the statistical supervision of my second manuscript and your patient help through the jungle of the trap-nest dataset. I think without your help I would have given up and looked for a different topic to analyse.

…My special thanks to my field assistants Daudi, Raymond, Johannes, Jumanne and Zakaria and to many more porters in Tanzania. I am incredibly grateful for the nice time together in the field with many funny moments, for your motivation, your interest, patience and care!!!

…Among many people working at Nkweseko, I thank specifically Wilbard, Nelson, Mama Tuma, Vero, and Steve for supporting my fieldwork with either food or driving. I especially thank Wilbard for creatively solving small and major issues, giving me the opportunity to comprehend Tanzanian mentality that I did not understand without you, inviting me home, your friendship, and your care when I was sick.

…I am very grateful to my Hiwis Anna, Anna, Ann-Kathrin, Charlotte, Christopher, Clemens, Janina, Kaddl, Laura, Leopold, Marc, Matthias, Nicole, Patrick, Philipp, Sandra, Sindy, Sophia, Verena, without you I would have never managed the amount of samples! Especially I want to thank Sindy, through your structured thinking you have created a decisive contribution of our trap-nest cosmos, Anna, for your fast and accurate work and Sophia and Kaddl, you have shown as much commitment as if it were your own projects; with you I could discuss any challenging issue and with you I had such a great, funny time! Thank you so much!!!

…I am also very thankful to Norbert, with you I did not only enjoy to talk about “god and the world”, but you also spend a lot of hours with my trap nests and greatly improved my work with your smart and practical ideas for particularities in working in the tropics.

…Also special thanks to Gudrun and Beate! I thank you a lot for being so patient and persistent with my *Lasioglossum* project and not losing the motivation to get as many DNA sequences as possible! I really enjoyed working with you in the lab! I also thank you, Mareen, for the analysis of CHC and your nice and reliable nature.

…I would also like to thank those who support the department administratively or technically. Birgit, for your compassion during all the years and Michaela, for your uncomplicated way in solving annoying bureaucratical issues. Thomas, for fixing computer problems despite the mess at my desk ;-), Amy, for instant (!) GIS help and your nice and friendly nature, Doris, for quickly running the GC/MS analysis of my bees and wasps. The department and work of PhD students would not work without you!

…A big thank to all my nice collegues in the department. I especially thank my former and recent office mates Sarah, Alice, Veronika, Bea, Fabienne and Myrthe for tolerating my chaos in a very relaxed manner! I thank you, for your help, advice and nice distractions whenever needed. I further thank you, Sarah and Bea, for helping me getting my 16 boxes of trap nests on the plane. I also thank Elena and Friederike for sharing scripts and your patient help with R. Anytime I needed breaks for chatting, I always felt welcome in your office.

…I also want to thank my Bachelor and Master students Ruth and Sophia. I thank you for your motivation and interest in your topics. I learned a lot about the ‘other side’ as supervisor and I am proud of how well you have completed your work!

…I thank Connal, for helping me and sharing your experience in the identification of bees. I enjoyed the joint working time very much! I further thank Eva (bees) and Daria (flies) for your help with identification, but above all, your friendship! I feed on each encounter with you.

…I further thank the Tanzanian authorities for permitting my research at Mt. Kilimanjaro, specifically the Tanzanian Commission for Science and

Technology (COSTECH), the Tanzanian Wildlife Research Institute (TAWIRI) and the Mount Kilimanjaro National Park (KINAPA).

…the funding of my PhD thesis was provided by the German Research Foundation (DFG), the woman promotion program of the Biocentre and a Scientia scholarship (funding program for women in science). I thank Ricarda Scheiner for your effort and advice for funding opportunities, I further thank the Scientia mentoring program for fruitful workshops and meetings, the coordinator Rose Liebert and my mentor Barbara Sponholz for your advice and support outside the department.

…I greatly thank Katrin, Franziska, Vanessa, Isa and Anja for your company in our self-founded writing and potential analysis groups beyond the Scientia mentoring program. The certainty of not sitting alone at my desk has motivated me tremendously to finish my work quickly. And that is why I especially enjoyed the final sprint of the writing phase, even though it is usually the most exhausting part of the thesis. I enjoyed your company and black humour so much that I already think about new writing projects to stay part of the group ;-). Thank you for lending ears, giving advice and wake-up calls and making me laugh!

…I warmly want to thank those people who influenced me on my way as biologist, during my studies and beyond and strengthened my love of ecology and tropical biology. I deeply want to thank Brigitte for passing my way. I learned a lot from you, scientifically and humanly and I will always remember that there is no need to treat people differently, depending on their position. I miss you and I wished you could see that I finally managed to finish my thesis… I thank Prof. Linsenmair for sharing hauntingly his knowledge about tropical biology and being always a scientific paragon. I thank Frauke for inspiring talks about conservation. And I deeply thank Dieter, for your empathetic, gracious kind, great, thrilling teaching and being always there for me whenever I needed advice!

…I deeply thank Kalli and Suse, for our nice lunch breaks under the apple tree- what an honour to share the lunch break with friends! I thank you, Suse, for your great experience and help with the trap nests, for rescuing me when I arrived with all my luggage in Frankfurt and for the great effort and trouble with getting

this big amount of trap-nest data organised into Excel. And thank you so much for your direct honest way, your open ear and personal advice. Kalli, I deeply thank you for our scientific discussions and your smart, but also practical ideas, proof-reading parts of my thesis, even in stressful times for yourself, our joint path and our nightly conversations at the university above all what the heart desires!

…I am very grateful to my friends Ruth, Max, Sophia and Vanessa for proof-reading parts of my thesis. Max, you incredibly encouraged and motivated me when I was at my lowest point. Thank you very much for your nice words! Ruth, I utterly thank you for not becoming tired of proof-reading parts of my thesis. You helped me so much, especially in the last time of writing and I think without your opinion, it would have taken me much longer to hand in my thesis. I deeply value your critical way and would always ask you if I would need an honest answer. Now I am looking so much forward to our joint unemployment. I greatly thank you for being there- in every possible manner!

…I deeply thank my fellows during fieldwork. I thank Adi, Anna, Armin, David, Doro, Ema, Flo, Friederike, Hannes, Hawa, Henry, Insa, Jerome, Joscha, Marion, Max, Natalia, Samuel, Sophia, Tim, Ulli for wonderful, funny, but also profound memories in Tanzania. I especially thank those who accompanied me in the worst nights of my life. I greatly thank Joscha and Flo for cheering me up and staying up with me several nights, Anna, Natalia and Hannes for taking care of me, I thank David for the probably longest massage ever done in front of an hospital, Doro and Ulli for organising medical and practical issues, Henry, for taking care when I was in hospital and above all, Marion. I infinitively thank you for your 100% countability. You are not even thinking about own plans, when it comes to taking a spontaneous trip back to Germany- then, you are just grabbing your passport and jacket and we are ready to go. Thank you for your unlimited selfless friendship in every moment!!!

…I am very grateful to my flatmate Simon, you did not only manage that I got up (which is quite a hard job), but we also enjoyed nearly every morning breakfast together, which kept my mood on a high level during the whole day and motivated me for work. Thank you for your patience to listen to the same stories all over again, your nice surprises, your friendship and your open and kind nature!

…A big thank you to all friends and family members who are not listed in specific here, escorting me on my way and providing distraction, motivation and opinions- that is, what is strengthening my back!

…A heartfelt thank to my parents and my sister. You raised me, my curiosity and my love of nature. I know that you suffered, either, because I went to Tanzania many times and I didn't make you worry any less about me at any point, or, because I withdrew and had little time, in order to better concentrate on my thesis. Thank you so much Mama, for your incredible willpower and strength, so that I did not feel alone during my greatest fears in Tanzania. Thank you, Papa, for showing me animals and plants and for making me aware of the wonders of nature since I was a little child on which my love of nature is built on today. Thanks to my sister Viola, you have always supported my plans for abroad, calmed down our parents and taught me a lot about myself. I also warmly thank my grandmother, you always showed interest about my work, bees and the Kilimanjaro and supplied me with articles. I know you wanted to see me finish and I really hope you can! Thank you all for your trust, patience and love!!!

…And finally, I thank you, Achim, from the bottom of my heart. With you, I could always feel free enough to go to Tanzania without a hint of a bad conscience. Despite the risks, you've left it all to my responsibility to decide for myself and you granted me everything. Thank you, for keeping my back extremely free during the main writing phase and doing all the annoying stuff at home alone: washing, cleaning, buying food and cooking! You helped me formatting this thesis and you were at my side in the last exhausting nights, although you had to work the next mornings. Thank you, for going with me through good and evil and for your solid love!!!